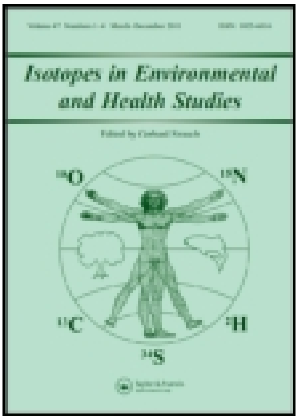

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Latitudinal distribution of nitrogen isotopic composition in suspended particulate organic matter in tropical/subtropical seas

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Natural nitrogen isotopic composition ($\delta^{15}N$) of suspended particulate organic matter (POM) and nitrogen fixation rates via $^{15}N_2$ assay were measured in surface waters along 120° E from 30° N to 30° S in the Asian marginal seas (the East/South China Seas and the Sulu/Celebes/Java Seas) and the northeastern Indian Ocean in November–December 2005 and March 2006. The POM $\delta^{15}N$ values ranged from −1.8 to 12.2‰ with an average of 3.6‰ and showed a decreasing trend towards the equator in both hemispheres. In parallel, the measured N_2 fixation rates showed an increase from the subtropical to the tropical seas. This implies that a higher contribution of ^{15}N-depleted POM was derived from enhanced N_2 fixation. Water temperature and the stability of water column were partly responsible for the observed variations in nitrogen fixation. The large-scale spatial variations in suspended POM $\delta^{15}N$ and N_2 fixation rates suggest that the suspended POM $\delta^{15}N$ may be a potential indicator of nitrogen fixation in surface waters in tropical/subtropical seas.

Keywords: Asian marginal seas; isotope biochemistry; isotope ecology; nitrogen-15; N_2 fixation; northeastern Indian Ocean

1. Introduction

N_2 fixation in pelagic environment plays a key role in marine nitrogen cycle, because it does not only relieve N deficiency in the open ocean, but also controls the ocean nitrogen budget and the sequestration of atmospheric carbon dioxide [1,2]. In the tropical and subtropical surface oceans, N_2 fixation can be discernible by the nitrogen isotopic composition (in terms of $\delta^{15}N$) of suspended particulate organic matter (POM). The shifts in surface ocean POM $\delta^{15}N$ values have been primarily interpreted as a result of the changes in the isotopic composition of the source nitrogen used by the phytoplankton (nitrate versus N_2) and/or the isotopic fractionation (preferential uptake of ^{14}N) associated with the assimilation processes. N_2 fixation is a process that adds ^{15}N-depleted combined nitrogen to local nitrogen pool in contrast with the uptake of

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nitrate, which typically results in isotopically heavier particulate organic nitrogen (PON). This is due to the differences in both the isotopic compositions of inorganic nitrogen sources and the magnitudes of isotopic fractionation therein [3–5].

δ^{15}N signature of suspended POM has been widely used as an indicator for the contribution of N_2 fixation in the tropical and subtropical sea regions [6–10]. However, little is known about the large-scale pattern of suspended POM δ^{15}N in the tropical and subtropical Asian marginal seas and adjacent Indian Ocean, and direct measurements of N_2 fixation rate are even more lacking. Nevertheless, a few sparse studies have indicated N_2 fixation in some of these waters [11–17]. Here, we present the first large-scale spatial variations in suspended POM δ^{15}N and N_2 fixation rates in tropical and subtropical Asian marginal seas and the northeastern Indian Ocean along a latitudinal transect. The feasibility of suspended POM δ^{15}N as an indicator of nitrogen fixation in surface water was evaluated.

2. Sampling and methods

Samplings ($n = 36$) were conducted onboard R/V *Xuelong* along a latitudinal transect within 28° N–27° S across the East China Sea, South China Sea, Sulu Sea, Celebes Sea, Java Sea and the northeastern Indian Ocean during the cruise of the 22nd Chinese Antarctic Research Expedition (CHINARE) in November–December 2005 (NE monsoon season) and March 2006 (transition period between NE and SW monsoons), respectively (Figure 1 and Table 1). Surface seawater suspended POM samples were collected at all stations, while measurements of N_2 fixation rate were conducted at 18 stations (Table 1). Temperature and salinity were measured using thermometer

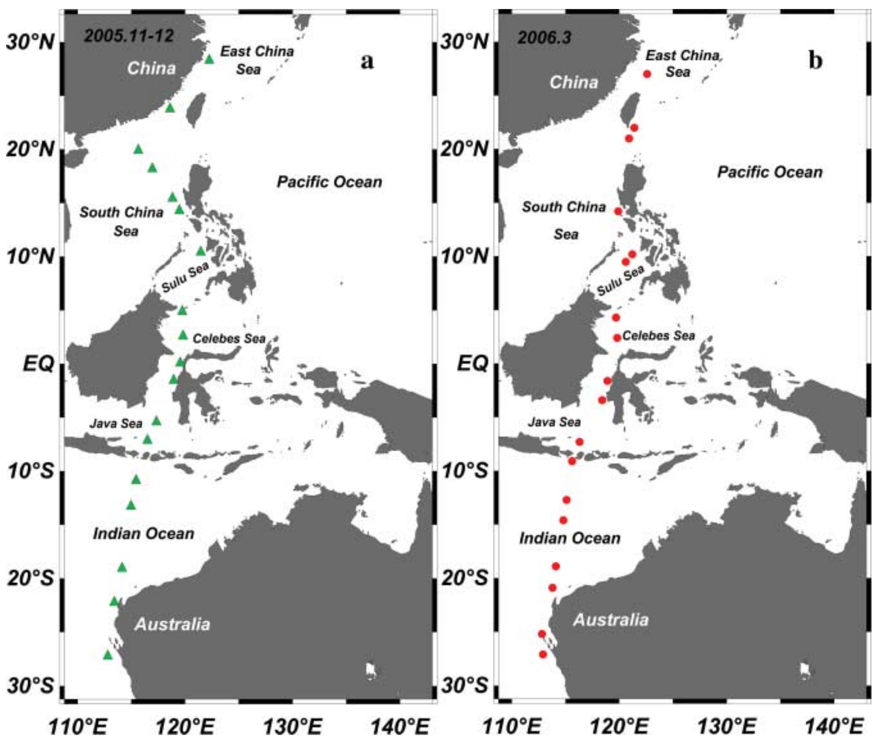

Figure 1. Maps showing sampling locations in the tropical and subtropical Asian marginal seas and the eastern Indian Ocean from 30° N to 30° S during (a) November–December 2005 and (b) March 2006.

Table 1. Sampling locations in the Asian marginal seas and the northeastern Indian Ocean along the 22nd CHINARE cruise track.

Region	Station	Latitude	Longitude (°E)	Sampling date (d/m/year)	Wind direction	Wind speed (kt)	T(°C)	PON (μmol dm^{-3})	δ^{15}N (‰)	N_2 fixation rate (nmol N m^{-3} h^{-1})
East China Sea	NJ-1	28.47° N	122.02	20 November 2005	NE	6	22.0	1.5	4.9	52
	NJ-36	27.00° N	122.60	23 March 2006	NE	7	19.2	1.6	0.6	nd
South China Sea	NJ-2	24.05° N	118.53	21 November 2005	NE	6	23.0	1.4	4.6	13
	NJ-3	20.13° N	115.53	25 November 2005	NE	5	26.8	0.6	4.6	0
	NJ-4	18.40° N	116.75	25 November 2005	NE	6	27.0	0.2	1.4	1
	NJ-5	15.47° N	118.80	26 November 2005	NE	2	30.0	0.3	1.2	6
	NJ-6	14.40° N	119.55	26 November 2005	NE	4	29.8	0.5	4.5	10
	NJ-33	14.20° N	119.90	21 March 2006	nd	nd	27.9	1.1	3.9	113
	NJ-34	21.00° N	120.90	22 March 2006	NW	3	27.6	1.1	2.6	18
	NJ-35	22.00° N	121.40	22 March 2006	Ely	4	27.9	1.2	3.0	31
Sulu Sea	NJ-7	10.61° N	121.37	27 November 2005	Nly	4	30.0	0.7	0.0	87
	NJ-31	9.50° N	120.60	20 March 2006	NE	5	28.7	1.2	−1.8	107
	NJ-32	10.20° N	121.20	20 March 2006	NE	3	28.2	1.2	−0.3	194
Celebes Sea	NJ-8	4.75° N	119.69	28 November 2005	SE	2	29.7	0.9	−1.1	39
	NJ-9	2.71° N	119.75	28 November 2005	Sly	4	30.2	0.6	−1.7	nd
	NJ-10	0.40° N	119.50	29 November 2005	Sly	3	29.8	1.1	1.2	170
	NJ-11	1.57° S	118.91	29 November 2005	SW	2	30.6	0.9	0.4	nd
	NJ-29	2.40° N	119.80	19 March 2006	NW	2	29.5	2.1	2.6	nd
	NJ-30	4.30° N	119.70	19 March 2006	NE	3	29.5	1.2	1.7	nd
	NJ-28	1.60° S	118.90	18 March 2006	NW	3	30.0	1.9	0.8	nd
Java Sea	NJ-12	5.30° S	117.21	30 November 2005	NW	3	30.5	0.6	2.2	nd
	NJ-13	6.97° S	116.40	30 November 2005	NW	4	30.7	0.8	0.4	85
	NJ-26	7.30° S	116.30	17 March 2006	NW	3	29.9	1.2	6.2	nd
	NJ-27	3.40° S	118.40	18 March 2006	Nly	3	29.9	1.4	3.3	nd
Indian Ocean	NJ-14	10.73° S	115.39	1 December 2005	S	3	29.5	0.7	10.1	nd
	NJ-15	13.25° S	114.98	1 December 2005	S	5	30.3	0.4	6.9	nd
	NJ-16	19.03° S	114.05	2 December 2005	SW	5	26.3	0.4	6.7	58
	NJ-17	22.21° S	113.48	3 December 2005	S	6	23.7	0.5	11.1	7
	NJ-18	27.03° S	112.91	4 December 2005	SW	5	21.7	0.4	12.2	19
	NJ-19	27.10° S	112.90	14 March 2006	SE	6	25.7	2.6	7.4	nd
	NJ-20	25.20° S	112.80	14 March 2006	Sly	6	26.5	1.4	5.7	nd
	NJ-21	20.90° S	113.80	15 March 2006	NE	2	29.7	1.6	8.9	nd
	NJ-22	18.90° S	114.10	15 March 2006	NE	3	29.9	0.7	3.7	nd
	NJ-23	14.60° S	114.80	16 March 2006	Nly	3	30.0	0.9	4.5	nd
	NJ-24	12.70° S	115.10	16 March 2006	NW	5	29.4	1.2	5.1	nd
	NJ-25	9.10° S	115.60	17 March 2006	NW	2	28.6	1.3	2.0	nd

Note: nd represents not determined. Ely, Nly and Sly represent easterly, northerly and southerly, respectively.

and salinity refractometer (ATAGO), respectively. No measurements of nutrient concentration or chlorophyll were conducted in the voyage. Weather information (wind direction and speed) during sampling was recorded by a shipboard Vaisala MILOS 500 automatic meteorological station (more detailed meteorological data are available on the CHINARE website: www.polar.gov.cn).

For measurements of natural ^{15}N abundance in suspended POM, seawaters (5 dm^3) were sampled on a precombusted (450°C, 2 h) Whatman GF/F membrane. For measurements of N_2 fixation rate [18], duplicate surface seawater samples were filled bubble-free into 600 cm^3 transparent glass bottles and spiked with 1.0 cm^3 $^{15}N_2$ (99 at% ^{15}N, Research Institute of Chemical Industry, China) via a septum using a gas-tight syringe (VICI Sample-Lok, 1 cm^3) and then sealed. The pressures across the septum were balanced. After gentle shaking, the bottles were placed in a deck incubator with flowing surface seawater for cooling under natural sunlight. After 24 h of incubation, N_2 fixation samples were filtered onto precombusted Whatman GF/F glass fibre filters. The filters containing POM were stored frozen (−20°C) while on the sea.

While in the land laboratory, PON content and ^{15}N abundance on the filters (with no acid treatment) were measured on a Finnigan MAT DELTA^{plus} XP isotope ratio mass spectrometer coupled to a Carlo Erba NC2500 elemental analyser (EA-IRMS). Nitrogen isotopic composition was presented as per mil deviation from standard in the conventional δ notation:

$$\delta^{15}\mathrm{N} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1000,$$

where R_{sample} and R_{standard} are the $^{15}N/^{14}N$ ratios of sample and the standard air N_2, respectively. The reproducibility of $\delta^{15}N$ measurements was better than 0.2‰.

Rates of N_2 fixation (NF, nmol N m^{-3} h^{-1}) were calculated as

$$\mathrm{NF}(\mathrm{nmol\ N\ m^{-3}h^{-1}}) = \frac{1}{\Delta t} \times \left(\frac{A_{\mathrm{PN}_f} - A_{\mathrm{PN}_0}}{A_{\mathrm{N}_2} - A_{\mathrm{PN}_0}}\right) \times \frac{\mathrm{PON}_0 + \mathrm{PON}_f}{2},$$

where Δt is the duration of incubation; PON is the concentration of PON; and A_{PNf}, A_{PN0} and A_{N_2} are the absolute abundance ratios of natural ($t = 0$) and final PON and the N_2 substrate, respectively. The values of $A_{\mathrm{PN_f}}$ and $A_{\mathrm{PN_0}}$ were provided by the EA-IRMS, and A_{N_2} values were calculated from the ^{15}N abundance of air N_2 and the solubility of N_2 gas in seawater [19].

3. Results

During sampling, northerly winds prevailed at most stations, and the wind speed in the semi-enclosed Indonesian Seas was relatively low compared with that in other regions in general (Table 1). Surface-suspended POM $\delta^{15}N$ values ranged from −1.8 to 12.2‰ (averaging 3.6‰), falling in the reported value range in the tropical and subtropical waters in the world oceans [7,17,20]. As shown in Table 1, POM was generally enriched in ^{15}N in the East China Sea, the South China Sea and the northeastern Indian Ocean (averaging 5.3‰), where the $\delta^{15}N$ values were similar to those reported for the other new N sources, nitrate supplied below the surface [21–23]. The maximum POM $\delta^{15}N$ values (∼10‰) were observed in the northeastern Indian Ocean. By comparison, isotopically light POM (−1.8 to 2.6‰, averaging 0.2‰) was observed in the low-latitude Sulu/Celebes Seas. At most of the remaining stations, POM $\delta^{15}N$ generally varied around 5‰.

N_2 fixation rates varied from 0 to 194 nmol N m^{-3} h^{-1} and fell in the range of the published results in the northeastern South China Sea and the Leeuwin Current coastal eddies (>30° S) in the eastern Indian Ocean [11,12]. Relatively high rates of N_2 fixation (>100 nmol N m^{-3} h^{-1}) were generally observed within a latitude range of 10° S–10°N, such as in the Sulu/Celebes Seas. To our knowledge, this is the first report of N_2 fixation rates in the Sulu/Celebes/Java Seas, although some past studies have revealed relatively high abundance of *Trichodesmium* spp. and *Richelia intracellularis* in these waters [24,25].

4. Discussion

Previous studies showed that the relatively low POM δ^{15}N values in the low-latitude oligotrophic sea regions are mainly due to the elevated significance of N_2 fixation [9,26,27]. The low POM δ^{15}N values in this study are similar to those found for POM in oligotrophic surface oceans, such as the Sargasso Sea and the central North Pacific, where N_2 fixation occurs actively [28–30]. The observed low POM δ^{15}N values are also similar to those reported for some well-known N_2 fixers, such as *Trichodesmium* spp. and diatom-symbiotic cyanobacteria [6,27]. It is worth noting that suspended POM δ^{15}N values above 10‰ are not common in the surface ocean. The exact reasons for such 'anomaly' in the shelf break off the west coast of Australia are not known. We speculate that the effect of denitrification (and subsequent upward supply of ^{15}N-enriched NO_3^- to the euphotic zone) along with lack of significant N_2 fixation may be a candidate. Indeed, both our results and those from the study in the Leeuwin Current waters (south of 30° S) neighbouring our stations revealed relatively low N_2 fixation rates [12]. As a result of preferential reduction of $^{14}NO_3^-$ during denitrification, δ^{15}N–NO_3^- may increase from ~5‰ in deep waters to 15–18‰ within the denitrifying layer core in the central Arabian Sea and the eastern tropical North Pacific [31,32]. However, studies on denitrification in this region are still few to date and thus prevent us from further discussion.

The most prominent feature of POM δ^{15}N is a decreasing trend towards the equator from the subtropics during both sampling periods in 2005 and 2006, suggesting that the observed latitudinal patterns may not be accidental (Figure 2(a)). In fact, similar large-scale POM δ^{15}N distributions have been found in the Atlantic and the North Pacific [7,9,20]. We suggest that the elevated contribution of N_2 fixation to the local combined N pool towards the low latitudes was responsible for the observed POM δ^{15}N distribution pattern, as elevated contribution of N_2 fixation generally results in ^{15}N-depleted signals. This idea is confirmed by the latitudinal distribution of N_2 fixation rate (Figure 2(b)). Dissolved N_2, the substrate for biological N_2 fixation, typically has a δ^{15}N value near 0‰, and the isotopic fractionation during N_2 fixation is relatively small, thus the organic nitrogen produced by N_2 fixation is only slightly depleted in ^{15}N compared with its substrate, with a δ^{15}N value of 0 to -1‰ relative to atmospheric N_2 [6]. By contrast, the major oceanic source of new N, nitrate, has a mean δ^{15}N value of ~5‰ [33]. Past studies have revealed that the Sulu/Celebes/Java surface waters are all highly oligotrophic even in monsoon seasons [25,34], implying that a large nitrogen isotopic fractionation during biological uptake of the combined nitrogen is unlikely and the subsequently produced POM will finally reflect the isotopic composition of the source nitrate supplied from below the euphotic zone [3,35]. Thus, ^{15}N-depleted POM in combination with relatively high rates of N_2 fixation point out that the Sulu/Celebes/Java Seas are more favourable for diazotrophs. By comparison, the POM δ^{15}N values around 5‰ at most stations in the East China/South China Seas and the waters between the Indonesian archipelago and the west Australia mainly reflect the isotopic composition of combined nitrogen, which is confirmed by the relatively low N_2 fixation rates. Hence, the large-scale latitudinal pattern of suspended POM δ^{15}N implies that the contribution from N_2 fixation increased from the subtropics to the tropics.

Indeed, some past studies have revealed latitudinal dependence of N_2 fixation in the tropical/subtropical oceans, where temperature probably plays an important role in modulating the large-scale distribution pattern of N_2 fixation [1,8,9,36]. Subtropical studies found that even during

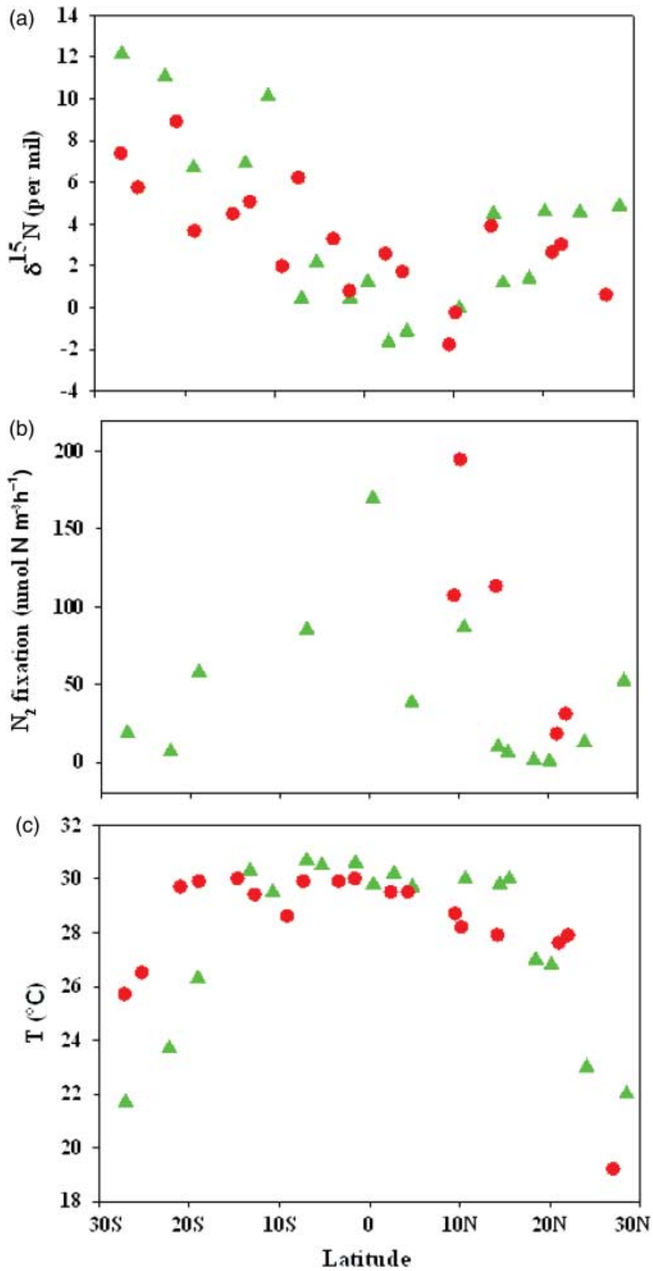

Figure 2. Latitudinal distributions of (a) POM δ^{15}N, (b) N_2 fixation rate and (c) temperature at the surface waters during the 2005 (▲) and 2006 (●) cruises.

the summer, when diazotrophs were relatively abundant and active, rates of N_2 fixation were still low compared with those in the tropical waters [4,8,37]. This idea was partly evidenced by the latitudinal distribution of sea surface temperature in this study (Figure 2(c)), which is consistent with the spatial pattern of N_2 fixation rate. Although the sea surface temperatures (19.2–30.7°C) at all the sampling stations generally fell in the reported temperature range for N_2 fixation, it is probable that warmer waters are more favourable for N_2 fixers (with a relatively high energy demand for the process) to flourish, thus adding stronger ^{15}N-depleted signals to the surface ocean. The large-scale variations in bioavailability of some N_2 fixation-limiting elements (such as iron and phosphorus) could also be important factors controlling N_2 fixation in the tropical and subtropical seas [8–10,38,39]. Compared with the northern South China Sea, high concentrations of dissolved iron were observed in the Sulu/Celebes Seas (1 versus 0.2 nM) [34,40]. This is consistent with the observed pattern of N_2 fixation rate in this study. In addition, key biological factors, such as the variations in diazotrophic composition in adaptation to the ambient environmental conditions, may also play a role in regulating N_2 fixation activity on a large spatial scale [36], but no concurrent analyses were conducted in this study.

It is interesting to note that relatively high rates of N_2 fixation were generally observed in the semi-enclosed Sulu/Celebes/Java Seas where wind speed was relatively low during the sampling period, revealing that water column stability may play a role in controlling N_2 fixation distribution. Past studies have shown that diazotrophs generally favour low wind in order to accumulate efficiently and flourish when other environmental conditions are also suitable [1,4]. The stability of water column in the tropical/subtropical Asian marginal seas and the Indian Ocean will be affected by the monsoon change. Studies have shown variability of N_2 fixation during monsoon cycles; however, the exact mechanism of monsoonal forcing is not clear [41,42].

5. Conclusions

The latitudinal distributions of surface POM $\delta^{15}N$ and the measured N_2 fixation rates across the Asian marginal seas and the northeastern Indian Ocean suggest the increased contribution of N_2 fixation towards the equator. Temperature may be a regulating factor in controlling N_2 fixation distribution, with warmer waters being more favourable for N_2 fixation. The low POM $\delta^{15}N$ values and the high N_2 fixation rates were observed in the semi-enclosed Sulu/Celebes/Java Seas where wind speed was relatively low during the sampling period, implying that the stability of water column may also affect marine N_2 fixation. Comprehensive studies combining diazotroph populations and bioavailability of limiting elements for N_2 fixation are needed to better elucidate the constraints of N_2 fixation in the tropical and subtropical seas.

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