

# Impact of glacial/interglacial sea level change on the ocean nitrogen cycle

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The continental shelves are the most biologically dynamic regions of the ocean, and they are extensive worldwide, especially in the western North Pacific. Their area has varied dramatically over the glacial/interglacial cycles of the last million years, but the effects of this variation on ocean biological and chemical processes remain poorly understood. Conversion of nitrate to  $N_2$  by denitrification in sediments accounts for half or more of the removal of biologically available nitrogen ("fixed N") from the ocean. The emergence of continental shelves during ice ages and their flooding during interglacials have been hypothesized to drive changes in sedimentary denitrification. Denitrification leads to the occurrence of phosphorus-bearing, N-depleted surface waters, which encourages  $N_2$  fixation, the dominant N input to the ocean. An 860,000-y record of foraminifera shell-bound N isotopes from the South China Sea indicates that  $N_2$  fixation covaried with sea level. The  $N_2$  fixation changes are best explained as a response to changes in regional excess phosphorus supply due to sea level-driven variations in shallow sediment denitrification associated with the cyclic drowning and emergence of the continental shelves. This hypothesis is consistent with a glacial ocean that hosted globally lower rates of fixed N input and loss and a longer residence time for oceanic fixed N—a "sluggish" ocean N budget during ice ages. In addition, this work provides a clear sign of sea level-driven glacial/interglacial oscillations in biogeochemical fluxes at and near the ocean margins, with implications for coastal organisms and ecosystems.

denitrification | nitrogen fixation | nitrogen isotopes | glacial cycles

Biological productivity in much of the ocean is limited by the supply of biologically available nitrogen ("fixed N") (1). Biological processes are central to the input and output of fixed N to and from the ocean:  $N_2$  fixation by cyanobacteria in surface waters appears to dominate the input of N to the ocean, whereas the main sink is biological reduction to  $N_2$  (generalized here as "denitrification") in sediments and in suboxic zones of the water column (2). Given this biologically determined input/output budget, the variation or constancy of the oceanic fixed N reservoir has broader implications for the potential of ocean life to regulate environmental conditions on a global scale. Because the "major nutrients" N and phosphorus (P) fuel the biological sequestration of  $CO_2$  in the deep ocean, changes in the oceanic fixed N reservoir have also been proposed as a driver of glacial/interglacial  $CO_2$  change (3, 4).

Sediment records show N isotopic evidence of reduced water column denitrification during the Last Glacial Maximum (LGM) and other cold phases of the glacial cycles relative to the current interglacial (the "Holocene") and past warm time intervals (5, 6). "Benthic" denitrification (that which occurs in seafloor sediments) is equally as or more important than water column denitrification in the removal of N from the global ocean, and it has been hypothesized to decrease during glacials (times of high land

ice volume) as well (7). This hypothesis is based on the generally rapid rate of denitrification in continental shelf sediments and on calculations that indicate the importance of shelf denitrification in the global ocean rate of denitrification (8). The continental shelves are characterized by high fluxes of organic matter to the sediments both because their shallow depth allows sinking matter to reach the bottom quickly and because the breakdown of organic matter in the shallow sediments returns nutrients immediately to the sunlit upper ocean. As a result, the nutrients supplied to the waters overlying the continental shelf drive multiple rapid cycles of productivity, sedimentation, and remineralization over its broad extent of shallow seafloor. During glacial maxima, the ~120-m decline in sea level converted the continental shelves into coastal land, removing much of this environment as a site of oceanic N loss. The greater mean depth and steepness of the seaward continental slope should render the slope far less efficient at returning the nutrients released from the sediments to the upper ocean. Thus, upon sea level lowering, the coastal environment would be less favorable as an environment for both coastal productivity and benthic N loss. However, because the direct impact of benthic N loss on the N isotopes is typically nil or very weak (9, 10), there have been, as yet, no direct tests of this hypothesis.

Since the first studies of the ocean N budget, it has been recognized that a balance is required between inputs (dominantly  $N_2$

## Significance

Biologically available nitrogen (fixed N) limits the fertility of much of the ocean. Of the processes that remove fixed N from the ocean, conversion to  $N_2$  in coastal sediments appears to dominate. This work provides the strongest data-based support for the long-standing hypothesis of changes in N loss along the ocean margin due to the cyclic drowning and emergence of the continental shelves. The data also imply strong local coupling of N loss to  $N_2$  fixation, the dominant N input to the ocean, thus suggesting a stable oceanic fixed N reservoir over glacial cycles. Finally, this work points to glacial/interglacial oscillations in the biogeochemical fluxes at and near the ocean margins that would have influenced the evolution of coastal species.

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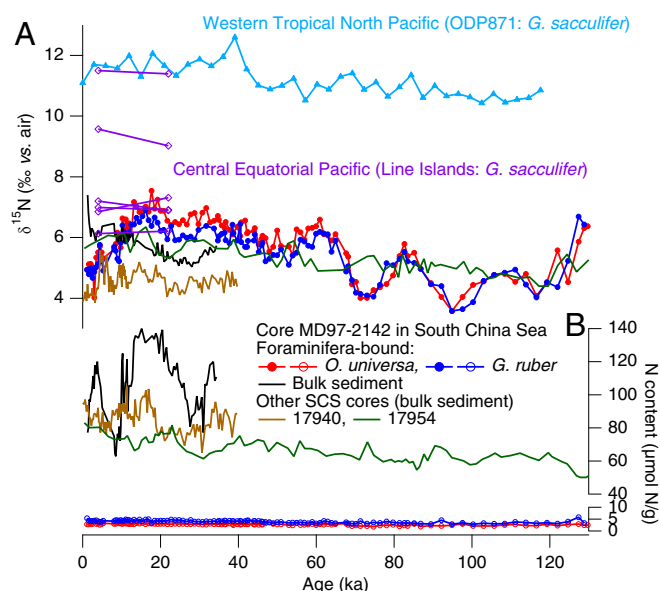
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**Fig. 2.** Compilation of the (A)  $\delta^{15}\text{N}$  and (B) N content of foraminifera-bound N and bulk sedimentary N records in the SCS and open Pacific. In SCS core MD97-2142, FB- $\delta^{15}\text{N}$  (filled circles) and N content (open circles) are shown for the planktonic foraminifera species *O. universa* (red circles) and *G. ruber* (blue circles) and for bulk sedimentary N (black line). Previously published bulk sedimentary  $\delta^{15}\text{N}$  and N content from SCS cores 17940 and 17954 are also shown (green and brown lines) (27, 28). One open Pacific data set is a last ice age/Holocene FB- $\delta^{15}\text{N}$  comparison in cores from along the Line Islands in the central equatorial Pacific (purple diamonds) (36). The latitudes of the five cores are 0.22°S, 1.27°N, 2.46°N, 2.97°N, 5.2°N, and 6.83°N. Each core corresponds to paired LGM and Holocene data, with the FB- $\delta^{15}\text{N}$  from both the LGM and the Holocene increasing northward from the equator; from the assemblage of cores, no LGM-to-Holocene difference is observed. A second open Pacific data set is a record of FB- $\delta^{15}\text{N}$  from the western tropical north Pacific [ODP Site 871 (5.56°N, 172.35°E); blue triangles]. This record shows no clear FB- $\delta^{15}\text{N}$  decrease from the last ice age to the Holocene, in contrast to SCS FB- $\delta^{15}\text{N}$ . The locations of the sediment cores are shown in Fig. S9.

range of the SCS. Therefore, the upward decline in nitrate  $\delta^{15}\text{N}$  observed in the SCS thermocline (Fig. 1C) is probably mostly generated within the SCS.

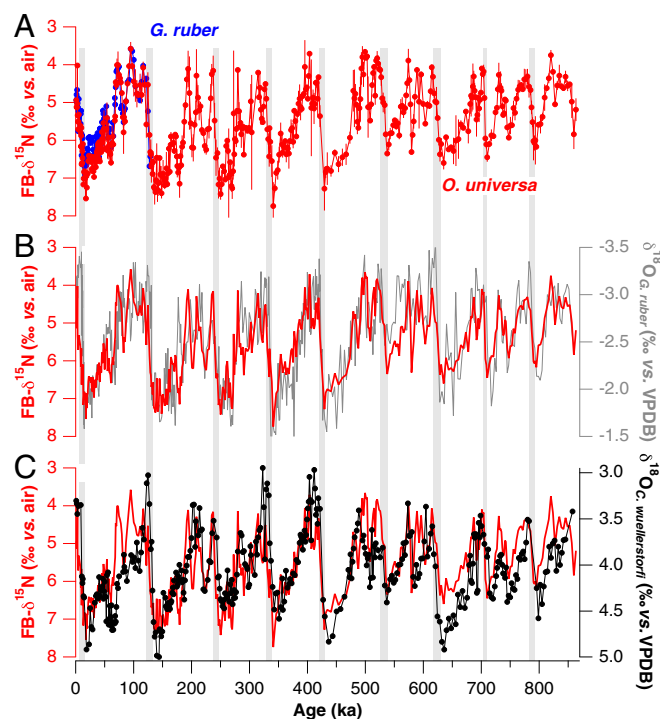
Nitrate from the shallow thermocline supplied by vertical mixing is the dominant N source to the tropical and subtropical surface ocean on an annual basis (25). Thus, the  $\delta^{15}\text{N}$  of the shallow thermocline nitrate is the dominant control on the  $\delta^{15}\text{N}$  of net biomass production in the surface ocean each year, which, in turn, sets the  $\delta^{15}\text{N}$  of the various species of planktonic foraminifera, the shells of which can be analyzed for the  $\delta^{15}\text{N}$  of their fossil-bound organic N (26). As a consequence, foraminifera-bound N has a lower  $\delta^{15}\text{N}$  in the modern SCS than in, for example, most of the equatorial Pacific (Fig. 2). Moreover, a higher rate of  $\text{N}_2$  fixation in the SCS would cause a further decline in foraminifera-bound  $\delta^{15}\text{N}$  (FB- $\delta^{15}\text{N}$ ), whereas slower  $\text{N}_2$  fixation would cause a  $\delta^{15}\text{N}$  rise.

## Results and Discussion

Here we report a record of FB- $\delta^{15}\text{N}$  in the SCS over the last 860 ky, covering eight major glacial cycles (*Methods*). The sediment core is from site MD97-2142 on the slope off Palawan Island (Fig. 1A, 12°41'N, 119°27'E, water depth of 1,557 m, sedimentation rate of 10 cm/ky, age model shown in Fig. S2). The full record uses a single planktonic species, *Orbulina universa*. To test the generality of the *O. universa* FB- $\delta^{15}\text{N}$  record, the FB- $\delta^{15}\text{N}$  of *Globigerinoides ruber* was also analyzed over the last glacial cycle (back to ~125 ka). FB- $\delta^{15}\text{N}$  is expected to be similar for these two euphotic zone-dwelling species (26), and the data fit this expectation (Figs. 2 and 3). Slightly lower  $\delta^{15}\text{N}$  is

observed for *G. ruber* than for *O. universa* during the last ice age, with an average offset of 0.39‰ for 20 ka to 60 ka compared with 0.25‰ for the entire overlapping period (Fig. 2). The same sense of divergence (with the  $\delta^{15}\text{N}$  of *O. universa* greater than that of *G. ruber*) is also observed in LGM samples from the Caribbean Sea (13), where it was tentatively interpreted to provide secondary support of the idea of reduced  $\text{N}_2$  fixation during the LGM (13); a similar explanation may apply in the SCS. In any case, the changes in interspecies FB- $\delta^{15}\text{N}$  difference are minor relative to the FB- $\delta^{15}\text{N}$  changes shared by the two species.

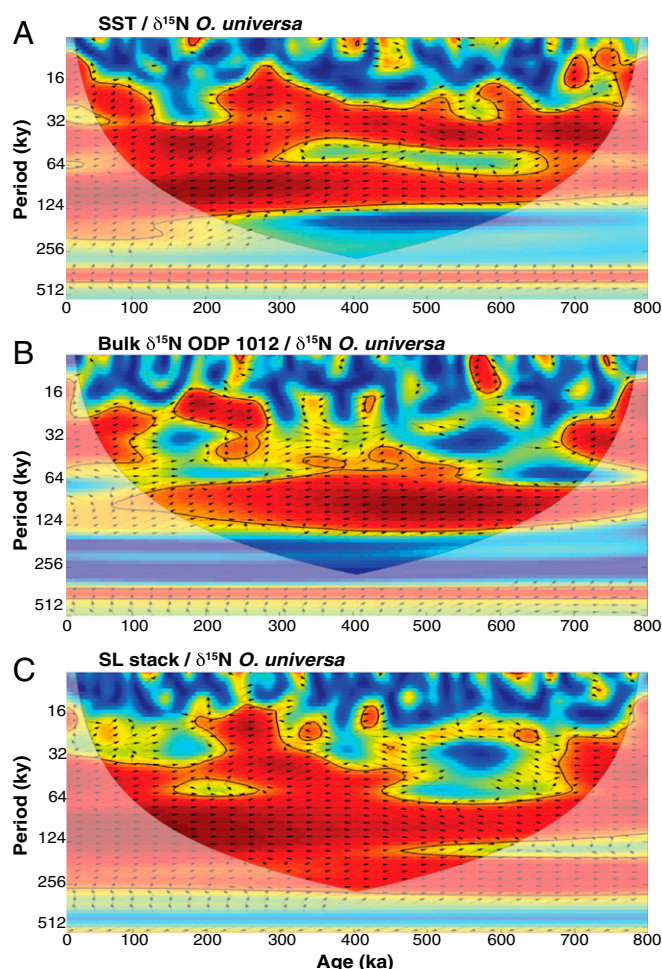
The FB- $\delta^{15}\text{N}$  records have no clear correspondence with the bulk sediment records from the SCS, which do not show systematic glacial/interglacial changes (Fig. 2). Several of the existing bulk sediment records from the SCS are substantially dissimilar from one another (Fig. 2A) (27). Moreover, although foraminifera-bound N content is low and stable over glacial cycles, bulk sediment N content varies substantially over time and across records (Fig. 2B). Similar observations regarding SCS bulk sedimentary N records have previously been attributed to diagenesis and to multiple sources of N to the bulk sediment (28). Variation in terrigenous input at our study site has been documented to be associated with sea level change over the glacial cycles, for example, with higher concentrations of *n*-alkanes coinciding with lower sea level (29). A general disconnect between FB- $\delta^{15}\text{N}$  and bulk sediment  $\delta^{15}\text{N}$  has been observed in the Caribbean Sea as well, where sedimentological data also point to terrestrial/shelf N inputs to the bulk sediments, especially in glacial intervals (13, 14). These findings argue against the utility



**Fig. 3.** Records of planktonic FB- $\delta^{15}\text{N}$  and planktonic and benthic foraminiferal calcite  $\delta^{18}\text{O}$  over the last eight glacial cycles. FB- $\delta^{15}\text{N}$  is plotted decreasing upward, such that  $\text{N}_2$  fixation increases upward. FB- $\delta^{15}\text{N}$  of *O. universa* (A–C, red) at MD97-2142 is similar to the FB- $\delta^{15}\text{N}$  of *G. ruber* (A, blue) during the last 130 ky. Error bars indicate 1 SD of the oxidation replicates (*Methods*). FB- $\delta^{15}\text{N}$  is highly correlated with the  $\delta^{18}\text{O}$  of *G. ruber* (B, gray) (64) and of *C. wuellerstorfi* (C, black). The glacial/interglacial change in the  $\delta^{18}\text{O}$  of the benthic foraminifera *C. wuellerstorfi* is typically considered to be mostly due to change in global ice volume (and hence sea level), with deep-water temperature change having a smaller effect. The light gray bars indicate the glacial terminations.







**Fig. 5.** Cross-wavelet coherence and phase relationship among records of  $N_2$  fixation, sea level, sea surface temperature, and water column denitrification. Squared wavelet coherence between two time series was computed using the methods of ref. 68. The 95% confidence level against red noise was calculated using the Monte Carlo method and is shown as a thick contour that encloses the significant sections. The light shading indicates the region possibly influenced by edge effects. Black arrows indicate the phase relationship between the two time series, with in-phase pointing right,  $FB-\delta^{15}N$  leading a given climate variable pointing down, and  $FB-\delta^{15}N$  lagging pointing up. The different records have been interpolated to an evenly spaced time series of 2 ky before the spectral analysis. (A) The SST record (29) from the same sediment core has high coherence with, but leads, the  $FB-\delta^{15}N$  of *O. universa* by around 4 ky during the last 400 ky at the dominant 41- and 100-ky bands, as indicated by the direction of the arrows, which is inconsistent with a causal connection in this case. (B) The bulk  $\delta^{15}N$  record from California margin (37) is coherent with  $FB-\delta^{15}N$  in the SCS at the period near 100 ky. (C) The sea level record stack (47–49) shows high coherence with  $FB-\delta^{15}N$  at a wide range of frequencies.

A changing rate of  $N_2$  fixation is the sole remaining mechanism with the potential to explain the cycles in  $FB-\delta^{15}N$  at this site in the SCS. We conclude that the  $\delta^{15}N$  of the shallow thermocline nitrate was lowered less by  $N_2$  fixation during glacials, due to an ice age reduction in the rate of this process. The amplitude of the SCS  $\delta^{15}N$  rise in the glacials is similar to that observed in the tropical western North Atlantic (13, 14), where  $N_2$  fixation also has a strong imprint on thermocline nitrate  $\delta^{15}N$  (20). The 3‰ amplitude of the glacial/interglacial  $FB-\delta^{15}N$  change in the SCS is comparable to the largest regional declines in ocean nitrate  $\delta^{15}N$  attributed to  $N_2$  fixation in the modern ocean (44, 45); this suggests that the ice age decline in  $N_2$  fixation rate was dramatic, most likely to less than half of the modern rate based on a two end-member mixing calculation (*Estimate for Glacial-Interglacial Changes in  $N_2$  Fixation Rate*).

A question that arises is how  $FB-\delta^{15}N$  glacial-interglacial variations of  $\sim 3\text{‰}$  could result when the modern nitrate  $\delta^{15}N$  decline from  $\sim 500$  m depth into the shallow SCS thermocline is only 1 to 2‰ (Fig. 1B). First, the Holocene does not represent the minimum observed  $FB-\delta^{15}N$ , so shallow thermocline nitrate  $\delta^{15}N$  is reconstructed to have been still lower during previous interglacials. Second, the role of  $N_2$  fixation in lowering the  $\delta^{15}N$  of subsurface nitrate is greater than indicated by the local vertical gradient in nitrate  $\delta^{15}N$  alone, as low  $\delta^{15}N$  N from  $N_2$  fixation spreads horizontally and vertically, as nitrate and sinking particulate nitrogen (45). This latter point also reinforces the arguments above against a hydrographic (e.g., vertical mixing) explanation for the observed  $FB-\delta^{15}N$  changes.

At all nine glacial terminations covered by our  $FB-\delta^{15}N$  record, a reconstructed increase in  $N_2$  fixation in the SCS coincides with decreases in planktonic and benthic  $\delta^{18}O_c$ , a rise in sea level and thus an increase in shelf area (Fig. 4 and Figs. S4 and S5), a rise in SST, and an apparent deglacial increase in water column denitrification in the eastern tropical Pacific (Figs. 3 and 4). The length of the SCS  $FB-\delta^{15}N$  record allows for the use of time series analysis to identify the correlations that are most consistent with a causal connection.

Variability in SST is highly coherent with that in  $FB-\delta^{15}N$  (Fig. 5A). However,  $FB-\delta^{15}N$  lags SST by more than 4 ky in the dominant 41- and 100-ky bands for the latter half of the record (Fig. 5A). Because the physiological and biochemical response of  $N_2$  fixers to SST would be effectively instantaneous, the lag argues against SST as the driver of the greatest  $FB-\delta^{15}N$  variations. Moreover, based on observed sensitivities (15), the reconstructed SCS SSTs fall into the optimal range for  $N_2$  fixation, and a 3 °C cooling would be far too small to explain the dramatic reduction in  $N_2$  fixation during glacials. Dust fluxes are lowest when reconstructed  $N_2$  fixation is highest, arguing against iron supply as the explanation for the reconstructed  $N_2$  fixation changes (Fig. 4F). This lack of positive correlation between  $N_2$  fixation and dust supply is consistent with high iron availability in the SCS even during interglacials, both from the margins and from atmospheric deposition.

There are three bulk sediment  $\delta^{15}N$  records from near water column zones of suboxia and that are adequately long to compare with our SCS  $FB-\delta^{15}N$  record (Figs. 4 C–E and 5B). These environments are characterized by high export production and relatively good preservation of sedimentary organic matter, such that the potential of bulk sediment  $\delta^{15}N$  to robustly record the  $\delta^{15}N$  of N export is greater than in most other ocean regions (46). Of these records, only ODP Site 1012 (37) from the California margin shows significant coherence (Figs. 4C and 5B). The anticorrelation of the records might be taken to suggest that enhanced water column denitrification in the eastern tropical North Pacific during interglacials was responsible for coincident  $N_2$  fixation in the SCS. However, the coherence is limited to periods near 100 ky, suggesting that observed similarities in the records reflect independent but similarly timed responses to glacial cycles.

The SCS  $FB-\delta^{15}N$  and  $\delta^{18}O_c$  records are similar in large-scale structure (Figs. 3 B and C), suggesting a connection between  $N_2$  fixation and sea level. A stack of sea level records (47–49) shows high coherence with the SCS  $FB-\delta^{15}N$  over a wide range of frequencies (Fig. 5C; significant against red noise with 95% confidence), as strong as the coherence between independent sea level reconstructions (Figs. S6–S8). Thus, the reconstructed glacial/interglacial changes in  $N_2$  fixation appear to require a mechanism that involves ice volume and/or sea level change. The correlation of markers of terrigenous input with  $FB-\delta^{15}N$  in MD972142, with greater terrigenous material when  $FB-\delta^{15}N$  is high (29), provides additional support for this interpretation (Fig. S3D). As no relatively direct, low-lag connection between ice volume and  $N_2$  fixation appears plausible for the SCS, the data argue for sea level as the dominant driver of  $N_2$  fixation change.



The extensive continental shelf area of the tropical western North Pacific adjacent to the SCS, the Sunda shelf in particular, appears to be an important locus of benthic denitrification (8). This shelf area was nearly completely lost during peak glacials (Fig. 1A). The reduction in shelf area has been proposed to reduce shelf sedimentary denitrification in the glacials (7), which, in turn, would lead to higher N/P (less excess P) in the upper water column. This change would have discouraged  $N_2$  fixation in the SCS and neighboring regions, explaining the remarkable coherency of the sea level records and our SCS FB- $\delta^{15}N$  record (Fig. 6).

The SCS FB- $\delta^{15}N$  record thus provides the most direct evidence to date for the long-hypothesized scenario in which sea level drives glacial cycles in benthic N loss along the continental margins. Such a mechanism implies that SCS  $N_2$  fixation responded to changes in nearby shelf area, as changes in N loss on distant shelves should have been compensated by  $N_2$  fixation in those regions.  $N_2$  fixation compensation for N loss might be confounded by changes in iron availability in other tropical/subtropical ocean regions. However, for regions such as the SCS that are characterized by high iron supply, local compensation for N loss changes is arguably to be expected.

Continental slopes are known to deposit substantial quantities of margin-derived organic matter at their base (50), and the resulting accumulation drives denitrification on the slope (31, 51, 52). It is possible that this process was accelerated during ice ages and, in part, replaced the sedimentary denitrification on the continental shelves. N loss on the slope may not lead to synchronous changes in  $N_2$  fixation because the N deficit would accumulate in deep water, not directly affecting the N/P of the nutrient supply to the locally overlying surface ocean. However, the funneling of organic matter into the deep ocean prevents the upper ocean nutrient recycling and other processes that render N loss so rapid on the shelves. Therefore, any increased N loss by denitrification on the slope is unlikely to have substantially compensated for the reduced N loss on the shallow margins.

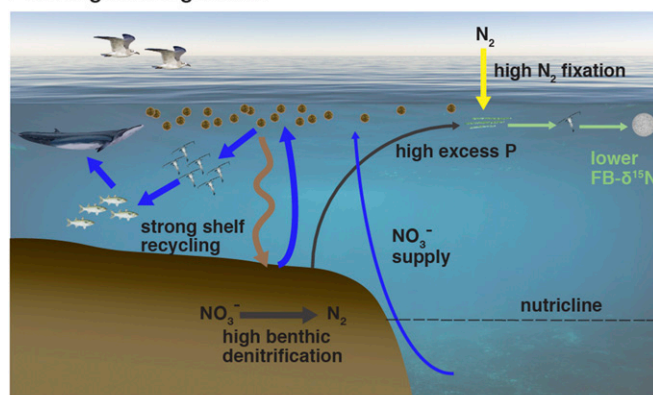
$N_2$  fixation slowed substantially during ice ages, as reconstructed here for the western tropical Pacific and previously for the North Atlantic, in both cases consistent with the response of  $N_2$  fixation to excess P supply as the dominant driver of the changes (13, 14). The correlation between SCS  $N_2$  fixation and sea level provides data-based support for the hypothesis of reduced sedimentary denitrification during ice ages (7, 53, 54), and bulk sediment  $\delta^{15}N$  records argue for reductions in water column denitrification as well (5, 6). With these lower rates of both input and loss, the residence time of fixed N in the ocean [currently ~3 ky (55, 56)] would have become longer and thus less distinct from the residence time of phosphorus [15 ky to 40 ky (57)], although the latter may also have changed over glacial cycles.

Benthic N loss on the continental margins reflects the high flux of organic matter to the coastal seabed (50–52), a consequence of both the shallow continental shelf and the high productivity of the coastal water column (Fig. 6). The high productivity is, in turn, supported by the shelf, which traps sinking organic matter and quickly returns nutrients to the sunlit surface ocean. Thus, the reduction in benthic N loss during ice ages implies a net decline in the organic matter supply to coastal ecosystems, especially those organisms that rely on the benthos. In part because of their extraordinarily high productivity and benthic activity, the modern continental shelves have tremendous importance for seafloor fauna, fish, and marine mammals. The reconstructed biogeochemical changes imply that these higher trophic levels would have suffered a notable decline in food supply during the low sea level stands of ice ages (Fig. 6), potentially impacting the evolution and current characteristics of coastal species and ecosystems (e.g., ref. 58).

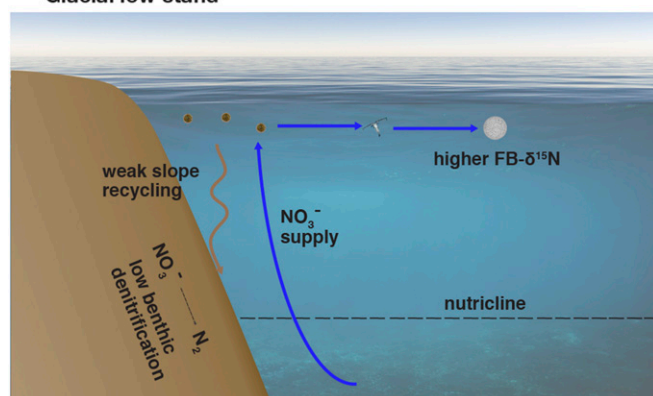
## Methods

**FB- $\delta^{15}N$  Analyses.** The protocol follows and is modified from that of refs. 13 and 14. The individual foraminifera species (250- to 425- $\mu m$ -size fraction, ~5 mg per sample) are picked manually and gently crushed under a dissecting microscope.

## A Interglacial high stand



## B Glacial low stand



**Fig. 6.** Inferred glacial/interglacial changes along the SCS margin. (A) During interglacial high sea level stands, organic matter decomposition on the shallow shelf promotes high coastal ocean productivity and rapid shelf denitrification. The denitrification, by consuming fixed N, causes the shelf water to have excess P. When this water is transported into the open SCS, phytoplankton growth draws down its nutrients, and its excess P causes N to become depleted before P. The availability of P in the absence of N enhances  $N_2$  fixation, which is reflected in a lowering of thermocline nitrate  $\delta^{15}N$  and thus lower FB- $\delta^{15}N$ . (B) The sea level-driven loss of the shallow shelf during glacials reduces productivity and sedimentary denitrification along the margin. The reduction in sedimentary denitrification rate is compensated by slower offshore  $N_2$  fixation, causing thermocline nitrate  $\delta^{15}N$  and FB- $\delta^{15}N$  to rise. Along the margin, the glacial reduction in shallow seafloor nutrient recycling and thus phytoplankton production would impact the upper trophic levels that thrive on the modern (interglacial) shelf. This mechanism, which explains the observed coupled changes in sea level and  $N_2$  fixation in the SCS, should also apply along other ocean margins.

Samples are first sonicated for 5 min in an ultrasonic bath using 2% polyphosphate solution to remove clay particles. To remove metal coatings, bicarbonate-buffered dithionite–citric acid solution is then added to each sample, and the samples are placed in a water bath at 80 °C for 1 h. The final cleaning step is oxidative: Basic potassium persulfate solution is added to each sample, and the samples are autoclaved (at 121 °C) for 1 h. The cleaned samples are rinsed in deionized water and dried overnight at 55 °C. This cleaning protocol typically preserves 60 to 75% of the initial foraminifera weight.

Cleaned foraminifera (~3 mg to 4 mg per sample) are weighed into a previously combusted glass vial and dissolved in 3N HCl. To convert the released organic N to nitrate, purified basic potassium persulfate oxidizing solution is added to the vials, which are then autoclaved for 1 h on a slow-vent setting. To lower the N blank associated with the oxidizing solution, the potassium persulfate is recrystallized three times. At the time of processing, 0.8 g of NaOH and 0.5 g of potassium persulfate are dissolved in 100 mL of deionized water. Organic standards are used to constrain the  $\delta^{15}N$  of the persulfate reagent blank. Three different organic standards were used: US Geological Survey (USGS) 40 ( $\delta^{15}N = -4.5\%$  vs. air), USGS 41 ( $\delta^{15}N = 47.6\%$  vs. air), and a laboratory standard made of a mixture of 6-aminocaproic acid

and glycine ( $\delta^{15}\text{N} = 5.4\%$  vs. air). A minimum of 18 organic standards and three to five blanks were analyzed per batch of samples.

To determine the N content of the samples, nitrate concentration is measured in the oxidation solution after autoclaving. The nitrate analysis is by reduction to nitric oxide using vanadium (III) followed by chemiluminescence detection (59). The blank is also quantified in this way. Consistent with our previous findings, *O. universa* and *G. ruber* had an average N content of 3 mmol to 4 mmol N per gram of cleaned calcite, yielding nitrate concentrations in the oxidation solutions of 10  $\mu\text{M}$  to 20  $\mu\text{M}$ , whereas the nitrate concentration of the blanks ranged between 0.3  $\mu\text{M}$  and 0.7  $\mu\text{M}$  (less than 5%, typically less than 2%, of the total N per sample).

The  $\delta^{15}\text{N}$  of the samples is determined using the denitrifier method in conjunction with gas chromatography and isotope ratio mass spectrometry (60, 61). The denitrifier method involves the transformation of dissolved nitrate and nitrite into nitrous oxide gas ( $\text{N}_2\text{O}$ ) via a naturally occurring denitrifying bacterial strain that lacks an active form of the enzyme  $\text{N}_2\text{O}$  reductase. Before adding the foraminifera samples to the bacteria, the sample solution is acidified to pH 3 to 7. The denitrifier *Pseudomonas chlororaphis* was used for this work. Normally, 5-nmol samples are added to 1.5 mL of bacterial concentrate after degassing of the bacteria. Along with the samples, the organic standards as well as replicate analyses of nitrate reference material International Atomic Energy Agency NO3 reference (IAEA-N3) ( $\delta^{15}\text{N} = 4.7\%$  vs. air) and a bacterial blank are also measured. The IAEA-N3 standards are used to monitor the bacterial conversion and the stability of the mass spectrometry, and the oxidation standards are used to correct for the oxidation blanks. If possible, samples were oxidized in duplicate, and oxidized samples were also sometimes analyzed by the denitrifier method in duplicate. The denitrifier method typically has a SD ( $1\sigma$ ) of less than 0.1‰ and is not reported here. The reported error is the SD estimated from the means of separate oxidations of cleaned foraminiferal material, which averaged 0.22‰ (57% were less than 0.2‰, and 93% were less than 0.5‰).

The data reported in this work will be accessible at National Centers for Environmental Information (NOAA) once the paper is published online.

**The  $\delta^{18}\text{O}$  Analyses on *Cibicides wuellerstorfi*.** Approximately 15 *Cibicides wuellerstorfi* individuals were picked from each sample. The samples were ultrasonicated first in 1 mL of deionized water for 3 s to 5 s, then in 0.2 mL of methanol for 3 s to 5 s. The samples were rinsed with deionized water two to

three times and dried in an oven at 60 °C overnight. The cleaned foraminifera samples were crushed, and 35 mg to 80 mg weighed into 4.5-mL vials. The  $\delta^{18}\text{O}$  were analyzed with a Thermo GasBench II coupled to a Thermo Delta V Plus mass spectrometer at Eidgenössische Technische Hochschule Zürich (62). The average of the SD of single  $\delta^{18}\text{O}$  measurements is  $\sim 0.04\%$ .

#### Nitrate Sampling and $\delta^{15}\text{N}$ Analyses at the South East Asian Time-Series Station and in the Open Western Pacific.

The South East Asian Time-Series (SEATS) station is located at 18°N and 116°E (Fig. 1A) in about 3,800 m of water. It was sampled four times between August 2012 and December 2013 in approximately seasonal intervals aboard R/V *Ocean Researcher I*. Two casts during August 2012 and eight casts from each of the other three cruises were sampled for nitrate  $\delta^{15}\text{N}$  analyses. The western subtropical Pacific transect is located along 23.5°N from 122.25°E to 126°E. Discrete water samples were collected from five open ocean stations in 2013 July on R/V *Ocean Research V*. All water samples were collected with General Oceanics GO-FLO bottles mounted onto a Rosette sampling assembly. From each depth, seawater was collected unfiltered in a rinsed 60-mL high-density polyethylene bottle and immediately frozen at  $-20^\circ\text{C}$ .

The concentration of nitrate plus nitrite was analyzed by reduction to nitric oxide using vanadium (III) followed by chemiluminescence detection (59). The  $\delta^{15}\text{N}$  of nitrate was determined using the denitrifier method, as described above. We use two international nitrate isotope reference materials, IAEA-N3 ( $\delta^{15}\text{N} = 4.7\%$  vs. air) and USGS-34 ( $\delta^{15}\text{N} = -1.8\%$  vs. air), to correct the data. The analytical precision for  $\delta^{15}\text{N}$  was 0.08‰. The error bars in Fig. 1C represent 1 SD of the nitrate  $\delta^{15}\text{N}$  analyzed at the same depth from the different casts, which averaged 0.20‰.

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# Supporting Information

Ren et al. 10.1073/pnas.1701315114

## Estimate for Glacial-Interglacial Changes in $N_2$ Fixation Rate

The deep thermocline nitrate in the SCS is transported from the western Pacific Ocean primarily through Luzon Strait. In the tropical latitudes of the western and central Pacific, nitrate in the lower thermocline (300 m to 600 m depth) has a  $\delta^{15}N$  between 7‰ and 8‰, with most of the nitrate between 7.0‰ and 7.5‰ (24, 32). Newly fixed N from  $N_2$  fixation, on the other hand, has a low  $\delta^{15}N$  close to that of atmospheric  $N_2$   $\delta^{15}N$  (−2‰ to 0‰ vs. air  $N_2$ ) (69–71), and it is taken as the dominant mechanism that lowers nitrate  $\delta^{15}N$  in the subsurface of the SCS and nearby regions to values below 7‰ (22). The current shallow subsurface nitrate  $\delta^{15}N$  at the northern SCS is 4.9‰ [average nitrate  $\delta^{15}N$  at 100 m depth from four cruises from 2012 to 2013 at SEATS) (Fig. 1C)]. Using a two end-member mixture of newly fixed N with a  $\delta^{15}N$  of −1‰ and the deep thermocline nitrate with a  $\delta^{15}N$  of 7‰ in the SCS [ $\delta^{15}N_{N_2 \text{ fixation}} \times \text{Fraction}_{N_2 \text{ fixation}} + \delta^{15}N_{\text{thermocline nitrate}} \times (1 - \text{Fraction}_{N_2 \text{ fixation}}) = \delta^{15}N_{\text{subsurface nitrate}}$ ], we estimate that about 26% of the nitrate at 100 m depth originates from  $N_2$  fixation in the SCS and the neighboring western Pacific margin.

The  $\delta^{15}N$  of both *G. ruber* and *O. universa* measured in the surface sediment is 4.9‰, the same as the 100-m-depth minimum in nitrate  $\delta^{15}N$  (Fig. 1C). This nitrate-to-foraminifera similarity is consistent with our previous findings from global compilations that FB- $\delta^{15}N$  is similar to the  $\delta^{15}N$  of the subsurface nitrate available for upward transport into the euphotic zone. We thus use past changes in the FB- $\delta^{15}N$  to reconstruct past changes in the subsurface nitrate  $\delta^{15}N$ , and, in turn, changes in  $N_2$  fixation rate. Alternative explanations for the FB- $\delta^{15}N$  changes involving circulation- or remote biogeochemistry-driven changes in the  $\delta^{15}N$  of the interior nitrate supplied to the SCS are discussed in *Results and Discussion*.

FB- $\delta^{15}N$  varies between 4‰ and 7‰ from peak interglacials to glacials. Using the average FB- $\delta^{15}N$  of *G. ruber* between 21 ka and 29 ka (~6.0‰), we estimate that recently fixed N contributes

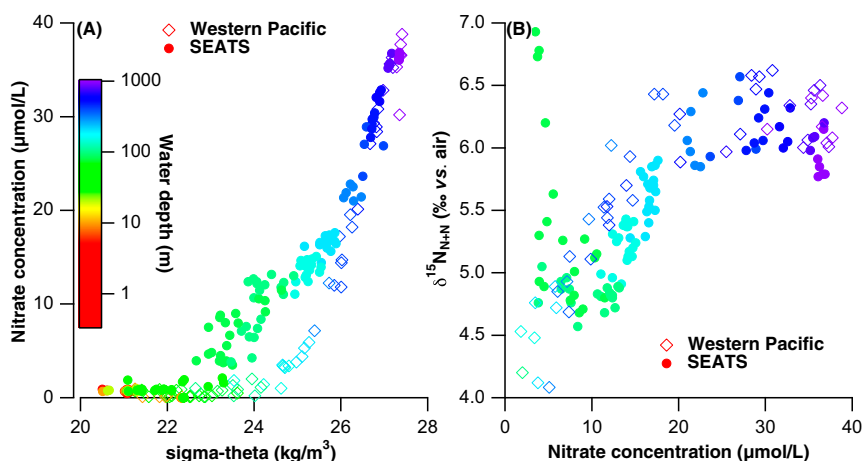
only about 13% of the shallow subsurface nitrate during the LGM. If we assume that the higher FB- $\delta^{15}N$  of the LGM is due to changes in  $N_2$  fixation alone, then the LGM  $N_2$  fixation rate is only 48% of its Holocene rate. In our previous study, this value is estimated to be 27% (40); this is because we had assumed that the intermediate-depth nitrate  $\delta^{15}N$  (~6.2‰) in the SCS was not affected by  $N_2$  fixation. However, the intermediate-depth nitrate  $\delta^{15}N$  in the SCS is lower than in the open western and central Pacific, suggesting that the entire water column in the SCS is influenced by nitrate from the remineralization of newly fixed N; this is to be expected given the strong vertical mixing in the deep SCS (72). The minimum interglacial FB- $\delta^{15}N$  value of 4‰ suggests that newly fixed N can contribute up to 38% of the subsurface nitrate. In summary, our record indicates that the  $N_2$  fixation rate in the SCS varies by threefold between ice ages and interglacials.

## Phase Relationship Between FB- $\delta^{15}N$ and Benthic Calcite $\delta^{18}O$

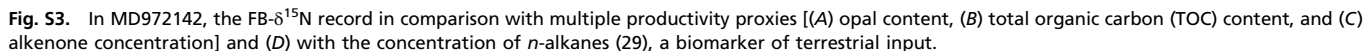
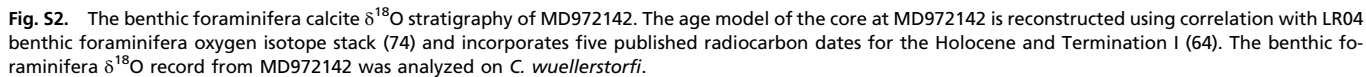
FB- $\delta^{15}N$  and benthic calcite  $\delta^{18}O$  show high coherency throughout the record. One possible exception involves the major glacial terminations (such as that before the Holocene; Fig. 3C and Fig. S8), during which a transient nitrate  $\delta^{15}N$  rise of ~1‰ may occur (40, 54). This  $\delta^{15}N$  rise would introduce an apparent lag in the deglacial FB- $\delta^{15}N$  decrease relative to whatever environmental parameter is predominantly driving the glacial/interglacial changes, potentially explaining the observed lag of FB- $\delta^{15}N$  relative to sea level at the terminations.

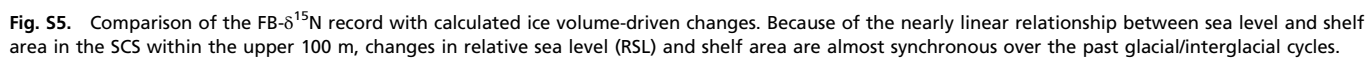
## Shelf Area and Sea Level in the SCS

The SCS hypsographic curve [using the ETOPO1 Ice Surface global relief model (73)] suggests that the shallow shelf area and sea level is close to linearly correlated within the upper 100 m (Fig. S4). As a result, changes in sea level and shelf area would have been almost synchronous, with little deviation from a linearly proportional relationship (Fig. S5).

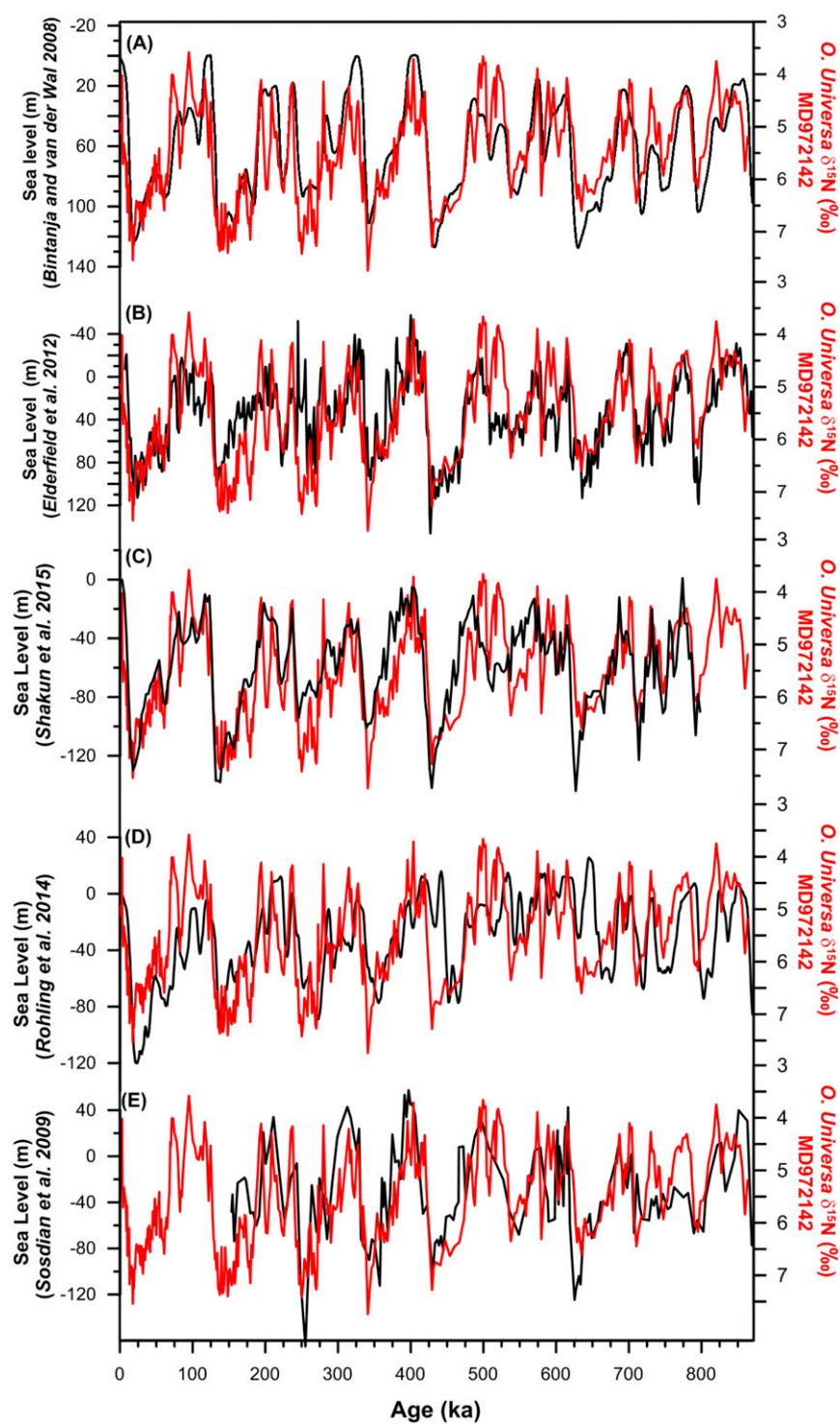


**Fig. S1.** Hydrographic evidence that the low nitrate  $\delta^{15}N$  in the shallow thermocline of the SCS derives from in situ remineralization of newly fixed N. (A) The nutrient concentration above 500 m is more elevated than in the open western Pacific on any given isopycnal. (B) The shallow SCS (between 100 m and 500 m) has a lower nitrate  $\delta^{15}N$  at a given nitrate concentration in comparison with the open western Pacific.









**Fig. S6.** The FB- $\delta^{15}\text{N}$  record in comparison with different sea level records. FB- $\delta^{15}\text{N}$  is plotted with the five individual sea level records (A–E) that are part of the sea level stack and that cover the last 800 ky (47, 48, 75–77).



