



## Supporting Online Material for

### **Foraminiferal Isotope Evidence of Reduced Nitrogen Fixation in the Ice Age Atlantic Ocean**

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## **Materials and Methods**

### **Sites of surface sediments and downcore materials**

The locations of all surface sediment samples and ODP Site 999A are shown in Fig. S1. All materials are from regions where essentially all nitrate imported into the euphotic zone is consumed, as indicated by mean annual surface nitrate concentration in Fig. S1.

### **Foraminifera-bound N isotope measurement**

#### *Protocol*

The protocol includes 1) chemical treatment of the foraminifera shells to remove external nitrogen contamination, followed by acid dissolution of the cleaned shells, 2) conversion of organic nitrogen released to nitrate by persulfate oxidation (*S1*), 3) measurement of nitrate concentration (in our work by reduction to nitric oxide and chemiluminescent detection (*S2*)), and 4) quantitative bacterial conversion of nitrate to nitrous oxide (*S3*), with measurement of the isotopic composition of the nitrous oxide by gas chromatography-isotope ratio mass spectrometry using a modified ThermoFinnigan GasBench II and DeltaPlus (*S4*).

To remove external nitrogen contamination, 5 to 10 mg of foraminifera tests per sample were gently crushed, cleaned first by 5 minutes ultrasonication in 2% sodium hexametaphosphate (pH 8), then rinsed twice with deionized water (DI). Samples were soaked in 13% sodium hypochlorite for 6 hours, with several agitations to expose surfaces, and then rinsed five times with DI. The remaining 3 to 5 mg of cleaned

foraminifera tests were subsequently completely dissolved in 6 N hydrochloric acid, releasing organic matter for analysis.

### *Cleaning tests*

Figure S1 shows the changes in nitrogen content and  $\delta^{15}\text{N}$  of foraminifera bound N for different periods of treatment with hypochlorite. After 6 hours of hypochlorite treatment, N content and  $\delta^{15}\text{N}$  reach stable, reproducible values. In Table S1, the hypochlorite cleaning protocol is compared with a different cleaning protocol based on the potassium persulfate/sodium hydroxide reagent used for conversion of organic N to nitrate (S1, S5). The two methods yield identical and reproducible results. Since the persulfate oxidation reaction is designed to be extremely harsh (S6), the consistency of the two protocols suggests complete removal of external nitrogen with the hypochlorite protocol. It also indicates integrity of the foraminifera-bound N in the cleaned samples.

N content measurements of foraminiferal samples (Fig. S2) from ODP 999A show that *O. universa* has lower test bound N compared to *G. ruber* and *G. sacculifer*, consistent with previous results (e.g., Table S1), while the three size fractions do not show consistent differences in their N content. The difference in N content among different species is consistent with earlier amino acid studies that observed lower amino acid content in *O. universa* than in the other two species (S7). There are no systematic variations in N content of the samples from different depths; most importantly, LGM and Holocene samples have similar N content, showing no substantial N loss over the past 30 ka.

### **Bulk sediment measurements**

The total N (TN), organic N ( $N_{\text{org}}$ ) and organic carbon ( $C_{\text{org}}$ ) contents as well as the isotopic composition ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}_{\text{org}}$ ) were analyzed using an elemental analyzer (NC2500 Carlo Erba) coupled with a ConFlo III interface on a ThermoFischer Scientific DeltaPlusXL mass spectrometer at the GeoForschungsZentrum, Potsdam.

For TN and  $\delta^{15}\text{N}$  determination, around 40 mg of sample material were loaded into tin capsules for combustion in the elemental analyzer. In general, the N content was calibrated against acetanilide whereas for the nitrogen isotopic composition, two ammonium sulphate standards (e.g. IAEA N-1 and N-2) were used. Because of the small amount of nitrogen in the samples (<20mg), a linearity correction was made using varied quantities of internal laboratory standards L1 (ammonium sulfate), T54 (organic rich shale) and Boden2 (soil). To test the influence of high calcium carbonate, T54 were mixed with marble powder in different concentration, without any effects on the isotopic composition. Replicate analysis of individual ODP 999A samples indicate a standard deviation  $\pm 0.3\text{‰}$  for  $\delta^{15}\text{N}$ . The analytical precision for N content is 5%.

$C_{\text{org}}$  content and  $\delta^{13}\text{C}_{\text{org}}$  were determined on decalcified samples, in this case requiring ~10 times less material. Decalcification involved weighing samples into silver capsules, addition of an adequate volume of 20% HCl to achieve complete decarbonation, and heating to dryness at 75°C. Calibration was performed using in-house urea and certified isotopic standards (USGS24, CH-7), and tested with an internal soil reference sample (Boden2). The standard deviation calculated for  $\delta^{13}\text{C}_{\text{org}}$  replicate analyses is 0.2‰. The analytical precision for  $C_{\text{org}}$  content is 5 %.

**Supporting Text:  $\delta^{15}\text{N}$  of sub-euphotic zone dwelling foraminifera**

Across the tropical and subtropical ocean, the concentration of suspended particulate organic N (PON) is relatively uniform in the euphotic zone but decreases sharply into the subsurface (S8). This pattern is consistent with net PON production in the euphotic zone and net consumption in the dark interior. Associated with the downward decrease in PON concentration, PON  $\delta^{15}\text{N}$  increases sharply into the interior, from values near 0‰ in the euphotic zone to  $\geq 5\text{‰}$  in the deep Atlantic (S9). This trend is not evident in Fig. 3, which only shows the  $\delta^{15}\text{N}$  of PON at depths above the sharp decline in PON concentration into the interior.

In our analyses of surface sediments, there is some evidence that foraminiferal species known to dwell partially or entirely in the thermocline (*G. menardii*, *N. dutertrei*, *G. truncatulanoides*, *H. pelagica*, *G. inflata*) are higher in  $\delta^{15}\text{N}$  than the euphotic zone dwellers (*G. ruber*, *G. sacculifer* and *O. universa*), although the data are not fully consistent in this regard, especially for *G. menardii* and *N. dutertrei* in the Atlantic. In the ODP Site 999 record, both *N. dutertrei* and *G. menardii* show elevated  $\delta^{15}\text{N}$  relative to the euphotic zone dwellers (Fig. S4). *N. dutertrei*  $\delta^{15}\text{N}$  shows an LGM-to-Holocene decrease very similar to that observed in *O. universa*, as would be expected if the  $\delta^{15}\text{N}$  increase from the base of euphotic zone into the interior has persisted since the LGM.

**Supporting Text: N supply to the euphotic zone, N fixation vs. subsurface nitrate**

The proportion of the N supply to the euphotic zone deriving from N fixation vs. subsurface nitrate (i.e. the ratio between these two N sources) is relevant to our surface sediment calibration. The greater this ratio, the more the  $\delta^{15}\text{N}$  of the total N supply to the euphotic zone falls below that of the subsurface nitrate supply, reaching the  $\delta^{15}\text{N}$  of

newly fixed N (~1‰) in the hypothetical end-member case in which all N derives from N fixation.

One approach to estimate this ratio is to compare the  $\delta^{15}\text{N}$  of N export with that of nitrate supply into the euphotic zone. The first application of this approach at the Bermuda Atlantic Time-series Site indicated no isotopic need for N input from N fixation (*S9*), and subsequent isotope measurement of nitrate and upper water column total dissolved N (largely dissolved organic N) did not substantially change this conclusion (*S1*). BATS is characterized by relatively rapid nitrate supply associated with deep winter mixing (*S10*), so it could be argued that this site is not representative of the relative importance of N fixation in the tropics. In this regard, the same approach for the Hawaii Ocean Time-series station ALOHA indicates that ~25% of the N supply derives from N fixation (*S11*), again suggesting that nitrate is the predominant N input to the oligotrophic ocean. One major uncertainty with this approach involves the  $\delta^{15}\text{N}$  of exported N; it is not clear how accurate sediment traps are at providing this value.

Bottle measurements of N fixation in the western tropical North Atlantic, when compared with estimates of nitrate supply derived from vertical diffusivity calculations or physical models, yield a range of estimates for the N fixation/nitrate supply ratio that includes much higher values, reaching above 1 (*S12*). The general conclusion from that study was that N fixation and subsurface nitrate supply are comparable in this region. However, the uncertainties in this approach are great, arguably deriving more from the uncertainty in nitrate supply than in N fixation.

Until estimates for the N fixation/nitrate supply ratio are more robust, it is difficult to account for it in our surface sediment comparisons. In our data to date, among

our study sites, there is no clear difference in  $\delta^{15}\text{N}$  relationship between subsurface nitrate and foraminifera-bound N. This suggests no dramatic difference in the N fixation/nitrate supply ratio among the sites. One possibility is that this ratio is uniformly low, as suggested by the N isotope budgets described above. However, given current uncertainties, this is no more than a speculation.

### **Supporting Text: Upper water column structure in the Caribbean during the LGM**

From the LGM to the Holocene, there were significant changes in tropical North Atlantic conditions, physical and biogeochemical. These should be considered for their potential to provide alternatives to lower LGM N fixation as the explanation for the observed foraminiferal  $\delta^{15}\text{N}$  changes.

There has been much work done on the mid-depth characteristics of the Atlantic during the LGM. The interior waters above 2 km depth were characterized by lower nutrient conditions than today, which is interpreted as the result of ventilation by Glacial North Atlantic Intermediate Water (GNAIW) (e.g., *S13*). The studies of greatest relevance here are those that involve conditions in the thermocline, above 1 km depth. One set of studies acquired benthic foraminiferal data from a depth transect of sediment cores from Little Bahama Bank to generate a vertical profile through the LGM thermocline and below (*S14*). Foraminiferal  $\delta^{18}\text{O}$  indicates that the vertical density gradient within the LGM thermocline was similar to today's, except at its base, which apparently shoaled from 1000 m to 800 m. This is consistent with a southward shift in the gyre system (*S14*). This might have allowed more rapid vertical mixing at the base of the thermocline, making it easier for high- $\delta^{15}\text{N}$  nitrate to be mixed upward. However, the low

$\delta^{15}\text{N}$  of modern thermocline nitrate begins at  $\sim 650$  m today, so this was unlikely to have been important for the  $\delta^{15}\text{N}$  of nitrate in the shallow ( $\sim 200$  m) thermocline.

From the same work, foraminiferal  $\delta^{13}\text{C}$  indicates that the lower thermocline had less respired dissolved inorganic carbon (DIC) during the LGM, although potential changes in gas exchange are an uncertainty in this interpretation. The  $\delta^{13}\text{C}$  minimum associated with the modern  $[\text{O}_2]$  minimum at  $\sim 800$  m was absent during the LGM, another sign of stronger ventilation from the North. Nutrient and respired DIC changes in the upper thermocline are uncertain, with  $\delta^{13}\text{C}$  data appearing inconsistent with Cd/Ca data (S15). The basic picture that emerges is that today's southern-sourced waters at 1000 m were replaced during the LGM with waters from the North with higher DIC  $\delta^{13}\text{C}$  and thus presumably less respired DIC. If anything, this would have lowered the  $\delta^{15}\text{N}$  of nitrate in the system, as the southern sourced water imports relatively high  $\delta^{15}\text{N}$  nitrate into the North Atlantic (S16). This is the opposite sense of change required to provide an alternative explanation for the  $\delta^{15}\text{N}$  change. Moreover, if nutrients also dropped in the shallow thermocline, this would have allowed a given amount of N fixation to drive a lower  $\delta^{15}\text{N}$  in shallow thermocline nitrate; again, this is the wrong sense of change to explain the N isotope data.

We are unaware of focused studies of photic zone vertical structure in the Caribbean during the LGM. The LGM-Holocene  $\delta^{18}\text{O}$  change for *G. ruber* at ODP Site 999 is  $\sim 1.7\text{‰}$  (S17), which is difficult to distinguish from the LBB shallow thermocline data. *G. ruber* Mg/Ca indicates that the Site 999 mixed layer was  $\sim 2^\circ\text{C}$  colder during the LGM, such that the  $\delta^{18}\text{O}$  data require that the mixed layer was saltier during the LGM (S17). Given the evidence for a more southerly ITCZ during the LGM (S18), the trade

winds over ODP Site 999 should have been stronger during the LGM; one might speculate that this deepened the mixed layer.

The  $\delta^{15}\text{N}$  difference between *G. ruber* and *O. universa* in the glacial section of our record might suggest to some readers a change in depth habitat for one or both of these forms. However, no clear scenario arises. *O. universa* is broadly distributed in the modern ocean (S19), so it seems unlikely to have changed its habitat markedly. Given its symbionts and the need for light, migration to deeper waters, which would work to raise its  $\delta^{15}\text{N}$  as observed, is implausible. Given its modern preference for warm, sunlit waters, *G. ruber* might have been driven to shallower depths during the LGM. However, it already dwells primarily in the surface mixed layer, and there is no distinct shallower habitat into which to migrate so as to diverge in  $\delta^{15}\text{N}$  from *O. universa*.

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**Supporting Table S1. Comparison of the bleach treatment and persulfate treatment**

		<i>O. universa</i> <sup>1</sup>		<i>Gr. inflata</i> <sup>2</sup>	
		N content (μmol/g)	δ <sup>15</sup> N (‰)	N content (μmol/g)	δ <sup>15</sup> N (‰)
Bleach Treatment	Average	4.10	3.65	4.78	7.22
	Standard deviation	0.26	0.2	0.32	0.24
	Numbers of measurements	10		5	
Persulfate Treatment	Average	3.97	3.63	5.08	7.18
	Standard deviation	0.36	0.30	0.61	0.36
	Number of measurements	10		5	

<sup>1</sup>*O. universa* are from Barbuda-Antigua (17°27.0'N, 61°02.0'W, 4030 m depth, 5-10 cm in gravity core EN18, >250 μm size fraction).

<sup>2</sup>*Gr. inflata* are from the southwest Pacific, east of New Zealand (37°15.64'S, 176°40.02'E, 663 m, 0-1 cm in multicores, >150 μm).

## Supporting Figure Captions

### Fig. S1.

Evolution of nitrogen content and  $\delta^{15}\text{N}$  in *O. universa* during the hypochlorite cleaning step. Filled symbols are  $\delta^{15}\text{N}$  (‰ vs. air), and open symbols are nitrogen content ( $\mu\text{mol Nitrogen} / \text{g cleaned foraminifera shell}$ ). Samples are from the North Atlantic near Barbuda-Antiqua ( $17^{\circ}27.0'\text{N}$ ,  $61^{\circ}02.0'\text{W}$ , 4030 m depth, 5-10 cm in gravity core EN18,  $>250 \mu\text{m}$  size fraction).

### Fig. S2.

N content of foraminifera samples as well as bulk sedimentary N content from ODP 999A for the last 30 ka. Dark blue circles: *O. universa*; Red circles: *G. sacculifer*; Green circles: *G. ruber*; light blue cross:  $>355\mu\text{m}$  foraminifera size fraction; pink cross: 250-355 $\mu\text{m}$  fraction; yellow cross: 125-250 $\mu\text{m}$  fraction; black squares: bulk sedimentary N. Error bars are standard deviations calculated from replicates where available. Bulk sedimentary N decreases into the Holocene (probably due to reduced clay-bound N; see Fig. S6 and S7), foraminifera-bound does not show an LGM-to-Holocene change.

### Fig. S3.

Locations of surface sediment samples (red crosses): the North Atlantic near Barbuda-Antiqua ( $17^{\circ}27.0'\text{N}$ ,  $61^{\circ}02.0'\text{W}$ , 4030 m depth), Little Bahama Banks ( $26^{\circ}02'\text{N}$ ,  $77^{\circ}31'$ - $78^{\circ}01'\text{W}$ ; 1049-1067 m) and Great Bahama Banks ( $24^{\circ}20'$ - $24^{\circ}30'\text{N}$ ,  $79^{\circ}11'$ - $79^{\circ}25'\text{W}$ , 600-728 m depth), the Makassar-Bali Basin east of Indonesia ( $6^{\circ}46.25'\text{S}$ ,  $116^{\circ}58.47'\text{E}$ , 419 m), and the southwest Pacific, east of New Zealand (A:  $36^{\circ}22.63'\text{S}$ ,  $177^{\circ}26.75'\text{E}$ ,

2252 m; B: 37°15.64'S, 176°40.02'E, 663 m). Also shown are ODP Site 999 (12°45'N, 78°44'W, 2827 m), in which the downcore records were measured (filled blue circle), and the downcore records. Map shading shows the mean annual surface nitrate concentration (S20). Also shown are the locations of the bulk sediment N isotope records plotted in Fig. 4 for comparison with the Site 999 data. Records from denitrification regions are in open black diamonds. The record from Cariaco basin is indicated by a filled purple diamond.

**Fig. S4.**

Foraminifera-bound  $\delta^{15}\text{N}$  of individual species in the Caribbean Sea from ODP 999A during the last 30 ka, as in Fig. 2 but with additional foraminifera species. Blue: *O. universa*; Red: *G. sacculifer*; Green: *G. ruber*; Purple: *Neogloboquadrina dutertrei*; Yellow: *Globorotalia menardii*. The  $\delta^{15}\text{N}$  of two thermocline dwelling foraminifera species, *N. dutertrei* and *G. menardii*, are higher in  $\delta^{15}\text{N}$  than the three euphotic zone dwellers measured in ODP Site 999. This is best explained by the globally observed increase in POM  $\delta^{15}\text{N}$  with depth below the euphotic zone (S21). *N. dutertrei*  $\delta^{15}\text{N}$  shows an LGM-to-Holocene decrease very similar to that observed in *O. universa*.

**Fig. S5.**

Calculation of North Atlantic thermocline nitrate  $\delta^{15}\text{N}$  (‰ vs. air) as a function of N fixation rate, to estimate the relative reduction in N fixation rate during the LGM. Nitrate  $\delta^{15}\text{N}$  is calculated from a two end-member mixture of (a) deep Atlantic nitrate, with a  $\delta^{15}\text{N}$  of 5.2‰ (from 1000 m at the Bermuda Atlantic Time-series Site (S1)) and (b) newly fixed N with a  $\delta^{15}\text{N}$  of -1‰ (S22, S23). As N fixation rate increases, recently fixed nitrate

becomes a larger fraction of the thermocline nitrate pool, the  $\delta^{15}\text{N}$  of which therefore decreases. The  $\delta^{15}\text{N}$  of modern thermocline nitrate is 2.6‰ (SI). The difference in  $\delta^{15}\text{N}$  between Holocene thermocline nitrate  $\delta^{15}\text{N}$  and the LGM value (vertical distance between the two horizontal lines) is taken from foraminifera-bound  $\delta^{15}\text{N}$  differences of *G. ruber*, the species with the smallest LGM-Holocene change. Based on the LGM-Holocene difference in the  $\delta^{15}\text{N}$  of thermocline nitrate, the LGM N fixation rate is 21% of its Holocene rate.

**Fig. S6.**

C/N ratio and  $\delta^{13}\text{C}$  of sedimentary organic matter from ODP 999A during the last 30ka. Red circles: C/N ratio. Blue triangles:  $\delta^{13}\text{C}$ . The  $\delta^{13}\text{C}$  of the sedimentary organic matter is 1 to 2 ‰ lower during the last ice age, consistent with a higher terrestrial input of organic matter. The opposite sense of change would be expected if  $\delta^{13}\text{C}$  were set solely by open ocean-derived organic matter, given atmospheric  $\text{CO}_2$  change (S24). A simple two end-member mixing calculation using the difference between the observed and expected  $\delta^{13}\text{C}$  changes suggests that a substantial fraction (~40%) of  $\text{C}_{\text{org}}$  in the LGM interval is terrestrial. The C:N ratio of organic matter is mostly less than 8 during the last ice age but centered around 9 during the interglacial, consistent with more clay-bound N input during the LGM. The opposite point-to-point correlation between  $\delta^{13}\text{C}$  and C/N within both the LGM and Holocene sections is likely due to sporadic terrestrial organic matter input with a low  $\delta^{13}\text{C}$  and high C/N.

**Fig. S7.**

TN and  $C_{\text{org}}$  from ODP 999A during the last 30 ka. Blue and red circles are TN and  $C_{\text{org}}$  results from LGM (4.745 – 10.32 ka) and the Holocene (14.542 – 30.903 ka), respectively. The regression lines for both LGM and Holocene data have y-intercepts significantly above the origin, indicating significant clay-bound N throughout the record (model-II least squares regression; 1 s.d. shown in parentheses). The higher y-intercept for the LGM data suggests a greater proportion of clay-bound N. The slopes of the trend lines are roughly a third of the N/C molar ratio in marine organic matter ( $\sim 0.14$ ), suggesting that variability in  $C_{\text{org}}$  content within each climatic interval derives at least partially from variable amounts of terrestrial organic matter, which has a low N/C ratio. This is supported by the point-to-point anti-correlation between C/N and  $\delta^{13}\text{C}$  within both the LGM and Holocene intervals (Fig. S6).

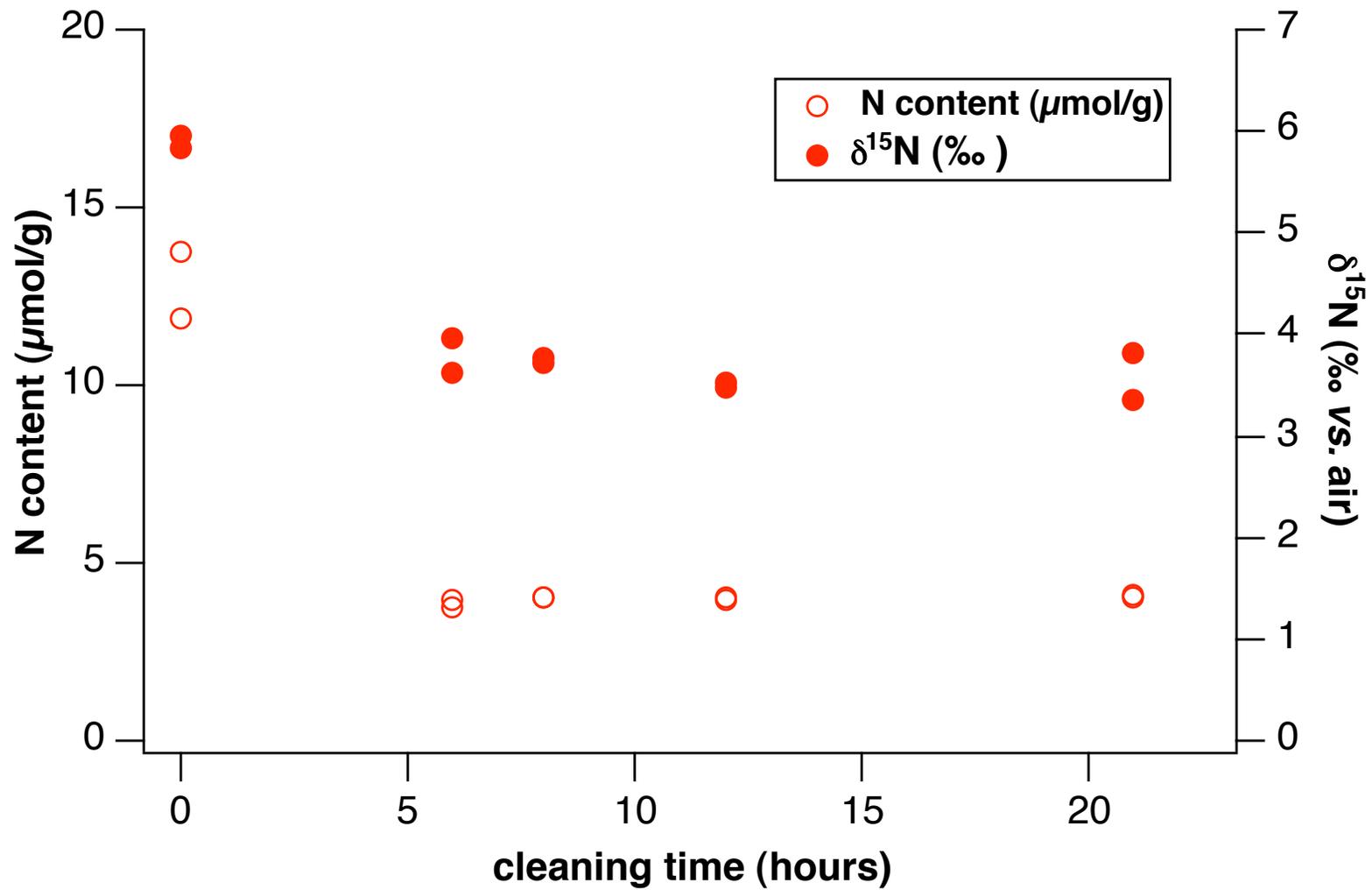


Fig. S1

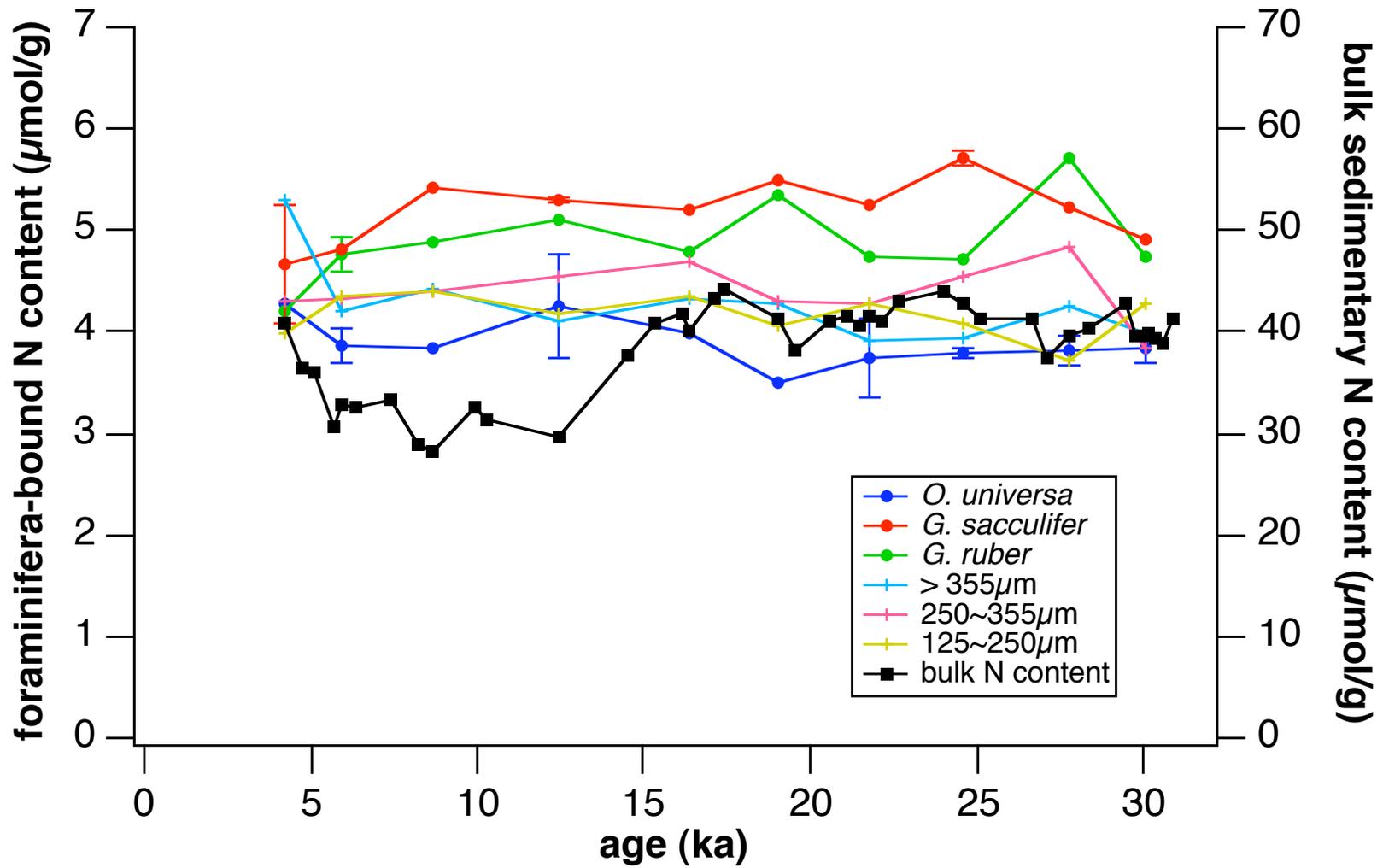


Fig. S2

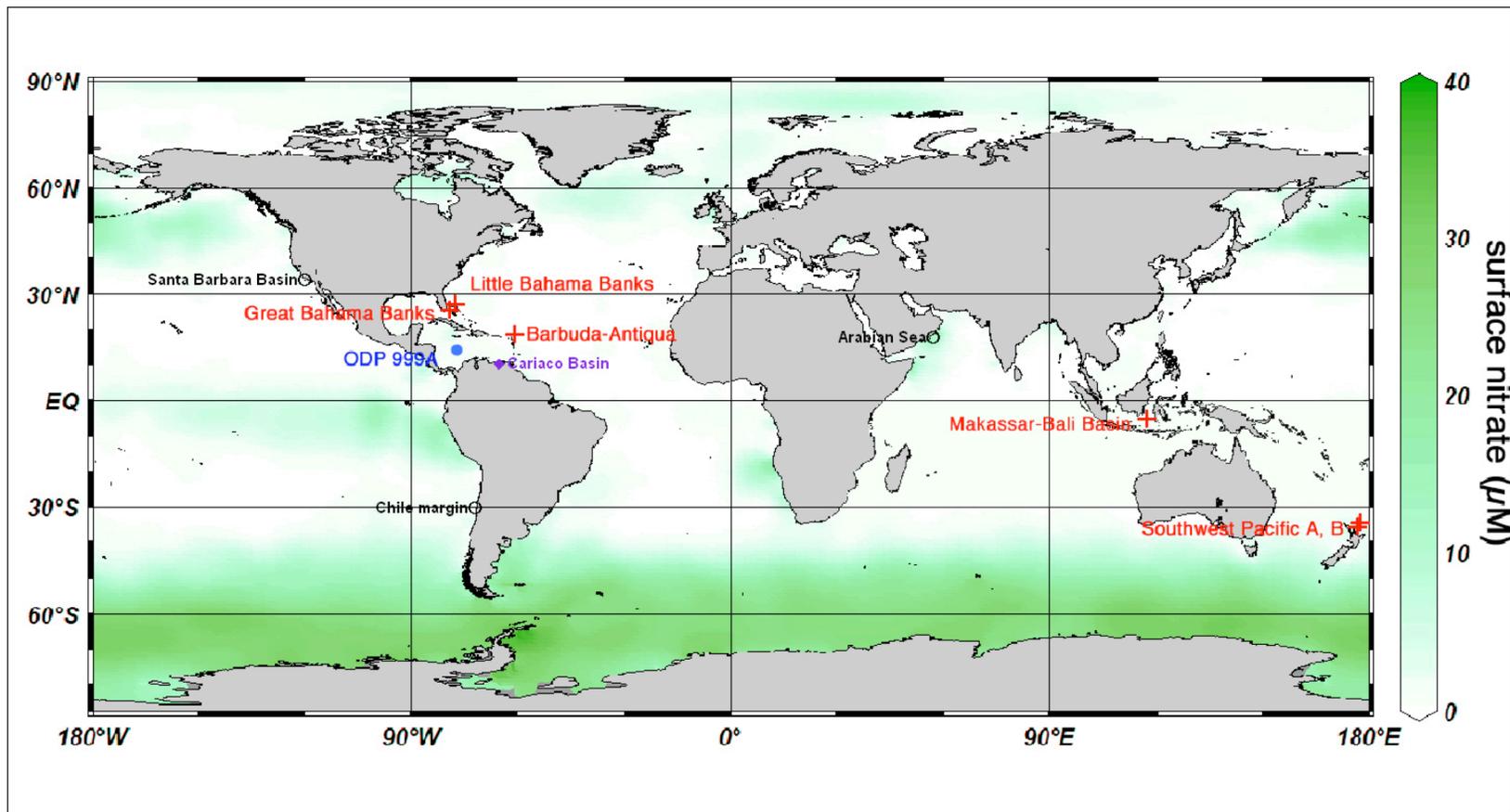


Fig. S3

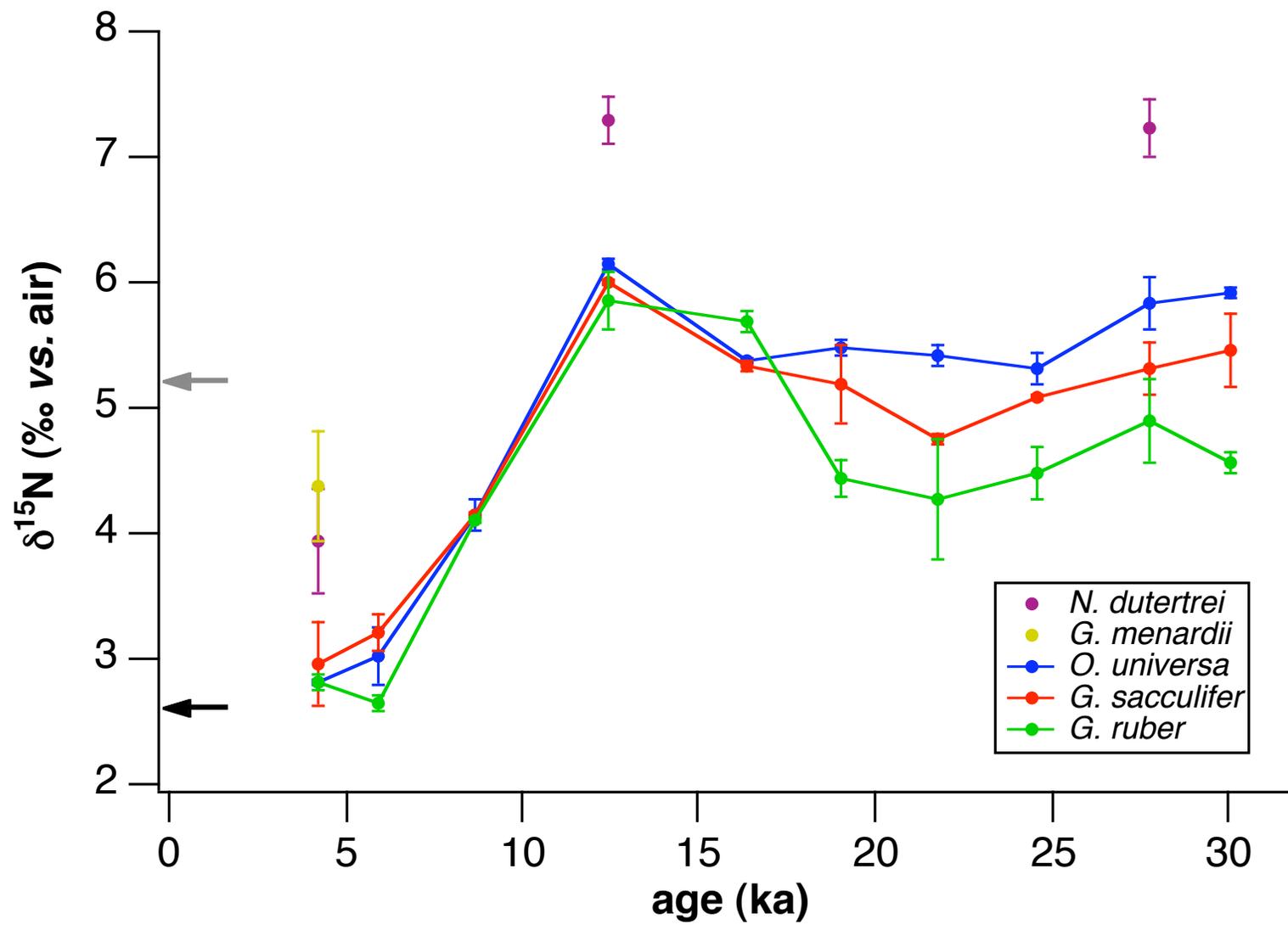
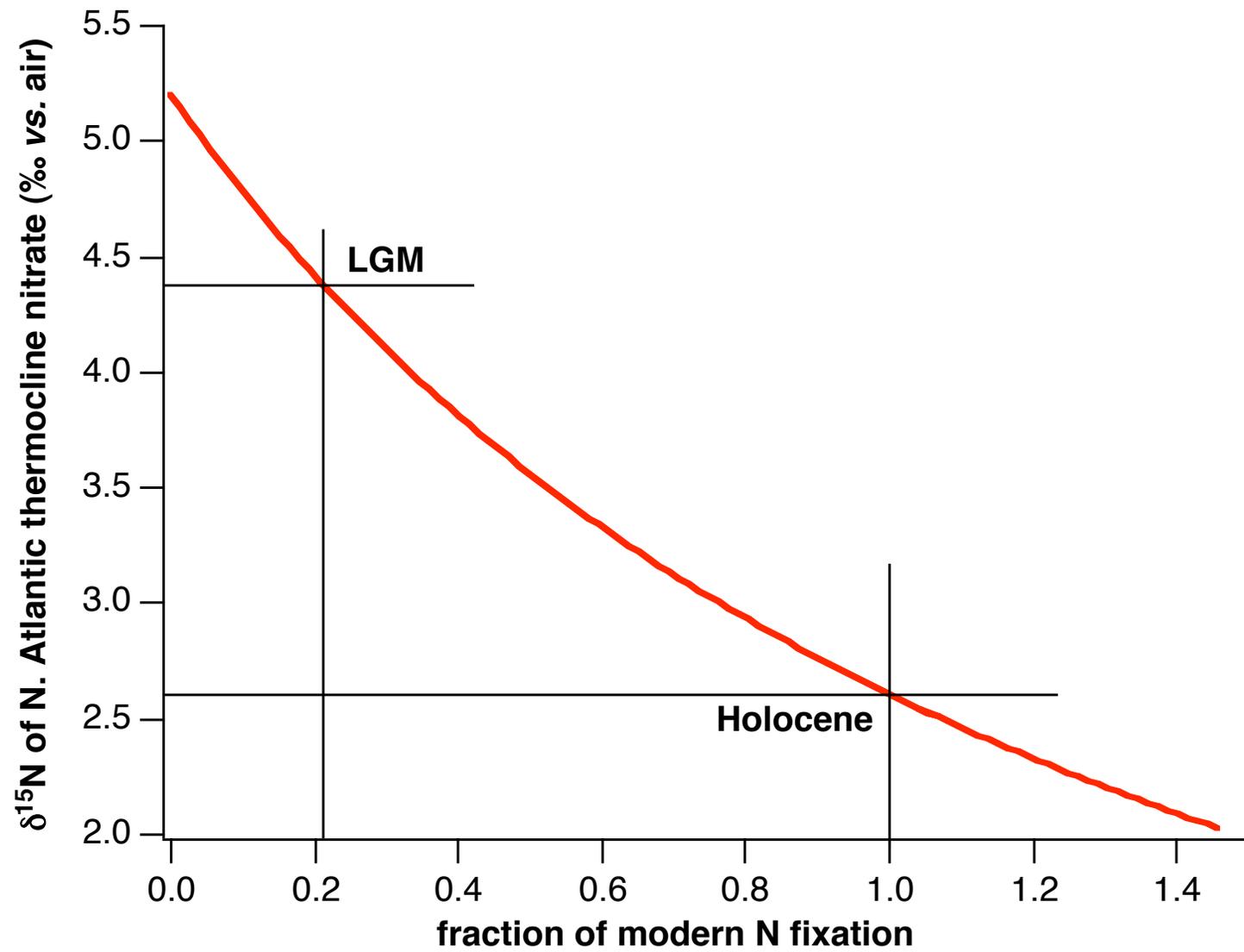


Fig. S4



**Fig. S5**

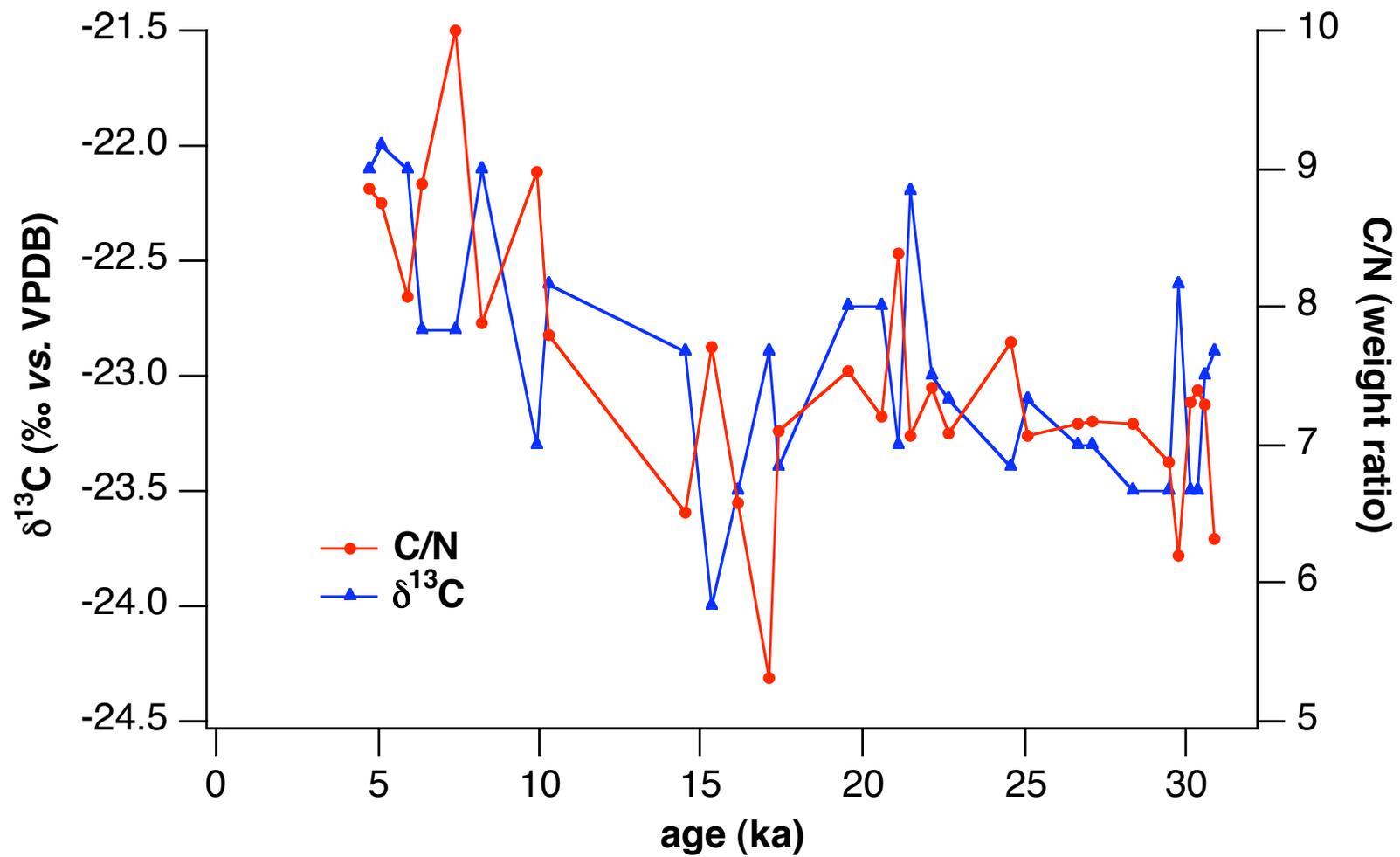


Fig. S6

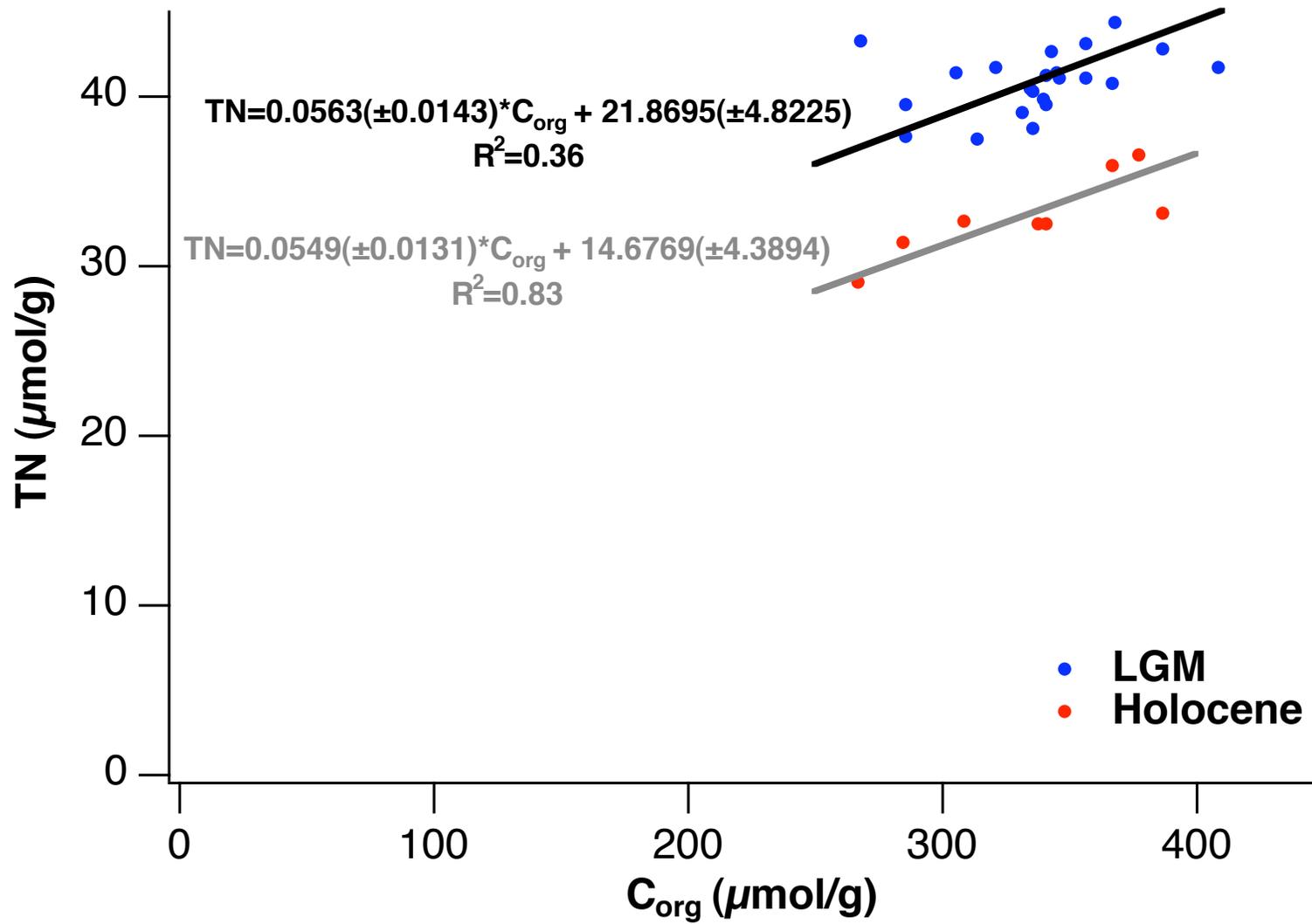


Fig. S7