

Revisiting nutrient utilization in the glacial Antarctic: Evidence from a new method for diatom-bound N isotopic analysis

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[1] Isotopic measurements of diatom-bound nitrogen, using a wet chemical oxidation combined with the “denitrifier” method for nitrate analysis, show significant offsets from previously published combustion-based measurements. This offset is attributed to a gaseous nitrogen blank associated with the diatom’s opal frustule. Moreover, experimentation with multiple chemical cleaning protocols demonstrates that diatom microfossils from the clay-rich sediments of the glacial Antarctic are more difficult to clean than Holocene materials. New downcore profiles from the Antarctic show no change in the diatom-bound N $^{15}\text{N}/^{14}\text{N}$ between the last glacial and the Holocene in the Atlantic sector, and the elevation of glacial diatom-bound $^{15}\text{N}/^{14}\text{N}$ relative to the Holocene in the Indian sector is smaller than in previous measurements. These data suggest no change in the degree of nitrate utilization in the Atlantic sector and at most a 20% increase (from ~25 to 45%) in the Indian sector. The new measurements suggest that, during the last ice age in the Atlantic sector of the Antarctic, the atmospheric source of biologically available iron was not so great as to become significant relative to the iron supply from below. Given the apparent spatial variability in the degree of nitrate drawdown, more work is required to develop an adequate picture of the glacial Antarctic nutrient field. *INDEX TERMS*: 4806 Oceanography: Biological and Chemical: Carbon cycling; 4845 Oceanography: Biological and Chemical: Nutrients and nutrient cycling; 4894 Oceanography: Biological and Chemical: Instruments and techniques; 9310 Information Related to Geographic Region: Antarctica; *KEYWORDS*: nutrient status, glacial/interglacial, Antarctic

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1. Introduction

[2] The nutrient-rich surface of the modern Antarctic Zone in the Southern Ocean releases carbon dioxide (CO_2) that had previously been sequestered in the ocean interior by the rain of biogenic debris out of the surface ocean in other regions. This “ CO_2 leak” in the Antarctic limits the degree to which the global biological pump is able to lower atmospheric CO_2 , and it results from the rapid exposure of CO_2 -rich water at the surface and incomplete consumption of the major nutrients (nitrate and phosphate) in these waters. Enhanced utilization of nitrate, such as might result from iron enrichment of the Antarctic [Martin, 1990], would have rendered the biological pump more efficient during the Last Glacial Maximum (LGM) by reducing the loss of CO_2 from the Antarctic [Knox and McElroy, 1984; Sarmiento and Toggweiler, 1984; Siegenthaler and Wenk, 1984]. Thus there is acute interest in paleoceanographic indices of nutrient status in the Antarctic surface over glacial/interglacial cycles.

[3] The $^{15}\text{N}/^{14}\text{N}$ of sinking organic matter reflects the utilization of nitrate (NO_3^-) in regions such as the Southern Ocean where NO_3^- is not completely consumed [Altabet and Francois, 1994]. Phytoplankton preferentially take up

^{14}N -bearing NO_3^- . As the initial NO_3^- supply is progressively consumed, the $^{15}\text{N}/^{14}\text{N}$ of the NO_3^- increases, leading to a related increase in the $^{15}\text{N}/^{14}\text{N}$ of the organic matter produced from the NO_3^- . There is ample evidence from the modern Southern Ocean that the N isotopic composition of the surface NO_3^- pool and surface sedimentary organic matter reflects the proportion of the NO_3^- supply from the subsurface that is consumed in the euphotic zone [Altabet and Francois, 1994; Francois et al., 1992; Sigman et al., 1999]. Thus measurements of higher $^{15}\text{N}/^{14}\text{N}$ in bulk sediment from glacial intervals in Antarctic sediment cores have been taken to indicate enhanced NO_3^- utilization during glacial episodes [Francois et al., 1997].

[4] In addition to the primary signal of NO_3^- assimilation by phytoplankton, the $^{15}\text{N}/^{14}\text{N}$ of sedimentary organic matter may be altered during sinking and burial [Altabet and Francois, 1994]. Isotopic measurements in relatively organic C-poor sediments from the open ocean suggest that isotopic alteration (^{15}N enrichment) occurs during the diagenesis of organic matter as it is incorporated into the sediment, leading to ^{15}N enrichment of the buried N relative to the organic matter flux to the seafloor [Altabet, 1996]. Studies of spatial variations in the equatorial Pacific and the Southern Ocean, where sediments are deposited at depth, suggest that the degree of alteration at the seafloor is consistently 2–5‰, such that variations in sediment $^{15}\text{N}/^{14}\text{N}$ are largely related to variations in $^{15}\text{N}/^{14}\text{N}$ of the

flux to the seafloor [Altabet and Francois, 1994; Farrell et al., 1995]. However, studies of continental margin sediments show little alteration of the bulk $^{15}\text{N}/^{14}\text{N}$ signal from surface to seafloor, presumably due to less intense alteration of the organic matter accumulating in the sediments [Altabet et al., 1999; Ganeshram et al., 2000; Kienast et al., 2002]. Thus changes in seafloor conditions may lead to significant changes in the degree of isotopic alteration of sedimentary N. Moreover, while rapidly sinking material undergoes relatively little isotopic alteration in the water column [Altabet and Francois, 1994], concerns have recently been raised that slowly sinking PN may undergo extensive isotopic alteration [Lourey et al., 2003].

[5] Because of mounting questions pertaining to alteration, researchers have begun to measure the isotopic composition of N bound within the silica frustules of diatoms [Crosta and Shemesh, 2002; Shemesh et al., 1993; Sigman et al., 1999]. This organic material plays a role in the construction of the silica frustule itself [Kroger et al., 2002] and is thought to be protected from alteration [Ingalls et al., 2003; Shemesh et al., 1993; Sigman et al., 1999]. If the diatom-bound N is protected, then it represents an N archive related to the primary photosynthetic signal that is free of diagenetic artifacts. The results of Sigman et al. [1999] suggested that ^{15}N -enriched material of diagenetic origin was removed by this approach, and that the sense of the downcore diatom-bound $^{15}\text{N}/^{14}\text{N}$ (or $\delta^{15}\text{N}$) trends were consistent with the bulk sediment measurements, suggesting that past interpretations based on bulk measurements were robust ($\delta^{15}\text{N} = (^{15}\text{N}/^{14}\text{N}_{\text{sample}}/^{15}\text{N}/^{14}\text{N}_{\text{reference}} - 1) \times 1000\%$ where the universal reference is atmospheric N_2).

[6] In this paper we describe a new method for measuring the $\delta^{15}\text{N}$ of diatom-bound nitrogen. The method development was initiated to reduce the required sample size to <10 mg of opal, in order to expand the utility of the microfossil-bound proxy to the study of sediment trap samples and opal-poor sedimentary records. Rather than using the traditional combustion method for measuring the N isotopic composition of the diatom-bound organic matter, we combine (1) a wet chemical oxidation with potassium persulfate to convert the organic N to NO_3^- with (2) the “denitrifier” method of isotopic analysis of NO_3^- [Sigman et al., 2001]. We refer to this combination as the persulfate-denitrifier method throughout [Knapp and Sigman, 2003].

[7] To our surprise, repeated experiments have shown an offset between the combustion-based diatom-bound $\delta^{15}\text{N}$ measurements and those from the persulfate-denitrifier method that can only be explained by the presence of an additional N pool associated with the solid opal samples. This additional N pool is probably adsorbed and/or trapped N_2 and can therefore be considered a contaminant related to the combustion-based measurement of diatom-bound $\delta^{15}\text{N}$ (see Appendix A for further discussion). The realization of a problem with the previous method led us to remeasure downcore profiles from the Southern Ocean that have been published previously [Crosta and Shemesh, 2002; Sigman et al., 1999]. Diatom-bound $\delta^{15}\text{N}$ profiles generated by the persulfate-denitrifier method show a reduced or absent increase into the LGM indicating that changes in NO_3^-

utilization are smaller, more local features than previously supposed. These results, in the context of lowered export productivity [Frank et al., 2000; Kumar et al., 1993; Mortlock et al., 1991], remain consistent with a stratified glacial Southern Ocean [Francois et al., 1997], in that the reduced export production of the glacial Antarctic was not apparently associated with reduced nutrient utilization during the last ice age. However, the new data fit with the argument of Lefevre and Watson [1999] that atmospheric Fe inputs were subordinate to upwelling as the dominant mechanism of iron supply even during the last ice age, such that NO_3^- utilization did not change greatly as the Antarctic stratified. Given these new findings, we reconsider the potential of Antarctic stratification to explain the reduction of atmospheric CO_2 during the last ice age. However, the apparent variability in the degree of nitrate consumption between the Atlantic and Indian sectors of the Antarctic suggests that more work is required to develop an adequate picture of the glacial Antarctic NO_3^- field.

2. Methodology

2.1. Materials

[8] The $\delta^{15}\text{N}$ of diatom-bound N was measured in a surface sediment (4–5 cm) transect (ANTARES) from 56° to 46°S, from the Antarctic zone across the Polar Front into the Subantarctic Zone of the Southern Ocean (Figure 1), as well as from three Southern Ocean depth profiles from south of the Antarctic Polar Front (APF). The profile from core NBP 96-4-2 MC4 (64°S, 170°E) is exclusively Holocene in age (R. F. Anderson, personal communication, 2003). The other two records extend to the LGM: piston core IO1277-10PC (52°S; 21°E) is from the Atlantic sector and piston core MD84-552 (55°S, 74°E) is from the Indian sector of the Southern Ocean (Figure 1). Estimation of the depth of the LGM in MD84-552 is based on the planktonic foraminiferal oxygen isotope stratigraphy (L.D. Labeyrie, personal communication, 1996). The LGM in IO1277-10PC is identified using *Euclampia Antarctica* abundance [Crosta and Shemesh, 2002].

2.2. Physical Separation and Cleaning

[9] Diatoms were physically separated from bulk sediment and cleaned of external organic material, following a protocol adapted from Sigman et al. [1999] as follows. After the physical separation, the resulting 10–150 μm fraction is chemically cleaned using a hot 30% hydrogen peroxide (H_2O_2) solution that is refreshed 3–4 times. Approximately 30 mL of H_2O_2 is added to a 50 mL centrifuge tube containing the diatom fraction. The tube is capped and then placed in a boiling water bath for ~40 min. The cap must be vented. After each oxidation, the sample is spun down in a centrifuge and the resulting supernatant is discarded. After the final oxidation, the sample must be rinsed thoroughly to remove any traces of nitrate. The use of H_2O_2 is the primary difference between our chemical cleaning method and Sigman et al.’s [1999] published protocol. The <150 μm opal fraction could potentially contain radiolarian fragments. Samples were checked visually for significant quantities of

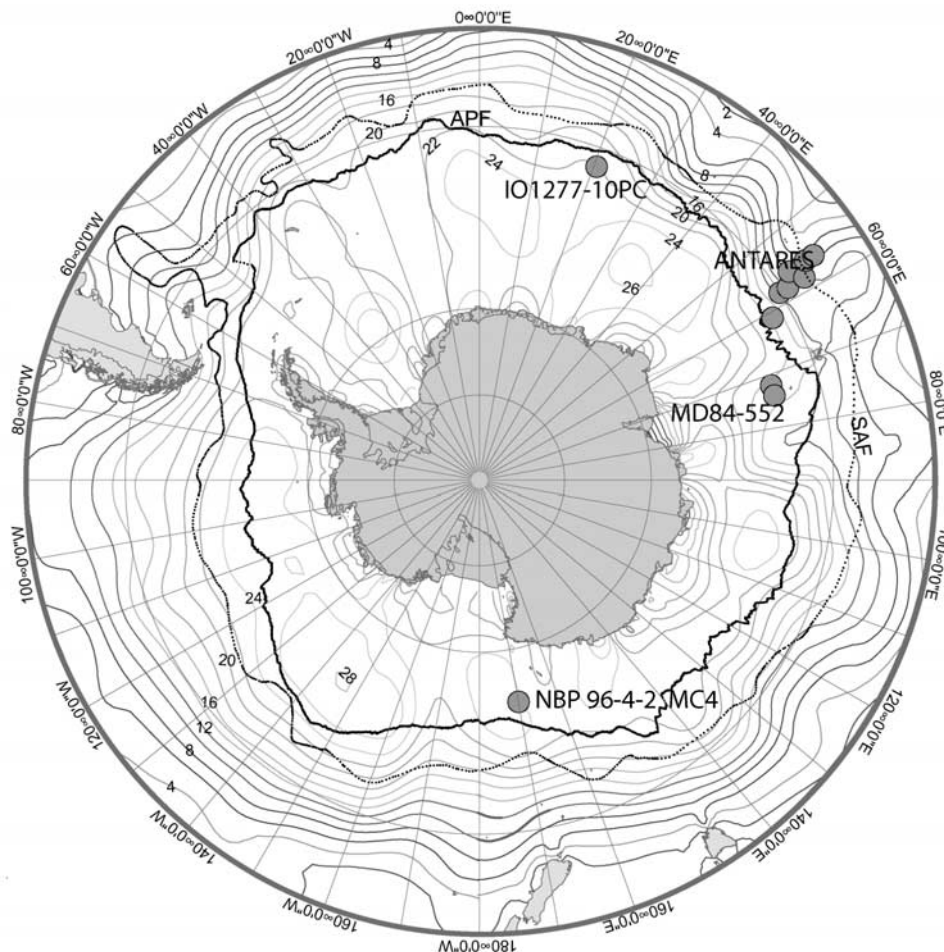


Figure 1. Polar stereographic view of the Southern Ocean showing the locations of sediment cores used in this study. Multicore NBP 96-4-2 MC4, from the west Pacific sector, is composed of Holocene diatomaceous ooze and was used to compare the combustion-based and persulfate-denitrifier techniques for measuring the $\delta^{15}\text{N}$ of diatom-bound N. The ANTARES cores provide a meridional transect across the Antarctic-to-Subantarctic transition to test for the existence of a link between surface nitrate concentrations and diatom-bound $\delta^{15}\text{N}$. Core MD84-552 from the Indian sector and cores IO 1277-10PC from the Atlantic sector were used to investigate diatom-bound $\delta^{15}\text{N}$ across the last glacial/interglacial transition. All of the downcore profiles are from sites to the south of the modern Antarctic Polar Front (APF, bold line; from Moore *et al.* [2000]). The ANTARES cores extend from the Antarctic Zone across the APF, the Polar Frontal Zone, the Subantarctic Front (SAF, dashed line; from Orsi *et al.* [1995]), and into the Subantarctic Zone. Annually averaged NO_3^- concentrations (μM) [Levitus *et al.*, 1994] in the Southern Ocean are contoured with a $2 \mu\text{M}$ contour interval. See color version of this figure in the HTML.

radiolarian, but these were rare in their occurrence. As a secondary check, a subset of samples from IO1277-10PC were sieved at $63 \mu\text{m}$. The diatom-bound $\delta^{15}\text{N}$ measured from the <63 and $<150 \mu\text{m}$ fractions were indistinguishable, suggesting that the larger radiolarians were not contributing significantly to the “diatom” signal or were not isotopically distinct from it.

[10] The NBP 96-2-4 MC4 profile, the ANTARES samples, and the MD84-552 profile had been cleaned previously with perchloric acid [Sigman *et al.*, 1999]. In this study, new samples from NBP 96-2-4 MC4 and MD84-552 were cleaned with H_2O_2 ; the results for these two cleanings are compared below. The persulfate-denitri-

fier measurements are for ANTARES samples cleaned previously with perchloric acid and then subsequently recleaned using the H_2O_2 treatment. The IO1277-10PC samples were physically separated and then cleaned using the H_2O_2 -based protocol.

[11] With the persulfate-denitrifier method for analysis of microfossil-bound, N differences among oxidative cleaning methods became apparent, requiring that we reconsider the cleaning protocol. We experimented with several cleaning methods before adopting the H_2O_2 treatment. These experimental treatments ranged from weak, using the detergent sodium dodecyl sulfate (SDS), to extremely harsh, where the samples are boiled in 72% H_4ClO_4 for 2 hours (Table 1).

Table 1. Chemical Cleaning Treatments

Treatment	Persulfate-Denitrifier		Combustion	
	Blank Corrected, $\mu\text{mol N/g}$	Blank Corrected, $\delta^{15}\text{N}$	Blank Corrected, $\mu\text{mol N/g}$	Blank Corrected, $\delta^{15}\text{N}$
Bulk, no treatment	25.1	5.5	34.1	4
SDS, 1 hour boiling	25.3	4.8		
70°C H ₄ ClO ₄ , overnight	14.4	3.5	27.5	1
SDS + 30% H ₂ O ₂ , ^a boiling	13.6	2.8		
H ₄ ClO ₄ soft ^b	14.2	2.7		
SDS + H ₄ ClO ₄ soft	14.6	2.9		
SDS + H ₂ O ₂ + H ₄ ClO ₄ soft	13.2	3.8		
H ₄ ClO ₄ hard ^c	12.4	3.8	20.5	0.5
SDS + H ₄ ClO ₄ hard	12.9	3.2		
SDS + H ₂ O ₂ + H ₄ ClO ₄ hard	10.8	5.5		

^aThree fresh aliquots of H₂O₂ were added, each after 1 hour of sample treatment in a boiling water bath. The sample was centrifuged and the spent H₂O₂ decanted prior to the addition of fresh H₂O₂.

^bIn the H₄ClO₄ soft treatment, the oxidation was considered complete as soon as the H₄ClO₄ was brought to boiling.

^cIn the H₄ClO₄ hard treatment, the sample was brought to boiling and simmered for two hours.

Various cleanings were performed first on a single sample (Table 1 and Figure 2) and then on samples of varying ages and sediment compositions (Figure 3). In the course of our experiments, we made two critical observations: (1) the chemical oxidation can go beyond removing the external organic material and begin to attack the microfossil-bound or “target” N (Table 1; Figure 2) and (2) diatoms from clay-rich sediments are more difficult to clean of external organic N (Figure 3). An initial decrease in both concentration and $\delta^{15}\text{N}$ occurs with progressively more aggressive cleaning of the same material, which we interpret as the removal of the external, ^{15}N -enriched N (Figure 2). Beyond this point, N concentrations continue to fall while the isotopic composition begins to rise. This is most pronounced in the sample that has been cleaned first in SDS, then in 30% H₂O₂, and finally boiled for two hours in 72% perchloric acid, which removed ~14 μmol of N per g of opal and resulted in a final $\delta^{15}\text{N}$ of 5.5‰. We interpret the secondary rise in $\delta^{15}\text{N}$ as the result of fractionation during the attack of diatom-bound N by the oxidizing reagent, probably because of opal dissolution during the extreme heating (72% perchloric acid boils at 203°C). The IO 1277-10PC depth profile of sample boiled for two hours in 72% perchloric acid (perchloric hard) shows high-amplitude variations in $\delta^{15}\text{N}$, ranging from 3.5–7.5‰, that are negatively correlated to N content (Figure 3), these variations are also taken to indicate alteration of the “target” N. The lowest $\delta^{15}\text{N}$ results from the SDS + H₂O₂ and SDS + “perchloric soft,” where the sample-bearing perchloric acid is brought to its boiling point and then cooled immediately (Figures 2 and 3); we interpret these cleanings to “completely” clean the microfossils of external organics without altering the target N. The SDS treatment was subsequently shown to have little effect in deep sea sediments and was abandoned. The cleaning of *Sigman et al.* [1999], using 72% perchloric acid heated to 70°C overnight, yields slightly higher N concentration and $\delta^{15}\text{N}$ than the H₂O₂ treatment, which we interpreted to be an indication of slightly weaker cleaning (Figure 2). Comparison of H₂O₂ treatment and the 70°C perchloric treatment in the NBP 96-4-2 MC4 and MD84-552 profiles yielded similar results. We adopted the H₂O₂

treatment over the mild perchloric treatments for safety reasons.

[12] In IO1277-10PC, using only a single aliquot of 30% H₂O₂ for diatom cleaning produced a significantly higher diatom-bound $\delta^{15}\text{N}$ and N content in deglacial/glacial sediments (from 550 to 950 cm depth) than in the Holocene section (Figure 3). However, this LGM/Holocene difference disappeared when the samples were cleaned further with subsequent additions of fresh oxidant, with the glacial samples collapsing to the same values as the Holocene samples (Figure 3). The apparent recalcitrance of the external organics in the glacial samples from IO1277-10PC may be related to the suggestion of *Lourey et al.* [2003] that the organic N in glacial age sediments was highly altered in the water column prior to deposition. Alternatively, the high Al content of the glacial sediments or some change in the conditions at the seafloor (e.g., lower bottom water O₂ during the last ice age [*Francois et al.*, 1997]) may be responsible. There was a small (~1‰) shift to higher $\delta^{15}\text{N}$ between the third and fourth additions of H₂O₂ in some of the middepth (500–700 cm) samples. This shift was not apparent in other samples but is still a cause for concern for future work. As a rule of thumb, we use the N content as an indication of sample cleanliness, although some variation (as much as 3–4 μmol N per gram of sediment) is typical. High outliers were targeted for additional H₂O₂ treatments. It is important to note that, with experience, we have found that very clay-rich samples (i.e., not from the Antarctic) are more difficult to clean. It seems that small amounts of residual clay adhere to the silica surfaces and impede the oxidative cleaning so that some samples may require up to 7 additions of fresh H₂O₂ (B. Brunelle, unpublished data, 2003). N from clays does not appear to affect the measuring $\delta^{15}\text{N}$. Examination under scanning electron microscope revealed no obvious differences between samples cleaned by various methods.

2.3. Concentration and Isotope Measurements

[13] Nitrogen concentration and $\delta^{15}\text{N}$ measurements were made on the cleaned opal samples using a persulfate oxidation technique to convert organic nitrogen (ON) to

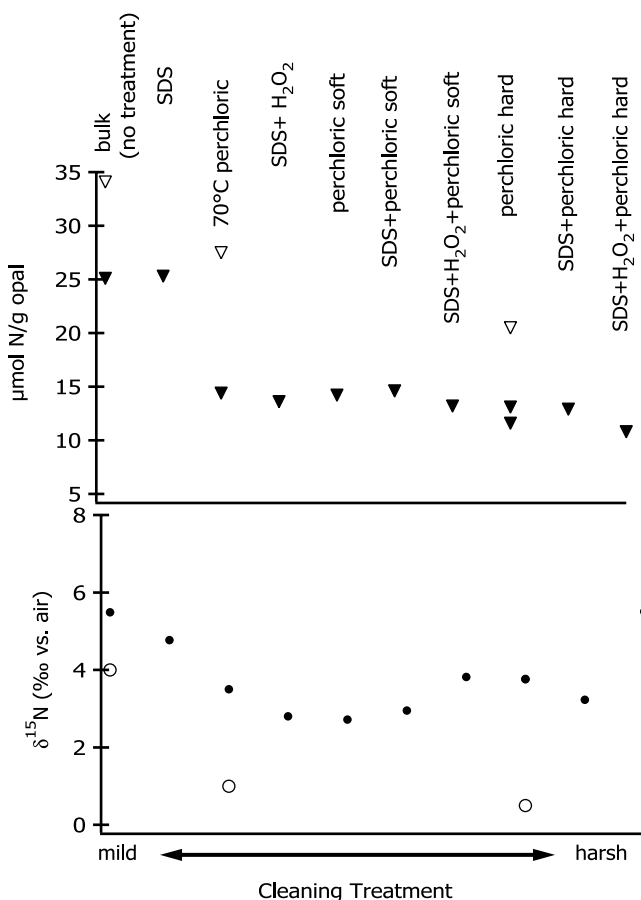


Figure 2. Diatom-bound N content (upper panel) and $\delta^{15}\text{N}$ (lower panel) for subsamples of sample NBP 96-4-2 MC4 14–16 cm are plotted against cleaning treatment. See Table 1 for full explanation of the cleaning treatments. The treatments are qualitatively ranked from mild (left) to harsh (right). Analyses by combustion (open symbols) and the persulfate-denitrifier method (filled symbols) are plotted for comparison. In samples ranging from no treatment through the “perchloric soft” treatment, N content, and $\delta^{15}\text{N}$ decline with increasing strength of cleaning. Beyond the perchloric soft treatments, N content continues to decline while $\delta^{15}\text{N}$ increases. The initial decreasing trend is taken to represent the removal of diagenetically altered, ^{15}N -enriched external organic material. With the “perchloric hard” treatments, where the cleanings involve prolonged exposure of the silica to very high temperature ($>200^\circ\text{C}$), there is an increase in $\delta^{15}\text{N}$ that likely results from the attack and isotopic alteration of the internal, diatom-bound N. See color version of this figure in the HTML.

NO_3^- combined with the “denitrifier method” for measuring the N isotopic composition of NO_3^- [Knapp and Sigman, 2003; Sigman et al., 2001]. Persulfate oxidation is a commonly utilized technique for measuring dissolved organic N in natural waters [Bronk et al., 2000]. The solution’s basic pH and oxidizing potential worked to both dissolve the opal, releasing the interstitial ON, and then quantitatively convert the ON to NO_3^- . A solution of 1.5 M sodium hydroxide (NaOH) and 0.22 M potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$; recrys-

tallized 3 times) was added to a precombusted glass vial containing 5–10 mg of cleaned opal. The vials were tightly capped and autoclaved for 55 min. NO_3^- concentrations were measured by chemiluminescence [Braman and Hendrix, 1989]. Several reagent blanks, generally less than $2 \mu\text{M}$ NO_3^- , were measured in each run, and they generally

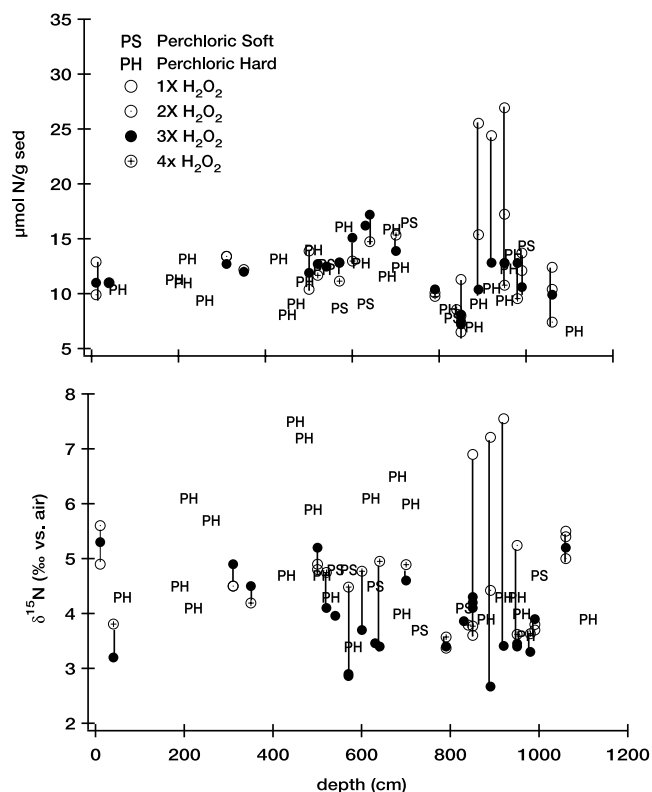


Figure 3. Downcore diatom-bound N content (upper panel) and $\delta^{15}\text{N}$ (lower panel) from different cleaning treatments on subsamples of sediment that range in age from Holocene to the last glacial period in IO1277-10PC. The LGM is located at approximately 820 cm depth [Crosta and Shemesh, 2002]. The perchloric hard (PH) analyses show high-amplitude variation in diatom-bound $\delta^{15}\text{N}$, likely due to alteration of the diatom-bound N during this treatment. The perchloric soft (PS) cleaned samples do not show this degree of variability. The H_2O_2 treated samples were cleaned with up to four aliquots of 30% H_2O_2 . Each of the samples shown here was subsampled before each subsequent addition of H_2O_2 and analyzed for diatom-bound N content and $\delta^{15}\text{N}$. The results show little change between additions of H_2O_2 in the youngest part of the core. The changes in N content and $\delta^{15}\text{N}$ demonstrate the continued removal of N during cleaning for samples between 550 and 620 cm and again between 830 and 950 cm. Glacial age samples show a significant decrease in diatom-bound N content and $\delta^{15}\text{N}$ after second and third additions of H_2O_2 and some show a small change after the fourth addition of H_2O_2 . The middepth samples show a slightly different pattern of change, where N content falls after the fourth addition of H_2O_2 but the $\delta^{15}\text{N}$ increases by up to 1.5‰.

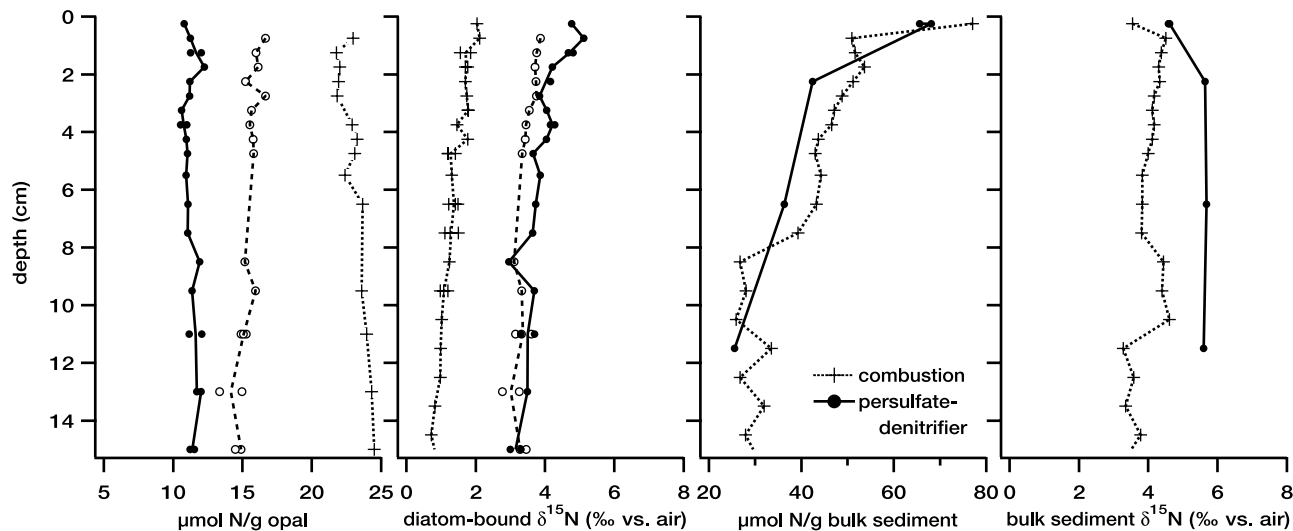


Figure 4. Diatom-bound N content and $\delta^{15}\text{N}$ from multicore NBP 96-4-2 MC4 from the Pacific sector of the Antarctic. The sediment of this core is entirely of Holocene age. Measurements by the combustion-based method (crosses) and persulfate-denitrifier method (circles) are shown for comparison for both diatom-bound (left panels) and bulk sediment N (right panels). Samples cleaned with 72% perchloric acid at 70°C overnight are shown with an open circle. Those cleaned by the H_2O_2 technique are plotted as filled circles. The physical separation steps were excluded for the NBP 96-2-4 MC4 samples due to the purity of the opal. The offset between the bulk, uncleaned sediment, and the diatom-bound material suggests that we are measuring the $\delta^{15}\text{N}$ of a different pool. See color version of this figure in the HTML.

accounted for less than 7% of the total NO_3^- concentration. The resulting NO_3^- in solution was then measured for $\delta^{15}\text{N}$ by the denitrifier, in which the NO_3^- is converted to N_2O to isotopic analysis [Sigman *et al.*, 2001]. The isotopic composition of the N_2O is measured by gas chromatography-isotope ratio mass spectrometry using a ThermoFinnigan GasBench II and Delta Plus. Isotope analyses were all standardized to potassium nitrate reference material IAEA-N3 (4.7‰) [Revesz *et al.*, 1997]. Long term precision for the total analysis, including cleaning, wet chemical oxidation and isotope measurement, is currently better than 0.5‰. Persulfate blank corrections were made for each sample and corrections were generally within precision.

[14] Previous workers have measured the $\delta^{15}\text{N}$ of diatom-bound organic matter by combustion methods, where an elemental analyzer is coupled to an isotope ratio mass spectrometer [e.g., Crosta and Shemesh, 2002; Sigman *et al.*, 1999]. The persulfate-denitrifier technique is more sensitive than the combustion method, allowing for a reduction in sample size for a single analysis from 80 to 100 mg using combustion [Sigman *et al.*, 1999] to ~5 mg of opal using the persulfate-denitrifier method, the handling of the microfossils and the scale of the oxidation preventing the use of even smaller quantities. More importantly, the persulfate-denitrifier method gives significantly different N content and isotope results for the same samples measured by combustion (Figures 2, 4, 5, and 6): N is typically lower by $\geq 5 \mu\text{mol N/g opal}$, and $\delta^{15}\text{N}$ is higher by 1–3‰. These offsets are also apparent in bulk, uncleaned sediment, although the isotopic discrepancy tends to be smaller, presumably due to the higher total N content of bulk sediment when compared to cleaned opal (Figure 4). One

explanation for this discrepancy is incomplete conversion of opal-bound ON during the persulfate oxidation step. Without quantitative conversion of the ON to NO_3^- , there could be fractionation of the N isotopes. Several experiments were performed to determine conversion efficiency. Initial tests demonstrated complete conversion of relatively high concentrations (50–150 μM) of simple organic compounds such as glycine and aminocaproic acid. Questions remained because the opal-bound proteins could be more difficult to oxidize to NO_3^- . Working with the diatom-bound material, additional oxidations were performed to see if there was any remaining organic N to be converted to NO_3^- . Measured concentrations remained unchanged after blank adjustment upon the addition of a second aliquot of persulfate reagent and a second autoclaving. In an experiment to test whether the opal was completely dissolving to release all of the bound ON, the silica was removed prior to the oxidation adding 25 μl of hydrofluoric acid (HF) to each sample. The samples were suspended in 100 μl deionized water in clean Teflon vials in an ice bath and dried completely following the HF addition. The remaining organic residue was oxidized using persulfate. The N yield and isotopic compositions remained unchanged.

[15] The consistency of these results left us confident in our ability to quantitatively convert the diatom-bound ON to NO_3^- , so that we began to suspect the earlier combustion-based measurements of Sigman *et al.* [1999]. One possibility is that there is an additional N pool associated with the diatom frustule that was measured during flash combustion of the solid diatom samples; given the differences between the two methods, this additional N pool is likely to be gaseous. The logic behind this inference is that both the

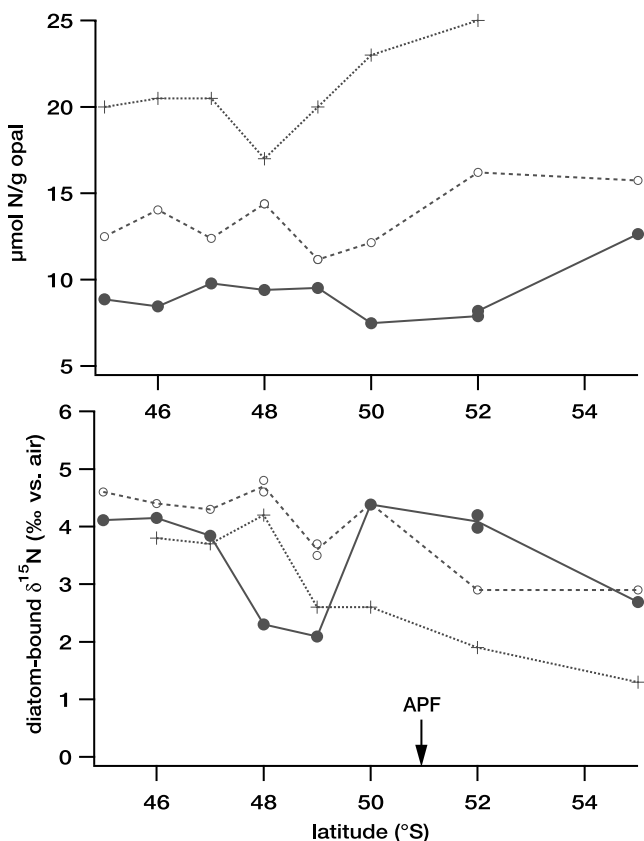


Figure 5. Diatom-bound N content and $\delta^{15}\text{N}$ from the ANTARES meridional transect across the APF (51°S) in the Indian sector of the Southern Ocean. All samples are taken from within the Holocene interval at 4–5 cm depth. Measurements by the combustion-based method (crosses) and the persulfate-denitrifier method (circles) are shown for comparison. Open circles represent samples cleaned with 72% perchloric acid at 70°C overnight and those same samples then recleaned with the H_2O_2 technique are shown as filled circles. The increases in $\delta^{15}\text{N}$ across the Antarctic Zone and then again across the Subantarctic correspond with decreasing surface nitrate (Figure 1), consistent with the expectation that diatom-bound $\delta^{15}\text{N}$ is sensitive to the $\delta^{15}\text{N}$ of surface nitrate and thus the degree of nitrate utilization by phytoplankton. The minimum around 49°S, at or near the Subantarctic Front, is likely due to regional differences in the isotope effect (ϵ). See color version of this figure in the HTML.

microfossil bound ON and the additional N would be measured in the combustion system, but any gaseous N adsorbed to the diatom frustules would be released during the dissolution and wet chemical oxidation. At first blush, it would seem that the contaminant N pool is N_2 , which has an isotopic composition of 0‰ in the atmosphere and $\sim 0.5\text{‰}$ in seawater [Brandes *et al.*, 1998], is easily adsorbed by silica, and could be trapped in diatom frustule pore spaces. Mass balance estimates of the isotopic composition of the additional N, using the combustion and persulfate-denitrifier results from the NBP 96-4-2 MC 4 Holocene profile

(Figure 4), give an average value of $-3.0\text{‰} \pm 0.8\text{‰}$ (1σ), which is lower than one would expect from air (see Appendix A for a more complete discussion). We tested the existence of a gaseous N blank in the combustion analysis by measuring the concentration of microfossil bound N with a Carlo Erba 2500 elemental analyzer, with and without the frustule, again using HF for frustule removal. Here 200–300 μl of HF was added to an in-house sedimentary opal standard that was cleaned by boiling in perchloric acid which was sample suspended in 200 μl of 30% HCl in a silver capsule on ice, subsequently dried down, resuspended in HCl to volatilize any remaining HF and dried down a second time. N concentration in the untreated solid sample was similar to those measured with combustion by Sigman *et al.* [1999], 17 $\mu\text{mol N g}^{-1}$ sediment, while the sample without the silica frustule contained only 12 $\mu\text{mol N g}^{-1}$ sediment, identical to the N content measured by the persulfate method. Six additional samples from IO1277-10PC were prepared for isotopic analysis by combustion on a Carlo Erba elemental analyzer/Thermo-Finnigan system at the GFZ Potsdam; three from the Holocene section and three from the glacial. As anticipated, the diatom-bound $\delta^{15}\text{N}$ of solid samples was always lower than the diatom-bound $\delta^{15}\text{N}$ of the samples without a frustule. Holocene solid samples averaged 0.8‰ while the samples treated with HF averaged 3.5‰ and the average for these same samples measured by the persulfate-denitrifier method is 4.2‰. Similarly, in the glacial interval, the average for the solid samples was 0.6‰, for the HF-treated samples it was 3.1‰, and as measured by persulfate-denitrifier it was 4.3‰. These results suggest that, indeed, the main difference between the combustion and persulfate-denitrifier analyses is related to the presence of the diatom frustule. While more work is needed, we suspect that the modest differences in results between HF treatment/combustion and the persulfate-denitrifier method are due to either (1) a N blank associated with HF, which was too small to characterize isotopically, or (2) the persistence of some opal through the HF treatment. There are also significant differences between the solid, combustion-based diatom-bound $\delta^{15}\text{N}$ values that we measured and those measured by Crosta and Shemesh [2002]. Both the large offset in absolute values and the lack of glacial-interglacial difference in our analyses are likely due to differences in the cleaning protocols. Crosta *et al.* [2002] report diatom-bound N concentrations in the range of 0.05–0.08% (35–60 $\mu\text{mol N/g opal}$) with the higher values in glacial sediments. The N content of their samples is on average 2–4 times higher than the N contents that we have measured with the persulfate-denitrifier method or than what Sigman *et al.* [1999] published as part of their combustion-based results. These differences underscore the importance of our new cleaning results and their demonstration of the variability in cleaning requirements between samples.

[16] BET surface area experiments which rely on the adsorption of N_2 have been previously conducted with biogenic silica-rich sediments [Hurd *et al.*, 1981, 1979]. These measurements indicate that, if the total specific pore volume of a gram of diatomaceous material held N_2 , then there would be between 0.25 and 0.5 cm^3 of N_2 or $\sim 20\text{--}$

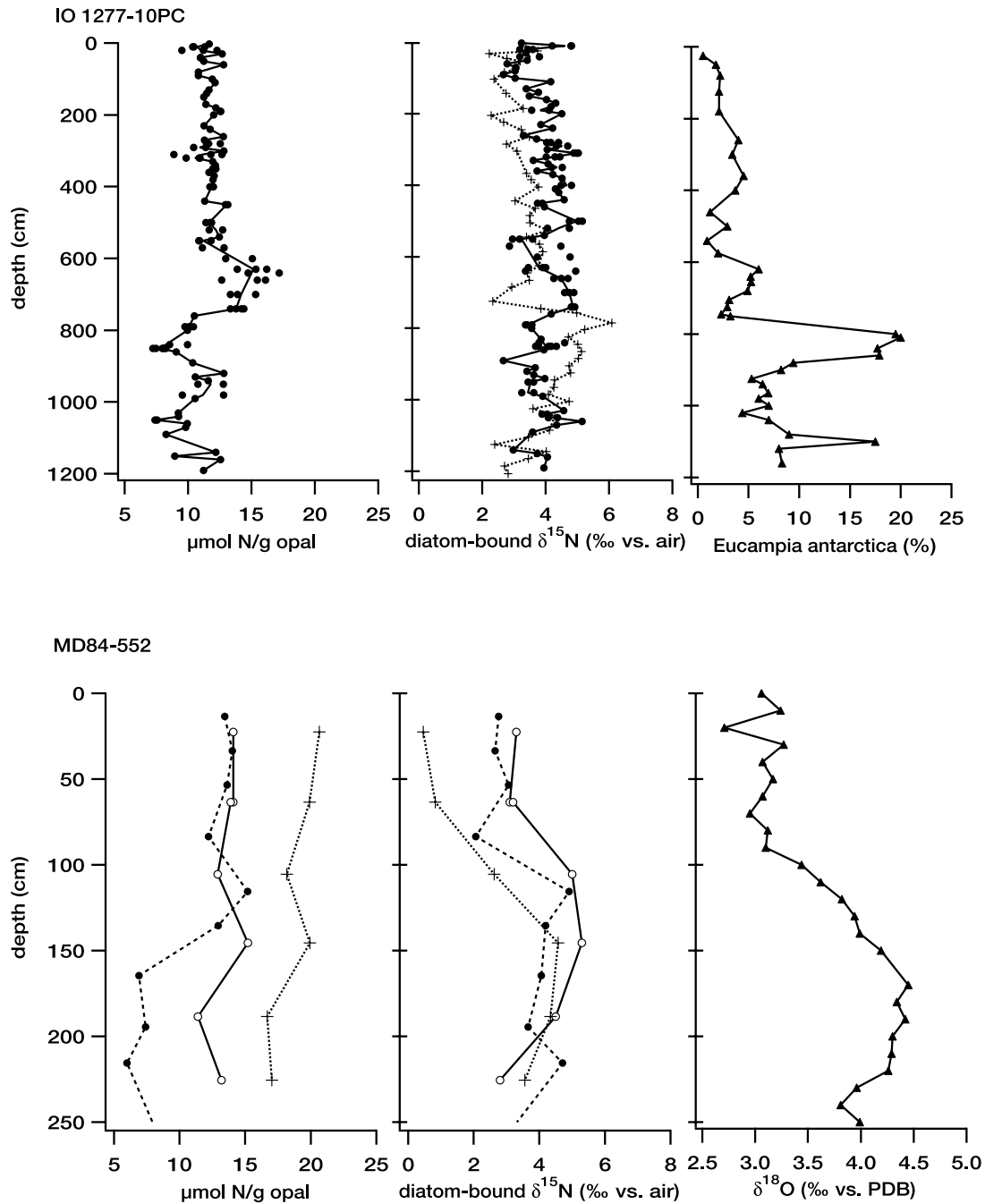


Figure 6. Downcore profiles of diatom-bound N content (left) and diatom-bound $\delta^{15}\text{N}$ (center) for Atlantic sector core IO1277-10PC (top) and Indian sector core MD84-552 (bottom). Measurements by combustion (crosses) and the persulfate-denitrifier method (circles) are shown for comparison. IO1277-10PC samples were cleaned using H_2O_2 (circles). The location of the LGM at 820 cm in IO 1277-10PC is based on the *Eucampia antarctica* stratigraphy (right) [Crosta and Shemesh, 2002]. There is no notable change in $\delta^{15}\text{N}$ across the glacial-interglacial transition, suggesting that nutrient utilization was not significantly different in the Atlantic sector of the Antarctic during the LGM. Combustion-based measurements (crosses) and persulfate-denitrifier measurements (circles) from MD84-552 are shown for comparison (bottom). Results from samples that were cleaned using the H_2O_2 technique are plotted as filled circles. Samples cleaned with 72% perchloric acid at 70°C overnight are shown with an open circle. Stratigraphic age control for MD84-552 is provided by *N. pachyderma* $\delta^{18}\text{O}$ (bottom right) (L. D. Labeyrie, personal communication, 1996). There is a clear difference in $\delta^{15}\text{N}$ between late Holocene and LGM samples, with higher values in the glacial interval, but the transition is late relative to the glacial-interglacial transition inferred from the oxygen isotopes. See color version of this figure in the HTML.

40 μmoles of N. Thus, if 10–25% of this N_2 was held tightly enough that purging of the sample with He on the elemental analyzer did not remove it prior to combustion, it could account for the differences observed when comparing the two methods. The internal pore volume of diatom opal increases with age due to dissolution and reprecipitation [Hurd *et al.*, 1981, 1979], which may facilitate trapping of N_2 within the frustule.

[17] In summary, the methods for oxidative cleaning and measurement of isotopic measurement of diatom-bound N described above are different from that of Sigman *et al.* [1999] so as to account for two critical observations. First, we demonstrate an offset between combustion-based and persulfate-denitrifier measurements that is best explained by the presence of a gaseous N blank associated with the diatom frustule. Second, we find differences in the ease with which interglacial and glacial Antarctic diatom microfossils are cleaned of their external organic matter. The earlier 70°C, 72% H_4ClO_4 cleaning treatment of Sigman *et al.* [1999] was not thoroughly tested for glacial age samples, so it is unclear whether that treatment is harsh enough for all sample types. In light of these methodological findings, we report new $\delta^{15}\text{N}$ data from two Southern Ocean cores that extend to the LGM.

3. Results and Interpretation

3.1. Measurements of Holocene Sediments

[18] In the Holocene NBP 96-4-2 MC4 profile, there is a large offset in both the isotopic and N content between the combustion and persulfate-denitrifier methods (Figure 4). Sigman *et al.* [1999] found that the diatom-bound $\delta^{15}\text{N}$ was similar to the $\delta^{15}\text{N}$ of the mean annual sinking flux in the Antarctic (0–1‰) and 3–5‰ lower than bulk sediment $\delta^{15}\text{N}$. Diatom-bound $\delta^{15}\text{N}$ generated by the persulfate-denitrifier method in NBP 96-4-2 MC 4 are 2–3‰ lower than the bulk sediment $\delta^{15}\text{N}$ measured by the persulfate-denitrifier method (Figure 4). Thus the offset between the bulk sediment and diatom-bound $\delta^{15}\text{N}$ is similar to that observed by Sigman *et al.* [1999]. However, diatom-bound $\delta^{15}\text{N}$ no longer matches the expected $\delta^{15}\text{N}$ of the bulk sinking flux. These data underscore the need to investigate the absolute relationship between diatom-bound $\delta^{15}\text{N}$ and the $\delta^{15}\text{N}$ of the surface nitrate.

[19] We have not tested the persulfate-denitrifier method for its effectiveness at oxidizing bulk sedimentary N, which includes the external, possibly more chemically recalcitrant N. However, the concentration difference between the two methods suggests that bulk sediment oxidation was complete in this case (Table 1; Figures 2 and 4). Thus our results suggest that combustion analyses of low-ON, high-opal sediments are also complicated by the gaseous N contaminant.

[20] In the ANTARES surface sediment transect, diatom-bound $\delta^{15}\text{N}$ increases across the Antarctic Zone across the Antarctic Polar Front (APF) into the Polar Frontal Zone, from 2.8‰ to 4.5‰ (Figure 5), corresponding to a northward decrease in surface water NO_3^- concentration (Figure 1). The meridional increase is interrupted in the vicinity of the Subantarctic Front (SAF) ($\sim 49^\circ\text{S}$), where

diatom-bound $\delta^{15}\text{N}$ drops to ~ 2.0 ‰ and then increases again toward the north across the SAF, as NO_3^- concentration decreases. The decline in diatom-bound $\delta^{15}\text{N}$ at the SAF is best explained by regional differences in the isotope effect of nitrate assimilation (ϵ), where ϵ is 2–3‰ higher in the Subantarctic than in the Antarctic (i.e., 7–8‰ and 5‰, respectively). This difference in ϵ between the Antarctic and the Subantarctic has been proposed previously on the basis of sinking flux $\delta^{15}\text{N}$, which has an unexpectedly small meridional gradient from the Polar Frontal Zone to the Subantarctic Zone [Lourey *et al.*, 2003]. An epsilon of 7–8‰ in the Subantarctic also appears to be required to explain seasonal variations in surface nitrate $\delta^{15}\text{N}$ in this zone (D. Sigman and T. Trull, unpublished data, 2004). This arising evidence for regional variations in the isotope effect is a concern for the use of N isotope records to reveal changes in utilization and requires that we study the meridional gradient of diatom-bound $\delta^{15}\text{N}$ in a region with a broader meridional extent for the zones between the Antarctic and the subtropics.

3.2. Paleoceanographic Profiles

[21] In the Atlantic Ocean record IO1277-10PC, there is no clear change in the $\delta^{15}\text{N}$ across the glacial-interglacial transition (Figure 6), with the diatom-bound $\delta^{15}\text{N}$ varying around 4 ± 1 ‰. This is in marked contrast to the combustion measurements that indicated a ~ 4 ‰ shift across the glacial/interglacial transition (Figure 6) [Crosta and Shemesh, 2002]. The diatom-bound $\delta^{15}\text{N}$ record from IO1277-10PC has significant “scatter,” which may be associated with the extremely high accumulation rate of this core but might be at least partially due to greater error in our early work with the new method. The $\delta^{15}\text{N}$ of diatom-bound organic matter in core MD84-552 from the Indian sector, as measured by the persulfate-denitrifier method, is ~ 2 ‰ higher in the deglacial than during the Holocene and ~ 1 ‰ higher during the LGM than the Holocene (Figure 6). This LGM/Holocene difference is much reduced (from 4‰ to 1‰) from the previous combustion-based analysis [Sigman *et al.*, 1999]. Moreover, the depth of the $\delta^{15}\text{N}$ transition relative to the planktonic foraminiferal $\delta^{18}\text{O}$ transition suggests that the N isotopic change occurs after initial deglaciation. However, there is no absolute age control for this core, and thus there is some uncertainty surrounding the age model in MD84-552. Taken together, the records reported here suggest some amount of spatial variation in the degree to which nitrate consumption has changed over the last glacial/interglacial transition.

4. Discussion

4.1. Paleoceanographic Significance

[22] There is proxy evidence for a decrease in export productivity south of the APF during glacial times [Francois *et al.*, 1997; Frank *et al.*, 2000; Kumar *et al.*, 1995; Mortlock *et al.*, 1991], while the previous isotope measurements on bulk sedimentary and diatom-bound N were taken to indicate higher nitrate utilization in the glacial Antarctic. For lower export production to coincide with more complete nitrate consumption requires that the supply of nitrate was reduced during glacial times, yielding

the inference of vertical stratification in the glacial Antarctic [Francois *et al.*, 1997].

[23] The diatom-bound $\delta^{15}\text{N}$ data reported here, which indicate that nitrate utilization was either the same or somewhat higher during glacial times, remains consistent with this interpretation, in that a glacial decrease in nitrate utilization would be required if the lower export production were to occur without a lower nutrient supply. However, these results do lead to a different picture for the biogeochemical conditions of the surface Antarctic during the last ice age and their effect on atmospheric CO_2 .

4.2. Spatial Heterogeneity in Nitrate Utilization

[24] The new data hint at some degree of spatial heterogeneity in the history of nitrate utilization within the Antarctic, with utilization increasing somewhat in the Indian sector but remaining constant in the Atlantic sector. Estimates of the extent of nitrate utilization, using the Rayleigh model equations for nitrate fractionation and the persulfate-denitrifier results, suggest that uptake in the Indian sector may have increased to $\sim 50\%$ during the glacial based on the integrated product equation or $\sim 35\%$ based on the instantaneous product equation [Sigman *et al.*, 1999]. While the estimated increase in nitrate utilization is significantly lower than previous estimates [Crosta and Shemesh, 2002; Sigman *et al.*, 1999], it nonetheless represents a clear increase in the degree of nitrate consumption and therefore the efficiency of the biological pump in the Indian Sector.

[25] Surface water in the ACC travels eastward at <20 cm/s (~ 17 km/day) between fronts and as fast as 40 cm/s (~ 35 km/day) in the frontal regions. At these rates, it takes >2 years for a parcel of water in the ACC to circumnavigate Antarctica. Since the $^{15}\text{N}/^{14}\text{N}$ of surface nitrate is reset toward deep ocean conditions each winter, we should not expect that the increased nitrate consumption in the Indian sector would be evident in $\delta^{15}\text{N}$ of diatom-bound N in Atlantic records. Thus the spatial variability in the nitrate uptake histories suggested by the diatom-bound N isotope data in the Atlantic and Indian sectors is at least physically possible given the celerity of the ACC.

4.3. The Inputs of Iron to the Glacial Antarctic

[26] The incomplete consumption of the major nutrients nitrate and phosphate in the modern Southern Ocean is generally attributed to iron limitation [Martin, 1990; Moore *et al.*, 2000]. From this perspective, an increase in the completeness of nitrate consumption requires an increase in the iron-to-nitrate ratio ($\text{Fe}:\text{NO}_3^-$). Upwelling from the ocean interior supplies both Fe and NO_3^- to phytoplankton, while atmospheric inputs typically supply Fe in a much higher ratio to bioavailable N. Thus an increase in the $\text{Fe}:\text{NO}_3^-$ supply ratio in the Antarctic could be driven by: (1) an increase in the $\text{Fe}:\text{NO}_3^-$ of the upwelling deep water, or (2) an increase in the relative importance of atmospheric Fe input, by increasing the atmospheric input and/or decreasing the deep water input. Lacking any information on the Fe chemistry of deep water, we concern ourselves only with (2).

[27] Ice core and sediment records from the Antarctic suggest that atmospheric iron deposition was perhaps 20 times higher during the last ice age [Petit *et al.*, 1999]. Despite this apparently large increase in deposition, compilations of global iron data suggest that atmospheric iron supply to the Antarctic likely makes up less than 10%, maybe less than 1%, of the total iron delivered to the surface Antarctic [Archer and Johnson, 2000; Lefevre and Watson, 1999], although there is debate over this ratio [Archer and Johnson, 2000; Fung *et al.*, 2000; Lefevre and Watson, 1999]. Lefevre and Watson [1999] argue that even a 20-fold increase in atmospheric Fe input during the LGM was unlikely to have had a great effect on the overall Fe budget in the Southern Ocean. Our diatom microfossil N isotope data from the Atlantic, which show no evidence for more complete nitrate consumption during the last ice age, are consistent with that argument.

[28] However, the Indian sector data do show an increase in NO_3^- utilization. Modern dust deposition maps compiled from observations and models suggest that the highest rates of deposition in the Atlantic sector and the lowest in the Pacific [Duce and Tindale, 1991; Mahowald *et al.*, 1999]. Taking the broadest interpretation of the model estimates of dust deposition during the LGM, there is a predicted intensification of atmospheric dust deposition that is focused in the Atlantic sector, with only a small (2–5 fold) increase in the Indian sector [Mahowald *et al.*, 1999]. Thus our tentative observation of increased nitrate utilization in the Indian sector of the Antarctic does not appear to fit with model-based expectations for the distribution of Fe input during the LGM. Before this level of interpretation can be fruitful, more work should be done to improve the quality and quantity of the downcore records.

4.4. Comparison With Other Proxies

[29] Planktonic foraminiferal Cd/Ca data, generally used as a proxy for surface water nutrient concentrations, show no difference between the Holocene and the LGM in profiles from both the Atlantic and Indian sectors of the Antarctic [Boyle, 1988; Keigwin and Boyle, 1989]. The new diatom-bound $\delta^{15}\text{N}$ profiles are now more consistent with these data (from the nutrient proxy point of view), in that they show no change in nutrient utilization between the last glacial and the Holocene in the Atlantic sector and a smaller change than previously in the Indian sector. However, the general change in the diatom-derived N isotope records may prove to be problematic for a recent hypothesis that worked to reconcile the N and Si isotope records in the Antarctic [Brzezinski *et al.*, 2002]. $\delta^{30}\text{Si}$, which is lower in glacial periods and thus suggests lower silicate utilization during the last ice age [DeLaRocha *et al.*, 1998], was reconciled with the N isotope data in support of higher nitrate utilization by proposing that the Si to NO_3^- uptake ratio was lower because of Fe-replete conditions in the glacial Antarctic [Brzezinski *et al.*, 2002; Hutchins and Bruland, 1998; Takeda, 1998]. Our Indian sector data remain consistent with this scenario, although the N isotope data show a reduced change from previous records, and with a less clear glacial/interglacial structure. However, the Atlantic sector N isotope data do not fit this view any longer, in that the new

data give no evidence of Fe fertilization during the last ice age.

4.5. Implications for Atmospheric CO₂

[30] Because the data reported above yield a picture of glacial/interglacial changes in nitrate consumption that is different from previous studies [Crosta and Shemesh, 2002; Francois *et al.*, 1997; Sigman *et al.*, 1999] we finish by considering the implications for the capacity of Antarctic stratification to explain the glacial reduction in atmospheric CO₂. Antarctic stratification can lower atmospheric CO₂ in three ways [Sigman and Haug, 2004] (excluding here the potential effects of Antarctic changes on the nutrient supply to the low latitude ocean [Sarmiento *et al.*, 2004; Sigman *et al.*, 2003]). First, a decrease in Antarctic overturning simply prevents deeply sequestered CO₂ from being upwelled into the surface ocean; if CO₂ is not brought to the surface, it has no opportunity to escape to the atmosphere, regardless of the Antarctic surface productivity [Toggweiler, 1999; Toggweiler *et al.*, 2003]. Second, if the decrease in overturning is not matched by an equivalent decrease in export production, a larger fraction of the CO₂ that is upwelled to the surface will be returned to the deep sea rather than escaping to the atmosphere. Third, any decrease in CO₂ release via the Antarctic will leave more CO₂ in the deep sea; this will drive CaCO₃ dissolution, rendering the ocean more alkaline and lowering atmospheric CO₂ further [Sigman *et al.*, 1998; Toggweiler, 1999]. In any stratification scenario, the first process is at work. The second process is the one that depends on the degree of nutrient consumption in the surface, with a higher degree of nutrient consumption leading to a greater decrease in atmospheric CO₂. The strength of the third process depends on the combined strength of the first and second processes.

[31] Previous results for diatom-bound δ¹⁵N argued for a pervasive and significant drawdown of nutrients in the glacial Antarctic surface. Our new results cast doubt on both the amplitude and the geographic extent of this drawdown. Compared with the earlier results, if Antarctic stratification were largely responsible for the glacial reduction in atmospheric CO₂, the new results would require a greater role for the simple reduction in deep water exposure at the Antarctic surface. As models show markedly different sensitivities to polar ocean changes [Archer *et al.*, 2003], it remains unclear whether physical reduction of the exposure of CO₂-charged deep water at the Antarctic surface is adequate to explain the amplitude of the glacial decrease in atmospheric CO₂; some model studies suggest that it is [Toggweiler, 1999].

5. Conclusions

[32] Persulfate-denitrifier diatom-bound δ¹⁵N analyses give lower N contents and higher δ¹⁵N values than the previous combustion-based measurements. This difference is inferred to be the result of a N blank associated with the solid silica frustule in the combustion-based method. Sigman *et al.*'s [1999] fundamental interpretation of the surface sediment data, that the diatom-bound N is likely a protected archive and that its δ¹⁵N appears to reflect surface nitrate utilization, are not changed. However, the persulfate-

denitrifier diatom-bound paleoceanographic profiles present a different history of nitrate utilization for the Antarctic between the Holocene and the LGM. Diatom-bound δ¹⁵N in the Indian sector decreases into the Holocene, indicating that the degree of nitrate utilization was higher during the glacial than at present. This trend is consistent with the results presented by Sigman *et al.* [1999] and Crosta and Shemesh [2002], but the magnitude of the shift is reduced from 4‰ to 1‰, indicating a much more modest (~+20%) increase in nitrate utilization. In more marked contrast, the persulfate-denitrifier data from the Atlantic sector indicate no change, where the combustion-based results indicated a 4–5‰ shift. Thus the persulfate-denitrifier results suggest at most a modest increase in nitrate utilization that is spatially variable within the Antarctic. While this view perhaps fits better with the view that it is difficult for changes in atmospheric Fe deposition to greatly impact the overall Fe supply the Antarctic [Lefevre and Watson, 1999], the pattern of spatial variation does not fit expectations based on modeled estimates of glacial atmospheric iron deposition. In short, the new diatom-bound δ¹⁵N data cast uncertainty on the role of polar phytoplankton in the modulation of glacial-interglacial CO₂ cycles. Nonetheless, the diatom-bound data remain consistent with proposed physical mechanisms for the drawdown of glacial CO₂ related to stratification of the high latitude surface ocean.

Appendix A: The N Blank in Combustion-Based Diatom-Bound N Analysis

[33] In all of the downcore profiles, the δ¹⁵N measured by the persulfate-denitrifier method is elevated with respect to the δ¹⁵N measured by combustion in Holocene samples, while the δ¹⁵N determined by the two methods is approximately equal for the LGM (Figures 4–6). The difference in the Holocene intervals, with combustion-derived δ¹⁵N lower than that derived from the persulfate-denitrifier method is consistent with the idea of a N blank with a lower δ¹⁵N than that of the diatom-bound organic N, such as would be expected from a contaminant N₂ pool. However, in glacial age sediments, the discrepancy between the two methods is much smaller. In the two LGM profiles, the combustion and persulfate-denitrifier records converge, and then cross, so that the persulfate-denitrifier-derived δ¹⁵N is equal to or slightly lower than the combustion-based δ¹⁵N during the glacial intervals. Concentration measurements between the two methods generally differ by 5–10 μmol N/g opal with the persulfate-denitrifier analysis always yielding less N than the combustion analysis. It should be noted that because of differences in the isolation and cleaning methods, the difference between the IO1277-10PC records cannot be uniquely attributed to this missing N pool. First, Crosta and Shemesh [2002] utilized a different size fraction (10–20 μm rather than < 150 μm). Second, we described above data indicating that samples from the last glacial maximum are more difficult to clean than other samples, a consideration that has not been reported previously (Figure 3).

[34] Overall, the concentration differences between combustion analyses and persulfate-denitrifier analyses do not change, so that the concentration profiles generated by the

two methods for any one core are effectively parallel when comparing samples prepared with the same cleaning technique. This suggests that the isotopic composition of the additional N pool in combustion analyses is somehow affected by the depth, age, or composition of the sediments. Mass balance estimates based on the MD84-552 samples cleaned by perchloric acid at 70°C overnight predict that this unknown N pool has an isotopic composition between -6 and 6‰, with a marked increase across the glacial-interglacial transition. A change of 12‰ is far larger than the documented range in $\delta^{15}\text{N}$ of water column N_2 ($\sim 0.5\text{‰}$ [Brandes *et al.*, 1998]). Thus we are challenged to find a mechanism by which cleaned microfossils could be left with such an isotopically variable gaseous N pool.

[35] It is likely that N_2 is the only gaseous N species abundant enough to account for the concentration differences between the combustion and persulfate-denitrifier results. If so, the N_2 pool must be strongly fractionated by some process. Adsorption/desorption of N_2 to the sediment surfaces or fractionation during diffusion are potential causes of fractionation that might explain the downcore $\delta^{15}\text{N}$ change in the unknown N pool. ^{15}N -bearing $^{29}\text{N}_2$ might be preferentially retained on the mineral surfaces rather than the lighter $^{28}\text{N}_2$, thus causing an ^{15}N enrichment in the solid over time. This is generally consistent with the downcore changes we calculate, where the isotopically heavier N is from deeper in the cores, but it is not clear why an adsorbed N_2 pool would be closed to subsequent exchange. It remains a possibility that the fractionation occurs upon desorption from the opal surface during the purge step in the elemental analyzer and that the seemingly systematic downcore changes in the $^{15}\text{N}/^{14}\text{N}$ are related to sediment type and quality of the frustule rather than to a signal captured during burial. Experimental work done suggests no isotopic effect from desorption of N_2 in firm during heating from -60 to -30°C [Grachev and Severinghaus, 2003]. There is a small equilibrium isotope effect, up to 1‰, in the $^{18}\text{O}/^{16}\text{O}$ and $^{13}\text{C}/^{12}\text{C}$ from CO_2 during adsorption onto mineral surfaces at low temperatures [Rahn and Eiler, 2001]. In short, we know of no compelling evidence that a large kinetic isotope effect of adsorption or desorption can adequately explain the large range of $\delta^{15}\text{N}$ that we predict, neither is this option precluded.

[36] It is perhaps more useful to consider N_2 that is sorbed or trapped deep in the opal structure. The internal pore space of a diatom is modified by dissolution and reprecipitation

of the silica frustule during burial. The end result of this process is a denser frustule with isolated internal pore volume rather than the porous, open framework of the original frustule [Hurd *et al.*, 1981]. The inferred N_2 may be sufficiently isolated within the opal for molecular diffusion to be an important process. Since molecular diffusivity is proportional to the square root of the molecular mass, the potential fractionation of N_2 due to diffusion is $\sim 17.7\text{‰}$. If this fractionation is well expressed, it may cause variations in the measured $\delta^{15}\text{N}$ of the trapped or adsorbed N_2 blank that correlate with variations in the nature of the diatom opal (e.g., its internal pore volume). Even if these variations are controlled by the nature of the opal, the actual cause of the isotopic variations may originate at nearly any stage up to and including the He purging step in an elemental analyzer system.

[37] The samples showing the largest combustion/wet oxidation $\delta^{15}\text{N}$ differences are consistently those with higher opal concentrations: those to the south of the APF in the case of the ANTARES transect and Holocene age samples in the case of the downcore profiles MD84-552 and to some degree IO 1277-10PC. In NBP 96-4-2 MC4, which is composed of entirely Holocene material and in which opal concentrations do not vary significantly, there is little variability in the offset between the measured $\delta^{15}\text{N}$ values and thus in the calculated $\delta^{15}\text{N}$ of the unknown N pool. It seems possible that the variation in its isotopic composition is somehow related to sediment composition, possibly related to opal accumulation or clay content, both of which change in relative abundance at the glacial-interglacial boundary, where the sharpest change in the calculated $\delta^{15}\text{N}$ of the unknown N pool occurs. Given the speculative nature of these suggestions, it is clear that more work needs to be done before we can adequately explain the discrepancy between the combustion and denitrifier methods.

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