Supplement to

Chaetoceros resting spores record low diatom-bound nitrogen isotope values: Evidence from laboratory culture and marine sediment

Isabel A. Dove et al.

S1. Description of laboratory culture experiments and data collected

Experiment 1 took place from December 2, 2020 through January 14, 2021. Vegetative cells were harvested on day 9 and CRS were harvested on day 43. Fluorescence, dissolved nitrate concentration, and $\delta 15N_{NO3}$ were measured throughout the experiment, and water samples were periodically inspected and preserved in a 2% acid Lugol's solution to monitor resting spore abundance.

Experiment 2 took place from June 23, 2021 through July 21, 2021. Vegetative cells were harvested on day 7 and CRS were harvested on day 28. The same measurements were taken as from experiment 1, in addition to ammonium and dissolved silica concentrations.

Experiment 3 took place from February 17, 2022 through March 25, 2022. Vegetative cells were harvested on day 6 and CRS were harvested on day 36. All measurements are described in the main text.

Data Table S1. Data collected throughout experiment 1.

Data Table S2. Data collected throughout experiment 2.

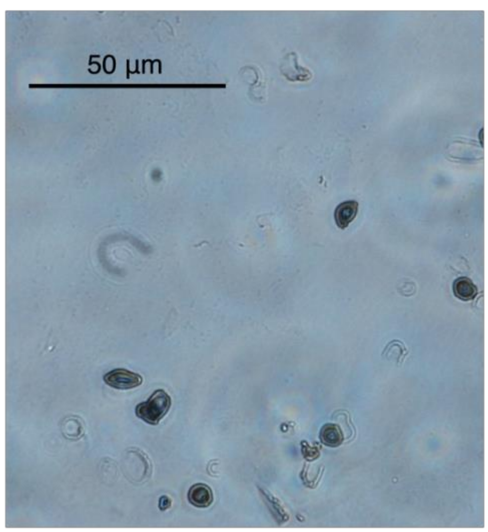

Figure S1. Photomicrograph of isolated CRS from experiment 3.

Figure S2. Photomicrograph of the <10 µm fraction from ODP Site 1098 sediment. Both vegetative *Chaetoceros* and CRS are present.

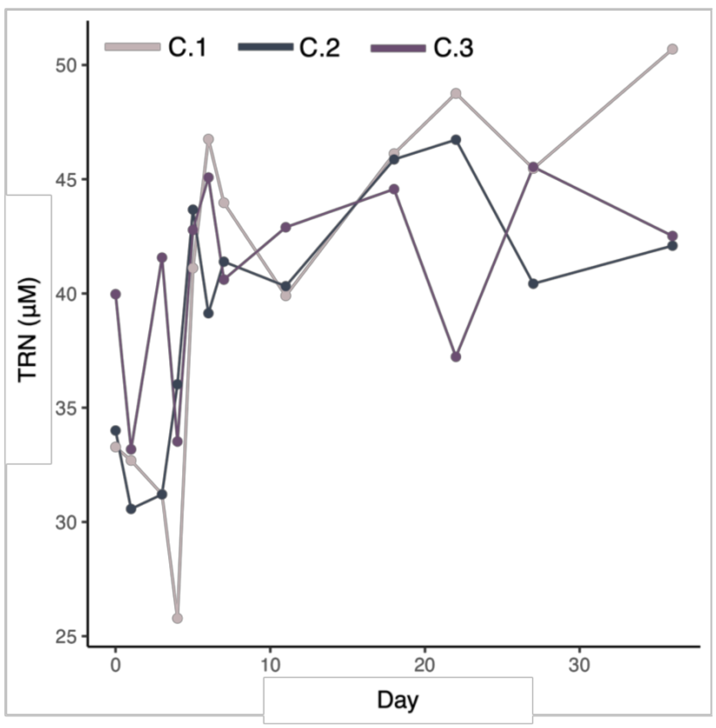

Figure S3. Total reduced nitrogen (TRN) concentrations in each carboy throughout experiment 3.

Table S1. $\delta^{15}N_{biomass}$ and $\delta^{15}N_{DB}$ measurements from experiments 1 and 2.

Experiment	Carboy	Type	$\delta^{15}N_{biomass}$ (‰)	$\delta^{15}N_{DB}$ (‰)
1	1	Vegetative	9.1 ± 0.3	11.0 ± 0.3
1	2	Vegetative	8.5 ± 0.3	11.3 ± 0.2
1	3	Vegetative	8.6 ± 0.2	11.6 ± 0.3
1	1	Mixed	8.3 ± 0.3	
1	2	Mixed	7.5 ± 0.3	
1	3	Mixed	7.5 ± 0.3	10.2 ± 0.4
2	1	Vegetative	8.5 ± 0.0	11.7 ± 0.1
2	2	Vegetative	8.3 ± 0.3	7.6 ± 0.1
2	3	Vegetative	8.3 ± 0.3	10.8 ± 1.4
2	1	Mixed	7.7 ± 0.3	11.8 ± 0.0
2	2	Mixed	8.4 ± 0.3	9.5 ± 0.2
2	3	Mixed	7.3 ± 0.3	7.9 ± 0.1

S2. Reduced N assimilation does not explain low $\delta^{15}N_{DB}$ values for CRS

A mass balance calculation is used to determine whether assimilation of reduced nitrogen with a low $\delta^{15}N$ value explains the low $\delta^{15}N_{DB}$ values measured in CRS relative to $\delta^{15}N_{DB}$ values measured in vegetative cells.

$$\Delta\delta^{15}N_{DB\text{-}CRS} = \delta^{15}N_{NO3} * (1\text{-}x) + \delta^{15}N_{TRN} * (x)$$
$$-4.4‰ = 9.6‰ * (1\text{-}x) + -2‰ * (x)$$

Using the average $\delta^{15}N_{NO3}$ value, a conservative estimate of a representative $\delta^{15}N_{TRN}$ value, and the smallest reasonable $\Delta\delta^{15}N_{DB}$ value (-7.0 + 2.6‰), we find that x=1.2. This calculation implies that 120% of nutrient-nitrogen assimilated by CRS would have to be reduced N in order to explain why CRS have a $\delta^{15}N_{DB}$ value 4.4‰ lower than that of vegetative *Chaetoceros*. Since this is physically impossible, another explanation for low $\delta^{15}N_{DB\text{-}CRS}$ values is required.

S3. Alternative glacial-interglacial ε_{DB} mass balance calculations

To ensure that our conclusions from the mass balance calculation of estimated community ε_{DB} values in glacial versus interglacial sediments are not biased by our chosen parameter of two-dimensional diatom surface area, we perform the same calculation using three-dimensional diatom surface area and diatom biovolume. We also repeat the calculation using two-dimensional diatom surface area estimates from another source: Cornet-Barthaux et al. (2007). Each calculation yields similar results and leads to the conclusion that, within the two sediment cores we examine, changes in CRS relative abundance between glacial-interglacial cycles will not meaningfully bias downcore $\delta^{15}N_{DB}$ records.

Table S2. Mass balance calculation of estimated community ε_{DB} values in glacial versus interglacial sediments using three-dimensional surface area (SA) estimates from Leblanc et al. (2012). The non-CRS ε_{DB} estimate is from Robinson et al. (2020). We assume that other diatoms comprise 10% of total diatom SA in all cases.

		ε_{DB} (‰)	Avg. SA (μm^2)	SA (%)
		TN057-13-PC4		
Glacial	CRS	2.4	363	4
	E. antarctica	-3.2	6784	33
	F. kerguelensis	-3.2	1780	14
	T. lentiginosa	-3.2	17944	40
	Other diatoms	-3.2		10
		Estimated community ε_{DB} (‰)		-3.0
Interglacial	CRS	2.4	363	0
	E. antarctica	-3.2	6784	4
	F. kerguelensis	-3.2	1780	31
	T. lentiginosa	-3.2	17944	55
	Other diatoms	-3.2		10
		Estimated community ε_{DB} (‰)		-3.2
		Glacial-interglacial ε_{DB} difference (‰)		**0.2**
		MD11-3353		
Glacial	CRS	2.4	363	0
	E. antarctica	-3.2	6784	5
	F. kerguelensis	-3.2	1780	10
	T. lentiginosa	-3.2	17944	75
	Other diatoms	-3.2		10
		Estimated community ε_{DB}		-3.2
Interglacial	CRS	2.4	363	0
	E. antarctica	-3.2	6784	6
	F. kerguelensis	-3.2	1780	19
	T. lentiginosa	-3.2	17944	64
	Other diatoms	-3.2		10
		Estimated community ε_{DB}		-3.2
		Glacial-interglacial ε_{DB} difference (‰)		**0.0**

Table S3. Mass balance calculation of estimated community ε_{DB} values in glacial versus interglacial sediments using biovolume (V) estimates from Leblanc et al. (2012). The non-CRS ε_{DB} estimate is from Robinson et al. (2020). We assume that other diatoms comprise 10% of total diatom V in all cases.

		ε_{DB} (‰)	Avg. V (μm^3)	V (%)
		TN057-13-PC4		
Glacial	CRS	2.4	2000	3
	E. antarctica	-3.2	40457	27
	F. kerguelensis	-3.2	5023	5
	T. lentiginosa	-3.2	171958	54
	Other diatoms	-3.2		10
		Estimated community ε_{DB} (‰)		-3.0
Interglacial	CRS	2.4	2000	0
	E. antarctica	-3.2	40457	3
	F. kerguelensis	-3.2	5023	12
	T. lentiginosa	-3.2	171958	74
	Other diatoms	-3.2		10
		Estimated community ε_{DB} (‰)		-3.2
		Glacial-interglacial ε_{DB} difference (‰)		**0.2**
		MD11-3353		
Glacial	CRS	2.4	2000	0
	E. antarctica	-3.2	40457	3
	F. kerguelensis	-3.2	5023	3
	T. lentiginosa	-3.2	171958	83
	Other diatoms	-3.2		10
		Estimated community ε_{DB}		-3.2
Interglacial	CRS	2.4	2000	0
	E. antarctica	-3.2	40457	5
	F. kerguelensis	-3.2	5023	7
	T. lentiginosa	-3.2	171958	78
	Other diatoms	-3.2		10
		Estimated community ε_{DB}		-3.2
		Glacial-interglacial ε_{DB} difference (‰)		**0**

Table S4. Mass balance calculation of estimated community ε_{DB} values in glacial versus interglacial sediments using two-dimensional surface area (SA) estimates from Cornet-Barthaux et al. (2007). The non-CRS ε_{DB} estimate is from Robinson et al. (2020). We assume that other diatoms comprise 10% of total diatom SA in all cases.

		ε_{DB} (‰)	Avg. SA (μm^2)	SA (%)
		TN057-13-PC4		
Glacial	CRS	2.4	199	8
	E. antarctica	-3.2	1082	21
	F. kerguelensis	-3.2	1074	34
	T. lentiginosa	-3.2	2818	26
	Other diatoms	-3.2		10
		Estimated community ε_{DB} (‰)		-2.7
Interglacial	CRS	2.4	199	1
	E. antarctica	-3.2	1082	2
	F. kerguelensis	-3.2	1074	60
	T. lentiginosa	-3.2	2818	27
	Other diatoms	-3.2		10
		Estimated community ε_{DB} (‰)		-3.1
		Glacial-interglacial εDB difference (‰)		**0.4**
		MD11-3353		
Glacial	CRS	2.4	199	1
	E. antarctica	-3.2	1082	4
	F. kerguelensis	-3.2	1074	28
	T. lentiginosa	-3.2	2818	57
	Other diatoms	-3.2		10
		Estimated community ε_{DB}		-3.1
Interglacial	CRS	2.4	199	1
	E. antarctica	-3.2	1082	4
	F. kerguelensis	-3.2	1074	45
	T. lentiginosa	-3.2	2818	40
	Other diatoms	-3.2		10
		Estimated community ε_{DB}		-3.2
		Glacial-interglacial ε_{DB} difference (‰)		**0.1**

Supplementary material references

Cornet-Barthaux, V., Armand, L., and Quéguiner, B.: Biovolume and biomass estimates of key diatoms in the Southern Ocean, Aquatic Microbial Ecology, 48, 295–308, https://doi.org/10.3354/AME048295, 2007.

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