

***Chaetoceros* Resting Spores Do Not Significantly Bias Sedimentary Diatom-bound Nitrogen Isotope Records Despite Distinctly Low Values**

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Introduction

Here we present additional details and data from laboratory culture experiments 1 and 2. Fluorescence and nutrient concentration data from experiments 1 and 2 are attached as Excel files. We also include photomicrographs of samples mentioned in the main text. Figure S4 shows the linear relationship between CRS surface area and relative abundance. Figure S5 shows a sample calculation of CRS bias to $\delta^{15}\text{N}_{\text{DB}}$ measurements as a function of CRS surface area contribution.



Figure S1. Photomicrograph of the <10 μm fraction from ODP Site 1098 sediment. Both vegetative *Chaetoceros* and CRS are present.

Text 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 $\delta^{15}\text{N}_{\text{NO}_3}$ 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.

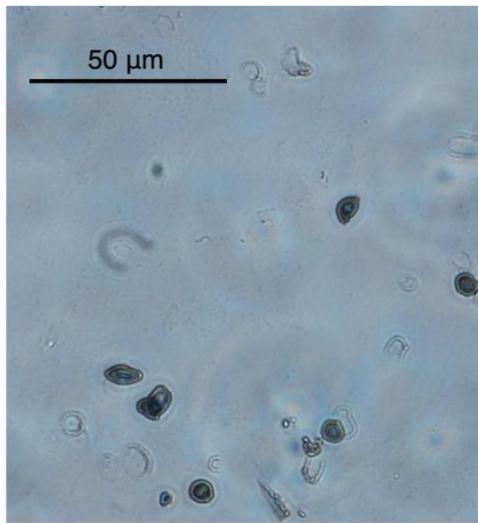


Figure S2. Photomicrograph of isolated CRS from experiment 3.

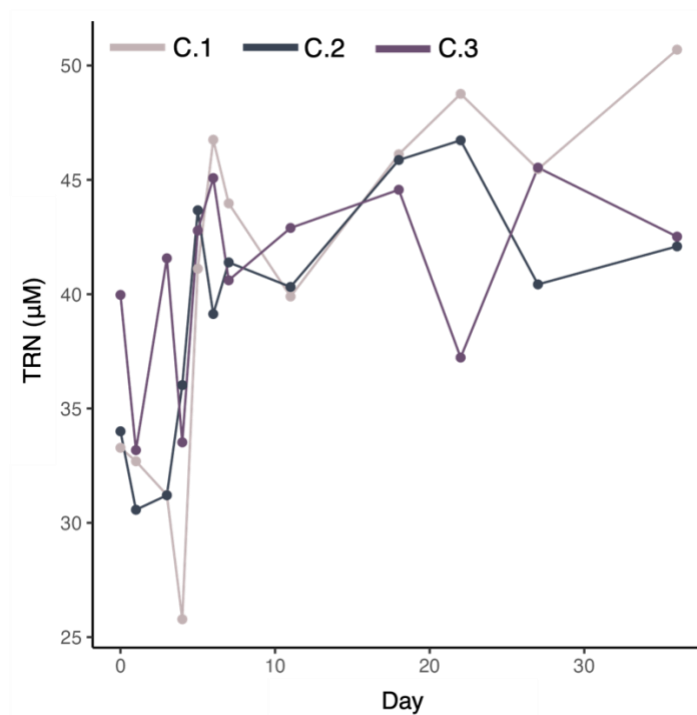


Figure S3. Total reduced nitrogen (TRN) concentrations in each carboy throughout experiment 3.

Table S1. $\delta^{15}\text{N}_{\text{biomass}}$, $\delta^{15}\text{N}_{\text{DB}}$, and N:Si measurements from experiments 1 and 2.

| Experiment | Carboy | Type | $\delta^{15}\text{N}_{\text{biomass}}$ (‰) | $\delta^{15}\text{N}_{\text{DB}}$ (‰) | $\mu\text{mol N} :$ g bSi |
|------------|--------|------------|---|--|------------------------------|
| 1 | 1 | Vegetative | 9.1 ± 0.3 | 11.0 ± 0.3 | 15.7 |
| 1 | 2 | Vegetative | 8.5 ± 0.3 | 11.3 ± 0.2 | 9.8 |
| 1 | 3 | Vegetative | 8.6 ± 0.2 | 11.6 ± 0.3 | 12.5 |
| 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 | 8.7 |
| 2 | 1 | Vegetative | 8.5 ± 0.0 | 11.7 ± 0.1 | 15.6 |
| 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 | 11.1 |
| 2 | 2 | Mixed | 8.4 ± 0.3 | 9.5 ± 0.2 | 21.4 |
| 2 | 3 | Mixed | 7.3 ± 0.3 | 7.9 ± 0.1 | 19.0 |

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

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

$$\Delta\delta^{15}\text{N}_{\text{DB-CRS}} = \delta^{15}\text{N}_{\text{NO}_3} * (1-x) + \delta^{15}\text{N}_{\text{TRN}} * (x)$$

$$-4.4\text{‰} = 9.6\text{‰} * (1-x) + -2\text{‰} * (x)$$

Using the average $\delta^{15}\text{N}_{\text{NO}_3}$ value, a conservative estimate of a representative $\delta^{15}\text{N}_{\text{TRN}}$ value, and the smallest reasonable $\Delta\delta^{15}\text{N}_{\text{DB}}$ value ($-7.0 + 2.6\text{‰}$), 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 $\delta^{15}\text{N}_{\text{DB}}$ value 4.4‰ lower than that of vegetative *Chaetoceros*. Since this is physically impossible, another explanation for low $\delta^{15}\text{N}_{\text{DB-CRS}}$ values is required.

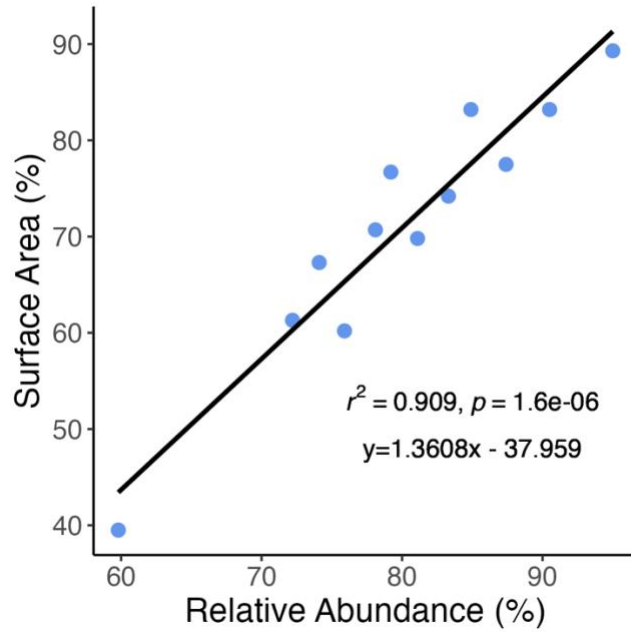


Figure S4. Linear relationship between CRS relative abundance and surface area contribution, based on 12 samples from ODP Core 1098B-5H-7.

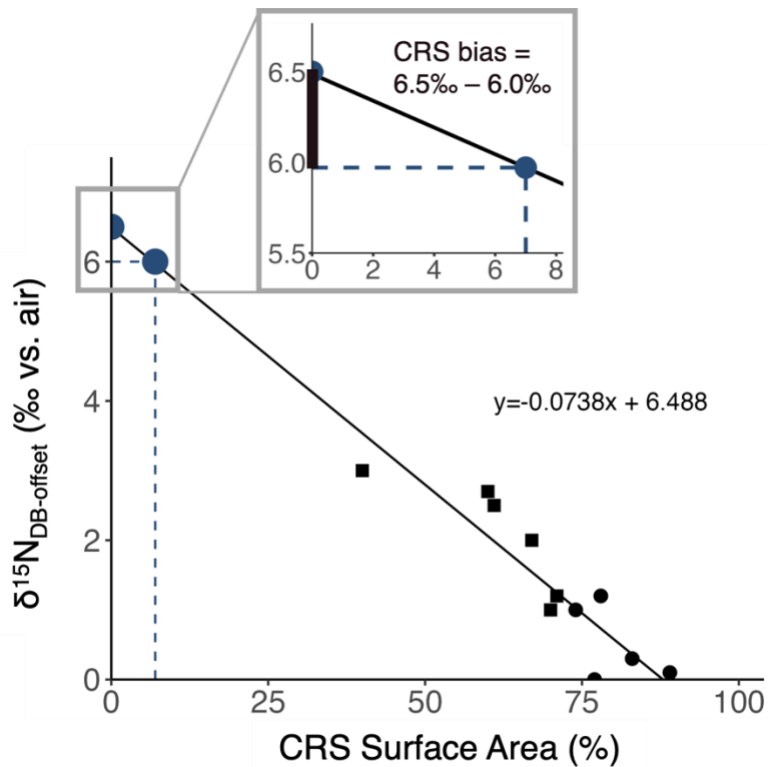


Figure S5. Graphical representation of expected $\delta^{15}\text{N}_{\text{DB}}$ bias in sediment core MD11-3353 due to the presence of CRS. A sample with a 7% CRS surface area contribution equates to a $\delta^{15}\text{N}_{\text{DB}}$ measurement 0.5‰ lower than a sample with no CRS.