The Effect of Light on Coupled N and O stable Isotope Fractionation During Nitrate Assimilation by Marine Phytoplankton

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Objectives

1. Determine the N-isotope effect associated with NO_3^- assimilation by an indigenous marine phytoplankton assemblage incubated under different light intensities.
2. Determine whether N and O isotopes of NO_3^- are fractionated at a ratio of one during its assimilation by indigenous plankton.

Introduction

The use of nitrogen stable isotopes to study the marine nitrogen cycle requires a comprehensive understanding of the mechanisms by which it is fractionated. Previous studies have demonstrated that nitrate is fractionated intracellularly by nitrate reductase (NR) followed by its propagation to the extracellular medium via cellular nitrate efflux[1-4]. The expression of the intrinsic isotope effect associated with NR has been proven by isolates in culture to be highly influenced by light[2], where the magnitude of the isotope effect has been found to result from the relative difference between uptake and efflux[1,4]. Light limitation has been found to impose a higher apparent isotope effect, likely due to a reduction of NR activity under low light while cellular nitrate uptake remains constant (Fig. 1).

Figure 2: Depth profiles of N and O stable isotopes associated with nitrate from N.E. Pacific. (Sigman & Casciotti, 2001)

Depth profiles of the N and O isotopic composition of nitrate (Fig. 2) suggest that N and O isotopes of nitrate are fractionated to the same extent by assimilation at the surface ocean. This observation is corroborated by phytoplankton culture studies, which established that the cultured isolates consistently fractionate N and O isotopes proportionately, with an O-to-N ratio of 1.

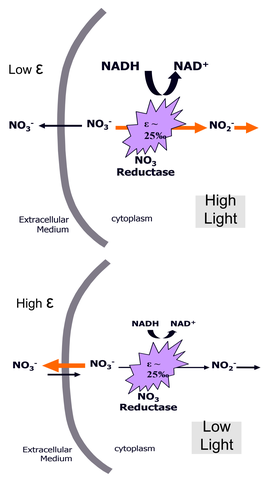

Figure 1: Schematic illustration of the putative effect of light intensity on NR activity and the isotope effect. Where the supply of NADH (or NADPH) to the NR impacts its activity such that the efflux to influx ratio changes.

Methods

- The natural phytoplankton assemblage was collected in Nov. 2005 at Jericho Beach, Vancouver, BC with a 20 μm plankton net.
- Collected algal assemblages consisted chiefly of winter diatoms, with *Skeletonema* sp. dominating the assemblage at the end of the experiments.
- Assemblages were re-suspended in sterile seawater amended with 10μm PO_4^-, 80μm SiO_3^{-2}, and 150μm NO_3^-, f/2 vitamins, and EDTA-trace metals.
- Triplicate assemblage culture were incubated in polycarbonate bottles at 12 ± 1°C under continuous light conditions at 16.6-23.2 μE (low light) and 70.6-132 μE (saturating light).
- Cultures were sub-sampled throughout 15 days of incubation
- Nitrate was measured on nitrate/nitrite analyzer[5].
- Nitrate isotopic ratios ($^{15}N/^{14}N$ and $^{18}O/^{16}O$) were analyzed with the "denitrifier method"[7,8] after nitrite was removed from samples[9].

N (and O) isotope ratios are expressed in delta notation:

$$\delta\ ^{15}N\ (‰) = \{(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{standard} - 1\} \times 1000 \quad [1]$$

The isotope effect, ε, is a function of the ratios of the reaction coefficients between heavy and light atom bearing substances.

$$^{15}\varepsilon\ (‰) = (^{14}k/^{15}k - 1) \times 1000 \quad [2]$$

N and O isotope effects were derived using the 'Rayleigh' model, where *f* is the fraction of nitrate remaining.

$$\delta\ ^{15}N_{nitrate} = \delta\ ^{15}N_{initial} - \varepsilon\ \{\ln(f)\} \quad [3]$$

Results

N-Isotope Effects at High and Low Light

- The isotope effect observed for the natural assemblage incubations ranged between 4.8-7.6‰ and 4.9-8.3‰ for high and low light, respectively.
- There were no discernable effects of light on isotope effects, as the variation among replicates was larger than between treatments, although isotope fractionation appeared slightly more variable at low light.

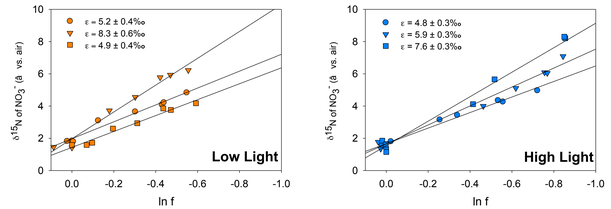

Figure 3: N-Isotope ratios as a function of nitrate consumption (ln *f*) for high and low light. Respective slopes approximate the N isotope effects for replicate incubations [Eq. 3].

Coupling of N and O Isotopes

- Although the isotope effects were different among replicates, O-to-N coupling was identical within treatments, however differed between treatments (Table 1).
- The pooled slopes for low and high light were 1.15 ± 0.03 and 1.00 ± 0.03, respectively. This revealed a significant difference in the co-variation in the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- between the high and low light treatments (P<0.001).

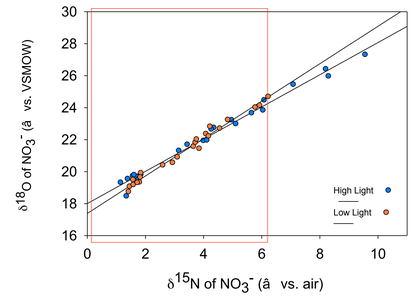

Figure 4: Comparison of the N vs. O slopes for both high and low light. The Red box indicates the data set that was included in the statistical analysis.

Treatment	Replicate	Slope (± S.E.)
High Light		
	A	1.08 ± 0.03
	B	1.07 ± 0.05
	C	0.97 ± 0.02
	Pooled (n=19)	**1.00 ± 0.03***
Low Light		
	A	1.17 ± 0.07
	B	1.17 ± 0.03
	C	1.15 ± 0.08
	Pooled (n=22)	**1.15 ± 0.03**

* Pooled slope for high light was restricted to those data points ranging between 0-6.1‰ for the $\delta^{15}N$ of NO_3^- to ensure that there was no bias when comparing the slopes of each treatment.

Table 1: Comparison of slopes for O vs. N and O isotope ratios among treatments. A student's *t*-test was used for the comparison of slopes (P<0.001)

Discussion

N-Isotope Effect at High and Low Light

There appeared to be no effect of light on the isotope effects. This suggests that the natural phytoplankton assemblage used in this experiment, which was collected in November, may have been adapted to low light such that NR activity is optimal at low light. Therefore, we would not expect a higher efflux if uptake and reduction by NR are coupled at low light (Fig.1).

Coupling of N and O Isotopes

Fractionation during nitrate assimilation was coupled with a O-to-N ratio of 1, as observed previously in culture, for only high light. At low light, O isotopes were more enriched than N isotopes relative to the expected 1:1. We hypothesize that this is to be due to the activity of nitrifiers at low light. Nitrification would act to enrich the $\delta^{18}O$ signal relative to the $\delta^{15}N$ of NO_3^- (Fig. 5). This hypothesis would explain why the high light cultures retained a 1:1 co-variation of N and O, since nitrification is inhibited by light.

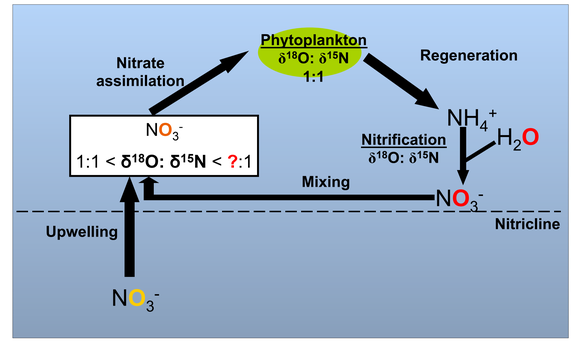

Figure 3: Schematic illustration of the coupling between N and O during nitrate assimilation and nitrification. The ratio between N and O is expected to deviate from 1:1 if a substantial amount of nitrate is being supplied to the mixed layer via nitrification.

Conclusion

- The similar isotope effects found between high and low light differed to those measured in the Southern Antarctic Zone and in culture. This may be, however, a function of the assemblage collected and be expressing bias towards low light. More experiments need to be conducted to determine whether adaptation to low light can yield insensitive responses to NR.
- The significant difference in the ratio between N and O found for high and low light show a promising potential use of coupled N and O isotope analysis of nitrate as a tracer for nitrification in the mixed layer. Although we are still in the process of testing these findings more rigorously, the quantification of nitrification in the mixed layer would help constrain export production in the ocean.

References

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