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Coupled nitrate N and O stable isotope fractionation by a natural marine plankton consortium

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The stable nitrogen (N) and oxygen (O) isotope ratios ($^{15}N/^{14}N$ and $^{18}O/^{16}O$, respectively) of nitrate (NO_3^-) were measured during incubations of freshly collected seawater to investigate the effect of light intensity on the isotope fractionation associated with nitrate assimilation and possible co-occurring regeneration and nitrification by *in situ* plankton communities. Surface seawater was collected off the coast of Vancouver, Canada in late fall and in late summer and was incubated under different laboratory light conditions for 10 and 30 days, respectively. In the late summer experiments, parallel incubations were supplemented with $^{15}NH_4^+$ and $H_2^{18}O$ tracers to monitor co-occurring nitrification. Differences in irradiance in the fall incubations resulted in slightly reduced nitrate consumption at low light but had no distinguishable impact on the N isotope effect ($^{15}\varepsilon$) associated with NO_3^- assimilation, which ranged between 5 and 8‰. The late-summer community incubations, in contrast, showed significantly reduced growth rates at low light and more elevated $^{15}\varepsilon$ of 11.9 ± 0.4‰, compared to 8.4 ± 0.3‰ at high-light conditions. The seasonal differences could reflect physiological adaptations of the fall plankton community to reduced irradiance, such that their incubation at low light did not elicit the increase in proportional cellular nitrate efflux required to raise the isotope effect. In both the fall and summer incubations, the ratio of the coincident rises in the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- was comparable to previous monoculture phytoplankton experiments, with a $\Delta\delta^{18}O{:}\Delta\delta^{15}N$ of ~1, regardless of light level. A decoupling of $\Delta\delta^{18}O{:}\Delta\delta^{15}N$ is expected if nitrification occurs concomitantly with nitrate assimilation. The lack of such decoupling is best explained by the absence of significant nitrification in any of our study's treatments, an interpretation supported by our inability to identify any tracer ^{15}N and ^{18}O uptake into the NO_3^- pool in the late-summer community incubations.

Keywords: nitrate isotopes, nitrate assimilation, nitrification, phytoplankton, marine ecology

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Introduction

The naturally occurring stable isotope ratios of nitrogen (N) and oxygen (O) in nitrate (NO_3^-), $^{15}N/^{14}N$ and $^{18}O/^{16}O$, are important tools for understanding the marine N cycle. The utility of the N and O isotopes of NO_3^- derives from the effects of biological transformations on the isotope ratios of the ambient NO_3^- pool in the marine environment (reviewed by Sigman et al., 2009b). During

biochemical reactions, substrate molecules bearing the heavier isotopologues of the elements tend to react more slowly than lighter isotopologues. As a result, in sunlit surface waters, the $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of NO_3^- increase as the NO_3^- pool is progressively consumed during photosynthesis by phytoplankton (e.g., Casciotti et al., 2002). Isotope ratios of NO_3^- are expressed here in delta (δ) notation in units of per mil (‰), where $\delta^{15}N$ is $[(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{air} - 1] \times 1000$, with "air" referring to atmospheric N_2, and $\delta^{18}O$ is $[(^{18}O/^{16}O)_{sample}/(^{18}O/^{16}O)_{VSMOW} - 1] \times 1000$, with "VSMOW" referring to Vienna Mean Standard Ocean Water.

With the appropriate background information, the isotopic ratios of NO_3^- can provide integrative constraints on the relative importance of various biogeochemical and physical transformations in the natural environment. For the isotopes of NO_3^- to be used in this way, the isotope systematics of NO_3^- production and consumption must be known or determinable from environmental data. In the case of NO_3^- assimilation, the amplitude of isotope fractionation is quantified by the kinetic isotope effect ε, a measure of the ratio of reaction rate constants (k) of heavy and light isotopologues: $^{15}\varepsilon = (^{14}k/^{15}k - 1) \times 1000$ (expressed in units of per mil, ‰) for the N isotopologues of NO_3^-, and $^{18}\varepsilon = (^{16}k/^{18}k - 1) \times 1000$ for the O isotopologues of NO_3^- (Mariotti et al., 1981). The $^{15}\varepsilon$ and $^{18}\varepsilon$ associated with NO_3^- assimilation are a critical aspect of the systematics needed to use these isotopes as a constraint on the extent of NO_3^- consumption in the upper ocean, today and in the past.

Ocean field studies suggest that the $^{15}\varepsilon$ (and $^{18}\varepsilon$) associated with NO_3^- assimilation is frequently 5–6‰ (Sigman et al., 1999; DiFiore et al., 2009), but with variation skewed toward higher values. For example, isotope effect estimates upwards of 8–9‰ are observed for the Subantarctic Zone (SAZ, ∼40–52°S) of the Southern Ocean (Karsh et al., 2003; DiFiore et al., 2006). Phytoplankton mono-culture indicate that $^{15}\varepsilon$ can cover a broad range, from 0 to 20‰ (Montoya and McCarthy, 1995; Waser et al., 1998a,b; Needoba et al., 2003; Granger et al., 2004), with most estimates ranging between 5 and 10‰. While the causes of the variability in $^{15}\varepsilon$ are not completely understood, light intensity has been identified as a potential environmental driver of $^{15}\varepsilon$ variations (Needoba and Harrison, 2004; Needoba et al., 2004; DiFiore et al., 2010). Phytoplankton cultures, particularly diatoms, display higher $^{15}\varepsilon$ when grown under low-light conditions (Wada and Hattori, 1978; Needoba and Harrison, 2004; Needoba et al., 2004). This dynamic appears to be manifested in the Southern Ocean, where a regional variation in $^{15}\varepsilon$ has been inferred from the relationship between surface nitrate $\delta^{15}N$ and ambient nitrate concentrations. The apparent $^{15}\varepsilon$ in the deeply mixed SAZ (40–52°S) is ∼8–9‰ , significantly higher than the $^{15}\varepsilon$ of ∼5‰ observed in the Polar Antarctic Zone (PAZ, ∼66°S) surface, where the summertime mixed layer is significantly shallower (often 20 m or less; DiFiore et al., 2010).

The sensitivity of $^{15}\varepsilon$ to light intensity might thus be used as a tool for identifying, and perhaps quantifying, light limitation on phytoplankton growth in the modern ocean. This dynamic, however, complicates the interpretation of N isotope data from sediment records intended to reconstruct past changes in nitrate consumption. For example, in the SAZ of the Southern Ocean, elevated $\delta^{15}N$ has been measured in diatom frustule-bound nitrogen and foraminifera-bound nitrogen from the last glacial maximum, which has been interpreted as indication of more complete NO_3^- consumption compared to the current interglacial period (Robinson et al., 2005; Martínez-García et al., 2014). This change was apparently coupled with higher export production in the SAZ, raising the possibility of ice age iron fertilization in the SAZ, which could partly explain the lower atmospheric CO_2 concentration during ice ages (Martin et al., 1990; Martínez-García et al., 2014). However, this interpretation of the N isotope record relies on the assumption that there have not been large changes in the isotope effect of NO_3^- assimilation. A better understanding of the effect of light and other processes on the amplitude of the isotope effect during NO_3^- assimilation by indigenous communities in the surface ocean is required in order to improve our understanding of modern surface ocean N dynamics and our ability to reconstruct NO_3^- consumption in the surface waters of the past ocean.

The isotopes of NO_3^- may also be affected by internal N cycling within the euphotic zone. As with the amplitude of the isotope effects of NO_3^- assimilation, such effects may provide new constraints on N cycling in the modern ocean, while at the same time they are likely to represent a complication in the use of the $\delta^{15}N$ of sediment and sedimentary components to reconstruct past nutrient consumption changes. The recycling of N within the euphotic zone by the remineralization of particulate organic nitrogen (PON) to NH_4^+ and its subsequent re-assimilation by phytoplankton has long been argued to impact the $\delta^{15}N$ of suspended particles (Altabet, 1988; Fawcett et al., 2011; Treibergs et al., 2014). Furthermore, the partial conversion of NH_4^+ to NO_3^- during nitrification in the surface ocean may also affect the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-, thereby altering the isotopic link between NO_3^- assimilation and N export. Although nitrification is believed to be inhibited by light, it has been observed at the base of the euphotic zone (0.1–1% light level; Ward, 1987) and may contribute a significant source of NO_3^- within the euphotic zone (Ward et al., 1989; Dore and Karl, 1996; Bianchi et al., 1997), adding to the input of NO_3^- from the ocean interior.

The O isotopes of NO_3^- hold the potential to provide constraints on the degree of nitrification in the mixed layer, offering insight into whether the upper ocean NO_3^- in a given region is supplied *via* upper ocean recycling or solely mixing with the underlying deep ocean (Granger et al., 2004; Wankel et al., 2007; Bourbonnais et al., 2009; Smart et al., 2015). An identical enrichment in ^{15}N- and ^{18}O- bearing NO_3^- is expected during both NO_3^- assimilation and denitrification such that $^{15}\varepsilon \approx {}^{18}\varepsilon$ ($\Delta\delta^{18}O \approx \delta^{15}N$) (Granger et al., 2004, 2008, 2010). It has been hypothesized that if NO_3^- is being regenerated within the mixed layer, then a deviation from the expected 1:1 $\Delta\delta^{18}O$:$\Delta\delta^{15}N$ ratio of NO_3^- reduction should manifest (Granger et al., 2004; Sigman et al., 2005; Wankel et al., 2007; DiFiore et al., 2009). This hypothesis is based on the expectation (Casciotti et al., 2002) and growing evidence (Sigman et al., 2009a; Buchwald et al., 2012) that the $\delta^{18}O$ of newly nitrified NO_3^- has a $\delta^{18}O$ similar to that of ambient H_2O, such that newly nitrified NO_3^- has a higher $\delta^{18}O$-NO_3^- than that of the NO_3^- lost during assimilatory NO_3^- reduction, given an assimilatory isotope effect of 5–10‰

and a δ^{18}O-NO_3^- for the deep NO_3^- supply to the euphotic zone of ∼2‰. In contrast, the δ^{15}N of newly nitrified NO_3^- is expected to be similar to, or lower than, that of the NO_3^- pool for most assumptions regarding isotope fractionation during remineralization and nitrification (DiFiore et al., 2009; Smart et al., 2015). The dual N and O isotopes of NO_3^- may thus provide a means to quantify the fraction of NO_3^- in the euphotic zone deriving from *in situ* nitrification. Application of this approach has found substantial evidence of euphotic zone nitrification in open ocean off of Monterey Bay (Wankel et al., 2007) and in the wintertime Antarctic Zone (Smart et al., 2015) but no evidence for it along the summertime Antarctic margin (DiFiore et al., 2009).

Here we investigate the impact of light limitation on the N and O isotope fractionation of NO_3^- during its assimilation by a natural marine plankton community. We aimed to: (1) determine whether the amplitude of the N and O isotope effect is sensitive to irradiance, as has been observed in mono-specific cultures (Wada and Hattori, 1978; Needoba and Harrison, 2004; Needoba et al., 2004) and inferred for regions of the upper ocean (DiFiore et al., 2010); and (2) assess whether the δ^{18}O and δ^{15}N rise in a 1:1 proportion as NO_3^- is assimilated, and/or if nitrification would lead to a deviation from this 1:1 $\Delta\delta^{18}$O:$\Delta\delta^{15}$N relationship when nitrification is promoted under low-light conditions.

Materials and Methods

Incubation Experiments

Natural phytoplankton community assemblages were collected from surface waters (∼3 m depth) on two separate occasions. Water for a first set of incubations was collected off a public peer at Jericho Beach in Vancouver in November ("late fall") of 2005. A second set of incubations was initiated with surface water collected in the Strait of Georgia, a semi-enclosed estuary located between the Canadian mainland of British Columbia and Vancouver Island near the mouth of the Fraser River in September ("late summer") 2006. Zooplankters were removed from the community assemblage using cheesecloth (#40, 24 × 20 threads per inch) on both occasions. Late fall water from Jericho Beach was collected in a 10-L acid-washed polypropylene carboy and reallocated into 9 transparent acid-washed 4 L polycarbonate bottles. Late summer water from the Strait of Georgia was collected in an acid-washed polycarbonate 60-L carboy and reallocated into acid-washed 4-L bottles, 12 transparent polycarbonate bottles and 3 opaque polypropylene bottles. Each incubation bottle was supplemented with 175 μM (fall) or 100 μM (summer) sodium nitrate ($NaNO_3$), 100 μM silicic acid (SiO_4), 10 μM potassium phosphate (K_3PO_4), and f/2 vitamins (Guillard, 1975). The relatively elevated nutrient additions were intended to increase the dynamic range of observed nitrate isotope ratios during the nitrate drawdown, in order to clearly distinguish differences in isotope effect amplitudes among light treatments and potential deviations in the $\Delta\delta^{18}$O:$\Delta\delta^{15}$N ratio of ∼1 from coincident nitrification. Initial sub-samples were collected for the analysis of NO_3^- concentrations for both incubations, as well as NO_2^- and NH_4^+ concentrations (for the summer incubations only) prior to their placement in an incubator set with different irradiance treatments.

Late-fall incubations were subjected to three irradiance treatments, which were monitored using a light meter (Biospherical Instruments Inc. Model QSL-100): 3 bottles (A–C) were incubated under high light (∼140 μmol quanta m^{-2} s^{-1}), 3 bottles (A–C) under medium light (∼70 μmol quanta m^{-2} s^{-1}), and 3 bottles in low light (∼25 μmol quanta m^{-2} s^{-1}). The irradiance conditions in the fall incubations correspond to respective light levels of 56%, 28%, and 10% of surface photosynthetically active light (PAR) during the highest incident solar radiation experienced in July (250 μmol quanta m^{-2} s^{-1}; Masson and Peña, 2009) when assuming a vertical attenuation coefficient (K) of 0.55 m^{-1} that is pertinent to the study area (Stockner et al., 1979). For comparison, *K* values of 0.02 m^{-1} and 2 m^{-1} represent light conditions in clear oligotrophic and turbid eutrophic regions, respectively. The high- and medium-simulated light levels were, however, both higher than the measured surface light level for Jericho Beach during our initial sample collection in November (∼45 μmol quanta m^{-2} s^{-1}), whereas the low-light level (∼25 μmol quanta m^{-2} s^{-1}) corresponds to ∼50% of actual surface PAR. Experimental light levels were adjusted with black meshed screening. Self-shading due to increasing algal biomass was not accounted for in this first set of incubations. Incubations were placed in a 12°C incubator with constant illumination and monitored for 10 days.

For the summer incubations, 6 bottles (A–C and G–I) were incubated under high-light (∼130 μmol quanta m^{-2} s^{-1}), 6 bottles (D–F and J–L) under low light (∼ 25 μmol quanta m^{-2} s^{-1}), and the 3 opaque bottles (M–O) with no light (0 μmol quanta m^{-2} s^{-1}). The irradiances in the late summer incubations correspond to light levels of 52% and 10% of PAR, respectively, during the highest incident solar radiation experienced in July in the Strait of Georgia (also 250 μmol quanta m^{-2} s^{-1} (Masson and Peña, 2009) using a *K* value of 0.73 m^{-1} (Stockner et al., 1979). The late summer incubations have a larger *K* value than the late fall incubations due to the presence of suspended particulate matter from the Fraser River effluent, which results in the high-light level (∼130 μmol quanta m^{-2} s^{-1}) corresponding to ∼75% of ambient surface PAR during our collection in September, while the light low-light treatment (∼25 μmol quanta m^{-2} s^{-1}) was ∼15% of ambient surface PAR. Light levels in the illuminated treatments were continually re-adjusted with black meshed screening throughout the length of the incubations to account for progressive self-shading due to increasing algal biomass. Incubations were placed in a 12°C incubator with constant illumination overnight.

One day after sampling of the late summer experiment (on Day 1), 100 μL of 95% $H_2^{18}O$ and of 100 nM 10% atom $^{15}NH_4^+$ were added to triplicates of each set of the light treatments (high light: G–I and low light: J–L) and to the three dark bottles (M–O), in order to detect nitrification rates in parallel to the un-amended triplicates of the light treatments (high-light: bottles A–C and low-light: bottles D–F). The resulting δ^{15}N values of the NH_4^+ pool in the spiked bottles were different among treatments, given overnight changes from an initial NH_4^+ concentration of 370 ±

6 nM among all treatments on Day 0, compared to 44 ± 28 nM, 287 ± 76 nM and 807± 19 nM on Day 1 in the high, low and no light treatments, respectively. These resulted in calculated $\delta^{15}N$ of NH_4^+ of approximately 18,050‰, 6720‰, and 2870‰, assuming a $\delta^{15}N$ of 0‰ for ambient NH_4^+. The $\delta^{18}O$ of H_2O in the spiked bottles was 237‰, compared to ≤0‰ in the ambient Strait of Georgia water. The summer incubations were monitored for 30 days.

Sample Collection

During the fall incubations, subsamples were collected at discrete time intervals throughout the 15-day experiment. Approximately 30 mL aliquots were collected and pre-filtered with a 25 mm pre-combusted glass fiber filter (GF/F), and the filtrate was stored frozen for analysis of [NO_3^-] and its N and O isotope ratios. Samples for microscopic identification of plankton populations were also collected on day 1 and day 9 of the incubations, and preserved with Lugol's solution.

During the summer incubations, subsamples were collected throughout the experiment for inorganic nitrogen concentrations (NO_3^-, NO_2^- and NH_4^+), N and O isotopes of NO_3^-, and chlorophyll-*a* concentrations. Aliquots were collected from each treatment bottle and filtered through a 13-mm diameter GF/F filter mounted on a Swinnex cartridge to collect chlorophyll. Filters were immediately frozen and stored. The filtrate was recovered in an acid-washed polypropylene bottle. NO_2^- concentrations in the filtrate were analyzed immediately upon collection, and the remaining filtrates for each of the samples were then frozen and stored for NO_3^- concentrations and isotope analyses at the end of the experiment. Samples collected simultaneously for NH_4^+ analysis were not filtered to avoid contamination, and NH_4^+ concentrations were measured immediately upon collection. Samples of the phytoplankton consortium were collected at two time points during the incubations and preserved with Lugol's solution for microscopic identification of the algal population.

Nutrient Analyses

NO_3^- (plus NO_2^-) concentrations were measured by reduction to nitric oxide (NO) in a hot vanadium-III solution followed by chemiluminescence detection (Braman and Hendrix, 1989) on an Antek 1750 NO_3^-/NO_2^- analyzer. Samples collected for NH_4^+ were immediately analyzed using the fluorometric NH_4^+ method (Holmes et al., 1999) on a Turner Design 700 fluorometer, for which our detection limit was 10 nM. NO_2^- concentrations were measured colorimetrically by reaction with Greiss reagents (sulfanilamide and NNED: N-1-naphthyleneethylenediamine), and measurement of absorbance at 543 nm. The detection limit was 0.1 μM. Any NO_2^- detected was subsequently removed prior to NO_3^- isotope analysis with ascorbate, and the resultant NO gas was purged with an inert gas stream (Granger et al., 2006).

Chlorophyll-a Analyses

To monitor phytoplankton growth, chlorophyll on the frozen filters was extracted with acetone, and the chlorophyll-*a* concentrations were determined by fluorescence (Turner 10-AU fluorometer). Growth rates and their associated statistical errors were calculated by determining the exponential phase slope of both chlorophyll-*a* and NO_3^- using a type-I regression model, which minimizes the normal deviates for the y-coordinate[1]. Chlorophyll-*a* concentrations were monitored for up to 30 days (in the summer incubations only), at which point fluorescence had ceased to increase among the illuminated summer treatments.

NO_3^- N and O Isotope Ratio Measurements

N and O isotope ratios of NO_3^- were measured using the "denitrifier method" (Sigman et al., 2001; Casciotti et al., 2002), where NO_3^- is reduced to nitrous oxide (N_2O) by denitrifying bacteria that lack the N_2O reductase enzyme. Normalization of the measured $^{15}N/^{14}N$ and $^{18}O/^{16}O$ to N_2 in air and VSMOW, respectively, was done using the international potassium nitrate reference material IAEA-N3, with an assigned $\delta^{15}N$ of +4.7‰ (Gonfiantini et al., 1995) and a $\delta^{18}O$ of 25.6‰ (Révész et al., 1997; Silva et al., 2000; Böhlke et al., 2003). The O isotope data were corrected for exchange with oxygen atoms from water during reduction of NO_3^- to N_2O (Casciotti et al., 2002), which is quantified by analysis of IAEA-N3 in ^{18}O-enriched water and was 5% or less for the analyses reported here. The reproducibility of the replicate isotope values in this study was within one standard deviation of 0.3‰ for $\delta^{15}N$ and 0.5‰ for $\delta^{18}O$.

N and O isotope effects ($^{15}\varepsilon$ and $^{18}\varepsilon$, respectively) were determined for each treatment by plotting the $\delta^{15}N$ and $\delta^{18}O$ against the natural logarithm of the residual NO_3^- or of the residual substrate fraction (f) according to Mariotti et al. (1981), where the slope of the linear regression line (Rayleigh linearization) approximates ε :

$$\delta^{15}N\left(\text{or } \delta^{18}O\right)_{reactant} = \delta^{15}N\left(\text{or } \delta^{18}O\right)_{initial} - \varepsilon\left\{\ln\left(f\right)\right\}$$

$\delta^{15}N_{initial}$ is the isotopic composition of the NO_3^- at the begin of the incubations and the $\delta^{15}N_{reactant}$ refers to the isotopic composition of the remaining NO_3^- concentration after some period of consumption (Mariotti et al., 1981). Regression slopes and the associated statistical errors for both the isotope effects and $\Delta\delta^{18}O{:}\Delta\delta^{15}N$ ratio were determined by a type-II least squares cubic regression model that minimizes the normal deviates of both the weighted x and y coordinates[1].

Results

Growth Rates and Inorganic Nitrogen Dynamics

Late Fall Incubations

In the fall incubations, NO_3^- consumption by Day 15 appeared to be most pronounced in the high-light incubations, with a NO_3^- drawdown of 104 ± 13 μM among replicates, compared to 87 ± 8 μM and 76 ± 5 μM for the medium- and low-light treatments, respectively (**Figure 1**). The differences in total nitrate drawdown were statistically different ($p = 0.03$, One-Way ANOVA; Supplementary Table 1). The exponential growth rates determined from the NO_3^- drawdown ranged between 0.3

[1] Peltzer, E. T. (2007). MATLAB® shell-scripts for linear regression analysis. mbari.org.

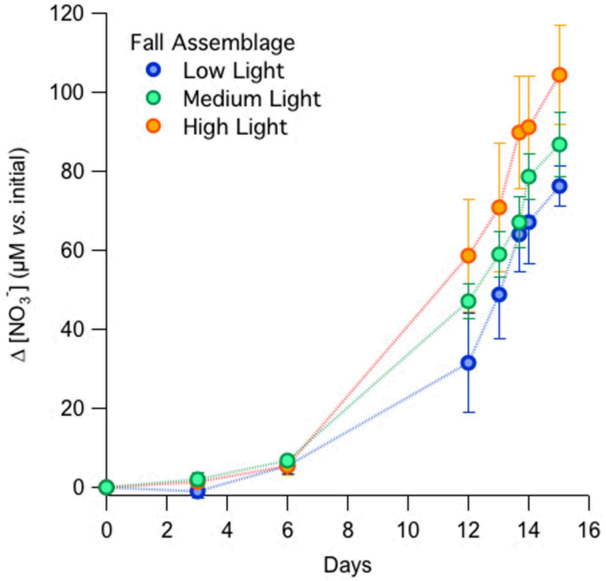

FIGURE 1 | Net NO_3^- consumption among light treatments in the fall incubations.

TABLE 1 | Mean specific growth rates, μ, and standard errors of the means (σ_M), of the (A) late fall and (B) late summer plankton incubations.

Light Level	*N* =	μ ± σ_M (d^{-1}) from Δ[NO_3^-]	μ ± σ_M (d^{-1}) from Δ[chl-*a*]
(A) FALL ASSEMBLAGE			
High	3	0.36 ± 0.09	–
Medium	3	0.31 ± 0.03	–
Low	3	0.30 ± 0.04	–
(B) SUMMER ASSEMBLAGE			
High	3	0.46 ± 0.07	0.38 ± 0.09
Low	3	0.29 ± 0.07	0.24 ± 0.07
Dark	2	N.d.	N.d.

Rates were calculated from the log-linear time-dependent increase in chlorophyll-a concentrations (late summer experiment only) and from the specific NO_3^- drawdown (log Δ[NO_3^-]). N = the number of replicate bottles. N.d. = not detected.

and 0.4 day^{-1} from the low- to high-light treatments, but were not statistically distinguishable among treatments (**Table 1A**; $p = 0.46$, One-Way ANOVA; Supplementary Table 1).

Microscopic identification of the preserved samples indicated that the phytoplankton in the late-fall experiment was largely comprised of the diatom *Skeletonema* spp., which is a common dominant phytoplankton species native to the Strait of Georgia (Harrison et al., 1983; Spies and Parsons, 1985). NO_2^- and NH_4^+ concentrations were not measured in the fall incubations.

Late Summer Incubations

During the summer incubations, chlorophyll-*a* concentrations increased fastest in the high-light treatment and not measurably in the dark treatment (**Figure 2A**; chl-*a* in the dark treatment incubations was measured only in bottle M). The chlorophyll-*a* increase in the high-light bottles was roughly exponential for the first 8 days, after which it reached a plateau to a variable mean. The chlorophyll-*a* increase in the low-light treatment was roughly exponential for the first 15 days, after which it appeared to slowdown. Phytoplankton growth in bottle F (low-light, unlabeled) was noticeably lower than in parallel low-light replicates.

In both the high- and low-light treatments, NO_3^- decreased over the 30 incubation days (**Figure 2B**). The overall rate of NO_3^- consumption was fastest in the high-light treatment, with a total drawdown of ~70–85 μM. A measurable NO_3^- drawdown was detectable after 5 days and a NO_3^- concentration plateau was reached around Day 10, consistent with the plateauing of chlorophyll-*a* concentration in the incubation. The onset of the NO_3^- drawdown in the low-light treatment began around Day 8, after which NO_3^- concentration decreased gradually, yielding a total drawdown of ~62–74 μM at the end of the incubation. Bottle F (low-light, unlabeled) displayed only a modest net decrease in NO_3^- concentration of 20 μM. In the dark treatment bottles (M and N bottles) of the incubations, NO_3^- remained at its initial concentration of ~100 μM throughout the incubation period (within measurement error). Nitrate was not measured for the dark "O" treatment.

Exponential growth rates were computed from the initial exponential increase in chlorophyll-*a* for the high- and low-light treatments, respectively, and were verified against the exponential decrease in NO_3^- concentrations (**Table 1B**). Exponential rates determined by chlorophyll-*a* were on the order 0.38 ± 0.07 day^{-1} among the high-light treatment bottles, and 0.24 ± 0.07 day^{-1} for the low-light treatment. Similarly, exponential rates determined from the specific NO_3^- drawdown (**Figure 2B**; **Table 1B**) were 0.46 ± 0.07 day^{-1} for the high-light treatment and 0.29 ± 0.07 day^{-1} at low light. Growth rates differed significantly between high and low light treatments, whether determined from the chlorophyll-*a* increase ($p = 0.01$; *t*-test; Supplementary Table 1) or from the corresponding nitrate drawdown ($p < 0.01$, *t*-test; Supplementary Table 1).

Nitrite (NO_2^-) was not detected in any of the summer treatments until Day 5 (**Figure 2C**), at which point concentrations began to increase in the high-light treatment to a maximum of 2.0 μM around Day 10, remaining relatively elevated thereon. Only a moderate increase in NO_2^- was recorded in the low-light incubations, first detectable on Day 8, and reaching concentrations comparable to those in high-light treatment after Day 15. NO_2^- concentrations in the dark treatment remained near or below detection throughout.

Ammonium concentrations remained below 0.1 μM during exponential growth in both light treatments (**Figure 2D**). At Day 10, an increase to ≥0.5 μM was observed in the high-light treatment. Concentrations of NH_4^+ in the low-light treatment remained near or slightly above detection until Day 18 and were not measured thereafter. In contrast to the light treatments, NH_4^+ concentrations in the dark treatment increased steadily from 0.4 on Day 0, to ~3 μM by Day 10, after which concentrations dropped again to 2 μM.

Skeletonema spp. was also the dominant algal group in the summer incubations. This species was prevalent in our initial algal consortia and remained so in all of the light treatments. Differences between the high- and low-light community compositions were not readily discernable by microscopy. These

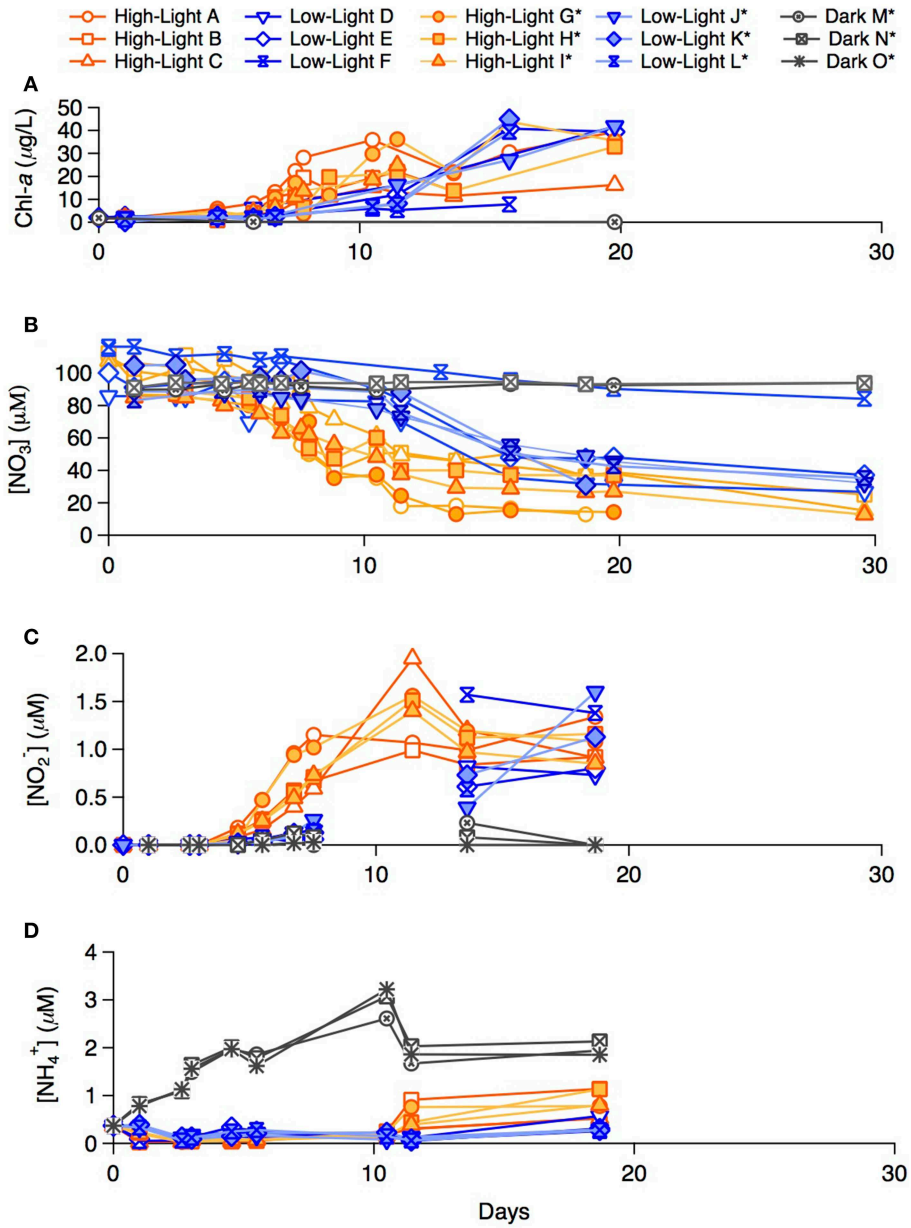

FIGURE 2 | Late summer incubation concentrations of (A) chlorophyll-*a*, (B) NO_3^-, (C) NO_2^-, (D) NH_4^+ for both the labeled and un-labeled treatments incubated under high-light, low-light, and dark conditions.

summer samples contained fragments of diatoms and other cells, suggesting that we collected a senescent phytoplankton assemblage. Early September, at which time the samples were collected, generally corresponds to the late summer period of maximum heterotrophic productivity in the Strait of Georgia (Albright, 1977).

N and O Isotopes of NO_3^-

In the high-, medium-, and low-light treatments of the fall incubations, NO_3^- $\delta^{15}N$ and $\delta^{18}O$ increased with decreasing NO_3^- concentrations, from a $\delta^{15}N$ of 1.5‰ to as high as 10‰ in high-light and concurrently from a $\delta^{18}O$ of 19‰ to as high as 28‰ (**Figure 3A**). The increase in both $\delta^{18}O$ as a function of the natural logarithm of NO_3^- were linear among all treatments (**Figure 3A**), and thus consistent with Rayleigh fractionation (consumption of a closed substrate pool with a constant isotope effect; Mariotti et al., 1981). The slopes of the regression lines in the Rayleigh plots indicated $^{15}\varepsilon$ and $^{18}\varepsilon$ values that ranged between 5 and 9‰ among all incubations; isotope effects did not vary coherently among treatments (**Table 2A**; Supplementary Table 1). The $\delta^{18}O$ increased in tandem with the corresponding $\delta^{15}N$, translating into $^{18}\varepsilon$ values similar to the corresponding $^{15}\varepsilon$.

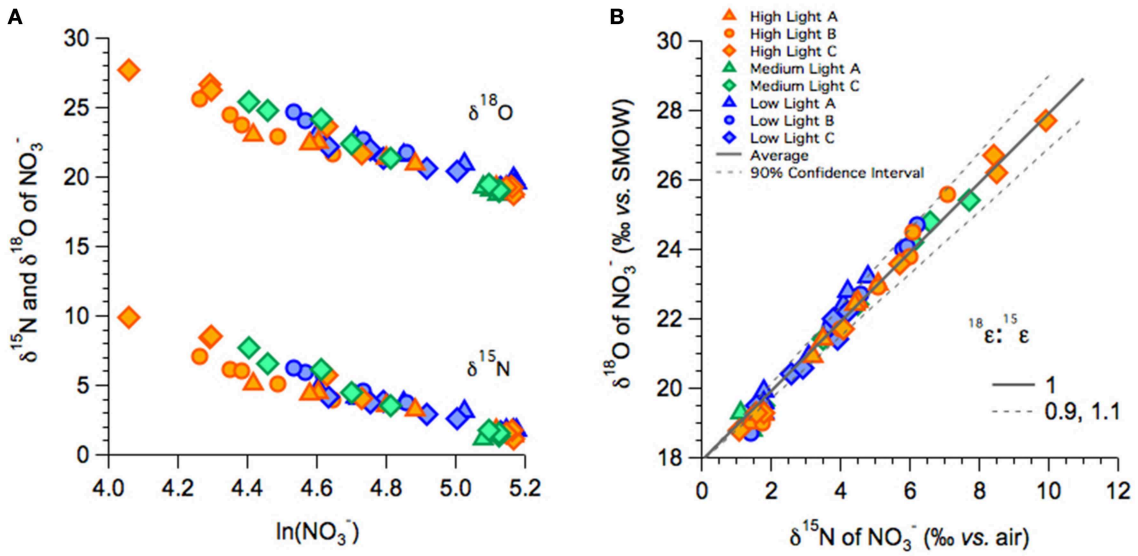

FIGURE 3 | NO_3^- isotope dynamics of fall incubations: (A) NO_3^- $\delta^{15}N$ plotted vs. the logarithm of the NO_3^- remaining for the high-, medium-, and low-light treatments. The respective slopes of the linear relationships approximate $^{15}\varepsilon$. **(B)** $\delta^{18}O$ vs. $\delta^{15}N$ of NO_3^- for the high-, medium-, and low-light treatments.

TABLE 2 | Mean N and O isotope effects of NO_3^- and $\Delta\delta^{18}O$:$\Delta\delta^{15}N$ change and associated standard error (σ_M) for each light treatment for the (A) late fall community incubations and (B) late summer community incubations.

Light Level	N =	$^{15}\varepsilon \pm \sigma_M$	$^{18}\varepsilon \pm \sigma_M$	$\Delta\delta^{18}O$: $\Delta\delta^{15}N \pm \sigma_M$
(A) FALL ASSEMBLAGE				
High	3	6.2 ± 1.5	6.9 ± 1.3	1.07 ± 0.08
Medium	1	8.4 ± 0.5	8.2 ± 0.6	0.96 ± 0.04
Low	3	6.2 ± 1.4	7.4 ± 1.8	1.18 ± 0.03
(B) SUMMER ASSEMBLAGE				
High	3	7.8 ± 0.6	8.4 ± 0.4	1.08 ± 0.04
*High**	2	9.4 ± 0.4	10.2 ± 0.5	1.08 ± 0.01
Low	2	11.7 ± 1.0	12.5 ± 2.8	1.03 ± 0.06
*Low**	3	12.1 ± 0.8	12.6 ± 0.6	1.05 ± 0.06

N = the number of replicate bottles. Treatments supplemented with $^{15}NH_4^+$ and $H_2^{18}O$ are denoted by an asterisk (*).

Plotted against each other, the change in $\delta^{18}O$ *vs.* that in $\Delta\delta^{15}N$ ($\Delta\delta^{18}O$:$\delta^{15}N$) yielded slopes near unity, 1.07 ± 0.08‰ for the high-light incubations, 0.96 ± 0.04 for a single medium-light treatment, and of 1.18 ± 0.03‰ for the low-light incubations (**Table 2A**; **Figure 3B**). The apparent $\Delta\delta^{18}O$:$\Delta\delta^{15}N$ differences between high- and low- light treatments were not statistically significant ($p = 0.07$; One-Way ANOVA; Supplementary Table 1).

The $NO_3^-\delta^{15}N$ and $\delta^{18}O$ in both the high- and low-light treatments of the summer incubations also increased in parallel to the decreasing NO_3^- concentrations, from 1.5 to 25‰ 25‰ for $\delta^{15}N$, and 20 to 40‰ for $\delta^{18}O$ (**Figures 4A,B**). Unlike the fall experiment, the corresponding $^{15}\varepsilon$ differed significantly between light treatments, averaging 7.8 ± 0.6‰ among high-light incubations vs. 11.7 ± 1.0‰ for the low-light incubations ($p < 0.01$; Two-Way ANOVA; **Figure 5**; **Table 2B**; Supplementary Table 1). The Two-Way ANOVA also indicated that the $^{15}\varepsilon$ in incubations supplemented with both $^{15}NH_4^+$ and $H_2^{18}O$ were comparable to those in the unlabeled incubations for both of the light treatments, averaging 9.4 ± 0.4‰ at high-light and 12.1 ± 0.8‰ at low-light ($p = 0.08$, Two-Way ANOVA; Supplementary Table 1).

The $^{18}\varepsilon$ values observed in the summer experiment were 8.4 ± 0.4‰ and 12.5 ± 2.8‰ for high- and-low light bottles, respectively, thus similar to the corresponding $^{15}\varepsilon$ values. The change of $\delta^{18}O$ vs. that of $\delta^{15}N$ ($\Delta\delta^{18}O$:$\Delta\delta^{15}N$) yielded statistically indistinguishable slopes of 1.08 ± 0.04‰ and 1.03 ± 0.06‰ for the *unlabeled* high- and low-light incubations, respectively ($p = 0.12$, Two-Way ANOVA; Supplementary Table 1). The $\Delta^{18}O$:$\Delta\delta^{15}N$ values of the parallel *labeled* incubations were 1.08 ± 0.01‰, and 1.05 ± 0.06‰ in high- and low-light respectively, thus comparable to the natural abundance treatments ($p = 1.00$, Two-Way ANOVA; Supplementary Table 1). In the dark incubations, which were supplemented with the $^{15}NH_4^+$ and $H_2^{18}O$ tracers, the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- remained unchanged for the entire 30 days, indicating undetectable nitrification in these incubations (**Figure 4C**).

Discussion

The late fall *vs.* late summer plankton community incubations displayed different responses to the level of irradiance. The low-light incubations of the fall plankton community had slower total nitrate consumption than the corresponding high-light incubations, yet no clear distinction in NO_3^- isotope dynamics was observed between low and high light. In the summer plankton community, a similar contrast in irradiance yielded significantly different growth rates and total NO_3^- consumption. Low light also resulted in higher N and O isotope effects compared to high light, and a distinction in recycled nutrient (NH_4^+ and NO_2^-) dynamics was also observed between low-

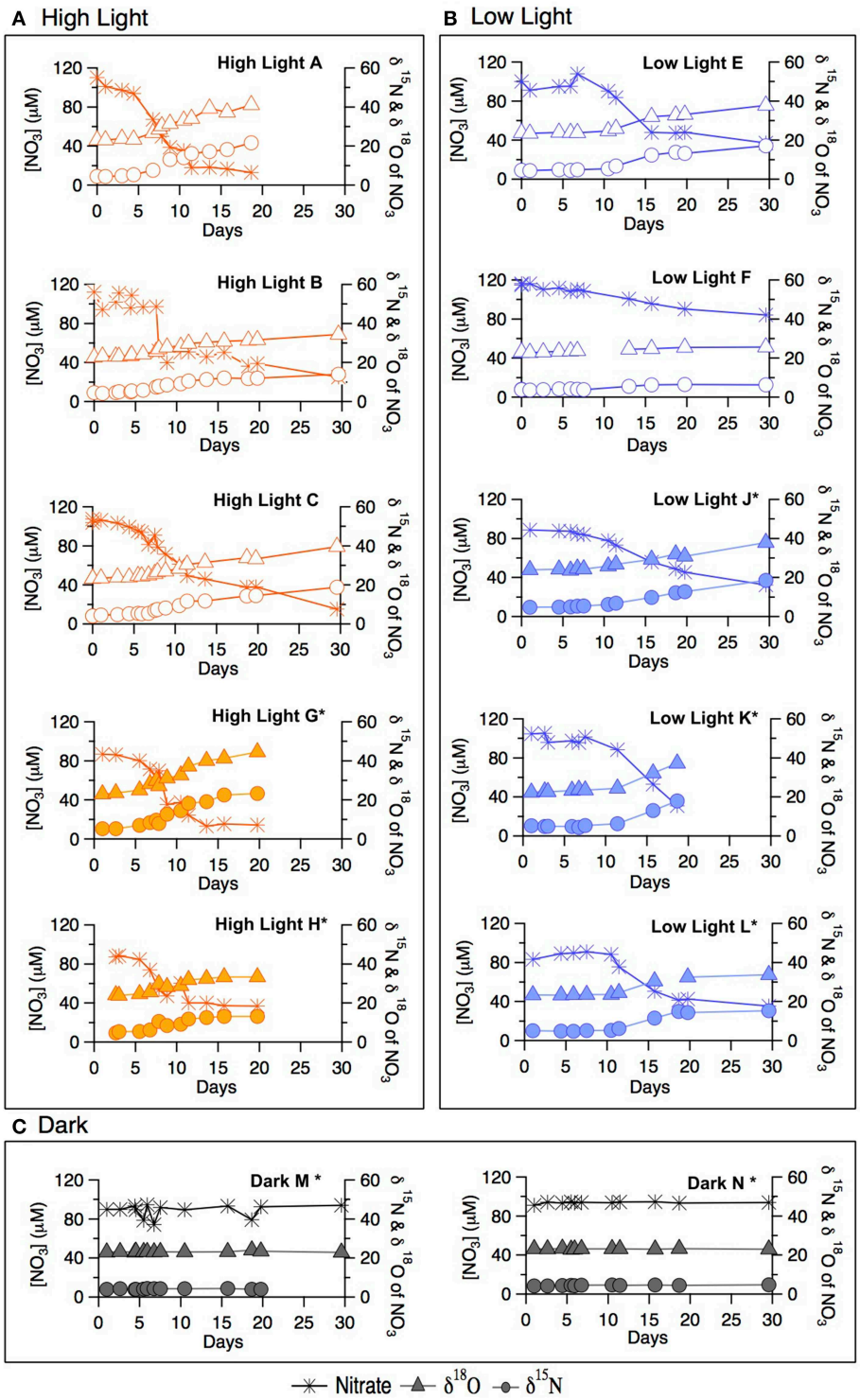

FIGURE 4 | Changes in NO_3^- concentrations during the late-summer incubations with the corresponding $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- for (A) high-light (orange symbols), (B) low-light (blue symbols), and (C) dark treatments (gray symbols). Labeled experiments (closed symbols) are denoted by an asterisk (*).

and high-light treatments. None of the irradiance treatments, however, appeared to foster detectable nitrification in either the fall or summer incubations. The respective growth dynamics of the fall and summer incubations, and the effect of light on the amplitude of the NO_3^- N isotope effects are discussed below, as are the implications for the interpretation of NO_3^-

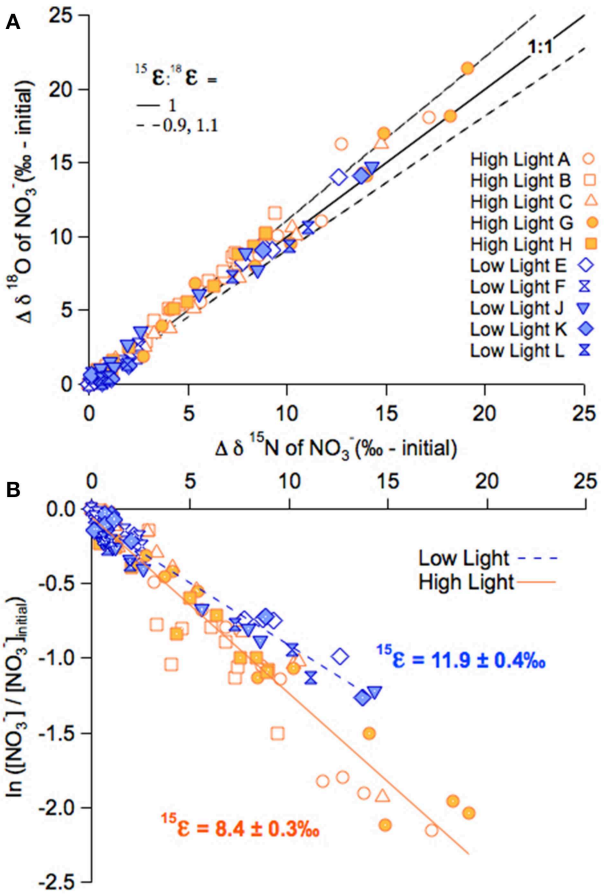

FIGURE 5 | NO_3^- isotope dynamics of the late summer incubations. (A) The change in NO_3^- $\delta^{18}O$ ($\delta^{18}O$ *minus* $\delta^{18}O_{initial}$) plotted on the corresponding change in $\delta^{15}N$ in the high-light and low-light summer incubations, for both unlabeled and labeled treatments. **(B)** NO_3^- $\delta^{15}N$ plotted vs. the logarithm of the fraction of NO_3^- remaining for the high-light and low-light summer incubations, for both labeled and unlabeled treatments. The respective slopes of the linear relationships approximate $^{15}\varepsilon$.

isotope distributions in the surface ocean. We also scrutinize our experimental design to assess whether nitrification coincident with net NO_3^- assimilation was detectable from the coupled N and O isotope ratios. We propose modifications that could yield a more sensitive test of an isotopic role for nitrification at low light.

Growth Dynamics at High and Low Light

The consumption of NO_3^- during the fall incubations was similar under all light regimes, and slowed around Day 15. Similarly, net phytoplankton growth in the summer incubations was no longer detected after Day 10 in the high-light treatment, and after Day 15 in the low-light treatment. The cessation of apparent growth was not due to macronutrient exhaustion, given the elevated NO_3^- concentrations remaining ($\geq$50 μM) and given the fact that SiO_4^{2-} and PO_4^{-3} were initially added in excess with respect to Redfield stoichiometry. The subsidence of NO_3^- consumption could be the result of self-shading by the growing algal populations, because we did not mitigate the corresponding changes in luminosity for the fall incubations. Alternatively, slowed NO_3^- consumption could reflect a relative increase in heterotrophic activity in the incubations, which could arise due to a number of potential factors, including viral lysis, and/or changes in seawater chemistry stemming from a pH increase due to carbon dioxide removal during photosynthesis, with consequent effects on phytoplankton physiology.

Senescence of the phytoplankton assemblage in the summer experiment was apparent *via* microscopy during the incubation period, which suggests that heterotrophy could have surpassed photosynthesis in spite of continual illumination. Chlorophyll-*a* concentrations after Day 10 in the high-light treatment proved very variable, which could also portend autotrophic-heterotrophic coupling. Analogous variability was not detected following the end of growth in the low-light cultures (after Day 15), although chlorophyll-*a* concentrations were not monitored with sufficient frequency after Day 15 to assess this with certainty.

The potential for net heterotrophy in the summer incubations, suggested by microscopic inspection and from the plateau in chlorophyll-*a* concentrations, was also evidenced by the NH_4^+ pool dynamics within each of the treatments. NH_4^+ concentrations in both light treatments remained near the limit of detection (10 nM) until net phytoplankton growth ceased, after which NH_4^+ increased modestly in the high-light treatment, whereas the concentration of NH_4^+ remained stable and did not increase comparably following the cessation of growth at low light (Day 15; **Figure 2D**). Together, these trends are best explained by the relative predominance of heterotrophic metabolism after the cessation of net phytoplankton growth, with some ammonium assimilation by phytoplankton (the lack of a continuous rise in ammonium requires that phytoplankton are still consuming it even after the step-up in concentration at ~Day 12). The regeneration of NH_4^+ *via* the decomposition of organic matter would provide a preferred source of N over NO_3^- for algal growth, because of the additional energy required for the intracellular reduction of NO_3^- to NH_4^+ (Bates, 1976; Thompson et al., 1989; Dortch, 1990).

The concentration of NH_4^+ in the dark treatment of the summer incubations increased steadily from the onset of the incubations, from 0.3 to a maximum of 3 μM at Day 10, indicating ammonification of organic material (**Figure 2D**). The subsequent drop in NH_4^+ to 2 μM in the dark treatment after Day 10 is difficult to explain. The absence of any increase in the $\delta^{15}N$ of NO_3^- of the $^{15}NH_4^+$-amended dark treatment, expected if any NH_4^+ had been oxidized, further rules out nitrification as a potential explanation for the observed decrease in NH_4^+, as will be discussed in a subsequent section. Moreover, the biological oxidation of 1 μM ammonium overnight would require a relatively high abundance of nitrifying bacteria, which is unlikely given their inherently slow maximum growth rates (e.g., Keen and Prosser, 1987). Hence, the rapid NH_4^+ decrease in the dark bottles after Day 10 remains elusive. Ammonium assimilation associated with heterotrophic bacterial growth on dissolved organic carbon is one possible explanation, but we lack the data to consider it further.

In the high- and low-light treatment bottles, the accumulation of NO_2^- during the summer incubations (**Figure 2C**) could,

hypothetically, derive from the oxidation of NH_4^+ by nitrifiers. Slower rates of NO_2^- oxidation compared to NH_4^+ oxidation at the base of the euphotic zone have been cited as the origin of the primary nitrite maximum (Ward et al., 1984; Buchwald and Casciotti, 2013). However, incomplete NO_3^- reduction by light-limited phytoplankton in ocean environments with fluctuating light levels has also been hypothesized to contribute to or dominate NO_2^- production at the nitrite maximum in the ocean's mixed layer (Collos, 1998; Lomas and Lipschultz, 2006; Mackey et al., 2011). The conspicuous absence of NO_2^- in the *dark* bottles, coupled with a lack of detectable $\delta^{15}N$ and $\delta^{18}O$ increase in the NO_3^- pool therein after isotope label amendment, suggests an absence of NH_4^+ oxidation to NO_2^-. Therefore, we posit that the accumulation of NO_2^- in the light bottles was due to intracellular NO_3^- reduction and cellular NO_2^- efflux at low-light (Martinez, 1991) rather than to nitrification.

The Influence of Light on the Amplitude of $^{15}\varepsilon$ and $^{18}\varepsilon$

The response of N (and O) isotope effect amplitudes to irradiance was qualitatively different in the fall *vs.* summer incubations. Decreased irradiance had no concerted effect on the $^{15}\varepsilon$ and $^{18}\varepsilon$ of the fall community incubations, whereas low light resulted in a sizeable increase in $^{15}\varepsilon$ and corresponding $^{18}\varepsilon$ of the summer community incubations. While these contrasting results are apparently contradictory, they nevertheless capture the range of possible responses to irradiance observed in mono-culture studies (Wada and Hattori, 1978; Needoba and Harrison, 2004; Needoba et al., 2004). In particular, Needoba and Harrison (2004) noted that among monocultures of four phytoplankton strains, two diatoms and a prymnesiophyte showed higher N isotope effects in light-limited vs. high-light cultures, whereas one diatom species exhibited no increase in $^{15}\varepsilon$ at low light. Species-specific differences in $^{15}\varepsilon$ as well as intra-species differences in $^{15}\varepsilon$ have been attributed to variation in the expression of the enzymatic isotope effect imposed by nitrate reductase, which is determined by the ratio of NO_3^- effluxing out of the cell relative to its uptake (Shearer et al., 1991; Granger et al., 2004; Needoba et al., 2004). The intrinsic N (and O) isotope effect(s) of the eukaryotic assimilatory NO_3^- reductase *in vitro* has been estimated to be ~27‰ (Karsh et al., 2012), whereas data on the N or O isotope effects imparted on NO_3^- during its active uptake at the cell surface indicate that they range between minor (up to 2‰; Karsh et al., 2013) and undetectable (Granger et al., 2010). N and O isotope discrimination of NO_3^- during its assimilation is thus incurred dominantly during intracellular reduction by nitrate reductase. However, the enzymatic ^{15}N-enrichment that is transmitted to the external medium through cellular efflux of the internal NO_3^- pool and the cellular-level isotope effect (i.e., the expression of the isotope fractionation in the external medium) depends on the ratio of uptake to efflux (i.e., "efflux model"; Shearer et al., 1991; Granger et al., 2004; Needoba et al., 2004), which may vary with changing physiological and/or environmental conditions.

In this context, the higher N and O isotope effects associated with the low-light treatments are diagnostic of a higher ratio of NO_3^- efflux to NO_3^- uptake by the phytoplankton (Needoba et al., 2004; Karsh et al., 2013). The increase in $^{15}\varepsilon$ associated with light limitation may reflect a specific physiological response of NO_3^- assimilation to this energetic constraint. Under low-light conditions, phytoplankton have lower internal concentrations of NADH/NADPH reductant that is generated during photosynthesis and utilized to fuel the NO_3^- reductase enzyme. As a result, lower NO_3^- reductase activity under low light would leave proportionally more of the intracellular NO_3^- pool to be released back into the external medium. Conversely, higher NO_3^- reductase activity due to an increase in the concentration of NADH/NADPH under higher light conditions would result in NO_3^- assimilation and a smaller quantity of stored intracellular NO_3^- remaining to be released in the extracellular environment (Needoba et al., 2004).

This explanation, however, does not address why phytoplankton would not simply down-regulate their NO_3^- uptake in parallel with the decrease in NO_3^- reduction to minimize the energetic investment for NO_3^- uptake and the subsequent energetic requirement for NO_3^- efflux. A hypothesis that has been posed for this behavior is that light-limited phytoplankton generate high internal NO_3^- concentrations in preparation for some imminent event of high light availability, promoting an inadvertent increase in cellular NO_3^- efflux due to the steeper concentration gradient from the cell interior relative to the medium (Needoba and Harrison, 2004; Needoba et al., 2004).

Our data do not offer a specific test of the physiological hypotheses for this dynamic, yet they do indicate that the range of NO_3^- isotope dynamics observed in response to light limitation in pure culture also applies to phytoplankton in natural marine consortia. The lack of a clear difference in the $^{15}\varepsilon$ amplitude between high- and low-light incubations of the fall community suggests that NO_3^- homeostasis of the phytoplankton was not affected by light limitation. The overall growth of the consortium was slowed modestly at lower light conditions compared to the late-summer community, which suggests that the assemblage was adapted to maintain near maximum fitness at relatively low light. The consortium may have comprised species that are adapted to lower incident light conditions in winter, and for whom changes in NO_3^- physiology would only be instigated at substantially lower light levels, if at all. Conversely, NO_3^- metabolism of the summer phytoplankton community was very sensitive to irradiance, showing clear decrease in growth rates and parallel increase in $^{15}\varepsilon$ at lower light levels, akin to the response of some phytoplankton species in culture.

Given the differential response observed here and in monocultures, the magnitude of a given community $^{15}\varepsilon$ is evidently not diagnostic of irradiance alone. At the surface ocean, notable exceptions to the "community" isotope effect on the order of 5‰ exist in the Subantarctic, where the mixed layer is deep and phytoplankton may easily be mixed between high and low light conditions (DiFiore et al., 2006). At the deeper mixed layer depths in the Subantarctic with mean light intensity of ~1.5 $MJ/m^{-2}d^{-1}$ (~80 μmol quanta m^{-2} s^{-1}), the observed $^{15}\varepsilon$ is 8–9‰. However, this range in $^{15}\varepsilon$ is similar to the range observed in our high-light treatments of ~130 μmol quanta m^{-2} s^{-1}. Overall, the existing data indicate that there

is a connection between light limitation and NO_3^- isotope effects, but a unique relationship appears clouded by differences in species composition and/or adaptive characteristics among phytoplankton assemblages.

Isotopic Impact of Nitrification

A secondary goal of this study was to test whether nitrification occurring simultaneously with net NO_3^- assimilation by phytoplankton manifests as an apparent alteration of the $\Delta\delta^{18}O{:}\Delta\delta^{15}N$ co-variation due to NO_3^- assimilation (Granger et al., 2004). Our expectation was that an indigenous plankton consortium incubated at low-light would host some degree of nitrification relative to high-light incubations and that this would be expressed as a positive deviation in the $\Delta\delta^{18}O{:}\Delta\delta^{15}N$ from a nominal value of 1 (Granger et al., 2004; Wankel et al., 2007; DiFiore et al., 2009; Smart et al., 2015). There were, however, no detectable differences in the $\Delta\delta^{18}O{:}\Delta\delta^{15}N$ among light treatments in either the fall or summer incubations (**Figure 5A**).

We explain the invariant $\Delta\delta^{18}O{:}\Delta\delta^{15}N$ among light treatments by very low to negligible nitrification rates in our incubations. This assessment is supported for the summer incubations, in which there were no detectable isotopic enrichments of NO_3^- in the parallel treatments supplemented with $^{15}NH_4^+$ and $H_2^{18}O$. Arguably, the rapid assimilation of the $^{15}NH_4^+$ tracer into biomass could have rendered the incubations less sensitive to nitrification than intended. Given the low concentrations of $^{15}NH_4^+$ in the light treatments ($\sim$10 nM during net phytoplankton growth), the supplemented $^{15}NH_4^+$ in the N pool could have been diluted to extinction relatively rapidly in the low- and high-light treatment bottles due to rapid assimilation and regeneration. However, the continual increase in ambient NH_4^+ in the dark labeled incubations, from 0.3 at the onset to 3 μM by Day 10, suggests that the $^{15}NH_4^+$ tracer therein was not entirely shunted into biomass, if at all. The elevated experimental NO_3^- added ($\geq$100 μM) to the incubations rendered the ^{15}N-label-isotope approach less sensitive to nitrification than customarily (e.g., Ward, 1987). Nevertheless, our calculations indicate that a nitrification rate as low as 10 nM d^{-1}, characteristic of near-shore systems (e.g., Ward, 1987), would have resulted in a detectable $\delta^{15}N$ increase of 1.0‰ over 10 days of incubations in the dark, given negligible assimilation of the $^{15}NH_4^+$ into biomass. A nitrification rate of 30 nM d^{-1} could have been detected overnight in the dark treatment, yielding a detectable NO_3^- $\delta^{15}N$ increase of 0.5‰ by Day 2 and of nearly 4‰ by Day 10. As prescribed, the ^{18}O-label approach was considerably less sensitive to nitrification given the isotopic enrichment of ambient H_2O (237‰). Still, our calculations suggest that a nitrification rate of $\sim$50 nM d^{-1}, would have resulted in a 1.0‰ increase in the $\delta^{18}O$ of nitrate over 10 days in the dark incubations; yet, this was not observed.

Therefore, the lack of any detectable increase in either the $\delta^{15}N$ or the $\delta^{18}O$ of NO_3^- in the dark incubations suggests that nitrification, if at all occurring, was slow. It follows that nitrification rates would have been still lower in the summer incubations with light, given the higher potential for competition for ammonium with phytoplankton and light inhibition of nitrification (Ward et al., 1984; Smith et al., 2014).

Our data thus fail to test mounting evidence from field studies that, in some regions of the surface ocean, nitrification does induce a decoupling of the nitrate N and O isotopes from a $\Delta\delta^{18}O{:}\Delta\delta^{15}N$ of $\sim$1 expected for NO_3^- consumption (Wankel et al., 2007; Smart et al., 2015). Nitrification rates in our experiments were likely $<$10 nM d^{-1}, which our experiments were not designed well to detect at natural abundance levels because of the elevated initial NO_3^- concentrations. In an attempt to extend the dynamic range of the NO_3^- isotope ratios, we used a large initial NO_3^- addition, which decreased the sensitivity of the coupled N and O isotope ratios to coincident nitrification. Future experiments to test whether coincident nitrification induces a decoupling of the nitrate N and O isotope ratios from the assimilatory $\Delta\delta^{18}O{:}\Delta\delta^{15}N$ ratio of 1 could be more sensitive if conducted with lower initial NO_3^- concentrations akin to field conditions. Experimental verification of this dynamic remains desirable, to validate the inferences gleaned from nitrate isotope distributions in the ocean environment.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2015.00028/abstract

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