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The influence of light on nitrogen cycling and the primary nitrite maximum in a seasonally stratified sea

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A B S T R A C T
In the seasonally stratified Gulf of Aqaba Red Sea, both NO3 release by phytoplankton and NH3 oxidation by nitrifying microbes contributed to the formation of a primary nitrite maximum (PNM) over different seasons and depths in the water column. In the winter and during the days immediately following spring stratification, NO3 formation was strongly correlated \( (R^2 = 0.99) \) with decreasing irradiance and chlorophyll, suggesting that incomplete NO3 reduction by light limited phytoplankton was a major source of NO3. However, as stratification progressed, NO3 continued to be generated below the euphotic depth by microbial NH3 oxidation, likely due to differential photoinhibition of NH3 and NO3 oxidizing populations. Natural abundance stable nitrogen isotope analyses revealed a decoupling of the \( \delta^{15}N \) and \( \delta^{18}O \) in the combined NO3 and NO2 pool, suggesting that assimilation and nitrification were co-occurring in surface waters. As stratification progressed, the \( \delta^{15}N \) of particulate N below the euphotic depth increased from \(-5\%\) to up to \(+20\%\).

N uptake rates were also influenced by light; based on \(^{15}N\) tracer experiments, assimilation of NO3, NO2, and urea was more rapid in the light \((434 \pm 24, 94 \pm 17, \text{ and } 1194 \pm 48 \text{ nmol N L}^{-1} \text{ day}^{-1} \) respectively) than in the dark \((58 \pm 14, 29 \pm 14, \text{ and } 476 \pm 31 \text{ nmol N L}^{-1} \text{ day}^{-1} \) respectively). Dark NH3 assimilation was \(314 \pm 31 \text{ nmol N L}^{-1} \text{ day}^{-1} \), while light NH3 assimilation was much faster, resulting in complete consumption of the \(^{15}N\) spike in less than \(7 \text{ h} \) from spike addition. The overall rate of coupled urea mineralization and NH3 oxidation \((14.1 \pm 7.6 \text{ nmol N L}^{-1} \text{ day}^{-1}) \) was similar to that of NH3 oxidation alone \((16.4 \pm 8.1 \text{ nmol N L}^{-1} \text{ day}^{-1}) \), suggesting that mineralization of labile dissolved organic N compounds like urea was not a rate limiting step for nitrification. Our results suggest that assimilation and nitrification compete for NH3 and that N transformation rates throughout the water column are influenced by light over diel and seasonal cycles, allowing phytoplankton and nitrifying microbes to contribute jointly to PNM formation. We identify important factors that influence the N cycle throughout the year, including light intensity, substrate availability, and microbial community structure. These processes could be relevant to other regions worldwide where seasonal variability in mixing depth and stratification influence the contributions of phytoplankton and non-photosynthetic microbes to the N cycle.

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1. Introduction

Nitrogen (N) is a limiting nutrient for primary producers in many marine environments, and nitrogen compounds are important energy sources for marine microbes. Nitrogen cycling in the surface ocean involves several key N transformation pathways (Fig. 1). The major source of new (external) N is the supply of nitrate \((\text{NO}_3^-)\) from deep mixing, advection, or diffusion (Zehr and Ward, 2002). N2 fixation and atmospheric deposition also provide new bioavailable N for phytoplankton growth in some regions (Sanudo-Wilhelmy et al., 2001; Gruber and Sarmiento, 1997; Montoya et al., 2004; Duce et al., 2008). Phytoplankton assimilate NH3, NO2, and NO3, collectively referred to as dissolved inorganic N (DIN), into their biomass during autotrophic growth, forming particulate and dissolved organic N (PON and DON) compounds. Organic N is released directly into the environment during cell lysis or excretion, and can be remineralized back to NH3 by microbes during ammonification (Hollibaugh and Azam, 1983;
in a stratified water column when NO$_3^-$ production exceeds its consumption, leading to formation of a primary NO$_2^-$ maximum (PNM, Lomas and Lipschultz, 2006). Two mechanisms have been proposed to describe how NO$_3^-$ maxima form. The first entails uncoupled oxidation of NH$_4^+$ and NO$_2^-$ during nitrification which leads to NO$_2^-$ buildup if the microbial populations responsible for each step are spatially segregated within the water column. This could occur if the populations have different sensitivities to light (Olson, 1981; Guerrero and Jones, 1996) or different demands for substrate. The second process involves NO$_2^-$ production during incomplete NO$_3^-$ assimilation by phytoplankton, particularly when light stressed (Collos, 1998; Lomas and Gilbert, 1999; Lomas and Lipschultz, 2006). NO$_2^-$ release by phytoplankton could occur if the cell does not receive enough light energy to complete the reduction of NO$_2^-$ into NH$_4^+$ (Collos, 1998), or in response to rapidly changing light conditions, possibly as a photoprotective mechanism (Lomas and Gilbert, 2000). Nitrite maxima throughout the world’s oceans are generally attributed to one of these two processes (Lomas and Lipschultz (2006) and references therein), although Dore and Karl (1996a,b) showed that vertical separation of reductive and oxidative microbial processes contributes to PNM formation in the Pacific Ocean. Whether these processes co-occur in other locations and, if so, how physical factors influence which process dominates and at what depth in the water column is not clear.

Isotopic analysis of coupled nitrogen ($^{15}$N) and oxygen ($^{18}$O) in NO$_3^-$ can be used for discriminating between biologically mediated N transformation processes, such as those giving rise to the PNM, since each process imparts a unique isotopic signature to both the N and O composition of the sample (Casciotti et al., 2002; Wankel et al., 2006). In processes such as assimilation (and denitrification under anaerobic conditions), the $^{15}$ONO$_3^-$ and $^{15}$NO$_3^-$ are viewed to be coupled, as they increase proportionally as NO$_3^-$ is consumed, with an O:N ratio of isotopic effects of $\sim 1$ (Granger et al., 2004, 2008, 2010).

In contrast, nitrification results in the decoupling of $^{18}$O and $^{15}$N of nitrate and as a result values will plot along a line with a slope greater than 1. This decoupling is a result of the processes of assimilation and nitrification competing for the NH$_4^+$ substrate (Wankel et al., 2007). The difference between the isotope effect of nitrification and that of assimilation will determine the isotopic composition of the NO$_3^-$ returned to the N pool. The greater the difference between the isotope effects of the two branching processes, the lower the $^{15}$ONO$_3^-$ becomes, whereas the oxygen signature is insensitive to the origin of the N in nitrification (Wankel et al., 2007, 2009b).

The goal of this work is to improve our understanding of the N cycle in the Gulf of Aqaba, Red Sea; a system with nutrient cycles that are similar to many other seasonally stratified subtropical seas (Labiosa et al., 2003). Prior observations in the Gulf have suggested that substrate availability has a strong influence on PNM dynamics, and that nitrification and NO$_3^-$ excretion are dominant in the summer and winter respectively (Meeder et al., in press). In this study, we seek to improve our understanding of how key physical, chemical and biological processes contribute to this seasonality and identify temporal and spatial trends in N transformation processes and rates. Our approach uses $^{15}$N tracer experiments together with natural abundance stable isotope measurements to quantify N transformation rates and determine the extent of N regeneration from organic matter. This combined approach characterizes different pathways in the N cycle over multiple temporal scales under both manipulated (experimental) and in situ conditions. Particular attention is given to processes influencing NO$_3^-$ maxima, and formation of the PNM is used as a framework to discuss the different N transformation processes occurring in the Gulf.

Fig. 1. The N cycle under oxic conditions, showing pathways and isotope effects of major N transformation processes (Casciotti, 2009 and references therein). "ND" indicates that the isotope effect has not been determined.
2. Materials and methods

2.1. Field site

The Gulf of Aqaba is a seasonally stratified, subtropical water body extending from the northern Red Sea. During the summer, thermal stratification leads to oligotrophic conditions and picocyanobacteria dominate the phytoplankton community (Lindell and Post, 1995; Mackey et al., 2007). During the mixed winter season, mesotrophic conditions prevail, favoring eukaryotic phytoplankton (Lindell and Post, 1995). A spring bloom generally occurs in March or April at the onset of stratification, in which eukaryotic phytoplankton (Lindell and Post, 1995) typically dominate and are later succeeded by a secondary bloom of *Synechococcus* (Lindell and Post, 1995; Mackey et al., 2009). Throughout the year the entire water column is highly oxygenated down to the sea floor.

2.2. In situ sampling

Monthly samples were collected from station A (29°28’N, 34°55’E) in the Northern Gulf of Aqaba as part of a monitoring program (http://www.iui-eilat.ac.il/NMP). Depth profiles were taken using a sampling CTD-Rosette (SeaBird) equipped with 12 L Niskin bottles. Depth profiles were also collected at station A before (March 18) and during (March 24 and 25) the spring bloom in 2008 as the water column transitioned from deep mixing to stratification (we refer to this as “in situ bloom monitoring” throughout the text).

2.3. 15N tracer experiments

To quantify N transformation rates, two 1-day 15N tracer experiments were conducted on back-to-back days. Surface water (1 m depth) was collected each day (during the start of the spring bloom) at ~02:00 h from an offshore station and transported to IUI within 1 h. Water was dispensed into acid-washed, sample-rinsed transparent polyethylene bottles (2 L per bottle, 15 bottles per treatment). Isotopically enriched N additions were made from 15N enriched salts (Icon Isotopes) at the following concentrations: 0.1 μmol L⁻¹ NO₃, 0.1 μmol L⁻¹ urea, 0.07 μmol L⁻¹ NO₂, or 0.005 μmol L⁻¹ NH₄NO₃ and urea were used during the 1st experiment and NO₂ and NH₄ were used in the 2nd experiment. The NO₂ transformation was repeated on the 2nd day, though only tₐ and t₂ time points were taken (see below for sampling schedule). Control (no addition) bottles were included in both experiments.

For each experiment, ten baseline samples were collected at ~04:00 h prior to adding the nitrogen spikes. Spikes were administered before dawn at approximately 05:00 h, and three bottles from each treatment were immediately sampled within 1 h of adding the spike. All remaining bottles (12 per treatment) were incubated in a flow-through tank that maintained ambient surface seawater temperature (~21°C). For each treatment, six bottles were incubated in the light under screening material (50% light attenuation), and six were incubated in the dark under a black cloth that yielded 100% light attenuation. Three light and three dark bottles were collected for each treatment at two time points. The first time point was at 12:00 h (7 h after the tracer was added) and the second time point was at 18:00 h (13 h after the tracer was added). Each time point took approximately 1 h to process. Sub-samples were collected for flow cytometry, total and dissolved nutrients, and particulate and dissolved 15N analyses as described below. Separate dedicated sets of equipment (e.g. funnels, filtration manifolds, forceps, etc.) were always used for processing isotopically enriched and control samples. All equipment was acid washed and thoroughly rinsed with seawater prior to use.

Addition of 15N tracer to low nutrient seawater can result in increased uptake rates relative to natural levels following Michaelis-Menten kinetics. We therefore limited our tracer additions to <10% of the ambient concentrations based on measurements of surface water that were taken 1–2 days prior to the experiments. However, measurements of the actual background concentrations for NO₃ (0.2 μmol L⁻¹), NO₂ (0.03 μmol L⁻¹), and NH₄ (0.025 μmol L⁻¹) were lower during the experiment than expected. Our measured rates may therefore overestimate the actual rates by 50%, 230%, and 20% for NO₃, NO₂, and NH₄, respectively based on Michaelis-Menten kinetics (Dugdale and Goering, 1967). Urea concentrations were assumed to be 10% of DON, typical of oligotrophic surface waters (Jackson and Williams, 1985; Eppley et al., 1977) and consistent with prior measurements for urea in the Gulf of Aqaba (A. Post, unpublished data). Our measured urea transformation rates could therefore underestimate the actual rates by a maximum of 90% if all DON was urea, however this is highly unlikely.

Despite the potentially large over or under estimates reported above we note that the rates calculated should still be within a typical range of values for the Gulf during this time of year because the 15N additions were based on real concentration levels measured within a few days of the experiment and the phytoplankton composition and abundance did not change significantly over that time (data not shown).

2.4. Particulate nitrogen 15N analysis

Samples for particulate N concentration and isotopic composition were collected for the in situ bloom monitoring and for the 15N tracer experiment. Samples were obtained by filtering 1 L aliquots of sample water through pre-combusted (500°C, 5 h) 25 mm glass fiber filters (GF/F, Whatman). Sample filters were analyzed at the Stable Isotope Facility at University of California, Davis using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS, Sercon Ltd., Cheshire, UK). Sample δ¹⁵N values were calculated by adjusting the measured values using an empirical calibration scale based on laboratory standards. Two laboratory standards (NIST 1547 and acetanilide) were analyzed every 12 samples. Laboratory standards were calibrated against NIST Standard Reference Materials (IAEA-N1, IAEA-N2, IAEA-N3, IAEA-CH7, and NBS-22). The standard deviation of repeated measurements for the method is 0.2‰.

2.5. δ¹⁵N of dissolved inorganic nitrogen

Water samples for dissolved NO₃ and NO₂ (N + N) isotopic composition were collected during the in situ bloom monitoring and during the 15N tracer experiment. Samples were filtered through pre-combusted (500°C, 5 h) glass fiber filters (GF/F, Whatman) by hand under low pressure using a syringe and Swinnex filter holder. Filtrate was immediately acidified to pH< 3 with trace metal grade hydrochloric acid and stored in the dark at room temperature until analysis. The δ¹⁵N and δ¹⁸O were determined using the method of McIlvin and Altabet (2005). Briefly, the samples were rendered alkaline by addition of excess MgO, and NO₃ was reduced to NO₂ by shaking overnight with activated cadmium (Cd). NO₂ was then reduced to nitrous oxide with sodium azide in an acetic acid buffer for 1 h, followed by neutralization with sodium hydroxide and analysis on a continuous flow isotope ratio mass spectrometer (IRMS). Data obtained by this method include contribution from NO₃ and NO₂, which we refer to in the text as N + N for simplicity. The isotopic composition of NO₂ alone was determined in the 15N tracer experiment samples by omitting the NO₃ reduction step.

All samples were calibrated and blank corrected using the international isotopic standards USGS 32, USGS 34, and USGS 35 for
NO$_3^-$ and three in house standards for NO$_2^-$. The reference scale for N and O isotopic composition were atmospheric N$_2$ and SMOW (standard mean ocean water), respectively. Standards were run before, after, and at 12–15 sample intervals during the run. Analytical precision measured from multiple determinations on standards was 0.2% for $\delta^{15}$N and 0.7% for $\delta^{18}$O. The detection limit for successful isotopic determination was $\sim$2 nmol N (corresponding to $\sim$130 nmol N L$^{-1}$) based on the volumes of sample we used. For samples falling below this concentration threshold in the $^{15}$N tracer experiment, it was possible to increase the N concentration by addition of a known quantity of standard NO$_3^-$ material because introduction of even a small fraction of $^{15}$N tracer into the NO$_2^-$ pool would measurably affect the isotopic composition of the mixture. This allowed us to calculate the isotopic composition of the sample from the measured composition of the mixture and the known composition of the standard based on conservation of mass. This process could not be used for natural abundance samples collected during the spring bloom because the isotope signals of the sample and the standards were too similar to determine an accurate value. Therefore, only the isotopic composition of the combined N + N was determined for those samples.

Since NO$_2^-$ was not removed, the Cd reduction method measured the combined isotope composition of NO$_3^-$ and NO$_2^-$ in our samples, and the isotopic values of samples containing a high proportion of NO$_2^-$ will therefore be affected by an analytical artifact. To get a conservative estimate of what the values of $\delta^{15}$NO$_3^-$ and $\delta^{18}$O$_{NO3}$ would be without the NO$_2^-$ signal, two corrections were applied. First, we assumed that all of the O atoms in NO$_2^-$ exchanged with the seawater for which the abiotic equilibrium isotope effect causes the O in NO$_2^-$ to become isotopically enriched by 14% relative to the surrounding water (Casciotti and McIlvin, 2007). This assumption is valid as samples were acidified immediately after collection and equilibration of oxygen atoms between water and nitrite is rapid at low pH (Casciotti and McIlvin, 2007). This would lead to $\delta^{18}$O values in NO$_2^-$ of $\sim$15.5–16.5‰ for the Gulf of Aqaba, where the $\delta^{18}$O of water is 1.5–2.5‰. We then used conservation of mass to determine what the $\delta^{18}$O would be if no NO$_2^-$ was present by subtracting out its signal using the NO$_3^-$ concentration data. Similarly for N, we calculated what the $\delta^{15}$N would be if no NO$_2^-$ was present by assuming all of the NO$_2^-$ in the sample was 12.8‰ lighter that NO$_3^-$ due to the inverse fractionation effect associated with nitrite oxidation (Casciotti et al., 2010). While nitrification is not necessarily the dominant process throughout the water column, it is likely to be an important process where NO$_3^-$ levels are high, so this assumption provides a conservative yet realistic correction. The $\delta^{15}$N of the combined N + N pool may therefore be lighter than expected for NO$_3^-$ alone. The influence of these processes is dependent on the portion NO$_3^-$ in the N + N pool. NO$_2^-$ comprised up to 21% of the N + N in some surface samples from the March 24 and 25 profiles; and the specific implications of this on our data are discussed along with the results. We note that a number of methods are now available to remove NO$_2^-$ material because of its inverse effect as associated with nitrite oxidation (Casciotti et al., 2010). While nitrification is not necessarily the dominant process throughout the water column, it is likely to be an important process where NO$_3^-$ levels are high, so this assumption provides a conservative yet realistic correction. The $\delta^{15}$N of the combined N + N pool may therefore be lighter than expected for NO$_3^-$ alone. The influence of these processes is dependent on the portion NO$_3^-$ in the N + N pool. NO$_2^-$ comprised up to 21% of the N + N in some surface samples from the March 24 and 25 profiles; and the specific implications of this on our data are discussed along with the results. We note that a number of methods are now available to remove NO$_2^-$ from samples prior to analysis (Granger and Sigman, 2009) such that the $\delta^{15}$N of NO$_3^-$ can be measured via the Cd reduction method without this analytical artifact.

2.6. N uptake and transformation rate calculations

N uptake rates were determined from particulate N samples collected at the beginning and end of the $^{15}$N tracer experiment. Uptake rates ($r$) were measured for NO$_3^-$, NO$_2^-$, NH$_4^+$ and urea using two equations based on a constant uptake model (Dugdale and Wilkerson, 1986):

$$r = \frac{c_0}{\tau} \times \frac{15N_{enr} - \langle F \rangle}{15N_{enr} - \langle F \rangle}$$

where $\langle F \rangle$ is the at.% $^{15}$N in the sample measured by a mass spectrometer as described above; $15N_{enr}$ is the at.% $^{15}$N in the initially labeled pool of NO$_3^-$, NO$_2^-$, NH$_4^+$ or Urea; $\langle F \rangle$ is the natural abundance of $^{15}$N (in at.%); and $\tau$ is the incubation time. The quantities $c_0$ and $c_0$ denote the particulate N concentration (µmol L$^{-1}$) at time $t$ and time zero respectively, and are used to calculate the absolute uptake rate, with units mass per volume per time (nmol N L$^{-1}$ day$^{-1}$). Eq. (1) can underestimate and Eq. (2) can overestimate the actual uptake rate if there is a significant change in the amount of particulate matter over the course of the experiment (Dugdale and Wilkerson, 1986). This effect is small for low uptake rates but can increase as uptake rates increase. We found that values from these equations agreed well for all but our two highest uptake rates. We therefore report an average of $\rho_t$ and $\rho_0$ as suggested by Dugdale and Wilkerson (1986).

Rates of NH$_4^+$ oxidation and combined urea mineralization and subsequent oxidation of the NH$_4^+$ generated were determined from the natural abundance of NO$_3^-$ measured at the 1 h time point in the $^{15}$N tracer experiment using the following equation:

$$r = \frac{c_0}{\tau} \times \frac{15N_{enr} - \langle F \rangle}{15N_{enr} - \langle F \rangle}$$

where $r$ is the net reaction rate, $\langle F \rangle$ is the at.% $^{15}$N in the sample measured by mass spectrometer as described above for the first time point; $15N_{enr}$ is the at.% $^{15}$N in the initially labeled pool of NH$_3^+$ or Urea; $\langle F \rangle$ is the natural abundance of $^{15}$N of NO$_3^-$ in the baseline sample water (in at.%); and $\tau$ is the incubation time. The quantities $c_0$ and $c_0$ denote the NO$_2^-$ concentration (µmol L$^{-1}$) at time $t$ and time zero (before additions were made), respectively.

Determination of rates based on enrichment experiments is based on the assumption that the labeled fraction represents a constant portion of the total substrate pool throughout the experiment. For example, if $^{15}$NO$_3^-$ tracer is added as 10% of the background NO$_3^-$ concentration at the start of the experiment, then the atomic percent of $^{15}$NO$_3^-$ should ideally remain 10% throughout the experiment for accurate measurements to be made. Transformation rates can then be calculated based on this relationship once the amount of label that gets transformed is measured (e.g. for every one $^{15}$N atom taken up, 9 $^{14}$N atoms also get taken up). These estimates are subject to error if rapid substrate regeneration occurs (Gilbert et al., 1982; Dugdale and Wilkerson, 1986). For example, if NO$_3^-$ is regenerated during an experiment, then the labeled fraction will continually get “diluted” over the course of the experiment. This effect becomes more pronounced in longer experiments. We were unable to quantify dissolved N transformations based on the 7 and 13 h time points in the $^{15}$N tracer experiment because the turnover rates were more rapid than we expected and dilution of the isotope label occurred, thus we use the 1 h point only.

2.7. Total and dissolved nutrients, chl a and Irradiance

Total N, NO$_3^-$ and NO$_2^-$ concentrations were collected during all in situ monitoring, as well as during the nutrient addition experiment and $^{15}$N tracer experiment. Concentrations of NO$_3^-$ and NO$_2^-$ were determined using colorimetric methods described by Hansen and Koroleff (1999) modified for a Flow Injection Autoanalyzer (FIA, Lachat Instruments Model QuickChem 8000) as described previously (Mackey et al., 2007). The precision of the methods was 0.05 µmol L$^{-1}$ for NO$_3^-$ and NO$_2^-$. The detection limit for these nutrients was 0.02 µmol L$^{-1}$. Ammonium samples from in situ field samples collected during the spring bloom progression were measured using the ortho-phthaldehyde method described by Holmes et al. (1999) with a precision of 0.02 µmol L$^{-1}$ and a detection limit
of 0.01 µmol L⁻¹. Total N was determined for March 24 and 25 and for the ¹⁵N tracer experiment on whole water samples without filtration. Samples were digested by persulfate oxidation, reduced in a copper–cadmium column, and analyzed colorimetrically following D’Elia et al. (1977). The detection limit was 1.4 µmol L⁻¹. Dissolved organic N (DON) was calculated by subtracting the particulate N and total inorganic N (NO₃⁻ + NO₂⁻ + NH₄⁺) from total N. Photosynthetically available radiation (PAR, 400–700 nm) was measured using a standard high-resolution profiling reflectance radiometer (Biopsherial PRR-800, data courtesy of D. Iluz). Chl a was measured fluorometrically using a Turner Fluorometer (Turner Designs 10-AU-005-CE) following 90% acetone extraction at 0 °C for 24 h as described previously (Mackey et al., 2009).

2.8. Flow cytometry

Flow cytometry was used to determine the abundance of phytoplankton and non-photosynthetic microbes in samples from in situ bloom monitoring, the nutrient addition experiment, and the ¹⁵N tracer experiment. Samples were preserved with 0.1% glutaraldehyde, flash frozen in liquid nitrogen, and stored at −80 °C until analysis. Cell abundances in samples from the in situ bloom monitoring and the ¹⁵N tracer experiment were measured using a LSRII flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). Before analysis SYTO 42 blue fluorescent nucleic acid stain (Invitrogen, Molecular Probes) was added at a final concentration of 8 µmol L⁻¹ and samples were incubated at room temperature for 5 min. The SYTO 42 stain has excitation and emission peaks at 433 nm and 460 nm respectively, and offers strong fluorescence enhancement upon binding nucleic acids such that the fluorescence signal from stained cells is maximized relative to background. Cell populations were identified using 90° light scatter, autofluorescence of photopigments, and SYTO 42 fluorescence.

Chlorophyll positive (phytoplankton) cells were identified as Synechococcus based on positive phycocyanin content. Prochlorococcus, picoeukaryotes (eukaryotic phytoplankton <2 µm in diameter) and nanophytoplankton (phytoplankton >2 µm in diameter) were identified based on their relative scatter and chlorophyll fluorescence levels. Non-photosynthetic cells were identified based on lack of chlorophyll fluorescence and positive SYTO 42 staining. Cell numbers were determined by spiking each sample with a known concentration of 1 µm fluorescent yellow green calibration beads (Polysciences).

3. Results

3.1. In situ monthly monitoring

Time series analyses of NO₃⁻ and NO₂⁻ depth profiles over representative 1-year periods showed a clear relationship with seasonal mixing and stratification (Fig. 2). In February 2008 the water column was mixed down to the seafloor before stratification occurred in March (Fig. 1A). In the winter (e.g., January–March), NO₃⁻ and NO₂⁻ levels were inversely related, with higher NO₃⁻ levels in the upper mixed layer than at depth. Primary NO₃⁻ maxima (PNM) began to take shape in March or April, which is the spring season when the water column first begins to stratify. In the summer (e.g., May–September), when the euphotic depth is approximately 100 m, PNM in the stratified water column were evident between 50 and 200 m. NO₃⁻ concentrations remained below detection throughout the euphotic zone, and increased gradually with depth below 100 m. This trend is typical of other years, although the actual NO₃⁻ and NO₂⁻ concentrations within and below the mixed layer vary with mixing depth. For example in 2003, when the mixing depth was only down to ~400 m, NO₃⁻ and NO₂⁻ concentrations differed from those in 2008, but still retained their inverse relationship in the winter and PNM formation the summer (Fig. 2B).

Monthly monitoring of chl a also showed seasonal changes (Fig. 2), with homogenous mixed layer profiles in the winter months and the formation of deep chlorophyll maxima (DCM) between 50 and 100 m in the stratified summer months. The PNM was located at or below the depth of the DCM in 2008 and 2003.

3.2. In situ spring bloom monitoring

To determine how changing physical, chemical, and biological water column characteristics influence N transformation rates, we compared nutrient, chlorophyll a, flow cytometry, and isotope data from three profiles taken during early stages of stratification in 2008. The first profile was taken when the water column

![Fig. 2. Depth profiles of NO₃⁻ (shaded area), NO₂⁻ (black line), and chl a (green line) for January–December in (A) 2008 when the water column mixed down to the seafloor, and (B) 2003 when the mixing depth was ~400 m. During winter mixing NO₃⁻ accumulates and chl a is homogenously distributed in the mixed layer, regardless of the mixing depth. During summer stratification a PNM forms at or below the DCM. The euphotic depth is ~60 m in winter and ~100 m in summer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](image-url)
retained many of its characteristics from previous deep mixing. The other profiles were taken on two consecutive days after stratification was established. Prior to the spring bloom in 2008, mixing depths extending to greater than 600 m as judged from nutrient (Fig. 3A) and density profiles (not shown).

### 3.2.1. Nutrients

Field sampling conducted on March 18 at the very onset of stratification (Fig. 3A) showed nearly homogenous NO$_3^-$ levels ($\sim 3$ mmol L$^{-1}$) throughout the water column, with a tendency towards lower concentrations in surface waters ($\sim 2$ mmol L$^{-1}$). In surface waters, NO$_2^-$ was higher ($0.23$ mmol L$^{-1}$) than throughout the rest of the euphotic zone ($\sim 0.18$ mmol L$^{-1}$), whereas NH$_4^+$ levels peaked at 100 m ($0.42$ mmol L$^{-1}$). Sampling conducted on 24 and 25 March 2008 (Fig. 3A) following stratification and during the spring bloom showed continued drawdown of NO$_3^-$ in surface waters, as well as the formation of a PNM peak between 200 and 250 m (reaching $0.59$ mmol L$^{-1}$ at 200 m on 25 March). Maximum NH$_4^+$ levels occurred above the NO$_3^-$ maxima at depths of 160–200 m, and reached $0.59$ mmol L$^{-1}$ at 200 m on 24 March. Particulate N levels increased in surface waters from 0.43 to 2.57 mmol N L$^{-1}$ between March 18–24, and decreased to $1.08$ mmol N L$^{-1}$ by March 25 (Fig. 3B). Total N was $12.1 \pm 0.7$ mmol N L$^{-1}$ ($n = 21$) for all depths in the water column (Fig. 3C).

### 3.2.2. Phytoplankton growth

Chl $a$ profiles from 18, 24, and 25 March 2008 (Fig. 3A) showed the progression of the phytoplankton bloom following stratification. On March 18, the chl $a$ profile was homogenous throughout the euphotic zone ($\sim 0.2$ mg m$^{-3}$), except in the upper 20 m where it increased to $\sim 0.5$ mg m$^{-3}$ (Fig. 3A). Chl $a$ maxima were apparent in both the 24 and 25 March profiles, reaching maximum concentrations of $0.8–0.9$ mg m$^{-3}$ between 40 and 60 m.

Flow cytometry measurements show that by March 24 and 25, phytoplankton populations were most abundant in the upper water column and were dominated by *Synechococcus* and nanophytoplankton (Fig. 4). Picoeukaryotes were present in smaller numbers (Fig. 4), and no substantial populations of *Prochlorococcus* were identified (data not shown). In the surface, *Synechococcus* reached $8.0 \times 10^4$ cm L$^{-1}$ and nanophytoplankton reached $2.0 \times 10^4$ cm L$^{-1}$. Both populations increased approximately two-fold between March 24 and 25 between depths of 60–120 m despite being below the 1% light level (60 m). The picoeukaryote population decreased from $3 \times 10^3$ to $0.8 \times 10^3$ cm L$^{-1}$ between March 24–25 in surface waters. Non-photosynthetic cells ranged from $5.00 \times 10^5$ to $2.00 \times 10^6$ cm L$^{-1}$ throughout the water column (Fig. 4).

### 3.2.3. Isotopes of dissolved $N + N$ and particulate $N$

Prior to stratification on March 18th the $\delta^{15}N_{NN}$ and $\delta^{18}O_{NN}$ were homogenous through the water column, averaging $2.6 \pm 0.08\%e$ and $6.7 \pm 0.17\%e$, respectively (Fig. 5A and B). These values are distinctly different from those expected for average open ocean deep water nitrate $\delta^{15}N$ (5%e; Sigman et al., 2000) and $\delta^{18}O$ (2%e; Knapp et al., 2008). As stratification progressed and the bloom developed, $\delta^{15}N_{NN}$ and $\delta^{18}O_{NN}$ values both increased in surface waters. $\delta^{15}N_{NN}$ reached peak values of $\sim 10%e$ at 60 and 20 m on March 24 and 25 respectively (Fig. 5A), whereas maximum $\delta^{18}O_{NN}$ values of $53%e$ and $40%e$ were seen at the
Fig. 4. Cell abundances of Synechococcus, nanophytoplankton, picoeukaryotes, and non-photosynthetic microbes on March 24 (closed circles) and 25 (open circles). Note that different scales are used for each group.

Fig. 5. Isotopic composition of N + N and PON on March 18, 24, and 25, showing (A) $\delta^{15}N-N+N$, (B) $\delta^{18}O-N+N$, and (C) $\delta^{15}N-PON$. Measured values for N + N are shown with open circles. Data with the correction applied to remove the NO$_3^-$ signal as described in the text are shown by the grey line for $\delta^{15}N-N+N$ and $\delta^{18}O-N+N$. 

surface (Fig. 5B). The $\delta^{15}$N also showed a subsurface peak of $\sim 11\%$ at 160 m. These values of $\delta^{15}$N and $\delta^{18}$O include an influence from NO$_2^-$, and may therefore differ from values that would be expected from NO$_3^-$ alone. As outlined above, an isotope mass balance calculation was used to correct for this artifact, the corrected data are plotted in Fig. 5A and B along with the actual measured data. The difference measured and corrected values is greatest for depths in the vicinity of the PNM, and is greater for $\delta^{15}$N than for $\delta^{18}$O. Despite this limitation, trends in vertical and temporal distributions are larger than can be explained by this artifact alone, hence showing true variability.

The dual isotope plot of $\delta^{18}$O and $\delta^{15}$N (Fig. 6) shows the tight clustering of values on March 18 as a result of the values being homogenous throughout the water column. If nitrate assimilation was the only process impacting the nitrate pool as stratification progressed, we would expect to see the values plot along a 1:1 line as isotopic fractionation during nitrate assimilation is known to produce a 1:1 increase in the $\delta^{15}$N and $\delta^{18}$O of nitrate (Granger et al., 2004). Instead by March 25 the ratios were close to 5:1 (Fig. 6C), suggesting a decoupling of the N and O isotopes.

Fig. 6. Relationships between $\delta^{18}$O and $\delta^{15}$N for (A) March 18, (B) March 24, and (C) March 25. Data points are color coded as follows: euphotic zone (red), sub-euphotic zone (orange), upper PNM (green), and disphotic zone (black). The lines show the 1:1, 3:1 and 5:1 slopes anchored to $\delta^{15}$N of 2.03$\%$ and $\delta^{18}$O of 5.35$\%$ representing deep water in this region (600 m, March 18th). Data with the correction applied to remove the NO$_2^-$ signal (as described in the text) plot in a similar distribution, but are not shown in the graph for clarity.

above. However, although the value of the slopes were not as high overall for any given day in the corrected data set Fig. 6 (gray circles), the increase in the slopes between days is still apparent.

The $\delta^{15}$N values of particulate matter on March 18 averaged $-4.7\%$ (Fig. 5C). Values increased as stratification was established. Within the upper 100 m, values ranged from 0.8$\%$ to 6.4$\%$, and increased with depth, reaching nearly 20$\%$ at 600 m.

3.3. $^{15}$N tracer experiment

At the start of the $^{15}$N tracer experiment the phytoplankton population was dominated by Synechococcus (1.24e$^3$ c mL$^{-1}$), followed by nanophytoplankton (4.66e$^3$ c mL$^{-1}$) and picoeukaryotes (4.2e$^3$ c mL$^{-1}$). Non-photosynthetic cells were approximately an order of magnitude more abundant than phytoplankton ($\sim 1.4e^6$ c mL$^{-1}$). There were no appreciable changes in the community composition of the water used on the 1st and 2nd day of the experiment (not shown).

In order to estimate fluxes of N between different N pools, we used isotope data from the $^{15}$N tracer experiment along with nutrient inventory mass balance. We sought to quantify rates for the following N transformations: (1) biological assimilation for NO$_3^-$, NO$_2^-$, NH$_4^+$, and urea; (2) oxidation of NH$_4^+$ and urea (via NH$_4^+$ intermediate) to NO$_3^-$ during nitrification; and (3) incomplete NO$_3^-$ reduction to NO$_2^-$ by phytoplankton. The rate of N transfer between the two pools can be estimated from tracer experiments if dilution of the $^{15}$N label by substrate regeneration is minimal during the experiment, as described above. Dilution of the isotope spike during substrate regeneration generates artificially low rate estimates because the ratio of tracer to unlabeled N becomes smaller than assumed based on initial concentrations of the substrate (i.e., the regenerated substrate “dilutes” the tracer as the experiment progresses). Rates will also be underestimated if the N product formed from the tracer is rapidly consumed by another process. These sources of error can be minimized by selecting appropriate time scales over which to calculate different rates (Gilbert et al., 1982), and these concerns are discussed for each rate estimate below.

3.4. Biological N assimilation

N uptake and assimilation rates were estimated in the $^{15}$N tracer experiment based on direct measurements of enrichment in the particulate matter for both light and dark treatments. Error from dilution of the $^{15}$N label due to substrate regeneration increases with longer incubation times, as does the likelihood that phytoplankton will excrete and re-assimilate the tracer (Gilbert et al., 1982; Bronk et al., 1994). However, assimilation rates immediately following tracer addition are generally higher than actual in situ rates, a problem that can be ameliorated by using a slightly longer incubation time. We used the 1, 7 and 13 h time points to calculate uptake rates; however, our calculated values could underestimate the assimilation rates by a factor of 2 due to dilution of the $^{15}$N label from regeneration of substrate (Gilbert et al., 1982), and by 50–74% due to excretion of the $^{15}$N label as DON following uptake (Bronk et al., 1994). The background urea concentration during the experiment was 1.0 ± 0.1 $\mu$mol L$^{-1}$. Urea uptake (1194 nmol N L$^{-1}$ day$^{-1}$) was approximately three-fold faster than NO$_3^-$ uptake ($\sim 434$ nmol N L$^{-1}$ day$^{-1}$) in the light (Table 1, Fig. 7A and B). Both urea and NO$_3^-$ uptake rates were higher in light bottles than in dark bottles (476 nmol N L$^{-1}$ day$^{-1}$ for urea and 58 nmol N L$^{-1}$ day$^{-1}$ for NO$_3^-$, Table 1, Fig. 7A and B). For the NH$_4^+$ treatment, all of the $^{15}$NH$_4^+$ spike was assimilated prior to the 7 h sampling in both light and dark bottles, so we only report uptake values based on the 1 h time point (314 nmol N L$^{-1}$ day$^{-1}$, Table 1). For NO$_2^-$, all of the $^{15}$NO$_2^-$ was assimilated before the 13 h
---

### Table 1

<table>
<thead>
<tr>
<th>N addition</th>
<th>Process</th>
<th>Experiment number</th>
<th>Time (h)</th>
<th>Light rate (nmol N L(^{-1}) day(^{-1})) (a)</th>
<th>Dark rate (nmol N L(^{-1}) day(^{-1})) (a)</th>
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<tbody>
<tr>
<td>NO(_3^{-})</td>
<td>Assimilation</td>
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<td>ND</td>
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<td>ND</td>
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<td>NO(_3^{-})</td>
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<tr>
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<td>1285 ± 32</td>
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<tr>
<td>Urea</td>
<td>Remineralization and oxidation to NO(_2^{-})</td>
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<td>1</td>
<td>ND</td>
<td>14.1 ± 7.6</td>
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<td>NH(_4^{+})</td>
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<td>ND</td>
<td>314 ± 31</td>
</tr>
<tr>
<td>NH(_4^{+})</td>
<td>Assimilation</td>
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<td>7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>NH(_4^{+})</td>
<td>Assimilation</td>
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<td>13</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>NH(_4^{+})</td>
<td>Oxidation to NO(_2^{-})</td>
<td>2</td>
<td>1</td>
<td>ND</td>
<td>16.4 ± 8.1</td>
</tr>
</tbody>
</table>

ND indicates that the rate was not determined. The second time interval of 13 h was not used for some samples because all of the \(^{15}\)N spike had been exhausted (taken up) within the first 7 h of incubation (see text). The rates in the light were not determined for 1 h time points because they were measured before dawn.

\(a\) Values reported are the mean ± standard error of triplicate measurements from independent bottles (i.e., three independent bottles per treatment per time point).

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3.5. Oxidation of NH\(_4^{+}\) and urea to NO\(_2^{-}\)

Oxidation rates of NH\(_4^{+}\) and urea (following mineralization to NH\(_4^{+}\)) were determined based on measurements of NO\(_2^{-}\) isotopic composition after \(^{15}\)N enriched spikes of NH\(_4^{+}\) or urea were added in the \(^{15}\)N tracer experiment. We calculated rates for the 1 h time point, but were unable to quantify rates from the 7 and 13 h time points because the \(^{15}\)N enrichments were too small or the turnover of the N pools was too rapid for accurate estimates to be made over these longer time scale (substrate regeneration affected the results). Oxidation of NH\(_4^{+}\) to NO\(_2^{-}\) occurred at a rate of 16.4 ± 8.1 nmol N L\(^{-1}\) day\(^{-1}\) (or 0.68 ± 0.34 nmol N L\(^{-1}\) h\(^{-1}\)). Mineralization of urea to NH\(_4^{+}\) with subsequent oxidation to NO\(_2^{-}\) occurred at a rate of 14.1 ± 7.6 nmol N L\(^{-1}\) day\(^{-1}\) (0.59 ± 0.32 nmol N L\(^{-1}\) h\(^{-1}\)).

3.6. Reduction of NO\(_3^{-}\) to NO\(_2^{-}\)

We were unable to measure reduction of NO\(_3^{-}\) to NO\(_2^{-}\} based on data from the \(^{15}\)N tracer experiment. For the 7 and 13 h time points, substrate regeneration caused dilution of the \(^{15}\)N label during the experiment and precluded accurate calculations from being made. In addition, since reduction of NO\(_3^{-}\) to NO\(_2^{-}\}) is driven by light, we were unable to measure this process in samples from the 1 h time point, because the samples were collected before dawn and received no light to initiate this process. Therefore, the rate of NO\(_3^{-}\) reduction based on the 1 h time point in the \(^{15}\)N tracer experiment was negligible, as expected.

However, incomplete reduction of NO\(_3^{-}\) and release of NO\(_2^{-}\) by light limited phytoplankton is a well documented phenomenon in both field and culture studies (Collos, 1998; Lomas and...
Lipschultz, 2006 and references therein). The rate of NO$_3$ reduction to NO$_2$ by phytoplankton is dependent on light and on phytoplankton abundance. Therefore, if phytoplankton were a significant source of NO$_2$ in a certain portion of the water column following stratification, then we would expect the change in NO$_2$ concentration to be correlated with both light and chlorophyll abundance over those depths. We therefore calculated a range of net NO$_2$ formation rates based on changes in the in situ NO$_2$ concentrations measured during the spring bloom between March 18–24, and tested if they were correlated with irradiance or chl $a$ concentrations. These NO$_2$ formation rates are “net accumulation” rates, and represent the combined input from all NO$_2$ sources (e.g. phytoplankton or NH$_4$ oxidation) as well as all NO$_3$ sinks (e.g. assimilation or NO$_2$ oxidation). While all of these processes can potentially influence the calculated rate at each depth, light and chlorophyll abundance will correlate most strongly with NO$_2$ formation over depths where incomplete reduction of NO$_3$ and expulsion of NO$_2$ by light limited phytoplankton is the dominant process.

We found that net NO$_3$ formation was strongly correlated with light between 60 and 200 m ($R^2 = 0.99$, Fig. 8B, Table 2) and ranged from 2.2 to 58 nmol L$^{-1}$ day$^{-1}$ (0.092–2.4 nmol L$^{-1}$ h$^{-1}$). Chl $a$ concentration was also correlated with NO$_2$ formation rates; however, this relationship was primarily because chl $a$ abundance is also controlled by light (Fig. 8C). To parse the independent effect of chl $a$ concentration on NO$_2$ formation rate, we compared the residual chl $a$ and NO$_2$ formation rate data after subtracting out the influence of light on each parameter according to the following procedure.

The influence of light on each parameter (chl $a$ concentration or NO$_2$ formation rate) was calculated based on the equations best fit as shown in Fig. 8B and C. The calculated value was subtracted from the actual measured value to obtain the residual value. The residual values are the portions of the actual chl $a$ and net NO$_2$

![Fig. 8](https://example.com/fig8.png)

**Table 2**

Net NO$_3$ production rates. NO$_3$ production was dominated by NO$_3$ reduction by phytoplankton at depths of 60, 80, 160, and 200 m, and by NH$_4$ oxidation by nitrifying microbes at 120 m (NH$_4$ oxidation, see Fig. 8D and text for explanation). Rates were calculated from the change in concentration of NO$_3$ between March 18 and March 24 at the onset of stratification, and are given on a per volume basis as well as on a per unit chl $a$ basis. No values were calculated for 180 m because this depth was not sampled on March 18, so no change in NO$_3$ concentration could be calculated.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Light attenuation (% of surface PAR)</th>
<th>Δ[NO$_3$] ($\text{nmol L}^{-1}$)</th>
<th>chl $a$ ($\mu$g L$^{-1}$)</th>
<th>NO$_2$ production rate ($\text{nmol L}^{-1}$ day$^{-1}$)</th>
<th>NO$_2$ production rate ($\text{nmol} \mu$g chl $a^{-1}$ day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
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<td>13</td>
<td>0.44</td>
<td>2.2</td>
<td>5.0</td>
</tr>
<tr>
<td>80</td>
<td>0.2</td>
<td>58</td>
<td>0.39</td>
<td>3.9</td>
<td>25</td>
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<tr>
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<td>0.00002</td>
<td>345</td>
<td>0.19</td>
<td>58</td>
<td>290</td>
</tr>
</tbody>
</table>

NA indicates “not applicable” because the source of NO$_2$ was NH$_4$ oxidizers rather than phytoplankton at this depth, so the rate was not normalized to chl $a$. 
formation rate measurements that are not accounted for by light. The residual values of chl a and net NO$_2$ formation rate were then plotted (Fig. 8D) to determine the relationship between chl a and net NO$_2$ formation rate. With the exception of one outlier point (showing a lower NO$_2$ formation rate than expected), a strong linear relationship existed between residual chl a levels and residual NO$_2$ formation rates (Fig. 8D). Interestingly, the outlier point coincided with an NH$_4$ peak at 120 m that got consumed between March 18 and 24 (Fig. 3A). The NO$_2$ formation rate at this depth did not correspond to chl a because a larger portion of the NO$_2$ at that depth was likely formed by NH$_4$ oxidation. If all of the NH$_4$ drawn down at this depth between March 18–24 was oxidized to NO$_2$, it would have contributed ~115 mmol NO$_2$ L$^{-1}$, or approximately 80% of the NO$_2$ inventory at that depth, enough that the NO$_2$ formation rate would no longer be correlated with chl a (e.g. because it is generated by non-photosynthetic microbes instead of phytoplankton). While the net NO$_2$ formation rate we calculated for 120 m contains some non-quantified input from NH$_4$ oxidation, the robust correlations between NO$_2$ formation and light and chl a at the other depths between 60 and 200 m strongly suggest that NO$_2$ reduction was the dominant NO$_2$ forming process at these depths. However, the net NO$_2$ formation rates we report are not necessarily equivalent to NO$_2$ reduction rates by phytoplankton; they likely underestimate real NO$_2$ reduction rates because they do not account for processes that remove NO$_2$, such as NO$_2$ oxidation during nitrification.

4. Discussion

The Gulf of Aqaba has predictable seasonal patterns of NO$_2$ distribution, and the spring bloom is a period in which water column N dynamics transition between two different steady states. The changing physical, chemical, and biological characteristics of the water column during the onset of stratification in 2008 gave rise to substantial changes in the N cycle such that new steady state nutrient inventories were established. As the water chemistry shifted toward this new steady state different processes became dominant, giving rise to a PNM over a period of several days. Below we discuss these changes in the N cycle and how they lead to formation of the PNM which is maintained throughout the summer stratified period.

4.1. NO$_2$ dynamics during the transition from mixing to stratification

The persistence of NO$_2$ in the ocean results from an imbalance in the processes that produce and consume NO$_2$ (Fig. 1). In the aerobic water column, NO$_2$ is produced by NH$_4$ oxidizing organisms during the first step of nitrification, and by phytoplankton during incomplete NO$_2$ assimilation. It is consumed by NO$_2$ oxidizers during the second step of nitrification, and by phytoplankton during assimilation. Nitrite accumulates when production exceeds consumption as long as dispersion rates are sufficiently low. In the Gulf of Aqaba in winter, NO$_2$ is present at measurable concentrations throughout the mixed layer, whereas in the summer NO$_2$ accumulates below the euphotic zone, forming a PNM (Fig. 2; Al-Qutob et al., 2002; Meeder et al., in press).

To determine the role of phytoplankton in NO$_2$ formation, we considered the following three observations. First, in winter NO$_2$ was observed throughout the mixed layer, which is the depth of the water column occupied by phytoplankton, regardless of the exact mixing depth (Fig. 1). NO$_2$ did not accumulate below the mixing depth where phytoplankton do not survive. The mixed layer is the portion of the water column homogenized by turbulent mixing; for example, the mixed layer extended to the sea floor (~700 m) in February 2008 (Fig. 2A), and to ~250 m in February 2003 (Fig. 2B). Phytoplankton can inhabit the whole mixed layer because water periodically gets mixed to the sunlit surface waters and allows for photosynthesis to occur (Smayda and Mitchell-Innes, 1974); they cannot grow in the permanent darkness of the deep water below the mixing depth. NH$_4$ oxidizers, on the other hand, can occupy and grow throughout the entire water column including deep waters below the mixing depth because they do not require sunlight to survive. Therefore, if the major source of the NO$_2$ in winter were NH$_4$ oxidizers, then the accumulation of NO$_2$ would not be confined exclusively to the mixed layer, as we observe (Fig. 2). Second, the inverse relationship between NO$_2$ and NO$_3$ in winter profiles is maintained regardless of shoaling or deepening of the mixed layer during winter (Fig. 2). This correlation suggests that NO$_3$ is the source of NO$_2$ generated within the mixed layer because as NO$_3$ is consumed NO$_2$ is produced. Third, the NO$_2$ and NO$_3$ inventories in the winter mixed layer agree well with the ratios of NO$_2$ to NO$_3$ observed during excretion by light limited phytoplankton following NO$_3$ uptake. Specifically, the fraction of NO$_3$ generated relative to NO$_2$ consumed in the mixed layer ranged from ~10% in 2003 (where ~0.4 μmol NO$_2$ L$^{-1}$ was generated and 4-6 μmol NO$_3$ L$^{-1}$ was consumed; Fig. 2A) to ~15% in 2008 (where ~0.3 μmol NO$_2$ L$^{-1}$ was generated and 2 μmol NO$_3$ L$^{-1}$ was consumed; Fig. 3A). These ratios are consistent with the range of ratios measured in cultures of light limited phytoplankton that expel a portion of the NO$_3$ they take up as NO$_2$ (Collos, 1998 and references therein). The non-nutritional uptake of NO$_2$ and release of NO$_3$ may be a mechanism by which certain phytoplankton regulate photosynthetic electron flow during periods when irradiance fluctuates (Lomas and Gilbert, 1999, 2000), e.g. during deep mixing. Based on the above observations, phytoplankton appear to be the major source of NO$_3$ during convective winter mixing. These findings agree with an incubation study by Al-Qutob and co-workers (2002), in which NO$_3$ was produced by phytoplankton following N additions, and with monitoring studies conducted in this region (Meeder et al., in press).

In a mixed water column, biological N transformation rates reflect the “average” light conditions because their products get distributed over the entire mixed layer. During winter in the Gulf of Aqaba, the mixing time (e.g. the time required for a parcel of water to complete one cycle of mixing from surface to the mixing depth and back to surface) is approximately 14 h, 22 h, and 29 h for mixing depths of 200 m, 400 m, and 600 m respectively based on typical heat flux and wind stress values for the region (S. Monsimuth, personal communication). The homogeneity of NO$_3$ in the mixed layer suggests that the mixing time is fast relative to the rates of NO$_2$ production and consumption such that no localized accumulation or drawdown of NO$_2$ is observed in the mixed layer.

In contrast, in a stratified water column organisms at any given depth are subject to relatively predictable light regimes. This allows different groups of organisms to populate depths they are best adapted to occupy. The PNM forms when stratification imposes a range of physical and chemical gradients on organisms, allowing different steady states to be reached between NO$_2$ production and consumption at different depths in the water column. This is evident from summer profiles of NO$_2$ from 2003 and 2008, where NO$_2$ accumulates at ~100 m, but not in surface or deep waters. These monthly “snapshots” provide information on steady state nutrient levels; they integrate and reflect the net result of all processes that produce and consume NO$_2$ at a given depth.

The individual contributions of specific N transformation processes on PNM formation can be discerned from the higher frequency monitoring data collected during the spring bloom. To focus our discussion, we define four principal regions of the water column based on light attenuation and major features of the PNM (Fig. 9). The “euphotic zone” (0–60 m during our study), extends from the surface to the compensation depth (i.e., the depth at
which light is attenuated to 1% of surface irradiance). The “sub-euphotic zone” (60–160 m during our study), extends to the top of the PNM. The “upper PNM” (180–225 m during our study), encompasses depths with substantial accumulation of NO₃. The “disphotic zone” extends from the depth where the NO₂ concentrations of the PNM starts decreasing down to the sea floor (below 225 m during our study). We note that the absolute depths given above for our study are not universal for all summers in the Gulf of Aqaba or for all water columns because they would change depending on the depth of the mixed layer prior to stratification, latitude, amount of chl a present, and other factors influencing light penetration. Below we describe how N cycling processes that produce and consume NO₂ generate conditions that give rise to the PNM.

4.1.1. Euphotic zone

The euphotic zone is the layer in which sufficient light is available for photosynthesis to exceed respiration, and where the majority of photosynthetic biomass is generated. Uptake of NO₃ and NH₄ is at times light dependent in natural phytoplankton populations, with the highest rates generally occurring in the surface ocean and decreasing with depth as light becomes attenuated (MacIsaac and Dugdale, 1972). This trend was observed in the euphotic zone of the Gulf where stratification became established. DIN uptake by phytoplankton was highest in surface waters and lower at the base of the euphotic zone (Fig. 3A).

Most of the available NO₃ and NO₂ in the euphotic zone of the Gulf of Aqaba was assimilated and converted into biomass (e.g. photosynthetic uptake) (Fig. 3) between March 18 and 25. However, results suggest that mineralization and subsequent nitrification of organic N played an important role in the euphotic zone, where DIN concentrations were low due to efficient phytoplankton uptake. Between March 18 and March 25 δ¹⁵N of N₂ fixation, which reflects the δ¹⁵N of atmospheric N₂ gas that is by definition zero. Measurements of N₂ fixation rates in the Gulf have ranged from below detection (Hadas and Erez, 2004) to low but measurable rates of 1–2 nmol L⁻¹ day⁻¹ (Foster et al., 2009). These rates are small compared to other N transformation rates measured for the Gulf (Table 1). However, we did not measure N₂ fixation or atmospheric deposition directly in this study, so a contribution from either cannot be confirmed or ruled out. Preferential export of ¹⁵N in particulate matter out of the euphotic zone (Altabet, 1988) can skew the δ¹⁵O of N₂ fixation relationship in surface waters, and is apparent from the increased δ¹⁵N of particulate N with depth as the bloom progressed (Fig. 5C), although fractionation during mineralization could also contribute to this signal.

4.1.2. Sub-euphotic zone

In this zone light is attenuated below the compensation threshold, and respiration by the entire microbial community is likely to exceed photosynthesis by phytoplankton. Regression analysis for depths in the sub-euphotic zone and down to 200 m showed that net NO₂ production rates correlated very strongly with decreasing irradiance (Fig. 8B). However, regression analysis of residual chl a and residual NO₂ production data (i.e. with the influence of irradiance removed) also showed a remarkably strong correlation (Fig. 8D), and suggested that NO₂ uptake and release as NO₂ by light limited phytoplankton was the dominant N transformation process in the sub-euphotic zone during the beginning of the bloom (March 18–24). These results agree with the findings of Dore and Karl (1996a) in the Pacific Ocean, where they suggest that the upper portion of the PNM is generated by phytoplankton NO₂ release and closely tracks the nitricline.

An exception occurred at 120 m, where a large portion of NO₂ was generated from NH₄ oxidation rather than NO₂ reduction based on regression statistics (Figs. 3A and 8D). The contribution of NH₄ oxidation to the NO₂ formation over this range of depths suggests that substrate limitation of NH₄ oxidation rates may be impacting NO₂ distribution in the water column (Ward, 1985). Our data shows that NO₂ formation from NH₄ oxidation can match or exceed NO₂ reduction where ample NH₄ is available. Indeed, the increasing slope of the best fit line for δ¹⁵O of N₂ fixation over this range of depths (Fig. 6, orange circles) indicates that nitrification was occurring within the sub-euphotic zone.
While the sub-euphotic zone is below the compensation depth, it is important to note that phytoplankton continue to take up nutrients and perform photosynthesis in this dim layer (these rates are simply exceeded by respiration rates). The $\delta^{15}$N$_{N_2}$ was elevated in the sub-euphotic zone with respect to deeper water as the bloom progressed (Fig. 5A), indicating that assimilation of N + N by phytoplankton or other microbes takes place. While seemingly counterintuitive that phytoplankton could be both a source and a sink for NO$_3^-$ in the sub-euphotic zone over the course of a bloom, several processes could lead to this outcome. First, intermittent changes in light intensity due to internal waves could lead phytoplankton at the base of the sub-euphotic zone to toggle between NO$_3^-$ assimilation and NO$_2^-$ excretion depending on their light requirements. Another factor is that the phytoplankton community is a diverse assemblage of different sub-populations, each with its own light requirements and N assimilation strategies. During the bloom succession occurs within the phytoplankton community, and different sub-populations coexist, compete, and eventually either survive or get out-competed. Therefore, while one sub-population may take up NO$_3^-$ and release NO$_2^-$ due to light limitation, another may be able to complete the assimilation of NO$_3^-$ into biomass. Between March 24–25 nanophytoplankton abundance increased in the sub-euphotic zone (Fig. 4). Nanophytoplankton include phytoplankton taxa such as diatoms, and monitoring conducted after our sampling period showed that the spring bloom became dominated by diatoms by the beginning of April (Iziz et al., 2009). Non-nutritional uptake of NO$_3^-$ has been observed in some marine diatoms (Lomas and Gilbert, 1999), and uptake (though not necessarily assimilation) by these comparatively large cells may have played a role in the drawdown of NO$_3^-$ and NO$_2^-$ in the sub-euphotic zone. Light-independent assimilation of NO$_3^-$ and NO$_2^-$ by non-photosynthetic microbes, which were abundant throughout the water column, could also have caused the high $\delta^{15}$N$_{N_2}$ values at these dim depths (Tupas et al., 1994).

4.1.3. The upper PNM

The upper PNM (180–225 m) is a dynamic region where NO$_3^-$ accumulates. Within this layer light is attenuated to levels too low for photosynthesis (Fig. 8A). During the first part of the bloom NO$_3^-$ dynamics in the upper PNM were similar to the sub-euphotic zone in that NO$_3^-$ production was strongly correlated with chl a levels, implicating phytoplankton as the main source of NO$_3^-$ (Fig. 8). However, over the next day, net NO$_3^-$ production continued within the upper region of the PNM and was no longer correlated to chl a (data not shown). The NO$_3^-$ produced by phytoplankton during the first part of the bloom was derived from NO$_3^-$ taken up during the mixed period (e.g. March 18) when, due to mixing, light was episodically high enough to support NO$_3^-$ uptake. Following stratification phytoplankton trapped below the euphotic depth would have expelled as NO$_3^-$ due to a lack of light energy needed to complete the assimilation process. However, by March 24 phytoplankton trapped within the upper PNM would have been without sufficient light for approximately 1 week. It is unlikely that these cells could initiate de novo uptake of fresh NO$_3^-$ and be the source of NO$_3^-$ generated at this depth between March 24 and 25. NH$_4^+$ oxidizers, on the other hand, would have access to an increasingly large pool of DON from which to access their NH$_4^+$ substrate following ammonification.

NO$_3^-$ can only accumulate if production exceeds consumption and dispersion is sufficiently low. The NO$_3^-$ accumulation in the upper PNM (180–225 m) during the second part of the bloom indicates that NO$_3^-$ production and consumption were decoupled, with production exceeding consumption. Nitrification was the major source of NO$_3^-$ in the upper PNM once phytoplankton NO$_2^-$ excretion had declined following the initial stages of stratification. The main NO$_3^-$ consuming process at these depths was NO$_2^-$ oxidation because photosynthetic NO$_3^-$ assimilation is light limited at these dark depths. The slope of $\delta^{15}$N$_{N_2}$/$\delta^{15}$N$_{N_3}$ values for N + N shows that nitrification was occurring over this range of depths by March 25 when stratification was firmly established (Fig. 6, green circles). Olson (1981) postulated that the greater sensitivity of NO$_2^-$ oxidizers than NH$_4^+$ oxidizers to light could be a mechanism by which PNM form. Guerrero and Jones (1996) added to this model, noting that NH$_4^+$ oxidizers recover more rapidly from photoinhibition than do NO$_2^-$ oxidizers. Based on these observations, NH$_4^+$ oxidizers are postulated to be more active in shallower regions of the water column than NO$_2^-$ oxidizers, and this spatial segregation of the populations leads to accumulation of NO$_2^-$.

The pattern of PNM formation in the Gulf of Aqaba is consistent with these hypotheses of differential photoinhibition and recovery based on the concentrations of NH$_4^+$, NO$_3^-$, and NO$_2^-$ throughout the water column. The NO$_3^-$ concentration data suggests that NO$_3^-$ oxidation was closely coupled to NH$_4^+$ oxidation only at depths below ~225 m, where production of NO$_3^-$ was observed concurrently with NH$_4^+$ and NO$_3^-$ consumption (Fig. 3A). At these dark depths no NO$_3^-$ accumulated, consistent with a lack of photoinhibition of either NH$_4^+$ or NO$_2^-$ oxidizers. In contrast, above the upper PNM (140–180 m) NH$_4^+$ accumulated and resulted in an NH$_4^+$ peak by March 25. The light levels at these depths may have been sufficiently high to inhibit NH$_4^+$ oxidation rates in keeping with the hypotheses discussed above, thereby allowing NH$_4^+$ to accumulate. However, within the upper PNM depths of 180–225 m, NO$_3^-$ and NO$_2^-$ accumulated concurrently, suggesting that NH$_4^+$ oxidation was continuing while NO$_2^-$ oxidation was slowing, an observation that could be explained by differential photosensitivity of the two nitrifier populations. However, recovery from photoinhibition must have been reversible over the diel cycle, as the isotope data strongly indicate a complete nitrification cycle within the euphotic zone and upper PNM in the Gulf of Aqaba.

4.1.4. The disphotic zone

The disphotic zone contains the lower portion of the PNM (225–300 m; Fig. 3A) as well as deep water. Within the disphotic zone, sunlight is attenuated to less than 0.001% of surface irradiance and phytoplankton are unable to perform photosynthesis. Therefore, non-photosynthetic microbial processes dominate the N cycle at these depths, and indeed, several observations are indicative of a nitrification-dominated system. As noted above, NH$_4^+$ and NO$_3^-$ were consumed while net NO$_3^-$ production occurred below 225 m, consistent with microbiologically mediated oxidation of NH$_4^+$ and NO$_3^-$ into NO$_2^-$ Microbial nitrification in the disphotic zone also refined the shape of the lower PNM during the onset of stratification by consuming a portion of the broad band of NO$_3^-$ that was generated during the beginning of stratification, and helped maintain the characteristic shape of the PNM throughout the summer. This can be seen on March 25, where the falling limb of the PNM took on a steeper slope than on March 24 (Fig. 3A) and was more similar to summer profiles from other years (Fig. 2).

The elevated $\delta^{15}$N of particulate N that was spread throughout the water column by March 25 (Fig. 5C) also suggests that a link exists between phytoplankton growth in the surface and mineralization/nitrification at depth. Active processes, such as selective zooplankton grazing and excretion, play an important role in packaging smaller suspended particles, such as phytoplankton cells, within the euphotic zone for export as sinking particles. As a result, sinking particles are generally higher in $\delta^{15}$N than suspended particles within the euphotic zone (Altabet, 1988). The transport of sinking particles occurred quickly, as the elevated $\delta^{15}$N$_{part}$ was already spread throughout the water column within days of the bloom initiating (Fig. 5C). The sinking of particulate matter from the surface to deep water is likely to be an important source of N.
that fuels nitrification in the disphotic zone throughout the stratified period, and recharges the NO$_3^-$ reservoir at depth.

Values for $\delta^{15}$N$_{PNM}$ and $\delta^{18}$O$_{PNM}$ in the disphotic zone varied little over the time period studied ($\delta^{15}$N$_{PNM}$ was $\sim 2.5$ and $\delta^{18}$O$_{PNM}$ was $\sim 6.5$), but were distinct from those observed for open ocean deep water nitrate; ($\delta^{15}$N 5% (Sigman et al., 2000) and $\delta^{18}$O 2% (Knapp et al., 2008). Low $\delta^{15}$N with respect to deep water NO$_3^-$ has also been observed in the Mediterranean Sea (Pantoja et al., 2002). Potential causes include $^{15}$N depleted sources such as $N_2$ fixation and atmospheric deposition along with the lack of water column denitrification and its associated large isotope effect ($\sim 25\%e$, Cline and Kaplan, 1975) and the restricted exchange of these systems with the open ocean. The higher $\delta^{18}$O$_{PNM}$ values may be partially due to the higher $\delta^{18}$O of water in the Gulf of Aqaba corresponding to its elevated salinity, and on a regional scale the higher $\delta^{18}$O signal in water would be transferred to NO$_3^-$ via nitrification (Casciotti et al., 2010). Atmospheric deposition in this region is another source of NO$_3^-$ with higher $\delta^{18}$O$_{PNM}$ (Wankel et al., 2009a).

4.2. N uptake and regeneration in the Gulf of Aqaba

Our measurements for uptake of NO$_3^-$ (26–434 mmol N L$^{-1}$ day$^{-1}$) and NH$_4^+$ (314 nmol N L$^{-1}$ day$^{-1}$) are in good agreement with uptake estimates from other studies for NO$_3^-$ (ranging from 48 to 526 nmol N L$^{-1}$ day$^{-1}$) and NH$_4^+$ (40–1536 nmol N L$^{-1}$ day$^{-1}$) in a range of environments and for different light intensities (Bronk et al., 1994; Wheeler and Kirchman, 1986; Probyn and Painting, 1985; McCarthy, 1972). Fewer measurements of NO$_3^-$ uptake are available; however, an approximation can be made based on the cell specific NO$_3^-$ uptake rate determined for Synechococcus 7803 (0.02 fmol cell$^{-1}$ h$^{-1}$; Lindell et al., 1998). This would correspond to a NO$_3^-$ uptake rate of $\sim 80$ nmol N L$^{-1}$ day$^{-1}$ based on the phytoplankton cell abundances measured during our study ($\sim 1.70\times 10^6$ cells L$^{-1}$), and is consistent with the range of uptake rates we measured in our $^{15}$N tracer experiment (29–94 nmol N L$^{-1}$ day$^{-1}$). Urea uptake rates encompass a much broader set of values in the environment, ranging from $<4$ to 86,400 nmol N L$^{-1}$ h$^{-1}$ (Kristiansen, 1983; Berg et al., 1997; Lomas et al., 2002; Berman and Bronk, 2003), and our measured rates of 296–1285 nmol N L$^{-1}$ h$^{-1}$ fall within that range. We note that spontaneous decomposition of urea into NH$_4^+$ can occur in the light and were determined to be $\sim 240$ mmol N L$^{-1}$ day$^{-1}$ in the Gulf of Aqaba (Kamennaya et al., 2008). However, this rate was measured following a relatively concentrated urea spike of 20 nmol N L$^{-1}$, compared to our dilute spike of 0.2 mmol N L$^{-1}$. If degradation kinetics are similar over this range of urea concentrations, then spontaneous degradation of urea to NH$_4^+$ could have caused an overestimation of $\sim 20\%$ for our urea uptake rates in the light.

The N cycle in the Gulf of Aqaba provides an example of a system with closely coupled N assimilation and regeneration during the stratified period. The increasing slope of the best fit line for $\delta^{15}$N$_{PNM}$-$\delta^{18}$O$_{PNM}$ (from 2:1 to 5:1; Fig. 6) indicates that regenerated organic matter is a major source of N for primary producers in the Gulf of Aqaba, because it shows a strong signature of uncoupled fractionation of N and O that is imparted during nitrification. This observation is consistent with other studies that have found high rates of primary productivity despite relatively low standing stocks of phytoplankton in the Gulf (Hase et al., 2006). Together these findings suggest that assimilation and nitrification compete for NH$_4^+$, and that primary productivity is tightly coupled to grazing food webs and microbial remineralization processes, which are a source of NH$_4^+$. Productivity is therefore partially supported by efficient sequestration of NH$_4^+$ within cells as soon as it becomes available, in addition to using NO$_3^-$ produced during nitrification.

During our monitoring of the spring bloom the concentration of DON increased by 1.1 mmol N L$^{-1}$ as DIN decreased by this amount (Fig. 3B). Labile DON could play an important role in the Gulf’s biogeochemical cycling of N and serve as an important nutrient resource for non-photosynthetic microbes and marine phytoplankton, similar to other areas of the ocean (Solomon et al., 2010; Palenik and Morel, 1990; Moore et al., 2002; Zubkov et al., 2003). The role of labile DON could be particularly important in ultra-oligotrophic marine environments where DIN concentrations are very low and the reservoir of DON can be over an order of magnitude larger than DIN, as was the case in the Gulf of Aqaba where DON reached $\sim 10$ mmol N L$^{-1}$ and the NO$_3^-$ concentration was 0.1–0.2 mmol L$^{-1}$ (March 25). Moreover, in some marine diatoms NH$_4^+$ and DON uptake rates increase with temperature while NO$_3^-$ uptake rates decrease (Lomas and Gilbert, 1999), suggesting that DON could be the preferred source of N for phytoplankton that bloom in warming surface waters as stratification becomes established.

In the Gulf of Aqaba where ammonification and nitrification are closely coupled, NH$_4^+$ generated during mineralization of DON should be considered when making measurements of NH$_4^+$ oxidation. Calculations based on $^{15}$N labeling data are complicated by rapid and closely coupled NH$_4^+$ production and consumption, and can result in rate underestimation. In this study the rate of coupled mineralization and NH$_4^+$ oxidation were measured in the $^{15}$N tracer experiment for urea, a labile form of DON. The overall rate of coupled urea mineralization and NH$_4^+$ oxidation ($14.1$ nmol N L$^{-1}$ day$^{-1}$) was remarkably similar to that of NH$_4^+$ oxidation alone based on our study ($16.4$ mmol N L$^{-1}$ day$^{-1}$) and other studies ($\sim 18–40$ mmol N L$^{-1}$ day$^{-1}$; Ward, 2005; Ward et al., 1982), suggesting that mineralization is not a rate limiting step for nitrification, at least when the DON pool is relatively large and labile. The composition and lability of DON changes based on community composition, grazing rates, mixing, and numerous other factors, although estimates suggest that complete DON turnover occurs on the order of 10 days in oligotrophic waters (Bronk et al., 1994). Rates measured for labile DON compounds, such as urea in this study, provide maximum potential rates for DON mineralization and nitrification. Actual rates will be lower, and mineralization may limit nitrification rates for NH$_4^+$ derived from more refractory forms of DON. More work is needed to characterize the DON pools in different waters and determine their influence on marine nitrification rates.

5. Conclusion

This study used isotope data from natural abundance samples in the Gulf of Aqaba together with tracer experiments to identify important processes in the N cycle and quantify their rates. The approach has highlighted the importance of regenerated N for supporting productivity in the Gulf of Aqaba, where efficient photosynthetic sequestration of N in surface waters is coupled to mineralization and nitrification of PON and DON throughout the water column. Export and regeneration (mineralization and nitrification) of particulate N to DIN at depth serves to recharge the NO$_3^-$ reservoir in deep water.

Several major light-sensitive processes contribute to the formation of PNM in the Gulf of Aqaba during the transition from mixing to stratification. Within the euphotic zone, phytoplankton assimilate N during growth by drawing down DIN levels sharply in the well-lit surface waters. Below the euphotic depth during the initial stages of stratification, a large inventory of NO$_3^-$ is generated from incomplete NO$_3^-$ reduction by trapped, light limited phytoplankton. NO$_3^-$ from this process is distributed over a range of depths, creating a broad band of NO$_3^-$ with a subsurface peak. Later, once
stratification is firmly established, net NO$_3$ is generated by NH$_4^+$ oxidizers over a narrower range of depths coinciding with the upper part of the PNM, and is consistent with differential light inhibition of NH$_4^+$ and NO$_2^-$ oxidizing communities. Deeper in the water column where light is negligible, NO$_2^-$ oxidation rates match NH$_4^+$ oxidation, and NO$_3^-$ gets drawn down, defining the lower portion of the PNM.

Mineralization and subsequent nitrification of organic material is an important source of N for primary producers in the Gulf of Aqaba, where NO$_3^-$ formed from nitrification of NH$_4^+$ and urea (following ammonification) at rates of similar magnitude. The similar magnitudes of assimilation rates for urea and NO$_3^-$ suggest that labile organic N is an important source of N for primary producers in this oligotrophic region during the stratified season. The rate and type of N transformation processes operating throughout the water column are strongly influenced by light, which determines the maximum depths for net photosynthesis and may contribute to inhibition of nitrifying communities.

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