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Coupled effect of substrate and light on assimilation and oxidation of regenerated nitrogen in the euphotic ocean

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Abstract

Nitrogen (N), as a critical element for microbial metabolisms, recycles rapidly in the euphotic ocean. Oxidation by nitrifiers is a competing pathway for phytoplankton assimilation of regenerated N (NH_4^+ and urea). Sharing the overlapping substrates may result in competitive exclusion, thus, niche separation for the two assemblages. Both pathways are sensitive to light, but whether light intensity will intensify or alleviate such resource competition in the euphotic zone remains poorly explored in the field at the community level. By using ^{15}N labeling techniques, paired kinetic responses of uptake and oxidation were conducted in single bottles under manipulated light intensities for both NH_4^+ and urea. We found light stimulated the maximum rate (R_m) and specific affinities (α_U) of both NH_4^+ and urea uptake. In contrast, light effects were opposite for oxidation kinetics (R_m and α_O). As irradiance increased, the rapid increase in α_U and concomitant decrease in α_O imply a distinctive competition advantage of photosynthetic organisms over oxidizers under substrate-limited environments. The ratio of α_U/α_O for NH_4^+ ranged from 0.8 to 3089 (5.8–46,788 for urea) showing a distinct increasing pattern as ambient light increases, demonstrating that phytoplankton overwhelms nitrifiers throughout the oligotrophic euphotic zone, driving down concentrations and maintaining short turnover times of the two regenerated N substrates. Moreover, phytoplankton relied equally on NH_4^+ and urea; yet, nitrifiers preferred NH_4^+ to urea. In the nitrate-depleted euphotic ocean, light acts as a crucial driver for utilization pathways of regenerated N and vertical niche separation.

Ammonium (NH_4^+) and urea, two major regenerated nitrogenous nutrients, turn over rapidly to sustain primary producers in the euphotic waters (e.g., Herbland 1976; Glibert 1982; Harrison et al. 1985; Price and Harrison 1988; Martens-Habbena et al. 2009; Yool 2011). Chemolithotrophic nitrifiers may compete with photoautotrophs for the use of regenerated nitrogen (N) (Ward et al. 1984; Ward 2008; Smith et al. 2014; Santoro et al. 2017). The two divergent consumption pathways (i.e., uptake and oxidation) may lead to distinct biogeochemical consequences. More specifically, uptake processes produce organic nitrogen, generate oxygen, and may elevate seawater pH, whereas the oxidation processes produce nitrate (NO_3^-) or N_2O, consume oxygen, and may reduce the pH (Ward 2008; Hsiao et al. 2014). Moreover, the end-product of NH_4^+ and urea oxidation, NO_3^-, if generated in the euphotic zone, may directly refuel new production (Dugdale and Goering 1967) thus complicating the assessment of the ratio of new production to total production (*f*-ratio; Eppley and Peterson 1979; Yool et al. 2007). Knowledge of mechanisms controlling the distribution (niche separation) of such distinctive consumption pathways of regenerated N driven by photoautotrophs and chemolithotrophic nitrifiers is of particular importance for a better understanding of the biogeochemical cycle of N-limited oceans.

Light, as the ultimate energy source for photoautotrophs, may inhibit ammonia-oxidizing microorganisms (AOM; e.g., Merbt et al. 2011; Qin et al. 2014; Levipan et al. 2016), resulting in a general downward increase of AOM abundance (e.g., Mincer et al. 2007; Santoro et al. 2010; Newell et al. 2013) and NH_4^+ oxidation rate with depth in the upper ocean (Olson 1981; Ward 1985, 2005; Santoro et al. 2010; Beman et al. 2012; Newell et al. 2013; Shiozaki et al. 2016). Besides light inhibition, phytoplankton competition for substrates is also a plausible cause of suppressing euphotic NH_4^+ oxidation in the

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euphotic zone (Ward 1985; Christman et al. 2011; Beman et al. 2012; Smith et al. 2014). Recently, the concentration of ambient NO_3^- was found to be an effective factor additionally to influencing the vertical distribution of NH_4^+ oxidation (Wan et al. 2018), as increased ambient NO_3^- level may modulate the phytoplankton community and then down regulate the affinity of phytoplankton toward NH_4^+, leaving more opportunity for nitrifiers to thrive. Besides NH_4^+, urea has long been shown to be a reactive N source for phytoplankton growth (e.g., McCarthy 1972; Antia et al. 1991; Berman and Bronk 2003; Casey et al. 2007; Painter et al. 2008; Solomon et al. 2010). Recent studies found that many AOM possess the enzyme urease that hydrolyses urea to NH_3 and CO_2 and thus can utilize urea for nitrification (Alonso-Sáez et al. 2012; Bayer et al. 2015; Santoro et al. 2017; Tolar et al. 2017). Presumably, competition between phytoplankton and nitrifiers for urea should also occur to a certain degree in the euphotic zone.

Despite the importance of the two regenerated N species and associated competing pathways in the euphotic zone, limited depth profiles of NH_4^+ and urea concentrations have been reported in the open ocean (Harrison et al. 1985; Price and Harrison 1988; Kudela and Cochlan 2000; Thomalla et al. 2011; Bury et al. 2012). Some have reported both NH_4^+ uptake and oxidation rates (Ward et al. 1984; Ward 1985, 2005; Horak et al. 2017; Peng et al. 2018; Wan et al. 2018); yet, to our knowledge, there are virtually no studies in which assimilation and oxidation of these two regenerated N sources were measured simultaneously in the field from the perspective of kinetics. Although light is a well-known factor to tune the activities of assimilation and oxidation of NH_4^+ and urea, oxidation rates reported in field incubations have been mostly measured under solely dark conditions (e.g., Santoro et al. 2010; Newell et al. 2013; Peng et al. 2018). Therefore, light effects on the kinetics of both assimilation and oxidation, as well as the preference for NH_4^+ and urea of natural assemblages in the field, remain unclear.

In this study, by using ^{15}N-labeling techniques, we measured ^{15}N flows from the substrates (NH_4^+ or urea) into particulate nitrogen (PN) and NO_x^- ($NO_2^- + NO_3^-$) in parallel in the subtropical western North Pacific Ocean (WNP). The overall goal was to (1) investigate effect of light on the affinity of phytoplankton and nitrifiers for NH_4^+ and urea at natural community levels in the euphotic zone and (2) assess the relative control of turnover of the regenerated N by field assemblages of phytoplankton and nitrifiers. Results provide insight into N recycling processes in the euphotic ocean.

Material and methods

Study site and sample collection

A cruise (R/V *Dongfanghong II*) was undertaken during late March to early May 2015 across the WNP (Fig. 1). Five stations (Sta.), covering a wide range of chlorophyll *a* (Chl *a*) and nutrient conditions (coastal Sta. C2; offshore Sta. K3, B9, A6, and B1; see parameters in Table 1), were selected for experimental incubations. Samples were taken from the layers near or above the depth of the chlorophyll maximum (DCM) and within the euphotic zone (circles in Fig. 1). A SeaBird 911 plus conductivity temperature depth (SBE) equipped with a rosette of 12 × 12-liter Niskin bottles, a SeaTech flash fluorometer and a photosynthetically active radiation (PAR) sensor (Li-Cor Biosciences, LI-193) was employed for water sampling and associated measurements.

Surface irradiance was monitored on-deck by using a light sensor CR1000. Coupled with observed daily maximum of surface PAR (sPAR) and the light attenuation curve of corresponding station, maximum light curves with depth for each station were constructed (Fig. 1). The euphotic zone is defined herein as the depth at which the light was 1% of sPAR (Kirk 1994).

Samples for measurements of NH_4^+, NO_2^-, and NO_3^- concentrations were collected in 125-mL acid-washed high-density polyethylene bottles. For urea concentration analysis, 40-mL seawater were collected directly from the Niskin bottles into precombusted brown glass vials. For particulate organic nitrogen (PN) and Chl *a*, 1-liter seawater samples were gently filtered onto 25-mm GF/F filters (precombusted at 450°C for 4 h). All the samples were frozen at −20°C until analysis, except NH_4^+, which was analyzed immediately onboard, and Chl *a* samples, which were held at −80°C until analysis.

Incubations for paired rate response to light change

To explore assimilation and oxidation rates as a function of light, water from selected depths (> 1% sPAR) was incubated with ^{15}N-labeled NH_4^+ and urea substrates. The source ^{15}N was 98 atom % enriched from Sigma-Aldrich. Additions were made to yield final concentrations of 10, 20, 100, 200, and 2000 nmol L^{-1} for NH_4^+ and 60, 300, 600, and 3000 nmol N L^{-1} for urea. Paired incubations in 1-liter Nalgene PC bottles (two replicates) were exposed to various light levels and darkness. The exception to this was Sta. A6, for which the urea tracer was added to water from 25 m and NH_4^+ tracer was added to that from 50 m.

Samples from Sta. K3 and B9 were incubated under natural irradiance in on-deck incubators with flow-through surface seawater to maintain a constant temperature during the incubations. Neutral density screens (Lee Filters) were used to adjust the light penetration to 9% and 50% to mimic in situ light condition. For Sta. A6, B1 (which was sampled on a rainy day), and C2 (which was sampled on a sunny day but the PAR sensor malfunctioned), incubations were carried out in a thermostatic incubator (GXZ-250A, Ningbo) at in situ temperatures with constant light intensities of 0, 35, and 75 μmol photons m^{-2} s^{-1} for all selected depths. The light levels of incubated water (the mean light intensity during on-deck incubation for K3 and B9 and the constant light set for A6, B1, and C2 in the laboratory incubator) fell below the maximum daily light of the corresponding sampling depth in order to avoid photodamage.

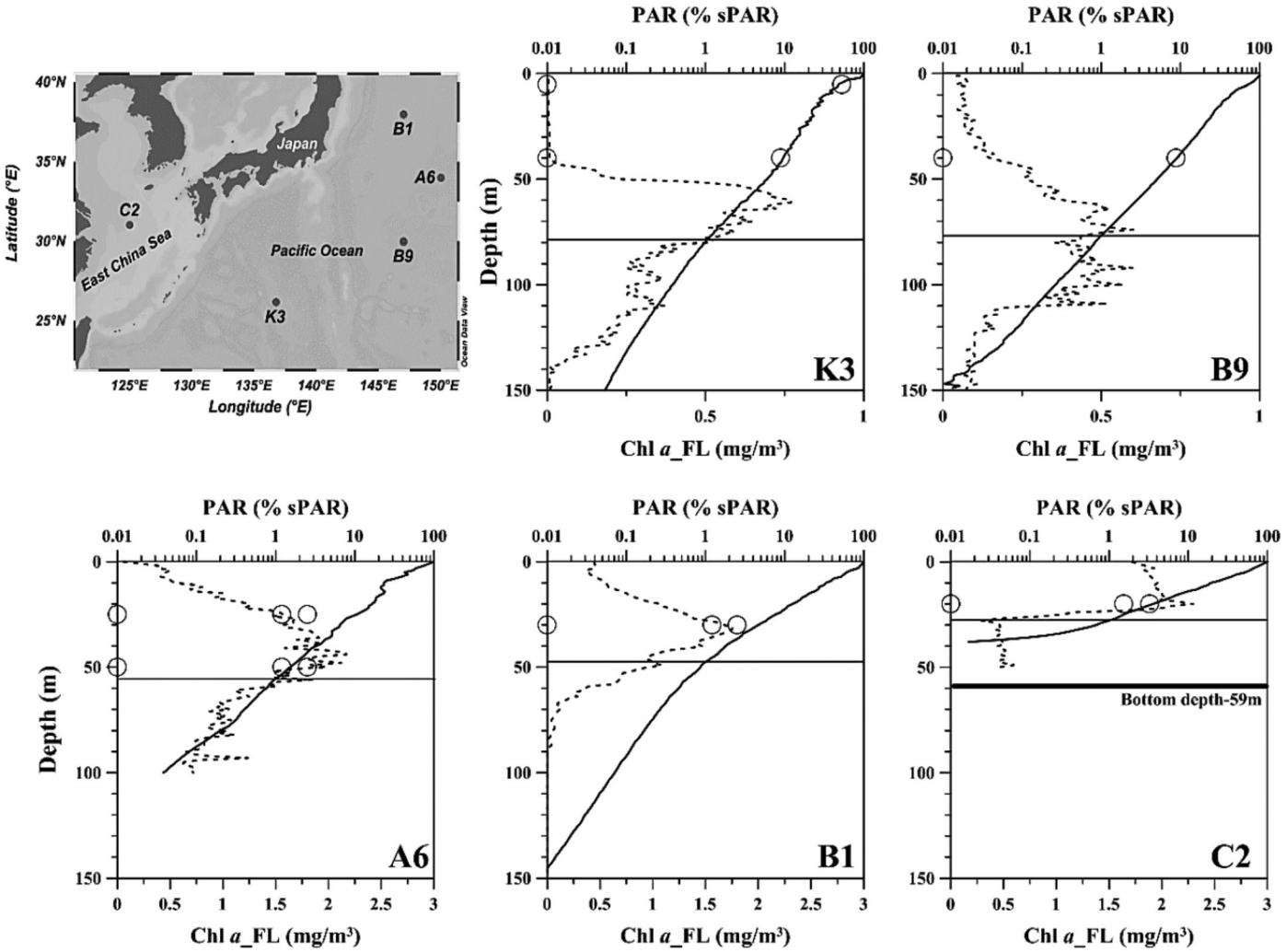

Fig. 1. The map of incubation stations and essential parameters in the spring cruise 2015. The vertical profiles of Chl a (mg/m^3) and light (% sPAR) were presented, respectively, in dashed and solid lines for five stations (name marked at lower right). The thin horizontal line represents 1% sPAR depth. The scattered circles represent samples taken from the selected depths but incubated under manipulated light conditions. The thick horizontal line in C2 panel is for the bottom depth. For A6, urea was applied for 25 m layer and ammonium was applied onto 50 m sample.

Table 1. Hydrographic data for selected stations/depths where light and substrate manipulated experiments were conducted.

Sta.	Depth (m)	% sPAR (%)	In situ *T* (°C)	Chl *a* (μg L^{-1})	DO (mg L^{-1})	NH_4^+ (nM)	Urea-N (nM)	NO_2^- (μM)	NO_3^- (μM)	PN (μM)
K3	5	48.0	23.3	<0.01	6.3	32	89	0.01	0.08	0.30
K3	40	9.6	21.1	0.01	6.8	29	121	0.01	0.07	0.28
B9	40	9.0	18.5	0.15	7.2	80	127	0.02	0.15	0.33
B1	30	4.7	13.5	1.67	8.0	205	124	0.56	9.51	1.24
A6	25	10.6	17.6	1.57	7.4	62	82	0.01	0.18	0.88
A6	50	1.5	17.5	1.99	7.4	39	134	0.12	0.73	0.45
C2	20	3.6	14.7	2.30	8.3	96	112	0.33	1.60	2.19

Chl *a*, chlorophyll *a*; DO, dissolved oxygen; PN, particulate nitrogen; sPAR, surface photosynthetically active radiation; Sta., station; T, temperature.

Incubations were terminated within 4–6 h after tracer addition. Immediately after tracer addition (t_0) and at the end of incubations, 900-mL were gently filtered through 25-mm GF/F filters to collect PN. All filters containing PN were rinsed with 0.22-μm filtered surface water to remove isotope that may have been adsorbed. From the same incubation bottle, 100-mL were

removed and filtered through 0.22 μm disposable syringe filters for filtrate collection. All filters and filtrates were frozen immediately after collection and kept frozen at −20°C for subsequent measurements.

Analysis of chemical and biological parameters

Concentrations of NH_4^+ were measured using the fluorometric *o*-phthaldialdehyde method (Zhu et al. 2013). To avoid contamination and biological alteration, ambient NH_4^+ concentrations were measured on-deck immediately after collection. Urea concentrations were measured using a 1-m length liquid waveguide capillary cell based on the colorimetric reaction with diacetyl monoxime (Chen et al. 2015). Detection limits were 1 nmol L^{-1} for both NH_4^+ and urea. Concentrations of NO_2^- and NO_3^- were determined by classical colorimetric methods with a Technicon AA3 Auto-Analyzer (Bran-Lube). The detection limits for NO_2^- and NO_3^- were 0.02 and 0.07 μmol L^{-1}, respectively (Dai et al. 2008). To measure PN concentrations, filters were first oxidized to NO_3^- by using purified persulfate as an oxidizing reagent (Knapp et al. 2005), then the NO_3^- concentrations were measured by the chemiluminescence method following the protocol of Braman and Hendrix (1989) with a detection limit of 10 nmol L^{-1}.

Chl *a* was extracted in 95% acetone at −20°C for 16–24 h, and the extracts were measured using a Turner Trilogy fluorometer (Welschmeyer 1994). The standard used for Chl *a* quantification was from the Danish Hydraulic Institute Water and Environment, Hørsholm, Denmark (Wang et al. 2016).

Isotope measurements and rate calculation

To determine uptake and oxidation rates for NH_4^+ and urea, the isotopic compositions of PN and NO_x^- collected at t_0 and after incubation were measured. PN was oxidized to NO_3^- and then converted into N_2O for isotope analysis by using the denitrifier method (Sigman et al. 2001; Casciotti et al. 2002; McIlvin and Casciotti 2011). Briefly, *Pseudomonas aureofaciens* (ATCC 13985) was harvested after 5–8 d of growth in culture by centrifugation at ~ 60 rpm in the dark at room temperature. Then, *P. aureofaciens* cells were concentrated 4–10 times by resuspending the cells in fresh NO_3^--free medium. Liquid samples (with ~ 20 nmol absolute amount of N) were individually aliquoted into 20 mL vials with 3-mL concentrated bacteria. NO_3^- and NO_x^- in the vials were quantitatively converted into N_2O, which was then measured by a Thermo Finnigan Gasbench-coupled isotope ratio mass spectrometer (IRMS, Delta V PLUS), to determine the N isotope composition ($\delta^{15}N$). Isotope standards of NO_3^-, USG34, IAEA3, and USGS32, were used to calibrate the $\delta^{15}N$-NO_x^-. Linearity of IRMS was affirmed by tuning a wide range of inflow of N_2O reference gas.

Rates of uptake and oxidation were calculated according to the net increments of ^{15}N in PN and in NO_x^-, respectively, over incubation periods. The effect of biological fractionation is assumed to be negligible during the tracer incubation experiments. The uptake and oxidation rates were determined according to:

$$R_S = \frac{r_t \times [P]_t - r_0 \times [P]_0}{T}, \quad (1)$$

where R is the uptake or oxidation rate based on ^{15}N accumulation in products (nmol N L^{-1} h^{-1}), S stands for substrate (NH_4^+ or urea) (nmol N L^{-1}), r_t and r_0 are the ^{15}N fractions (defined as $^{15}N/[^{15}N + {}^{14}N]$) at the end and the start, respectively, in the product pool of incubation; $[P]_t$ and $[P]_0$ are the product (PN for uptake and NO_x^- for oxidation) concentrations (nmol N L^{-1}) at the end and the start, respectively, of incubation, and T is the length of incubation (h). It was assumed that the isotope dilution effect of $^{15}NH_4^+$ tracer was minimal (Xu et al. 2017) in the incubations of < 24 h, although Glibert et al. (1982) showed that this effect can be substantial even in very short incubations. To confirm the validity of our assumption, we measure the time courses of ^{15}N abundance in products during incubation and examined the linearity of ^{15}N increase in product over time (see below).

Photosensitivity index and specific affinity

To evaluate substrate kinetics under various light intensities, the ratio of the rate (uptake or oxidation) in the light to the paired rate in the dark was calculated, yielding a photosensitivity index (PI; $PI = R_L/R_D$). Values of PI > 1 and < 1 indicate positive and negative light effects on the N transformation process, respectively. Furthermore, the greater the PI value deviates from 1, either positively or negatively, indicates a stronger light sensitivity for the associated assemblages.

The maximum rates (R_m, nmol N L^{-1} h^{-1}) and half-saturation constants (K_m, nmol N L^{-1}) were determined based on the Michaelis–Menten (M-M) equation:

$$R = \frac{R_m \times S}{K_m + S}. \quad (2)$$

A nonlinear regression was applied to derive the kinetic parameters by using SigmaPlot 12.0 Software (Ligand binding module, one-site saturation).

The ratio of R_m/K_m, i.e., the initial slope of the M-M curve at low substrate concentrations, α, the affinity coefficient (Healey 1980; Aksnes and Egge 1991), was calculated and has a unit of h^{-1}. Under extremely low substrate conditions, S in denominator can be ignored, therefore, the "instant" uptake or oxidation rate becomes $\alpha \times S$, in which α is also the reaction constant in the first-order reaction.

Results

Hydrographic data

Light decreased exponentially with depth with various attenuation coefficients among stations (Fig. 1). Chl *a* ranged from undetectable (< 0.01 μg L^{-1}) to 2.3 μg L^{-1} (Fig. 1). Samples from selected depths were oxygenated (DO > 6.3 mg L^{-1}) with a temperature range of 13.5°C to 23.3°C (Table 1). Concentrations of NH_4^+ ranged from 29 to 205 nmol N L^{-1}

(on average 87 ± 66 nmol N L^{-1}), while those of urea-N ranged from 82 to 134 nmol N L^{-1} (on average 113 ± 19 nmol N L^{-1}), and those of NO_2^- were around the detection limit except those at stations B1 (0.56 μmol N L^{-1}) and C2 (0.33 μmol N L^{-1}; Table 1). Similar to NO_2^-, NO_3^- concentrations were higher for samples in B1 (9.51 μmol N L^{-1}) and C2 (1.60 μmol N L^{-1}) than in other stations (< 0.73 μmol N L^{-1}).

Kinetic responses under different light

Time series incubations showed that ^{15}N flowed simultaneously from labeled substrate to the two products (^{15}N-PN and ^{15}N-NO_x^-) but in different rates (see slopes in Supporting Information Fig. S1). The ^{15}N in product pool increased linearly over time within 24 h incubation under all substrate concentration additions. Such a nice linearity suggests that the effect of dilution and community change on rate determination in our case can be ignored. Moreover, values of the coefficient of variation (%) of duplicate rates for all uptake and oxidation incubations were within ± 15% over a wide range (3–6 orders of magnitude) of measured rates (Supporting Information Fig. S2).

Substrate kinetics of uptake and oxidation for NH_4^+ and urea-N primarily followed the M-M curve (Fig. 2; Supporting Information Fig. S3). The results of samples collected from 40 m at Sta. K3 are presented as an example (Fig. 2). Both NH_4^+ and urea-N uptake rates increased with increasing ^{15}N addition until substrate saturation. Uptake rates were consistently higher in the light than in the dark regardless of substrate levels (Fig. 2a,b). In contrast, kinetics of oxidation (K3-40 m) showed an opposite response to light. Both NH_4^+ and urea oxidation rates in the light were lower than those in the dark for all substrate additions (Fig. 2c,d).

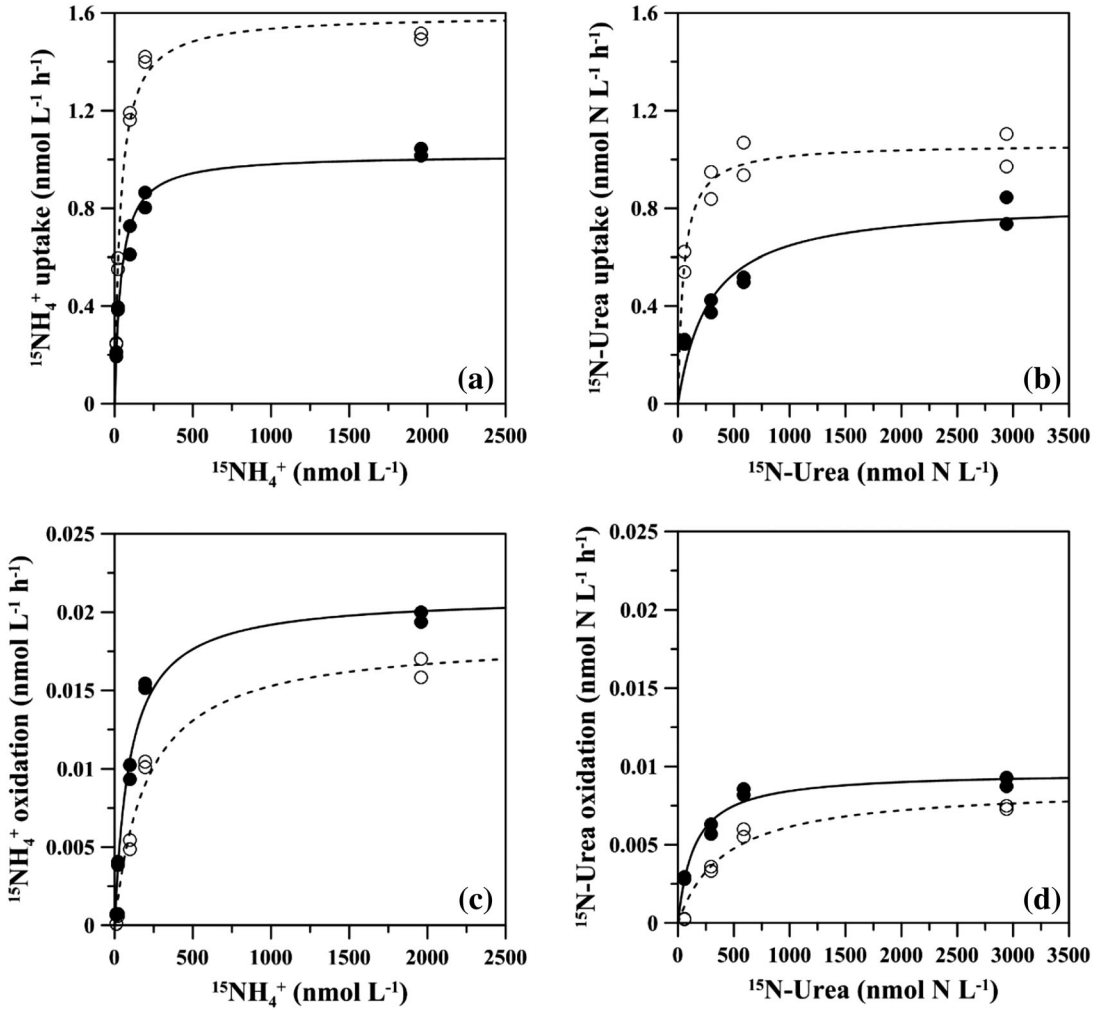

Fig. 2. The dependence of N (NH_4^+ and urea) uptake **(a and b)** and oxidation **(c and d)** rates on substrate concentration for samples from 40 m depth at Sta. K3. Measured ^{15}N transformation rates (nmol L^{-1} h^{-1}; circles) are shown as a function of total ^{15}N concentration (^{15}N in tracer + ^{15}N in ambient substrate; nmol N L^{-1}). A Michaelis–Menten function was applied to fit the data. The open and solid dots were for light and dark incubations, respectively. The dashed and solid lines stand for the curve fitting for results of light and dark incubations. See Supporting Information Fig. 3 for comparable data from other sampling sites.

Maximum rates of NH_4^+ uptake and oxidation in the light were 2.14 ± 0.48 and 0.75 ± 0.07 greater than those in the dark, respectively. Both R_m of NH_4^+ uptake in the light (0.82–18.09 nmol N L^{-1} h^{-1}) and in the dark (0.50–5.94 nmol N L^{-1} h^{-1}) were significantly higher than the R_m of NH_4^+ oxidation rates in corresponding conditions (0.005–2.28 nmol N L^{-1} h^{-1} in the light and 0.008–2.96 nmol N L^{-1} h^{-1} in the dark; Supporting Information Table S1). The response pattern of R_m for urea-N resembled these of NH_4^+ (Supporting Information Table S2).

The ranges of K_m of NH_4^+ uptake and oxidation (including light and dark) were 30–1018 and 76–2112 nmol N L^{-1}, respectively (Supporting Information Table S1). The K_m values of urea-N uptake and oxidation (including light and dark) were 30–552 and 63–7505 nmol N L^{-1} (Supporting Information Table S2), respectively.

Discussion

In principle, comparisons of the kinetic parameters of R_m and K_m derived from the M-M curves of N uptake (e.g., Kanda et al. 1985; Sahlsten 1987; Harrison et al. 1996; Kudela and Cochlan 2000) and NH_4^+ oxidation (e.g., Martens-Habbena et al. 2009; Horak et al. 2013; Newell et al. 2013) in field or culture samples can be used to understand/infer the roles of respective processes in N flow/allocation in ecosystem. However, as Healey (1980) pointed out, neither K_m nor R_m is robust enough to provide sufficient understanding regarding competitive advantages between taxa or between conditions or processes. As shown in Healey (1980), two species of phytoplankton having similar K_m but significantly different R_m in N uptake does not suggest their similar affinity for substrate but implies the phytoplankton with higher R_m has higher uptake rates relative to the one with lower R_m at all substrate concentrations. Therefore, Healey (1980) emphasized that the comparison of K_m for different species competing the same substrate can be used only when they have similar R_m. Accordingly, Healey (1980) suggested α, the ratio R_m/K_m as a better indicator of the competitive advantage between and among taxa, with a higher α implying a higher rate at lower substrate concentrations. In other word, a species with higher α may have a higher competitiveness in the process and conditions being considered as competition drives nutrient concentrations down. The value of α has also been emphasized and/or applied by Aksnes and Egge (1991), Fan et al. (2003), Martens-Habbena et al. (2009), and Kana and Glibert (2016). The latter paper emphasized the comparative strength of this parameter given the variability of kinetic parameters within taxa, not just between taxa.

Herein, substantial changes in α were observed as a function of light (Supporting Information Tables S1 and S2). For uptake, changes in α were a result of changes in R_m rather than K_m, but for oxidation, both R_m and K_m variability contributed to changes in α. When irradiance increased, α of uptake (α_U) for both NH_4^+ and urea increased rapidly with a synchronous decrease in α of oxidation (α_O) for any given depth (Fig. 3). This remarkable plasticity in α values following short-term changes in light indicates that both processes are sensitive to light. Light affects species affinity for NH_4^+ or urea and therefore their competitive capability for acquisition of nitrogenous resources.

Light effects over a wide range of substrate concentrations were compared by using the proposed photosensitivity indicator, i.e., R_L/R_D. By introducing substrate as a variable into simulated light/dark M-M curves, values of PI over the full range of substrate concentration for individual process were obtained (Fig. 4). For all cases, the R_L/R_D ratios of NH_4^+ and urea-N uptake were > 1, whereas the corresponding ratios of NH_4^+ and urea-N oxidation were <1, illustrating again the light stimulates NH_4^+ and urea-N uptake rates but inhibits oxidation rates. Furthermore, the PI was relatively constant when substrate was saturated (substrate > 1000 nM N) but deviated from a constant value, i.e., positive for uptake and negative for oxidation, under low substrate conditions (except C2-20 m with insignificant deviation and A6-25 m with diminishing urea uptake). Apparently, substrate level plays a role in photosensitivity. This phenomenon is similar to that reported by Horrigan and Springer (1990) who found that inhibition of NH_4^+ oxidation by light was more pronounced for oceanic AOB isolates than for estuarine isolates in laboratory culture. Conversely, in the euphotic Delaware River, Lipschultz et al. (1985) found no significant change in either NH_4^+ uptake or nitrification to further substrate addition ($NH_4^+ > 20$ μM) regardless of light conditions. Such a phenomenon of magnified photosensitivity at low substrate may facilitate the oligotrophic phytoplankton community in outcompeting nitrifiers in the euphotic ocean that may contribute to the downward increasing pattern for the oxidation rates.

At substrate saturated conditions, the reduction in R_m of oxidation processes in the light was directly caused by light inhibition rather than substrate competition from phytoplankton since the substrate was more than sufficient for both photosynthesizers and ammonia oxidizers. By contrast, under low-concentration conditions, it is unclear whether the reduction in oxidation rate in the light incubation was induced indirectly by enhanced substrate consumption by phytoplankton or directly by light. A back-of-the-envelope calculation shows that the maximum substrate reduction caused by phytoplankton consumption was 26%, which is much smaller than the inhibition in the corresponding oxidation rate (23–95% with an average of 54% $\pm$ 25%; Supporting Information Fig. S4). Apparently, the light-stimulated phytoplankton competition was not responsible for the reduction of oxidation rate in our cases.

To resolve the forcing function of light inhibition in substrate-limited oligotrophic oceans, the α derived from the rate responses to substrate changes under manipulated lights were compared. According to the first-order reactions at low substrate, i.e., $R_I = \alpha_I \times S$ (the subscript I represents light

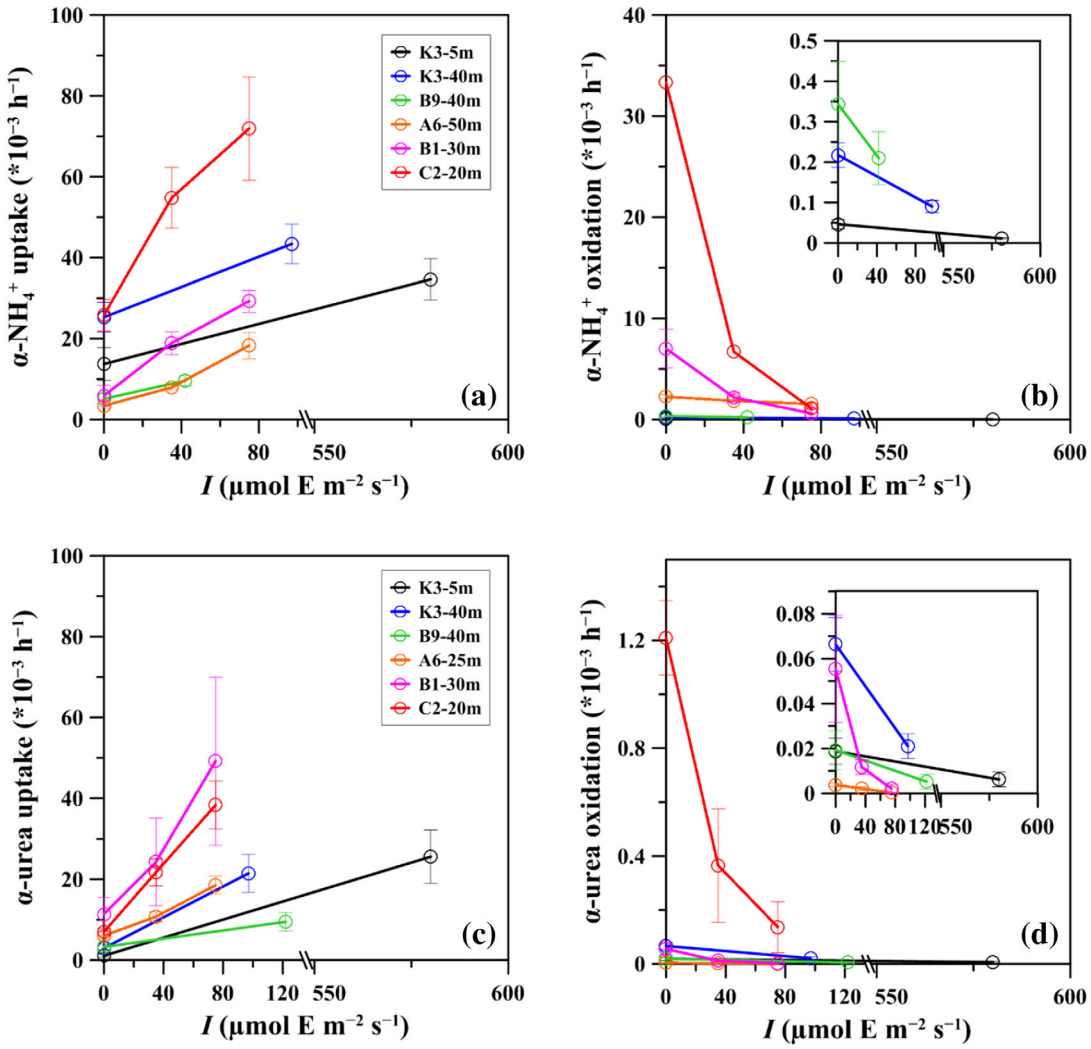

Fig. 3. The initial slope α ($\times 10^{-3}$ h^{-1}) of the Michaelis–Menten curves of **(a)** NH_4^+ uptake, **(b)** NH_4^+ oxidation, **(c)** urea uptake, and **(d)** urea oxidation against light intensity (I, μmol E m^{-2} s^{-1}) during incubations.

intensity), the observed "instant rate" increase (or decrease) was apparently caused by the instant change in α induced by light. Thus, our results show clearly that α values of both phytoplankton and ammonia oxidizers were regulated by light (a function of light) but in opposite ways.

In water columns shallower than 1% sPAR depth (Fig. 5a), the $\alpha_{U\text{-}NH4+}$ were consistently higher than $\alpha_{O\text{-}NH4+}$ by factors of > 4 (α_U/α_O ranged from 4.3 to 3089, except for A6-50 m, B1-30 m, and C2-20 m in the dark incubation; Supporting Information Table S1). Moreover, the ratios were much higher in the light than those in the dark incubations. Coupled with the positive trend of α_U/α_O for NH_4^+ against sPAR (Fig. 5a), the above comparison of α values illustrates that phytoplankton overwhelmed nitrifiers in general throughout the euphotic zone, being supportive of the report by Smith et al. (2014). The three exceptions in these data, with α_U/α_O values of ~ 1, occurred in layers of various depths with higher levels of NO_3^-. This finding was consistent with recent observations that the ambient NO_3^- level may determine the community structure of phytoplankton and regulate the affinity of phytoplankton for NH_4^+ leaving rooms for nitrifiers to thrive (Wan et al. 2018).

In the rate measurements, we cannot rule out the possibility of overestimation of phytoplankton uptake due to bacteria uptake retaining on GF/F filters (e.g., Lee et al. 1995; Kirchman and Wheeler 1998). For the data herein, a constant bacterial uptake rate over a diel cycle was assumed, as uptake by bacteria appears not to be light sensitive (Schmitz Fontes et al. 2018). Accordingly, the measured uptake rates would be overestimated by a constant offset regardless of the manipulated light intensity, though such an offset would be site dependent. The influence of bacterial uptake may thus reduce the ratio of $\alpha_U/\alpha_{O\text{-}NH4+}$ to a certain degree, but bacterial uptake would not influence the observed pattern in log scale.

Consistent results were obtained for urea-N uptake vs. oxidation (Fig. 5b; Supporting Information Table S2) but

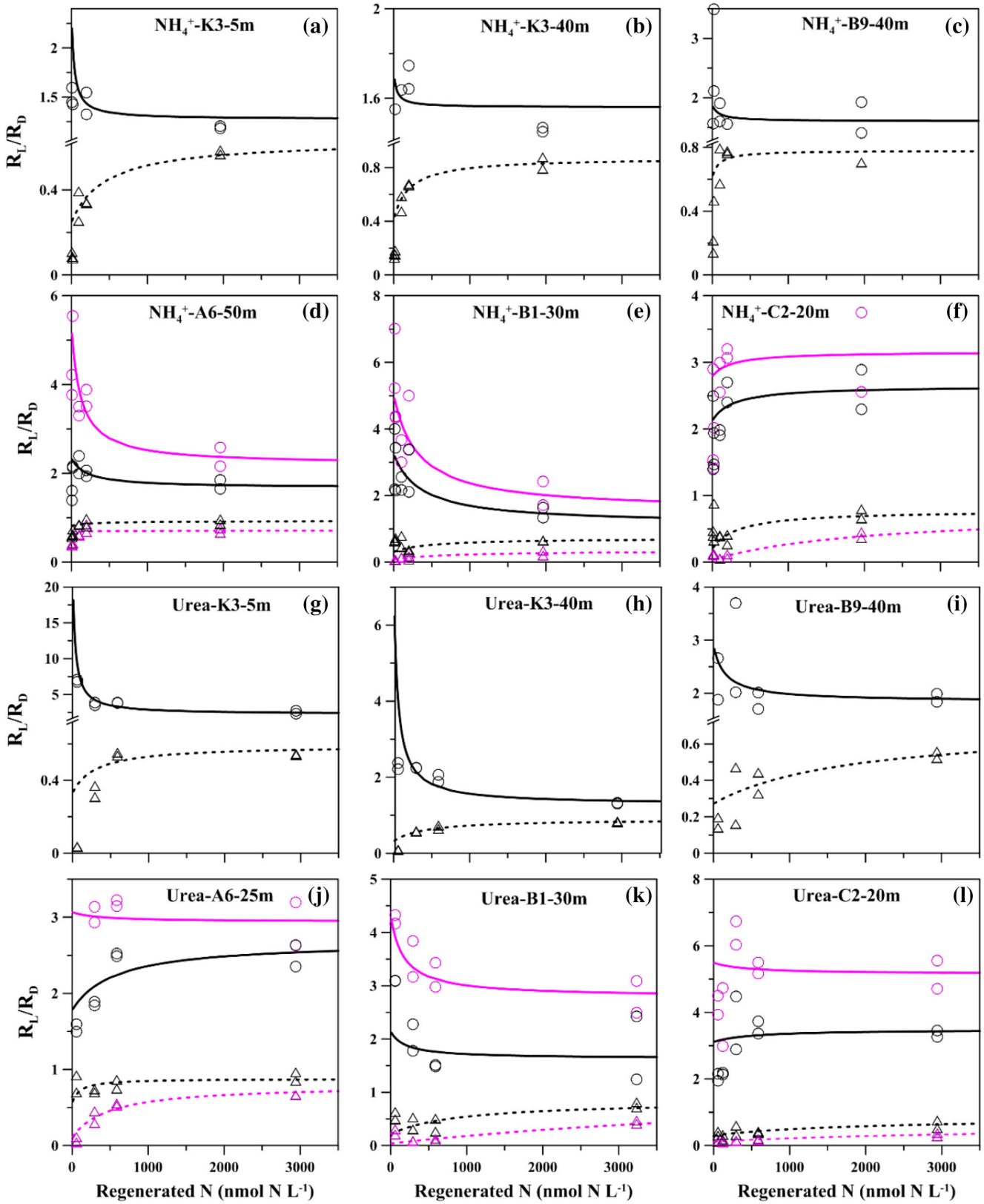

Fig. 4. Relationship between R_L and R_D (R_L/R_D, ratio of rate in light to that in the dark for uptake or oxidation) as a function of substrate (NH_4^+ or urea-N) concentrations. The solid lines were for uptake, and the dashed lines were for oxidation. The discrete circles and triangles stand for direct measurements. The dark and pink color stands for the ratio of low light to dark rates and high light to dark rates, respectively.

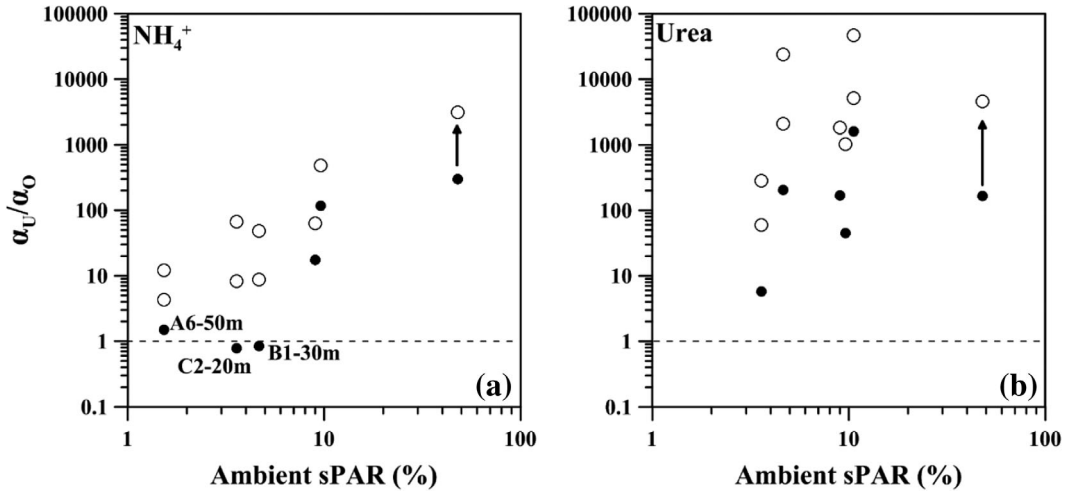

Fig. 5. Scatter plots of α_U/α_O for **(a)** NH_4^+ and **(b)** urea-N relative to ambient light. Subscripts U and O represent uptake and oxidation, respectively. The open and solid circles represent incubations in light and dark conditions. The dashed line represents the α_U/α_O ratio of 1. Black arrow represents light stimulation.

the ratios of $\alpha_U/\alpha_{O\text{-urea}}$ were > 5 without exception (ranging from 5.8 to 46,788; note that insufficient water was available for the lowest ambient light for urea incubations). The $\alpha_U/\alpha_{O\text{-urea}}$ varied over a wider range as light varied than did the $\alpha_U/\alpha_{O\text{-NH4+}}$, suggesting that there was a stronger differential light effect on urea allocation to phytoplankton and nitrifiers. Such a phenomenon was not caused by a higher affinity of phytoplankton for urea but rather by a lower preference of the nitrifiers for urea (see below). Nevertheless, phytoplankton have an absolute competitive advantage over their counterpart nitrifying rivals in terms of acquiring the two regenerated N forms in the euphotic zone.

In the oligotrophic ocean constrained by first-order reactions, the turnover time is $1/\alpha$. Based on the light responses described above, the turnover time (T) of NH_4^+ or urea-N was also light dependent. Although the most accurate way to estimate the turnover time would be the integration of α over a diel cycle, here an approximation was made based on the assumption of the day time of late spring of 16 h:

$$T\,(\mathrm{d}) = \frac{\mathrm{S}}{16(R_{UL}+R_{OL})+8\times(R_{UD}+R_{OD})} = \frac{\mathrm{S}}{16(\alpha_{UL}\times S+\alpha_{OL}\times S)+8\times(\alpha_{UD}\times S+\alpha_{OD}\times S)} = \frac{1}{16(\alpha_{UL}+\alpha_{OL})+8\times(\alpha_{UD}+\alpha_{OD})}, \quad (3)$$

where the subscript U and O represents process of uptake and oxidation and the L and D stand for light and dark, respectively. Based on this calculation, the turnover times of NH_4^+ and urea ranged from 0.6 to 5.0 and 1.1 to 5.6 d, respectively, which were comparable to the reported turnover times of NH_4^+ and urea in the subtropical North Pacific (Kanda et al. 1985) and tropical Atlantic (Herbland 1976). As the sampling scheme herein covered a wide range of geographic and nutritional conditions that may have led to differences in community and microbial biomass, the lack of clear patterns for NH_4^+ or urea turnover time against ambient light is reasonable (Fig. 6a,b).

The processes that dominated the turnover of regenerated N pool can also be estimated. The fractional contribution of the oxidation process to the turnover of NH_4^+ and urea showed a significant increasing trend with decreasing ambient light and the maximum contribution may reach 23% and 3.7%, respectively (Fig. 6c,d). In this study, as in many other studies (e.g., Price and Harrison 1988; Casey et al. 2007; Santoro et al. 2010; Shiozaki et al. 2016), the DCM often appeared at around the depth of 1–10% sPAR. Accordingly, ~ 20% of the regenerated NH_4^+ would be transformed into NO_3^- at this subsurface depth. To what extent such oxidation of regenerated N may contribute to new production and whether there is geographic difference in its contribution warrant further studies.

Lastly, the preferences for NH_4^+ relative to urea as N and energy sources, respectively, for photoautotrophs and nitrifiers were estimated by using M-M regression parameters (Supporting Information Tables S1 and S2). For all stations, R_m for urea uptake were similar in magnitude to those of NH_4^+ uptake, with an average ratio of $R_{m\text{-urea}}/R_{m\text{-NH4+}}$ of 0.86 ± 0.40 (one-way analysis of variance [ANOVA], $p > 0.05$, $n = 12$). The regressed K_m for NH_4^+ and urea uptake rates ranged from 30 to 1018 nmol N L^{-1} (Supporting Information Table S1) and 30 to 552 nmol N L^{-1} (Supporting Information Table S2), respectively, in manipulated light and dark incubations and these corresponding data points also did not show statistical differences (one-way ANOVA, $p = 0.50 > 0.05$). Accordingly, the values of α for urea uptake were comparable with the corresponding NH_4^+ uptake in most cases (one-way ANOVA, $p = 0.23 > 0.05$; Fig. 7a). Overall, results broadly suggest that urea might be as equally important as NH_4^+ in serving as an N source for phytoplankton growth. In addition, the similar

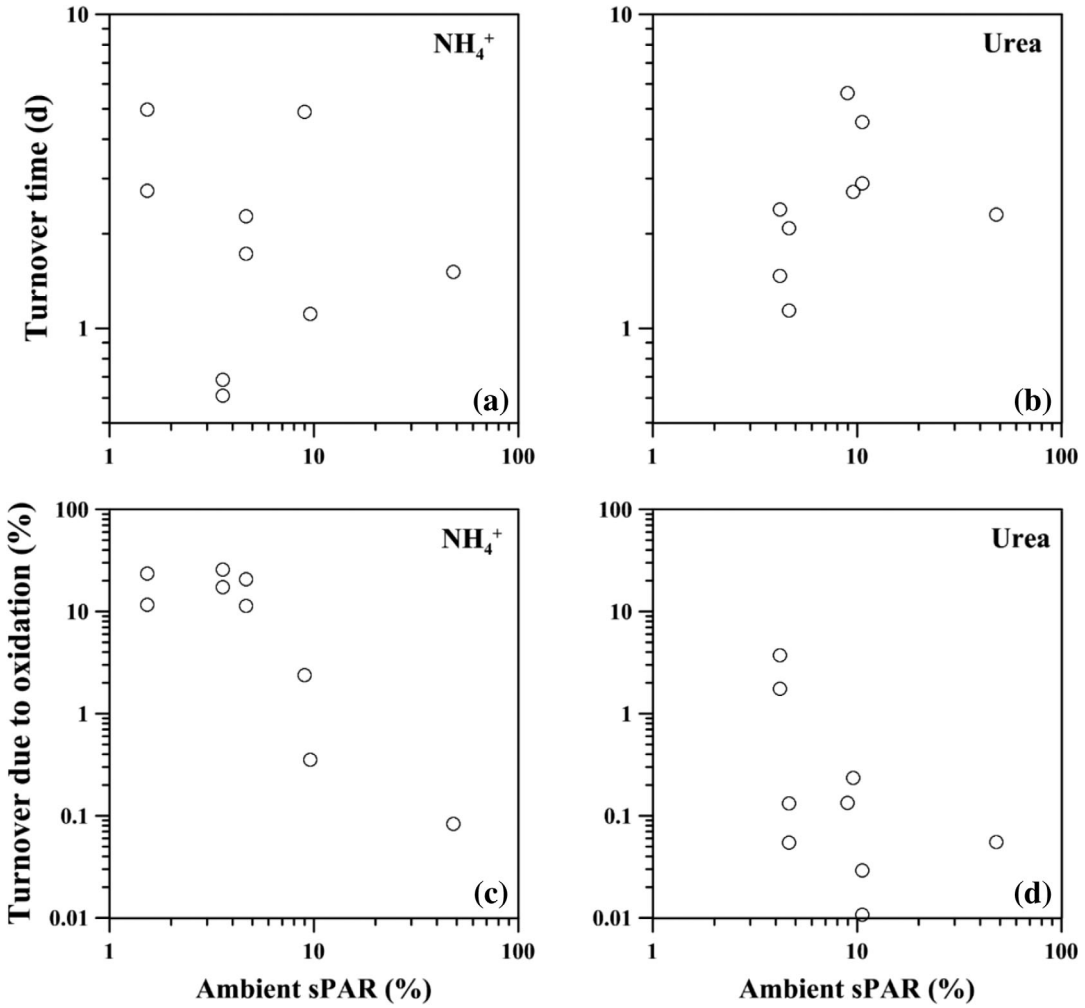

Fig. 6. Estimated turnover time of **(a)** NH_4^+ and **(b)** urea, and the fractional contribution (%) to turnover with respect to **(c)** NH_4^+ oxidation and **(d)** urea-N oxidation as a function of ambient light.

turnover time of NH_4^+ and urea (2.6 ± 2.0 vs. 2.8 ± 1.4 d) with respect to uptake may also point to the importance of urea, as previous studies (e.g., McCarthy 1972; Harrison et al. 1985; Berman and Bronk 2003; Solomon et al. 2010). The abundant urea transporters detected by proteomic analysis in the same cruise (Li et al. 2018) was also supportive of the importance of urea.

In contrast to uptake, the R_m values for urea-N oxidation were substantially lower than those for NH_4^+ oxidation (one-way ANOVA, $p = 0.018 < 0.05$; $n = 12$), but the K_m values for urea oxidation (63–7506 nmol N L^{-1}) were not significantly different from those of NH_4^+ oxidation (76–2112 nmol N L^{-1}) for all stations (one-way ANOVA, $p = 0.11$, $n = 12$), suggesting urea oxidation rates are probably lower than NH_4^+ oxidation at all substrate level. The higher α for NH_4^+ oxidation (Fig. 7b) further confirmed that nitrifiers may be more inclined to utilize NH_4^+ as a direct energy source, which may also explain the previously reported observations that NH_4^+ oxidation rates were always higher than oxidation rates of urea-N in coastal Georgia and the South Atlantic Bight (Tolar et al. 2017).

Conclusions

This study showed that uptake and oxidation rates of regenerated forms of N were jointly influenced by light and substrate concentrations in the euphotic zone. As light intensified, NH_4^+ and urea-N uptake by phytoplankton were stimulated, whereas their oxidation rates by nitrifiers were suppressed regardless of whether substrate concentrations were limiting or saturated. Light shifted the kinetic parameters, R_m and α, thus modulating the affinity of phytoplankton and nitrifiers for substrates. According to the change in α per unit light intensity and their enhanced photosensitivity under low substrate levels, phytoplankton have a stronger competitive advantage than nitrifiers over a natural diurnal cycle for both NH_4^+ and urea-N, particularly in low substrate conditions

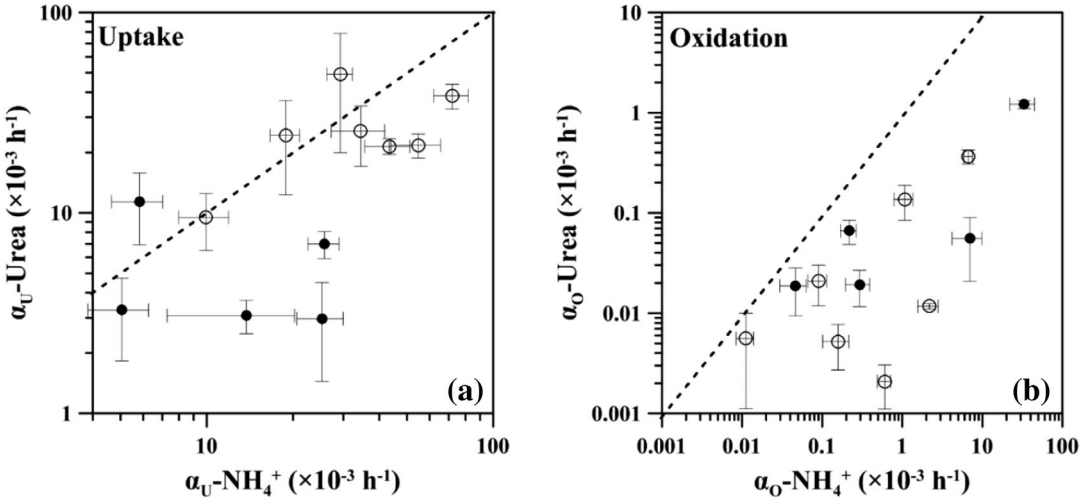

Fig. 7. Scatter plots of specific affinity, α, of **(a)** urea uptake vs. NH_4^+ uptake and **(b)** urea oxidation vs. NH_4^+ oxidation. The open and solid circles represent incubations in the light and in the dark. The dashed line is the line with a slope of 1.

at depth where there was > 5% sPAR and where NO_3^- is vanishingly low. Such differential photosensitivity may be important in contributing to the success of phytoplankton as the main contributor in turning over NH_4^+ and urea-N and in maintaining regenerated production in the surface ocean. Moreover, this study has shown, consistent with previous findings, that urea-N may be of equal importance as NH_4^+ for phytoplankton growth in the euphotic ocean, suggesting that urea should be included in determination of regenerated production and in *f*-ratio calculations as well. However, urea-N was a less preferable energy source relative to NH_4^+ for nitrifiers at least in the euphotic ocean. This information may contribute to N-based ocean biogeochemical models.

Based on this study, there are many questions that are yet to be resolved, and many opportunities for future studies. The community structure of phytoplankton and nitrifiers needs to be further resolved with respect to the types of relationships shown here. Applications of stable isotope pairing and flow cytometry to extract the light response of individual species from communities may help to resolve taxon-specific N utilization and oxidation rates and are the next step to advance our knowledge of N recycling in the euphotic ocean.

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Conflict of Interest

None declared.

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