Inorganic and organic nitrogen cycling in the Southern California Bight

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Abstract

On the basis of mass balance calculations performed for nitrogen (N) uptake experiments in the Southern California Bight (SCB), it has been suggested that a significant portion of dissolved inorganic N (DIN) uptake results in the production of dissolved organic N (DON). To investigate this process, the fate of ammonium (NH$_4^+$) and nitrate (NO$_3^-$) uptake was quantified within the euphotic zone at three coastal stations in the SCB using $^{15}$N tracer techniques. Several trends in the fate of DIN and the production of DON were observed. First, production of particulate N (PN), from both NH$_4^+$ and NO$_3^-$, was quantitatively more important in near surface waters, while DON release dominated within the nitriline. Second, the percentage of gross N uptake released as DON was generally higher when NO$_3^-$, rather than NH$_4^+$, was the substrate. Third, the percentage of N released as DON was higher at night, relative to the day. Fourth, rates of DON release were significantly correlated to NH$_4^+$ regeneration, suggesting that similar mechanisms are responsible for both processes—presumably grazing. The results of this study indicate that the DON pool is a sink for DIN uptake on the time scale of hours. One implication of this finding is that new production estimates based on $^{15}$NO$_3^-$ uptake rates will likely underestimate particle flux out of the surface layer because the rate of NO$_3^-$ uptake is underestimated due to loss of DO$^{15}$N during the incubation. On time scales of months to years, however, the N that is taken up as NO$_3^-$ and released as DON will likely contribute to export flux via incorporation of the dissolved phase during seasonal mixing into sinking particles or transport. The export of DON on these time scales argues for the use of gross uptake rates to calculate $f$-ratios.

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1. Introduction

The Southern California Bight (SCB) has been the site of research into the marine nitrogen (N) cycle for decades (Eppley et al., 1979a,b; Ward, 1987; reviewed by Eppley, 1992). In the SCB, nutrient regimes range from eutrophic to oligotrophic with intermittent zones of upwelling, making it an ideal area to pursue research questions on new and regenerated production. New production is of particular interest because of its relationship to export flux and the sequestration of carbon (C) in marine sediments over long time-scales (Chester, 2003).

One common way to estimate new production in the SCB and elsewhere is to use $^{15}$N tracer
techniques to quantify NO$_3^-$ uptake rates. Implicit in this approach is the assumption that the uptake of $^{15}$N-label results in the production of particulate N (PN). However, a significant flux into dissolved organic N (DON) during $^{15}$N tracer studies has been hypothesized for decades based on deficits in $^{15}$N mass balances (Glibert et al., 1982; Laws, 1984; Ward et al., 1989; Slawyk et al., 1990). In an extreme case, Eppley and Renger (1992) documented missing $^{15}$N seven-fold greater than the N incorporated into biomass. More recently, several researchers, in a range of environments, have found that an important fate of NO$_3^-$ uptake is DON release, in addition to PN production (Bronk and Glibert, 1991; reviewed by Bronk, 2002; Varela et al., 2003). Prior research findings in the SCB provide evidence that DON release may be quantitatively important in the SCB as well. Ward et al. (1989) used $^{15}$N tracer techniques to measure uptake of NO$_3^-$ and NO$_2^-$ in the SCB and consistently observed a gradual loss of up to 98% of the $^{15}$N-label, added as either NO$_3^-$ or NO$_2^-$, over the course of a 24 h incubation. They hypothesized that a N pool, other than the PN, NO$_3^-$, and NO$_2^-$ pools measured, was a sink for the missing $^{15}$N—a likely candidate was the DON pool. The study presented here was undertaken to address this hypothesis, and to quantify the significance of DON release by planktonic assemblages in the SCB.

1.1. Site description

Two offshore stations, 205 (April 1994) and 305 (October 1992), and one nearshore station, 303 (October 1992), were occupied. The depth of the water at the offshore station occupied in April (33°18’.7” N, 118°9’.6” W, 53 km from shore) and the offshore station occupied in October (33°45’.0” N, 118°47’.0” W, 46 km from shore) was approximately 920 m. The stations were under the influence of the California Current; however, water temperatures at these stations are typically higher than those observed in the main flow of the Current. The depth of the water at the nearshore station (33°35’.0” N, 118°31’.0” W, 5.6 km from shore) was approximately 50 m, and was located over the narrow continental shelf; this shelf region typically has higher chlorophyll a (chl a) and particulate matter concentrations relative to the offshore regions of the SCB (Mullin, 1986).

All of these stations were located within the California Cooperative Oceanic Fisheries Investigations (CalCOFI) program grid, which has included measurements of chl a and primary production since 1969 and measurements of plankton standing stocks since 1974 as part of the Southern California Bight Study. All of the stations were within the region of the “Northern Inshore” grouping defined by Venrick (1998). Venrick (1998) found that diatoms dominated the phytoplankton community in the region of our stations during April 1993 and 1995; we assume that the community composition was similar during our cruises.

The Inner Bight region of the SCB, where these experiments were conducted, has been extensively studied and is characterized annually by oligotrophic conditions as defined by biomass and nutrient data (reviewed in Azam, 1986; Eppley and Holm-Hansen, 1986; Williams, 1986). The water column within the euphotic zone at these sites has two layers; a surface layer, where inorganic N concentrations are near the limit of detection, and a nitracline layer, where NO$_3^-$ concentrations increase significantly with increasing depth.

1.2. Research objectives

The specific objectives of this study were to quantify rates of (1) net and gross NH$_4^+$ and NO$_3^-$ uptake and (2) DON release resulting from uptake of both NH$_4^+$ and NO$_3^-$. We use “DON release” to refer to the production of DON from labeled dissolved inorganic N (DIN), regardless of the mechanism involved. Used in this way, the term “release” does not simply imply passive release by phytoplankton but includes both exudation and grazing losses. These rates were characterized within the two layers of the euphotic zone, the surface layer and the nitracline. To accomplish these objectives, vertical profiles were performed at the three stations during two cruises. Ambient nutrient and chl a concentrations were quantified and N flux rates were measured using $^{15}$N tracer techniques. Additional NH$_4^+$ and NO$_3^-$ uptake experiments were also carried out in 20 L carboys, at an offshore and a nearshore site on the October cruise, to allow a larger set of variables to be measured simultaneously.

2. Methods

2.1. Field sampling

Water was collected at each station using 10 or 30 L Niskin or Go-Flo bottles, with five to six
sample depths chosen to span the range of N and light environments within the euphotic zone (Table 1). The euphotic zone was defined as the surface down to the 1% light depth, which was assumed to be 2.7 times the Secchi depth. Day incubations were initiated approximately at midday, and night incubations were initiated at dusk.

2.2. Ambient conditions

Water from each depth was filtered through precombusted (450°C for 2 h) Whatman GF/F filters. The filter was retained and used to measure the concentration of chl a after grinding the filter in acetone and allowing the ground filter to extract in acetone overnight (Parsons et al., 1984). The filtrate was frozen for later determination of nutrient concentrations; all samples for the project were run within 18 months of collection and generally within 2–3 months. Concentrations of NO$_3^-$ and NO$_2^-$ were measured with a Technicon AutoAnalyzer and concentrations of NH$_4^+$ were measured manually with the phenol/hypochlorite method (Grasshoff et al., 1999). Concentrations of DON, defined as organic N passing through a 0.2 μm Supor filter, were measured with UV oxidation using a 1200 watt Hg vapor lamp, H$_2$O$_2$ as an oxidant, and 18 h of irradiation (Armstrong and Tibbitts, 1968; Bronk et al., 2000). Concentrations of PN and particulate C (PC) were measured on precombusted GF/F filters collected at the end of the incubation; PN and PC filters were analyzed with a Control Equipment CHN Analyzer (Grasshoff et al., 1983).

2.3. N uptake and NH$_4^+$ regeneration rates

Rates of NH$_4^+$ and NO$_3^-$ uptake were measured with $^{15}$N tracer techniques using 0.1 μM additions for both NH$_4^+$ and NO$_3^-$ incubations (Bronk and Ward, 1999); additions were 5–95% of the ambient pool. Experiments were done in 4 L polycarbonate bottles under simulated in situ light conditions, and samples were incubated for 4–6 h in on-deck flow-through incubators (Ward and Bronk, 2001). At the end of each incubation, samples were filtered through precombusted GF/F filters which were subsequently dried at 50°C and ampoulated using the micro-Dumas method (Barsdate and Dugdale, 1965). The PN atom % enrichments were determined using either a Jasco emission spectrometer (model N-150; Fiedler and Proksch, 1975) or a Europa 20/20 mass spectrometer with an ANCA preparatory unit. A subset of PN samples that were run on both instruments had a mean CV of 7.2% with neither instrument consistently producing higher or lower values; these data include between-sample variability and analytical variability. The filtrate from the NH$_4^+$ incubations was collected and frozen for later determination of $^{15}$N atom % enrichment of the NH$_4^+$ using steam distillation (Glibert et al., 1982).

2.4. DON isolation and DON production rates

The DON pool was isolated using the protocol described in Bronk and Ward (1999). Briefly, at the end of each incubation, an aliquot from each of the $^{15}$NH$_4^+$ and $^{15}$NO$_3^-$ incubations was passed through a 0.2 μm Supor filter and frozen for later isolation of the DON pool. In the lab, DON was isolated with a series of chemical manipulations designed to remove the $^{15}$N-labeled inorganic NH$_4^+$ or NO$_3^-$ present in the sample. To isolate the DON pool from NH$_4^+$ incubations, vacuum distillation was used (Glibert et al., 1982). In the case of DON from NO$_3^-$ incubations, filtrate was heated in the presence of

<table>
<thead>
<tr>
<th>Station</th>
<th>Description</th>
<th>Location</th>
<th>Date</th>
<th>Time</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>205</td>
<td>Offshore</td>
<td>33.17° N 118.09° W</td>
<td>19-04-1994</td>
<td>Night</td>
<td>Vertical profile</td>
</tr>
<tr>
<td>205</td>
<td>Offshore</td>
<td>33.17° N 118.09° W</td>
<td>20-04-1994</td>
<td>Day</td>
<td>Vertical profile</td>
</tr>
<tr>
<td>305</td>
<td>Offshore</td>
<td>33.45° N 118.47° W</td>
<td>11-10-1992</td>
<td>Day</td>
<td>Vertical profile</td>
</tr>
<tr>
<td>305</td>
<td>Offshore</td>
<td>33.45° N 118.47° W</td>
<td>12-10-1992</td>
<td>Day</td>
<td>Vertical profile</td>
</tr>
<tr>
<td>305</td>
<td>Offshore</td>
<td>33.45° N 118.47° W</td>
<td>12-10-1992</td>
<td>Night</td>
<td>Vertical profile</td>
</tr>
<tr>
<td>305</td>
<td>Offshore</td>
<td>33.45° N 118.47° W</td>
<td>14-10-1992</td>
<td>Day</td>
<td>20 L carboy</td>
</tr>
<tr>
<td>303</td>
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<td>33.53° N 118.31° W</td>
<td>15-10-1992</td>
<td>Day</td>
<td>Vertical profile</td>
</tr>
<tr>
<td>303</td>
<td>Nearshore</td>
<td>33.53° N 118.31° W</td>
<td>15-10-1992</td>
<td>Day</td>
<td>20 L carboy</td>
</tr>
</tbody>
</table>

*Ambient concentrations were measured but no rate measurements were done.*
DeVarda’s Alloy to convert NO$_3^-$ to NH$_4^+$, which was then lost through volatilization. The remaining DON concentrates were UV oxidized for 18 h (Armstrong and Tibbitts, 1968); after the oxidation, all DON in the sample were in the form of NO$_3^-$. The NO$_3^-$ was reduced to NO$_2^-$ by shaking the sample with spongy cadmium for 1.5 h (Jones, 1984) and then the NO$_2^-$ produced was isolated with the organic extraction method of Olson (1981). The isolated DON, now in the form of an azo dye dissolved in methylene chloride, was concentrated by evaporation in a fume hood, spotted onto a precombusted GF/F filter, and the $^{15}$N atom % enrichment was measured with an emission spectrometer. Recovery of DON from NH$_4^+$ and NO$_3^-$ incubations was 89 $\pm$ 35.4% and 62.0 $\pm$ 21.7%, respectively. The CV for replicate DON atom % analyses was 3.8 $\pm$ 3.3 for NH$_4^+$ incubations and 6.6 $\pm$ 6.7 for NO$_2^-$ incubations.

Atom % enrichments of DON were corrected for possible residual NH$_4^+$ or NO$_3^-$ present in the final isolated DON fraction. Removal of NH$_4^+$ and NO$_3^-$ with the method used is 100% as determined using wet chemical analyses of NH$_4^+$ and NO$_3^-$. However, these wet chemistry analytical methods have limits of detection of $\sim 0.03–0.05 \mu$M. To guarantee that rates of DON release were not overestimated due to a small but analytically undetectable amount of labeled inorganic N remaining in the isolated DON fraction, a correction was performed as described in Bronk and Glibert (1991). The $^{15}$N atom % enrichment of any calculated residual NH$_4^+$ or NO$_3^-$ was taken to be the same as that measured at the end of the incubation.

Individual $^{15}$N incubations were not routinely duplicated for the vertical profiles, though all chemical analyses of the various N concentrations and $^{15}$N atom % enrichments were performed in duplicate or better. A propagation of error analysis was done to estimate the error associated with rate measurements in the vertical profiles (Bevington, 1969); this method provides a conservative estimate of the variance.

2.5. Large carboy experiments

One carboy was set up for each treatment (NH$_4^+$ and NO$_3^-$) using surface seawater at both the offshore and nearshore stations in October. All rate determinations from the 20 L carboy experiments were run in duplicate and subsamples were analyzed with the same procedures used on the samples from the vertical profiles, except with respect to DON isolation (see below). In the 20 L carboys, uptake by cells that passed through the GF/F filter (nominal pore size 0.7 $\mu$m) was also measured. In this size fraction, $^{15}$N-label can be incorporated through direct uptake of $^{15}$N-labeled NH$_4^+$ or NO$_3^-$ or via uptake of recently released $^{15}$N-labeled DON (Bronk and Glibert, 1994). To estimate the uptake of $^{15}$N-label by plankton that passed through the GF/F filter, DON in the GF/F filtrate was isolated and the $^{15}$N atom % enrichment was determined and compared to the DON in the 0.2 $\mu$m filtrate as described in Bronk and Glibert (1994). The $^{15}$N in this fraction would include any $^{15}$N in the DON pool as well as any $^{15}$N in organisms that passed through the GF/F filter. Microscopy confirmed that these organisms were primarily bacteria. To calculate the mass of $^{15}$N present in this bacteria pool, the $^{15}$N present in the DON pool ($<0.2 \mu$m filtrate) was subtracted from the $^{15}$N in the combined DON + bacteria pool (GF/F filtrate) as discussed in Bronk and Glibert (1994). We note that this does not represent total bacterial uptake but only incorporation of label into those cells that were not trapped on the GF/F filter.

In the 20 L carboy samples, DON was isolated using ion retardation resin as described in Bronk and Glibert (1991, 1993). The ion retardation resin (BioRad AG 11 A8) attracts small charged molecules while allowing DON to pass. We note that the manufacturer has changed the production process of the BioRad AG 11 A8 resin such that it now retains variable amounts of DON; neither the original resin nor the resin we used in this study retained DON (reviewed by Bronk, 2002).

2.6. Nitrogen rate calculations

Net uptake rates of NH$_4^+$ and NO$_3^-$ were calculated according to Dugdale and Goering (1967) with NH$_4^+$ uptake rates corrected for isotopic dilution (Glibert et al., 1982). Rates of NH$_4^+$ regeneration were calculated according to Glibert et al. (1982). To calculate gross NH$_4^+$ and NO$_3^-$ uptake rates, the gross atom % enrichment of the PN, which included $^{15}$N measured in both the PN and the extracellular DON pools, was calculated (Bronk et al., 1994). In Bronk et al. (1994) rates of gross N uptake and DON release were corrected for the small amount of N released as DON during the incubation. To make this correction, an iterative process was used to first estimate DON release...
without the correction, and then this estimate was used to further refine the DON release rate. This correction was found to be insignificant and so was not done here or on other recently published studies including Bronk et al. (1998), Bronk and Ward (1999), and Ward and Bronk (2001). To calculate the final gross uptake rate, the gross PN atom % enrichment was substituted for the net PN atom % enrichment used in the traditional uptake equation (Bronk et al., 1994, 1998). The rate of DON release was determined as the difference between the gross and net uptake rate of \( \text{NH}_4^+ \) or \( \text{NO}_3^- \) (Bronk et al., 1994, 1998). Note that this method of calculation (Bronk et al., 1994) is identical to the later protocol introduced by Slawyk et al. (1998) to calculate the rate of DIN loss \( (\rho_{\text{DIN}}) \). The following equations were used in the calculations:

\[
\text{Net uptake rate} = \rho = \frac{\text{PN at} \%}{\text{DIN at} \% \times \text{Time}} \times [\text{PN}],
\]

\[
\text{Gross uptake rate} = \rho_G = \frac{(\text{PN at} \% \times [\text{PN}]) + (\text{DON at} \% \times [\text{DON}])}{\text{DIN at} \% \times \text{Time}},
\]

\[
\text{DON release rate} = \rho_G - \rho = \frac{\text{DON at} \% \times [\text{DON}]}{\text{DIN at} \% \times \text{Time}},
\]

where PN, DIN, and DON at % are the \(^{15}\text{N}\) atom % enrichments of PN, DIN and DON pools, respectively. Time is the incubation time. Brackets [ ] denote concentrations. Daily rates were calculated as the day rate multiplied by 14 h (light period) plus the night rate multiplied by 10 h (dark period).

We investigated the use of \(^{15}\text{N}\) mass balances as a way of estimating rates of gross uptake and DON release as an alternative to the labor intensive, and therefore expensive, process of quantifying the rates directly. In this approach, the \(^{15}\text{N}\) in the PN and substrate (\( \text{NH}_4^+ \) or \( \text{NO}_3^- \)) pools were summed and compared to the \(^{15}\text{N}\) added at the start of the experiment. The assumption was made that any missing \(^{15}\text{N}\) was transferred to the DON pool, and a DON atom % and a corrected PN atom % enrichment were then calculated in the same fashion as if \(^{15}\text{N}\) in the DON pool had been measured directly (Bronk and Ward, 1999). This procedure produced gross uptake rates that were overestimated by a mean factor of 4.1, relative to gross uptake rates measured directly, when all incubations were combined in the analysis. Similarly, estimates of DON release rates calculated by mass balance were significantly overestimated in all cases (mean factor of 15). We conclude that \(^{15}\text{N}\) mass balances cannot be used to estimate rates of gross N uptake or DON release.

3. Results

Vertical profiles of ambient N concentrations, rates of net and gross N uptake, DON release, and \( \text{NH}_4^+ \) regeneration are presented below for all three stations. These results are discussed in relation to characteristics in the surface layer (\( \sim \text{upper 25 m} \)) in contrast to characteristics within the nitriline, the deepest two sample points at each site. Results are also presented from \(^{15}\text{NH}_4^+\) and \(^{15}\text{NO}_3^-\) incubations in 20 L carboys conducted in October.

3.1. Vertical profiles of ambient conditions

Concentrations of \( \text{NO}_3^- \) and \( \text{NO}_2^- \) were depleted in the upper \( \sim 20\text{m} \) at all three study sites (Fig. 1A–F). The top of the nitracline was at 27 m at the offshore station in April, 23 m at the offshore station in October, and 20 m at the nearshore station during this study, based on more detailed vertical profiles (Fig. 1 or data not shown). Concentrations of \( \text{NH}_4^+ \) were very low but measurable in the near-surface waters in all vertical profiles. At the offshore station in April, \( \text{NH}_4^+ \) concentrations increased several-fold within the nitracline coincident with increases in \( \text{NO}_2^- \) concentrations (Fig. 1F and I). Concentrations of DON were relatively constant throughout the water column at all three stations, ranging from 4.7 to 7.3 \( \mu \text{M} \) (Fig. 1J–L). A deeper, more detailed profile during the day at the offshore station in October showed additional variability in the DON concentrations above the nitracline and a clear subsurface accumulation of DON (Fig. 2).

3.2. Vertical profiles of N flux rates

At both offshore stations, rates of gross \( \text{NO}_3^- \) uptake and DON release increased several-fold within the nitracline (Figs. 3 and 4). The dominant N form producing biomass (i.e. PN) in both April and October was \( \text{NH}_4^+ \). Net \( \text{NH}_4^+ \) uptake rates averaged 5 and 12 times higher than parallel net \( \text{NO}_3^- \) uptake rates at the offshore stations in April.
and October, respectively (Figs. 3 and 4). Note that NH$_4^+$ uptake rates have been corrected for isotope dilution while NO$_3^-$ uptake rates have not; if nitrification were occurring in the water column, the nitrate uptake rate would be underestimated, thereby contributing to the difference between NH$_4^+$ and NO$_3^-$ uptake. At all sites, the percentage of gross uptake released as DON tended to increase with depth. In contrast to the offshore stations, DON release from NH$_4^+$ exceeded its release from NO$_3^-$ at the nearshore station, while net uptake of NH$_4^+$ peaked at 20 m, DON release remained high at depths greater than 20 m (Fig. 5).

Flux rates were integrated within the upper ~23 m (depending on the profile as noted above) and then within the nitracline to the base of the euphotic zone, defined as the 1% light depth and represented by the deepest sample collected at each profile. Several trends emerged. First, the production of PN was higher in the surface layer while the production of DON was higher in the nitracline (Fig. 6). Second, a higher percentage of N was released as DON when NO$_3^-$ was the substrate, relative to NH$_4^+$ (Fig. 6). Third, in seven of eight cases, the percentage of DON released from both NH$_4^+$ and NO$_3^-$ was higher at night than during the
Specific uptake rates, for both NH$_4^+$ and NO$_3^-$/C0, were also higher during the day than at night, except for the 1 m NH$_4^+$ uptake sample in April (data not shown). Fourth, with all rates taken together, rates of net NH$_4^+$ uptake were positively correlated to gross NH$_4^+$ uptake ($r^2 = 0.92$), but rates of net NO$_3^-$ uptake did not correlate well with gross NO$_3^-$ uptake. However, when only the surface layer was considered, net and gross NO$_3^-$ uptake rates were closely correlated ($r^2 = 0.91$) at the offshore station in April and the nearshore station in October (Figs. 4 and 6).

In general, NH$_4^+$ regeneration exceeded both net and gross NH$_4^+$ uptake at all depths and the ratio of regeneration to uptake increased with depth (data not shown). Rates of NH$_4^+$ regeneration were greater than rates of DON release at all depths, representing upwards of 86% of the N released within the euphotic zone (data not shown). The ratio of integrated NH$_4^+$ regeneration: DON release, however, was higher in the surface waters (19.6–31.6) than within the nitratacline (6.5–6.6). Rates of NH$_4^+$ regeneration and DON release, resulting from NH$_4^+$ uptake, were significantly correlated ($r^2 = 0.73; n = 19; p < 0.001$).

3.3. Large carboy experiments

Inorganic N concentrations were three times higher in the nearshore carboys than in the offshore carboy (Table 2). Concentrations of DON, chl a, and PN were similar at both stations, but the C:N ratio of the particulate material was significantly higher nearshore (Table 2). Consistent with the vertical profiles (Fig. 4 and 5), gross uptake rates of NH$_4^+$ were significantly higher than NO$_3^-$ at both sites (Table 3). Rates of NH$_4^+$ and NO$_3^-$ uptake into the bacterial size fraction, defined as the 0.2–0.7 µm fraction, were over three times higher offshore (Table 3). Bacterial uptake of NH$_4^+$ and NO$_3^-$, as a percentage of total NH$_4^+$ and NO$_3^-$ uptake (defined as uptake into cells >0.2 µm), was seven times higher offshore (Table 3). More DON was released as a result of NH$_4^+$ uptake, relative to NO$_3^-$ uptake, at both sites (Table 3). Rates of DON release were similar in magnitude at both sites though the percentage of gross N uptake that was released as DON was two times higher offshore than nearshore. In contrast, rates of NH$_4^+$ regeneration were similar at both sites (Table 3).

3.4. Turnover times

Turnover times estimated from depth profile experiments for DIN, PN and DON ranged from less than a day to several weeks. In general, turnover times were shorter in October than in April (Table 4). The NH$_4^+$ pool (and NO$_3^-$ in October) had the shortest turnover times and the DON pool had the longest (Table 4).

4. Discussion

The objectives of this study were to quantify rates of net and gross NH$_4^+$ and NO$_3^-$ uptake and DON release in the SCB. The results show that, in all experiments at all depths, DON was an important fate for DIN uptake. In this section, we compare our results to those from other systems, highlight the importance of grazing in DON release, present evidence for bacterial DIN and DON uptake, and discuss how DON release affects export flux and its measurement.

4.1. DON release across systems

In this study, the percentage of N uptake released as DON was generally higher in NO$_3^-$ incubations compared to NH$_4^+$ incubations, and DON release as a percentage of gross N uptake was relatively low in the surface layer but increased significantly within the nitratacline; both observations are consistent with findings in Monterey Bay, CA (Bronk and Ward,
In the Gulf of Lyons (NW Mediterranean), however, the magnitude of DON release decreased with depth and did not vary consistently with N substrate—an average of 26% and 24% of NH₄⁺ and NO₃⁻/C₀, respectively, was taken up and released as DON (Diaz and Raimbault, 2000). Furthermore, in the Gulf of Lyons, net and gross uptake rates were closely correlated in both NH₄⁺ (slope = 0.74; \( r^2 = 0.95 \)) and NO₃⁻/C₀ incubations (slope = 0.79; \( r^2 = 0.93 \); Diaz and Raimbault, 2000). In the SCB, rates of net and gross NH₄⁺ uptake were also closely correlated (slope = 0.82; \( r^2 = 0.92 \)), but a linear relationship was not observed between net and gross NO₃⁻ uptake. If only the surface waters are considered for the SCB data, however, the relationship was much closer to linear (\( r^2 = 0.91 \)) providing evidence that within the nitracline, NO₃⁻ uptake and biomass production were uncoupled.

At both offshore sites where day and night rates were measured, the percentage of gross N uptake resulting in the production of DON was higher at night than during the day (Fig. 7). A similar trend was also observed during three diel studies in Chesapeake Bay where rates of DON release were higher at dusk than at other times of the day (Bronk et al., 1998). In addition to the vertical profiles and large carboy studies reported here, we also did size-fractionation experiments on different days of each cruise and reported the results previously (Ward and Bronk, 2001). In the size-fractionation studies, DON release rates were higher at night than during the day in seven of 10 experiments in October but in

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**Fig. 3.** Vertical profiles of day and night NH₄⁺ gross (●) and net (○) uptake rates and NO₃⁻ gross (●) and net (○) uptake rates and DON release resulting from uptake of NH₄⁺ (○) and NO₃⁻ (●) at Station 205 in April. Error bars denote standard deviations; where error bars are not seen, errors are smaller than the symbols.
only one of seven experiments in April (Ward and Bronk, 2001). We also note dramatic differences in concentrations and rates measured on the days when vertical profiles were performed when compared to days when the size-fractionation experiments were done. The ratio of the concentration or rate measured during the size-fractionation experiment divided by the concentration or rate measured at the closest depth during the vertical profile varied from a mean of 1.2–6.0 for NO$_2^-$ concentrations, 1.5–6.3 for NO$_3^-$ concentrations, 1.2–3.3 for DIN uptake, 5.2–15.1 for DON release and 0.6–3.0 for NH$_4^+$ regeneration. These differences likely reflect changes in the water column, including an apparent shallowing of the nitracline, as well as the large variability inherent in biological rate measurements. Similar to DON release rates, NH$_4^+$ regeneration rates were also higher at night than in the day during the April cruise presented here; an incomplete NH$_4^+$ regeneration profile precluded any day/night comparison in October. Ward and Bronk (2001) found that NH$_4^+$ regeneration was higher at night than during the day in three of four size-fractionation experiments performed in the SCB in April and five of six experiments in October. DON release, resulting from NH$_4^+$ uptake, and NH$_4^+$ regeneration were also found to be significantly correlated ($r^2 = 0.73; n = 19; p < 0.001$). The Model II regression slopes suggest that DON release was approximately 40% of the measured NH$_4^+$ regeneration rate. Similar trends were also observed during our size-fractionation
experiments in the SCB and Monterey Bay (Ward and Bronk, 2001) as well as in a study off the coast of Spain (Varela et al., 2003). In Japanese coastal waters, DON release was approximately 59% of NH$_4^+$ regeneration (Hasegawa et al., 2000b).

Fig. 5. Vertical profiles of day NH$_4^+$ gross (■) and net (○) uptake rates and NO$_3^-$ gross (●) and net (○) uptake rates and DON release resulting from uptake of NH$_4^+$ (○) and NO$_3^-$ (●) at nearshore Station 303 in October; uptake rates were not measured at night. Error bars denote standard deviations; where error bars are not seen, errors are smaller than the symbols.

Fig. 6. The percentage of integrated gross nitrogen uptake that resulted in the production of PN (■) or DON (□) in the surface layers and within the nitracline layer for the offshore vertical profiles in April and October.

Fig. 7. The percentage of integrated gross nitrogen uptake that resulted in the production of DON in the surface layers and within the nitracline during the day (○) and at night ( ■). Error bars represent a propagation of error analysis.
Table 2
Ambient conditions in the 20 L carboys containing offshore and nearshore water from 10 m in the Southern California Bight in October

<table>
<thead>
<tr>
<th></th>
<th>Offshore</th>
<th>Nearshore</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_4^+$ (μM)</td>
<td>0.08 ± 0.00</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>NO$_3^−$/NO$_2^−$ (μM)</td>
<td>0.03 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>DON (μM)</td>
<td>7.1 ± 0.4</td>
<td>6.7 ± 0.05</td>
</tr>
<tr>
<td>chlorophyll a (μg L$^{-1}$)</td>
<td>0.23 ± 0.01</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>PC (μmol C L$^{-1}$)</td>
<td>9.17 ± 0.50</td>
<td>15.4 ± 0.50</td>
</tr>
<tr>
<td>PN (μmol N L$^{-1}$)</td>
<td>1.14 ± 0.11</td>
<td>1.25 ± 0.13</td>
</tr>
<tr>
<td>C:N of particulate</td>
<td>8.0</td>
<td>12.3</td>
</tr>
</tbody>
</table>

Table 3
Gross and net nitrogen uptake and DON release rates for the offshore and nearshore 20 L carboy experiments performed in the Southern California Bight in October

<table>
<thead>
<tr>
<th></th>
<th>Offshore</th>
<th>Nearshore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross uptake (&gt;0.7 μm) (nM h$^{-1}$)</td>
<td>39.1 ± 0.4</td>
<td>71.3 ± 1.0</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>4.1 ± 0.2</td>
<td>8.7 ± 0.1</td>
</tr>
<tr>
<td>Gross bacterial uptake (0.2–0.7 μm) (nM h$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>1.9 ± 0.9</td>
<td>0.4 ± 0.7</td>
</tr>
<tr>
<td>NO$_3^−$</td>
<td>0.7 ± 0.7</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Bacterial uptake: total uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>4.7%</td>
<td>0.6%</td>
</tr>
<tr>
<td>NO$_3^−$</td>
<td>14.7%</td>
<td>2.4%</td>
</tr>
<tr>
<td>DON release (nM h$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>from NH$_4^+$ uptake</td>
<td>10.3 ± 0.4</td>
<td>11.9 ± 0.6</td>
</tr>
<tr>
<td>from NO$_3^−$ uptake</td>
<td>2.4 ± 0.2</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>DON release: Gross uptake in the 0.2 μm fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>25.1%</td>
<td>16.6%</td>
</tr>
<tr>
<td>NO$_3^−$</td>
<td>50.0%</td>
<td>24.7%</td>
</tr>
<tr>
<td>NH$_4^+$ regeneration</td>
<td>23.4 ± 2.8</td>
<td>27.8 ± 3.4</td>
</tr>
</tbody>
</table>

Standard deviations come from replicate bottles.

4.2. Evidence for the importance of grazing to DON release

The robust correlations between DON release and NH$_4^+$ regeneration, and the tendency for higher DON production rates at night, suggest that both NH$_4^+$ regeneration and DON release are likely influenced, on some level, by the same process—the most likely of which is grazing (reviewed in Nagata, 2000 and Bronk, 2002; Hasegawa et al., 2000a, 2001). The importance of grazing in DOM production was predicted in a study by Jackson and Eldridge (1992) where inverse modeling was used to estimate the rates of N and C uptake and release, DON and DOC production, and grazing in the microbial and protozoan size fractions. The model indicates that grazing is responsible for a major flux of material from phytoplankton through the detrital pool and from there into DOC and DON. Jackson and Eldridge (1992) conclude that exudation or passive DON release is not important, but that grazing-induced fluxes into DOM are at least as large as direct phytoplankton biomass consumption by grazers. Unfortunately, there are few direct measurements of DON release during grazing though there are a number of studies that measure grazer mediated release of DOC (Dagg, 1974; Lampert, 1978; Urban-Rich, 1999). One study in Japanese coastal waters that was specific to DON showed that, when chl a concentrations are high (>6 μg chl L$^{-1}$), the addition of copepods increases the rate of DON release, but the relationship does not hold at lower chl a levels (Hasegawa et al., 2000a). In another study, Hasegawa et al. (2000b) used an isotope dilution approach to demonstrate that grazing by microzooplankton was an important source of DON.

It is important to stress that DON release during grazing is separated from release during excretion where dissolved organic products are released after ingestion. In our experiments the flow of $^{15}$N-label was traced into the phytoplankton and then released from the cells either directly or as a result of sloppy feeding. We assumed that the shorter incubations times used (~3 h) would largely prevent us from including excretion in our release rates because there would not be enough time for the $^{15}$N-label to be taken up by a phytoplankton cell, consumed by a grazer, and then metabolized and excreted; we are unaware of data supporting or refuting this assumption, however. If this assumption is not correct, then a portion of the release we measured may be due to excretion. Indeed, results from excretion studies are similar to what we observed. At the BATS station, for example, increases in concentrations of DON and NH$_4^+$ were monitored over time to estimate net release rates. In experiments with copepods, DON release is 21% of the total N excreted (DON and NH$_4^+$ release combined). Combining all data from the SCB study presented here, DON was 11.5 ± 10.0% of total N regeneration.
4.3. Bacterial N uptake

In this study, the amount of $^{15}$N recovered in the bacterial fraction was used to estimate rates of N uptake into that fraction. Bronk and Glibert (1994) showed that a significant portion of $^{15}$N-label can be incorporated by the 0.2–0.7 \( \mu \)m size fraction, assumed to include primarily heterotrophic bacteria. The incorporation can occur via direct uptake of inorganic N or via uptake of recently released $^{15}$N-labeled DON (Bronk and Glibert, 1994). In general, the amount of N that accumulates in the bacterial fraction is usually larger when NH$_4^+$, rather than NO$_3^-$, is the substrate (Bronk and Glibert, 1994). This trend likely reflects the preference by bacteria for NH$_4^+$ over NO$_3^-$ that has been observed in a number of studies (Kirchman, 1994; reviewed in Kirchman, 2000). In the offshore large carboy experiment presented here, only slightly more $^{15}$N could have come from direct uptake of $^{15}$NO$_3^-$ or from recently released DO$^{15}$N. DON release rates were five times higher when NH$_4^+$ was the substrate resulting in higher concentrations of recently released DON in NH$_4^+$ incubations. Therefore, the larger amount of $^{15}$N that appeared in the bacterial fraction when NO$_3^-$ was the substrate likely reflects higher rates of bacterial utilization of NO$_3^-$ rather than recently released DON. Bacterial uptake is increasingly recognized as an important sink for NO$_3^-$ in marine surface waters (Wheeler and Kirchman, 1986; Horrigan et al., 1988; Kirchman and Wheeler, 1998; Allen et al., 2002).

4.4. DON release and export flux

The recognition that appreciable amounts of N uptake result in the production of DON rather than PN is important when considering export production. With the exception of isolated sites of downwelling and during seasonal overturn, N must be packaged into particles of sufficient size and density to sink out of the euphotic zone. Transfer of N to a dissolved fraction, particularly one with longer turnover times such as DON (Table 4), will retain N within the more biologically active surface waters for a longer period of time. Here we focus on three

<table>
<thead>
<tr>
<th>Cruise</th>
<th>Depth (m)</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>DON</th>
<th>PN</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>1</td>
<td>0.75</td>
<td>0.09</td>
<td>89.7</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.50</td>
<td>0.29</td>
<td>30.2</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>1.50</td>
<td>0.00</td>
<td>156.9</td>
<td>2.61</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>6.90$^a$</td>
<td>0.65</td>
<td>7.1$^a$</td>
<td>0.57$^a$</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>11.17</td>
<td>0.45</td>
<td>19.2</td>
<td>1.16</td>
</tr>
<tr>
<td>mean ± std</td>
<td>4.37 ± 4.53</td>
<td>0.30 ± 0.26</td>
<td>60.6 ± 62.5</td>
<td>1.36 ± 0.78</td>
<td></td>
</tr>
<tr>
<td>October</td>
<td>1</td>
<td>&lt; 0.01</td>
<td>0.21</td>
<td>46.8</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>&lt; 0.01</td>
<td>0.14</td>
<td>35.1</td>
<td>1.19</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>0.37</td>
<td>0.22</td>
<td>32.1</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>&lt; 0.01</td>
<td>0.54</td>
<td>24.8</td>
<td>1.63</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.53</td>
<td>0.40</td>
<td>7.1</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>0.45</td>
<td>0.22</td>
<td>3.3</td>
<td>0.28</td>
</tr>
<tr>
<td>mean ± std</td>
<td>0.30 ± 0.35</td>
<td>0.31 ± 0.15</td>
<td>24.9 ± 16.8</td>
<td>0.94 ± 0.45</td>
<td></td>
</tr>
</tbody>
</table>

$^a$NO$_3^-$ uptake and DON release from NO$_3^-$ uptake were from night experiments only.

Table 4
Turnover times of NH$_4^+$, NO$_3^-$, and dissolved organic nitrogen (DON) estimated during two cruises in the Southern California Bight

Turnover times of NH$_4^+$ and NO$_3^-$ were estimated using gross uptake rates. DON turnover times were estimated by combining rates of DON release estimated in incubations with NH$_4^+$ and NO$_3^-$. Turnover times for particulate nitrogen (PN) were calculated using ambient PN concentrations and the combined gross NH$_4^+$ and NO$_3^-$ daily uptake rates.

Turnover times of NH$_4^+$ over NO$_3^-$ that has been observed in a number of studies (Kirchman, 1994; reviewed in Kirchman, 2000). In the offshore large carboy experiment presented here, only slightly more $^{15}$N-label was incorporated into the bacterial fraction when NO$_3^-$ was the substrate relative to NH$_4^+$; the recovered $^{15}$N could have come from direct uptake of $^{15}$NO$_3^-$ or of recently released DO$^{15}$N. DON release rates were five times higher when NH$_4^+$ was the substrate resulting in higher concentrations of recently released DON in NH$_4^+$ incubations. Therefore, the larger amount of $^{15}$N that appeared in the bacterial fraction when NO$_3^-$ was the substrate likely reflects higher rates of bacterial utilization of NO$_3^-$ rather than recently released DON. Bacterial uptake is increasingly recognized as an important sink for NO$_3^-$ in marine surface waters (Wheeler and Kirchman, 1986; Horrigan et al., 1988; Kirchman and Wheeler, 1998; Allen et al., 2002).
aspects of DON release that will affect export production and its estimation: the relative magnitudes of DON release from NH$_4^+$ versus NO$_3^-$, the fate of recently released DON, and the location of DON release in the water column.

The $f$-ratio is the ratio of new production (commonly assumed to be the rate of NO$_3^-$ uptake) divided by the sum of new and regenerated production (commonly assumed to be the rate of NO$_3^-$ uptake plus NH$_4^+$ uptake and, at times, urea uptake; Dugdale and Goering, 1967; Eppley and Peterson, 1979). Though the common application of the $f$-ratio tends to oversimplify a very complex system it is still a useful index for quickly describing the reliance of a system on different N forms and as a relative indicator of export flux. DON release is important to consider when calculating the $f$-ratio because it will affect the balance of new versus regenerated production.

In this study, more biomass was produced as a result of NH$_4^+$ utilization, while NO$_3^-$ uptake yielded relatively more DON production at all stations. At our offshore sites, $f$-ratios increased by up to a factor of four when gross uptake rates were used, reflecting the substantial loss of DON observed when NO$_3^-$ was the substrate. Three earlier studies focused on NO$_3^-$ uptake rates and new production at the same sites occupied in this study: Eppley and Renger (1986), Ward et al. (1989), and Small et al. (1989). The rates measured using $^{15}$N tracers and 24 h time-courses were based on the accumulation of $^{15}$N-label in the cells and are therefore approximations of net NO$_3^-$ uptake rates (Eppley and Renger, 1986; Ward et al., 1989)). Rates in these studies that were based on NO$_3^-$ concentration changes measured with chemiluminescent detection, however, measured NO$_3^-$ disappearance regardless of the ultimate fate of the N and are, therefore, approximations of gross NO$_3^-$ uptake rates, in the absence of nitrification (Eppley and Renger, 1986; Small et al., 1989). New production estimates made using net uptake rates were 8.3–10.5 mg N m$^{-2}$ d$^{-1}$ compared to new production estimates of 17.8–55.5 mg N m$^{-2}$ d$^{-1}$ made using gross uptake rates (data pooled from Eppley and Renger, 1986; Ward et al., 1989; Small et al., 1989; this study).

The question then becomes which $f$-ratio is the appropriate one to use in discussions of export flux. We submit that the answer depends on the fate of the released DON and the time scale under consideration. The primary fate of DON is generally thought to be bacterial utilization. Bacteria do not have appreciable sinking rates such that any N incorporated into their biomass would be retained within the surface layer. On the order of days to weeks, therefore, it would be unlikely that the N taken up as NO$_3^-$ and released as DON would contribute to export flux. As a result, the traditionally determined net uptake rates, which measure only PN production, would be the appropriate rates to use in calculating the $f$-ratio. On the order of months to years, however, it is much more likely that N, released as DON during NO$_3^-$ uptake, would be exported to depth. In specific, DON could be transported to depth in three ways—diffusion-driven flux, seasonal overturn and uptake into sinkable particles.

In our deeper more detailed casts, we observed a surface accumulation of DON particularly at the base of the euphotic zone (Fig. 2). This accumulation is similar to the “semi-labile” pool observed in DOC profiles (Kirchman et al., 1993; Carlson and Ducklow, 1995) and its presence suggests that DON uptake and production are uncoupled. A surface enrichment of DON has been observed in a large number of environments (reviewed in Bronk, 2002), and elevated DON concentrations at the surface suggest that DON can be exported to depth during seasonal mixing (Toggweiler, 1989; Hopkinson et al., 1997; Hansell, 2002). Vidal et al. (1999) calculated vertical gradient-driven fluxes of DON in the equatorial Atlantic using vertical profiles of DON concentrations and found that surface DON did appear to be transported to depth.

Incorporation of DON into sinkable particles is another important mechanism for NO$_3^-$ uptake released as DON to reach the deep ocean. Historically, it was believed that phytoplankton production was fueled by inorganic N and that DON did not contribute significantly to phytoplankton N nutrition. Although many phytoplankton species were known to have the ability to take up a variety of organic compounds (Berg et al., 1997; reviewed by Antia et al., 1991, Bronk, 2002, and Berman and Bronk, 2003) most studies were done using cultures and large (mM) substrate additions (reviewed in Bronk and Flynn, in press). At the concentrations of DON found in nature, however, it was thought that phytoplankton could not compete with bacteria for the organic substrates. More recent work, however, has shown that many phytoplankton can and do obtain N from organic substrates (reviewed in Bronk, 2002; Berman and Bronk, 2003). If recently
released DON is reincorporated into autotrophic biomass than export out of the surface layer via direct sinking or repackaging into fecal pellets becomes much more likely. In this regard, DON differs markedly from DOC because C can be respired, significantly reducing the likelihood it will be packaged into sinkable particles.

DON production would be especially significant if it occurred deeper in the water column. Diatoms, which have appreciable sinking rates, are common at the base of the euphotic because of the abundant NO$_3^-$ (Goldman, 1988). If diatoms could also use recently released DON as a N source, then this is another mechanism for getting DON exported from the surface layer. In contrast, if diatoms are losing a substantial amount of the N they take up as DON, then the affect on the magnitude of N flux out of the euphotic could be great because this N is not packaged into sinkable particles (i.e. diatom biomass) but instead accumulates at the base of the euphotic zone (e.g. Fig. 2). The accumulated DON could, however, be transported to depth during seasonal overturn (Hansell, 2002). The high rates of DON release in the nitracline measured (Fig. 4) and the apparent DON accumulation (Fig. 2) observed supports the contention that DIN uptake at the base of the photic zone does not translate wholly into sinkable PN.

In summary, on the time scale of months to years, DON release, which occurs as a result of new N uptake, could contribute to export flux via incorporation into sinkable particles or seasonal mixing. As a result, gross N uptake rates should be used when calculating the $f$-ratio.

5. Conclusions

A number of studies investigating N uptake and new production in the SCB found circumstantial evidence for substantial DON release including missing $^{15}$N in isotope mass balances (Eppley and Renger, 1986; Ward et al., 1989). Here we provide direct evidence for DON release and its relative magnitude throughout the euphotic zone. When we incorporate our findings into what is known about N cycling in the SCB, the picture emerging is one where N uptake in the surface waters is dominated by NH$_4^+$ uptake (Eppley et al., 1979a, b; this study) and the primary fate of DIN uptake is PN production. NH$_4^+$ is the most important regenerated N form (the ratio of NH$_4^+$ regeneration to DON release was 20–30) and the DON pool is turning over on the order of weeks to months. Deeper in the water column, approaching the nitracline, the importance of NO$_3^-$ increases as a N source, and the dominant fate of NO$_3^-$ uptake is DON production resulting in an accumulation of DON, which has a turnover time on the order of days. NH$_4^+$ was still the most important regenerated N form but the ratio of NH$_4^+$ regeneration to DON release decreased to six. The strong correlation between NH$_4^+$ regeneration and DON release and the higher rates of DON release measured point to grazing as an important mechanism for DON release. Finally, in a broader sense, significant production of DON during standard incubation experiments implies that uptake rates based solely on $^{15}$N accumulation in PN underestimate the rate of phytoplankton N assimilation.

Acknowledgments

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References


