Nitrogen isotopic evidence for a shift from nitrate- to diazotroph-fueled export production in the VAHINE mesocosm experiments

Angela N. Knapp[1*], Sarah E. Fawcett[2,3], Alfredo Martínez-Garcia[4], Nathalie Leblond[5], Thierry Moutin[5], and Sophie Bonnet[5]

[1]Earth, Ocean, and Atmospheric Science Department, Florida State University, 117 N Woodward AVE, Tallahassee, FL, 32306, USA

[2]Department of Geosciences, Guyot Hall, Princeton University, Princeton, NJ 08544, USA

[3]Department of Oceanography, University of Cape Town, Rondebosch, 7701 South Africa

[4]Max Plank Institute for Chemistry, Hahn-Meitner-Weg 1, 55128 Mainz, Germany

[5]Mediterranean Institute of Oceanography (MIO) – IRD/CNRS/Aix-Marseille University, IRD Noumea, 101 Promenade R. Laroque, BPA5, 98848 Noumea Cedex

*Correspondence to: A.N. Knapp (anknapp@fsu.edu)

Abstract:

In a coastal lagoon with a shallow, 25 m water column off the southwest coast of New Caledonia, large-volume (~50 m^3) mesocosm experiments were undertaken to track the fate of newly fixed nitrogen (N). The mesocosms were intentionally fertilized with 0.8 μM dissolved inorganic phosphorus (DIP) to stimulate diazotrophy. N isotopic evidence indicates that the dominant source of N fueling export production shifted from subsurface nitrate (NO_3^-) assimilated prior to the start of the 23-day experiments to N_2 fixation by the end of the experiments. While the $\delta^{15}N$ of the sinking particulate N (PN_{sink}) flux changed during the experiments, the $\delta^{15}N$ of the suspended PN (PN_{susp}) and dissolved organic N (DON) pools did not. This is consistent with previous observations that the $\delta^{15}N$ of surface ocean N pools is less responsive than that of PN_{sink} to changes in the dominant source of new N to surface waters. In spite of the absence of detectable NO_3^- in the mesocosms, the $\delta^{15}N$ of PN_{sink} indicated that NO_3^- continued to fuel a significant fraction of export production (20 to 60%) throughout the 23-day experiments, with N_2 fixation dominating export after about two weeks. The low rates of organic N export during the first 14 days were primarily supported by NO_3^-, and phytoplankton abundance data suggest that sinking material primarily comprised large diatoms. Concurrent molecular and taxonomic studies indicate that the diazotroph community was dominated by diatom-diazotroph assemblages (DDAs) at this time. However, these DDAs represented a minor fraction (<5%) of the total diatom community and contributed very little new N via N_2 fixation; they were thus not important for driving export production, either directly or indirectly. The unicellular cyanobacterial diazotroph, a *Cyanothece*-like UCYN-C, proliferated during the last phase of the experiments when N_2 fixation, primary production, and the flux of PN_{sink} increased significantly, and $\delta^{15}N$ budgets reflected a predominantly diazotrophic source of N fueling export. At this time, the export flux itself was likely dominated by the non-diazotrophic diatom, *Cylindrotheca closterium*, along with a lesser contribution from other eukaryotic phytoplankton and aggregated UCYN-C cells. Despite comprising a small fraction of the total biomass, UCYN-C was largely responsible for driving export production during the last ~10 days of the experiments both directly (~5 to 22% of PN_{sink}) and through the rapid transfer of its newly fixed N to other phytoplankton; we infer that this newly fixed N was transferred rapidly through the

dissolved N (including DON) and PN_{susp} pools. This inference reconciles previous observations of invariant oligotrophic surface ocean DON concentrations and $\delta^{15}N$ with incubation studies showing that diazotrophs can release a significant fraction of their newly fixed N as some form of DON.

1 Introduction

One of the primary pathways by which carbon dioxide (CO_2) is removed from the atmosphere is via photosynthesis, through which it is converted into organic carbon. In the ocean, this process is known as the "biological pump" since upon death, the phytoplankton that fixed inorganic carbon into biomass in surface waters are eventually transported to depth (either via passive sinking or as a byproduct of grazing), thereby "pumping" the carbon to deep waters where it remains isolated from the atmosphere on hundred to thousand year time scales. In broad regions of the surface ocean, the scarcity of the essential macronutrient, nitrogen (N), limits photosynthesis and thus the capacity of the biological pump to remove CO_2 from the atmosphere (Falkowski, 1997). Consequently, there is considerable interest in quantifying fluxes of N to the ocean, as well as in understanding the fate of that N once it enters the ocean.

In addition to lesser contributions from rivers and atmospheric deposition, the dominant source of N to the ocean is biologically-mediated di-nitrogen (N_2) fixation (Gruber, 2004). Marine cyanobacteria, bacteria, and archaea that can access the abundant dissolved N_2 gas pool as a source of assimilative N are known as diazotrophs and have a competitive advantage over other microbes and phytoplankton that require an exogenous source of N such as nitrate (NO_3^-), ammonium (NH_4^+), and/or dissolved organic N (DON). Several geochemical signals that accumulate in the thermocline of the oligotrophic gyres are thought to result from diazotrophic activity in overlying surface waters. In particular, diazotrophic biomass has elevated N to phosphorus (P) ratios (~25:1 to 50:1) (Holl and Montoya, 2008; Krauk et al., 2006; Kustka et al., 2003; White et al., 2006) relative to typical (i.e., "Redfieldian") marine biomass (~16:1 N:P) (Falkowski, 2000; Redfield, 1958; Sterner and Elser, 2002). The death of diazotrophs and subsequent remineralization of their biomass in the thermocline can cause NO_3^- to phosphate (PO_4^{3-}) concentration ratios >16:1 in regions associated with high rates of N_2 fixation (Gruber and Sarmiento, 1997; Hansell et al., 2004). Combining inventories of elevated subsurface NO_3^-:PO_4^{3-} concentration ratios with timescales over which the signal has accumulated has been used to estimate basin-scale rates of marine N_2 fixation (Deutsch et al., 2001; Eugster and Gruber, 2012; Gruber and Sarmiento, 1997). Additionally, the NO_3^-

accumulating in the thermocline as a result of diazotrophic activity has a N isotopic composition ("$\delta^{15}N$") of ~-2 to 0‰ (Carpenter et al., 1997; Hoering and Ford, 1960; Minagawa and Wada, 1986), which is distinct from that of mean ocean NO_3^-, ~5‰ (Sigman et al., 2009) ("$\delta^{15}N$", where $\delta^{15}N = \{[(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{reference}] - 1\}*1000$, with atmospheric N_2 as the reference). Consequently, regions of the ocean associated with elevated rates of N_2 fixation show an accumulation of low-$\delta^{15}N$ NO_3^- in the same water masses that host elevated NO_3^-:PO_4^{3-} concentration ratios; the accumulation of this low-$\delta^{15}N$ NO_3^- has also been used to estimate basin-scale N_2 fixation rates (Knapp et al., 2008).

While geochemical evidence indicates that the high N:P ratios and low $\delta^{15}N$ of diazotrophic biomass is ultimately incorporated into the upper thermocline of the tropical and subtropical ocean, the pathways by which these geochemical signatures are transferred from the surface to subsurface ocean remain enigmatic. For example, the conspicuous marine diazotroph *Trichodesmium* spp. is thought to be grazed by only a small number of zooplankton (O'Neill and Roman, 1994; Roman, 1978), suggesting that this diazotroph may not be transferred up the food web in the same way as other phytoplankton. Additionally, due to the presence of buoyant gas vacuoles, *Trichodesmium* spp. may not sink as efficiently as other phytoplankton, potentially explaining why its biomass is often not observed in sediment traps (Walsby, 1992). This renders sinking upon death, another common fate of phytoplankton biomass, a less likely pathway by which newly fixed N may leave surface waters.

Instead of being transferred up the food web to higher trophic levels or being removed via sinking, newly fixed N has been thought to play a critical role in supporting the microbial loop through the release of DON and NH_4^+. Programmed cell death (Berman-Frank et al., 2004), grazing (Glibert and Bronk, 1994), and direct release (Capone et al., 1994) have been invoked as mechanisms by which *Trichodesmium* spp. may release DON and NH_4^+ to surrounding waters. While consumption of this diazotroph derived N (DDN) would retain its low-$\delta^{15}N$ signature in the event that the DDN consumers eventually sink into the thermocline, it leaves unclear the mechanism by which an

elevated NO_3^-:PO_4^{3-} concentration ratio accumulates in the thermocline, since the micro-organisms consuming the DDN would likely do so at or close to Redfield stoichiometry. Moreover, there is little field evidence of DON concentrations increasing, or the $\delta^{15}N$ of DON decreasing, in regions (Knapp et al., 2011) or periods (Knapp et al., 2005) of high N_2 fixation relative to regions and/or times with low rates of N_2 fixation. Consequently, while many pathways have been explored, the fate of newly fixed N remains obscure.

One geochemical tool that has been used to track the fate of DDN, as well as to quantify its contribution to export production, is the upper ocean $\delta^{15}N$ budget. Comparing the distinct $\delta^{15}N$ of subsurface NO_3^- and newly fixed N, the two dominant sources of new N to surface waters, with the $\delta^{15}N$ of the export flux ("PN_{sink} $\delta^{15}N$") provides an integrative measure of the relative contributions of subsurface NO_3^- and N_2 fixation to export production (e.g., (Altabet, 1988; Casciotti et al., 2008; Dore et al., 2002; Karl et al., 1997; Knapp et al., 2005; Liu et al., 1996)). Assigning newly fixed N a $\delta^{15}N$ of -1‰, the fractional importance of N_2 fixation for supporting export production (x) in an upper ocean $\delta^{15}N$ budget can be expressed as:

$$PN_{sink}\ \delta^{15}N = x(-1‰) + (1 - x)(NO_3^-\ \delta^{15}N) \qquad \text{Eq. 1}$$

Rearranging and solving for x yields:

$$x = (NO_3^-\ \delta^{15}N - PN_{sink}\ \delta^{15}N)/(1 + NO_3^-\ \delta^{15}N) \qquad \text{Eq. 2}$$

Multiplying the fraction of export production supported by N_2 fixation (x) by the PN_{sink} mass flux provides a time-integrated N_2 fixation rate that can be compared with $^{15}N_2$ incubation-based N_2 fixation rate measurements (Knapp et al., 2016). We note that the $\delta^{15}N$ of NO_3^- in the equations above more accurately refers to the $\delta^{15}N$ of NO_3^- + nitrite (NO_2^-); however, NO_2^- concentrations are typically extremely low throughout the oxidized water column, so for brevity, we refer to NO_3^-+NO_2^- measurements as NO_3^- measurements.

N isotope budgets in stratified, oligotrophic gyres consistently indicate that subsurface NO_3^- supports at least 75%, and often >90%, of export production, even during the stratified summer season (Altabet, 1988; Casciotti et al., 2008; Fawcett et al., 2011; Knapp et al., 2016; Knapp et al., 2005). This is inconsistent with biological assays indicating that N_2 fixation supports a higher, and often dominant, fraction of tropical and subtropical export production (e.g., (Capone et al., 2005; Montoya et al., 2004)). The disagreement between the results of these biological assays and the $\delta^{15}N$ budgets (as well as the lack of response in the concentration and/or $\delta^{15}N$ of oligotrophic surface ocean DON) raises the following questions: are upper ocean $\delta^{15}N$ budgets an appropriate tool for tracking the fate of DDN?, and is the $\delta^{15}N$ of sinking organic matter diagnostic for the source of N fueling export production?

To address the fate of DDN and to quantify the contribution of newly fixed N to export production, large volume (~50 m^3) mesocosms were deployed in a region of the southwest Pacific known to support diazotrophy during the austral summer (Bonnet et al., 2015; Dupouy et al., 2000; Rodier and Le Borgne, 2010, 2008) and where PO_4^{3-} availability appears to ultimately control N_2 fixation rates (Moutin et al., 2008; Moutin et al., 2005). In order to better track the fate of DDN, these mesocosms were intentionally fertilized with ~0.8 μM DIP to stimulate diazotrophic activity and thus amplify the biogeochemical signals of N_2 fixation. Here, we report the results of $\delta^{15}N$ budgets from inside the manipulative mesocosm experiments and discuss how the geochemical signals correspond to contemporaneous shifts in diazotroph and phytoplankton community composition.

2 Methods

2.1 Experimental design and sample collection

A detailed description of the VAHINE mesocosm experiments is provided elsewhere (Bonnet et al., 2016b). Briefly, three 2.3 m diameter, 15 m deep (~50 m^3) cylinders of impermeable, transparent plastic sheeting (subsequently referred to as M1, M2, and M3) were deployed in shallow waters (25 m water column depth) of the oligotrophic Noumea

lagoon, 28 km from New Caledonia (Bonnet et al., 2016b). Screw-top plastic bottles (250 mL) were attached to the bottom of the mesocosms to collect PN_{sink}. These "sediment trap" samples were collected daily by SCUBA divers and "swimmers" were removed from them prior to analysis. Water column samples were collected daily at 6 m depth from each of the three mesocosms throughout the 23-day experiment. Discrete samples for nutrients including NO_3^-+NO_2^- and NH_4^+, suspended particulate N (PN_{susp}), and total N (TN = PN_{susp} + DON + NO_3^-+NO_2^- + NH_4^+) were collected by pumping water via PVC tubing connected to a teflon pump (Astii) into 50 L polyethylene carboys atop a floating platform. The 50 L carboys were transferred to the R/V *Alis* and immediately subsampled on deck for the discrete samples described above. Finally, samples were also collected at a control site near the mesocosms (subsequently referred to as "Noumea lagoon waters") to monitor biogeochemical conditions outside of the manipulative mesocosm experiments.

To stimulate diazotrophy, DIP was added on the evening of the fourth day of the experiments to reach a final concentration of ~0.8 μM in each mesocosm. As described in (Bonnet et al., 2016b), this was achieved by pumping a 20 L concentrated DIP stock solution throughout the 15 m water column of each mesocosm.

2.2 Nitrogen concentration and $\delta^{15}N$ analyses

A detailed description of the sample collection, analysis, and results of dissolved inorganic N measurements made in the VAHINE mesocosm experiments is described elsewhere (Berthelot et al., 2015; Bonnet et al., 2016b). Briefly, the concentration of NH_4^+ was determined using a fluorometric method (Holmes et al., 1999) with a detection limit of 0.01 μM, the concentration of NO_3^-+NO_2^- was determined using colorimetric methods (Strickland and Parsons, 1968) with a detection limit of 0.01 μM, and the concentration of PN_{susp} was determined by wet oxidation (Pujo-Pay and Raimbault, 1994) with a quantification limit of 0.06 μM. The $\delta^{15}N$ of PN_{susp} was determined by filtering seawater through a pre-combusted, acid-washed Whatman GF/F (nominal pore size of 0.7 μm), followed by high-temperature combustion of the filter coupled with isotope ratio

mass spectrometry using a Delta Plus Thermo Fisher Scientific mass spectrometer as described in (Berthelot et al., 2015).

The concentration of total N (TN) for samples collected at the same time and in the same manner as the DIN samples described above was determined by persulfate oxidation (Solorzano and Sharp, 1980) with adaptations (Knapp et al., 2005), and the resulting NO_3^- was measured by chemiluminescence (Braman and Hendrix, 1989). DON concentration was determined by subtracting the concentrations of PN_{susp}, NH_4^+, and NO_3^-+NO_2^- (reported in (Berthelot et al., 2015)) from the measured TN concentration of each sample with a propagated error of ± 0.5 μM. The $\delta^{15}N$ of NO_3^-+NO_2^- was measured using the denitrifier method (Casciotti et al., 2002; McIlvin and Casciotti, 2011; Sigman et al., 2001) with a typical standard deviation of ± 0.2 ‰. The $\delta^{15}N$ of TN was determined via persulfate oxidation of TN to NO_3^- (Knapp et al., 2005) and subsequent analysis of NO_3^- $\delta^{15}N$ by the denitrifier method, with a propagated error for DON $\delta^{15}N$ calculated using a Monte Carlo method (Press et al., 1992) of ± 0.6‰. Finally, the $\delta^{15}N$ of PN_{sink} was measured using a Thermo Scientific Flash 2000 Elemental Analyzer coupled with a Delta Plus Thermo Scientific mass spectrometer. The average standard deviation for the standards analyzed was ± 0.06 ‰.

3 Results

The intentional DIP fertilization of the mesocosms on the fourth evening of the VAHINE experiments lends temporal structure to the 23-day course of observations. Three distinct phases are evident based on multiple biogeochemical metrics: days 1 to 4, prior to DIP fertilization (subsequently referred to as "P0"), which largely reflect "background" conditions; days 5 to 14, after DIP fertilization (subsequently referred to as "P1"), characterized by a much longer DIP turnover time than P0; and finally, days 15 to 23 (subsequently referred to as "P2"), during which DIP concentrations decreased and biomass, primary production, and N_2 fixation rates increased (Berthelot et al., 2015; Bonnet et al., 2016b). Differences in the community composition of phytoplankton and diazotrophs (Leblanc et al., 2016; Turk-Kubo et al., 2015) were also evident among the three phases of the VAHINE experiments. Consequently, the results described below are

evaluated within the temporal context of the observed biogeochemical changes in the mesocosms.

3.1 DON concentration and $\delta^{15}N$

Concentrations of DON within the mesocosms showed no significant change over the course of the 23-day experiments (Fig. 1a). Average DON concentrations in M1, M2, and M3 were 5.4 ± 0.3 μM, 5.3 ± 1.1 μM, and 5.5 ± 0.6 μM, respectively. These concentrations are consistent with previous observations from surface waters of other oligotrophic ocean regions (Knapp et al., 2011; Letscher et al., 2013), as well as with the DON concentration of 5.4 μM in Noumea lagoon waters measured outside the mesocosms. However, while our observation of invariant DON concentrations over the duration of the experiments are largely similar to those reported by Berthelot et al. (2015), the DON concentration that we measure for three samples collected at the end of P2 diverge from those of Berthelot et al. (2015) who report decreasing DON concentrations (of ~0.9 μM) in all three mesocosms during P2 compared to P0 and P1. For comparison, Fig. 1a shows DON concentration measurements from this study overlain upon those of Berthelot et al. (2015). We note that our DON sampling resolution was not as high as that of Berthelot et al. (2015), so it is possible that we missed the decrease in DON concentration in the mesocosms. The Berthelot et al. (2015) DON concentration decrease, which they attribute to consumption by phytoplankton and/or heterotrophic bacteria, was not accompanied by a decrease in DOC concentration, which given typical C:N ratios for marine dissolved organic matter (DOM) of 12 to 14 (Benner, 2002), would be expected to decline by ~9 to 12 μM. Since both TN sample sets were collected at the same time and in the same manner, and since the DON concentration calculated by mass balance in both cases used the same DIN and PN_{susp} measurements reported by (Berthelot et al., 2015), the only measurement contributing to the discrepancy is that of TN. We note that slightly different reagents were used to chemically oxidize TN to NO_3^-; while (Berthelot et al., 2015) used the wet-oxidation method of (Pujo-Pay and Raimbault, 1994), the TN measurements reported here were made with a reagent that contained no boric acid and a higher sodium hydroxide concentrations (Fawcett et al., 2011; Knapp et al., 2005). In addition, the potassium persulfate was recrystallized four

times, then rinsed with GC-grade methanol to speed drying and remove N contamination to facilitate isotopic analysis (Fawcett et al., 2014; Higgins et al., 2009). We cannot explain the discrepancy between the DON concentration measurements from samples collected at the end of P2 in this study and those reported by Berthelot et al. (2015); given that our samples were also measured for DON $\delta^{15}N$ (discussed below), we interpret the data presented in this study in the context of our DON concentration measurements rather than those of Berthelot et al. (2015). We note, however, that regardless of the DON concentration used, the conclusions from our $\delta^{15}N$ budget remain the same (see section 4.1 below).

Similar to the concentration of DON, the $\delta^{15}N$ of DON showed no significant change over the course of the experiments (Fig. 1b, Table 1). The average DON $\delta^{15}N$ in M1, M2, and M3 was 4.7 ± 1.0‰, 4.7 ± 0.4‰, and 5.3 ± 1.0‰, respectively. The $\delta^{15}N$ of DON in the VAHINE mesocosms is similar to that reported previously for the North Pacific gyre (4.7‰), where the similarity of the $\delta^{15}N$ of DON to the $\delta^{15}N$ of subsurface NO_3^- was interpreted to reflect the dominance of subsurface NO_3^- for fueling export production in the North Pacific gyre (Knapp et al., 2011).

3.2 PN_{susp} $\delta^{15}N$

The concentration of PN_{susp} (along with the concentrations of suspended particulate organic carbon (PC_{susp}) and phosphorus (PP_{susp})) increased over the course of the experiments (Fig. 1c), most notably during P2, consistent with the observed increase in carbon and N_2 fixation during P2 (Berthelot et al., 2015). However, the $\delta^{15}N$ of PN_{susp} in the mesocosms did not show any significant change with time, and was largely similar to the $\delta^{15}N$ of PN_{susp} in the lagoon waters (Fig. 1d, Table 1). The average $\delta^{15}N$ of PN_{susp} in M1, M2, and M3 was 3.3 ± 0.8‰, 3.4 ± 1.4‰, and 3.8 ± 1.5‰, respectively, while the $\delta^{15}N$ of PN_{susp} outside the mesocosms was 3.3 ± 1.3‰.

3.3 PN_{sink} $\delta^{15}N$

In contrast to the concentration of DON and the $\delta^{15}N$ of DON and PN_{susp}, the $\delta^{15}N$ of PN_{sink} changed significantly over the course of the experiments (Fig 2a). Evaluating the PN_{sink} $\delta^{15}N$ collected in all three mesocosms during P0, P1, and P2 with the Kruskal-Wallis rank-sum test for non-parametric data (Triola, 2001) shows that the mean $\delta^{15}N$ of PN_{sink} for each time period (P0, P1 and P2) is significantly different ($p<0.005$). Considering the mesocosms individually, the $\delta^{15}N$ of PN_{sink} for each time period was significantly different for M2 ($p<0.005$) and potentially for M3 ($0.1>p>0.05$), but not for M1 ($0.9>p>0.1$). The average PN_{sink} $\delta^{15}N$ in M1, M2, and M3 during P0 was 3.9 ± 0.1‰, 4.4 ± 0.3‰, and 4.2 ± 0.2‰, respectively, decreasing to 2.9 ± 0.5‰, 3.2 ± 0.4‰, and 3.0 ± 0.3‰ during P1 and 2.2 ± 1.9‰, 1.4 ± 1.2‰, and 3.3 ± 1.9‰ during P2 (Fig. 2a). All PN_{sink} $\delta^{15}N$ measurements for the mesocosms are reported in Supplementary Table 1.

4 Discussion

4.1 The fate of newly fixed N – pools or fluxes?

As described above, a primary goal of the VAHINE project was to track the fate of newly fixed N in manipulative mesocosm experiments (Bonnet et al., 2016b). The $\delta^{15}N$ of PN_{susp} in the euphotic zone has often been used to infer the dominant N form fueling primary production, particularly in oligotrophic systems where NO_3^- and NH_4^+ are typically effectively completely consumed in surface waters (Altabet, 1988; Altabet and McCarthy, 1985; Capone et al., 2005; Treibergs et al., 2014). However, bulk PN_{susp} includes compositionally-distinct N-containing particles: diverse living autotrophs and heterotrophs as well as detrital organic matter (Fawcett et al., 2011; Rau et al., 1990). In the oligotrophic Sargasso Sea, each of these groups has been shown to have a distinct $\delta^{15}N$ signature, with the $\delta^{15}N$ of bulk PN_{susp} recording their mass-weighted average (Fawcett et al., 2011; Fawcett et al., 2014; Treibergs et al., 2014). The $\delta^{15}N$ of PN_{susp} is also altered by the consumption and production of N forms recycled in surface waters (e.g., NH_4^+), the fluxes of which can often greatly exceed the external supply of N to the euphotic zone (e.g., via N_2 fixation or NO_3^- mixed up from below) (Altabet, 1988; Knapp et al., 2011). Thus, while the $\delta^{15}N$ of PN_{susp} may provide some indication of the primary N

source supporting the upper ocean ecosystem, it is unlikely to be a good indicator of the dominant N form fueling export production (Altabet, 1988).

This appears to be the case in the VAHINE mesocosms. The $\delta^{15}N$ of PN_{susp} remained roughly constant throughout the 23-day experiments and did not significantly differ from the $\delta^{15}N$ of PN_{susp} in the lagoon waters where rates of N_2 fixation were relatively constant in the absence of DIP fertilization (Fig. 1d, Table 1). During P1, N_2 fixation added ~0.1 μM N to the mesocosms (Berthelot et al., 2015). Assuming a $\delta^{15}N$ of -1 ‰ for this DDN (Carpenter et al., 1997; Hoering and Ford, 1960; Minagawa and Wada, 1986), its accumulation as PN_{susp} (assuming an average PN_{susp} $\delta^{15}N$ of 3.0 ‰ on day 5) would lower the $\delta^{15}N$ of this pool by ~0.4‰. However, the $\delta^{15}N$ of PN_{susp} did not decline and, if anything, increased by day 14 (average $\delta^{15}N$ of 3.7 ‰), further indicating that DDN did not accumulate significantly in the PN_{susp} pool in the mesocosms. This pattern was even more pronounced during P2: while N_2 fixation added ~0.25 μM N and the concentration of PN_{susp} increased by 0.25 to 0.74 μM (Berthelot et al., 2015), the $\delta^{15}N$ of PN_{susp} remained unchanged (Fig. 1d, Table 1). Thus, while DDN may have passed through the PN_{susp} pool, its transit was sufficiently rapid (<1 day) that the $\delta^{15}N$ of N_2 fixation never accumulated in PN_{susp}.

We note that both the concentration and $\delta^{15}N$ of PN_{susp} in the lagoon waters were high (i.e., 0.8 ± 0.1 μM and 3.3 ± 1.3 ‰) relative to euphotic zone PN_{susp} in similar oligotrophic regions such as near Bermuda and Hawaii (e.g., PN_{susp} concentration and $\delta^{15}N$ of 0.2 to 0.3 μM and -1 to 1 ‰; (Altabet, 1989, 1988; Casciotti et al., 2008; Dore et al., 2002; Fawcett et al., 2011; Fawcett et al., 2014). The high background PN_{susp} concentrations observed in the Noumea lagoon have been previously attributed to anthropogenically-driven eutrophication related to untreated sewage release from New Caledonia (Fichez et al., 2010). While the site of the VAHINE mesocosms located 28 km off the coast was selected to be as representative of the open ocean as possible, it was still at the entrance to the lagoon where the water quality is affected by ocean water inflow, land-derived inputs, and anthropogenic inputs such as industrial and waste water

discharge (Labrosse et al., 2000). The high $\delta^{15}N$ of PN_{susp} may also be at least partly due to this "island effect" as NO_3^- deriving from human waste is typically high in $\delta^{15}N$ (5 ‰ to 20 ‰; (McClelland and Valiela, 1998; Swart et al., 2013; Townsend-Small et al., 2007). However, subsurface NO_3^- $\delta^{15}N$ in this region is 6.5 ‰ (this study; (Yoshikawa et al., 2015)), such that its assimilation by phytoplankton would also serve to elevate the $\delta^{15}N$ of PN_{susp}. In sum, the high $\delta^{15}N$ of PN_{susp} requires the assimilation of NO_3^- even if the source of that NO_3^- is uncertain. More importantly, the invariant $\delta^{15}N$ of PN_{susp} throughout the mesocosm experiments confirms that while fluxes of DDN may have passed through the PN_{susp} pool on <1 day time scales, DDN did not accumulate as PN_{susp}. This observation is consistent with previous work showing low seasonality in the $\delta^{15}N$ of the PN_{susp} pool in spite of changes in the sources and fluxes of new N to oligotrophic surface waters (e.g., (Altabet, 1988)).

Similarly, the stability of the DON concentration and $\delta^{15}N$ (as well as the consistently low concentrations of NO_3^-+NO_2^- and NH_4^+; (Berthelot et al., 2015)) in the mesocosms could be interpreted as indicating that very little DDN was transferred to the dissolved pools during the experiments. These observations are in contrast to previous studies documenting the release of significant quantities of dissolved N during N_2 fixation. For example, elevated DON and/or NH_4^+ concentrations have been observed in the waters surrounding *Trichodesmium* blooms (Devassy et al., 1978; Karl et al., 1997; Lenes et al., 2001) and in aging *Trichodesmium* cultures (Mulholland and Capone, 2001), and *Trichodesmium* has been shown to directly release upwards of 50% of its newly fixed N as DON and/or NH_4^+ (Bonnet et al., In press; Capone et al., 1994; Glibert and Bronk, 1994; Mulholland et al., 2004) with a low $\delta^{15}N$ (Meador et al., 2007). While the VAHINE mesocosms were dominated by diazotrophs other than *Trichodesmium* (Turk-Kubo et al., 2015), it is still possible that DDN was released during the experiments but was so rapidly taken up by other (N-limited) organisms that it never accumulated in the dissolved pool. Indeed, when N_2 fixation rates increased towards the end of P1 and into P2, diatoms without diazotrophic symbionts rapidly increased 3- to 6-fold in all mesocosms, the non-diazotrophic cyanobacterium, *Synechococcus*, increased ~10-fold,

and small (<35 μm) eukaryotic phytoplankton increased 2- to 4-fold (Leblanc et al., 2016). Given that the mesocosm bags were impermeable to an external physical N supply (e.g., upwelled or advected NO_3^-), the mostly likely N source fueling the observed phytoplankton growth during P2 was DDN. This is supported by short-term (24 to 72 h) experiments conducted during the VAHINE study that were designed to track the fate of DDN. They showed the accumulation of ^{15}N originating from $^{15}N_2$ fixation in the dissolved N pool and in the biomass of non-diazotrophic diatoms and picoplankton (0.2 to 2 μm size fraction) on day 17 and 19 of the mesocosm experiments (Bonnet et al., 2016a). The total N supplied by N_2 fixation during P2, when N_2 fixation rates were highest (average of 27.3 ± 1.0 nmol N L^{-1} d^{-1} over the three mesocosms; Berthelot et al., 2015), was ~0.25 μM. This quantity of N amounts to <5 % of the ambient DON concentration, such that the addition of any portion of this DDN to the DON pool, regardless of whether it was subsequently consumed by phytoplankton, would not have been evident above the background DON concentration or $\delta^{15}N$. However, it is clear that DDN did not accumulate as NH_4^+ since, while NH_4^+ concentrations increased slightly during P2 (from ~0.01 μM to 0.06 μM; Berthelot et al., 2015), they were still extremely low throughout the experiments.

In contrast to the invariant $\delta^{15}N$ of the PN_{susp} and DON pools, the $\delta^{15}N$ of PN_{sink} significantly decreased over the course of the experiments (Fig. 2a, Table 1). The unique experimental design of the mesocosms provided a closed system that prevented the resupply of nutrients via lateral or vertical exchange, such that N_2 fixation is the only new N source that could drive changes in the $\delta^{15}N$ of PN_{sink}. Moreover, the effectively complete NO_3^- consumption that occurred in these waters prior to the initiation of the experiments (Berthelot et al., 2015) simplifies $\delta^{15}N$ budget calculations by removing the need to consider a potentially variable isotope effect (or indeed, any isotope effect) for NO_3^- assimilation; only the initial $\delta^{15}N$ of the NO_3^- is required.

We use Eq. (2) to evaluate the contribution of N_2 fixation to export production in the mesocosms, taking the $\delta^{15}N$ of subsurface NO_3^- to be that measured in the outside waters

that are thought to flush the lagoon (6.5‰ at 200 m). The average fractional contribution of N_2 fixation to export production within the three mesocosms increased over the course of the experiments; N_2 fixation supported 32 ± 4 %, 47 ± 6 %, and 56 ± 24 % of export production during P0, P1, and P2, respectively (Fig. 2b, Table 1). In spite of the range in PN_{sink} $\delta^{15}N$, especially in P2, the mean $\delta^{15}N$ of PN_{sink} is significantly different between each time period; the fraction of export production supported by N_2 fixation during each time period is thus also significantly different. We note that the apparent fractional contribution of N_2 fixation to export production suggested by the $\delta^{15}N$ of PN_{sink} in the VAHINE experiments is high relative to geochemical studies conducted in other tropical and subtropical open ocean regions (<10-25 %; Altabet, 1998; Knapp et al., 2005; Casciotti et al., 2008). However, the intentional fertilization of the mesocosms with DIP, the lack of external N sources other than N_2 fixation to the water column, and the 15 m mesocosm water column that was both significantly shallower and less turbulent than that of the open ocean study sites all likely favored diazotrophy in the mesocosms. Direct comparison of the fractional significance of N_2 fixation to export production in the VAHINE experiments with observations from open ocean sites should thus be made with caution.

Given the potential for especially large gradients in the $\delta^{15}N$ of NO_3^- in the upper thermocline of the South Pacific (Casciotti et al., 2013; Yoshikawa et al., 2015), and the possibility that the island provided a source of NO_3^- of unknown (albeit high) $\delta^{15}N$ to the lagoon, the results of our $\delta^{15}N$ budget are best used to evaluate relative changes in the sources of N fueling export production. Regardless of the uncertainty in the absolute contribution of N_2 fixation to export production at any one time point, the relative shift in the $\delta^{15}N$ of PN_{sink} is significant and clearly indicates that export production in the mesocosms was initially fueled primarily by NO_3^- that had been assimilated prior to the start of the experiments, with N_2 fixation becoming the dominant driver of export by the end of the experiments.

During P0, the rates of primary production and N_2 fixation were low, although N_2 fixation appears to have been slightly higher than during P1 (Berthelot et al., 2015). In

addition, there was no observable increase in PN_{susp} concentration during P1, indicating that little to no growth occurred during this phase of the experiments. Given the mean PN_{sink} $\delta^{15}N$ of 4.1 ± 0.3 ‰ during P0, we hypothesize that the sinking flux (which was also low; ~0.07 mmol N m^{-2} d^{-1}; Fig. 2a) likely constituted mainly large cells that, due to the lack of nutrients and turbulence that characterized the mesocosm enclosures, were unable to grow and instead sank rapidly out of surface waters. This is supported by: i) a small but detectable decline in the concentration of PC_{susp} during P0 (Berthelot et al., 2015); ii) taxonomy data from the mesocosms showing a sharp decline in the abundance of the initially dominant, large and chain-forming diatom species (e.g., *Thalassionema spp*., *Leptocylindrus spp*., and *Chaetoceros spp*.) between days 2 and 5 (Leblanc et al., 2016), and iii) calculations using Stokes' law, modified specifically for diatoms by (Miklasz and Denny, 2010), that predict that diatoms with a diameter of 50 to 100 μm will sink at speeds >10 m day^{-1}, allowing them to easily sink out of the 15 m-deep mesocosms on the timescale of a day. Given that diatoms have a strong tendency towards NO_3^- assimilation (Dortch, 1990; Fawcett and Ward, 2011; Goericke, 2002), the preferential sinking out of large diatoms that had consumed predominantly NO_3^- prior to the commencement of the experiments can explain the slightly higher $\delta^{15}N$ of PN_{sink} during P0 than P1 (average of 4.1 ± 0.3 ‰ versus 3.0 ± 0.4 ‰), even though N_2 fixation was slightly higher during P0.

Throughout most of P1, N_2 fixation rates, primary production, and the sinking flux remained low and constant (Berthelot et al., 2015; Fig. 2a). Along with the relatively invariant $\delta^{15}N$ of PN_{sink} during this period, these observations suggest that PN_{sink} comprised mostly aggregated suspended material that had been present in surface waters since the beginning of the experiments rather than newly generated biomass. Indeed, the $\delta^{15}N$ of PN_{sink} throughout P1 is indistinguishable from that of PN_{susp} (3 ‰; Figs. 1d and 2a, Table 1). Thus, despite the lack of NO_3^- in the mesocosms, more than half of the export production that occurred during P1 was supported by NO_3^- that had been assimilated by phytoplankton prior to the start of the experiments (Eq. 1). N_2 fixation rates began to increase by day 11 or 12 in all mesocosms; this was quickly followed by an increase in PN_{susp} concentrations, as well as an increase in the magnitude of the sinking

flux and a decrease in its $\delta^{15}N$, consistent with both an increased supply of N to the mesocosms and a low $\delta^{15}N$ for that N.

To confirm that the decrease in the $\delta^{15}N$ of PN_{sink} is best explained by N_2 fixation, we compared the N_2 fixation rate derived from the $\delta^{15}N$ budget (Eq. (1) and (2), above) with the $^{15}N_2$ incubation-based N_2 fixation rates (Berthelot et al., 2015) (Table 2). The time-integrated DDN that accumulated as PN_{sink} over the course of the 23-day experiments in each of the mesocosms corresponds to 52 % to 75 % of the $^{15}N_2$ incubation-based N_2 fixation flux integrated over the same time period (Table 2). In spite of the uncertainty associated with both analyses, including the different time scales over which each metric may integrate N_2 fixation fluxes and the possibility that some of the DDN accumulated in the DON and/or PN_{susp} pools below analytical detection limits, we conclude that the primary fate of newly fixed N in the VAHINE mesocosm experiments was to be converted into the PN_{sink} flux. We note that the net DON consumption at the end of P2 proposed by (Berthelot et al., 2015) does not change the results of our $\delta^{15}N$ budgets given the suggestion by the authors that the primary fate of this DON was to accumulate in the PN_{susp} pool. This represents a redistribution of N between surface pools separate from the PN_{sink} flux, such that it would not affect our $\delta^{15}N$ budgets. While there is no reason that the consumed DON had to be retained in the PN_{susp} pool, the isotopic data indicate that if the (Berthelot et al., 2015) DON concentration data are correct, then, as the authors propose, the fate of this DON has to primarily be retention in the PN_{susp} pool. The isotope data also suggest that the majority of the DDN in the mesocosms was fairly rapidly exported, either directly by sinking diazotrophs, or indirectly after being cycled through the dissolved N pool and assimilated by non-diazotrophic plankton in the PN_{susp} pool that then sank into the sediment traps (Bonnet et al., 2016a), rather than being retained in surface waters. This is consistent with prior work using $\delta^{15}N$ budgets to quantify the significance of DDN for supporting export production (Altabet, 1988; Casciotti et al., 2008). The results presented here demonstrate that the $\delta^{15}N$ of the PN_{sink} flux, compared to the $\delta^{15}N$ of DON and/or the PN_{susp} pool, is the most appropriate tool for evaluating the

fate of newly fixed N on relatively short timescales, since it records the $\delta^{15}N$ of the sources of new N fueling export production with the most fidelity.

4.2 NO_3^-- and N_2 fixation-driven export production in the context of changing phytoplankton and diazotroph community composition

The shift from NO_3^- to N_2 fixation as the dominant source of N fueling export production during the VAHINE mesocosm experiments is paralleled by observed changes in the composition of the phytoplankton and diazotroph communities (Leblanc et al., 2016; Turk-Kubo et al., 2015). In particular, the diazotroph that dominated inside the mesocosms prior to DIP fertilization (i.e., during P0), as well as immediately following DIP fertilization (i.e., during P1), was *Richelia* associated with the diatom *Rhizosolenia* (Het-1), a diatom-diazotroph assemblage (DDA) that was also common in the Noumea lagoon waters (Turk-Kubo et al., 2015). However, a *Cyanothece*-like group-C unicellular cyanobacterial diazotroph (hereafter, "UCYN-C") came to dominate the diazotroph community inside the mesocosms during P2. This diazotroph was rarely observed outside the mesocosms, suggesting that the experiment itself created favorable conditions for the success of this ecotype, which has never been observed at high abundances in the marine water column (Turk-Kubo et al., 2015). It is possible that the microbial community response to DIP fertilization created conditions suitable for UCYN-C growth inside the mesocosms (see below; (Turk-Kubo et al., 2015)).

During P0, the diatom community was numerically dominated by non-diazotrophic species such as *Leptocylindrus spp*. and *Chaetoceros spp*., with DDAs comprising a minor fraction (i.e., <5%) of total diatom abundance, and becoming even less abundant during P1 (Leblanc et al., 2016). Thus, while DDAs may have been responsible for the low levels of N_2 fixation detected during P0 and P1, they were not sufficiently abundant to be important drivers of export production; rather, we suggest that the small amount of export that occurred during P0 and P1 was fueled by large (non-DDA) diatoms and aggregating PN_{susp} that bore the high $\delta^{15}N$ of earlier NO_3^- consumption (see above).

The increase in the rate of N_2 fixation observed towards the end of P1 (days 11 to 12) was rapidly followed by a 2- to 10-fold increase in the abundance of non-diazotrophic diatoms, driven almost exclusively by *Cylindrotheca closterium*, which reached maximum abundance on days 15 to 16 and then declined to P1 levels by days 18 to 20 (Leblanc et al., 2016). Beginning on day 11 to 15, the abundance of both *Synechococcus* and small eukaryotic phytoplankton (<35 μm) also increased, although less rapidly than the diatoms. Unlike the large diatoms, these two groups continued to grow until the end of the experiments (Leblanc et al., 2016). Molecular data suggest that UCYN-C were the dominant diazotrophs responsible for the elevated rates of N_2 fixation during late P1 and throughout P2 (Turk-Kubo et al., 2015). We hypothesize that the subsequent rapid transfer of DDN to the dissolved pool fueled the observed growth of *C. closterium* and other phytoplankton during this time period. This is supported by a short-term $^{15}N_2$ labeled-DDN transfer experiment performed by (Bonnet et al., 2016a) on days 17 and 19 in which nanoSIMS (nanoscale secondary ion mass spectrometry) analyses revealed that non-diazotrophic plankton (diatoms and picoplankton) became significantly enriched in ^{15}N after 24 to 72 h due to their assimilation of DDN transferred from the diazotrophs in the mesocosms. Regardless of the form of this DDN (i.e., NH_4^+ or DON), it would retain the low-$\delta^{15}N$ characteristic of N_2 fixation, thereby lowering the $\delta^{15}N$ of the phytoplankton that consumed it. Since the $\delta^{15}N$ of PN_{susp} did not decline significantly during P2 but the $\delta^{15}N$ of PN_{sink} did, it follows that the sinking flux likely comprised a contribution from both UCYN-C and the DDN-fueled phytoplankton. The isotope data also suggest that while the *C. closterium* and other phytoplankton that consumed the DDN may have briefly contributed to the PN_{susp} pool, they did not reside in the PN_{susp} pool for >1 day (i.e., the timescale of mesocosm sampling) before sinking. This is analogous to the DDN passing briefly and undetectably through the dissolved N pool before it was rapidly consumed by phytoplankton. UCYN-C are small cyanobacteria (5.7 ± 0.8 µm; (Bonnet et al., 2016a)), but they were observed to aggregate into 100 to 500 μm particles that sank rapidly, constituting 22.4 ± 5 % of the PC_{sink} flux at the height of the UCYN-C bloom (day 17) and ~5 % as the bloom decayed (Bonnet et al., 2016a). In addition to their direct contribution to export, UCYN-C provided the N that fueled phytoplankton growth during P2, such that this organism was responsible for driving most of export production in the

mesocosms, albeit largely indirectly. One implication of these results is that the phenomenon of newly fixed N being released to the dissolved pool is apparently not unique to *Trichodesmium* spp.. Another implication of the indirect control of diazotrophs on export production, if relevant to the open ocean, is that while the transfer of DDN to depth via non-diazotrophic phytoplankton ultimately leads to a decline in the $\delta^{15}N$ of thermocline NO_3^-, it will not increase the NO_3^-:PO_4^{3-} concentration ratio of these subsurface waters.

5 Conclusions

The goal of the VAHINE project was to track the fate of newly fixed N in large-volume, DIP-fertilized mesocosm experiments. Consistent with previous work, we found no evidence of newly fixed N accumulating in the surface DON or PN_{susp} pools. Instead, the $\delta^{15}N$ of the PN_{sink} flux decreased over the course of the experiments in proportion to increasing rates of N_2 fixation. These observations are consistent with the traditional oceanographic paradigm that new fluxes of N to the surface ocean are balanced by the dominant flux out of surface waters, the sinking particulate flux (Eppley and Peterson, 1979). Moreover, they suggest that upper ocean $\delta^{15}N$ budgets that include measurements of PN_{sink} are the best metric for tracking the fate of DDN and for diagnosing the dominant N source fueling export production. While at-sea collections of PN_{sink} are expensive and logistically challenging, our results underscore the value of PN_{sink} $\delta^{15}N$ measurements and emphasize their critical role in constraining the location, magnitude, and timing of marine N_2 fixation fluxes.

This work provides isotopic evidence not only for newly fixed N leaving surface waters via the sinking flux, but also strongly suggests that DDN was first rapidly cycled through the dissolved N and PN_{susp} pools before being transferred to the sinking flux. While prior $\delta^{15}N$ budget studies have shown the rapid transfer of low-$\delta^{15}N$ N from surface to subsurface waters, the unique design of the mesocosm experiments that received no other external N source to support phytoplankton growth after several weeks of isolation requires that the low-$\delta^{15}N$ PN_{sink} flux observed during P2 was fueled by DDN. Daily water column measurements of dissolved organic and inorganic N concentrations (and

the $\delta^{15}N$ of DON) indicate that DDN did not accumulate in these or the PN_{susp} pools for >1 day timescales. While the $\delta^{15}N$ budget suggests that N_2 fixation was the primary source of N fueling export production during P2, phytoplankton abundance data show that non-diazotrophic phytoplankton, including large diatoms and *Synechococcus*, "bloomed" during P2 (Leblanc et al., 2016), accumulating in numbers too large to be supported by recycled forms of N that did not derive from N_2 fixation. Assuming that these non-diazotrophic phytoplankton had no other means of acquiring N than via the UCYN-C population that also increased significantly during P2, it is extremely likely that DDN was transferred from UCYN-C to the non-diazotrophic phytoplankton that drove most of the export production, along with a small direct contribution (~5 to 22%) from aggregated UCYN-C cells (Bonnet et al., 2016a). Indeed, such a DDN transfer to the non-diazotrophic pool was directly observed in a companion nanoSIMS-$^{15}N_2$ study conducted on days 17 and 19 of the experiments when UCYN-C was blooming but diatom abundances were already declining (Bonnet et al., 2016a); there is no reason that the same mechanism did not fuel the growth of diatoms earlier in P2. The diatoms that grew during P2, including *C. closterium*, reportedly have the ability to survive in low nutrient environments with seed populations that remain poised to thrive when supplied with a pulse of nutrients, and then sink out of surface waters under calm conditions due to their size (Kingston, 2009; Margalef, 1978; Wasmund et al., 2014). This is consistent with observations from the VAHINE experiments. In addition, *C. closterium* abundances have been observed to increase dramatically after *Trichodesmium* blooms in the South West Pacific (Bonnet et al., Under review). Our study provides some of the first evidence for DDN being rapidly transferred through the dissolved pool to other phytoplankton that then dominate the sinking flux instead of being transferred to the subsurface by diazotrophs sinking directly out of surface waters.

Our findings are consistent with prior work showing that diazotrophs release newly fixed N to the dissolved pool (Capone et al., 1994; Glibert and Bronk, 1994; Mulholland et al., 2006; Mulholland et al., 2004), as well as with numerous studies that have failed to observe DDN accumulating in surface ocean N pools (Fawcett et al., 2011; Fawcett et al., 2014; Knapp et al., 2005; Knapp et al., 2011). The results of the VAHINE experiments

reconcile some of these observations, but also leave open the question of the composition of the DDN that is released to the dissolved pool. Additionally, the experiments raise the question of how microbes and phytoplankton stay "poised" to rapidly assimilate DDN, and why they sink out of surface waters when they acquire DDN, with no retention or accumulation of that DDN in the upper ocean N pools. In other words, why is the fate of DDN so disproportionately biased towards sinking?

Acknowledgments

Funding for A.N.K. was provided by NSF-OCE #1537314, for S.E.F. from the University of Cape Town URC fund, the Grand Challenges Program of Princeton University, and NSF-OCE #1136345 to B. Ward and D. Sigman. Funding for this research was provided by the Agence Nationale de la Recherche (ANR starting grant VAHINE ANR-13-JS06-0002), INSU-LEFE-CYBER program, GOPS, IRD and M.I.O. The authors thank the captain and crew of the R/V *Alis* and acknowledge the SEOH divers service from the IRD research center of Noumea (E. Folcher, B. Bourgeois, and A. Renaud) and from the Observatoire Océanologique de Villefranche-sur-mer (OOV, J.M. Grisoni) as well as the technical service of the IRD research center of Noumea for their helpful technical support. C. Guieu, F. Louis, and J.M. Grisoni from OOV are warmly thanked for the mesocosm design and their useful advice for deployment. The authors also thank D. Sigman and G. Haug for providing analytical support for N concentration and isotope measurements.

REFERENCES

Altabet, M. A.: Particulate New Nitrogen Fluxes in the Sargasso Sea, Journal of Geophysical Research-Oceans, 94, 12771-12779, 1989.

Altabet, M. A.: Variations in Nitrogen Isotopic Composition between Sinking and Suspended Particles - Implications for Nitrogen Cycling and Particle Transformation in the Open Ocean, Deep-Sea Research Part a-Oceanographic Research Papers, 35, 535-554, 1988.

Altabet, M. A. and McCarthy, J. J.: Temporal and Spatial Variations in the Natural Abundance of N-15 in Pon from a Warm-Core Ring, Deep-Sea Research Part a-Oceanographic Research Papers, 32, 755-772, 1985.

Benner, R.: Chemical Composition and Reactivity. In: Biogeochemistry of Marine Dissolved Organic Matter, Hansell, D. A. and Carlson, C. A. (Eds.), Academic Press, New York, 2002.

Berman-Frank, I., Bidle, K. D., Haramaty, L., and Falkowski, P. G.: The demise of the marine cyanobacterium, Trichodesmium spp., via an autocatalyzed cell death pathway, Limnology and Oceanography, 49, 997-1005, 2004.

Berthelot, H., Moutin, T., L'Helguen, S., Leblanc, K., Helias, S., Grosso, O., Leblond, N., Charriere, B., and Bonnet, S.: Dinitrogen fixation and dissolved organic nitrogen fueled primary production and particulate export during the VAHINE mesocosm experiment (New Caledonia lagoon), Biogeosciences, 12, 4099-4112, 2015.

Bonnet, S., Berthelot, H., Turk-Kubo, K., Cornet-Barthaux, V., Fawcett, S., Berman-Frank, I., Barani, A., Dekaezemacker, J., Benavides, M., and Capone, D.: Diazotroph derived nitrogen supports diatom growth in the South West Pacific: a quantitative study using nanoSIMS, Limnology and Oceanography, In press. In press.

Bonnet, S., Berthelot, H., Turk-Kubo, K., Fawcett, S., Rahav, E., L'Helguen, S., and Berman-Frank, I.: Dynamics of N2 fixation and fate of diazotroph-derived nitrogen during the VAHINE mesocosm experiment, Biogeosciences, 13, 2653-2673, 2016a.

Bonnet, S., Grisoni, J.-M., Moutin, T., Folcher, E., Bourgeois, B., and Renaud, A.: Introduction to the project VAHINE: VAriability of vertical and tropHIc transfer of fixed N2 in the south wEst Pacific, Biogeosciences, 13, 2803-2814, 2016b.

Bonnet, S., Rodier, M., Turk-Kubo, K. A., Germineaud, C., Menkes, C., Ganachaud, A., Cravatte, S., Raimbault, P., Campbell, E., Quéroué, F., Sarthou, G., Desnues, A., Maes, C., and Eldin, G.: Contrasted geographical distribution of N2 fixation rates and nifH phylotypes in the Coral and Solomon Seas (southwestern Pacific) during austral winter conditions, Global Biogeochemical Cycles, 29, - n/a, 2015.

Braman, R. S. and Hendrix, S. A.: Nanogram Nitrite and Nitrate Determination in Environmental and Biological-Materials by Vanadium(Iii) Reduction with Chemi-Luminescence Detection, Analytical Chemistry, 61, 2715-2718, 1989.

Capone, D. G., Burns, J. A., Montoya, J. P., Subramaniam, A., Mahaffey, C., Gunderson, T., Michaels, A. F., and Carpenter, E. J.: Nitrogen fixation by Trichodesmium spp.: An important

source of new nitrogen to the tropical and subtropical North Atlantic Ocean, Global Biogeochemical Cycles, 19, 2005.

Capone, D. G., Ferrier, M. D., and Carpenter, E. J.: Amino-Acid Cycling in Colonies of the Planktonic Marine Cyanobacterium Trichodesmium-Thiebautii, Applied and Environmental Microbiology, 60, 3989-3995, 1994.

Carpenter, E. J., Harvey, H. R., Fry, B., and Capone, D. G.: Biogeochemical tracers of the marine cyanobacterium Trichodesmium, Deep-Sea Research Part I-Oceanographic Research Papers, 44, 27-38, 1997.

Casciotti, K. L., Buchwald, C., and McIlvin, M.: Implications of nitrate and nitrite isotopic measurements for the mechanisms of nitrogen cycling in the Peru oxygen deficient zone, Deep Sea Research Part I: Oceanographic Research Papers, 80, 78-93, 2013.

Casciotti, K. L., Sigman, D. M., Hastings, M. G., Bohlke, J. K., and Hilkert, A.: Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method, Analytical Chemistry, 74, 4905-4912, 2002.

Casciotti, K. L., Trull, T. W., Glover, D. M., and Davies, D.: Constraints on nitrogen cycling at the subtropical North Pacific Station ALOHA from isotopic measurements of nitrate and particulate nitrogen, Deep-Sea Research Part Ii-Topical Studies in Oceanography, 55, 1661-1672, 2008.

Deutsch, C., Gruber, N., Key, R. M., Sarmiento, J. L., and Ganachaud, A.: Denitrification and N(2) fixation in the Pacific Ocean, Global Biogeochemical Cycles, 15, 483-506, 2001.

Devassy, V. P., Bhattathiri, P. M. A., and Qasim, S. Z.: Trichodesmium-erythraeum phenomenon, Indian Journal of Marine Sciences, 7, 168-186, 1978.

Dore, J. E., Brum, J. R., Tupas, L. M., and Karl, D. M.: Seasonal and interannual variability in sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean, Limnology and Oceanography, 47, 1595-1607, 2002.

Dortch, Q.: The interaction between ammonium and nitrate uptake in phytoplankton, Marine Ecology Progress Series, 61, 183-201, 1990.

Dupouy, C., Neveux, J., Subramaniam, A., Mulholland, M. R., Montoya, J. P., Campbell, L., Carpenter, E. J., and Capone, D. G.: Satellite captures trichodesmium blooms in the southwestern tropical Pacific, EOS, 81, 13-16, 2000.

Eppley, R. W. and Peterson, B. J.: Particulate organic-matter flux and planktonic new production in the deep ocean, Nature, 282, 677-680, 1979.

Eugster, O. and Gruber, N.: A probabilistic estimate of global marine N-fixation and denitrification, Global Biogeochemical Cycles, 26, GB4013, 2012.

Falkowski, P. G.: Evolution of the nitrogen cycle and its influence on the biological sequestration of CO2 in the ocean, Nature, 387, 272-275, 1997.

Falkowski, P. G.: Rationalizing elemental ratios in unicellular algae, Journal of Phycology, 36, 3-6, 2000.

Fawcett, S. and Ward, B.: Phytoplankton succession and nitrogen utilization during the development of an upwelling bloom, Marine Ecology Progress Series, 428, 13-31, 2011.

Fawcett, S. E., Lomas, M., Casey, J. R., Ward, B. B., and Sigman, D. M.: Assimilation of upwelled nitrate by small eukaryotes in the Sargasso Sea, Nature Geoscience, 4, 717-722, 2011.

Fawcett, S. E., Lomas, M. W., Ward, B. B., and Sigman, D. M.: The counterintuitive effect of summer-to-fall mixed layer deepening on eukaryotic new production in the Sargasso Sea, Global Biogeochemical Cycles, 28, 86-102, 2014.

Fichez, R., Chifflet, S., Douillet, P., Gérard, P., Gutierrez, F., Jouon, A., Ouillon, S., and Grenz, C.: Biogeochemical typology and temporal variability of lagoon waters in a coral reef ecosystem subject to terrigeneous and anthropogenic inputs (New Caledonia), Marine Pollution Bulletin, 61, 309-322, 2010.

Glibert, P. M. and Bronk, D. A.: Release of Dissolved Organic Nitrogen by Marine Diazotrophic Cyanobacteria, Trichodesmium Spp, Applied and Environmental Microbiology, 60, 3996-4000, 1994.

Goericke, R.: Top-down control of phytoplankton biomass and community structure in the monsoonal Arabian Sea, Limnology and Oceanography, 47, 1307-1323, 2002.

Gruber, N.: The dynamics of the marine nitrogen cycle and its influence on atmospheric CO2 variations. In: The Ocean Carbon Cycle and Climate, Follows, M. a. O., T. (Ed.), Kluwer Academic, Dordrecht, 2004.

Gruber, N. and Sarmiento, J. L.: Global patterns of marine nitrogen fixation and denitrification, Global Biogeochemical Cycles, 11, 235-266, 1997.

Hansell, D. A., Bates, N. R., and Olson, D. B.: Excess nitrate and nitrogen fixation in the North Atlantic Ocean, Marine Chemistry, 84, 243-265, 2004.

Higgins, M. B., Robinson, R. S., Casciotti, K. L., McIlvin, M. R., and Pearson, A.: A Method for Determining the Nitrogen Isotopic Composition of Porphyrins, Analytical Chemistry, 81, 184-192, 2009.

Hoering, T. C. and Ford, H. T.: The Isotope Effect in the Fixation of Nitrogen by Azotobacter, Journal of the American Chemical Society, 82, 376-378, 1960.

Holl, C. M. and Montoya, J. P.: Diazotrophic growth of the marine cyanobacterium Trichodesmium IMS101 in continuous culture: Effects of growth rate on N(2)-fixation rate, biomass, and C : N : P stoichiometry, Journal of Phycology, 44, 929-937, 2008.

Holmes, R. M., Aminot, A., Kerouel, R., Hooker, B. A., and Peterson, B. J.: A simple and precise method for measuring ammonium in marine and freshwater ecosystems, Canadian Journal of Fisheries and Aquatic Sciences, 56, 1801-1808, 1999.

Karl, D., Letelier, R., Tupas, L., Dore, J., Christian, J., and Hebel, D.: The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean, Nature, 388, 533-538, 1997.

Kingston, M. B.: Growth and motility of the diatom Cylindrotheca Closterium: Implications for commercial applications, Journal of the North Carolina Academy of Science, 125, 138-142, 2009.

Knapp, A. N., Casciotti, K. L., Berelson, W. M., Prokopenko, M. G., and Capone, D. G.: Low rates of nitrogen fixation in eastern tropical South Pacific surface waters, Proceedings of the National Academy of Sciences, doi: 10.1073/pnas.1515641113, 2016. 2016.

Knapp, A. N., DiFiore, P. J., Deutsch, C., Sigman, D. M., and Lipschultz, F.: Nitrate isotopic composition between Bermuda and Puerto Rico: Implications for N(2) fixation in the Atlantic Ocean, Global Biogeochemical Cycles, 22, 2008.

Knapp, A. N., Sigman, D. M., and Lipschultz, F.: N isotopic composition of dissolved organic nitrogen and nitrate at the Bermuda Atlantic time-series study site, Global Biogeochemical Cycles, 19, 2005.

Knapp, A. N., Sigman, D. M., Lipschultz, F., Kustka, A. B., and Capone, D. G.: Interbasin isotopic correspondence between upper-ocean bulk DON and subsurface nitrate and its implications for marine nitrogen cycling, Global Biogeochemical Cycles, 25, 2011.

Krauk, J. M., Villareal, T. A., Sohm, J. A., Montoya, J. P., and Capone, D. G.: Plasticity of N : P ratios in laboratory and field populations of Trichodesmium spp, Aquatic Microbial Ecology, 42, 243-253, 2006.

Kustka, A. B., Sanudo-Wilhelmy, S. A., Carpenter, E. J., Capone, D., Burns, J., and Sunda, W. G.: Iron requirements for dinitrogen- and ammonium-supported growth in cultures of Trichodesmium (IMS 101): Comparison with nitrogen fixation rates and iron: carbon ratios of field populations, Limnology and Oceanography, 48, 1869-1884, 2003.

Labrosse, P., Fichez, R., Farman, R., and Adams, T.: New Caledonia. In: Seas at the Millenium, An Environmental Evaluation, Sheppard, C. (Ed.), Elsevier, Amsterdam, 2000.

Leblanc, K., Cornet, V., Caffin, M., Rodier, M., Desnues, A., Berthelot, H., and Heliou, J.: Phytoplankton community structure in the VAHINE MESOCOSM experiment, Biogeosciences Discussion, doi: 10.5194/bg-2015-605, 2016. 2016.

Lenes, J. M., Darrow, B. P., Cattrall, C., Heil, C. A., Callahan, M., Vargo, G. A., Byrne, R. H., Prospero, J. M., Bates, D. E., Fanning, K. A., and Walsh, J. J.: Iron fertilization and the Trichodesmium response on the West Florida shelf, Limnology and Oceanography, 46, 1261-1277, 2001.

Letscher, R. T., Hansell, D. A., Carlson, C. A., Lumpkin, R., and Knapp, A. N.: Dissolved organic nitrogen in the global surface ocean: Distribution and fate, Global Biogeochemical Cycles, 27, 141-153, 2013.

Liu, K. K., Su, M. J., Hsueh, C. R., and Gong, G. C.: The nitrogen isotopic composition of nitrate in the Kuroshio Water northeast of Taiwan: Evidence for nitrogen fixation as a source of isotopically light nitrate, Marine Chemistry, 54, 273-292, 1996.

Margalef, R.: Life-forms of phytoplankton as survival alternatives in an unsustainable environment, Oceanologica Acta, 1, 493-509, 1978.

McClelland, J. W. and Valiela, I.: Linking nitrogen in estuarine producers to land-derived sources, Limnology and Oceanography, 43, 577-585, 1998.

McIlvin, M. R. and Casciotti, K. L.: Technical Updates to the Bacterial Method for Nitrate Isotopic Analyses, Analytical Chemistry, 83, 1850-1856, 2011.

Meador, T. B., Aluwihare, L. I., and Mahaffey, C.: Isotopic heterogeneity and cycling of organic nitrogen in the oligotrophic ocean, Limnology and Oceanography, 52, 934-947, 2007.

Miklasz, K. A. and Denny, M. W.: Diatom sinking speeds: Improved predictions and insight from a modified Stokes' law, Limnology and Oceanography, 55, 2513-2525, 2010.

Minagawa, M. and Wada, E.: Nitrogen Isotope Ratios of Red Tide Organisms in the East-China-Sea - a Characterization of Biological Nitrogen-Fixation, Marine Chemistry, 19, 245-259, 1986.

Montoya, J. P., Holl, C. M., Zehr, J. P., Hansen, A., Villareal, T. A., and Capone, D. G.: High rates of N-2 fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean, Nature, 430, 1027-1031, 2004.

Moutin, T., Karl, D. M., Duhamel, S., Rimmelin, P., Raimbault, P., Van Mooy, B. A. S., and Claustre, H.: Phosphate availability and the ultimate control of new nitrogen input by nitrogen fixation in the tropical Pacific Ocean, Biogeosciences, 5, 95-109, 2008.

Moutin, T., Van Den Broeck, N., Beker, B., Dupouy, C., Rimmelin, P., and Le Bouteiller, A.: Phosphate availability controls Trichodesmium spp. biomass in the SW Pacific Ocean, Marine Ecology Progress Series, 297, 15-21, 2005.

Mulholland, M. R., Bernhardt, P. W., Heil, C. A., Bronk, D. A., and O'Neil, J. M.: Nitrogen fixation and release of fixed nitrogen by Trichodesmium spp. in the Gulf of Mexico, Limnology and Oceanography, 51, 1762-1776, 2006.

Mulholland, M. R., Bronk, D. A., and Capone, D. G.: Dinitrogen fixation and release of ammonium and dissolved organic nitrogen by Trichodesmium IMS101, Aquatic Microbial Ecology, 37, 85-94, 2004.

Mulholland, M. R. and Capone, D. G.: Stoichiometry of nitrogen and carbon utilization in cultured populations of Trichodesmium IMS101: Implications for growth, Limnology and Oceanography, 46, 436-443, 2001.

O'Neill, J. M. and Roman, M. R.: Ingestion of the cyanobacterium Trichodesmium spp. by pelagic harpacticoid copepods Macrosetella, Miracia and Oculosetella, Hydrobiologia, 292-293, 235-240, 1994.

Press, W. H., Teukolsky, S. A., Vetterling, W. T., and Flannery, B. P.: Numerical Recipes in C: The art of scientific computing, 2nd edition, Cambridge University Press, 1992.

Pujo-Pay, M. and Raimbault, P.: Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters, Marine Ecology Progress Series, 105, 203-207, 1994.

Rau, G. H., Teyssie, J.-L., Rassoulzadegan, F., and Fowler, S. W.: 13C/12C and 15N/14N variations among size-fractionated marine particles: implications for their origin and trophic relationships, Marine Ecology Progress Series, 59, 33-38, 1990.

Redfield, A. C.: The Biological Control of Chemical Factors in the Environment, American Scientist, 46, 205-221, 1958.

Rodier, M. and Le Borgne, R.: Population and trophic dynamics of Trichodesmium thiebautii in the SE lagoon of New Caledonia. Comparison with T. erythraeum in the SW lagoon, Marine Pollution Bulletin, 61, 349-359, 2010.

Rodier, M. and Le Borgne, R.: Population dynamics and environmental conditions affecting Trichodesmium spp. (filamentous cyanobacteria) blooms in the south-west lagoon of New Caledonia, Journal of Experimental Marine Biology and Ecology, 358, 20-32, 2008.

Roman, M. R.: Ingestion of Blue-Green-Alga Trichodesmium by Harpactacoid Copepod, Macrosetella-Gracilis, Limnology and Oceanography, 23, 1245-1248, 1978.

Sigman, D. M., Casciotti, K. L., Andreani, M., Barford, C., Galanter, M., and Bohlke, J. K.: A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater, Analytical Chemistry, 73, 4145-4153, 2001.

Sigman, D. M., DiFiore, P. J., Hain, M. P., Deutsch, C., Wang, Y., Karl, D. M., Knapp, A. N., Lehmann, M. F., and Pantoja, S.: The dual isotopes of deep nitrate as a constraint on the cycle and budget of oceanic fixed nitrogen, Deep-Sea Research Part I-Oceanographic Research Papers, 56, 1419-1439, 2009.

Solorzano, L. and Sharp, J. H.: Determination of Total Dissolved Nitrogen in Natural-Waters, Limnology and Oceanography, 25, 751-754, 1980.

Sterner, R. W. and Elser, J. J.: Ecological stoichiometry: the biology of elements from molecules to the biosphere. In: Ecological stoichiometry: the biology of elements from molecules to the biosphere., 2002.

Strickland, J. D. H. and Parsons, T. R.: A practical handbook of seawater analysis, Fisheries Research Board of Canada, Ottowa, 1968.

Swart, P. K., Anderson, W. T., Altabet, M. A., Drayer, C., and Bellmund, S.: Sources of dissolved inorganic nitrogen in a coastal lagoon adjacent to a major metropolitan area, Miami Florida (USA), Applied Geochemistry, 38, 134-146, 2013.

Townsend-Small, A., McCarthy, M. J., Brandes, J. A., Yang, L. Y., Zhang, L., and Gardner, W. S.: Stable isotopic composition of nitrate in Lake Taihu, China, and major inflow rivers Hydrobiologia, 581, 135-140, 2007.

Treibergs, L. A., Fawcett, S. E., Lomas, M. W., and Sigman, D. M.: Nitrogen isotopic response of prokaryotic and eukaryotic phytoplankton to nitrate availability in Sargasso Sea surface waters, Limnology and Oceanography, 59, 972-985, 2014.

Triola, M. F.: Elementary Statistics, Addison Wesley Longman, New York, NY, 2001.

Turk-Kubo, K. A., Frank, I. E., Hogan, M. E., Desnues, A., Bonnet, S., and Zehr, J.: Diazotroph community succession during the VAHINE mesocosms experiment (New Caledonia Lagoon), Biogeosciences Discussion, 12, 1-37, 2015.

Walsby, A. E.: The gas vesicles and buoyancy of Trichodesmium. In: Marine Pelagic Cyanobacteria: Trichodesmium and other Diazotrophs, Carpenter, E. J., Capone, D. G., and Rueter, J. G. (Eds.), Springer, Dordrecht, Netherlands, 1992.

Wasmund, N., Nausch, G., and Hansen, A.: Phytoplankton succession in an isolated upwelled Benguela water body in relation to different initial nutrient conditions, Journal of Marine Systems, 140, 163-174, 2014.

White, A. E., Spitz, Y. H., Karl, D. M., and Letelier, R. M.: Flexible elemental stoichiometry in Trichodesmium spp. and its ecological implications, Limnology and Oceanography, 51, 1777-1790, 2006.

Yoshikawa, C., Makabe, A., Shiozaki, T., Toyoda, S., Yoshida, O., Furuya, K., and Yoshida, N.: Nitrogen isotope ratios of nitrate and N* anomalies in the subtropical South Pacific, Geochemistry, Geophysics, Geosystems, 16, 1439-1448, 2015.

Figure captions and tables

Figure 1. VAHINE water column DON concentration measurements from this study in color overlain upon those from Berthelot et al. (2015), in gray (a), DON $\delta^{15}N$ (b), PN_{susp} concentration (c), and PN_{susp} $\delta^{15}N$ (d) from within M1 (red filled inverted triangles), M2 (blue filled squares), M3 (green filled circles), and in the lagoon waters outside the mesocosms ("X" symbols). Error bars represent propagated error for DON concentration and DON $\delta^{15}N$, and ±1 S.D. for PN_{susp} concentration. No replicate measurements of PN_{susp} $\delta^{15}N$ were made, so no error bars are shown. Shaded regions indicate P0 (days 1 through 4) and P2 (days 15 through 23), with the unshaded region in between indicating P1 (days 5 through 14).

Figure 2. VAHINE PN_{sink} mass flux in M1 (red solid line), M2 (blue dotted line), and M3 (green dashed line), and PN_{sink} $\delta^{15}N$ in M1 (red filled inverted triangles), M2 (blue filled squares), and M3 (green filled circles) (a) and the corresponding contribution of N_2 fixation to export production (b). Shaded regions indicate P0 (days 1 through 4) and P2 (days 15 through 23), with the unshaded region in between indicating P1 (days 5 through 14). PN_{sink} $\delta^{15}N$ error bars represent an average measurement S.D. of ±0.06‰, and error bars for the fractional contribution of N_2 fixation to the PN_{sink} flux reflect the ±0.06‰ range associated with the PN_{sink} $\delta^{15}N$ measurements.

Table 1. Average concentrations (± 1 S.D.) (μM) and $\delta^{15}N$ (‰) for organic N pools and fluxes in the VAHINE mesocosms during P0 (days 1 through 4), P1 (days 5 through 14), and P2 (days 15 through 23), as well as in the lagoon waters outside the mesocosms. Additionally, the average (± 1 S.D.) fraction of export supported by N_2 fixation based on $\delta^{15}N$ budget calculations, as well as the average (± 1 S.D.) N_2 fixation rate for each time period based on both $\delta^{15}N$ budget calculations and $^{15}N_2$ incubations, are reported. Note that DON concentration and $\delta^{15}N$ for the lagoon and P0 are based on one measurement, so no standard deviation is included. Dissolved inorganic N pool concentrations were low (i.e., <0.1 μM) and invariant throughout the experiment (Berthelot et al., 2015).

Table 1.	lagoon	P0	P1	P2
[DON] (μM)	5.3	5.4	5.3 ± 0.3	5.2 ± 0.7
DON $\delta^{15}N$ (‰)	5.5	3.2	5.0 ± 0.7	4.8 ± 0.7
$[PN_{susp}]$ (μM)	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	1.3 ± 0.4
PN_{susp} $\delta^{15}N$ (‰)	3.3 ± 1.3	3.2 ± 1.5	3.4 ± 1.5	3.7 ± 0.9
PN_{sink} $\delta^{15}N$ (‰)	N/A	4.1 ± 0.3	3.0 ± 0.4	2.3 ± 1.8
% export from N_2 fixation	N/A	32 ± 4%	47 ± 6%	56 ± 24%
$\delta^{15}N$ budget N_2 fix. rate (μmol N m^{-2} d^{-1})	N/A	23 ± 8	51 ± 41	329 ± 298
$^{15}N_2$ fix incub. N_2 fix. rate (μmol N m^{-2} d^{-1})	137 ± 52	259 ± 88	150 ± 61	411 ± 127

Table 2. Comparison of time-integrated diazotroph derived N (DDN) for each mesocosm based on $\delta^{15}N$ budget calculations and $^{15}N_2$ fixation incubation rates.

Table 2.	M1	M2	M3
$\delta^{15}N$ budget DDN (μM)	0.29	0.28	0.20
$^{15}N_2$ incubation [N] (μM)	0.41	0.38	0.38
$\delta^{15}N$ budget/$^{15}N_2$ incubation	71%	75%	52%

Figure 1. DON concentration (a), DON $\delta^{15}N$ (b), PN_{susp} concentration (c), and PN_{susp} $\delta^{15}N$ (d) from the VAHINE mesocosm experiments.

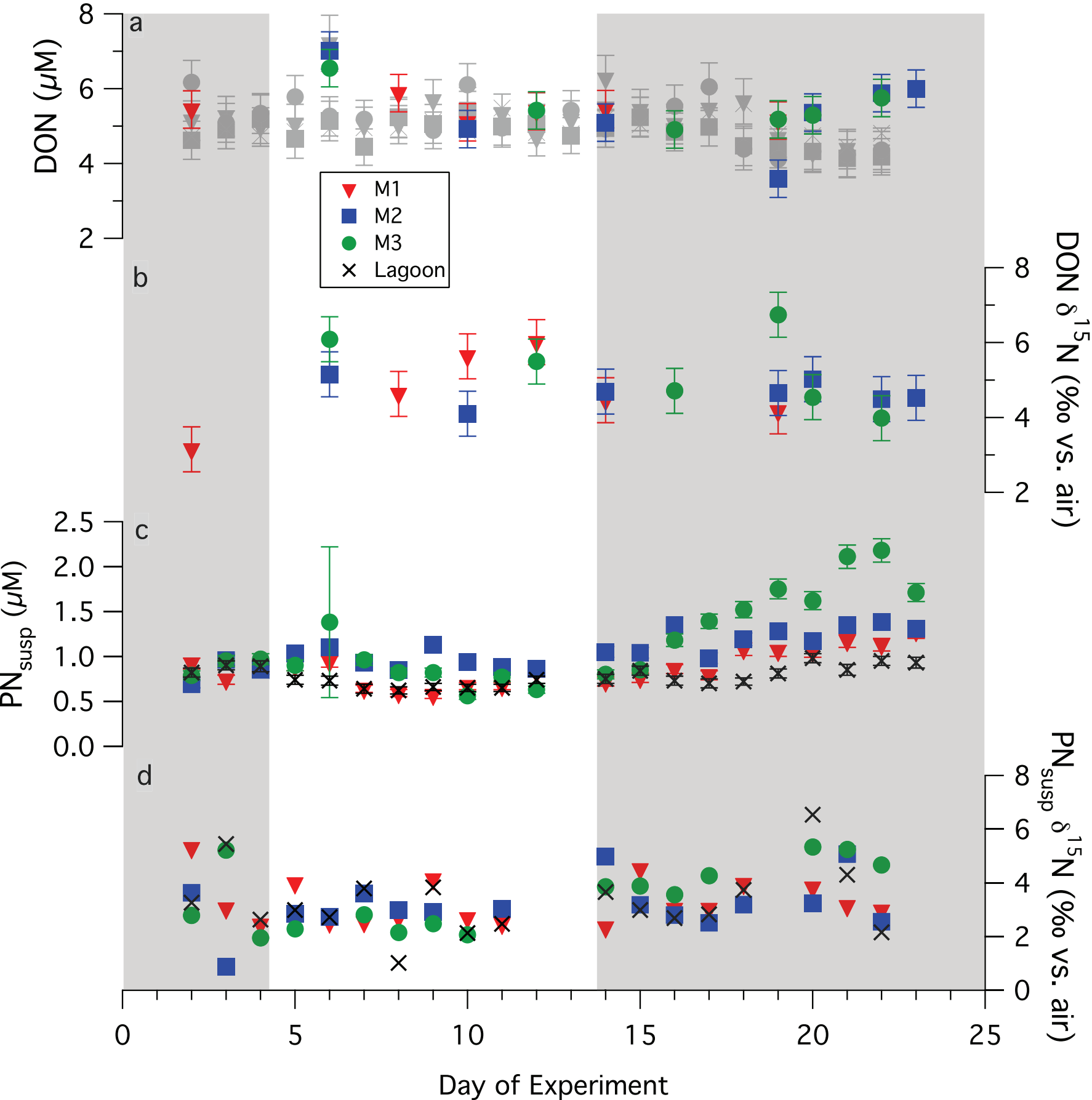
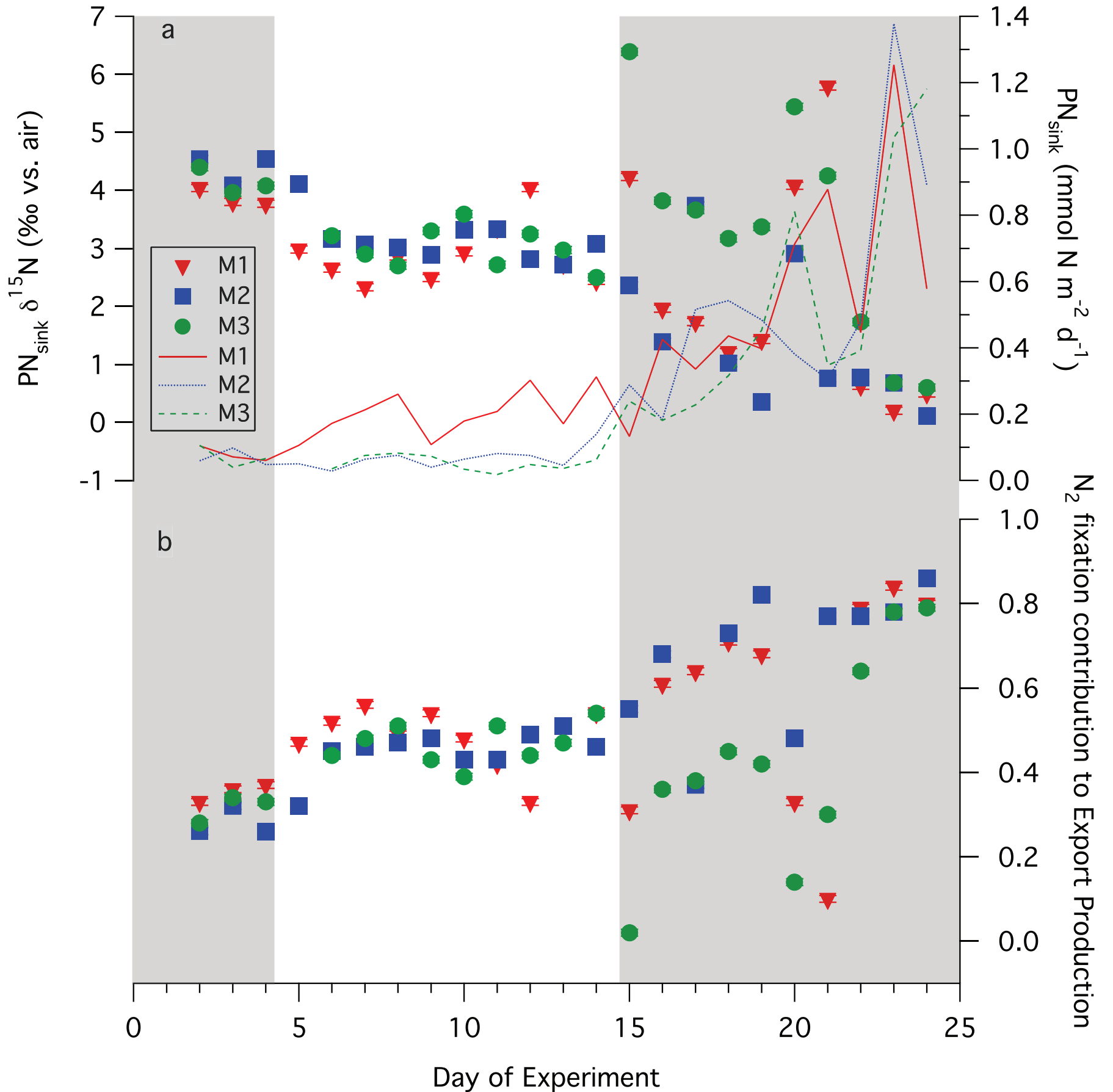


Figure 2. PN_{sink} mass flux and $\delta^{15}N$ (a) and $\delta^{15}N$ budget-based estimates of fractional contribution of N_2 fixation to export production (b).



Supplementary Information Table 1. Measurements of DON concentration ("[DON]") (μM) and DON $\delta^{15}N$ (‰), and PN_{sink} $\delta^{15}N$ (‰), made for the VAHINE mesocosms.

Day	M1 [DON] 6m	M1 DON $\delta^{15}N$ 6m	M2 [DON] 6m	M2 DON $\delta^{15}N$ 6m	M3 [DON] 6m	M3 DON $\delta^{15}N$ 6m	M1 $\delta^{15}N$ PN_{sink}	M2 $\delta^{15}N$ PN_{sink}	M3 $\delta^{15}N$ PN_{sink}
1									
2	5.4	3.2					4.0	4.5	4.4
3							3.8	4.1	4.0
4							3.8	4.5	4.1
5							3.0	4.1	
6			7.0	5.1	6.5	6.1	2.7	3.2	3.2
7							2.3	3.1	2.9
8	5.9	4.6					2.9	3.0	2.7
9							2.5	2.9	3.3
10	5.1	5.6	4.9	4.1			2.9	3.3	3.6
11							3.4	3.3	2.7
12	5.4	6.0			5.4	5.5	4.0	2.8	3.3
13							2.7	2.7	3.0
14	5.5	4.5	5.1	4.7			2.4	3.1	2.5
15							4.2	2.4	6.4
16					4.9	4.7	2.0	1.4	3.8
17							1.7	3.7	3.7
18							1.2	1.0	3.2
19	5.1	4.2	3.6	4.7	5.2	6.7	1.4	0.4	3.4
20			5.4	5.0	5.3	4.5	4.1	2.9	5.4
21							5.8	0.8	4.2
22			5.9	4.5	5.7	4.0	0.6	0.8	1.7
23			6.0	4.5			0.2	0.7	0.7
24							0.5	0.1	0.6