Transfer of diazotroph-derived nitrogen to the planktonic food web across gradients of N_2 fixation activity and diversity in the Western Tropical South Pacific

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Abstract.

Biological dinitrogen (N_2) fixation provides the major source of new nitrogen (N) to the open ocean, contributing more than atmospheric deposition and riverine inputs to the N supply. Yet the fate of the diazotroph-derived N (DDN) in the planktonic food web is poorly understood. The main goals of this study were to i) quantify how much of DDN is released to the dissolved pool during N_2 fixation and how much is transferred to bacteria, phytoplankton and zooplankton, ii) to compare the DDN release and transfer efficiencies under contrasting N_2 fixation activity and diversity in the oligotrophic waters of the Western Tropical South Pacific (WTSP) Ocean. We used nanometer scale secondary ion mass spectrometry (nanoSIMS) coupled with $^{15}N_2$ isotopic labelling and flow cytometry cell sorting to track the DDN transfer to plankton, in regions were the diazotroph community was either dominated by *Trichodesmium* or by UCYN-B. After 48 h, ~20-40 % of the N_2 fixed during the experiment was released to the dissolved pool when *Trichodesmium* dominated, while the DDN release was not quantifiable when UCYN-B dominated. ~7-15 % of the total fixed N (net N_2 fixation + release) was transferred to non-diazotrophic plankton within 48 h, with higher transfer efficiencies (15 ± 3 %) when UCYN-B dominated as compared to when *Trichodesmium* dominated (9 ± 3 %). The pico-cyanobacteria *Synechococcus* and *Prochlorococcus* were the primary beneficiaries of the DDN transferred (~65-70%), followed by heterotrophic bacteria (~23-34 %). The DDN transfer in bacteria was higher (34 ± 7 %) in the UCYN-B-dominating experiment compared to the *Trichodesmium*-dominating experiments (24 ± 5 %). Regarding higher trophic levels, the DDN transfer to the dominant zooplankton species was less efficient when the diazotroph community was dominated by *Trichodesmium* (~5-9 % of the DDN transfer) than when it was dominated by UCYN-B (~28 ± 13 % of the DDN transfer). To our knowledge, this study provides the first quantification of DDN release and transfer to phytoplankton, bacteria and zooplankton communities in open ocean waters. It reveals that despite UCYN-B fix N_2 at lower rates compared to *Trichodesmium* in the WTSP, the DDN from UCYN-B is much more available and efficiently transferred to the planktonic food web than the DDN originating from *Trichodesmium*.

1 Introduction

Nitrogen (N) is one of the basic building blocks of life, though much of the global Ocean surface (~70 %) is oligotrophic and characterized by low N availability, which limits primary productivity and phytoplankton growth (Falkowski, 1997; Moore et al., 2013). In these N-depleted areas of the tropical and subtropical ocean, biological dinitrogen (N_2) fixation (the reduction of atmospheric N_2 into bioavailable ammonia) sustains the major part of new production and organic matter export (Bonnet et al., 2009; Caffin et al., 2018; Capone et al., 2005; Karl et al., 2012). At the global scale, N_2 fixation is the major source of new N to the ocean, before atmospheric deposition and riverine inputs (100-150 Tg N yr^{-1}, Duce et al., 2008; Gruber, 2008). N_2 fixation is performed by prokaryotic organisms termed diazotrophs, which include the non-heterocystous filamentous cyanobacterium *Trichodesmium* (Capone et al., 1997; Carpenter, 1983), heterocystous cyanobacteria living in symbiosis with diatoms (or diatom-diazotroph associations, termed DDAs; Villareal, 1994), unicellular cyanobacteria termed UCYN (subdivided in Group A, B and C based on the *nifH* gene sequence, Zehr et al., 1998, 2008; Zehr and Turner, 2001) , diverse non-cyanobacterial bacteria (Bombar et al., 2015; Moisander et al., 2014; Riemann et al., 2010), and archaea (Löscher et al., 2014). Although considerable efforts have been deployed over the past decades to quantify N_2 fixation, identify the major players, and assess their biogeographical distribution in relation with environmental drivers, the fate of new N provided by N_2 fixation in the ocean, its role on the planktonic food web structure and large-scale biogeochemical fluxes is still poorly understood.

Early studies have reported high dissolved organic N (DON) and ammonia (NH_4^+) concentrations during and following *Trichodesmium* blooms in the Indian Ocean (Devassy et al., 1978, 1979; Glibert and O'Neil, 1999), suggesting that *Trichodesmium* release part of the recently fixed N_2 (hereafter referred to as diazotroph-derived N, DDN) to the dissolved pool, which could subsequently be consumed by the surrounding plankton communities. The first direct release measurements were performed in the early 1990s and showed that *Trichodesmium* colonies isolated from the tropical Atlantic Ocean release ~50 % of the recently fixed N_2 (Glibert and Bronk, 1994). Accumulations of DON and NH_4^+ have subsequently been confirmed near *Trichodesmium* blooms in the Pacific (Karl et al., 1992, 1997) and Atlantic Oceans (Lenes et al., 2001), although not systematic (Bonnet et al., 2016a; Hansell and Carlson, 2001), and in senescent *Trichodesmium* cultures (Mulholland and Capone, 2000), possibly related to the *Trichodesmium* Programmed Cell Death (PCD, Berman-Frank et al., 2004). This DDN release has been attributed i) to endogenous processes such as the dissipation of excess electrons linked to an excess of light (Wannicke et al., 2009) or to a means for the filamentous diazotrophs to transfer fixed N from N_2-fixing cells to vegetative cells (Mulholland and Capone, 2000) and ii) to exogenous processes such as viral lysis (Hewson et al., 2004; Ohki, 1999) or 'sloppy feeding' by copepods (O'Neil, 1999).

Numerous studies performed in culture (Hutchins et al., 2007; Karl et al., 1992, 1997) and in the field (Benavides et al., 2013b; Konno et al., 2010; Mulholland and Bernhardt, 2005) focused on quantifying this release, and most of them were performed on *Trichodesmium* and were based on the difference between the measurement of gross N_2 fixation (through the acetylene reduction method, Capone (1993)) and net N_2 fixation rates (through the $^{15}N_2$ isotope labelling method, Montoya et

al. (1996)) (Mulholland et al., 2004). It was thus shown that the DDN released to the dissolved pool averages ~50 % (10 to 80 %) of total N_2 fixation. The estimates based on this approach have then be questioned since the discovery of the methodological underestimation of net $^{15}N_2$ rates when the $^{15}N_2$ tracer is injected as a bubble in the incubation bottles (Mohr et al., 2010), leading to a potential overestimation of the DDN release. An alternative approach based on the direct measurement of the ^{15}N enrichment of both the particulate and dissolved pools (Glibert and Bronk, 1994; Slawyk and Raimbault, 1995) after incubation with $^{15}N_2$ present the advantage of providing ratio of particulate N_2 fixation versus DDN release, without being affected by potential underestimations issues. The studies based on this approach reveal that the proportion of DDN released to the dissolved pool ranges from 10 % to >80 % of total N_2 fixation measured in field (Benavides et al., 2013b; Berthelot et al., 2017; Glibert and Bronk, 1994; Konno et al., 2010). The release appears to be higher when *Trichodesmium* dominates the diazotroph community (Berthelot et al., 2017; Bonnet et al., 2016a; Glibert and Bronk, 1994) than when UCYN dominate (Benavides et al., 2013a; Bonnet et al., 2016b). The DDN release is generally much lower in (<5 %) in monospecific cultures (Berthelot et al. 2015, Benavides et al. 2013) than in field experiment suggesting that external factors such as sloppy feeding and viral lysis have a strong influence on the DDN release by diazotrophs in field.

The DDN released in the surface ocean is potentially available for surrounding planktonic communities, but its fate in the planktonic food web has poorly been quantified mainly due to methodological locks. Devassy et al. (1979) who reported high DON and NH_4^+ concentrations near *Trichodesmium* blooms were also the first to observe that during the decline of the *Trichodesmium* blooms, diatom abundances increased, followed by a succession of cladocerans, dinoflagellates, green algae and finally copepods. In the tropical Atlantic Ocean, high abundances of non-diazotrophic phytoplankton have also been observed following *Trichodesmium* blooms (Mulholland et al., 2004), and more recently, Chen et al. (2011) showed a positive correlation between abundances of *Trichodesmium* and diatoms in the Kuroshio Current. These studies suggest a link between diazotroph blooms and non-diazotrophic organisms. Studies based on size-fractionation carried out during a *Trichodesmium* bloom incubated in the presence of $^{15}N_2$ report that ~10 % of the fixed N_2 by *Trichodesmium* (recovered in the size fraction >30 µm) was rapidly transferred to non-diazotrophic organisms (recovered in the <30 µm fraction; Bryceson and Fay (1981)). Using similar methods, other studies suggested that during *Trichodesmium* blooms (Garcia et al., 2007; Mulholland et al., 2004) and *Nodularia* and *Aphanizomenon* blooms (Ohlendieck et al., 2000), 5 to 10 % of the DDN is transferred to the picoplankton compartment. However, the methods based on size fractionation do not discriminate the DDN transfer towards the non-diazotroph picoplankton to the potentially active N_2 fixation within picoplankton (in particular the UCYN-A, one of the most abundant diazotroph in our ocean (Luo et al., 2012)). This method therefore potentially overestimates the DDN transfer and is not applicable to study the DDN transfer associated with UCYN. Moreover, it is not possible with size fractionation methods to determine which populations (e.g. autotrophic vs. heterotrophic plankton, small vs. large plankton) have benefited the most from this source of new N. The recent use of high-resolution nanometer scale secondary ion mass spectrometry (nanoSIMS) coupled with $^{15}N_2$ isotopic labeling and flow cytometry cell sorting (Bonnet et al., 2016a, 2016b; Berthelot et al., 2016;) has proved its efficiency in the

quantification of the DDN transfer to specific groups of phytoplankton and bacteria. Applying this method during *Trichodesmium* blooms in the coastal Western Tropical South Pacific (WTSP), Bonnet et al. (2016a) revealed that after 48 h 13 ± 2 % to 48 ± 5% of the fixed N_2 was released to the dissolved pool and 6 ± 1 % to 8 ± 2 % of this DDN was transferred to non-diazotrophic plankton, mainly diatoms (45 ± 4 % to 61 ± 38 %) and bacteria (22 ± 27 % to 38 ± 12 %). A mesocosm experiment performed in the New Caledonian lagoon during a UCYN-C bloom (Bonnet et al., 2016b) revealed after 48 h 16 ± 6 % of the fixed N_2 was released to the dissolved pool and 21 ± 4 % of this DDN was transferred to non-diazotrophic plankton, mainly picoplankton (18 ± 4 %) and diatoms (3 ± 2 %). Finally, a comparative study between *Trichodesmium* vs. UCYN blooms simulated thanks to culture isolates (Berthelot et al., 2016), revealed that the DDN transfer to non-diazotrophic plankton is twice as high with *Trichodesmium* as with UCYN. The differences of DDN release and transfer rates observed between the different field experiments and the different diazotrophs suggest that these processes strongly depend on the physiological state of diazotrophs and the environment. Yet, the transfer of DDN to different groups of plankton from different diazotroph (*Trichodesmium* vs. UCYN) in the open ocean, where most of global marine N_2 fixation takes place, has never been investigated.

Regarding higher trophic levels, the low ^{15}N isotopic signature ($\delta^{15}N$) of zooplankton reveals that N_2 fixation can significantly contribute to zooplankton N requirements in high N_2 fixation areas (Aberle et al., 2010; Landrum et al., 2011; Loick-Wilde et al., 2012; Mompeán et al., 2013; Montoya et al., 2002; Sommer et al., 2006; Wannicke et al., 2013; Hunt et al., 2016). Few studies reports active grazing of *Trichodesmium* by some specific copepods (Micro- and Macrosetella sp., O'Neil et al. (1996); O'Neil and Roman (1992)). However, *Trichodesmium* has been shown to be toxic for most of the grazers (Guo and Tester, 1994; Hawser et al., 1992; Hawser and Codd, 1992) and the low $\delta^{15}N$ signature found in zooplankton (indicative of DDN consumption) where *Trichodesmium* thrive most likely originates from indirect transfer mediated by recycling processes (Capone et al., 1994; Capone and Montoya, 2001; Letelier and Karl, 1996) rather than from direct grazing. A recent study based on $^{15}N_2$ labelling in the coastal WTSP (Hunt et al., 2016) reveals that the DDN is less efficiently transferred to zooplankton when *Trichodesmium* and DDA dominate the diazotroph community than when UCYN-C dominate, suggesting that the DDN transfer efficiency to zooplankton strongly depends on the diazotroph involved in the N_2 fixation. To our knowledge, this has never been investigated so far in the open ocean.

The WTSP Ocean has recently been identified as a hot spot of N_2 fixation (Bonnet et al., 2017) and is characterized by trophic and N_2 fixation gradients (Moutin et al., 2017), with oligotrophic waters characterized by high N_2 fixation rates (631 ± 286 µmol N m^{-2} d^{-1}) mainly associated with *Trichodesmium* in the western part, and ultra-oligotrophic waters characterized by low N_2 fixation rates (85 ± 79 µmol N m^{-2} d^{-1}) mainly associated with UCYN in the eastern part (Bonnet et al., this issue; Stenegren et al., this issue). We performed a series of experiments under contrasting situations (either when *Trichodesmium* or UCYN was dominating the diazotroph community) to study the fate of DDN in the planktonic food web, with the following specific goals: (1) quantify the proportion of DDN released to the dissolved pool relative to total N_2 fixation, (2) quantify the DDN transfer to the non-diazotrophic phytoplankton and bacteria, and (3) quantify the DDN transfer to zooplankton.

2 Material and Methods

2.1 Experimental setup for DDN transfer experiments in phytoplankton and heterotrophic bacteria

This study was carried out during the OUTPACE (Oligotrophic to UlTra oligotrophic PACific Experiment) cruise (DOI: http://dx.doi.org/10.17600/15000900), which took place in February-March 2015 (austral summer) on-board the R/V *L'Atalante*. Samples were collected along a ~4000 km west to east zonal transect along ~19°S starting in New Caledonia and ending in French Polynesia, crossing Melanesian archipelago waters (hereafter referred to as MA waters) around New Caledonia, Vanuatu, Fiji up to Tonga and South Pacific Gyre waters located at the western boundary of the South Pacific Gyre (hereafter referred to as GY waters) (see Moutin et al. (2017) for details). Three experiments are reported here (hereafter named E1, E2 and E3). Two were performed at stations located in MA waters: station LD A : 19°12.8'S - 164°41.3'E, and station LD B: 18°14.4'S - 170°51.5'W, where *Trichodesmium* accounted for 95 and 100 % of the total diazotroph community quantified by quantitative PCR (Stenegren et al., 2017). The third experiment was performed in GY waters: station LD C: 18°25.2'S - 165°56.4'W, where UCYN accounted for 82 % of the total diazotroph community (Stenegren et al., 2017).

The experiments were designed according to Bonnet et al. (2016a, 2016b) and Berthelot et al. (2016). For experiments E1 and E2, seawater was collected by the surface underway pumping system at 6 m-depth. For E3, seawater was collected at 55 m-depth using Niskin bottles mounted on a CTD rosette. For all experiments, seawater was collected into 8 HCl-washed-sample rinsed (three times) 4.5 L polycarbonate bottles equipped with septum caps. 5 mL of $^{15}N_2$ gas (98.9 atom% ^{15}N, Cambridge isotopes) were injected into all bottles using a gas-tight syringe. The purity of the $^{15}N_2$ Cambridge isotopes stocks was previously checked by Dabundo et al. (2014) and more recently by Benavides et al. (2015) and Bonnet et al. (2016b), who concluded that the purity is satisfying (2×10^{-8} mol:mol N of $^{15}N_2$) and therefore do not alter the results presented below. The bottles were shaken 30 times to facilitate the $^{15}N_2$ dissolution and, except for the T0 set of bottles (see below), were incubated for 48 h in on-deck incubators covered with blue screening (50 % surface irradiance for E1 and E2 and 15 % surface irradiance for E3) and cooled with circulating surface seawater. At T0 and after incubation, a set of 4 bottles were collected and subsampled for the following measurements (see below for methods): N_2 fixation rates, DDN release, quantification of diazotrophs, heterotrophic bacteria and pico-, nano- and microphytoplankton enumeration, organic and inorganic nutrient concentrations, and ^{15}N-enrichment on diazotrophic and non diazotrophic plankton. Unless otherwise stated, each parameter reported below was measured in triplicates.

2.2 Net N_2 fixation rates and DDN released to the dissolved pool

N_2 fixation rates were measured using the $^{15}N_2$ isotopic tracer technique (adapted from Montoya et al. (1996)), as described in Bonnet et al. (this issue).

The DDN released to the dissolved pool under the form of NH_4^+ and DON during the N_2 fixation process was quantified using the three step diffusion method extensively described in Berthelot et al. (2015) and derived from Slawyk

and Raimbault (1995). As the NO_3^-pool was negligible, the total dissolved N (TDN) pool was defined as the sum of DON and NH_4^+ pools (TDN = DON + NH_4^+). After incubation with the $^{15}N_2$ tracer, 300 mL of the filtrate passed through pre-combusted Whatman GF/F filters were collected in 500 mL Duran Schott borosilicate flasks, poisoned with $HgCl_2$ (300 µL, final concentration 20 mg L^{-1}) and stored at 4°C in the dark until analysis. At the end of each step, NH_4^+ and DON fraction were recovered on acidified pre-combusted Whatman GF/F filters, dried 24 h at 60 °C and stored in pre-combusted glass tubes until analysis on an Elemental Analyser coupled to Isotope Ratio Mass Spectrometer (EA-IRMS, Integra2 Sercon Ltd) as described in Berthelot et al., (2015).

2.3 Inorganic and organic nutrient analyses

NH_4^+ concentrations were measured fluorimetrically according to Holmes et al. (1999) on a FP-2020 fluorimeter (Jasco, detection limit = 3 nM). NO_3^- and nitrite (NO_2^-) concentrations were measured using standard colorimetric procedures (Aminot and Kérouel, 2007) on a AA3 AutoAnalyzer (Seal-Analytical). DON concentrations were measured by the wet oxidation method according to Pujo-Pay and Raimbault (1994).

2.4 Plankton abundance determination

The abundance of *Trichodesmium* and UCYN-B was determined microscopically: 2.2 L of each triplicates $^{15}N_2$-amended 4.5 L bottles were gently filtered onto 2 µm pore size, 25 mm diameter Millipore polycarbonate filters and fixed with paraformaldehyde (2 % final concentration) for 1 h. *Trichodesmium* were enumerated on the entire surface of the filter at a x100 magnification with a Zeiss Axio Observer epifluorescence microscope fitted with a green (510-560 nm) excitation filter. The number of cells per trichome was counted on 20 trichomes for each experiment; we counted an average of 85 and 115 cells $trichome^{-1}$ for E1 and E2, respectively. UCYN-B were counted on 40 fields (1.3 mm^2 fields; 0-2800 UCYN-B per field) scanned and analyzed with the ImageJ1 software.

Samples for micro-phytoplankton identification and enumeration were collected in each of the triplicate 4.5 L incubated bottles (except for E1 were only one replicate was available) in five 50 mL sterile polypropylene tubes and preserved in acidic Lugol's solution (0.5 % final concentration). Diatoms were enumerated from a 250 mL subsample following the Utermohl method (Hasle, 1978), using a Nikon TE2000 inverted microscope equipped with phase-contrast and a long distance condenser. Diatoms were identified to the lowest possible taxonomic level in one of the three replicates.

Pico, nano-phytoplankton and heterotrophic bacteria abundances were determined by flow cytometry. After incubation, 1.8 mL were subsample from triplicate 4.5 L bottles into cryotubes, fixed with paraformaldehyde (200 µL, 4 % final concentration) for 5 min at room temperature, flash-frozen in liquid N_2, and stored at -80 °C until analysis on a FACSCalibur (BD Biosciences, San Jose, CA) according to Marie et al. (1999), at the PRECYM flow cytometry platform (https://precym.mio.univ-amu.fr/). Phytoplankton communities were clustered as *Prochlorococcus* spp. cell like, *Synechococcus* spp. cell like, nano-eukaryotes cell like, pico-eukaryotes cell like, and UCYN-B cell like. Truecount beads were used to determine the volume analyzed.

2.5 Cell sorting and sampling for nanoSIMS analyses

For flow cytometry cell sorting and subsequent analysis using nanoSIMS, 1 L of one of the 4.5 L bottles was filtered onto 0.2 µm pore size 47 mm polycarbonate filters to preconcentrate the cells and facilitate cell sorting. Filters were placed in a 5 mL cryotube® filled with 0.2 µm filtered seawater and PFA (2% final concentration), and incubated 1 h at room temperature in the dark. The cryovials were vortexed ~20 s to detach the cells from the filter and were stored at -80 °C until analysis. Cell sorting was performed using a Becton Dickinson Influx™ (BD Biosciences, Franklin Lakes, NJ) high speed cell sorter at the PRECYM platform, as described in Bonnet et al. (2016a, Supp. Info.). Planktonic groups (pico, nano-phytoplankton, bacteria and UCYN-B (for E3 only)) were separated using the same clusters as for the phytoplankton abundance determination. At the issue of the cell sorter, the cells were directly dropped onto a 0.2 µm pore size, 13 mm diameter polycarbonate filter (Millipore) connected to a low pressure pump in order to concentrate them on a surface as small as possible. The filters were stored at -80 °C until nanoSIMS analyses.

To recover large phytoplanktonic cells (*Trichodesmium* and diatoms), 1 L of the same 4.5 L bottle was filtered on 10 µm pore size 25 mm polycarbonate filters. The cells were fixed with PFA (2 % final concentration), incubated 1 h at ambient temperature and stored at -20 °C until nanoSIMS analyses.

2.6 NanoSIMS analyses

Just before nanoSIMS analyses, filters were thawed at ambient temperature and sputtered with gold and palladium to ensure conductivity. Analyses were performed on a NanoSIMS N50 (Cameca, Gennevilliers, France) at the French National Ion MicroProbe Facility as previsouly described in Bonnet et al. (2016a, 2016b) and Berthelot et al. (2016). Briefly, high density cells area were retrieved using the nanoSIMS optical camera (Fig.1 f.). Samples were pre-sputtered prior to analyses for at least 2 min to remove surface contaminants and increase conductivity with a ~22 pA Cesium primary beam. For the analysis, a ~1.2 pA Cesium (16 KeV) primary beam focused onto ~100 nm spot diameter was scanned across a 256×256 or 512×512 pixel raster (depending on the image size, which ranged from 20 µm × 20 µm to 40 µm × 40 µm) with a counting time of 1 ms per pixel. Negative secondary ions ($^{12}C^-$, $^{13}C^-$, $^{12}C^{14}N^-$, $^{12}C^{15}N^-$ and $^{28}Si^-$) were collected by electron multiplier detectors, and secondary electrons were imaged simultaneously. A total of 20 serial quantitative secondary ion images were generated to create the final image. Individual cells were easily identified in nanoSIMS secondary electron, $^{12}C^-$, $^{12}C^{14}N^-$ and $^{28}Si^-$ images that were used to define regions of interest (ROIs) around individual cells. A total of ~1500 ROIs was analyzed. For each ROI, the ^{15}N-enrichment was calculated. ~80 cells on average were analyzed for each plankton group and for each experiment (see Table 1 SI).

2.7 Cell-specific N content and DDN transfer calculations

To determine cell-specific N contents, cell sizes of *Trichodesmium* and dominant diatoms were directly measured on each sample collected for microscopy (see section 2.4). For *Trichodesmium*, cell length and width were measured on 25 to 50

cells per sample at x400 magnification with a Zeiss Axio Observer epifluorescence microscope. For diatoms, the cell cross section and apical and transapical dimensions were measured on at least 20 cells using a Nikon TE2000 inverted microscope equipped with phase contrast and a long-distance condenser. For UCYN-B, cells diameters were directly measured on the nanoSIMS images and further confirmed on microscopic images. The biovolumes (BV) of *Trichodesmium*, UCYN-B and diatoms were estimated following the geometric model of each cell type (Sun and Liu, 2003). The cellular carbon (C) contents were determined by using the relation between BV and C content according to Verity et al. (1992) for *Trichodesmium* and UCYN-B, and according to Eppley et al. (1970) and Smayda (1978) for diatoms. The N content was calculated based on a C:N ratios of 6 for *Trichodesmium* (Carpenter et al., 2004), 5 for UCYN-B (Dekaezemacker and Bonnet, 2011; Knapp et al., 2012), and a typical Redfield ratio of 6.6 for diatoms.

For *Synechococcus* and *Prochlorococcus* we used the C content reported in Buitenhuis et al. (2012) (255 and 36 fg C $cell^{-1}$, respectively). For nano-eukaryotes we used the C content reported in Grégori et al. (2001), and converted into N content according to the C:N Redfield ratio of 6.6 (leading to 3.2, 0.45 and 219 fmol N $cell^{-1}$ for *Synechococcus*, *Prochlorococcus* and nano-eukaryotes). For bacteria, an average N content of 2.1 fg N $cell^{-1}$ (Fukuda et al., 1998) was used. For the pico-eukaryotes, the cellular N content of 9.2 ± 2.9 fmol N $cell^{-1}$ was used as reported in Gregori et al. (2001).

The cell-specific N_2 fixation rates and DDN transfer rates (in nmol N L^{-1} 48 h^{-1}) to non-diazotrophic phytoplanktonwas calculated for each plankton group analyzed as follows:

$$DD^{15}N = \frac{^{15}Nex}{Nsr} \times N_{con} \times A$$

where ^{15}Nex (atom %) is the excess ^{15}N enrichment of the individual cells measured by nanoSIMS after 48 h of incubation relative to the time zero value, N_{sr} (atom %) is the excess ^{15}N enrichment of the source pool (N_2) in the experimental bottles determined by MIMS, N_{con} is the cellular N content (in nmol N $cell^{-1}$) of each cell and A is the abundance of the specific plankton group (in cell L^{-1}). Incertitude was estimated on each variable and the final incertitude was estimated using the propagation error rule.

2.8 Experimental setup for DDN transfer experiments in zooplankton

The DDN transfer to zooplankton was measured in four experiments performed at the same stations where the E1, E2 and E3 experiments were performed (hereafter names Zoo-1, Zoo-3 and Zoo-4) plus an additional station **(Zoo-2)** located between LDA and LDB (SD9, 20°57'S – 178°39'E). *Trichodesmium* was dominating the diazotroph community in the Zoo-1, Zoo-2, and Zoo-3 experiments, and UCYN-B was dominating the Zoo-4 experiment. The experiments consisted in incubations of freshly collected zooplankton in the presence of the natural planktonic assemblage pre-labelled with $^{15}N_2$ as a food source. In parallel to the experiments describes above, 6 additional HCl-washed-sample rinsed (three times) 1 L polycarbonate bottles equipped with septum caps were collected by the underway pumping system at 6 m-depth for Zoo-1, Zoo-2 and Zoo-3 and with Niskin bottles at 55 m-depth for Zoo-4. All bottles were amended with 1 mL of $^{15}N_2$ (98.9 atom% ^{15}N, Cambridge

isotopes). The bottles were shaken 30 times to facilitate the $^{15}N_2$ dissolution and incubated in on-deck incubators for 24 h-36 h as described above.

The incubation was stopped by filtering the bottles on 0.2 µm pore size 47 mm membrane filters in such a way that the ^{15}N enrichment of the food source provided to zooplankton (hereafter referred to as ^{15}N pre-labelled plankton) does not increase during the course of the experiment. For each experiment, the initial ^{15}N enrichment of the ^{15}N pre-labelled plankton was analyzed in triplicates by EA-IRMS. Plankton was then re-suspended in 6 1 L bottles filled with 0.2 µm filtered surface seawater collected at the same station. Meanwhile, zooplankton was collected using repeated net tows (120 mesh size) before dawn. Animals were recovered on a 120 µm sieve and placed into 4.5 L polycarbonate bottles filled with 0.2 µm filtered surface seawater in the dark for at least 6 h in order to allow them to empty their guts. Living animals were visually identified and the individuals belonging to the genus *Clausocalanus*, which largely dominated the zooplankton community at all stations (Carlotti et al., this issue), were handpicked and 12 animals were dispatched into each of the three 1 L bottles containing the ^{15}N pre-labelled plankton before being incubated in on-deck incubators for 24 h as described above. The three other bottles were immediately filtered after the introduction of animals, first through a 120 µm mesh in order to recover the animals and secondly through precombusted (4 h, 450 °C) GF/F filters, which were used to quantify the isotopic signature of the ^{15}N pre-labelled plankton at the beginning of the experiment, together with the initial NH_4^+ concentrations in the incubation bottles. After 24 h, the triplicate bottles containing the mixture of ^{15}N pre-labelled plankton and zooplankton were filtered in the same way. In addition, the filtrate was recovered as described in section 2.2 in order the measure the NH_4^+ concentration and the ^{15}N enrichment in the dissolved pool by using the two steps diffusion method. The recovered animals were placed on GF/F filters, which were analyzed by EA-IRMS as described above in section 2.2.

3 Results

3.1 Description of the biogeochemical context at the studied stations

Regarding the chlorophyll *a* and nutrient concentrations, the four studied stations were divided into two main sub-regions (Table 1): i) stations of E1/Zoo-1, Zoo-2 and E2/Zoo3 were located in the oligotrophic MA waters characterized by surface chlorophyll *a* concentrations of 0.159-0.377 µg L^{-1} and NO_3^- and PO_4^- concentrations below 50 nmol L^{-1}, ii) station E3/Zoo-4 located in the ultra-oligotrophic GY waters presenting lower chlorophyll *a* concentrations than in MA waters (0.053 µg L^{-1}), NO_3^- concentration below 50 nmol L^{-1}, and PO_4^- concentration ~110 nmol L^{-1}.

Trichodesmium dominated the diazotroph community (95 – 100 % of total *nifH* gene copies detected by qPCR, Stenegren et al., this issue) at 6 m-depth in the MA waters (E1/Zoo-1, Zoo-2 and E2/Zoo3), while UCYN-B dominated (82 % of *nifH* gene copies) at 55 m-depth in the GY waters (E3/Zoo-4 experiment). MA waters were characterized by a higher abundance of pico-phytoplankton (*Synechococcus* and *Prochlorococcus*) and bacteria abundances (125-200 and 271-424 10^{11} cells m^{-2}, respectively) than GY waters (~110 and ~290, 10^{11} cells m^{-2}, respectively; Bock et al., this issue).

3.2 N_2 fixation and DDN released to the dissolved pool

Net N_2 fixation rates were 20.1 ± 13.4, 49.9 ± 2.4 and 3.2 ± 0.3 nmol N L^{-1} 48 h^{-1} for the E1, E2 and E3 experiments, respectively (Fig. 2). The DDN released to the dissolved pool (the sum of DON and NH_4^+) was 14 ± 9 and 8 ± 2 nmol N L^{-1} 48 h^{-1} in E1 and E2, and was below quantification limits in E3. Considering gross N_2 fixation as the sum of net N_2 fixation and DDN release rates (Mulholland et al., 2004), the DDN released to the dissolved pool accounted for 40 ± 27 and 14 ± 4 % of gross N_2 fixation in E1 and E2, respectively (Fig. 4). DON accounted for the major part of the ^{15}N released and accounted for ~93 and ~96 % of the total N release in E1 and E2, respectively.

3.3 Cell-specific ^{15}N enrichment and DDN transfer to non diazotrophic plankton

NanoSIMS analyses performed on individual trichomes (E1 and E2) and UCYN-B cells (E3) revealed significant ($p<0.05$) ^{15}N-enrichment after 48 h of incubation (Fig. 1 a, b) compared to T0 samples (0.371 ± 0.005 atom%, n=5), indicating active N_2 fixation during the experiments: 1.946 ± 0.837 atom% (n=32) in E1, 1.523 ± 0.477 atom% (n=25) in E2 and 4.707 ± 0.210 atom% (n=192) in E3 (Fig. 3). Cell-specific N_2 fixation rates of *Trichodesmium* were 252.7 ± 50.5 fmol N $cell^{-1}$ 48 h^{-1} in E1 and 341.5 ± 68.3 fmol N $cell^{-1}$ 48 h^{-1} in E2, and cell-specific rates of UCYN-B were 18.6 ± 3.8 fmol N $cell^{-1}$ 48 h^{-1} in E3.

NanoSIMS analyses performed on non-diazotrophic plankton (diatoms and cell-sorted *Synechococcus* (Fig. 1 d), *Prochlorococcus*, bacteria, pico- and nano-eukaryotes (Fig. 1 e)) also revealed significant ^{15}N-enrichment as compared to T0 values ($p<0.05$) (Fig. 3). The ^{15}N-enrichment of the non-diazotrophic plankton (all groups pooled together) was not statistically different ($p>0.05$) between E1 and E2. However, it was significantly lower ($p<0.05$) in E3 compared to E1 and E2.

Over the 48 h of the experiment, 10 ± 2 % of the total DDN was transferred to non-diazotrophic plankton in E1, 7 ± 1 % in E2, and 15 ± 3 % in E3 (Fig.4). DDN was mainly transferred to pico-cyanobacteria (*Synechococcus* and *Prochlorococcus*), accounting for 73 ± 15 %, 68 ± 14 % and 65 ± 13 % of the total transfer into non-diazotrophs in E1, E2 and E3, respectively (Fig. 4). The transfer into heterotrophic bacteria accounted for ~~(~~25 ± 5 %, 23 ± 5 % and 34 ± 7 % of the total transfer, in E1, E2 and E3, respectively ~~(Fig. 4)~~. Lastly, 50 ± 40 %, 79 ± 4 % and 85 ± 9 % of the newly fixed $^{15}N_2$ remained in the pool of diazotrophs (corresponding to the major group of diazotrophs detected at each stations and analysed by nanoSIMS (*Trichodesmium* or UCYN-B), other potential diazotrophs that have not been targeted by qPCR (such as non-cyanobacterial diazotrophs), and other groups of non-diazotrophic plankton to which $^{15}N_2$ was transferred but that were not analyzed by nanoSIMS due to their very low abundance) for E1, E2 and E3, respectively.

3.4 DDN transfer to zooplankton

Before incubation with zooplankton, the isotopic enrichment of the ^{15}N pre-labelled plankton was not significantly different in the experiments Zoo-1, Zoo-2 and Zoo-3 (dominated by *Trichodesmium*) averaging 1.035 ± 0.091 atom% (n=9). The

isotopic enrichment was lower in the experiment Zoo-4 (dominated by UCYN-B) averaging 0.385 ± 0.005 atom% (n=3). After 24 h of incubation with zooplankton, the ^{15}N enrichment of the ^{15}N pre-labelled plankton decreased down to 0.431 ± 0.014 atom% on average in Zoo-1, Zoo-2 and Zoo-3, and down to 0.372 ± 0.010 atom% in Zoo-4. Meanwhile, the ^{15}N enrichment of zooplankton increased as compared to T0 values (0.383 atom% on average) and reached 0.482, 0.376, 0.513 and 0.368 atom% on average in Zoo-1, Zoo-2, Zoo-3 and Zoo-4, respectively. As the ^{15}N enrichment of the initial source food (^{15}N pre-labelled plankton) was different between the four stations/experiments, and in order to compare the results obtained among experiments, we normalized the values as the percentage of initial amount of ^{15}N atoms in excess in the ^{15}N pre-labelled plankton transferred to the different compartments (i.e. conserved in the ^{15}N pre-labelled plankton pool, transferred to the zooplankton pool and to the NH_4^+ pool) at the end of the incubation. In the experiments where *Trichodesmium* dominated the diazotroph community (Zoo-1, Zoo-2, and Zoo-3), 19 ± 7 % to 48 ± 21 % of the initial $DD^{15}N$ remained in the phytoplankton pool, 5 ± 5 % to 9 ± 13 % was transferred to the zooplankton and 0.4 ± 0.3 to 7 ± 3 % was transferred to the NH_4^+ pool (Fig. 5). In the Zoo-4 (where UCYN dominated the diazotroph community, Table 1), a greater proportion of $DD^{15}N$ was conserved in the phytoplankton (76 ± 34 %) but a greater transfer to the zooplankton was also observed (28 ± 8 %, Fig. 5). The recovery of the initial $DD^{15}N$ was comprised between 29 % and 100 %, suggesting that the remaining fraction was released to the DON pool. Interestingly, the recovery of the $DD^{15}N$ was in surplus in the Zoo-4 experiment (112.5 ± 8.5%), suggesting that the $DD^{15}N$ transfer in the DON pool is close to zero.

4 Discussion

4.1 DDN release to the dissolved pool

The quantity and quality of N released by diazotrophs to the dissolved pool during N_2 fixation potentially plays a key role in shaping the planktonic and microbial food webs. In this study, *Trichodesmium* released 14 ± 4 % to 40 ± 57 % of the newly fixed N into the dissolved pool, which is in agreement with values reported in the literature for field studies (Mulholland, 2007; Bonnet et al., 2016a). DON accounted for ~95 % of the DDN released by *Trichodesmium* (Fig. 2), accordingly with contributions measured in culture (80 - 90 %; Berthelot et al., 2015) and in the field (Berthelot et al., 2016). The low contribution of NH_4^+ to the DDN release does not mean that it was not released, but is likely the results of immediate consumption by surrounding plankton, which shows a great affinity for NH_4^+. Similarly, part of the DON released by *Trichodesmium* was probably uptaken by heterotrophic and mixotrophic plankton (Bronk et al., 2007) but a significant fraction was likely refractory (not easily available for organisms) leading to the observed accumulation in the dissolved pool. If not refractory, the DON would likely have been immediately assimilated as the region where these experiments were performed are strongly limited by N availability (Van Wambeke et al., 2008; this issue; Bonnet et al., 2008).

In the E1 experiment, we noticed a large variability of N_2 fixation and DDN release rates among the three replicates, which explains the high standard deviations (Fig. 2): two replicates exhibited net N_2 fixation rates ~25-30 nmol N L^{-1} 48 h^{-1} and DDN release rates ~7-10 nmol N L^{-1} 48 h^{-1}, whereas in the third replicate, the DDN release (~24 nmol N L^{-1} 48 h^{-1})

exceeded net N_2 fixation rates (5 nmol N L^{-1} 48 h^{-1}). This can be attributed to the decline of *Trichodesmium* in this replicate as we counted much more degraded trichomes in the third replicate. This suggests that decaying *Trichodesmium* release DDN more efficiently than healthy *Trichodesmium*, which has already been observed by Bonnet et al. (2016a). This may also explain why the DDN transfer to non-diazotrophic plankton was slightly higher in E1 (10 ± 2 %) than in E2 (7 ± 1 %), despite both stations were dominated by *Trichodesmium*.

Conversely to E1 and E2, the DDN released by UCYN-B (E3), was not quantifiable in our study. However, significant DDN transfer into non-diazotrophic plankton was detected (15 ± 3 % of the total fixed N, Fig. 4), suggesting that the DDN released to the dissolved pool is likely immediately transferred to surrounding communities. To our knowledge, this is the first report of DDN release in the field in the presence of a diazotroph community dominated by UCYN-B. Bonnet et al., (2016b) report low release from UCYN-C in coastal waters of the WTSP (16 ± 6 % of total N_2 fixation) compared to *Trichodesmium* (13 ± 2 % to 48 ± 5 %; Bonnet et al., 2016b). This seems to indicate that the DDN from UCYN is generally lower than the DDN from *Trichodesmium*. Several hypotheses may explain the differences observed between *Trichodesmium* and UCYN. DDN compounds released by UCYN may be more bio-available than the DDN released by *Trichodesmium*, limiting its accumulation. The lack of accumulation in E3 could also be due to the more severe N limitation of planktonic communities in the ultra-oligotrophic waters as compared to MA waters (Van Wambeke, this issue), and to the nature of the resident community. *Prochlorococcus* was dominating the planktonic community at LD C (E3) and is known to have a high affinity to its small surface to volume ratio (Partensky et al., 1999). PCD causing *Trichodesmium* bloom demise can also be involved in the relatively high DDN release and accumulation during *Trichodesmium* dominated experiments (Bar-Zeev et al., 2013). Exogenous factors, such as viral lyses (Fuhrman, 1999) and sloppy feeding (O'Neil and Roman, 1992b; Vincent et al., 2007) are also suspected to enhance the DDN release. These factors were found to excert a higher pressure in the MA waters where *Trichodesmium* dominated compared to ultra-oligotrophic waters (Bock et al., this issue), where UCYN-B dominated. Finally, part of the discrepancy might be due to a methodological artefact: different sampling procedures between E1 and E2 (pump) and E3 (Niskin bottles) as the pump is suspected to induce mechanical stress to the cells which may have potentially affected the DDN release.

The DDN release plays a key role in the transfer of N from diazotrophs to the surrounding non-diazotrophs, only it is not a good indicator of the DDN transfer efficiency as we observed that DDN transfer to non-diazotrophs was higher when the release was low (E3) than when it was high (E1 and E2). This has already been observed in coastal waters of the WTSP by (Berthelot et al., 2016).

4.2 DDN transfer efficiency and pathways in the WTSP

Here we report for the first time data on the transfer of DDN to the planktonic food web under contrasting diazotroph community composition in the open ocean. We reveal that 7 ± 1 % to 15 ± 3 % of the DDN is transferred to the non-diazotrophic plankton (Fig. 4) at short time scales (48 h), which is in the same order of magnitude than the transfer (~10 %) reported in coastal waters of the WTSP (Bonnet et al., 2016a; Berthelot et al., 2016). In terms of efficiency, despite UCYN-B

fix at lower rates compared to *Trichodesmium*, the DDN originating from UCYN-B is more efficiently transferred to non-diazotrophic plankton (15 ± 3 % of total fixed N in the E3 experiment, Fig. 4) compared to the DDN originating from *Trichodesmium* (10 ± 2 % and 7 ± 1 % in the E1 and E2 experiments, respectively). This results is in accordance with the fact that we did not detect any accumulation of ^{15}N-labelled N forms in the dissolved pool (see section above).

Several studies have proven that a fraction of the DDN release is transferred to surrounding non-diazotrophic plankton, and one of them (Bonnet et al., 2016a) conclude that diatoms are the major beneficiaries of the DDN originating from *Trichodesmium* and develop extensively during/after *Trichodesmium* blooms in the coastal WTSP ocean. Despite *Trichodesmium* is rarely recovered in sediment traps (Chen et al., 2003; Walsby, 1992), these authors hypothesize a tight coupling between *Trichodesmium* blooms and export of organic matter as diatoms are efficient exporters of organic carbon to depth (Nelson et al., 1995). In contrast, in the present study, > 90 % of the DDN was transferred to picoplankton (*Synechococcus*, *Prochlorococcus* and bacteria), whatever the station studied (Fig. 4). The cyanobacteria *Synechococcus* and *Prochlorococcus* were the primary beneficiaries (73 ± 15 %, 68 ± 14 % and 65 ± 13 % of the DDN transfer, Fig. 4), which is consistent with Bonnet et al. (this issue) who observed a positive correlation between N_2 fixation rates and the abundance of *Synechococcus* and *Prochlorococcus* in the WTSP. We attributed this difference between the present study and the Bonnet et al., (2016a) study to the phytoplanktonic populations present in ambient waters at the time of the experiments. In the Bonnet et al., (2016a) study, diatoms were accounting for ~30 % of the non-diazotrophic phytoplankton biomass at T0, whereas, diatoms were scarce (1 % of the non-diazotrophic phytoplankton biomass) in our offshore experiments, i.e. too low to show a significant increase in 48 h, even if they benefited from the DDN. However, in E2 (LD B), the diatom abundances were the highest of the three experiments (Leblanc et al., this issue) and the bloom at this station was mainly composed of diatoms and *Trichodesmium*, suggesting that *Trichodesmium* contributed to sustain this bloom.

In the E1 and E2 experiments, where *Trichodesmium* was the dominant diazotroph (Stenegren et al., this issue), the DDN was preferentially transferred to *Synechococcus*, while it was preferentially transferred to *Prochlorococcus* in E3 where UCYN-B was the dominant diazotroph (Stenegren et al., this issue). This suggests a possible coupling between *Synechococcus* and *Trichodesmium* as ever mentioned by Campbell et al. (2005), who report higher *Synechococcus* abundances inside *Trichodesmium* blooms compared to surrounding waters, while it is not the case for other plankton groups. This difference may also be linked with the communities present at the time of the experiments: *Prochlorococcus* accounted for ~65 % of pico-phytoplankton in E1 and E2, while it accounted for ~80 % in GY in E3. While the transfer of DDN to *Prochlorococcus* and *Synechococcus* together was roughly equivalent for E1 and E2 (~70 %, Fig. 4), *Synechococcus* abundances increased by 150 % in E1, and *Prochlorococcus* increased by 12 % during the time course of the experiment, while none of the populations increased in abundance in E2 (Fig. 1 Supp. Info.). This result is in agreement with Bock et al. (this issue) who report an increase of the grazing pressure with the decrease of the oligotrophic degree as E1 was performed in more oligotrophic waters than E2.

Besides *Prochlorococcus* and *Synechococcus*, heterotrophic bacteria were the second beneficiaries of the DDN, especially when the diazotroph community was dominated by UCYN-B (34 ± 7 % of the DDN transfer, Fig. 4). In this

experiment, bacteria abundances increased by 70 % on average (Fig. 1 Supp. Info), which is in agreement with Berthelot et al., (2016) and Bonnet et al., (2016a; 2016b), who reported similar bacterial increases in the coastal WTSP. When *Trichodesmium* was the dominant diazotroph, 23-25 % was transferred to bacteria, whose abundance increased by 135 % and 15 % in E1 and E2, consistent with Sheridan et al. (2002), who reported higher bacterial abundances in *Trichodesmium* blooms than in surrounding waters. The significant DDN transfer from *Trichodesmium* to bacteria concurs with *Trichodesmium* and bacteria association that has been largely highlighted in the last decades (Hmelo et al., 2012; Paerl et al., 1989; Sheridan et al., 2002). That is in accordance with Van Wambeke et al. (this issue), who report that N_2 fixation fuels 3-35 % of bacterial production in MA waters. Then, we could not discriminate the DDN transfer to pico- and nano-eukaryotes, but for diatoms, the DDN transfer represented a low contribution to the overall transfer into non-diazotrophs in this region of the open ocean.

4.3 Transfer of DDN to zooplankton

Regarding higher trophic levels, the experiments performed here show that the DDN transfer to the major group of zooplankton present in this ecosystem (the copepod *Clausocalanus*) was less efficient (Fig. 5) when the diazotroph community was dominated by *Trichodesmium* (~5-9 %) than when it was dominated by UCYN-B (~28 %). This result is consistent with a previous study based on analogous $^{15}N_2$ labelling method in coastal waters of the WTSP (Hunt et al., 2016), which also report a higher DDN transfer efficiency in the presence of UCYN.

Regarding the DDN transfer from UCYN-B, although the transfer experiments to phytoplankton and bacteria (E3) and zooplankton (Zoo-4) were not performed in the same incubation bottles, they consistently report lower ^{15}N-enrichments in all the studied pools as compared to the experiments performed when *Trichodesmium* dominated, but in fine, the DDN transfer efficiency was more important in the presence of UCYN. We observed that a larger fraction of DDN was conserved in the UCYN-B pool than in the *Trichodesmium* pool, and a larger part of the DDN was missing (likely associated to the DON pool) with *Trichodesmium* than with UCYN-B (Fig. 5). These observations are consistent with the transfer experiments E1, E2, and E3 which show that *Trichodesmium* released more DDN in the dissolve pool (DON + NH_4^+) than UCYN-B. The DDN released in the NH_4^+ pool did not presented significant differences between *Trichodesmium* (Zoo-1, Zoo-2 and Zoo-3) and UCYN-B (Zoo-4), and in all experiments, the DDN contribution was low in the NH_4^+ pool, as it was immediately assimilated by surrounding organisms as explained in section 4.1. We suggest that the DDN transfer was higher with UCYN than with *Trichodesmium* since the UCYN-B can be directly grazed due to their small size (2-3 µm) as mentioned in Hunt et al. (2016), who revealed high UCYN abundances in the copepods guts based on qPCR data, while less *Trichodesmium* were measured. This pleads for a direct transfer of DDN from UCYN-B to zooplankton and an indirect transfer from *Trichodesmium* through non-diazotrophs. At the ecosystem level, even if the DDN transfer efficiency (~15 %) to zooplankton from UCYN-B is higher than the one of *Trichodesmium*, the ultimate quantity of DDN transferred to secondary producers is higher when *Trichodesmium* dominates, as cell-specific N_2 fixation rates of *Trichodesmium* (~250-340 fmol N $cell^{-1}$ 48 h^{-1}) are far higher than those of UCYN-B (~19 fmol N $cell^{-1}$ 48 h^{-1}). This result is in agreement with

Carlotti et al. (this issue) results based on ^{15}N isotopic data showing that ~50-95 % and ~10-40 % of the zooplankton N content originates from N_2 fixation in MA and GY waters, respectively. Finally, the DDN transferred to zooplankton, either directly or indirectly, may be released in the dissolved pool as NH_4^+, providing additional NH_4^+ from DDN in the environment that is likely assimilated by organisms in N-depleted waters. Thus, zooplankton N release appears as another DDN transfer pathway to the microbial communities in the WTSP.

Zooplankton can contribute to organic matter export by production of sinking fecal pellets, active transport to depth and carcasses export. These processes are increasingly recognized as important vectors of organic matter export, and the magnitude of their contributions to organic matter export are highly dependent on regionally variable plankton community structure (Steinberg and Landry, 2017). In the WTSP, where N_2 fixation sustains most of the new primary production (Caffin et al., 2018) and an important fraction of the DDN is transferred to zooplankton, it might play a key role on the export production and hence the CO_2 sink which is the WTSP.

5. Conclusion and ecological impact of N_2 fixation in the WTSP

N_2 fixation acts as a natural N fertilizer in the ocean, releasing DDN in the dissolved pool, which is available for surrounding marine organisms. To our knowledge, this study provides the first quantification of DDN transfer to phytoplankton, bacteria and zooplankton communities in open ocean waters. The main interest of this study was to compare DDN transfer and release under contrasting N_2 fixation activity and diversity.

Here, we reveal that *Trichodesmium* released more DDN than UCYN-B, but a significant part of the DDN released by *Trichodesmium* accumulated in the dissolved pool, while the DDN released by UCYN-B immediately assimilated by the surrounding plankton communities. The DDN transfer efficiency to non-diazotrophic plankton was higher when UCYN-B dominated the diazotroph community than when *Trichodesmium* dominated. In the open ocean, most N_2 fixation is performed by *Trichodesmium* (Capone et al., 1997 Luo et al., 2012), thus on a global scale most of the DDN transfer can be attributed to *Trichodesmium*. The regions where UCYN are the dominant diazotrophs generally present lower N_2 fixation rates than the ones where *Trichodesmium* dominates, but UCYN provide a continuous source of DDN to surrounding plankton communities. The DDN was transferred to pico-plankton, which dominated the WTSP, suggesting that N_2 fixation fueled the growth of biomass in the N-depleted environment. This is consistent with Caffin et al., (2018), who revealed that N_2 fixation provides > ~~more than~~ 90 % of the new N to the photic layer of the WTSP. On a larger scale view, the simulation performed by Dutheil et al. (this issue) predicts that diazotrophs support a large part of PP (~15 %) in LNLC regions of the Pacific Ocean, comprising the WTSP.

Overall, this study indicates that N_2 fixation plays a key role on the marine biomass production, the structure of the planktonic food web, and finally on the export of organic matter towards the deep ocean. The DDN can be exported to the deep ocean by different pathways: i) the direct of export of diazotrophs, ii) the export of non-diazotrophs which benefited from the DDN, and iii) the export of zooplankton which benefited from the DDN. The direct export of diazotrophs accounted for ~ 30 % of total C export at LD A (E1), 5 % at LD B (E2) and < 0.1 % at LD C (E3) (Caffin et al., 2018).

Using a $\delta^{15}N$ budget, Knapp et al., (This issue) found that 50-80 % of exported material was sustained by N_2 fixation (this includes both direct and indirect export of DDN). Thus, N_2 fixation has ineluctably a key role on the biological carbon pump, as mentioned in Moutin et al. (this issue) who reveal a significant biological "soft tissue" carbon pump in the MA waters almost exclusively sustained by N_2 fixation, and acting as a net sink ~~for~~ of atmospheric CO_2 in the WTSP.

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Table 1: Environmental conditions at stations where experiments were performed. Station position, depth of sampling, concentrations of Chl a, NO_3^-, NH4+, DON, PO_4^-, DOP, dominant phytoplankton communities and dominant diazotrophs.

Experiment		E1 / Zoo-1	Zoo-2	E2 / Zoo-3	E3 / Zoo-4	References
Position	Lat. - Lon.	19°12.8'S 164°41.3'E	20°57'S 178°39'E	18°14.4'S 170°51.5'W	18°25.2'S 165°56.4'W	
Depth	m	6	6	6	55	
Chl a	µg L^{-1}	0.197	0.159	0.377	0.053	Dupouy et al., this issue
NO_3^-	nmol L^{-1}	30	< 10	20	20	
NH_4^+	nmol L^{-1}	5	2	8	1	
DON	µmol L^{-1}	6.20	5.20	6.00	5.15	
PO_4^-	µmol L^{-1}	10	10	20	110	
DOP	µmol L^{-1}	0.15	0.17	0.17	0.15	
Prochlorococcus	10^{11} cells m^{-2} integrated on the upper photic layer	122 ± 31		183 ± 27	110 ± 9	Bock et al., this issue
Synechococcus		3 ± 2		16 ± 9	0.5 ± 0.2	
Bacteria		271 ± 73		424 ± 108	290 ± 32	
Dominant diazotroph	% nifH gene copies	*Trichodesmium* 95 %	*Trichodesmium* 99 % *	*Trichodesmium* 100 %	UCYN-B 82 %	Stenegren et al., this issue

Figure captions

Figure 1: NanoSIMS images showing the ^{15}N-enrichment (a,b,d,e) after 48 h of incubation in the presence of $^{15}N_2$ for *Trichodesmium* (a), UCYN-B (b), Nano-Eukaryotes (d) and *Synechococcus* (e). The ROIs are represented with white line. NanoSIMS images showing the secondary electrons channel of UCYN (c) and optical camera image of *Prochlorococcus* spotted on the filter before NanoSIMS analyses (f).

Figure 2: N_2 fixation rates (dark grey, nmol N L^{-1} 48 h^{-1}), DDN release nmol N L^{-1} 48 h^{-1}) as NH_4^+ (light grey) and DON (white) for each experiment (E1, E2 and E3). Error bars represent the standard deviation of triplicate incubations.

Figure 3: Left panels: box-plot of the 15N-enrichment measured in diazotrophs (*Trichodesmium* for E1 and E3, and UCYN-B for E3). Right panels: ^{15}N-enrichment measured in non-diazotrophic plankton: *Synechococcus*, *Prochlorococcus*, Bacteria, Diatoms, Pico-Eukaryotes and Nano-Eukaryotes for each experiment. Black dotted line indicates the natural isotopic enrichment.

Figure 4: DDN fate after 48 h for each experiment. Left pie charts: Orange: DDN remained in diazotrophs (orange), yellow: DDN released to the dissolved pool, Dark blue: DDN transferred to non diazotrophic plankton Right pie charts, from dark blue to light blue: Relative DDN transferred to *Synechococcus*, *Prochlorococcus*, bacteria, diatoms, pico-eukaryotes and nano-eukaryotes in E1 (top), E2 (middle) and E3 (bottom pie chart)..

Figure 5: DD^{15}N transferred (%) in the NH_4^+ pool (white), zooplankton (light grey) and remained in the phytoplankton pool (dark grey) after 24 h of incubation. Error bars represent the standard deviation of triplicate incubations and the propagated analytical errors

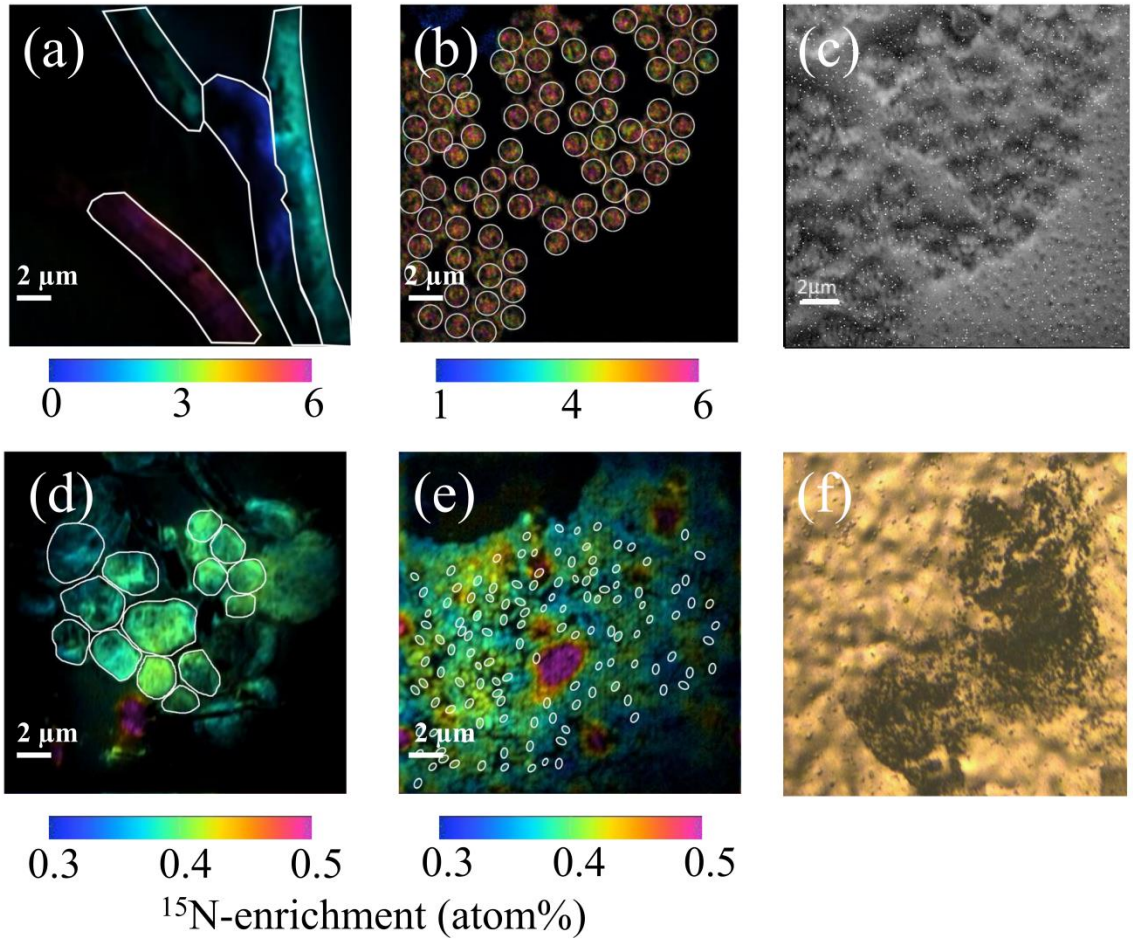
(a)
2 µm
(b)
2 µm
(c)
2µm
0
3
6
1
4
6
(d)
2 µm
(e)
2 µm
(f)
0.3
0.4
0.5
0.3
0.4
0.5
15N-enrichment (atom%)

Figure 1

5

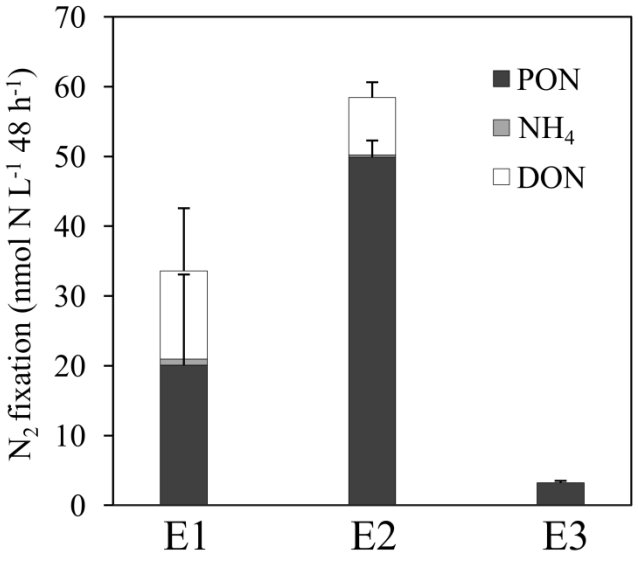
70
60
50
40
30
20
10
0
N_2 fixation (nmol N L^{-1} 48 h^{-1})
PON
NH_4
DON
E1
E2
E3

Figure 2

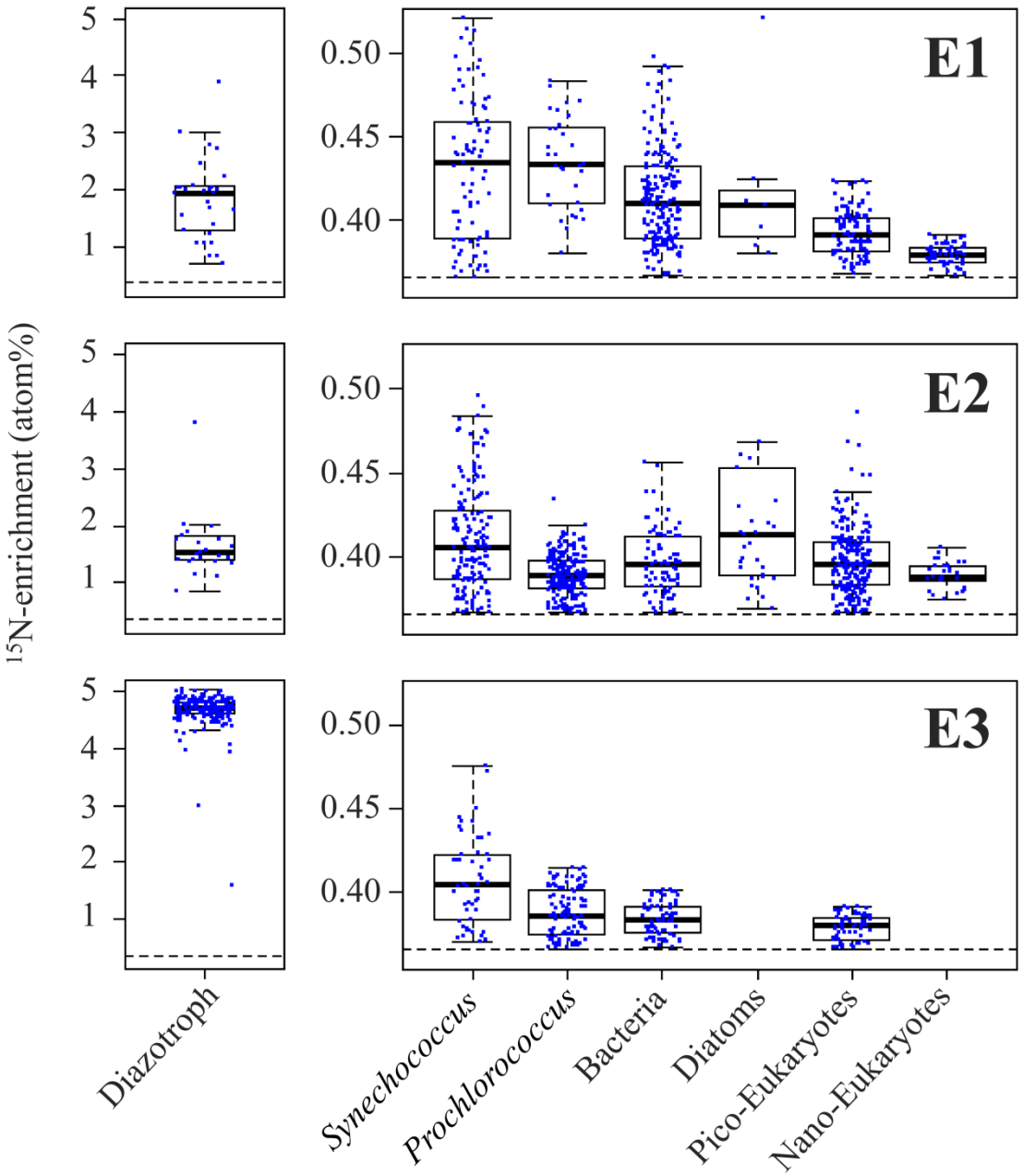
E1
E2
E3
^{15}N-enrichment (atom%)
Diazotroph
Synechococcus
Prochlorococcus
Bacteria
Diatoms
Pico-Eukaryotes
Nano-Eukaryotes

Figure 3

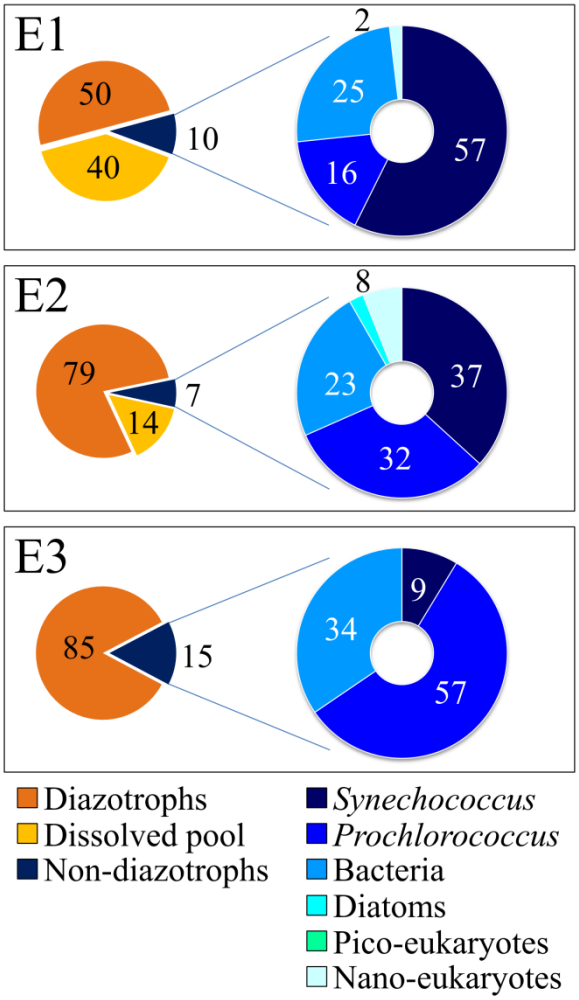
E1
50
40
10
2
25
16
57
E2
79
14
7
8
23
32
37
E3
85
15
34
9
57
Diazotrophs
Dissolved pool
Non-diazotrophs
Synechococcus
Prochlorococcus
Bacteria
Diatoms
Pico-eukaryotes
Nano-eukaryotes

Figure 4

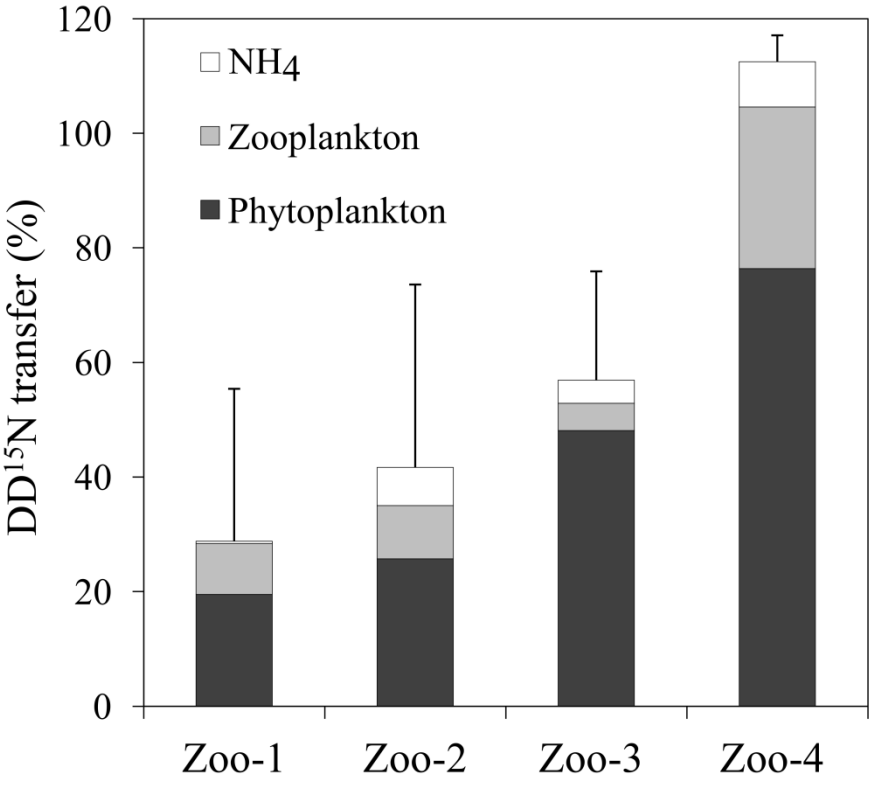
120
100
80
60
40
20
0
DD15N transfer (%)
NH4
Zooplankton
Phytoplankton
Zoo-1
Zoo-2
Zoo-3
Zoo-4

Figure 5