

Dinitrogen fixation and dissolved organic nitrogen fueled primary production and particulate export during the VAHINE mesocosms experiment (New Caledonia lagoon)

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Abstract

In the oligotrophic ocean characterized by nitrate (NO_3^-) depletion in surface waters, dinitrogen (N_2) fixation and dissolved organic nitrogen (DON) can represent significant nitrogen (N) sources for the ecosystem. Here we deployed in New Caledonia large in situ mesocosms in order to investigate (1) the contribution of N_2 fixation and DON use to primary production (PP) and particle export and (2) the fate of the freshly produced particulate organic N (PON) i.e. whether it is preferentially accumulated and recycled in the water column or exported out of the system. The mesocosms were fertilized with phosphate (PO_4^{3-}) in order to prevent phosphorus (P)-limitation and promote N_2 fixation. The diazotrophic community was dominated by diatoms-diazotrophs associations (DDAs) during the first part of the experiment for 10 days (P1) followed by the unicellular N_2 -fixing cyanobacteria UCYN-C the last 9 days (P2) of the experiment. N_2 fixation rates averaged 9.8 ± 4.0 and $27.7 \pm 8.6 \text{ nmol L}^{-1} \text{ d}^{-1}$ during P1 and P2, respectively. NO_3^- concentrations ($< 0.04 \mu\text{mol L}^{-1}$) in the mesocosms were a negligible source of N indicating that N_2 fixation was the main driver of new production all along the experiment. The contribution of N_2 fixation to PP was not significantly different ($p > 0.05$) during P1 ($9.0 \pm 3.3\%$) and P2 ($12.6 \pm 6.1\%$). However, the e -ratio that quantifies the efficiency of a system to export particulate organic carbon ($\text{POC}_{\text{export}}$) compared to PP ($e\text{-ratio} = \text{POC}_{\text{export}}/\text{PP}$) was significantly higher ($p < 0.05$) during P2 ($39.7 \pm 24.9\%$) than during P1 ($23.9 \pm 20.2\%$), indicating that the production sustained by UCYN-C was more efficient at promoting C export than the production sustained by DDAs. During P1, PON was stable and the total amount of N provided by N_2 fixation ($0.10 \pm 0.02 \mu\text{mol L}^{-1}$) was not significantly different ($p > 0.05$) from the total amount of PON exported ($0.10 \pm 0.04 \mu\text{mol L}^{-1}$), suggesting a rapid and probably direct export of the recently fixed N_2 by the DDAs. During P2, both PON concentrations and PON export increased in the mesocosms by a factor 1.5–2. Unlike in P1, this PON production was not totally explained by the new N provided by N_2 fixation. The use of DON, whose concentrations decreased significantly ($p < 0.05$) from $5.3 \pm 0.5 \mu\text{mol L}^{-1}$ to $4.4 \pm 0.5 \mu\text{mol L}^{-1}$, appeared to be the missing N source. The DON consumption ($\sim 0.9 \mu\text{mol L}^{-1}$) during P2 is

higher than the total amount of new N brought by N_2 fixation ($\sim 0.25 \mu\text{mol L}^{-1}$) during the same period. These results suggest that while DDAs mainly rely on N_2 fixation for their N requirements, both N_2 fixation and DON can be significant N-sources for primary production and particulate export following UCYN-C blooms in the New Caledonia lagoon and by extension in the N-limited oceans where similar events are likely to occur.

1 Introduction

Nitrogen (N) availability constitutes one of the most limiting factors for marine primary production (PP) (Moore et al., 2013). About 80% of the global ocean surface is depleted in dissolved inorganic N (nitrate (NO_3^-) and ammonium (NH_4^+) $< 1 \mu\text{mol L}^{-1}$) and characterized by low PP, low biomass and low particulate matter export fluxes (Longhurst, 2007). In these Low Nitrate, Low Chlorophyll (LNLC) ecosystems, the strong stratification of the photic surface layer prevents the mixing with NO_3^- -replete deep waters and imposes on phytoplanktonic communities to rely on alternative N sources for growth. These sources comprise the virtually inexhaustible dissolved dinitrogen (N_2) pool ($\sim 400 \mu\text{mol L}^{-1}$), and the large ($\sim 5 \mu\text{mol L}^{-1}$) but mainly refractory dissolved organic N (DON) pool.

The first pool (N_2) is only accessible to prokaryotic organisms possessing the *nifH* gene and able to reduce the N_2 gas molecule into bioavailable NH_4^+ . This process called N_2 fixation (or diazotrophy) is responsible for the main external source of N for the upper ocean (Gruber and Galloway, 2008; Mahaffey, 2005) and fuels PP in LNLC ecosystems (e.g. Dugdale and Goering, 1967; Karl et al., 1997). However, the fate of recently fixed N_2 in the planktonic food web and its potential impact on carbon export are poorly understood. Moreover, this fate may differ according to the diazotrophic species involved in N_2 fixation. The widely distributed filamentous cyanobacterium *Trichodesmium* spp., one of the main contributors to global N_2 fixation (Capone et al., 1997), is rarely found in sediment traps (Chen et al., 2003; Walsby, 1992) indicating that *Trichodesmium* spp. has a low direct export efficiency. However, a recent study performed in the South West Pacific indicates that N fixed by *Trichodesmium* spp. is preferentially and rapidly (within few days) transferred to

diatoms and bacteria (Bonnet et al., 2015b), potentially resulting in indirect carbon export. Conversely, diatoms-diazotrophs associations (DDAs) drive an efficient biological carbon pump in the Amazon River plume (Subramaniam et al., 2008) and in the North Pacific Gyre (Dore et al., 2008; Karl et al., 2012), indicating an efficient export of the N fixed by these organisms. Finally, unicellular N₂-fixing cyanobacteria (UCYN), are presumably the most abundant in the global ocean and are also major contributors to global N₂ fixation (e. g. Moisaner et al., 2010; Montoya et al., 2004). However, little is known regarding the fate of the N fixed by UCYN, whether it is directly or indirectly exported out of the euphotic zone or recycled in surface waters (Thompson and Zehr, 2013).

The second N pool (DON) may constitute a significant N source for planktonic communities but remains poorly constrained (Bronk, 2002). The DON pool is a “Black box” composed of various chemical products more or less refractory with their own specific turnover time (Bronk et al., 2007). The persistence of high DON concentrations in surface oceanic waters has formerly led to consider that it is unavailable for the marine biota. However, the determination of DON concentrations is submitted to high analytical uncertainties (Czerny et al., 2013) that may hide low but ecologically relevant changes in concentrations resulting from consumption of the labile or semi labile fraction of the DON pool. Furthermore, due to the heterogeneous composition of DON, isotopic labeling experiments using tracers are difficult to conduct, which explains the lack of information on the fluxes transiting in and out of the DON pool (Bronk, 2002; Bronk et al., 2007). Although heterotrophic bacteria are presumably the main users of this organic pool, it has been shown that primary producers can also use it to meet their N requirements (Antia et al., 1991; Berman and Bronk, 2003). Similarly to fixed N₂, the fate of DON assimilated by marine plankton will depend on the consumers, whether they would remineralize or export the particulate organic matter produced.

Studying the fate of N in the ocean is complex as it requires to follow the biogeochemical characteristics, the succession of planktonic species and the potential export from the same water mass for several weeks. In the open ocean, such studies are further complicated by physical processes (e.g. lateral advection) that spread the water masses. Here, we isolated a part of the water column from physical dispersion using in situ large mesocosms (52 m³)

equipped with sediment traps in order to overcome this issue. The objectives of this study were (1) to investigate the contribution of N_2 fixation and DON use on PP and particle export, and (2) to trace the fate of these N sources in the ecosystem, i.e. whether the freshly produced particulate organic N (PON) is accumulated or exported out of the system.

5 The mesocosms were deployed in the subtropical New Caledonian lagoon (South West Pacific), characterized by LNLC conditions (Fichez et al., 2010), where high N_2 fixation rates and abundances of *Trichodesmium* spp. and UCYN communities have been reported (Biegala and Raimbault, 2008; Garcia et al., 2007; Rodier and Le Borgne, 2008, 2010). Phosphate (PO_4^{3-}) availability has previously been reported to control N_2 fixation in the
10 South West Pacific (Moutin et al., 2005, 2008). In order to avoid PO_4^{3-} limitation and to create favorable conditions for diazotrophs growth, the mesocosms were fertilized with PO_4^{3-} at the beginning of the experiment. Diazotrophs developed extensively in the mesocosms during the 23-days experiment (Turk et al., 2015). The diazotroph community was dominated by DDAs during the first half of the experiment (from day 5 to day 14, hereafter called P1),
15 then shifted towards a large dominance of UCYN from group C (closely related to *Cyanothece* sp.) which developed extensively during the second half of the experiment (from day 15 to day 23, hereafter called P2). PP and N_2 fixation rates were monitored for the 23 days together with C, N and P pools dynamics in the water column and in export material. The results are discussed under the light of the shift of dominance between the two diazotrophic
20 communities.

2 Material and method

2.1 Study site and mesocosm description

25 Three mesocosms were deployed at the exit of the oligotrophic New Caledonian lagoon ($22^{\circ}29.1'S-166^{\circ}26.9'E$), 28 km off the coast of New Caledonia from 13 January 2013 (day 1) to 4 February 2013 (day 23). The site was 25 m depth on sandy bottom, protected from the dominant trade winds (southeast sector) and characterized by a high influence of

oceanic oligotrophic waters coming from outside the lagoon through the Boulari passage (Ouillon et al., 2010). The complete description of the mesocosms design is detailed in Bonnet et al. (2015c). Briefly, the enclosures were cylindrical bags 2.3 m in diameter and reaching about 15 m deep into the water. The bags were maintained 1 m above the surface to prevent external water inclusions. They were supported by a polyethylene frame and kept at the surface with floats. The bags were straightened by weights at the bottom of the mesocosms. After deployment, the mesocosms were left opened at the bottom for 24 h to insure a total homogeneity in the water column. On day 1, the bottom was closed with a sediment trap consisting in a funnel shape end fitted with a 3'' adapter for fastening a collection bottle for the daily sinking material.

The PO_4^{3-} fertilization was conducted in the evening of day 4. The fertilization consisted in an addition in each mesocosm of 20 L of a filtered seawater solution enriched with KH_2PO_4 (41.6 mM) leading to a final concentration of $\sim 0.8 \mu\text{mol L}^{-1}$ in the mesocosms. To insure homogenization, the solution was added to each mesocosm using a polyethylene tubing connected to a Teflon pump lifted regularly from the bottom to the surface of the mesocosms.

2.2 In situ monitoring and water sampling

CTD casts and water collection were conducted daily in each of the three replicate mesocosms (hereafter called M1, M2, and M3) and in surrounding waters. Seawater sampling was performed from a floating platform moved around the mesocosms. CTD casts were performed at 10 a.m. (local time) in each mesocosm and in surrounding waters using a memory probe SBE 911 plus (Sea-Bird Electronics, Inc.) equipped with conductivity, turbidity, fluorimetry, temperature and dissolved oxygen sensors. The CTD was handled with a speed of $0.2\text{--}0.3 \text{ m s}^{-1}$. The water was collected just before the CTD casts at three depths in each mesocosm (1, 6 and 12 m) using an air-compressed Teflon pump (AstiPure™) connected to a polyethylene tubing. Samples for particulate and dissolved organic and inorganic matter (C, N and P) were first collected in 50 L polypropylene carboys at the three depths and sub-sampled back on the R/V *Alis* moored 1 nautical mile away from the mesocosms site.

Samples for N₂ fixation rate and PP determination were directly collected from the pump in polycarbonate bottles (4.5 L) for each depth in each mesocosm and in surrounding waters. Sinking material was collected every day by divers from sediment traps as described in Bonnet et al. (2015c).

2.3 Primary production rates and phosphate turnover time

PP rates and PO₄³⁻ turnover time (T_{PO_4} i.e. the ratio of PO₄³⁻ concentration and uptake), were measured using the ¹⁴C/³³P dual labeling method (Duhamel et al., 2006). 60 mL bottles were amended with ³³P and ¹⁴C and incubated for 3 to 4 h on a mooring line close to the mesocosms site at the sampling depths. Incubations were stopped by adding 50 µL of KH₂PO₄ solution (10 mmol L⁻¹) in order to reduce to a minimum the ³³P assimilation by dilution effect and by keeping them in the dark to stop DIC uptake. Samples were then filtered on 0.2 µmol L⁻¹ polycarbonate membrane filters, and placed into scintillation vials with 250 µL of HCl 0.5 M. After 12 h, 5 mL of scintillation liquid (ULTIMA Gold MV, PerkinElmer Inc.) were added to each vial before the first count on a Packard Tri-Carb 2100TR scintillation counter. The activity of ³³P and ¹⁴C was separated using a second count made 5 months later taking into account the half-life of the ³³P: 25.38 days (Duhamel et al., 2006). PP and T_{PO_4} were calculated according to Moutin et al. (2002).

2.4 N₂ fixation rates

Samples for N₂ fixation incubations were collected in 4.5 L polycarbonate bottles. The latter were amended with ¹⁵N₂ enriched seawater according to the protocol developed by Mohr et al. (2010). Briefly, the ¹⁵N₂ enriched seawater was prepared daily from 0.2 µmol L⁻¹ filtered seawater collected from the same site in a 4.5 L polycarbonate bottle. Seawater was first degassed through a degassing membrane (Membrana, Minimodule, flow rate fixed at 450 mL min⁻¹) connected to a vacuum pump (< 200 mbar absolute pressure) for at least 1 h. It was then tightly closed with no head space with a silicone septum cap and amended with 1 mL of ¹⁵N₂ (98.9% Cambridge isotope) per 100 mL. The bottle was then shaken

5 vigorously and incubated overnight at 3 bars (20 m depth) to promote $^{15}\text{N}_2$ dissolution. Incubation bottles were then amended with 5 % (vol :vol) of $^{15}\text{N}_2$ enriched seawater and closed without headspace with silicone septum caps. The latter were incubated on an in situ mooring line close to the mesocosms at the appropriate sampling depths for 24 h. After
10 incubation, 12 mL of incubated water was sampled in Exetainers on 10 replicate samples and analyzed using a Membrane Inlet Mass Spectrometer (Kana et al., 1994) to estimate the final enrichment of the $^{15}\text{N}_2$ pool during the incubation. The measured final $^{15}\text{N}/^{14}\text{N}$ ratio of the N_2 in the incubation bottles was 2.4 ± 0.2 atom% ($n = 10$). Samples were then filtered on combusted (450 °C, 4 h) GF/F filters and stored at -20 °C for the duration of
15 the cruise. Every day, T0 samples were spiked with $^{15}\text{N}_2$ and immediately filtered in order to determine the initial background of $^{15}\text{N}/^{14}\text{N}$ ratio of PON for calculation of N_2 fixation rates. Filters were then dried at 60 °C during 24 h prior to analysis using a mass spectrometer (Delta plus, Thermo Fisher Scientific) coupled with an elemental analyzer (Flash EA, Thermo Fisher Scientific) for the PON concentrations and PON ^{15}N enrichment determination. Standard deviation was 0.004 μmol for PON and 0.0001 atom% for the $^{15}\text{N}/^{14}\text{N}$ isotopic ratio. The fluxes were defined as significant when ^{15}N enrichment was higher than three times the standard deviation obtained on T0 samples. The fluxes were calculated according to the equation detailed in Montoya et al. (1996). A recent study (Dabundo et al., 2014) reports potential contamination of commercial $^{15}\text{N}_2$ gas stocks with ^{15}N -enriched
20 NH_4^+ , NO_3^- and/or nitrite (NO_2^-), and nitrous oxide. The $^{15}\text{N}_2$ Cambridge Isotopes stocks analyzed contained low concentrations of ^{15}N contaminants, and the potential overestimated N_2 fixation rates modeled using this contamination level would range from undetectable to 0.02 $\text{nmol N L}^{-1} \text{d}^{-1}$. These rates are in the lower end of the range of rates measured in this study and we thus considered that this issue did not affect the results reported here.

25 **2.5 Chlorophyll *a*, inorganic and organic matter analyses**

Samples for chlorophyll *a* (Chl *a*) concentrations determination were collected by filtering 550 mL of seawater on GF/F filters. Filters were directly stored in liquid nitrogen. Chl *a* was extracted in methanol and measured by fluorometry (Herbland et al., 1985).

Samples for total organic carbon (TOC) concentrations were collected in duplicate at only one depth (6 m) in each mesocosm and in surrounding waters in precombusted (450 °C, 4 h) 12 mL sealed glassware flasks, acidified with H₃PO₄ and stored in the dark at 4 °C until analysis. Samples were analyzed on a Shimadzu TOCV analyzer with a typical precision of 2 μmol L⁻¹. Samples for particulate organic carbon (POC) concentrations were collected by filtering 2.3 L of seawater through precombusted (450 °C, 4 h) GF/F filter and determined using the combustion method (Strickland and Parsons, 1972) on an EA 2400 CHN analyzer. Filters were not acidified to remove inorganic carbon as it is assumed to be < 10 % of the total particulate C (Wangersky, 1994). Dissolved organic carbon (DOC) concentrations were calculated as the difference between TOC and POC concentrations. The DOC precision calculated from the analytical precision of each term according to the errors propagation law was 5 μmol L⁻¹.

Samples for NH₄⁺ were collected in 40 mL glass vials and analyzed by the fluorescence method according to Holmes et al. (1999) on a trilogy fluorometer (Turner Design). The detection limit was 0.01 μmol L⁻¹. Samples for NO₃⁻, NO₂⁻, PO₄³⁻, total N (TN) and total P (TP) concentrations determination were collected in 40 mL glass bottles and stored at -20 °C until analysis. NO₃⁻, NO₂⁻ and PO₄³⁻ concentrations were determined on a segmented flow analyzer according to Aminot and K erouel (2007). The detection limit was 0.01 and 0.005 μmol L⁻¹ for NO₃⁻ + NO₂⁻ and PO₄³⁻, respectively. TN and TP concentrations were determined according to the wet oxidation procedure described in Pujo-Pay and Raimbault (1994). The precision was 0.5 μmol L⁻¹ and 0.02 μmol L⁻¹ for TN and TP, respectively. Samples for PON and particulate organic P (POP) concentrations were collected by filtering 1.2 L of water on precombusted (450 °C, 4 h) and acid washed (HCl, 10 %) GF/F filters and analyzed according to the wet oxidation protocol described in Pujo-Pay and Raimbault (1994) with a precision of 0.06 and 0.007 μmol L⁻¹ for PON and POP, respectively. DON concentrations were calculated from TN concentrations subtracted by PON, NO₃⁻, NO₂⁻ and NH₄⁺ concentrations. Dissolved organic P (DOP) concentrations were calculated from TP concentrations subtracted by POP and PO₄³⁻ concentrations. The precision calculated

according to the propagation law of analytical precision associated with each parameter was 0.5 and 0.03 $\mu\text{mol L}^{-1}$ for DON and DOP, respectively.

Samples from sediment traps were collected daily and preserved in a 5% buffered solution of formaldehyde and stored at 4 °C until analysis. All the swimmers were handpicked from each sample, and were found to be a negligible source of particulate matter compared to the total particulate matter exported (< 5%). Samples were then desalted using ultrapure water (Milli-Q grade) and frozen dried. The daily amount of POC exported ($\text{POC}_{\text{export}}$), and PON exported ($\text{PON}_{\text{export}}$) were measured using a CHN analyzer (Perkin Elmer 2400). The POP exported ($\text{POP}_{\text{export}}$) was measured after mineralization using nitric acid and further determination of mineralized P according to Pujo-Pay and Raimbault (1994).

2.6 Data presentation and statistical analyses

The build-up of an elemental mass balanced budget is theoretically accessible as all the stocks and fluxes were sampled in the mesocosms. However, the attempts of closing an elemental mass budget in similar mesocosms studies were limited by the large analytical uncertainties on the organic pool determination (Czerny et al., 2013; Guieu et al., 2014). Nevertheless, as the only incoming (N_2 fixation) and export fluxes ($\text{PON}_{\text{export}}$) of N were characterized accurately, we were able to calculate the change in TN content ($\Delta\text{TN}_{\text{calc}}$) of a mesocosm. It was defined as:

$$\Delta\text{TN}_{\text{calc}} = \Sigma\text{N}_{2,\text{fix}} + \Sigma\text{PON}_{\text{export}} \quad (1)$$

where $\Sigma\text{N}_{2,\text{fix}}$ and $\Sigma\text{PON}_{\text{export}}$ are the cumulated average over depth N_2 fixation rates and $\text{PON}_{\text{export}}$. This approach does not discriminate the different N pools in the water column but allows a precise evaluation of the TN variation in the mesocosm and a direct comparison of the N_2 fixation and the $\text{PON}_{\text{export}}$.

The values of fluxes and concentrations presented in the text are averaged over the three depths (no significant differences have been evidenced between depths, paired Friedman test, $\alpha = 0.05$). Statistical differences between each mesocosm or between the mesocosms

and surrounding waters were tested using the paired non parametric Wilcoxon signed-rank test ($\alpha = 0.05$) for each parameter presented. If no significant differences between the mesocosms were detected, the values were averaged between the mesocosms. The associated uncertainties were calculated as the analytical precision cumulated to the standard deviation of each term according to the propagation of errors law. Differences between P1 and P2 were tested using the non parametric Kruskal–Wallis test ($\alpha = 0.05$).

3 Results

3.1 Hydrological background

The detailed description of hydrological and inorganic nutrients conditions during the experiment is presented in Bonnet et al. (2015c). Briefly, seawater temperature increased inside the mesocosms and in surrounding waters from 25.5 to 26.7 °C over the course of the experiment. The water column was well mixed in the mesocosms as temperature and salinity were homogeneous with depth over the course of the experiment. The sum of NO_3^- and NO_2^- concentrations averaged over depth in the mesocosms were below $0.04 \mu\text{mol L}^{-1}$ the day before the PO_4^{3-} fertilization (day 4) and decreased to $0.01 \mu\text{mol L}^{-1}$ towards the end of the experiment. NH_4^+ concentrations were close to the detection limit of $0.01 \mu\text{mol L}^{-1}$ from day 1 to day 18 and increased in all the mesocosms up to $0.06 \mu\text{mol L}^{-1}$ toward the end of the experiment. Prior to the PO_4^{3-} fertilization, PO_4^{3-} concentrations in the mesocosms ranged from 0.02 to $0.05 \mu\text{mol L}^{-1}$. The day after the fertilization, PO_4^{3-} concentrations reached $\sim 0.8 \mu\text{mol L}^{-1}$ in all mesocosms. Then, the concentrations decreased steadily, tending to initial concentrations ($0.02\text{--}0.08 \mu\text{mol L}^{-1}$) at the end of the experiment. In surrounding waters, NO_3^- remained below $0.20 \mu\text{mol L}^{-1}$ and PO_4^{3-} averaged $0.05 \mu\text{mol L}^{-1}$ all along the experiment.

3.2 Phosphate turn over time

The evolution of T_{PO_4} was closely related to the dynamics of PO_4^{3-} concentrations. Before the PO_4^{3-} fertilization, T_{PO_4} decreased from 1.0 ± 0.1 d on day 3 to 0.4 ± 0.1 d on day 4 in all the mesocosms (Fig. 1A). At the start of P1, T_{PO_4} dramatically increased in all mesocosms reaching 35.7 ± 15.7 , 30.1 ± 8.6 and 35.8 ± 10.5 d in M1, M2 and M3, respectively. T_{PO_4} then decreased steadily in all the mesocosms reaching 1 d on day 14, day 19 and day 21 in M1, M2 and M3, respectively. At the end of the experiment (day 23), T_{PO_4} values were the lowest reached over the experiment in all the mesocosms with values below 0.2 d. In surrounding waters, T_{PO_4} was stable around 1.8 ± 0.7 d from the start of the experiment to day 15 and then decreased to reach 0.5 ± 0.1 d on day 23 (Fig. 1A).

3.3 N_2 fixation and primary production rates

Before the PO_4^{3-} fertilization, N_2 fixation rates inside the mesocosms were 17.4 ± 7.3 $\text{nmol N L}^{-1} \text{d}^{-1}$ and decreased the days following the fertilization (Fig. 1B). During P1, the average N_2 fixation rates in the mesocosms were 9.8 ± 4.0 $\text{nmol N L}^{-1} \text{d}^{-1}$. During P2, N_2 fixation rates in the mesocosms were significantly ($p < 0.05$) higher than during P1 averaging 27.7 ± 8.6 $\text{nmol N L}^{-1} \text{d}^{-1}$. N_2 fixation rates were not significantly different ($p > 0.05$) between the three mesocosms all along the experiment. In surrounding waters, N_2 fixation rates did not show any clear pattern along the experiment and averaged 9.2 $\text{nmol N L}^{-1} \text{d}^{-1}$, ranging from 1.9 to 29.3 $\text{nmol N L}^{-1} \text{d}^{-1}$ (Fig. 1B).

The day before the PO_4^{3-} fertilization, PP was not significantly different ($p > 0.05$) between the three mesocosms and averaged 0.4 ± 0.1 $\mu\text{mol C L}^{-1} \text{d}^{-1}$ (Fig. 1C). During P1, PP increased steadily in the mesocosms to reach 0.9 ± 0.1 $\mu\text{mol C L}^{-1} \text{d}^{-1}$ at the end of P1. During P2, while T_{PO_4} was decreasing, PP continued to increase in the mesocosms with values generally exceeding 1.5 $\mu\text{mol C L}^{-1} \text{d}^{-1}$. During P2, PP was significantly higher in M3 than in M1 and M2 ($p < 0.05$). In surrounding waters, PP was stable before and during P1 at 0.9 ± 0.3 $\mu\text{mol C L}^{-1} \text{d}^{-1}$ and increased during P2 reaching 1.5 ± 0.2 $\mu\text{mol C L}^{-1} \text{d}^{-1}$ on day 23. Over the whole experiment, PP in the mesocosms was not significantly different

from surrounding waters ($p > 0.05$) except in M3 during P2 ($p < 0.05$). Assuming that all the diazotrophs are primary producers and have a C : N fixation ratio of 6.6 (Redfield, 1934), we calculated that N_2 fixation sustained $10.8 \pm 5.0\%$ (range 3.7–32.2%) of PP in the mesocosms after the PO_4^{3-} fertilization and $5.7 \pm 2.0\%$ (range 2.2–9.1%) in surrounding waters.

5 The contribution of N_2 fixation to PP was not significantly different ($p > 0.05$, $n = 57$) during P1 ($9.0 \pm 3.3\%$) and P2 ($12.6 \pm 6.1\%$).

3.4 Chl *a* and particulate organic matter dynamics

The day before the fertilization, Chl *a* concentrations in the mesocosms were $0.21 \pm 0.05 \mu\text{g L}^{-1}$ (Fig. 2). During P1, Chl *a* did not show any clear pattern; concentrations were
10 in the 0.12 to $0.28 \mu\text{g L}^{-1}$ range. During P2, Chl *a* increased in all the mesocosms but to a greater extent in M3 compared to M1 and M2 and reached maximal depth-averaged concentrations of 0.55 ± 0.01 , 0.47 ± 0.08 and $1.29 \pm 0.22 \mu\text{g L}^{-1}$ in M1, M2 and M3, respectively. Before and during P1, Chl *a* concentrations in surrounding waters were close to the concentrations in the mesocosms and ranged between 0.09 and $0.28 \mu\text{g L}^{-1}$. During P2, they
15 increased but to a lower extent than in the mesocosms with daily averaged concentrations of $0.42 \pm 0.03 \mu\text{g L}^{-1}$ on day 23.

The day before the PO_4^{3-} fertilization, POC concentrations ranged from 9 to $15 \mu\text{mol L}^{-1}$ (Fig. 3A). During P1, POC concentrations did not show any clear pattern in the mesocosms and concentrations ranged from 6 to $13 \mu\text{mol L}^{-1}$. During P2, POC concentrations
20 increased in M3 reaching $18 \mu\text{mol L}^{-1}$ on day 21, whereas they remained stable in M1 and M2. POC concentrations in surrounding waters were stable all along the experiment and were significantly lower ($p < 0.05$) than in the mesocosms. Initial PON concentrations were about $0.9 \mu\text{mol L}^{-1}$ and remained relatively stable during P1 (Fig. 3B). During P2, PON concentrations increased in all the mesocosms by a factor 1.5 in M1 and M2, and by a factor
25 2 in M3 at day 23 reaching $2 \mu\text{mol L}^{-1}$. PON concentrations also increased outside but to a lesser extent with values remaining below $1 \mu\text{mol L}^{-1}$. POP concentrations showed the same pattern than PON concentrations. The day before the PO_4^{3-} fertilization, POP concentrations were not significantly different ($p > 0.05$) between the mesocosms and averaged

0.05 $\mu\text{mol L}^{-1}$ (Fig. 3C). During P1, the concentrations in the mesocosms remained relatively stable. During P2, POP concentrations increased in all the mesocosms but to a higher extent in M3, reaching 0.07, 0.07 and 0.12 $\mu\text{mol L}^{-1}$ in M1, M2 and M3, respectively. Particulate POC/PON ratio decreased during the experiment from the initial averaged ratio of 12 to 8 at the end of the experiment, but remained higher than the Redfield ratio (6.6).

3.5 Dissolved organic matter dynamics

DOC concentrations ranged from 50 to 74 $\mu\text{mol L}^{-1}$ (average value of $60 \pm 4 \mu\text{mol L}^{-1}$) over the course of the experiment without any clear trend over the 23 days (Fig. 4A). Furthermore no significant differences were measured between the mesocosms and surrounding waters ($p > 0.05$). Before the PO_4^{3-} fertilization, DON concentrations averaged $5.2 \pm 0.5 \mu\text{mol L}^{-1}$ on day 4 (Fig. 4B). Concentrations remained stable during P1 in and out the mesocosms. During P2, DON concentrations decreased significantly ($p < 0.05$) in the three mesocosms. The decrease was of 0.7 ± 0.5 , 0.8 ± 0.5 and $1.0 \pm 0.5 \mu\text{mol L}^{-1}$ between day 17 and day 23 in M1, M2 and M3, respectively, and was not significantly different between the mesocosms ($p > 0.05$). DON concentrations were not significantly different in surrounding waters and in the mesocosms up to day 17 ($p > 0.05$). From this day, even though a significant decrease ($p < 0.05$) in DON concentrations was also observed in surrounding waters, the resulting concentrations were significantly higher outside than in the mesocosms ($p < 0.05$).

The DOP dynamics was similar to the DON dynamics: during P1, DOP concentrations were on average $0.14 \pm 0.03 \mu\text{mol L}^{-1}$ and remained stable up to day 14, day 16 and day 17 for M1, M2 and M3, respectively (Fig. 4C). After these days, DOP concentrations significantly decreased ($p < 0.05$) and reached $0.09 \pm 0.01 \mu\text{mol L}^{-1}$ on average. DOP concentrations also decreased in surrounding waters from day 18, but to a lesser extent than in the mesocosms.

3.6 Export fluxes and their coupling with primary production and N₂ fixation

Before the PO₄³⁻ fertilization (day 4), the exported fluxes were not significantly different ($p > 0.05$) between the three mesocosms (104 ± 35 , 6.5 ± 1.7 and 0.35 ± 0.09 nmol L⁻¹ d⁻¹ on average for POC_{export}, PON_{export} and POP_{export}, respectively) (Fig. 5). The daily exported particulate matter remained relatively stable during P1 averaging 164 ± 141 , 10.2 ± 7.1 and 0.9 ± 1.3 nmol L⁻¹ d⁻¹ for POC_{export}, PON_{export} and POP_{export}, respectively. During P2, the daily export fluxes increased continuously in all the mesocosms to a higher extent than during P1 peaking at 1197 ± 257 and 106.1 ± 20.1 nmol L⁻¹ d⁻¹ for C and N, respectively. The daily POP_{export} ranged from 1.0 to 18.4 nmol L⁻¹ d⁻¹.

The *e*-ratio, defined as the amount of exported carbon (POC_{export}) relative to the fixed carbon (PP), was significantly higher ($p < 0.05$, $n = 57$) during P2 ($39.7 \pm 4.9\%$) than during P1 ($23.9 \pm 20.2\%$). Integrated N₂ fixation rate over P1 was 0.10 ± 0.02 μmol L⁻¹ on average for all the mesocosms and did not significantly differ from the integrated PON_{export} of 0.10 ± 0.04 μmol L⁻¹ (Fig. 6). The resulting change on the TN pool in the mesocosms remained between -0.01 and 0.01 μmol L⁻¹ and was not significantly different from 0 (sign test, $p > 0.05$). Time integrated N₂ fixation rate over P2 was 0.25 ± 0.06 μmol L⁻¹ and the PON_{export} was 0.45 ± 0.04 μmol L⁻¹ (Fig. 6). The resulting change of the TN_{calc} pool in the mesocosms remained not significantly different from 0 (sign test, $p > 0.05$) up to day 18 but deviated negatively from 0 (sign test, $p < 0.05$) from day 19 to day 23 (Fig. 6). At the end of P2, the decrease of the TN_{calc} pool was 0.20 ± 0.04 μmol L⁻¹.

4 Discussion

4.1 Phosphate availability sustained N₂ fixation and in turn new primary production

N₂ fixation is limited by micronutrients availability such as iron (Fe) (Dekazemacker et al., 2013; Monteiro et al., 2011; Moore et al., 2009; Shiozaki et al., 2014), temperature (Breitbarth et al., 2007; Fu et al., 2014; Mulholland and Bernhardt, 2005), light (Garcia et al.,

2013; Kranz et al., 2010) and ultimately by PO_4^{3-} availability (Dore et al., 2008; Karl et al., 1997; Moutin et al., 2008). During this experiment, PO_4^{3-} fertilization was carried out as P limitation would have prevented diazotrophs growth. This fertilization associated with the relatively high micronutrient concentrations (e.g. Fe) (Ambatsian et al., 5 1997), high seawater temperature ($>25^\circ\text{C}$) and the maintenance of the water mass in the photic layer, provided optimal conditions for diazotrophs growth. As expected, diverse diazotroph phylotypes developed extensively in the mesocosms with abundances of 1.10^5 to 5.10^5 nifH copies L^{-1} (Turk et al., 2015). Furthermore, N_2 fixation rates in the mesocosms were high ($18.5 \pm 13.1 \text{ nmol N L}^{-1} \text{ d}^{-1}$) compared to those in surrounding waters ($9.2 \pm 4.8 \text{ nmol N L}^{-1} \text{ d}^{-1}$) (Fig. 1B) and are among the highest reported in the literature (Luo et al., 2012).

The contribution of N_2 fixation to PP ($10.8 \pm 5.0\%$) in the mesocosms and in surrounding waters ($5.7 \pm 2.0\%$) was in the upper range of previous studies in the Pacific Ocean (Raimbault and Garcia, 2008; Shiozaki et al., 2013) and the Mediterranean Sea (Bonnet et al., 2011; Ridame et al., 2013). Prior to PO_4^{3-} fertilization, NO_3^- concentrations were $<0.04 \mu\text{mol L}^{-1}$. As there was no external supply of NO_3^- , the potential consumption of the initial NO_3^- in the mesocosms represented $<11.5\%$ of the integrated N_2 fixation rates over P1 and P2 ($0.35 \pm 0.08 \mu\text{mol L}^{-1}$) (Fig. 6). Thus, N_2 fixation supplied nearly all the new production during the experiment. These results indicate that in a N depleted system, diazotrophs can provide enough new N to sustain high PP (exceeding $2 \mu\text{mol C L}^{-1} \text{ d}^{-1}$) and biomass (up to $1.42 \mu\text{g L}^{-1}$ of Chl *a*), as long as PO_4^{3-} does not limit N_2 fixation.

4.2 The relative efficiency of different diazotrophs to export particulate matter

Only few studies have focused on the direct coupling between N_2 fixation and particulate export (Dore et al., 2008; Karl et al., 2012; White et al., 2012). To our knowledge, the only study comparing the export efficiency of different diazotrophs reports that DDAs blooms could contribute up to 44% of the direct export in the North East Pacific, while UCYN (Group A) and *Trichodesmium* spp. could account for only 0 to 10% of the export (White et al., 2012). The scarcity of data is due to methodological issues associated with the use of

sediment traps in the open ocean due to (1) the patchy distribution of N_2 fixers that are not necessarily collected by the sediment traps, and (2) the temporal lag between the production and the export which is difficult to assess (Nodder and Waite, 2001). The mesocosm approach was designed to overcome these experimental limitations. The shallow depth of the traps (~ 15 m) and the absence of NO_3^- normally supplied via the nitracline prevent any comparison with open ocean studies. Nevertheless, the mesocosm approach enables a comparison of the export efficiency under contrasting ecological situations. In this case, the period dominated by DDAs (P1) is compared with the period dominated by UCYN-C (P2).

During P1, the biomass was stable in the mesocosms and the amount of recently fixed N_2 was equal to the amount of exported PON, suggesting a tight coupling between the two processes (Fig. 6). It has been shown that large aggregates of the diatom *Rhizosolenia* spp., representing the majority of DDAs during P1 (Turk et al., 2015), can sink at high rates (Villareal et al., 1996). This suggests that during this experiment, the recently fixed N_2 by DDAs remained within the symbiotic association and was quickly exported in the settling particles. This agrees with Karl et al. (2012) who showed that DDAs support the export pulses regularly observed in late summer in the tropical North Pacific Ocean.

During P2, the increase in PON concentrations during P2 (Fig. 3B) suggests that part of the freshly produced biomass remained in the water column. The accumulation of PON probably favored remineralization processes, explaining the increase in NH_4^+ concentrations. This may have enhanced the transfer of the recently fixed N_2 to the non diazotrophic plankton as demonstrated by Bonnet et al. (2015a) and explains the development of picocyanobacteria (Leblanc et al., 2015). Additionally, the total amount of N provided by N_2 fixation did not account for all the exported PON during P2 (Fig. 6), implying that an additional N source played a significant role in promoting the export. The only alternative N source is DON which, indeed, exhibited a significant decrease in concentration of $0.9 \pm 0.7 \mu\text{mol L}^{-1}$ in the mesocosms during P2 (see section 4.3 for further discussion on DON consumption). Assuming that DON and N_2 fixation are the only possible sources of N in the mesocosms, we calculated that a DON use of $0.9 \mu\text{mol L}^{-1}$ would have supported $\sim 78\%$ of the PON

production during P2, and potentially fueled the PON export to the same extent. This is in agreement with Torres-Valdes et al. (2009) and Letscher et al. (2013) who showed that DON pool is a dynamic contributor of the N cycle able to supports up to 40% of the vertical PON export in the oligotrophic gyres of the Pacific and Atlantic Oceans. A quantification of the diazotrophs in the sediment traps, performed on day 19, shows that $\sim 10\%$ of the UCYN-C biomass in the mesocosms was exported this day, explaining $\sim 7\%$ of the PON export (Bonnet et al., 2015a). Thus, the recently fixed N_2 by UCYN-C can directly be exported, but is probably more efficiently transferred to non diazotrophic plankton through mineralization processes.

The contrast between P1 and P2 is also observed using the e -ratio. The production driven by UCYN-C was more efficient in promoting POC export than the production driven by DDAs. During P1, it is probable that the recently fixed C by DDAs remained within the symbiotic association and sunk with the recently fixed N_2 constituting a direct and net C export. During P2, the higher efficiency of C export strongly suggests that the DON ultimately fueled PP which, in turn, increased POC export. Additionally, when UCYN-C dominated, an enhanced N remineralization may have enabled more C to be fixed per unit of fixed N_2 leading to a higher e -ratio. A proportionally higher N remineralization following high PP and N_2 fixation rates is supported by similar findings in the western North Pacific warm pool (Shiozaki et al., 2013).

4.3 The unexpected high dissolved organic matter consumption

The use of dissolved organic compounds and their implications on PP in the open ocean has long been demonstrated (Antia et al., 1991). The use of DOP by plankton communities in the oligotrophic ocean has been observed in the North Pacific Ocean (Bjorkman and Karl, 2003) and in the Atlantic Ocean (Lomas et al., 2010; Mather et al., 2008) and generally occurs under PO_4^{3-} limitation. In this study, the decrease in DOP concentrations during P2 occurred when T_{PO_4} reached the lowest levels, confirming the ability of the planktonic community to use DOP under low PO_4^{3-} availability. More surprisingly, the significant and rapid decrease in DON concentrations (Fig. 4) observed during the development of UCYN-

C (P2) in the mesocosms was associated with a rapid increase in PP (Fig. 1C), biomass (Figs. 2 and 3) and bacterial production (BP) (Van Wambeke et al., 2015), suggests high consumption of DON directly or indirectly by primary producers. In the open ocean, DON is mainly refractory. Nevertheless, it is now recognized that a fraction of the DON is labile and can directly support phytoplankton growth, while a semi labile fraction can be mineralized by bacterioplankton (Antia et al., 1991; Bronk, 2002; Bronk et al., 2007). In this study, we propose three hypotheses that could explain the observed decrease in DON concentrations during P2: (i) bacterial mineralization of DON triggered by high PP, (ii) direct uptake of DON by primary producers including UCYN-C and (iii) abiotic photo-degradation of DON into NH_4^+ .

(i) The increase in PP driven by high N_2 fixation rates during P2 led to an increase in bacterial production (Van Wambeke et al., 2015). The significant negative correlation between BP and DON concentrations (Spearman rank correlation, $r = -0.35$; $p < 0.001$) indicate significant consumption of DON by bacterial mineralization. Diazotrophs are known to over-fix C relative to N (Mulholland et al., 2007), which may explain why the POC : PON ratio was above the Redfield ratio during the experiment. The resulting N deficit for bacterial mineralization may have been found in the labile or semi labile DON pool. This hypothesis is supported by Van Wambeke et al. (2015) who showed that BP was limited by N availability in the mesocosms during the experiment. Based on BP data and assuming a bacterial growth efficiency of 10 to 30 % (del Giorgio and Cole, 1998) and a C : N ratio of 6.6 in bacteria cells (Fukuda et al., 1998), bacterial respiration would have led to a DON consumption of 0.2 to $0.7 \mu\text{mol L}^{-1}$ during P2, supporting at least part of the DON removal of $\sim 0.9 \mu\text{mol L}^{-1}$ reported here.

(ii) An alternative explanation for the decrease in DON concentrations is direct consumption by primary producers. Cyanobacteria are known to use DON compounds such as urea (Collier et al., 2009; Painter et al., 2008) to such an extent that DON has been reported to be the main source of N fueling cyanobacterial blooms in coastal waters (Glibert et al., 2004). The DON decrease occurring during the development of UCYN-C, whose abundances reached 5.10^5 nifH copies L^{-1} (Turk et al., 2015), questions their ability to use

DON to meet their N requirements. Direct uptake of glutamate and amino acids (constitutive components of the DON pool) has been reported in natural and laboratory populations of *Trichodesmium* spp. (Mulholland and Capone, 1999; Mulholland et al., 1999). Furthermore, large decrease in DON concentrations were observed after blooms of the diazotroph *Aphanizomenon ovalisporum* in Lake Kinneret (Berman, 1997). The hypothesis of a direct use of DON by *A. ovalisporum* was confirmed in culture experiment where the development of this diazotroph was stimulated by DON additions (Berman, 1997, 1999). To our knowledge, no direct uptake measurements of DON compounds have been performed on UCYN. However, the ureA gene implicated in the urea assimilation has been identified in the cyanobacterial diazotrophic strain *Cyanothece* PCC 7822 (Bandyopadhyay et al., 2011) closely related to the UCYN-C cluster. These pieces of evidences suggest that in addition to N₂ fixation, UCYN-C might be able to use the DON pool to meet their N requirements.

(iii) Finally, photo-degradation could be a possible sink of DON in surface waters (Bronk, 2002). A field study performed in the ultraoligotrophic eastern basin of the Mediterranean Sea indicates a production of NH₄⁺ from DON of 0.2–2.9 nmol N L⁻¹ d⁻¹ in surface waters (Kitidis et al., 2006). Taking into account the highest rates reported above, this process cannot explain more than 10 % of the observed DON removal. Moreover, the DON decrease occurred only during P2 whereas photo-degradation would be occurring continuously over the experiment.

The first two hypotheses (i and ii) are more likely to explain the DON decrease during P2. None of these two hypotheses can be excluded even though direct proof of large uptake of DON by UCYN-C is lacking. Thus, in this study the DON use was directly or indirectly triggered by the UCYN-C activity.

5 Conclusions

This study confirms that in the South West Pacific, N₂ fixation is a biogeochemically relevant process able to provide sufficient new N to drive new PP, biomass accumulation and organic matter export as long as P is not limiting. The fate of the recently fixed N appears to

be closely related to the diazotrophic community involved in N_2 fixation. A strong coupling of N_2 fixation and PON export occurred when DDAs dominated the diazotrophic community suggesting their direct export. When the community was dominated by UCYN-C, biomass accumulation was observed together with an efficient particulate export. A significant decrease in DON concentrations was observed during the same period indicating a direct or indirect use of DON by UCYN-C. Thus, in addition to fueling primary production, UCYN-C appear to be able to enhance regenerated production based both on the transfer of recently fixed N_2 toward non fixing planktonic groups and on the use of the DON pool. This use of DON exceeded the new N provided by N_2 fixation even though the N_2 fixation rates were among the highest reported in literature for the global ocean. These results suggest that DON has to be considered as a dynamic pool in LNLC areas as it may provide significant amounts of N and contribute significantly to particulate export.

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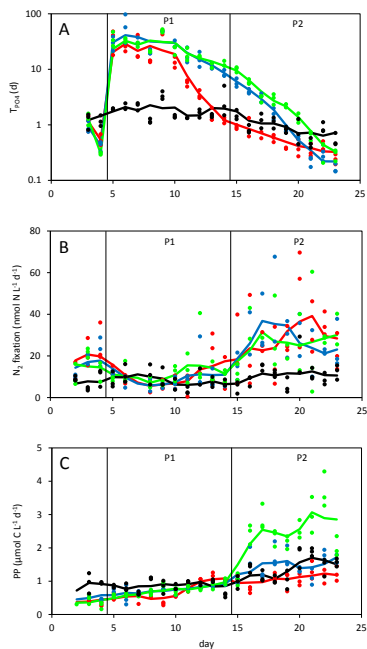


Figure 1. Temporal evolution of **(A)** phosphate turn over time (T_{PO_4} , d), **(B)** dinitrogen fixation (N_2 fixation) rates ($nmol\ N\ L^{-1}\ d^{-1}$), **(C)** primary production (PP) rates ($\mu mol\ C\ L^{-1}\ d^{-1}$) in the mesocosms M1 (red), M2 (blue) and M3 (green) and in surrounding waters (black). The three dots of each color represent the measured values on the three sampled depths. The solid lines are the three days running mean value. P1 and P2 denote the two phases of the experiment when the diazotrophic community was dominated by diatoms-diazotrophic associations and unicellular N_2 -fixing cyanobacteria (group C), respectively.

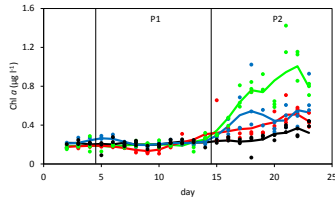


Figure 2. Temporal evolution of Chlorophyll *a* (Chl *a*) concentrations ($\mu\text{g L}^{-1}$) in the mesocosms and surrounding waters. The color code is identical to Fig. 1. The three dots of each color represent the measured values on the three sampled depths. The solid lines are the three days running mean value. P1 and P2 denote the two phases of the experiment when the diazotrophic community was dominated by diatoms-diazotrophic associations and unicellular N_2 -fixing cyanobacteria (group C), respectively.

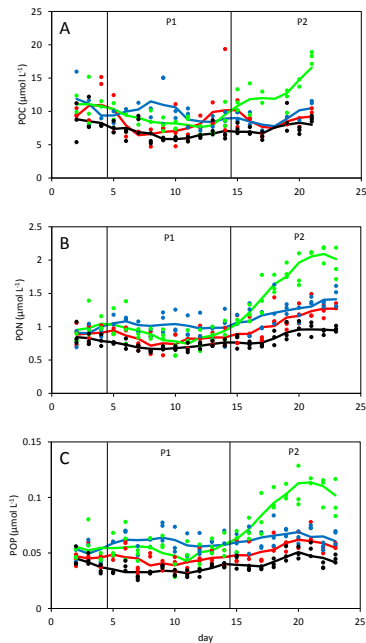


Figure 3. Temporal evolution of **(A)** particulate organic carbon (POC), **(B)** particulate organic nitrogen (PON), **(C)** particulate organic phosphorus (POP) concentrations ($\mu\text{mol L}^{-1}$) in the mesocosms and surrounding waters. The color code as is identical to Fig. 1. The three dots of each color represent the measured values on the three sampled depths. The solid lines are the three days running mean value. P1 and P2 denote the two phases of the experiment when the diazotrophic community was dominated by diatoms-diazotrophic associations and unicellular N_2 -fixing cyanobacteria (group C), respectively.

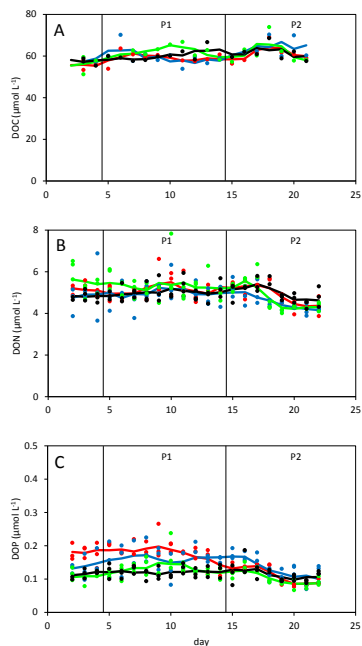


Figure 4. Temporal evolution of **(A)** dissolved organic carbon (DOC), **(B)** dissolved organic nitrogen (DON), **(C)** dissolved organic phosphorus (DOP) concentrations ($\mu\text{mol L}^{-1}$) in the mesocosms and surrounding waters. The color code is identical to Fig. 1. The three dots of each color represent the measured values on the three sampled depths. The solid lines are the three days running mean value. P1 and P2 denote the two phases of the experiment when the diazotrophic community was dominated by diatoms-diazotrophic associations and unicellular N_2 -fixing cyanobacteria (group C), respectively.

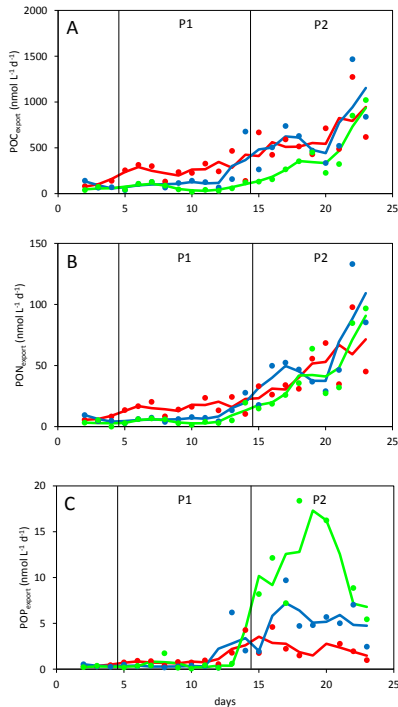


Figure 5. Temporal evolution of **(A)** particulate organic carbon exported ($\text{POC}_{\text{export}}$), **(B)** particulate organic nitrogen exported ($\text{PON}_{\text{export}}$) and **(C)** particulate organic phosphorus exported ($\text{POP}_{\text{export}}$) fluxes ($\text{nmol L}^{-1} \text{d}^{-1}$) in the mesocosms expressed in equivalent water volume. The color code is identical to Fig. 1. The solid lines are the three days running mean value. P1 and P2 denote the two phases of the experiment when the diazotrophic community was dominated by diatoms-diazotrophic associations and unicellular N_2 -fixing cyanobacteria (group C), respectively.

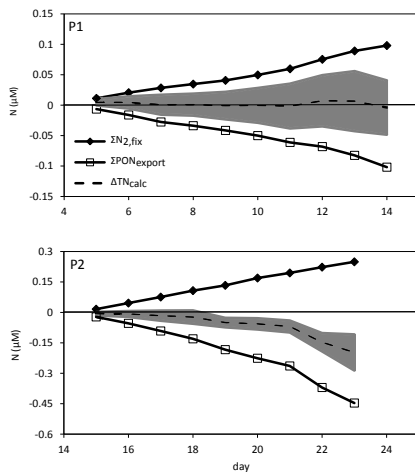


Figure 6. Integration of dinitrogen fixation rates ($\Sigma\text{N}_{2,\text{fix}}$) and particulate organic nitrogen exported ($\Sigma\text{PON}_{\text{export}}$) during P1 (dominance of diatoms-diazotrophic associations) and P2 (dominance of unicellular N_2 -fixing cyanobacteria group C) in the mesocosms together with the calculated change in Total N content ($\Delta\text{TN}_{\text{calc}}$) defined as the difference between $\Sigma\text{N}_{2,\text{fix}}$ and $\Sigma\text{PON}_{\text{export}}$. The shaded areas represent the uncertainty associated with the $\Delta\text{TN}_{\text{calc}}$ change.