Noumea, February 24th 2016

Revisions [Manuscript doi:10.5194/bgd-12-19579-2015]

Dear Reviewer,

We thank you for the constructive comments and suggestions, which have improved the manuscript. We have addressed the concerns below, and in a revised manuscript. A point by point response is given in the attached file (comments are in italics with our replies below), and all new text in the corresponding manuscript is track change mode.

Best Regards,

Sophie Bonnet

Reviewer 1.

Currently there is no flow to the description of experimental procedures and analytical protocols, which are randomly presented and make interpretation and understanding of the paper difficult. Please describe: 1) time series observations within the mesocosms; 2) DDN experiment and 3) Investigation of sediment traps, before detailing analytical methods.
The methods section has been reorganized as suggested by separating experimental procedures and analytical protocols. Some sentences have also been added to guide the reader.

If I understand the DDN transfer experiment correctly, and it is difficult at times to follow due to poor organisation of the methods, then I do not believe that N2 fixation rates can be presented after 24 hours of incubation. Surely the premise of this experiment is that 15N enriched nitrogen is being released into incubation bottles and being assimilated by the microbial community. How then, after 24 hours, do you differentiate between 15N which has been recently fixed from N2 from 15N enriched DDN? Certainly the "N2 fixation" rates presented at T72h and quite likely at T48h will be a combination of fixed 15N-N2 and assimilated 15N-DDN.
We agree that the way the release was presented was misleading. We have thus changed Figure 5a and the $^{15}N_2$ uptake data are now presented as cumulated uptake over the experimental study period (72 h). $^{15}N_2$ uptake includes both N_2 fixation and the uptake of ^{15}N-labelled DDN by non-diazotrophic plankton, especially after 24 h. Consequently, we no longer talk about N_2 fixation in the framework of the DDN experiment but about $^{15}N_2$ uptake. Similarly, the ^{15}DDN measured in the TDN pool either come from direct release during N_2 fixation, and/or from remineralization of diazotrophic biomass or biomass grown on ^{15}DDN. We thus no longer talk about release but about 'DDN quantified in the TDN pool'. The results and discussion sections as well as the legend of Figure 5 have been modified accordingly.

A more thorough discussion of the P requirements for N2 fixation needs to take place with reference to other published work and likely N:P stoichiometry. Currently the discussion here suggests P limiting conditions in the lagoon and mesocosms prior to DIP addition, yet rates of 9 nmolN L-1d-1 in the lagoon and 18 nmolN L-1d-1 over the first few days of the experiment do not suggest a resource limited community of diazotrophs. 30 nM phosphate does not

constitute “extremely low DIP”. Presentation of N and P data as a figure or table should be included within this manuscript. The negative correlations observed between both DIP and N2 fixation and DOP and N2 fixation is counter intuitive and should be investigated further.
We agree and the term ‘extremely’ has been removed. Moreover, NO_3^-, DIP, DON and DOP data have been included to the paper and are now presented in Table 3 as average concentrations over the three main periods (P0, P1, P2).
Diazotrophs can both be impacted by the DIP concentrations and have an impact on DIP via assimilation. We believe that the correlations observed between N2 fixation and DIP concentrations are not necessarily unexpected. N2 fixation rates increased while DIP concentrations were still relatively high. During spring blooms in temperate waters, the uptake of nitrate is negatively correlated with the nitrate concentrations, and nitrate concentrations become limiting at the end of the bloom. We have modified the sentence page 20 line 12 as follows: ‘Yet, in all three mesocosms, N_2 fixation rates were negatively correlated with DIP concentrations and DIP turnover time and positively correlated with APA (Table 2). Below, we describe the scenario that likely occurred in the mesocosms, which likely explains these correlations’.

P19581 L2 How is a spatial analysis enabled from a mesocosm experiment?
We agree and the word ‘spatial’ has been removed

L11 favourable for N2 fixation – and therefore probably not limited by P availability?
We removed ‘indicating that the waters surrounding New Caledonia are particularly favorable for N2 fixation’ as it was redundant with the previous sentence. The sections related to the effect of DIP on N_2 fixation have been expanded later in the MS so no more details are given in the abstract.

P19584 L16 22_28.855’S; 166_26.724’E or 22.481_S; 166.445_E ?
The real position was 22°28.855’S; 166°26.724’E. The minutes have been added to the text.

P19585, P19587 Please provide batch/lot number for 15N-N2 cylinder and details of how the (potential) contamination level was assessed.
The potential contamination level was assessed by the Dadundo group on one of our batches. The method is very long to explain and is not in the scope of this MS. However, some details have been incorporated in the Methods section as follows: ‘To verify this, one of our $^{15}N_2$ Cambridge Isotopes batches (18/061501) was checked for contamination following the method described in Dabundo et al. (2014); it was 1.4 x 10^{-8} mol of $^{15}NO_3^-$ per mol of $^{15}N_2$ and 1.1 x 10^{-8} mol NH_4^+ per mol of $^{15}N_2$. The application of this contamination level to our samples using the model provided by Dabundo et al. (2014) indicates that our rates may only be overestimated by ~0.05 %, confirming that our present results were unaffected by possible $^{15}N_2$ stock contamination’.

P19588 L4, L22 The DDN experiment has not been introduced at this point, and so please detail the DDN experiment earlier or re-phrase this section.
With the reorganization of the methods section, this kind of problem does not occur anymore.

L23 This is the first mention of 13C. Methods and reason for use should be introduced previously.
The DDN experiment is now presented before this mention of ^{13}C and refers to ^{13}C labelling in our bottles: ‘Full bottles were immediately amended with the dissolved $^{15}N_2$ gas (98.9 atom% ^{15}N, Cambridge Isotopes Laboratories, Inc) as described above (dissolution method),

and with 1 mL of 80 g L^{-1} $NaH^{13}CO_3$ solution (99 atom% ^{13}C, Cambridge Isotopes Laboratories, Inc) and incubated in situ…'

P19589 L2 Identify here that het-1 and het-2 are the DDAs mentioned elsewhere, as this is not specified explicitly.
We have added 'the two DDAs Richelia associated with both Rhizosolenia (het-1; (Church et al., 2005)) and Hemiaulus (het-2; (Foster et al., 2007)) diatoms…'

L9 inhibitors of what? Please describe this better
We have added the following precisions: 'Extracts were tested for the presences of PCR inhibitors, compounds sometimes present in DNA extracts from the environment or introduced in the extraction process that reduce PCR efficiency, using either the UCYN-B or the UCYN-C assay. If recovery of the spiked standard template in the sample extract was <98%, the sample was considered inhibited, and diluted 1:10 with 5 kD filtered milliQ water'.

L19 Which UCYN-C bloom? The reader does not know about this yet!
The UCYN-C bloom was mentioned in the Introduction section. In the current version it is also now mentioned in the Experimental procedures section before its mention in the DDN experiment.

L25 ..at the appropriate sampling depth? Isn't this always 6m as stated on L20?
Yes they were incubated at 6 m. It has been replaced 'and incubated in situ on the mooring line at 6 m-depth close to the mesocosms'

P19590 L1 How was "DDN released in the dissolved pool" determined? This whole section needs some reorganisation to group analytical methods together and experimental procedures together. Methods for N2 fixation and qPCR have already been presented, methods for DDN determination, cell counts and nanosims follow.
This was misleading in the previous section as this section was not separated from the 'experimental procedures'. A full dedicated section is now provided in the section 2.3 Analytical protocols (i.e. section 2.3.3 Quantification of the net release of DDN to the dissolved pool during the DDN transfer experiment).

L22 What denitrifying bacteria? First time these have been mentioned. Denitrifier method doesn't appear until L26.
The paragraph has been reordered as follows: 'The concentration of the resultant NO_3^- (i.e., TDN + the POR-associated N blank) was measured by chemiluminescence (Braman and Hendrix, 1989), after which the TDN isotopic composition was determined using the 'denitrifier method', wherein denitrifying bacteria that lack N_2O reductase quantitatively convert sample NO_3^- to N_2O (Casciotti et al., 2002; Sigman et al., 2001). The denitrifying bacteria (see below) are extremely sensitive to pH; care was thus taken to lower sample pH to 7-8 after POR oxidation via the addition of 12N ACS grade HCl'.

P19591 L11-15 I assume this describes procedure for picoplankton analysis? This should be stated.
Yes, this was indicated in the title of the section. We have also modified the first sentence as follow to state that: 'After each incubation period, 3.6 mL from each $^{15}N_2$-lableled 4.5 L bottle were subsampled into cryotubes, fixed with paraformaldehyde (2 % final concentration), flash

frozen in liquid N_2, and stored at -80°C until analysis. Picoplankton analyses were carried out at the PRECYM flow cytometry platform…'

L23-24 two subsets of bacteria (: : :: : :: : :: : :: : :..) were optically (Gasol et al., 1999). Something is missing here.
The sentence has been replaced by 'Based on these criteria, two subsets of bacteria (referred to low- and high nucleic acid-containing, or LNA and HNA, respectively) were optically resolved in all samples based on their green fluorescence intensity (Gasol et al., 1999)'.

P19597 L15 onwards Why is sediment trap data only presented for days 17 and 19? Surely this biases your conclusion that UCYNs are more efficiently exported than DDAs as this was a period of UCYN dominance? If sediment trap data is available for other days it should be included to allow comparison of export rates between the different periods of the experiment.
Sediment traps were collected daily with the main objective to measure for POC, PON and POP export and make budgets in our mesocosms. When the UCYN-C bloom occurred, we discovered (by microscopic analyses onboard) that cells were aggregated in the water column and decided to collect some aliquots of the traps at the height of the UCYN-C bloom to figure out whether or not these large aggregates could be exported to the traps. So we only have qPCR analyses in traps at that period (days 17 and 19). Of course they were taken at the height of the UCYN-C bloom, and it is likely that the contribution of UCYN-C to total POC export was lower at other periods of the experiment.

L24 Something cannot be described as "often" when the analysis is only described on 2 days. How about: UCYN-B was detected in all mesocosms on both days (except for M1 day 19) ??
We agree and the sentence has been replaced as advised: 'UCYN-B was detected in all mesocosms on both days (except in M1 day 19)….'

P19598 L3 sediments exist on the sea bed, change to sedimenting material (or similar description).
The term 'sediment' has been replaced by 'sedimenting material'

P19598 L27 There is no previous mention of 13C analysis by nanosims and no method Presented
The following section has been modified accordingly in the section '2.3.5 NanoSIMS analyses and ^{13}C ^{15}N assimilation rates during the DDN transfer experiment': 'All scans were first corrected for any drift of the beam during acquisition, and C and N isotope ratio images were created by adding the secondary ion counts for each recorded secondary ion for each pixel over all recorded planes and dividing the total counts by the total counts of a selected reference mass. 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 (ROI) around individual cells (^{28}Si data are not presented here). For each ROI, the ^{15}N and ^{13}C enrichments were calculated'.

P19600 L 15 The main difference between the mesocosms and lagoon was the modified DIP, however rates in the mesocosms were approximately twice those measured in the lagoon before DIP was added. How is this explained?
In this sentence, we meant that N_2 fixation rates were twice those measured in lagoon waters but this is over the whole 23 days of the experiment and not during P0. This was mentioned in the results section but we also modified the sentence in the discussion for clarity as follows: 'Averaged over the 23 days of the experiment, N_2 fixation rates in the mesocosms were ~ 2

fold higher (18.5±1.1 nmol N L^{-1} d^{-1}) than those measured in lagoon waters (9.2±4.7 nmol N L^{-1} d^{-1})'
However, N_2 fixation rates were indeed higher in the mesocosms compared to lagoon waters during P0. We added the following possible explanation page 20 line 18: 'During P0 (day 2 to 4), N_2 fixation rates were higher in the mesocosms than in the lagoon waters, possibly due to the reduction of turbulence in the water column facilitated by the closing of the mesocosms (Moisander et al., 1997) and/or to the reduction of the grazing pressure in the mesocosms as total zooplankton abundances were slightly lower (by a factor of 1.6) in the mesocosms compared to the lagoon waters (Hunt et al., 2016)'.

P19603 L15-16 Sentence does not make sense. Something like: : : :in our experiments did not utilise diazocytes to separate diazotrophy from photosynthesis.
The sentence has been modified as advised 'Here, UCYN-C cells fixed both ^{13}C and ^{15}N proportionally, which suggests they did not utilize diazocytes to separate diazotrophy from photosynthesis in our experiments'.

P19605 L7-9 This conclusion is possibly biased, as on days 17 and 19 when sedimenting material was collected UCYN-C was the dominant diazotroph. The carbon export potential is a significant factor, with great relevance. Is it possible that these aggregations were influenced by stress of containment within mesocosms?
We agree with this comment and the following sentence has been added page 23 line 12 to nuance our results in mesocosms: 'Aggregation processes may have been favored by the low turbulence in the mesocosms and it would be necessary to confirm that such processes also occur in the open ocean'.
Another sentence has been added in the conclusion section: 'Here, we demonstrate for the first time that UCYN can efficiently contribute to POC export in oligotrophic systems, predominantly due to the aggregation of small (5.7±0.8 µm) UCYN-C cells into large aggregates, which increase in size (up to 500 µm) with depth. Our results suggest that these small (typically 3-7 µm) organisms should be considered in future studies to confirm if processes observed in mesocosms are applicable to open ocean systems'.
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Reviewer 2.

Page 5, Line 14-20: Could do a little more here to put your experiment in context with the spatial and temporal studies you site. This experiment is different in that it looks at temporal changes, but over a short, rather than annual cycle
We agree with this comment and a sentence has been added page 4 line3: 'However, fairly little attention has been paid to sub-seasonal variability in N_2 fixation and its biogeochemical drivers and consequences'. Moreover the previous sentence has been replaced by: 'Our goal was to study the high frequency temporal dynamics of N_2 fixation over short time scales (sampling every day for 23 days), in relation to hydrological parameters, biogeochemical stocks and fluxes, and the dynamics of phytoplanktonic and bacterial communities in the same water mass'.

Mesocosm description: perhaps a small thing, but were the enclosures open to the air? It sounds like they were from the rest of the description, but the term bag is throwing me off.
Yes they were open to the air. This has been added page 5 line 12 and the word 'bag' has been replaced by the word 'enclosure': 'They consisted of large enclosures open to the air made of two 500 µm-thick films of polyethylene (PE)'

Sampling strategy: The Teflon membrane air pumps described for sampling can be a little rough with the water if they are set to pump at a high rate, which might damage cells and affect the biogeochemical rates being measured. Were they set to a gentle flow rate?
Yes the Teflon pump has been set to a gentle flow rate for sampling in the mesocosms. We have been using this kind of pump for a long time and could check in past studies that fragile cells like diatoms or Trichodesmium colonies were not destroyed by this sampling strategy.

N2 fixation rate measurements: were data from the mass spec corrected using low N content standards, as the del15N value typically changes when the mass decreases?
Yes of course, we often perform 'linearity tests' on our mass spec, i.e. we measure 3 replicates of a known IAEA reference molecule at various PN. At very low PN i.e. in ultra-oligotrophic regions (below 0.2 µM), the delta ^{15}N is very variable and not reliable. The Vahine samples from the lagoon had by far higher PN values (between 0.6 and 1.2 µM) and were in the range in which delta ^{15}N values are reliable and independent from the PN value.

UCYN microscopy: for clarity, it could be helpful to note here that UCYN-A will not be visible in this analysis
This has been added in the section 2.2.3. Phenotypic characterization of UCYN in the water column and the sediment traps: 'Note that UCYN-A cannot be observed by standard epifluorescent microscopy'.

NanoSIMS: you mention that you analyzed diatoms and UCYN-C here. The diatoms can of course be identified with microscopy, but for the UCYN-C, did you identify them first with epifluorescence microscopy? And did you know that no UCYN-B was present because of the qPCR data? What about UCYN-A? Please clarify this aspect of the method description
Our goal was to analyse the major diazotrophs at the time of the DDN experiment as well as the major groups of non-diazotrophic plankton to study the DDN transfer. As UCYN-C accounted for 90±29 % of bulk N_2 fixation during that period, we specifically targeted UCYN-C for nanoSIMS analyses but we cannot exclude that some UCYB-B were analyzed as well despite they were present at very low abundances, i.e. almost two orders of magnitude less abundant than UCYN-C (Fig. 5) in the analysed samples.
The following sentence has been added to the method section page 13 line 23: 'Diatoms were easily recognized on the CCD (charge coupled device) camera of the nanoSIMS, as were UCYN-C that formed large aggregates of cells, facilitating their recognition for nanoSIMS targeted analyses. However, we cannot exclude the possibility that some UCYN-B were analysed, despite being present at very low abundances, i.e., almost two orders of magnitude less abundant than UCYN-C (Fig. 5) in the analysed samples'.

Section 3.1: You define three periods of your experiment, P0, P1, and P2. P1 and P2 have the line at _15 days, but I don't really understand what that means. Did you use a different line between P1 and P2 for different measurements? Why is it not clearly defined?
Actually P0, P1 and P2 were defined by previous companion papers in the SI based on C, N, P pools and fluxes (Berthelot et alk., 2015) and based on qPCR data on nifH (Turk-Kubo et al., 2015). When looking at our N_2 fixation data, these 3 main periods could be defined and we used this terminology as all the other papers of the SI to ensure homogeneity and our descriptions. Exactly the same measurements were performed during the three periods.
The sentence has been modified in the text page16 line 7 to clarify this point 'Based on our data on N_2 fixation dynamics, we could identify three main periods during the experiments. These three periods were also defined by Berthelot et al. (2015b) based on biogeochemical

characteristics and by Turk-Kubo et al. (2015) based on changes in abundances of targeted diazotrophs'..

Sediment trap data: methods say samples were collected daily. I see that you focus on the data from days 17 and 19 because the microscopy matches up with that, but were data for other days also analyzed?
Please see response to Reviewer 1: Sediment traps were collected daily with the main objective to measure for POC, PON and POP export and make budgets in our mesocosms. When the UCYN-C bloom occurred, we discovered (by microscopic analyses onboard) that cells were aggregated in the water column and decided to collect some aliquots of the traps at the height of the UCYN-C bloom to figure out whether or not these large aggregates could be exported to the traps. So we only have qPCR analyses in traps at that period (days 17 and 19). Of course they were taken at the height of the UCYN-C bloom, and it is likely that the contribution of UCYN-C to total POC export was lower at other periods of the experiment.

Page 20, lines 1-9: I'm not totally on board with this calculation of export efficiency. The cells that are in the water column on day 17 and 19 are not the same cells that will be in the sediment traps on those days, but perhaps the material from 1 or 2 days before or more. What do you think the sinking rate of these different types are and can you then look at the data from those days before? Or were the qPCR abundances similar in the previous days so that this is a real number? Seems that this is a more complicated issue than what you have done to calculate efficiency – maybe there is more you can do with information available or maybe you need to put some caveats in your explanation.
The sinking rates of UCYN-C were not measured in the study but were estimated by the model presented by Gimenez et al., (2015, Vahine SI) to be 10 m/day at the end of the experiment when the UCYN-C bloom occurred. As sediment traps from day 19 (for example) integrate the export between day 18 and day 19, we agree that it is more accurate to consider water column qPCR data from day 18 than from day 19 in our calculations. This is what we did, which changes slightly the results. The paragraph is now: 'Using the volume of each mesocosm (Bonnet et al., 2016) and the total nifH copies for each diazotroph phylotype in the sedimenting material and in the water column the day before the collection of the sediment traps (Turk-Kubo et al., 2015) (assuming a sinking velocity of ~10 m day^{-1}, Gimenez et al. (2016)), we estimated the export efficiency for each phylotype. For UCYN-C, 4.6 % and 6.5 % of the cells present in the water column were exported to the traps per 24 h on day 17 and 19, respectively (assuming one nifH copy per cell). For het-1, 0.3 and 0.4 % of cells were exported into the traps on day 17 and 19, for het-3, 15.5 % and 10.5 % were exported, and for UCYN-B, 37.1 % and 15.5 % of UCYN-B were exported on day 17 and 19, respectively'.

DDN transfer experiment: The N2 fixation rates from the bulk values here are problematic to me. If you incubate bottles with isotope for 24, 48, and 72 hours, there should be an increasing total amount of fixed N in the bottles (as either PON or dissolved N). The hourly rates at the different timepoints don't entirely make sense then. What does it mean that the rate went up at the 72 hour timepoint? This value is averaged over the entire period; do you mean that because the 72 hour averaged value went up compared to the 48 hour value, that it must have actually gone up in the final 24 hours of the incubation? I'm not saying that the numbers don't have some sort of meaning, but that I think you need to make it more clear what they actually mean. I also question the calculation of the % gross N2 fixation that is released as DN. Especially when considering the 72 hour incubation, some DN that was released is then taken up by other organisms (non-diazotrophs, as your data shows). So the TDN pool doesn't represent all of the DN release over the course of the incubation. The

release of DN is likely quite a lot higher than what you have calculated because of this. Not sure how to deal with the issue, but it should be addressed.
Please see response to reviewer 1: We agree that the way the release was presented was misleading. We have thus changed Figure 5a and the $^{15}N_2$ uptake data are now presented as cumulative uptake over the experimental study period (72 h). $^{15}N_2$ uptake includes both N_2 fixation and the uptake of ^{15}N-labelled DDN by non-diazotrophic plankton, especially after 24 h. Consequently, we no longer talk about N_2 fixation in the framework of the DDN experiment but about $^{15}N_2$ uptake. Similarly, the ^{15}DDN measured in the TDN pool either come from direct release during N_2 fixation, and/or from remineralization of diazotrophic biomass or biomass grown on ^{15}DDN. We thus no longer talk about release but about 'DDN quantified in the TDN pool'. The results and discussion sections as well as the legend of Figure 5 have been modified accordingly.
Moreover, the ^{15}DDN measured in the TDN pool does not reflect the release by diazotrophs that may be higher as a part of this DDN has been uptaken by surrounding planktonic communities. This has been added to the discussion section page 25 line 3: 'The amount of DDN measured in the TDN pool during the 72 h DDN transfer experiment is higher than that reported for culture studies of Cyanothece populations (1.0±0.3 to 1.3±0.2 % of gross N_2 fixation (Benavides et al., 2013; Berthelot et al., 2015a)). The DDN measured in the TDN pool reflects the DDN release by diazotrophs during N_2 fixation and is likely underestimated here as a fraction of this DDN has been taken up by surrounding planktonic communities'.

Page 23, line 21: You can't exactly say that a specific cutoff for DIP turnover indicates DIP limitation, since limitation means control of productivity or biomass, and you haven't specifically done the experiment to compare these two values. You can say that more rapid cycling indicates deficiency. Also, the references for this 1 day value don't really fit the statement. I would look at Zohary and Robarts (L&O 1998) or Flonnes Flaten (DSRII 2005) for references that specifically address the bulk DIP turnover and how it relates to DIP limitation.
We replaced the previous sentence by 'In all cases, the increase in UCYN-C abundance coincided with low DIP turnover time, indicative of DIP deficiency (Berthelot et al., 2015b; Moutin et al., 2005)'

Page 25, line 25: Do you think that the UCYN-C grow as individual cells and then aggregated into the large clumps? Or do you think that as they divided, the presence of TEP kept the divided cells together in an aggregate? I suspect it is the latter, especially as you note that the currents were probably reduced, or maybe a little of both. UCYNB in culture has aggregates grow in size over time. Also, could you calculate roughly what the sinking rates might be in comparison to the potential growth rate of the cells? Might help resolve this question.
We believe that both are possible, i.e. UCYN-C cells grew as individual cells and aggregated afterwards, likely at the start of the bloom. It is also likely that divided inside the aggregates themselves. Please also see response to the comment above regarding sinking rates.

Page 29, lines 14-16: Remember also that smaller cells with higher surface area to volume ratios will outcompete larger cells for the DIP available – I do not think that this is just related to DOP usage.
The section has been modified as follows: 'We hypothesize that picoplankton were more competitive for DDN under low DIP conditions as small cells with high surface to volume ratios are known to outcompete larger cells for the available DIP (Moutin et al., 2002). Moreover, some prokaryotes from the 0.2-2 µm size-fraction can utilize DOP compounds (Duhamel et al., 2012)'.

Page 30, line 6: are the aggregates forming because of the reduced currents in the bags? If so, is that representative of what happens in the natural system? Please address, maybe not here, but somewhere.
We agree with this comment and have added the following sentence in the section '4.3 UCYN aggregation and export': 'Aggregation processes are probably enhanced by the low turbulence in the mesocosms and it would be necessary to confirm that such processes also occur in open ocean systems'.

Page 30, lines 12-16: I'd like to see this on a stronger note – what are the implications of this finding?!
The whole conclusion section has been modified. In particular the end of the discussion has been amended as follows: ''Moreover, the experimental and analytical approach used in this study allowed for the quantification of the actual transfer of DDN to different groups of non-diazotrophic plankton in the oligotrophic ocean. Our nanoSIMS results coupled with $^{15}N_2$ isotopic labelling revealed that a significant fraction of DDN (21±4 %) is quickly (within 24 h) transferred to non-diazotrophic plankton, which increased in abundance simultaneously with N_2 fixation rates. A similar nanoSIMS study performed during a Trichodesmium bloom (Bonnet et al., Accepted) revealed that diatoms were the primary beneficiaries of DDN and developed extensively during and after Trichodesmium spp. blooms. Diatoms are efficient exporters of organic matter to depth (Nelson et al., 1995). These studies show that plankton grown on DDN in the oligotrophic ocean drive indirect export of organic matter out of the photic zone, thus revealing a previously unaccounted for conduit between N_2 fixation and the eventual export to depth of DDN from the photic zone'.

Figure 2: The standard ODV scale is a little hard to read, with the alternating bright and light colors. I suggest using one of the scales that goes from white to a color.
Figure 2 has been redrawn using a different color code.

Reviewer 3.

As I understood, the scope of the manuscript and experiment is to provide a time series and temporal variability in N2 fixation rates. This should be mentioned already in the abstract.
This is now mentioned in the first sentence of the abstract 'N_2 fixation rates were measured daily in large (~50 m^3) mesocosms deployed in the tropical South West Pacific coastal ocean (New Caledonia) to investigate the temporal variability in N_2 fixation rates in relation with environmental parameters and study the fate of diazotroph-derived nitrogen (DDN) in a low nutrient, low chlorophyll ecosystem'.

What does the abbreviation VAHINE stand for? Please add!
This has been added page 4 line 6: 'In the framework of the VAHINE (VAriability of vertical and tropHIc transfer of diazotroph derived N in the south wEst Pacific) project…'

1) Page 19584, line 7: Short term fate of to me <24 hours.
The term 'short' has been removed and this whole section modified (see response to comment 2 below).

How do you distinguish between direct 15N2 fixation and recycling and re-uptake of 15N derived from of N2 fixation?
Please see comment to reviewer 1 and 2:

First, the way the release was presented was misleading. We have thus changed Figure 5a and the $^{15}N_2$ uptake data are now presented as cumulated uptake over the experimental study period (72 h). $^{15}N_2$ uptake includes both N_2 fixation and the uptake of ^{15}N-labelled DDN by non-diazotrophic plankton, especially after 24 h. Consequently, we no longer talk about N_2 fixation in the framework of the DDN experiment but about $^{15}N_2$ uptake. Similarly, the ^{15}DDN measured in the TDN pool either come from direct release during N_2 fixation, and/or from remineralization of diazotrophic biomass or biomass grown on ^{15}DDN. We thus no longer talk about release but about 'DDN quantified in the TDN pool'. The results and discussion sections as well as the legend of Figure 5 have been modified accordingly.
Moreover, the ^{15}DDN measured in the TDN pool does not reflect the release by diazotrophs that may be higher as a part of this DDN has been taken up by surrounding planktonic communities. This has been added to the discussion section: 'The amount of DDN measured in the TDN pool during the 72 h DDN transfer experiment is higher than that reported for culture studies of Cyanothece populations (1.0±0.3 to 1.3±0.2 % of gross N_2 fixation; (Benavides et al., 2013; Berthelot et al., 2015a)). The DDN measured in the TDN pool reflects the DDN release by diazotrophs during N_2 fixation and is likely underestimated here as a fraction of this DDN has been uptaken by surrounding planktonic communities'.

2) Please add a list of accompanied manuscripts which deal with the VAHNE mesocosm experiment and their individual scope (I understand that there were a couple more).
There are 16 articles in the Vahine SI (please see http://www.biogeosciences.net/special_issue193.html). We have modified this introduction section to introduce the papers dealing with DDN transfer. The new section is now: 'Over the course of this 23-day mesocosm experiment, diatom-diazotroph associations (DDAs) were the most abundant N_2 fixers during the first half of the experiment (days 2 to 14), while a bloom of the unicellular N_2-fixing cyanobacteria from Group C (UCYN-C) occurred during the second half of the experiment (days 15 to 23) (Turk-Kubo et al., 2015). Berthelot et al. (2015b) described the evolution of the C, N, and P pools and fluxes during the experiment and investigated the contribution of N_2 fixation and DON uptake to primary production and particle export. They also explored the fate of the freshly produced particulate organic N, i.e., whether it was preferentially accumulated and recycled in the water column or exported out of the system. Complementary to this approach, Knapp et al. (2015) report the results of a $\delta^{15}N$ budget performed in the mesocosms to assess the dominant source of N (i.e., NO_3^- versus N_2 fixation) fueling export production during the 23-day experiment. In the present study, we focus specifically on the fate of DDN in the ecosystem during the UCYN-C bloom by studying i) the direct export of diazotrophs into the sediment traps, and ii) the transfer of DDN to non-diazotrophic plankton using high-resolution nanometer scale secondary ion mass spectrometry (nanoSIMS) coupled with $^{15}N_2$ isotopic labelling during a 72 h-process experiment'.

3) Please structure analytical methods and experimental procedures together.
As suggested by Reviewer 1, the methods section has been reorganized by separating experimental procedures and analytical protocols. Some sentences have also been added to guide the reader.

4) Did you clean the walls of the mesocosm - cell wall growth can be a major difficulty and introduce errors in the overall element budget.
We did not clean the walls of the mesocosms during the experiment has it would have introduced artificial export of organic matter. However it is true that at the end of the experiment a biofilm started to be visible on the walls of the mesocosms. The way this biofilm

may affect the elemental budget has been fully discussed in the companion paper Knapp et al., 2015 (Vahine SI). We believe that it does not affect the results presented in the present paper and is not discussed here.

5) A schematic overview concerning samples taken and sub experiments done would be useful maybe put Fig. 1 in supplements and add it here.
We have fully restructured the Methods section, which should now be clearer for the reader. Consequently, we did not include a new Figure describing the protocols.

6) How did you calculate DIP turnover?
The DIP turnover time was calculated as the ratio of DIP concentration and uptake as described in Duhamed et al., (2006). The full DIP turnover time is presented in the companion paper Berthelot et al., (2015, Vahine SI).

7) Page 19581, line 10: The authors state, that their values are in the upper range of rates reported for the global ocean- that is not surprising as they added DIP to fuel production.
We modified the sentence as follows: 'These later rates measured after the DIP fertilization are higher than the upper range reported for the global ocean'.

8) What was the batch number of 15N2 gas used?
9) Page 19587, line 16:- Please give details on how you testes for contamination.
The batch number has been added. The potential contamination level was assessed by the Dadundo group on one of our batches. The method is very long to explain and is not in the scope of this MS. However, some details have been incorporated in the method section as follows: 'To verify this, one of our $^{15}N_2$ Cambridge Isotopes batches (18/061501) was checked for contamination following the method described in Dabundo et al. (2014); it was 1.4 x 10^{-8} mol of $^{15}NO_3^-$ per mol of $^{15}N_2$ and 1.1 x 10^{-8} mol NH_4^+ per mol of $^{15}N_2$. The application of this contamination level to our samples using the model provided by Dabundo et al. (2014) indicates that our rates may only be overestimated by ~0.05 %, confirming that our present results were unaffected by possible $^{15}N_2$ stock contamination'.

10) 15N enrichment in bottle done for the bubble method- why did you not analyze the 15N enrichment using MIMS like you did for the Mohr method and use measured value in the calculation instead of the theoretical one?
We agree that it would have been better to measure the ^{15}N enrichment value when using the bubble method as we did for the $^{15}N_2$ enriched seawater method but unfortunately we did not. We did that on a recent cruise and will be able to compare the theoretical value to the actual measured one for future studies.

11) How did you identify organisms in the NanoSIMS picture- by additional microscopic identification and marking with laser?
Please see response to Reviewer 2: Our goal was to analyse the major diazotrophs at the time of the DDN experiment as well as the major groups of non-diazotrophic plankton to study the DDN transfer. As UCYN-C accounted for 90±29 % of bulk N_2 fixation during that period, we specifically targeted UCYN-C for nanoSIMS analyses but we cannot exclude that some UCYB-B were analyzed as well despite they were present at very low abundances, i.e. almost two orders of magnitude less abundant than UCYN-C (Fig. 5) in the analysed samples.

The following sentence has been added to the method section: 'Diatoms were easily recognized on the CCD (charge coupled device) camera of the nanoSIMS, as were UCYN-C that formed large aggregates of cells, facilitating their recognition for nanoSIMS targeted analyses. However, we cannot exclude the possibility that some UCYN-B were analysed, despite being present at very low abundances, i.e., almost two orders of magnitude less abundant than UCYN-C (Fig. 5) in the analysed samples'.

12) Please add a table with abundances measured.
13) Figure 3- What sustained C-fixation in A1 below 200 m and was there any light available at that depth?
15) Fig. 1. SSHA is not an acronym for Aviso sea level anomaly- please correct!
16) Fig. 3: Please enlarge numbers and legends- it s hard to read.
17) Fig. 6. Please delete repetition of "N2 fixation and O2 and N2 fixation and O2"
I think these five comments do not refer to our paper…

14) Page 19605, line 9. Please explain the calculation of e ratio in methods.
The definition of the e ratio has been directly included to the sentence: 'This observation was further confirmed by the e ratio, which quantifies the efficiency of a system to export POC relative to primary production (e ratio = POC export/PP), and was significantly higher ($p<0.05$)…'

Dynamics of N_2 fixation and fate of diazotroph-derived nitrogen in a Low Nutrient Low Chlorophyll ecosystem: results from the VAHINE mesocosm experiment (New Caledonia)

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Abstract

N_2 fixation rates were measured daily in large (~50 m^3) mesocosms deployed in the tropical South West Pacific coastal ocean (New Caledonia) to investigate the spatial and temporal variability in N_2 fixation rates in relation with environmental parameters~~dynamics of diazotrophy~~ and study the fate of biologically fixed dinitrogen or diazotroph-derived nitrogen (DDN) in a low nutrient, low chlorophyll ecosystem. The mesocosms were ~~intentionally~~ fertilized with ~0.8 µM dissolved inorganic phosphorus (DIP) to stimulate diazotrophy. Bulk N_2 fixation rates were replicable between the three mesocosms, averaged 18.5±1.1 nmol N L^{-1} d^{-1} over the 23 days, and increased by a factor of two during the second half of the experiment (days 15 to 23) to reach 27.3±1.0 nmol N L^{-1} d^{-1}. These later rates measured after the DIP fertilization are higher than the upper range reported for the global ocean.~~, indicating that the waters surrounding New Caledonia are particularly favourable for N_2 fixation.~~ During the 23-days of the experiment, N_2 fixation rates were positively correlated with seawater temperature, primary production, bacterial production, standing stocks of particulate organic carbon, nitrogen and phosphorus, and alkaline phosphatase activity, and negatively correlated with DIP concentrations, DIP turnover time, nitrate, and dissolved organic nitrogen and phosphorus concentrations. The fate of DDN was investigated during ~~the~~ a bloom of the unicellular diazotroph, UCYN-C, that occurred during the second half of the experiment. Quantification of diazotrophs in the sediment traps indicates~~s~~ that ~10 % of UCYN-C from the water column ~~were~~ was exported daily to the traps, representing as much as 22.4±5.5 % of the total particulate organic carbon (POC) exported at the height of the UCYN-C bloom. This export was mainly due to the aggregation of small (5.7±0.8 µm) UCYN-C cells into large (100-500 µm) aggregates. During the same time period, a DDN transfer experiment based on high-resolution nanometer scale secondary ion mass spectrometry (nanoSIMS) coupled with $^{15}N_2$ isotopic labelling revealed that 16±6 % of the DDN was released to the dissolved pool and 21±4 % was transferred to non-diazotrophic plankton, mainly picoplankton (18±4 %) followed by diatoms (3±2 %). This is consistent with the observed dramatic increase in picoplankton and diatom abundances, primary production, bacterial production, and standing stocks of POC~~particulate organic carbon~~, particulate organic N (PON), and P (POP)~~nitrogen and phosphorus~~ during the second half of the experiment. These results offer insights into the fate of DDN during a bloom of UCYN-C in low nutrient, low chlorophyll ecosystems.

1 Introduction

Next to light, nitrogen (N) is the major limiting factor for primary productivity in much of the low-latitude surface ocean (Falkowski, 1997; Moore et al., 2013). Nitrate (NO_3^-) is the dominant form of fixed nitrogen (N) in seawater and derives from the remineralization of sinking organic N in the dark ocean. ~~It~~ NO_3^- is supplied to photic waters by upward mixing and transport, and constitutes the main source of fixed N for photosynthetic organisms in the temperate and high latitude ocean. In the oligotrophic tropical and subtropical oceans, vertical mixing and transport of NO_3^- is generally low and surface waters are often depleted in NO_3^-.

In these ocean deserts, specialized organisms termed N_2-fixers (or diazotrophs) are able to use N in its simplest and most abundant form on Earth and in seawater, namely dinitrogen (N_2). ~~These diazotrophs~~ Diazotrophs possess the nitrogenase enzyme, which cleaves the strong triple bond of the N_2 molecule to form bioavailable ammonium (NH_4^+) which is assimilated as aminoacids enabling biomass growth and division. N_2 fixation thus introduces a source of new bioavailable N to surface waters, and is considered to be the most important external source of N ~~for~~ to the ocean, ~~before~~ more significant than atmospheric and riverine inputs (Gruber, 2004).

The dynamics of microbial communities such as diazotrophs can change abruptly in the ocean in response to small perturbations or environmental stressors. In particular, N_2 fixation has been described as a very 'patchy' process in the ocean (Bombar et al., 2015). Many factors control the distribution and activity of diazotrophs such as temperature (Bonnet et al., 2015; Moisander et al., 2010; Raveh et al., 2015; Staal et al., 2003), nutrient availability (mainly phosphate and iron) ~~availability~~ (e.g., (Mills et al., 2004)), pCO_2 ((e.g. (Levitan et al., 2007)), ambient concentrations of fixed N (NO_3^- and NH_4^+) (e.g., (Knapp et al., 2012), as well as physical forcing (e.g., (Fong et al., 2008)). Most studies dedicated to understanding the controls on marine N_2 fixation have been undertaken along large oceanic transects; these are particularly valuable and have recently led to the compilation of a global ocean database of diazotrophy (Luo et al., 2012). Spatial variability in N_2 fixation is thus far better documented and understood than temporal variability, despite the intimate connections between time and space scales in the ocean. Time-series stations with near-monthly observations set up in the late 1980's under the international JGOFS program in the subtropical North Atlantic, Pacific, and Mediterranean Sea have provided valuable data regarding the controls on N_2 fixation and its role in biogeochemical cycles on seasonal and inter-annual timescales (Dore et al., 2008; Garcia et al., 2006; Grabowski et al., 2008; Karl et al., 2012; Knapp

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et al., 2005; Orcutt et al., 2001), and have also revealed novel diazotrophic microorganisms (Zehr et al., 2008) with unexpected metabolic strategies such as UCYN-A cyanobacteria that lack the oxygen-producing photosystem II complex (Tripp et al., 2010). However, fairly little attention has been paid to sub-seasonal variability in N_2 fixation and its biogeochemical drivers and consequences.

In the framework of the VAHINE (VAriability of vertical and tropHIc transfer of diazotroph derived N in the south wEst Pacific) project, we deployed three large volume mesocosms (~50 m^3, Fig. 1) in the tropical South West Pacific coastal ocean, a region known to support diazotrophy during the austral summer (Dupouy et al., 2000; Rodier and Le Borgne, 2010, 2008). Our goal was ~~in order~~ to study the high frequency temporal dynamics of N_2 fixation ~~at high frequency~~ over short time scales (sampling every day for 23 days) rather than seasonal cycles, in relation to hydrological parameters, biogeochemical stocks and fluxes, and the dynamics of phytoplanktonic and bacterial communities in the same water mass.

The mesocosm approach allowed us to investigate the fate of the recently fixed N_2 and its transfer from diazotrophs to non-diazotrophic organisms in this oligotrophic marine ecosystem. Diazotrophs can typically release from 10 to 50 % of their recently fixed N_2 (or diazotroph derived N, hereafter called DDN) as dissolved organic N (DON) and ammonium (NH_4^+) (Glibert and Bronk, 1994; Meador et al., 2007; Mulholland et al., 2006). This exudate is potentially available for assimilation by the surrounding planktonic communities. However, such transfer of DDN to the surrounding planktonic community and its potential impact on export production is poorly understood and rarely quantified.

Over the course of this 23-day mesocosm experiment, diatom-diazotroph associations (DDAs) were the most abundant N_2 fixers during the first half of the experiment (days 2 to 14), while a bloom of the unicellular N_2-fixing cyanobacteria from Group C (UCYN-C) occurred during the second half of the experiment (days 15 to 23) (Turk-Kubo et al., 2015). (Berthelot et al., 2015b) described the evolution of the C, N, and P pools and fluxes ~~along~~during the experiment and investigated the contribution of N_2 fixation and DON ~~use~~ uptake to primary production and particle export. They also explored the fate of the freshly produced particulate organic N, i.e., whether it was preferentially accumulated and recycled in the water column or exported out of the system. ~~The contribution of N_2 fixation to primary production and export was investigated during the two phases of the experiment in a companion paper by Berthelot et al. (2015b).~~

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Complementary to this approach (Knapp et al., 2015) report the results of a $\delta^{15}N$ budget performed in the mesocosms to assess the dominant source of N (~~from~~i.e., NO_3^- ~~and/or~~versus N_2 fixation) fueling export production ~~along~~during the 23-day~~s~~ experiment. In the present study, ~~Here,~~ we focus specifically on the ~~short term~~ fate of DDN in the ~~mesocosms~~ ecosystem during the UCYN-C bloom by studying i) the direct export of diazotrophs into the sediment traps, and ii) the transfer of DDN to non-diazotrophic plankton using high-resolution nanometer scale secondary ion mass spectrometry (nanoSIMS) coupled with $^{15}N_2$ isotopic labelling during a 72 h-process experiment.

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2 Methods

2.1 Mesocosm description and sampling strategy

Three replicate large-volume mesocosms (surface 4.15 m^2, volume ~50 m^3, Fig. 1) were deployed in the oligotrophic New Caledonian lagoon, 28 km off the coast of Noumea (latitude: 22°28,855' S; longitude: 166°26,724' E) from January 13th to February 6th 2013. They consisted of large ~~bags~~ enclosures open to the air made of two 500 µm-thick films of polyethylene (PE) and vinyl acetate (EVA, 19 %), with nylon meshing in between to allow for maximum resistance and light penetration (produced by HAIKONENE KY, Finland). The mesocosm bags were 2.3 m in diameter and 15 m in height, and were equipped with removable sediment traps that enabled the collection of sinking material once a day (Fig. 1b). To alleviate any potential phosphorus limitation of diazotrophy in the mesocosms, the bags were intentionally fertilized with ~0.8 µmol L^{-1} of dissolved inorganic phosphorus (DIP) four days after the start of the experiment. A more detailed description of the mesocosms setup, the selection of the study site, and the deployment strategy can be found in Bonnet et al. (2016).

Vertical CTD profiles were performed every morning in each of the three mesocosms (hereafter referred to as M1, M2, and M3) and in the surrounding waters (hereafter referred to as lagoon waters) using a SBE Seabird CTD. All discrete samples for the parameters described below were collected daily at 7 am at three depths (1, 6, and 12 m) in each mesocosm and in the lagoon waters using braided PVC tubing (Holzelock-Tricoflex, inner diameter = 9.5 mm) connected to a Teflon PFA pump (St-Gobain Performance Plastics) activated by pressurized air. Finally, sediment trap samples were collected daily from each mesocosm by SCUBA divers.

2.2 Experimental procedures

2.2.1. ~~Sampling,~~ N_2 fixation measurements within the mesocosms~~,~~ and method~~s~~ intercomparison

Seawater samples for N_2 fixation rate measurements were dispensed into HCl-washed 4.5 L polycarbonate bottles that were sealed with septa and amended with $^{15}N_2$-enriched seawater (Mohr et al., 2010; Wilson et al., 2012), hereafter called the $^{15}N_2$ dissolution method. Briefly, the $^{15}N_2$-enriched seawater was prepared from 0.2 µm-filtered seawater (Sartobrand (Sartorius) cartridges) collected from the same site in a 4.5 L polycarbonate HCl-washed 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) for at least 1 h. The bottle was then closed with a septum cap and amended with 1 mL of $^{15}N_2$ (98.9 atom% ^{15}N, Cambridge Isotopes Laboratories, Inc) per 100 mL of seawater. The bottle was shaken vigorously to fragment the $^{15}N_2$ bubble, and incubated overnight at 20 m depth at the study site (3 bars) to promote $^{15}N_2$ dissolution. The experimental bottles were amended with 5 % vol:vol $^{15}N_2$ enriched seawater (i.e., 225 mL), sealed without headspace with silicon septum caps, and incubated for 24 h on an *in situ* mooring line located close to the mesocosms at the appropriate sampling depth. After 24 h, 12 mL of the incubated seawater were subsampled into Exetainers®. These were preserved upside down in the dark at 4 °C and analyzed less than 6 months after the experiment using a Membrane Inlet Mass Spectrometer (MIMS) (Kana et al., 1994) to quantify the ^{15}N enrichment of the N_2 pool in the incubation bottles. The MIMS analyses yielded an average atom% ^{15}N for the N_2 pool of 2.4±0.2 (n=10). After collection of the Exetainer® subsamples, 2.2 L from each experiment bottle were filtered under low vacuum pressure (<100 mm Hg) onto a pre-combusted (4 h at 450 °C) GF/F filter (25 mm diameter, 0.7 µm nominal porosity) for 'bulk' N_2 fixation rate determination. The remaining volume (2.2 L) was pre-filtered through a 10 µm pore-size polycarbonate filter, and collected on a pre-combusted GF/F filter for analysis of the pico- and nanoplanktonic (<10 µm) N_2 fixation rates. Filters were stored at -20 °C until the end of the VAHINE experiment, then dried for 24 h at 60 °C before mass spectrometric analysis. Every day, an extra 2.2 L bottle was filled with mesocosm surface water (from ~1 m), spiked with $^{15}N_2$, and immediately filtered to determine the natural ^{15}N enrichment of the particulate organic N (PON), which is required for calculations of N_2 fixation rates (see analytical protocols below). ~~PON content and PON ^{15}N enrichment was were determined using a Delta plus Plus Thermo Fisher~~

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~~Scientific isotope ratio mass spectrometer (Bremen, Germany) coupled with an elemental analyzer (Flash EA, ThermoFisher Scientific). N_2 fixation rates were calculated according to the equations detailed in Montoya et al. (1996). Rates were considered significant when the ^{15}N enrichment of the PON was higher than three times the standard deviation obtained from T0 samples. The standard deviation was 0.004 µmol L^{-1} for PON and 0.0001 atom% for the ^{15}N enrichment.~~

In the present study, we decided to use the $^{15}N_2$ dissolution method to measure N_2 fixation rates as several authors (Großkopf et al., 2012; Mohr et al., 2010; Rahav et al., 2013; Wilson et al., 2012) have reported an underestimation of rates when using the bubble method (i.e., when the $^{15}N_2$ gas is injected directly in the incubation bottle using a syringe, see below) due to incomplete equilibration of the $^{15}N_2$ gas between the headspace and the seawater in the incubation bottles compared to theoretical calculations. However, the differences observed between the two methods appear to depend on the environmental conditions (Shiozaki et al., 2015). Here, we performed an inter-comparison of both methods on day 11 in surface waters (from ~1 m) collected from M1. Briefly, seawater samples from M1 were dispensed into twelve HCl-washed 4.5 L polycarbonate bottles as described above and closed with septum caps. Six bottles were spiked with 4 mL $^{15}N_2$ (98.9 atom% ^{15}N, Cambridge isotopes Laboratories, Inc) via a gas-tight syringe, hereafter called the bubble method. Each bottle was shaken 20 times to fragment the $^{15}N_2$ bubble and facilitate its dissolution. The six remaining bottles were treated as described above for the dissolution method. ~~The~~ All twelve bottles were then incubated for 24 h in an on-deck incubator at irradiances corresponding to the sampling depth using screening, and cooled with circulating surface seawater.

A recent study (Dabundo et al., 2014) reports potential contamination of some commercial $^{15}N_2$ gas stocks with ^{15}N-enriched NH_4^+, NO_3^- and/or nitrite (NO_2^-), and nitrous oxide (N_2O). Dabundo et al. (2014) analysed various brands of $^{15}N_2$ gas and found that the Cambridge Isotopes stock (i.e., the one used in this study) contained low concentrations of ^{15}N contaminants, and the potential overestimation of N_2 fixation rates modeled using this contamination level would range from undetectable to 0.02 nmol N L^{-1} d^{-1}. The rates measured in this study ranged from 0.5 to 69.6 nmol N L^{-1} d^{-1} suggesting that, if present, stock contamination of the magnitude reported by (Dabundo et al., 2014) would be too low to affect the results described here. To verify this, one of our ~~we analyzed one of our~~ $^{15}N_2$ Cambridge Isotopes batches (18/061501) was checked for

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contaminations following the method described in Dabundo et al. (2014);. It and found that the contamination of the $^{15}N_2$ gas stock was 1.4 x 10^{-8} mol of $^{15}NO_3^-$ per mol of $^{15}N_2$, and 1.1 x 10^{-8} mol NH_4^+ per mol of $^{15}N_2$. The application of this contamination level to our samples using the model provided by described in Dabundo et al. (2014) indicates that our rates may only be overestimated by ~0.05 %, confirming and confirmed that our present results were unaffected by possible $^{15}N_2$ stock contamination.

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2.2.3.3 Phenotypic characterization of UCYN in the water column and the sediment traps by microscopy

In order toTo investigate the direct export of UCYN-C cells during the bloom of UCYN-C that occurred duringin the second half of the experiment, a detailed phenotypic characterization of UCYN-C was performed at the height of the UCYN-C bloom (days 17 and 19), both in the water column and in the sediment traps. In parallel, UCYN-C and other diazotroph phylotypes were quantified in the sediment traps on days 17 and 19 (analytical protocols are detailed below in section 2.3).

Seawater samples for microscopic analyses were collected every day from 1, 6, and 12 m in each mesocosm in 4.5 L polycarbonate bottles as described above. Samples were immediately filtered onto 2 µm 47 mm polycarbonate filters that were fixed with paraformaldehyde (4 % final concentration) and incubated for 15 minutes at room temperature, then stored at -80 °C until microscopic analysis. Formalin-fixed sediment trap samples were homogenized and 2 ml were filtered onto 2 µm polycarbonate filters for further microscopic analyses. To further characterize the phenotype of UCYN (free living cells *versus* colonies) in the mesocosms as a function of depth, we performed a detailed microscopic analysis on days 17 and 19 in M2 (during the bloom of UCYN-C and during the DDN transfer experiment described below). Note that UCYN-A cannot be observed by microscopy. Filtered samples from each depth (1, 6, and 12 m) and from the sediment traps (~15 m) were visualized using a Zeiss Axioplan (Zeiss, Jena, Germany) epifluorescence microscope fitted with a green (510-560 nm) excitation filter, which targeted the UCYN phycoerythrin-rich cells. For each filter, 47 photographs of various sections of the filter were taken at random. Each fluorescent particle was automatically delimited as a region of interest (ROI) using an in-house imageJ script. The photographs were then scanned visually to remove ROIs that did not correspond to UCYN cells or UCYN aggregated cells. The area of each

ROI was converted to equivalent volume assuming a spherical shape for all the aggregates. The volume of individual cells was determined from the average volume of the ROI represented by only one cell. The resultant cell volume was then used to compute the number of cells in each aggregate.

2.2.3 DDN transfer experiment

The fate of the fixed N_2 during the UCYN-C bloom (that occurred from days 15 to 23 ~~XX to day XX~~) was investigated on days 17 to 20 in M2 at 6 m. In addition to N_2 fixation measurements, seawater was sampled as described above into twelve additional 4.5 L HCl-washed polycarbonate bottles equipped with septum caps. Full bottles were immediately amended with the dissolved $^{15}N_2$ gas (98.9 atom% ^{15}N, Cambridge Isotopes Laboratories, Inc) as described above (dissolution method), and with 1 mL of 80 g L^{-1} $NaH^{13}CO_3$ solution (99 atom% ^{13}C, Cambridge Isotopes Laboratories, Inc) and incubated *in situ* on the mooring line at 6 m-depth close to the mesocosms. After 24 h, 36 h, and 72 h of incubation (hereafter referred to as T24 h, T36 h, and T72 h), three replicate $^{15}N_2$ labelled bottles were recovered from the mooring line and subsampled for the analysis of bulk N_2 fixation rates, DDN released to the dissolved pool, abundance of targeted diazotrophs using qPCR, picophytoplankton and bacterial counts, and nanoSIMS analyses on UCYN-C and non-diazotrophs (diatoms and the 0.2-2 µm fraction) to assess the DD^{15}N transfer from diazotrophs to non-diazotrophs. All analytical protocols are detailed below in section 2.3. Three 4.5 L bottles were kept as unamended controls (i.e., without $^{15}N_2$ addition) and were immediately subsampled for the same parameters.

2.3 Analytical protocols

2.3.1 Mass spectrometry analyses

PON content and PON ^{15}N enrichment were determined using a Delta Plus Thermo Fisher Scientific isotope ratio mass spectrometer (Bremen, Germany) coupled with an elemental analyzer (Flash EA, ThermoFisher Scientific). N_2 fixation rates were calculated according to the equations detailed in Montoya et al. (1996). Rates were considered significant when the ^{15}N enrichment of the PON was higher than three times the standard deviation obtained from T0 samples. The standard deviation was 0.004 µmol L^{-1} for PON and 0.0001 atom% for the ^{15}N enrichment.

2.3.14 Quantification of diazotrophs using qPCR in sediment traps and during the DDN transfer experiment

During the bloom of UCYN-C (days 17 and 19), immediately after sediment trap samples were collected and prior to their fixation with formalin, trap material was homogenized and fresh aliquots of 1 mL were subsampled from each jar (trap from M1, M2, and M3) and filtered onto 0.2 µm Supor (Pall-Gelman) filters, flash frozen in liquid N_2, and stored at -80 ^{o}C until analysis. For the DDN transfer experiment ~~(see below)~~, after each incubation period, 2 L from each triplicate ^{13}C and $^{15}N_2$-labeled 4.5 L bottle were subsampled and filtered through 0.2 µm Supor (Pall-Gelman) filters using gentle peristaltic pumping, and stored as described above. The abundance of eight diazotrophic phylotypes was determined using Taqman® qPCR assays: unicellular cyanobacterial groups A1 (UCYN-A1; (Church et al., 2005)), A2 (UCYN-A2; (Thompson et al., 2014)), B (UCYN-B or *Crocosphaera* spp.; (Moisander et al., 2010)), and C (UCYN-C; (Foster et al., 2007)), the filamentous, colonial cyanobacteria *Trichodesmium* spp. (Church et al., 2005), the two DDAs *Richelia* associated with both *Rhizosolenia* (het-1; (Church et al., 2005)) and *Hemiaulus* (het-2; (Foster et al., 2007)) diatoms, *Calothrix* associated with *Chaetoceros* (het-3; (Foster et al., 2007)), as well as a heterotrophic phylotype of gamma proteobacteria (γ-24474A11; (Moisander et al., 2008)). All procedures are described extensively in the companion paper by (Turk-Kubo et al., 2015). Briefly, DNA was extracted using a Qiagen DNeasy kit with modifications to recover high quality genomic DNA from cyanobacteria including a freeze thaw step, agitation and a proteinase K digestion. Extracts were tested for the presence of inhibitors using either the UCYN-B or UCYN-C qPCR assay, and if recovery of the spiked qPCR standard was <98 %, the sample was considered inhibited, and diluted 1:10 with 5kD filtered milliQ water. All extracts from the sediment traps showed inhibition when undiluted, and no inhibition when diluted 1:10. DNA extracts from the DDN transfer experiment showed no inhibition. All qPCR reactions were carried out on diluted extracts as described in (Goebel et al., 2010). The limit of detection (LOD) and limit of quantitation (LOQ) was 250 and 2000 *nif*H copies mL^{-1}, respectively, for the sediment trap samples. The LOD and LOQ for DDN transfer experiment samples was 29 and 229 *nif*H copies L^{-1}, respectively.

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~~2.5 DDN transfer experiment and nanoSIMS analyses~~

~~The fate of the fixed N_2 during the UCYN-C bloom was investigated on days 17 to 20 in M2 at 6 m. In addition to N_2 fixation measurements, seawater was sampled as described above into twelve additional 4.5 L HCl washed polycarbonate bottles equipped with septum caps. Full bottles were immediately amended with the dissolved $^{15}N_2$ gas (98.9 atom% ^{15}N, Cambridge Isotopes Laboratories, Inc) as described above (dissolution method), and with 1 mL of 80 g L^{-1} $NaH^{13}CO_3$ solution (99 atom% ^{13}C, Cambridge Isotopes Laboratories, Inc) and incubated *in situ* on the mooring line at the appropriate sampling depth close to the mesocosms. After 24 h, 36 h, and 72 h of incubation (hereafter referred to as T24 h, T36 h, and T72 h), three replicate $^{15}N_2$ labelled bottles were recovered from the mooring line and subsampled for the analysis of bulk N_2 fixation rates, DDN released in the dissolved pool, abundance of targeted diazotrophs using qPCR as described above, picophytoplankton and bacterial counts, and nanoSIMS analyses on UCYN-C and non-diazotrophs (diatoms and the 0.2-2 µm fraction) to assess the DD^{15}N transfer from diazotrophs to non-diazotrophs. Three 4.5 L bottles were kept as unamended controls (i.e., without $^{15}N_2$ addition) and were immediately subsampled for the same parameters.~~

2.3.2 Quantification of the net release of DDN to the dissolved pool during the DDN transfer experiment

~~*Net release of DDN to the N dissolved pool.*~~ After each incubation period, 60 mL from each $^{15}N_2$-labeled 4.5 L bottle were subsampled and filtered through pre-combusted (4 h, 450 °C) GF/F filters and immediately frozen for later quantification of ^{15}N release (i.e., DDN release) to the total dissolved N pool (TDN; i.e., the sum of NO_2^-, NO_3^-, NH_4^+, and DON) ~~pool~~. The dissolved N was oxidized to NO_3^- using the persulfate oxidation method of (Knapp et al., 2005) with the amendments of Fawcett et al. (2011). Briefly, 1 mL of potassium persulfate oxidizing reagent (POR) was added to duplicate 5 mL aliquots of each subsample in 12 mL pre-combusted glass Wheaton vials, and to triplicate vials containing varying quantities of two L-glutamic acid standards, USGS-40 and USGS-41 (Qi et al., 2003) used to ensure complete oxidation and quantify the POR-associated N blank. The POR was made by dissolving 6 g of sodium hydroxide and 6 g of four-times recrystallized, methanol-rinsed potassium persulfate in 100 mL of ultra-high purity water (DIW). Sample vials were capped tightly after POR addition, and autoclaved at 121°C for 55 minutes on a slow-vent setting. The entire oxidation protocol was performed in duplicate (yielding a total of 4 oxidized aliquots for each subsample).

~~The denitrifying bacteria (see below) are extremely sensitive to pH; care was thus taken to lower sample pH to 7-8 after oxidation via the addition of 12N ACS grade HCl.~~ The concentration of the resultant NO_3^- (i.e., TDN + the POR-associated N blank) was measured ~~via~~ by ~~chemiluminescent~~ chemiluminescence ~~analysis~~ (Braman and Hendrix, 1989), after which the TDN isotopic composition was determined using the 'denitrifier method', wherein denitrifying bacteria that lack N_2O reductase quantitatively convert sample NO_3^- to N_2O (Casciotti et al., 2002; Sigman et al., 2001). The denitrifying bacteria (see below) are extremely sensitive to pH; care was thus taken to lower sample pH to 7-8 after POR oxidation via the addition of 12N ACS grade HCl. The ^{15}N enrichment of the N_2O was measured by GC-IRMS using a Delta V isotope ratio mass spectrometer and custom-built on-line N_2O extraction and purification system. The international reference materials, IAEA-N3, USGS-34, USGS-32, and an in-house N_2O standard were run in parallel to monitor bacterial conversion and mass spectrometry, and each oxidized sample was analyzed twice. The final TDN concentration and ^{15}N atom% were corrected for the N blank associated with the POR. The DDN released to the TDN pool was calculated according to: ^{15}N release (nmol L^{-1} d^{-1}) = ($^{15}N_{ex}$ x TDN_{con})/N_{sr}, where $^{15}N_{ex}$ is the atom% excess of the TDN for a given time point; the TDN_{con} is the TDN concentration measured at each time point, and N_{sr} is the ^{15}N enrichment of the source pool (N_2) in the experimental bottles (i.e., 2.4±0.2 atom% ^{15}N; see above).

2.3.3 Picophytoplankton and bacteria counts during the DDN transfer experiment

~~*Picophytoplankton and bacteria counts.*~~ After each incubation period, 3.6 mL from each $^{15}N_2$-lableled 4.5 L bottle were subsampled into cryotubes, fixed with paraformaldehyde (2 % final concentration), flash frozen in liquid N_2, and stored at -80°C until analysis. Picoplankton~~Flow cytometry~~ analyses were carried out at the PRECYM flow cytometry platform (https://precym.mio.univ-amu.fr/). Samples were analyzed using a FACSCalibur (BD Biosciences, San Jose, CA). For heterotrophic bacterial abundance (BA), 1.8 mL of seawater was fixed with formaldehyde (2 % final concentration, 15 minutes incubation at room temperature in the dark), frozen and stored in liquid N_2 until analysis in the laboratory. After thawing, 0.3 mL of each samples was incubated with SYBR Green II (Molecular Probes, final conc. 0.05 % [v / v], for 15 minutes at room temperature in the dark), for the nucleic acid staining, according to Marie et al. (2000). Cells were characterized by 2 main optical signals: side scatter (SSC), related to cell

size, and green fluorescence (530/40), related to nucleic acids staining. Based on these criteria, two subsets of bacteria (referred to low- and high nucleic acid-containing, or LNA and HNA, respectively) were optically resolved in all samples based on their green fluorescence intensity (Gasol et al., 1999). Just before analysis, 2 µm beads (Fluoresbrite YG, Polyscience), used as an internal control, and TruCount beads (BD Biosciences), used to determine the volume analyzed, were added to the samples. To assess autotrophic picoplankton abundances, the red fluorescence (670LP, related to chlorophyll *a* content) was used as trigger signal and phytoplankton cells were characterized by 3 other optical signals: forward scatter (FSC, related to cell size), side scatter (SSC, related to cell structure), and the orange fluorescence (580/30, related to phycoerythrin content). The 2 µm beads (Fluoresbrite YG, Polyscience) were also used to discriminate picoplankton (< 2 µm) from nanoplankton (> 2 µm) populations. The flow rate was estimated by weighing 3 tubes of samples before and after a 3 minutes run of the cytometer. The cells concentration was determined from both Trucount beads and flow rate measurements. All data were collected in log scale and stored in list mode using the CellQuest software (BD Biosciences). Data analysis was performed *a posteriori* using SUMMIT v4.3 software (Dako). on a FACScalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ) at the Regional Flow Cytometry Platform for Microbiology (PRECYM) (https://precym.mio.univ-amu.fr/). Standard protocols (Marie et al., 1999) were used to enumerate phytoplankton and heterotrophic prokaryotes. Samples were thawed at room temperature in the dark, homogenized by gentle shaking, and filtered through 20 µm strainers in order to avoid large aggregates clogging the instrument fluidics. Just before analysis, 1 mL of sample was transferred into a flow cytometry tube, and 10 µL of a 2 µm fluorosphere (Fluoresbryte™, Polysciences) solution were added. These beads were used both as an internal control, and to discriminate cell clusters. Flow cytometric analyses of heterotrophic prokaryotes required pre-staining with a fluorescent nucleic acid probe, SYBR® Green I (Sigma, Germany), at a 1:1000 v/v final dilution of the commercial solution (excitation 488 nm/emission 530 nm). Prior to analysis, samples were incubated with SYBR® Green I for 15 minutes in the dark at room temperature. Side scatter (SSC) was used as trigger signal and SYBR® Green I green fluorescence was collected in the green range of 510-550 nm. Combining SYBR® Green I fluorescence and light scattering unambiguously distinguishes cells from inorganic particles, detritus, and free DNA (Marie et al., 1999). ~~Two subsets of bacteria (referred to low- and high nucleic acid-containing, or LNA and HNA,~~

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~~respectively) were optically resolved in all samples based on their green fluorescence intensity (Gasol et al., 1999).~~

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2.3.4 Microscopic cell counts during the DDN transfer experiment

Microscopic cell counts. In parallel with the picoplankton counts, diatoms, dinoflagellates, and ciliates were enumerated from 100 mL subsamples collected from each mesocosm that were preserved in Lugol's solution following the Utermöhl method (Hasle, 1978). Cells were counted on a Nikon Eclipse TE2000-E inverted microscope equipped with phase-contrast and a long distance condenser. All groups were quantified in each sample, and diatoms were identified to the lowest possible taxonomic level to examine potential community composition changes and help us to prioritize nanoSIMS analyses.

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2.3.5 NanoSIMS analyses and ^{13}C and ^{15}N assimilation rates during the DDN transfer experiment

nanoSIMS analyses. After each incubation period (24, 36 and 72 h), 250 mL from each labeled 4.5 L bottle were subsampled, fixed with 25 mL of paraformaldehyde (2 % final concentration) and incubated for 24 h at 4 °C, then filtered successively through 25 mm diameter 10 µm, 2 µm, and 0.2 µm pore size polycarbonate filters and rinsed with 0.2 µm filtered seawater. All filters were then sputtered with gold and palladium to ensure conductivity prior to nanoSIMS analyses. Diatoms and UCYN-C were analysed on the 10 µm filters, and the picoplanktonic (0.2-2 µm) fraction was analysed on the 0.2 µm filters. Diatoms were easily recognized on the CCD (charge coupled device) camera of the nanoSIMS, as ~~well as~~were UCYN-C that ~~were typically forming~~formed large aggregates of cells, facilitating their recognition for nanoSIMS targeted analyses. However, we cannot exclude the possibility that some UCYN-B were analysed, despite ~~they were their~~ being present at very low abundances, i.e., almost two orders of magnitude less abundant than UCYN-C (Fig. 5) in the analysed samples. Several analyses were performed for each group of cells of interest (an average of ~25 cells analysed for UCYN-C and diatoms, and between 62 and 140 cells analysed for the 0.2-2 µm fraction per time point) to assess the variability of their isotopic composition. A total of ~400 individual cells were analysed by nanoSIMS in this experiment to ensure the robustness of the data. NanoSIMS analyses were performed on a N50 (Cameca, Gennevilliers France) at the French National Ion MicroProbe

Facility according to methods previously described (Bonnet et al., Accepted). A 1.3-3 pA 16 keV Cesium (Cs^+) primary beam focused onto a ~100 nm spot diameter was scanned on a 256 x 256 or 512 x 512 pixel raster (depending on the raster areas, which ranged from 15 µm x 15 µm to 50 µm x 50 µm) with a counting time of 1 ms per pixel. Samples were implanted with Cs^+ prior to analysis to remove surface contaminants and increase conductivity. For diatoms, the pre-implant was longer and with higher voltage (2-5 min, 17 pA) to penetrate the silica shell. Negative secondary ions $^{12}C^-$, $^{13}C^-$, $^{12}C^{14}N^-$, $^{12}C^{15}N^-$, and $^{28}Si^-$ were detected with electron multiplier detectors, and secondary electrons were imaged simultaneously. Ten to fifty serial quantitative secondary ion mass planes were generated and accumulated in the final image. Mass resolving power was ~8000 in order to resolve isobaric interferences. Data were processed using the look@nanosims software package (Polerecky et al., 2012). All scans were first corrected for any drift of the beam during acquisition, and C and N isotope ratio images were created by adding the secondary ion counts for each recorded secondary ion for each pixel over all recorded planes and dividing the total counts by the total counts of a selected reference mass. ~~were generated by dividing the $^{12}C^{15}N^-$ ion count by the $^{12}C^{14}N^-$ ion count for each pixel over all recorded planes, averaged for all pixels and planes.~~ 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 (ROI) around individual cells (^{28}Si data are not presented here). For each ROI, the ^{15}N and ^{13}C enrichments were calculated.~~For each ROI, the atom% ^{15}N was calculated.~~

^{13}C and ^{15}N assimilation rates were calculated for individual cells analysed by nanoSIMS. Our goal was to determine the biological compartment to which the ^{15}N had been transferred. These were performed after 24 h of incubation. Calculations were performed as follows (Foster et al., 2011; Foster et al., 2013): Assimilation (mol N $cell^{-1}$ d^{-1}) = ($^{15}N_{ex}$ x N_{con})/N_{sr}, where $^{15}N_{ex}$ is the excess atom% of the individual cells measured by nanoSIMS after 24 h of incubation; the N_{con} is the N content of each cell determined as described below, and N_{sr} is the ^{15}N enrichment of the source pool (N_2) in the experimental bottles (i.e. 2.4±0.2 atom% ^{15}N in this experiment). The cell-specific N assimilation rate was then multiplied by the cell number enumerated for each group of phytoplankton and bacteria by microscopy and flow cytometry. Standard deviations were calculated using the variability of ^{15}N enrichment measured by nanoSIMS on replicate cells and the standard deviation of the estimated cellular N content (see below) of UCYN-C, non-

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diazotrophic phytoplankton, and bacteria. Final standard deviations were calculated according to propagation of errors laws.

To determine the N_{con} of diatoms, cell cross section, apical and transapical dimensions were measured on the dominant diatom species present in the mesocosms and analysed by nanoSIMS to calculate biovolumes. All dimensions were measured on at least 20 cells using a Nikon Eclipse TE2000-E inverted microscope equipped with phase-contrast and a long distance condenser. Dimensions were entered into the international diatom data base (Leblanc et al., 2012) in which bio-volumes are calculated following the geometric model of each cell type as described in (Sun and Liu, 2003). Carbon (C) content (C_{con}) was then calculated for the species of interest using the equations of (Eppley et al., 1970) and (Smayda, 1978). For *Synechococcus* spp. and picoeukaryotes, we used C_{con} data from Fu et al. (2007) (249±21 fg C $cell^{-1}$) and Yentsch and Phinney (1985) (2100 fg $cell^{-1}$), respectively. C_{con} was then converted to N_{con} using the Redfield ratio of 6.6:1 (Redfield, 1934). For bacteria, an average N_{con} of 5.8±1.5 fg N $cell^{-1}$ (Fukuda et al., 1998) was used. For UCYN-C, cell dimensions were measured and the bio-volume was calculated based on the equations reported in Sun and Liu (2003). C_{con} was then calculated using the relationship between bio-volume and C_{con} (Verity et al., 1992) (22 pg $cell^{-1}$). C_{con} was then converted to N_{con} (2.3 pg $cell^{-1}$) using a ratio of 8.5:1 (Berthelot et al., 2015a).

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2.46 Statistical analyses

Spearman correlation coefficients were used to examine the relationships between N_2 fixation rates, hydrological, biogeochemical, and biological variables in the mesocosms (n=57 to 61, α=0.05). The methods used to analyze the parameters reported in the correlation table are described in detail in companion papers in this issue (Berthelot et al., 2015b; Bonnet et al., 2016; Leblanc et al., 2016; Turk-Kubo et al., 2015).

A non-parametric Mann-Whitney test (α=0.05) was used to compare the means of N_2 fixation rates obtained using the dissolution and the bubble method, as well as to compare the means of N_2 fixation between the different phases of the experiment, mean isotopic ratios between $^{15}N_2$-enriched and natural abundance of N (0.366 atom%), and mean isotopic ratios between T24 h and T72 h in the DDN transfer experiment.

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3 Results

3.1 N_2 fixation rates in the mesocosms

Bulk N_2 fixation rates averaged 18.5±1.1 nmol N L^{-1} d^{-1} ~~over~~ throughout the 23 days of the experiment in the three mesocosms (all depths averaged together) (Table 1). The variance between the three mesocosms was low, and the temporal dynamics of the rates were similar (Fig. 2, Table 1), indicating good replicability between the mesocosms. Based on our data on N_2 fixation dynamics, we could identify ~~T~~three main periods ~~over the course of~~during the experiments. ~~were identified based on the N_2 fixation dynamics; t~~These three periods were also ~~identified~~ defined by Berthelot et al. (2015b) based on biogeochemical characteristics and by Turk-Kubo et al. (2015) based on changes in abundances of targeted diazotrophs. During the first period (P0; from day 2 to 4, i.e., prior to the DIP fertilization), the average bulk N_2 fixation rate for the three mesocosms was 17.9±2.5 nmol N L^{-1} d^{-1} (Fig. 2a). These N_2 fixation rates decreased significantly ($p<0.05$) by ~40 % from day 5 to ~15 (hereafter called P1) to 10.1±1.3 nmol N L^{-1} d^{-1}, then increased significantly ($p<0.05$) from day 15 until the end of the experiment (day 15 to 23, hereafter called P2) to an average of 27.3±1.0 nmol N L^{-1} d^{-1} (Fig. 2a). Maximum rates were reached during P2 (between days 18 and 21) with 69.7, 67.7 and 60.4 nmol N L^{-1} d^{-1} in M1 (12 m), M2 (6 m) and M3 (12 m), respectively. From day ~15 to 21, N_2 fixation rates were higher at 12 m depth than in the surface. The difference was significant in M2 and M3 ($p<0.05$), but not in M1 ($p>0.05$). Size fractionation experiments indicate that 37±7 % of the measured N_2 fixation was associated with the <10 µm size fraction (Fig. 2b), and N_2 fixation rates in this fraction followed the same temporal trend as bulk N_2 fixation. These data indicate that for the experiment as a whole, the majority (~63 %) of the N_2 fixation was associated with the >10 µm fraction. N_2 fixation rates measured in the lagoon waters were half those measured in the mesocosms, and were on average 9.2±4.7 nmol N L^{-1} d^{-1} over the 23 days of the experiment.

The Spearman correlation matrix (Table 2) indicates that N_2 fixation was positively correlated with seawater temperature in the mesocosms, which was not the case in lagoon waters, although temperature was exactly the same inside and outside the mesocosms (from 25.4°C to 26.8°C) (Bonnet et al., 2016). N_2 fixation in the mesocosms was also positively correlated with particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate organic phosphorus (POP) (except in M2) concentrations, Chl *a* concentrations, primary production, bacterial production, alkaline phosphatase activity (APA), and *Synechococcus*, picoeukaryote and

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nanoeukaryote (except in M2) abundances. N_2 fixation was negatively correlated with NO_3^-, DIP, DON, dissolved organic phosphorus (DOP) (except in M2) concentrations, and DIP turn-over time.

The intercomparison between the bubble and dissolution methods performed on day 11 in M2 indicates that rates determined for the 6 replicates were 7.2±0.8 nmol N L^{-1} d^{-1} and 6.4±2.0 nmol N L^{-1} d^{-1} for the dissolution method and the bubble method, respectively, demonstrating that, at least in this study, N_2 fixation rates were not significantly different ($p>0.05$) between the two methods.

3.2 Phenotypic characterization of UCYN by microscopy

The average size of the UCYN-C cells present in the mesocosms was 5.7±0.8 µm (n=17). Both free-living and aggregated UCYN-C cells were observed in the water columns of the mesocosms~~, water columns~~. However, the detailed microscopic analysis performed on day 17 and day 19 in M2 (during the bloom of UCYN-C) (Fig. 3) indicates that the proportion of free-living cells (ROI characterized by one cell or two cells defined as dividing cells) was low (<1 % on day 17 and <5 % on day 19). The average number of UCYN-C cells per aggregate increased with depth (Fig. 3a), with the size of the aggregates reaching 50-100 µm at 6 m and 100-500 µm at 12 m depth. On day 17, the number of cells per aggregate averaged 162, 74, and 1273 at 1, 6, and 12 m, respectively. On day 19, the aggregates were much smaller (~50 µm) with only 4, 11, and 19 cells per aggregate. The sediment traps contained extremely high densities of UCYN-C cells with the average number of cells per aggregate 60 to 50,000 times higher than that measured in the water column aggregates (Fig. 3b-e).

3.3 Quantification of diazotrophs in sediment traps

qPCR analysis confirmed that UCYN-C was the most abundant diazotroph in the sediment traps on days 17 and 19, with abundances reaching 2.7 x 10^8 to 4 x 10^9 *nif*H copies L^{-1} (Fig. 4a). UCYN-C accounted for 97.4 to 99.2 % of the total *nif*H pool quantified in the traps. Abundances were higher in M2 and M3 (1.8 x 10^9 in M2 and 3 x 10^9 *nif*H copies L^{-1} in M3) compared to M1 (2.5 x 10^8 *nif*H copies L^{-1}) on day 19. Het-1 and het-3 were always recovered in the sediment traps, albeit at lower abundances (1.8 to 8.6 x 10^6 *nif*H copies L^{-1} for het-1 and 4.9 x 10^6 to 2.8 x 10^7 *nif*H copies L^{-1} for het-3) (Fig. 4b). They represented between 0.1 and 1.8 % of the targeted

*nif*H pool. UCYN-B was ~~often~~ detected in all mesocosms on both days (except in M1 on day 19), and UCYN-A2 and *Trichodesmium* were detected in M2 on day 17 but at low abundances (0.05 % of the total *nif*H pool) compared to the other phylotypes. Het-2 was never detected in the traps, and neither was γ24774A11 or UCYN-A1.

Using the volume of each mesocosm (Bonnet et al., 2016) and the total *nif*H copies for each diazotroph phylotype in the sedimenting material and in the water column ~~(Turk-Kubo et al., 2015)~~ the day before the collection of the sediment traps (Turk-Kubo et al., 2015) (~~considering~~assuming a sinking velocity of ~10 m day^{-1}, Gimenez et al. (2016))~~and in the sediments~~, we ~~were able to calculate~~ estimated the export efficiency for each phylotype~~:~~. ~~In the~~~~for~~For UCYN-C, 4.6~~10.0~~ % and 6.5~~9.5~~ % of the cells present in the water column were exported to the traps per 24 h ~~in the traps~~ on day 17 and 19, respectively (assuming one *nif*H copy per cell). For het-1, 0.3 and 0.4~~2~~ % of cells were exported into the traps on day 17 and 19~~both days~~, for het-3, 15.5~~3.2~~ % and 10.5~~4.7~~ % were exported, and for UCYN-B, 3~~.~~7.1 % and 15.5~~45.6~~ % of UCYN-B were exported on day 17 and 19, respectively. ~~These results indicate that UCYN were more efficiently exported than DDAs in this experiment.~~

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3.4. DDN transfer experiment performed on day 17

Net $^{15}N_2$ uptake~~fixation rates~~ was~~ere~~ 24.1±2.8 nmol N L^{-1} ~~d^{-1}~~ during the first 24 h of the DDN transfer experiment performed from days 17 to 20 (Fig. 5a). As expected, ~~I~~integrated $^{15}N_2$ uptake ~~over time logically~~ increased over the course of the experiment to reach~~Rates decreased at 48 h to~~ 28~~19~~.8~~2~~±4.3~~2.8~~ nmol N L^{-1} ~~d^{-1}~~ at T48 h and ~~increased to~~ 126.8~~42.2~~±35.5~~11.8~~ nmol N L^{-1} ~~d^{-1}~~ at T72 h. The DDN ~~released~~ quantified in~~to~~ the TDN pool ranged from 6.2±2.4 nmol N L^{-1} ~~d^{-1}~~ at T24 h to 9.6~~3.2~~±1.6~~0.5~~ nmol N L^{-1} ~~d^{-1}~~ at T72 h. Considering gross N_2 fixation as the sum of net N_2 fixation and DDN release (Mulholland et al., 2004), the DDN released to the TDN pool accounted for 7.1±1.2 to 20.6±8.1 % of gross N_2 fixation.

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During the 72 h targeted experiment (Fig. 5b) the diazotroph assemblage reflected that of the mesocosms from which they were sampled: UCYN-C dominated the diazotrophic community, comprising on average 62 % of the total *nifH* pool. The other most abundant phylotypes were UCYN-A2 and het-2, which represented 18 and 13 % of the total *nif*H pool, respectively. UCYN-A1, UCYN-B, het-1, het-3, and *Trichodesmium* were also detected but together they comprised

less than 8 % of the total targeted community. Phylotype abundances remained relatively stable throughout the 72 h of the experiment.

NanoSIMS analyses performed on individual UCYN-C at 24 h (Fig. 6) revealed significant ($p<0.05$) ^{13}C (1.477±0.542 atom%, n=35) and ^{15}N (1.515±0.370 atom%, n=35) enrichments relative to natural abundance, indicating that UCYN-C were actively photosynthesizing and fixing N_2. The correlation between ^{13}C enrichment and ^{15}N enrichment was significant ($r=0.85$, $p<0.01$, Fig. 6b). NanoSIMS analyses performed on diatoms and picoplankton (Fig. 5c) also revealed significant ($p<0.05$) ^{15}N enrichment of non-diazotrophic plankton, demonstrating a transfer of DDN from the diazotrophs to other phytoplankton. Both diatoms and picoplanktonic cells were significantly ($p<0.05$) more enriched at the end of the experiment (T72 h) (0.489±0.137 atom%, n=12 for diatoms; 0.457±0.077 atom%, n=96 for picopankton) than after the first 24 h (0.408±0.052 atom%, n=23 for diatoms; 0.389±0.014 atom%, n=63 for picoplankton). Finally, the ^{15}N enrichment of picoplankton and diatoms was not significantly different ($p>0.05$) during the DDN experiment.

4 Discussion

4.1 The bubble vs. the dissolution method: an intercomparison experiment

The inter-comparison experiment performed on day 11 reveals slightly lower, yet insignificantly different ($p>0.05$)~~,~~ average N_2 fixation rates when using the bubble method compared to the dissolution method. This result is in accordance with some comparisons made by Shiozaki et al. (2015) in temperate waters of the North Pacific. ~~However, one~~Although~~h~~However, a ~~might expect a~~ lower degree of dissolution of the $^{15}N_2$ bubble may occur in warm tropical waters such as those near New Caledonia ~~may occur~~ compared to the cooler, temperate North Pacific waters. In calculating N_2 fixation rates using the dissolution method, we used the value of 2.4±0.2 atom% for the ^{15}N enrichment of the N_2 pool as measured by MIMS. For the bubble method, we used the theoretical value of 8.4 atom% calculated for seawater with a temperature of 25.5 °C and salinity of 35.3 (as was the case on day 11). If we assume that equilibration was incomplete in our experiment using the bubble method, i.e., 75 % instead of 100 % as shown by Mohr et al. (2010), we calculate higher~~, yet insignificant~~albeit still insignificantly ~~so~~ ($p>0.05$)~~,~~ N_2 fixation rates for the bubble method (8.3±2.8 nmol N L^{-1} d^{-1}) compared to the dissolution method (7.2±0.8 nmol N

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L^{-1} d^{-1}), confirming that equivalent results are obtained with both methods.), although the difference is still not significant (p>0.05).).

4.2 The temporal dynamics of N_2 fixation in the mesocosms

Average N_2 fixation rates measured in the lagoon waters (outside the mesocosms, 9.2±4.7 nmol N L^{-1} d^{-1}, Table 1) are of the same order of magnitude as those reported for the Noumea lagoon during austral summer conditions (Biegala and Raimbault, 2008). They are within the upper range of rates reported in the global ocean database (Luo et al., 2012). Indeed, open ocean cruises performed offshore of New Caledonia in the Coral and Solomon Seas (e.g., (Bonnet et al., 2015; Garcia et al., 2007) also suggest that the South West Pacific Ocean is one of the areas with the highest N_2 fixation rates in the global ocean.

Averaged out over the 23 days of the experiment, In the mesocosms, rates were on average twice N_2 fixation rates in the mesocosms were ~ 2 fold higher as high (18.5±1.1 nmol N L^{-1} d^{-1}) as than those measured in lagoon waters (9.2±4.7 nmol N L^{-1} d^{-1}) –.on average over the whole experiment,. with theThe maximum observed rates of >60 nmol N L^{-1} d^{-1} ranking from days 18-21 XX are among the highest reported for marine waters (Luo et al., 2012). The enriched DIP The concentrations providedDIP concentration was the predominant difference between the ambient lagoon waters and those of the mesocosms were the modified DIP concentrations – .The mesocosms were fertilized with DIP on day 4, reaching ambient concentrations of ~0.8 µmol L^{-1} compared to lagoon waters in which DIP concentrations were typically <0.05 µmol L^{-1}. According to our experimental assumption, diazotrophy would be promoted by high concentrations of DIP. Yet, in all three mesocosms, N_2 fixation rates were negatively correlated with DIP concentrations and DIP turnover time and positively correlated with APA, suggesting that DIP deficiency may have induced favorable conditions for N_2 fixation (Table 2). Below, we describe the scenario that likely occurred in the mesocosms, which explains these unexpected negative correlations.

During P0 (day 2 to 4), N_2 fixation rates were higher in the mesocosms than in the lagoon waters, possibly due to the reduction of turbulence in the water column facilitated by the closing of the mesocosms (Moisander et al., 1997) and/or to the reduction of the grazing pressure in the mesocosms as total zooplankton abundances were slightly lower (by a factor of 1.6) in the mesocosms compared to those in the lagoon waters (Hunt et al., 2016). The most abundant

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diazotrophs in the mesocosms at P0 were het-1 and *Trichodesmium*, which were probably the most competitive groups under the initial conditions, i.e., NO_3^- depletion (concentrations were ~~below~~ 0.04±0.02 µmol L^{-1}, Table 3) and extremely low DIP concentrations (0.03±0.01 µmol L^{-1}, Table 3). *Trichodesmium* is able to use organic P substrates (DOP pool) under conditions of DIP deficiency (Dyhrman et al., 2006; Sohm and Capone, 2006). 24 h after the DIP fertilization (day 5), N_2 fixation rates in the mesocosms decreased by ~40 %, reaching rates comparable ~~rates as~~to those measured in lagoon waters during P1 (day 5 to 14). Enhanced DIP availability likely enabled non-diazotrophic organisms with lower energetic requirements and higher growth rates to outcompete the diazotrophs in the mesocosms via utilization of recycled N derived from recent N_2 fixation. This is supported by the observation that nano-eukaryotes and non-diazotrophic cyanobacteria such as *Prochlorococcus* sp. increased in abundance during P1 (Leblanc et al., 2016) in the three mesocosms ~~while~~ when N_2 fixation rates declined (Fig. 2).

During P2 (day 15 to 23), N_2 fixation rates increased dramatically in all three mesocosms. This period was defined by a high abundance of UCYN-C, which were present in low numbers in the lagoon and within the mesocosms during P0 and P1 (Turk-Kubo et al., 2015). The increase in UCYN-C abundance was synchronous with a decrease in DIP concentrations in the mesocosms (Turk-Kubo et al., 2015): UCYN-C abundance first increased in M1 (day 11), ~~subsequently~~ then in M2 (day 13), and finally in M3 (day 15). In all cases, the increase in UCYN-C abundance coincided with low ~~the day on which the~~ DIP turnover time, ~~dropped below 1 d,~~ indicative of DIP ~~limitation~~ deficiency (Berthelot et al., 2015b; Moutin et al., 2005). Under NO_3^- depletion and low DIP availability, UCYN-C appeared to be the most competitive diazotroph in the mesocosms, as they exhibited the highest maximum growth rates compared to those calculated for the other diazotrophic phylotypes for the same period (Turk-Kubo et al., 2015). Some *Cyanothece* strains possess the genes ~~enabling the~~required for utilization of organic P substrates such as phosphonates (Bandyopadhyay, 2011). Thus, UCYN-C, which were the major contributors to N_2 fixation during P2 (see below), may have used DOP as a P source during this period, consistent with the negative correlation observed between N_2 fixation rates and DOP concentrations (except in M2, Table 2), and driving the significant decline in DOP concentrations observed in all three mesocosms during P2 (Berthelot et al., 2015b; Moutin et al., 2005).

While temperature was not correlated with N_2 fixation in the lagoon, ~~in the mesocosms~~ we observed a significant positive correlation between these parameters in the mesocosms (Table 2),

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probably because some diazotrophic phylotypes present in the mesocosms and absent in the lagoon waters were particularly sensitive to seawater temperature. UCYN-C reached high abundances inside the mesocosms, but was virtually absent in the lagoon waters outside the mesocosms. Turk-Kubo et al. (2015) showed that UCYN-C abundance was positively correlated with seawater temperature, suggesting that the optimal temperature for UCYN-C growth is above 25.6 °C. This result is consistent with culture studies performed using three UCYN-C isolates from the Noumea lagoon that are closely related to the UCYN-C observed here, indicating maximum growth rates at around 30°C and no growth below 25 °C (Camps, Turk-Kubo, Bonnet, Pers. comm.). Temperature above 25.6 °C and up to 26.7°C were reached ~~since~~ on day 12 ~~up to the end in~~and were maintained through to the end of the mesocosm experiment, possibly explaining why UCYN-C was not ~~evident~~ observed during P0 (when temperature was 25.4°C) even though DIP turn-over time was ~1 d (Berthelot et al., 2015b; Moutin et al., 2005). If low DIP concentrations ~~(turn-over time less than 1 d)~~ and seawater temperatures greater than 25.6 °C are prerequisites for UCYN-C growth, an obvious question is why they did not thrive (despite being present at low abundances) in the lagoon waters during P2 when similar conditions prevailed. ~~Below, we~~We consider three possible explanations that are discussed extensively in Turk-Kubo et al. (2015): first, it is possible that UCYN-C are sensitive to turbulence, which was likely reduced in the mesocosms compared to the lagoon waters that are susceptible to trade winds and tides. Second, grazing pressures on UCYN-C may have been reduced as total zooplankton abundances were slightly lower (by a factor of 1.6) in the mesocosms compared to those in the lagoon waters (Hunt et al., 2016). Third, the water masses outside the mesocosms changed with tides and winds~~, .~~; ~~T~~thus,~~so~~ it is possible that UCYN-C were absent from the water mass encountered outside the mesocosms when we sampled for this experiment.

In the mesocosms, the cell specific $^{15}N_2$ fixation rate measured on day 17 (M2) for UCYN-C was 6.3±2.0 x 10^{-17} mol N $cell^{-1}$ d^{-1}. Multiplying this rate by the abundance of UCYN-C indicates that UCYN-C accounted for 90±29 % of bulk N_2 fixation during that period. This is consistent with the positive correlation observed between N_2 fixation rates and UCYN-C abundances in M2 (Table 2). In M1 and M3, the correlation was also positive ~~despite being~~yet insignificant~~,~~. This may have been due to ~~which may be due to~~ the low number of UCYN-C data points, ~~that~~ thus decreas~~es~~ing the sensitivity of the statistical test. Coupling between ^{13}C and ^{15}N incorporation in the mesocosms was significant (r=0.85, p<0.01) (Fig. 6b) and contrasts with results reported by

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Berthelot et al. (2016) for UCYN-C, in which ^{13}C and ^{15}N enrichment (and thus inorganic C and N_2 fixation) was ~~spatially~~ uncoupled in the cells. Based on their observations, these authors suggest that the heterogeneity in the ^{15}N and ^{13}C enrichments ~~is~~ can be explained by a specialization of some cells ~~such as diazocytes (similar to those~~that induces variability in cell-specific ^{15}N-enrichment e.g., diazocytes that contain the nitrogenase enzyme in ~~the case of~~ the colonial filamentous *Trichodesmium* sp~~.) that. induce variability in cell specific 15N enrichment~~. ~~In the present study,~~Spatial partitioning of N_2 and C fixation by colonial unicellular types was also suggested as evidence for diazocyte-like ~~-~~formation ~~also~~ in colonial C. watsonii-like (Foster et al., 2013). Here, ~~-~~UCYN-C cells ~~were fixing~~fixed both ^{13}C and ^{15}N proportionally, which suggests that the UCYN-C in our experiments did not ~~not utilize diazocytes to separate diazotrophy from photosynthesis~~specialize ~~some cells as diazocytes.~~separate these processes spatially or temporally ~~This is supported by a previous study showing that diazocyte formation by UCYN depends on the phenotype considered (Foster et al., 2013).~~

4.3 UCYN aggregation and export

Throughout the 23 days of the experiment, the majority of N2 fixation (63 %) occurred in the >10 µm size fraction, even during P2 when the small (5.7±0.8 µm) unicellular UCYN-C dominated the mesocosm diazotrophic community ~~in the mesocosms~~. These findings can be explained by the aggregation of UCYN-C cells into large (>10 µm) aggregates (Fig. 7) that were retained on 10 µm filters (Fig. 3). These large UCYN-C aggregates probably formed in part due to the presence of sticky TEP (Berman-Frank et al., 2016) or other extracellularly-released proteins, and ~~will~~ were characterized by ~~have~~ a high sinking velocity due to their large size (up to 500 µm in diameter) and a density ~~that is~~ greater than that of seawater (Azam and Malfatti, 2007). Their aggregation and subsequent sinking ~~in~~ within the mesocosms likely explains why volumetric N_2 fixation rates were higher at 12 m than at the surface during P2, as well as why the size of the aggregates increased with depth~~, ,~~ and why numerous large-size aggregates and extremely high abundances of UCYN-C were recovered in the sediment traps. ~~It has to be noted that a~~Aggregation processes may have been favored by the low turbulence in the mesocosms and it would be necessary to confirm that such processes also occur in open ocean systems.

Colonial phenotypes of UCYN (UCYN-B) have been observed in the water column of the North Tropical Pacific (ALOHA station) (Foster et al., 2013), but to our knowledge, this is the first time

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that UCYN have been detected in sediment traps. Contrary to published data (e.g. (White et al., 2012)), here we demonstrate a greater export efficiency of UCYN (~10 % exported to the traps within 24 h) compared to the export of DDAs (efficiency of 0.24 to 4.7 %). Diatoms sink rapidly and DDAs have been found in sediment traps at Station ALOHA (Karl et al., 2012; Karl et al., 1997; Scharek et al., 1999a; Sharek et al., 1999b), in the Gulf of California (White et al., 2012), and in the Amazon River plume (Subramaniam et al., 2008). In our study, we observed limited export of het-1 (*Richelia* in association with *Rhizosolenia*) and het-3 (*Calothrix*) during P2, while het-2 (*Richelia* associated with *Hemiaulus*) was never recovered in the sediment traps. This is likely because *Hemiaulus* has a lower sinking rate than *Rhizosolenia* due do its smaller size, or may be more easily grazed by zooplankton than *Rhizosolenia* or *Calothrix*, which are known to be toxic to crustaceans (Höckelmann et al., 2009). We observed only rare occurrences of *Trichodesmium* ~~was very rarely exported in this study~~export in this study~~,~~ probably due to its extremely limited presence and low growth rates in the mesocosms. Direct comparisons of our export results with findings from open ocean studies should be made cautiously as our mesocosms were ~~both~~ shallower (15 m) than typical oceanic export studies (>100 m) and were also probably characterized by reduced turbulence (Moisander et al., 1997).

We estimate ~~in M2~~ that the direct export of UCYN-C ~~in M2~~ accounted for 22.4±5.5 % of the total POC exported in each mesocosm at the height of the UCYN-C bloom (day 17) and decreased to 4.1±0.8 % on day 19 (Fig. 4c, Fig. 7). ~~–~~This calculation is based on the total ~~particulate organic C (~~POC~~)~~ content measured in the sediment traps (Berthelot et al., 2015b), our C_{con} for UCYN-C estimated as described above, and published C_{con} for other diazotrophs. The corresponding export of het-1, het-3, *Trichodesmium*, and UCYN-B on day 17 based on published C_{con} (Leblanc et al., 2012; Luo et al., 2012), and using an average of three *Richelia* and *Calothrix* symbionts per diatom, accounted for 6.8±0.5, 0.5±0.02, 0.3±0.3, and 0.1±0.01 % of the POC export on day 17, respectively, and for 4.2±1.7, 0.04±0.03 of the POC export on day 19 (the contribution of *Trichodesmium* and UCYN-B ~~did not show any contribution~~ to POC export on day 19 was negligible). Thus, our data emphasize~~s~~ that despite their small size relative to DDAs, UCYN-C are able to directly export organic matter to depth by forming densely~~-~~–populated aggregates that can rapidly sink. This observation ~~was~~ is further confirmed by the *e* ratio, which quantifies the efficiency of a system to export POC relative to primary production (*e* ratio = POC export/PP)~~,~~ and was significantly higher ($p<0.05$) during P2 (i.e., during the UCYN-C bloom;

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39.7±24.9 %) than during P1 (i.e., when DDAs dominated the diazotrophic community; 23.9±20.2 %) (Berthelot et al., 2015b). ~~This~~ It ~~is~~ is also consistent with the significantly ($p<0.05$) higher contribution of N_2 fixation to export production during P2 (56±24 %, and up to 80 % at the end of the experiment) compared to P1 (47±6 %, and never exceeded 60 %) as estimated by Knapp et al. (2015) using a $\delta^{15}N$ budget for the mesocosms. ~~This proportion~~ Our calculated contribution of N_2 fixation to export production is very high compared to other tropical and subtropical regions where diazotrophs are present (10 to 25 %; e.g., (Altabet, 1988; Knapp et al., 2005)). ~~Yet~~However, ~~but~~ it is consistent with the high~~er~~ rates of N_2 fixation measured in the enclosed mesocosms compared to those from the lagoon and other tropical pelagic studies (Luo et al., 2012). The direct export of UCYN-C and other diazotrophs cannot solely explain the high *e* ratio estimated for P2. We thus hypothesize that a fraction of the DDN export that occurred during P2 was transferred indirectly via primary utilization by non-diazotrophic plankton cells that were eventually exported to the sediment traps (Fig. 7).

4.4 DDN transfer to non-diazotrophic phytoplankton and ecological implications

The amount of ~~fractional release of~~ DDN measured in the TDN pool during the 72 h DDN transfer experiment is higher than that reported for culture studies of *Cyanothece* populations (1.0±0.3 to 1.3±0.2 % of gross N_2 fixation~~;~~ (Benavides et al., 2013; Berthelot et al., 2015a)). The DDN measured in the TDN pool reflects the DDN release by diazotrophs during N_2 fixation and is likely underestimated here as a fraction of this DDN has been ~~uptaken~~taken up by surrounding planktonic communities. In our experiment, other diazotrophs were present in addition to *Cyanothece*, and they may have also ~~have~~ contributed to the dissolved pool. Moreover, ~~in contrast to pure culture studies. Moreover, u~~Uunlike in culture studies, field experiments~~,~~ are also impacted by other exogenous factors such as viral lysis (Fuhrman, 1999) and sloppy feeding (O'Neil and Roman, 1992; Vincent et al., 2007), which may enhance N release.

This DDN release plays a critical role in the N transfer between diazotrophs and non-diazotrophs. The cell-specific uptake rates of DDN during the DDN transfer experiment were calculated for each cell analysed by nanoSIMS (diatoms and cells from the 0.2-2 µm fraction). By multiplying cell-specific N uptake rates by the cellular abundance of each group on a particular day, we could identify the specific pool (diazotrophs, dissolved pool, non-diazotrophs) into which the $DD^{15}N$ was transferred after 24 h, and the extent to which this $^{15}N_2$ accumulated. The results are

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summarized in Fig. 7. After 24 h, 52±17 % of the newly fixed $^{15}N_2$ remained in the UCYN-C biomass, 16±6 % had accumulated in the dissolved N pool, and 21±4 % had been transferred to non-diazotrophic plankton. In addition, 11 % of the newly fixed $^{15}N_2$ accumulated in a pool that we refer to as 'others' (corresponding to diazotrophs other than UCYN-C and potential non-diazotrophs to which $^{15}N_2$ was transferred; these cells were not analysed by nanoSIMS due to their very low abundance). Uncertainties take into account both the variability of the ^{15}N enrichment determined on ~25 cells per group by nanoSIMS, and the uncertainty in the N content per cell measured or taken from the literature.

Within the fraction of DDN transferred to the non-diazotrophs after 24 h (21 %), we calculat~~ed~~ that 18±4 % was transferred to picoplankton, and only 3±2 % was transferred to diatoms (Fig. 7). The ^{15}N enrichment of picoplankton and diatoms was not significantly different ($p>0.05$) in this study, but as picoplankton dominated the planktonic community in the mesocosms at the time of the DDN transfer experiment, they were the primary beneficiaries of the DDN. This is consistent with the positive correlation between N_2 fixation rates, *Synechococcus*, and pico-eukaryote abundances in the mesocosms (Table 2), as well as with the observed dramatic increase in *Synechococcus* and pico-eukaryotes abundances (by a factor of >2 between P1 and P2) (Leblanc et al., 2016). Diatom abundances also increased in the mesocosms by a factor of 2 between P1 and P2 (largely driven by *Cylindrotheca closterium*), but this increase occurred earlier than the picoplankton increase, i.e., at the end of P1 (days 11-12). Maximum diatom abundances were reached on day 15-16 at the very beginning of P2, and then declined by day 18 to reach abundances similar to those observed during P1. These results suggest that diatoms were the primary beneficiaries of DDN in the mesocosms at the start of P2, when N_2 fixation rates and UCYN-C abundances increased dramatically. This is consistent with a previous DDN transfer study performed in New Caledonia (Bonnet et al., Accepted) during which diatoms (mainly *Cylindrotheca closterium*) advantageously competed and utilized DDN released during *Trichodesmium* blooms. When the present DDN transfer experiment was performed (days 17 to 20), diatom abundances had already declined, likely due to DIP limitation (DIP turnover time was ~~be~~low, i.e below 1d). We hypothesize that picoplankton were more competitive for DDN under low DIP conditions as small cells with high surface to volume ratios are known to outcompete larger cells for the available DIP ~~available~~ (Moutin et al., 2002). Moreover, some prokaryotes from the 0.2-2 µm size-fraction can utilize DOP compounds (Duhamel et al., 2012).

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In this study, we could not discriminate the DDN transfer to pico-autotrophs from that to pico-heterotrophs, but it is likely that both communities took advantage of the DDN, as both primary production (Berthelot et al., 2015b) and bacterial production (Van Wambeke et al., 2015) were positively correlated with N_2 fixation rates (Table 2) and increased dramatically following the increase in N_2 fixation during P2. The standing stocks of POC, PON, and POP were also positively correlated with N_2 fixation rates, ~~confirming~~ suggesting that DDN sustained productivity in the studied system.

5 Conclusions

While studies on the fate of DDN in the ocean are rare, the contribution of DDN to particle export based on the $\delta^{15}N$ signatures of exported material indicate that N_2 fixation can efficiently contribute to export production in the oligotrophic ocean (Dore et al., 2008). The export of DDN may either be direct, through the sinking of diazotrophs, or indirect, through the transfer of DDN to non-diazotrophic plankton in the photic zone~~,~~ that ~~is~~are subsequently exported.

Trichodesmium is rarely recovered in sediment traps (REF) and most of the research dedicated to the export of diazotrophs has focused on DDAs (Karl et al., 2012) due to their high sinking velocity. Here, we demonstrate for the first time that UCYN can efficiently contribute ~~(up to 22.4±5.5 % at the height of the bloom)~~ to POC export in oligotrophic systems, predominantly due to the aggregation of small (5.7±0.8 µm) UCYN-C cells into large aggregates, which increase~~ing~~ in size (up to 500 µm) with depth. ~~These results indicating~~ Our results suggest that these small (typically 3-7 µm) organisms should be considered in future~~–~~ studies to confirm if processes observed in mesocosms are applicable to the open ocean. ~~This export was predominantly due to the aggregation of small (5.7±0.8 µm) UCYN-C cells into large aggregates, increasing in size (up to 500 µm) with depth.~~

Moreover, the experimental and analytical approach used in this study allowed for the quantification of the actual transfer of DDN to different groups of non-diazotrophic plankton in the oligotrophic ocean. ~~, and therefore reveals another level of complexity to the processes that occur between N_2 fixation and the eventual export of organic matter. A study based on high~~Our~~-~~resolution nanometer scale secondary ion mass spectrometry (~~nanoSIMS~~)~~ results coupled with $^{15}N_2$ isotopic labelling revealed that a significant fraction of DDN (21±4 %) is quickly (within 24 h) transferred to non-diazotrophic plankton, which increased in abundance simultaneously with

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N_2 fixation rates~~, a part of it having potentially contributed, in turn to indirect export of organic matterproduction~~. A similar nanoSIMS study performed during a *Trichodesmium* bloom (Bonnet et al., Accepted) reveal~~s~~ed that diatoms were the primary beneficiaries of DDN and developed extensively during and after *Trichodesmium* spp. blooms. Diatoms are efficient exporters of organic matter to depth (Nelson et al., 1995). ~~and both t~~These studies show that plankton grown on DDN in the oligotrophic ocean ~~may thus drive the~~drive ~~-~~indirect export of organic matter out of the photic zone, thus ~~. Thus,~~ revealing a previously ~~unseen level of complexity in the pathways that~~unaccounted for conduit between ~~occur between~~ N_2 fixation and the eventual export to depth of DDN from the photic zone.

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Author contribution: S. Bonnet designed the experiments and S. Bonnet and H. Berthelot carried them out with help from E. Rahav. S. Bonnet, H. Berthelot, K.A. Turk-Kubo, S. Fawcett and S. L'Helguen analyzed the samples. I. Berman-Frank took part in experimental planning, preparation, and implementation of the project. S. Bonnet prepared the manuscript with contributions from all co-authors.

Figure captions

Figure 1. (a) Mesocosms (~50 m^3) deployed in the framework of the VAHINE project. (b) Sediment traps screwed onto the base of the mesocosms and were sampled daily by SCUBA divers.

Figure 2. (a) Horizontal and vertical distributions of bulk N_2 fixation rates (nmol N L^{-1} d^{-1}), and (b) <10 µm N_2 fixation rates (nmol N L^{-1} d^{-1}) in M1, M2, M3, and lagoon waters. Note that N_2 fixation rates in the <10 µm fraction were not measured (lower right panel). The grey bars indicate the timing of the DIP ~~spike~~ addition on day 4.

Figure 3. (a) UCYN-C cells per aggregate in M2 on day 17 and 19. (b to e) Green excitation (510-560 nm) epifluorescent replicate micrographs of UCYN-C on day 17 taken at 1 m depth (x40) (b), 6 m depth (x40) (c), 12 m depth (x40) (d), and in the sediment traps (x10) (e). Scale bar 20 µm (b to d) and 100 µm (e).

Figure 4. (a) Abundance of UCYN-C (*nif*H copies L^{-1}) and (b) other *nif*H phylotypes (UCYN-A2, UCYN-B, *Trichodesmium*, het-1, het-3) (*nif*H copies L^{-1}) recovered in the sediment trap on day 17 and 19. (c) Proportion of POC export~~ed~~ associated with diazotrophs in the sediment traps on day 17 in M2 (height of UCYN-C bloom).

Figure 5. Results from the DDN transfer experiment performed from day 17 to 20 in M2. (a) Temporal changes in $^{15}N_2$ uptake ~~the rate (nmol N L^{-1} N d^{-1}) of N_2 fixation~~ (white, nmol N L^{-1}) and quantification of DDN in the dissolved pool~~release~~ (grey) over the course of the experiment. Error bars represent the standard deviation of three independent replicate incubations. (b) Temporal changes in diazotroph abundance determined by qPCR (*nif*H gene copies L^{-1}) during the same experiment. Error bars represent the standard deviation of triplicate incubations. (c) Summary of the nanoSIMS analyses. Measured ^{13}C and ^{15}N atom% values of non-diazotrophic diatoms (white) and picoplankton (grey) as a function of incubation time. The horizontal dashed line indicates the natural abundance of ^{15}N (0.366 atom%), and the error bars represent the standard deviation for the several cells analysed by nanoSIMS.

Figure 6. (a) Green excitation (510-560 nm) epifluorescent micrographs of UCYN-C, (b) ^{13}C and ^{15}N isotopic enrichment (atom%) in individual UCYN-C cells ~~at~~ on day 17 in M2, (c, d) nanoSIMS images showing the ^{13}C (c) and ^{15}N (d) enrichment of individual UCYN-C cells after 24 h of incubation. The white outlines show regions of interest (ROIs), which were used to estimate the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios.

Figure 7. Cartoon summary of the simplified pathways of N transfer in the first trophic level of the food web and the potential impact on the sinking POC flux at the height of the UCYN-C bloom in the VAHINE mesocosm experiment.

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Table 1. N_2 fixation rates (nmol N L^{-1} d^{-1}) measured in the mesocosms and in lagoon waters. Table shows the range, median, mean, contribution of the <10 µm fraction to total rates (%), and the number of samples analysed (n).

	Range	Median	Mean	% <10 µm	n
M1	0.5-69.7	15.9	19.7	38	61
M2	3.0-67.7	15.1	18.1	43	57
M3	2.9-60.4	14.2	17.7	29	59
Average mesocosms	2.1-65.9	15	18.5	37	177
Lagoon waters	1.9-29.3	8.7	9.2	n.a	61

Table 2. Spearman correlation matrix of N_2 fixation rates and hydrological parameters, biogeochemical stocks and fluxes, and planktonic communities (n=66). The significant correlations ($p<0.05$) are indicated in bold. n.a- not available.

	Parameter	M1	M2	M3	Lagoon waters
Hydrological parameters	Temperature	**0.394**	**0.319**	**0.347**	0.228
	Salinity	0.211	0.213	**0.266**	-0.122
Biogeochemical stocks and fluxes	NO_3^-	**-0.539**	**-0.302**	**-0.341**	0.145
	NH_4^+	0.152	0.103	0.006	0.197
	DIP	**-0.613**	**-0.569**	**-0.482**	-0.116
	DON	**-0.329**	**-0.413**	-0.235	-0.180
	DOP	**-0.563**	-0.157	**-0.316**	-0.243
	PON	**0.575**	**0.293**	**0.494**	0.077
	POP	**0.514**	0.001	**0.439**	0.036
	POC	**0.399**	**0.352**	**0.356**	-0.061
	Chl *a*	**0.660**	**0.656**	**0.656**	0.220
	Primary production	**0.443**	**0.498**	**0.445**	**0.268**
	Bacterial production	**0.708**	**0.408**	**0.471**	0.189
	T-DIP	**-0.670**	**-0.603**	**-0.564**	-0.190
	APA	**0.575**	**0.568**	**0.273**	-0.062
Planktonic communities	HNA	**0.317**	-0.043	**0.458**	n.a
	LNA	0.262	-0.021	0.000	n.a
	Prochlorococcus	**0.429**	-0.122	0.138	n.a
	Synechococcus	**0.699**	**0.434**	**0.499**	n.a
	Pico-eukaryotes	**0.614**	**0.563**	**0.414**	n.a
	Nanoeukaryotes	**0.477**	0.002	**0.442**	n.a
	Diatoms	-0.099	0.456	-0.200	n.a
	Dinoflagellates	0.242	-0.392	-0.321	n.a
	UCYN-A1	0.545	-0.521	-0.503	0.200
	UCYN-A2	0.127	**-0.631**	0.248	0.333
	UCYN-B	0.083	**0.696**	0.467	0.101
	UCYN-C	0.373	**0.621**	0.515	-0.167
	Trichodesmium	-0.145	0.147	0.285	-0.117
	DDAs	-0.036	-0.264	-0.527	0.262
	γ-24774A11	0.327	0.497	**-0.750**	**0.733**

Table 3. Average NO_3^-, DIP, DON, and DOP concentrations (µmol L^{-1}) measured over the P0, P1 and P2 periods. NO_3^- and DIP concentrations were determined using a segmented flow analyzer according to (Aminot and Kerouel, 2007). The detection limit was 0.01 and 0.005 µmol L^{-1} for NO_3^- and DIP, respectively. DON and DOP concentrations were determined according to the wet oxidation procedure described in (Pujo-Pay and Raimbault, 1994) and (Berthelot et al., 2015b).

	Average P0	Average P1	Average P2
NO_3^-	0.04±0.02	0.03±0.01	0.02±0.01
DIP	0.03±0.01	0.48±0.20	0.08±0.05
DON	5.19±0.37	5.22±0.54	4.73±0.49
DOP	0.14±0.01	0.16±0.03	0.12±0.02

Code de champ modifié

Code de champ modifié

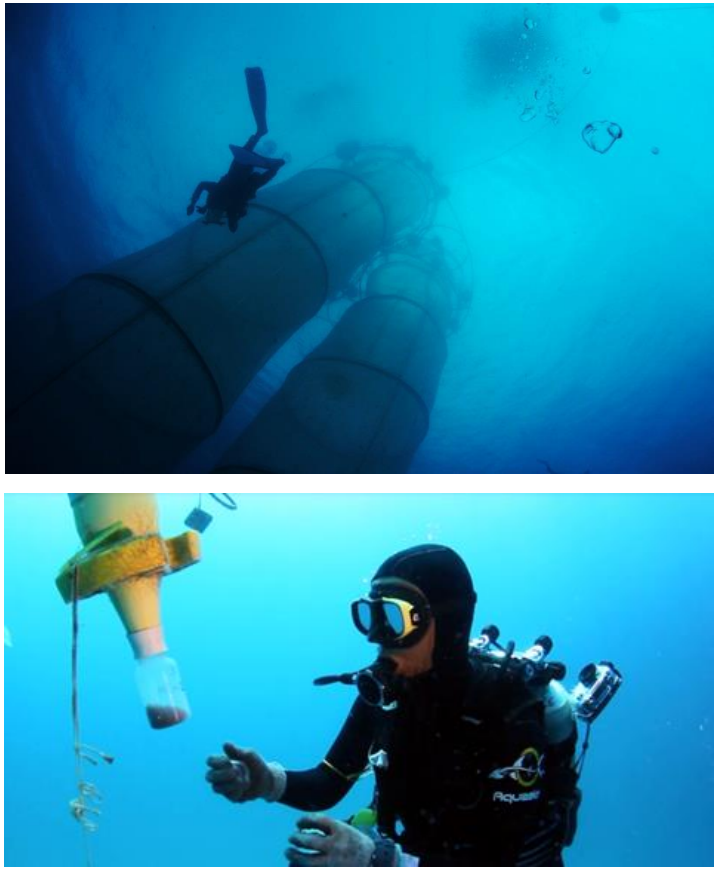

Figure 1.

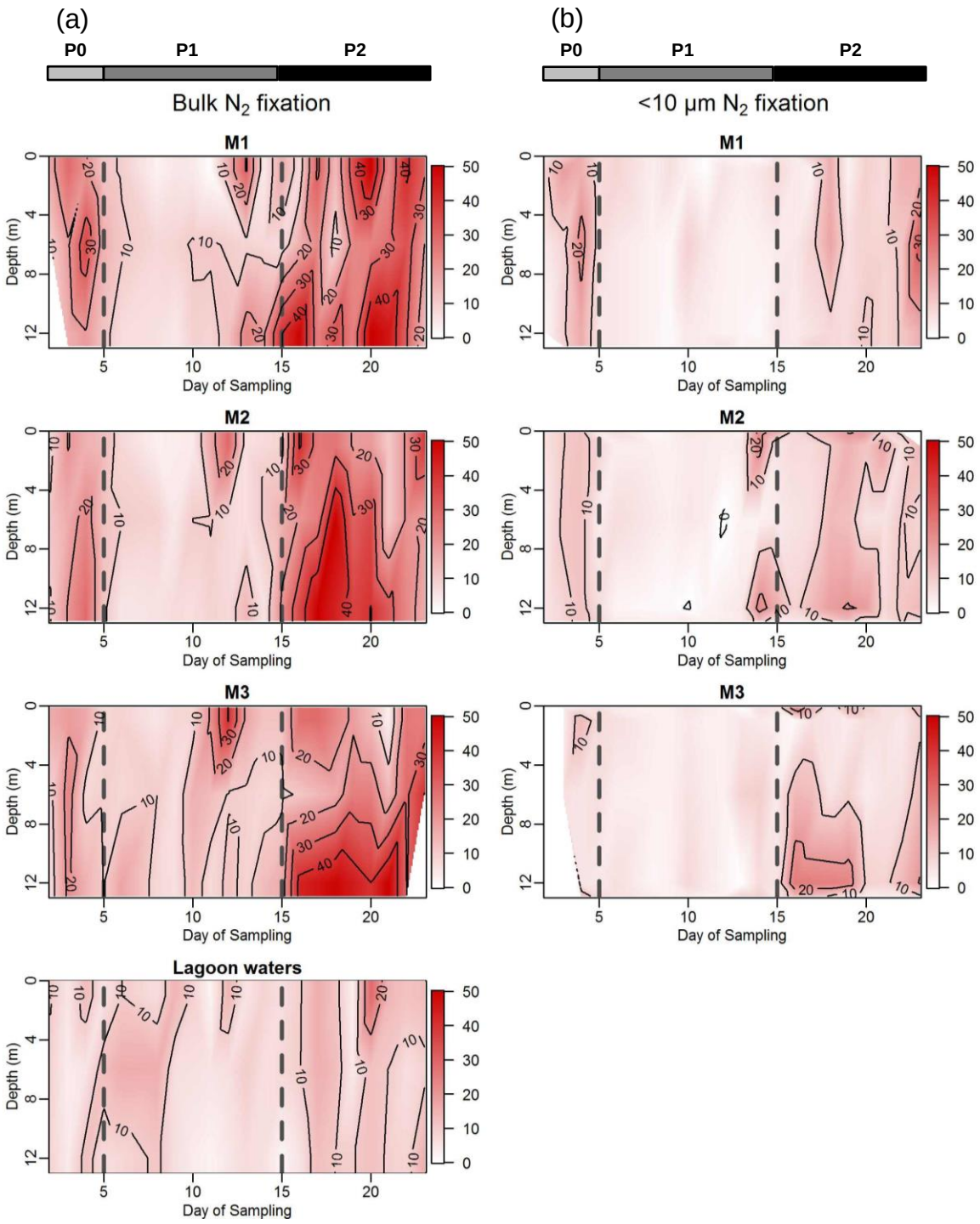
(a)
P0
P1
P2
Bulk N_2 fixation
M1
M2
M3
Lagoon waters
(b)
P0
P1
P2
<10 µm N_2 fixation
M1
M2
M3
Depth (m)
Day of Sampling

Figure 2.

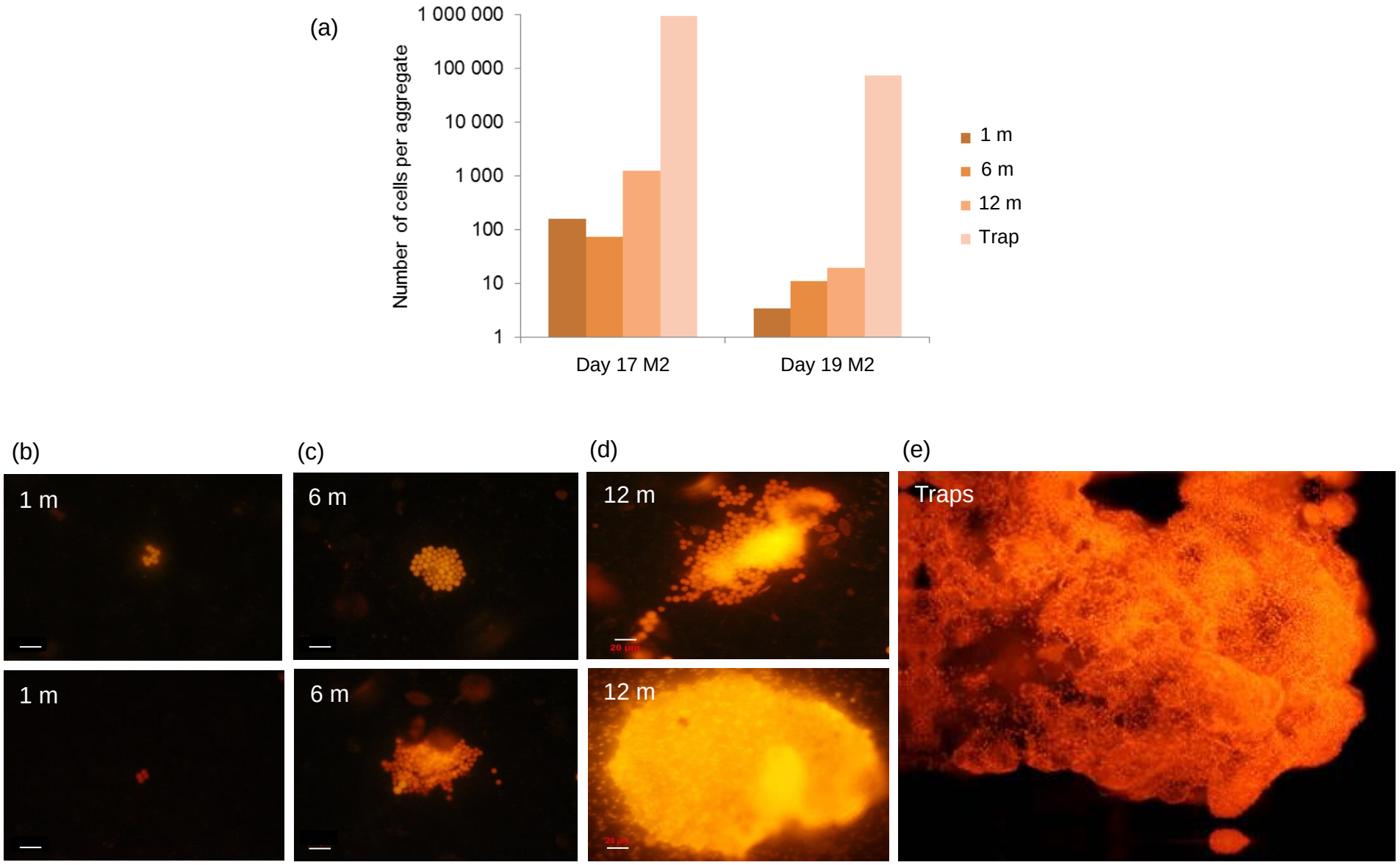
(a)
1 000 000
100 000
10 000
1 000
100
10
1
Number of cells per aggregate
Day 17 M2
Day 19 M2
1 m
6 m
12 m
Trap
(b)
(c)
(d)
(e)
1 m
1 m
6 m
6 m
12 m
12 m
Traps

Figure 3.

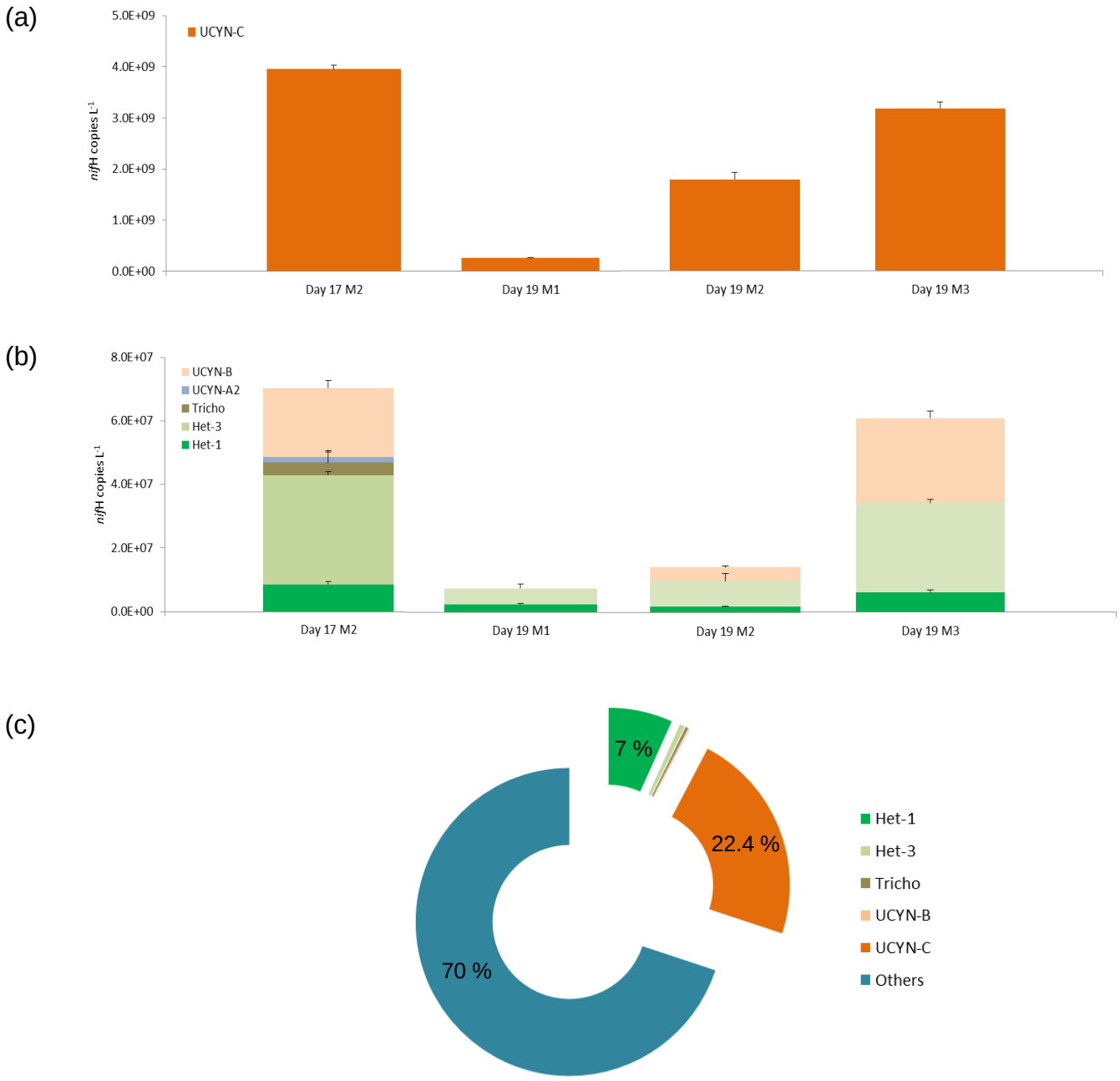
(a)
UCYN-C
5.0E+09
4.0E+09
3.0E+09
2.0E+09
1.0E+09
0.0E+00
nifH copies L-1
Day 17 M2
Day 19 M1
Day 19 M2
Day 19 M3
(b)
UCYN-B
UCYN-A2
Tricho
Het-3
Het-1
8.0E+07
6.0E+07
4.0E+07
2.0E+07
0.0E+00
nifH copies L-1
Day 17 M2
Day 19 M1
Day 19 M2
Day 19 M3
(c)
7 %
22.4 %
70 %
Het-1
Het-3
Tricho
UCYN-B
UCYN-C
Others

Figure 4.

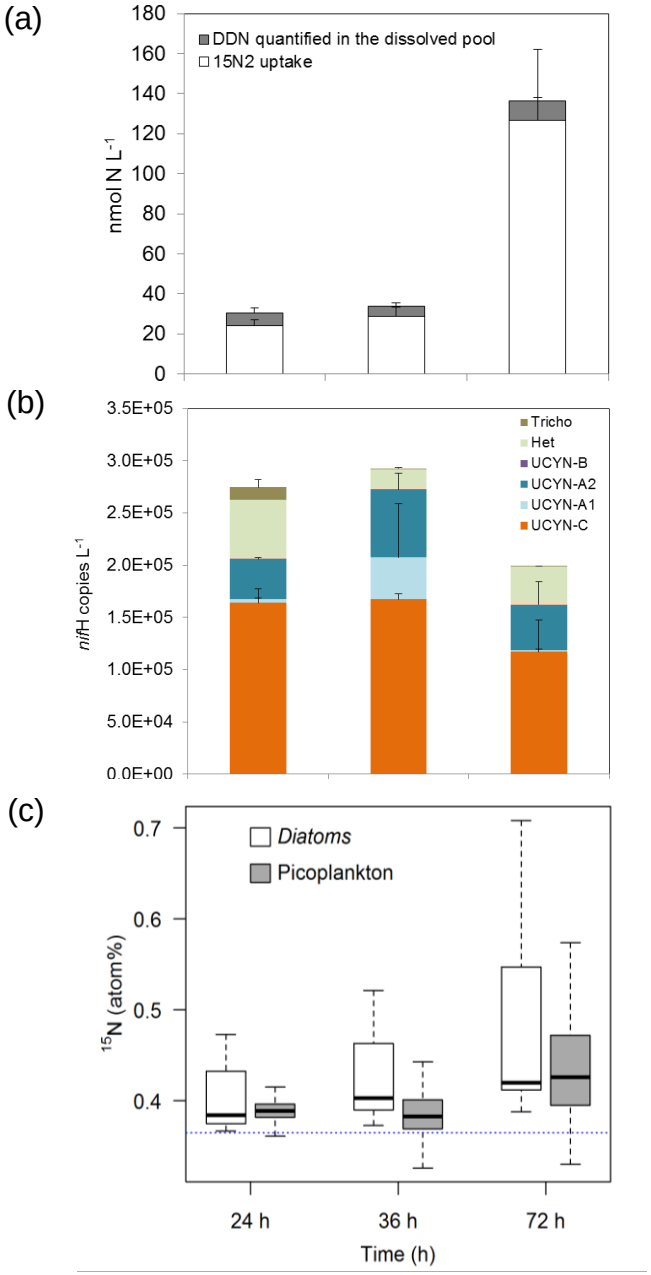
(a)
DDN quantified in the dissolved pool
15N2 uptake
nmol N L-1
180
160
140
120
100
80
60
40
20
0
(b)
Tricho
Het
UCYN-B
UCYN-A2
UCYN-A1
UCYN-C
nifH copies L-1
3.5E+05
3.0E+05
2.5E+05
2.0E+05
1.5E+05
1.0E+05
5.0E+04
0.0E+00
(c)
Diatoms
Picoplankton
15N (atom%)
0.7
0.6
0.5
0.4
24 h
36 h
72 h
Time (h)

Figure 5.

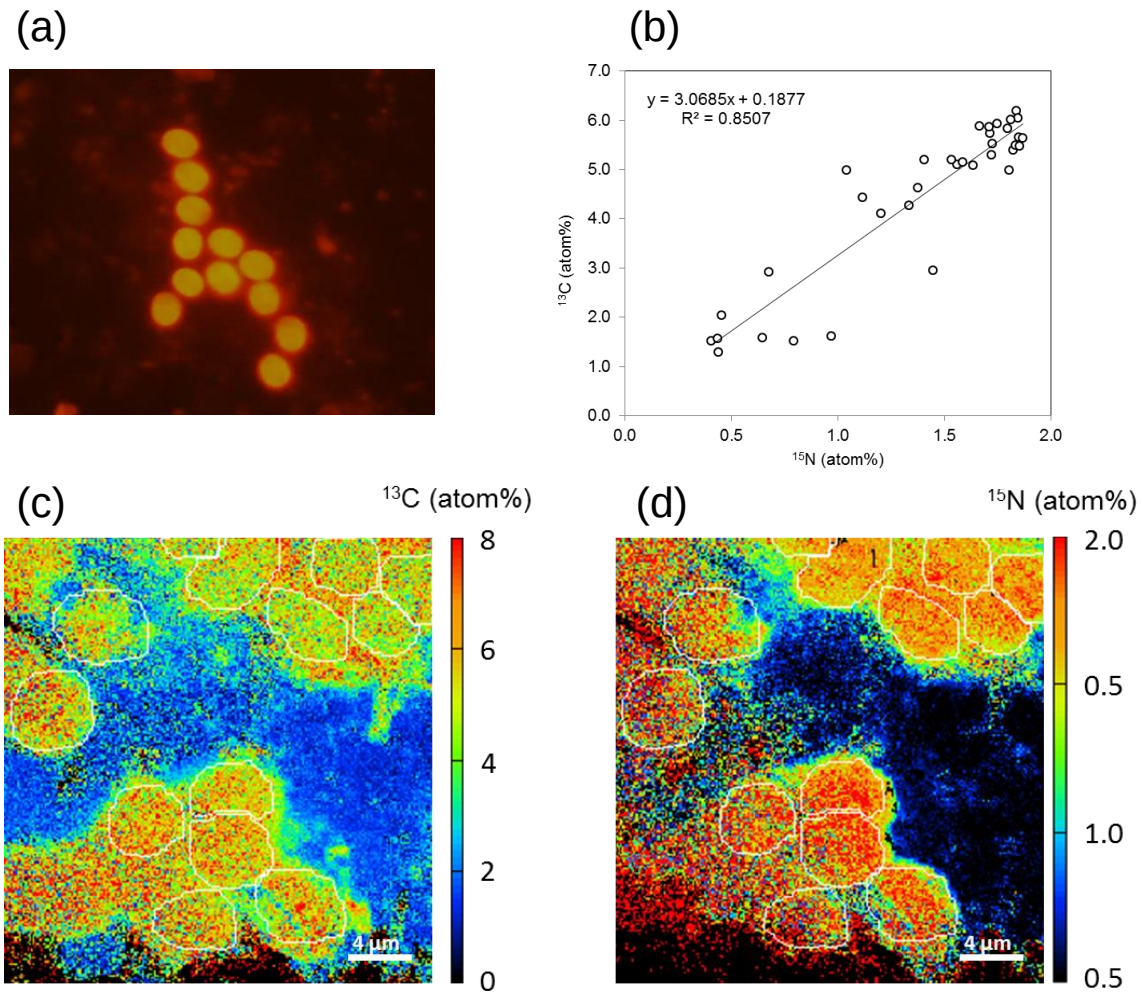
(a)
(b)
y = 3.0685x + 0.1877
R² = 0.8507
13C (atom%)
15N (atom%)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.0
0.5
1.0
1.5
2.0
(c)
13C (atom%)
8
6
4
2
0
4 µm
(d)
15N (atom%)
2.0
0.5
1.0
0.5
4 µm

Figure 6.

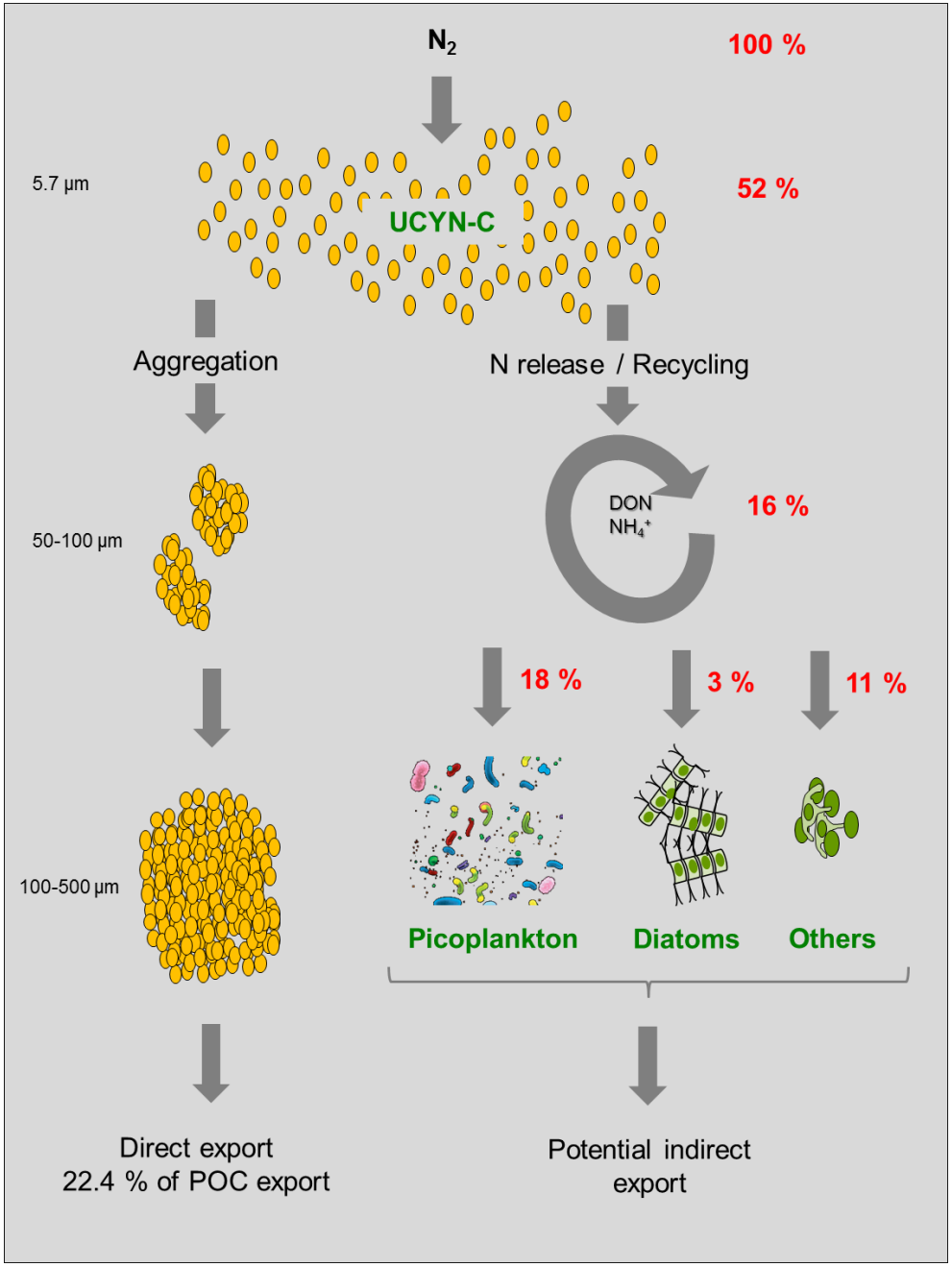
N_2
100 %
5.7 µm
UCYN-C
52 %
Aggregation
N release / Recycling
50-100 µm
DON
NH_4^+
16 %
18 %
3 %
11 %
100-500 µm
Picoplankton
Diatoms
Others
Direct export
22.4 % of POC export
Potential indirect
export

Figure 7.