Bleaching forces coral's heterotrophy on diazotrophs and *Synechococcus*

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Abstract

Coral reefs are threatened by global warming, which disrupts the symbiosis between corals and their photosynthetic symbionts (Symbiodiniaceae), leading to mass coral bleaching. Planktonic diazotrophs, or dinitrogen (N_2) fixing prokaryotes are abundant in coral lagoon waters and could be an alternative nutrient source for corals. Here we incubated untreated and bleached coral colonies of *Stylophora pistillata* with a $^{15}N_2$-pre-labelled natural plankton assemblage containing diazotrophs. $^{15}N_2$ assimilation rates in Symbiodiniaceae cells and tissue of bleached corals were 5 and 30-fold higher, respectively, than those measured in untreated corals, demonstrating that corals incorporate more nitrogen derived from planktonic diazotrophs under bleaching conditions. Bleached corals also preferentially fed on *Synechococcus*, picophytoplanctonic cells rich in nitrogen, instead of *Prochlorococcus* and

picoeukaryotes that are poorer in nitrogen content. By providing an alternative source of labile nitrogen, both the incorporation of nitrogen derived from planktonic diazotrophs and the ingestion of *Synechococcus* may have profound consequences for coral bleaching recovery, especially for the many coral reef ecosystems characterized by high abundance and activity of planktonic diazotrophs.

Key words: Scleractinian corals – Diazotrophic plankton – *Synechococcus* – pico-nanoplankton – Bleaching – Heterotrophy – N_2 fixation – New Caledonia

Introduction / Materials & methods / Results and discussion

Coral reefs are currently under threat by global warming, which disrupts the symbiosis between corals and their endosymbiotic dinoflagellates of the family Symbiodiniaceae [1], leading to mass coral bleaching [2]. When corals bleach, they lose part of their photosynthetic symbionts that provided them with nitrogen (Muscatine and D'Elia 1978) and seawater warming also decreases coral nitrogen acquisition capacity [3]. Several studies have reported an increase in the consumption of meso- and macroplankton by corals when exposed to thermal stress, potentially sustaining a critical supply of nutrients needed for recovery following bleaching [4–6]. The ability of corals to feed on smaller planktonic fractions, i.e. pico- (0.2 to 2 µm) and nanoplankton (2 to 20 µm) has also been documented [7], but the increase in the ingestion of bacteria and picoflagellates on bleached corals has only been observed in one study [8]. Among these size fractions, planktonic dinitrogen (N_2)-fixing prokaryotes (subsequently referred to as planktonic diazotrophs) are very abundant in coral lagoon waters [9, 10]. They reduce atmospheric N_2 into bioavailable ammonium (NH_4^+), providing sufficient nitrogen stocks for the development of the planktonic food web in oligotrophic waters [11]. The assimilation of nitrogen derived from planktonic diazotrophs has been recently demonstrated in corals [12]. According to Benavides et al. (2016), ^{15}N-enrichment in corals after their incubation with ^{15}N-labelled natural diazotrophic assemblages could be due to three different processes: *(i)* direct feeding on planktonic diazotrophs digested within the coelenteron, *(ii)* uptake of ^{15}N-dissolved nitrogen compounds fixed by the planktonic diazotrophs and released extracellularly, *(iii)* ingestion of non-diazotrophic plankton enriched in ^{15}N as a result of diazotroph-derived nitrogen transfer (Bonnet et al 2016). While several studies have demonstrated that N_2 fixation by coral symbiotic diazotroph communities increases in bleached corals (Bednarz et al 2017, 2019), the acquisition of nitrogen derived from planktonic diazotrophic activity has never been investigated in corals facing thermal stress. To determine if bleached corals also benefit from planktonic diazotrophs, we incubated colonies of the branching coral *S. pistillata* with a $^{15}N_2$-pre-labelled (24 h) natural plankton assemblage containing planktonic diazotrophs (pre-filtered through a 100 µm mesh to exclude larger cells) as described in Benavides *et al.* (2016). In parallel, N_2 fixation within endosymbiotic diazotrophs in colonies of the same species was measured by incubating colonies in ^{15}N-enriched filtered seawater. Coral colonies collected in the New Caledonian lagoon were acclimated to experimental conditions for three weeks. They were progressively bleached over 18 days (by a gradual temperature increase up to 31°C) or left at ambient temperature (28°C) as a control (subsequently referred to as untreated corals, see **Supplementary Information for details**). The $\delta^{15}N$ isotopic values were measured in symbionts, coral tissues and plankton before and after incubation (12 h). Nitrogen assimilation rates were calculated as previously described [13]. The contribution of ^{15}N-enrichment levels from endosymbiotic diazotrophic communities was minor (see results in the **Supplementary Information**). Conversely, after the incubation with ^{15}N-labelled natural diazotrophic assemblages significant ^{15}N-enrichments were found in the Symbiodiniaceae of both untreated and bleached corals. This suggests that Symbiodiniaceae used nitrogen originating from the planktonic diazotrophs [12, 14, 15]. Nitrogen assimilation rates in Symbiodiniaceae and bleached corals tissue increased by 5- (0.6512 ± 0.3890 µg N cm^{-2} h^{-1}; n = 5; Mann-Whitney-Wilcoxon test, $P < 0.05$) and 30-fold (0.0057 ± 0.0028 µg N cm^{-2} h^{-1}; n = 5; Mann-Whitney-Wilcoxon test, $P < 0.01$) respectively, compared to those measured in the untreated corals (0.1330 ± 0.2465 and 0.0002 ± 0.0004 µg N cm^{-2} h^{-1}) (**Fig. 1**). This demonstrates that corals could incorporate more nitrogen coming from planktonic diazotrophs under bleaching conditions than untreated corals. By providing an alternative source of labile nitrogen, the increased incorporation of nitrogen derived from planktonic diazotrophs may have profound consequences for coral bleaching recovery, particularly in coral reef ecosystems characterized by high abundance and activity of planktonic diazotrophs. Such kind of reefs are widespread, and can be found in the Western South Pacific (*e.g.* New Caledonia, Papua New Guinea, and Australian Great Barrier Reef) [9, 10, 16, 17], but also in Hawaii and in the Caribbean and Red Seas [18–20]. After 12 h of incubation, the assimilation rates were 100 times greater in Symbiodiniaceae than in coral tissues, regardless of the treatment (n = 10 for each compartment; Mann-Whitney-Wilcoxon test, P = 0.019). This observation is consistent with the results obtained by several authors (e.g. [23], [12], [24], [15, 25] ,[26]) who demonstrated that symbionts can immediately take up and store nitrogen-derived compounds that are then transferred to the host's tissue. We conducted quantitative PCR (qPCR) assays to determine planktonic diazotroph abundances (UCYN-A1, UCYN-C and *Trichodesmium, i.e.* the most important phylotypes in the lagoon [9, 27]) in the incubation medium at the beginning and at the end of incubation by targeting the *nifH* gene, a common biomarker for diazotrophs. These assays revealed (i) a significant abundance of diazotrophs in the incubation medium at the beginning of the experiment (UCYN-A1, UCYN-C and *Trichodesmium* abundances were respectively 4.14 ± 5.35 10^2, 0.97 ± 1.26 10^1 and 8.63 ± 6.03 10^2 *nifH* gene copies L^{-1}), and (ii) a decrease in the abundance of UCYN-A1 (1 µm) and

UCYN-C (4-8 µm) in all tanks containing corals (n = 3) compared to the controls without corals, confirming that corals fed on these two types of preys. While UCYN-A1 are ~1 µm in size, their association with a picoeukaryote host (Thompson et al 2012) could increase their size to 7-10 µm and thus improve their chances of being consumed by corals. Pico-, nano-eukaryotes and bacterial abundances were further assessed by flow cytometry at the start and end of incubations to quantify their ingestion by both bleached and untreated corals. During the 12 h of incubation *Prochlorococcus* was quantitatively the major prey ingested, followed by *Synechococcus* and picoeukaryotes in both treatments and confirming the ability of corals to feed on picoplankton [e.g. 9, 29; see **Supplementary Information**]. One of the most notable results of this study is that the ingestion rates of *Synechococcus* were 1.6 times higher in bleached corals ($3.79 \pm 0.64\ 10^4$ cell $cm^{-2}\ h^{-1}$) than in untreated corals ($2.38 \pm 0.24\ 10^4$ cell $cm^{-2}\ h^{-1}$, Mann-Whitney-Wilcoxon test, $P = 0.028$; **Fig.2**). Until now, studies have shown that corals can regulate their heterotrophic feeding capacities in zooplankton (> 50 µm) [5] and in picoflagellates and bacteria (Tremblay et al 2012) in response to bleaching. For the first time, our results show that thermally stressed corals are able to increase not only their consumption of planktonic diazotrophs and plankton that likely benefited from N_2 fixation [9], but also more specifically their ingestion of a very specific taxonomic group of picoplankton: the ubiquitous marine cyanobacterium *Synechoccoccus*. Surprisingly, bleached colonies of *S. pistillata* preferentially selected *Synechococcus* cells, which were not the most abundant in the medium during our incubation, but are known to be rich in nitrogen and also to benefit from nitrogen released by surrounding diazotrophs in the natural environment [29–31]. So far, this type of selective feeding on *Synechococcus* cells has only been shown under controlled conditions in colonies of *Porites astreoide*s [32]. Additional experiments are needed to determine which chemosensory cues are at the origin of this selection (Lenhoff and Heagy 1977).

Without their symbionts supplying them with nutrients [33], corals thriving within an oligotrophic environment have an urgent need for nitrogen. Our results demonstrate that, unlike in a previous study (Bednarz et al., 2017), bleached corals do not meet this nitrogen requirement through the activity of their endosymbiotic diazotrophs but through nitrogen derived from planktonic diazotrophs and plankton that benefited from N_2 fixation. The amount of nitrogen coming from planktonic diazotrophs and *Synechococcus* for bleached corals, compared to the other nitrogen sources can be estimated. *S. pistillata* is able to take up ammonium and nitrate (at *in situ* concentrations) at a rate of 2 ng cm^{-2} h^{-1} (Grover et al 2002, 2003) and Hoegh-Guldberg and Williamson (1999) also estimated that the uptake of nitrogen in the form of dissolved free amino acids was ca. 60 ng N cm^{-2} h^{-1}. Hence, the maximal amount of total dissolved nitrogen taken up is ca. 0.062 µg N cm^{-2} h^{-1}. We thus estimate that for the bleached corals in our study nitrogen coming from diazotrophic plankton and *Synechococcus* (0.658 µg N cm^{-2} h^{-1}) brings ten times more nitrogen than what corals take up in dissolved nitrogen when they still contain Symbiodiniaceae. This specific feeding also represents a non-negligible source of carbon for corals devoid of Symbiodiniaceae. Studying the fate of nitrogen derived from planktonic diazotrophs within coral holobionts holds great potential to improve our understanding of nutritional interactions driving coral function and resilience in the context of climate change. Benefiting from N_2 fixation could become a common strategy for coral recovery facing bleaching, as both the activity and geographical distribution of diazotrophs will likely increase with future raising sea surface temperature [21, 22].

Supplementary information is available at ISME's website

Conflict of interest

The authors declare no conflict of interest.

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Figure 1: Nitrogen assimilation rates (µg N cm^{-2} h^{-1}) in Symbiodiniaceae (A) and coral tissue (B) in untreated and bleached corals after 12 h of exposure to $^{15}N_2$-enriched natural plankton assemblage (mean ± SD; n = 5 for each treatment). Horizontal line in each boxplot indicates the median and black dots represent the outlier samples. Stars indicate statistically significant differences.

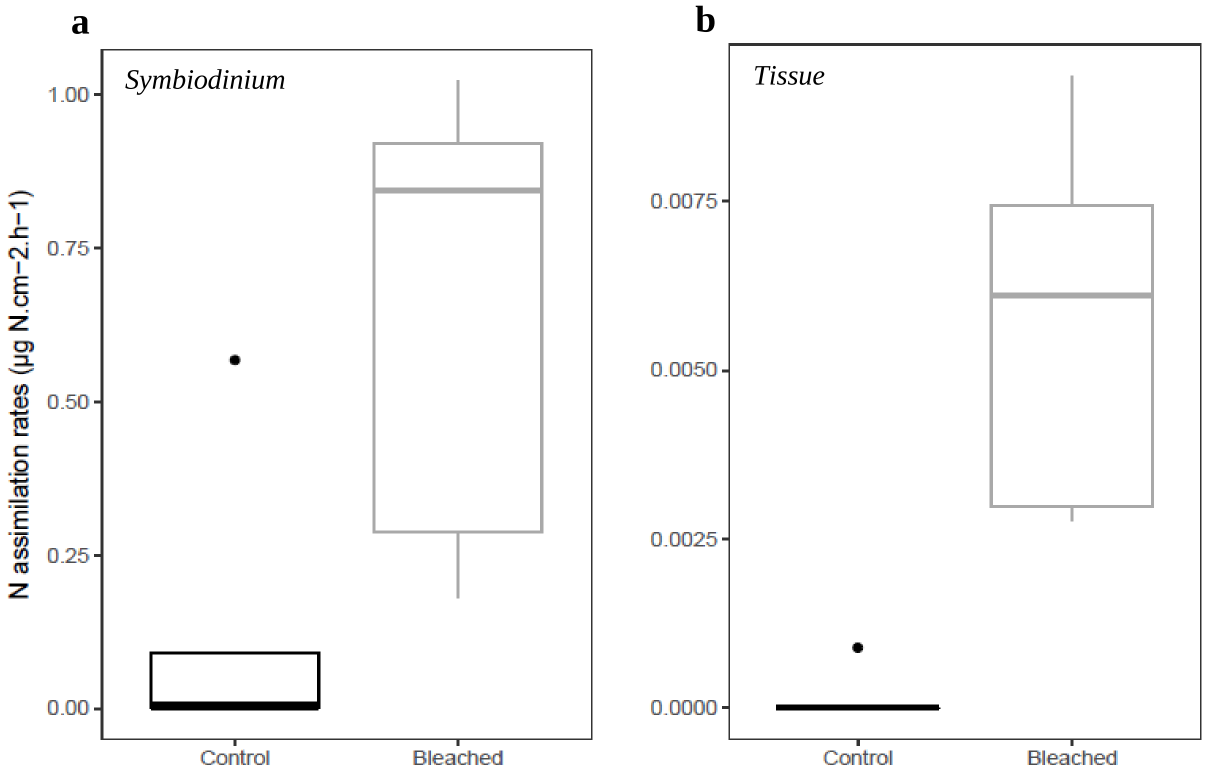
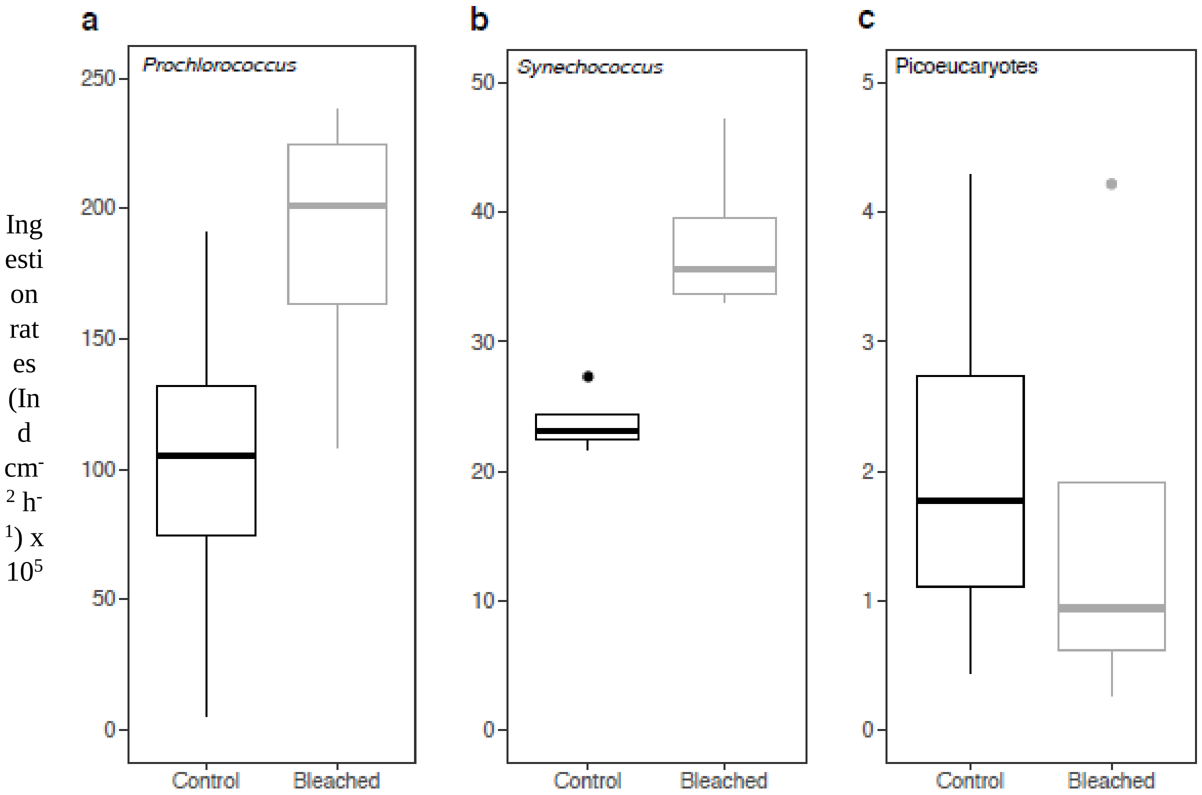


Figure 2: Ingestion rates (cell cm^{-2} h^{-1}) of *Prochlorococcus* (A), *Synechococcus* (B) and picoeukaryotes (C) in untreated and bleached corals (mean ± SD; n = 5 for each treatment). Horizontal line in each boxplot indicates the median and black dots represent the outlier samples. Stars indicate statistically significant differences.



References

1. LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, et al. Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. *Curr Biol* 2018; **28**: 2570–2580.
2. Hoegh-Guldberg O. Climate change, coral bleaching and the future of the world's coral reefs. *Mar Freshw Res* 1999; **50**: 839–866.
3. Godinot C, Houlbrèque F, Grover R, Ferrier-Pagès C. Coral uptake of inorganic phosphorus and nitrogen negatively affected by simultaneous changes in temperature and pH. *PLoS One* 2011; **6**: 1–10.
4. Palardy JE, Rodrigues LJ, Grottoli AG. The importance of zooplankton to the daily metabolic carbon requirements of healthy and bleached corals at two depths. *J Exp Mar Bio Ecol* 2008; **367**: 180–188.
5. Grottoli AG, Rodrigues LJ, Palardy JE. Heterotrophic plasticity and resilience in bleached corals. *Nature* 2006; **440**: 1186–1189.
6. Goldberg WM. Coral Food, Feeding, Nutrition, and Secretion : A Review. 2018.
7. Houlbrèque F, Ferrier-Pagès C. Heterotrophy in tropical scleractinian corals. *Biol Rev* . 2009. , 1–17
8. Tremblay P, Naumann MS, Sikorski S, Grover R, Ferrier-Pagès C. Experimental assessment of organic carbon fluxes in the scleractinian coral Stylophora pistillata during a thermal and photo stress event. *Mar Ecol Prog Ser* 2012; **453**: 63–77.
9. Turk-Kubo KA, Frank IE, Hogan ME, Desnues A, Bonnet S, Zehr JP. Diazotroph community succession during the VAHINE mesocosm experiment (New Caledonia lagoon). *Biogeosciences* 2015.
10. Messer LF, Brown M V., Furnas MJ, Carney RL, McKinnon AD, Seymour JR. Diversity and activity of diazotrophs in great barrier reef surface waters. *Front Microbiol* 2017; **8**: 1–16.
11. Bonnet S, Berthelot H, Turk-Kubo K, Cornet-Barthaux V, Fawcett S, Berman-Frank I, et al. Diazotroph derived nitrogen supports diatom growth in the South West Pacific: A quantitative study using nanoSIMS. *Limnol Oceanogr* 2016.
12. Benavides M, Houlbrèque F, Camps M, Lorrain A, Grosso O, Bonnet S. Diazotrophs: a non-negligible source of nitrogen for the tropical coral Stylophora pistillata. *J Exp Biol* 2016.
13. Montoya JP, Voss M, Kahler P, Capone DG. A Simple , High-Precision , High-

Sensitivity Tracer Assay for N2 Fixation. *Appl Environ Microbiol* 1996; **62**: 986–993.

14. Benavides M, Bednarz VN, Ferrier-Pagès C. Diazotrophs: Overlooked Key Players within the Coral Symbiosis and Tropical Reef Ecosystems? *Front Mar Sci* 2017; **4**.

15. Bednarz VN, Grover R, Maguer J-FF, Fine M, Ferrier-Pagès C, The M, et al. The assimilation of diazotroph-derived nitrogen by scleractinian corals depends on their Metabolic Status. *MBio* 2017; **8**: 1–14.

16. Messer LF, Mahaffey C, Robinson CM, Jeffries TC, Baker KG, Isaksson JB, et al. High levels of heterogeneity in diazotroph diversity and activity within a putative hotspot for marine nitrogen fixation. *ISME J* 2016; **10**: 1499–1513.

17. Bonnet S, Caffin M, Berthelot H, Moutin T. Hot spot of N $_2$ fixation in the western tropical South Pacific pleads for a spatial decoupling between N $_2$ fixation and denitrification. *Proc Natl Acad Sci* 2017; **114**: E2800–E2801.

18. Luo Y-W, Doney SC, Anderson LA, Benavides M, Berman-Frank I, Bode A, et al. Database of diazotrophs in global ocean: abundance, biomass and nitrogen fixation rates. *Earth Syst Sci Data* 2012; **4**: 47–73.

19. Foster RA, Paytan A, Zehr JP. Seasonality of N_2 fixation and *nifH* gene diversity in the Gulf of Aqaba (Red Sea). *Limnol Oceanogr* 2009; **54**: 219–233.

20. Rahav E, Bar-Zeev E, Ohayon S, Elifantz H, Belkin N, Herut B, et al. Dinitrogen fixation in aphotic oxygenated marine environments. *Front Microbiol* 2013; **4**: 1–11.

21. Boyd PW, Doney SC. Modelling regional responses by marine pelagic ecosystems to global climate change. *Geophys Res Lett* 2002; **29**: 53-1-53–4.

22. Breitbarth E, Oschlies A, Laroche J. Physiological constraints on the global distribution of Trichodesmium – effect of temperature on diazotrophy. 2007; 53–61.

23. Pernice M, Meibom A, Den Heuvel A Van, Kopp C, Domart-Coulon I, Hoegh-Guldberg O, et al. A single-cell view of ammonium assimilation in coral-dinoflagellate symbiosis. *ISME J* 2012; **6**: 1314–1324.

24. Krueger T, Bodin J, Horwitz N, Loussert-Fonta C, Sakr A, Escrig S, et al. Temperature and feeding induce tissue level changes in autotrophic and heterotrophic nutrient allocation in the coral symbiosis – A NanoSIMS study. *Sci Rep* 2018; **8**: 12710.

25. Cardini U, van Hoytema N, Bednarz VN, Rix L, Foster RA, Al-Rshaidat MMDD, et al. Microbial dinitrogen fixation in coral holobionts exposed to thermal stress and bleaching. *Environ Microbiol* 2016; **18**: 2620–2633.

26. Bednarz, Vanessa N.Ferrier-Pagès C, Grover R, Bednarz VN, Rabouille S, van de Water JAJM, Maguer J-F. Diazotrophic community and associated dinitrogen fixation

within the temperate coral Oculina patagonica. *Environ Microbiol* 2019; **21**: 480–495.

27. Henke BA, Turk-Kubo KA, Bonnet S, Zehr JP. Distributions and abundances of sublineages of the N2-fixing cyanobacterium Candidatus Atelocyanobacterium thalassa (UCYN-A) in the New Caledonian coral lagoon. *Front Microbiol* 2018; **9**: 1–14.
28. Fanny Houlbrèque, Eric Tambutté, Denis Allemand et Christine Ferrier-Pagèsreque F, Houlbrèque F, Tambutté E, Allemand D, Ferrier-Pagès C, Houlbrèque, Fanny, Eric Tambutté DA and CF-P. Interactions between zooplankton feeding, photosynthesis and skeletal growth in the scleractinian coral Stylophora pistillata. *J Exp Biol Exp Biol* 2004; **207**: 1461–1469.
29. Berthelot H, Bonnet S, Grosso O, Cornet V, Barani A. Transfer of diazotroph-derived nitrogen towards non-diazotrophic planktonic communities: A comparative study between Trichodesmium erythraeum Crocosphaera watsonii and Cyanothece sp. *Biogeosciences* 2016; **13**: 4005–4021.
30. Bertilsson S, Berglund O, Karl DM, Chisholm SW. Elemental composition of marine *Prochlorococcus* and *Synechococcus* : Implications for the ecological stoichiometry of the sea. *Limnol Oceanogr* 2003; **48**: 1721–1731.
31. Jacquet S, Delesalle B, Torréton JP, Blanchot J. Response of phytoplankton communities to increased anthropogenic influences (southwestern lagoon, New Caledonia). *Mar Ecol Prog Ser* 2006; **320**: 65–78.
32. McNally SP, Parsons RJ, Santoro AE, Apprill A. Multifaceted impacts of the stony coral Porites astreoides on picoplankton abundance and community composition. *Limnol Oceanogr* 2017; **62**: 217–234.
33. Muscatine, L CFD. The uptake, retention, and release of ammonium by reef corals. 1978; **23**: 725–734.