








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 tissues 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*, nitrogen-rich picophytoplanktonic cells, instead of *Prochlorococcus* and picoeukaryotes, which have a lower cellular nitrogen content. By providing an alternative source of bioavailable 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.

Introduction

Coral reefs are currently under threat from 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 provide them with nitrogen [3] and seawater warming also decreases coral

nitrogen acquisition capacity [4]. Several studies have reported an increase in the consumption of mesoplankton and macroplankton by corals when exposed to thermal stress, potentially sustaining a critical supply of nutrients needed for recovery following bleaching [5–7]. The ability of corals to feed on smaller planktonic fractions, i.e., picoplankton (0.2–2 μm) and nanoplankton (2–20 μm) has also been documented [8], but the increase in the ingestion of bacteria and picoflagellates by bleached corals has only been observed in one study [9]. Among these size fractions, planktonic dinitrogen (N_2)-fixing prokaryotes (subsequently referred to as planktonic diazotrophs) are very abundant in coral lagoon waters [10, 11]. 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 [12]. The assimilation of nitrogen derived from planktonic diazotrophs has been recently demonstrated in corals [13]. According to [13], ^{15}N -enrichment in corals after their incubation with $^{15}N_2$ -labelled natural diazotrophic assemblages could be due to three different processes: (1) direct feeding on planktonic diazotrophs digested within the coelenteron, (2) uptake of ^{15}N -dissolved nitrogen compounds fixed by the planktonic diazotrophs and released extracellularly, and (3) ingestion of nondiazotrophic plankton enriched in ^{15}N as a result of

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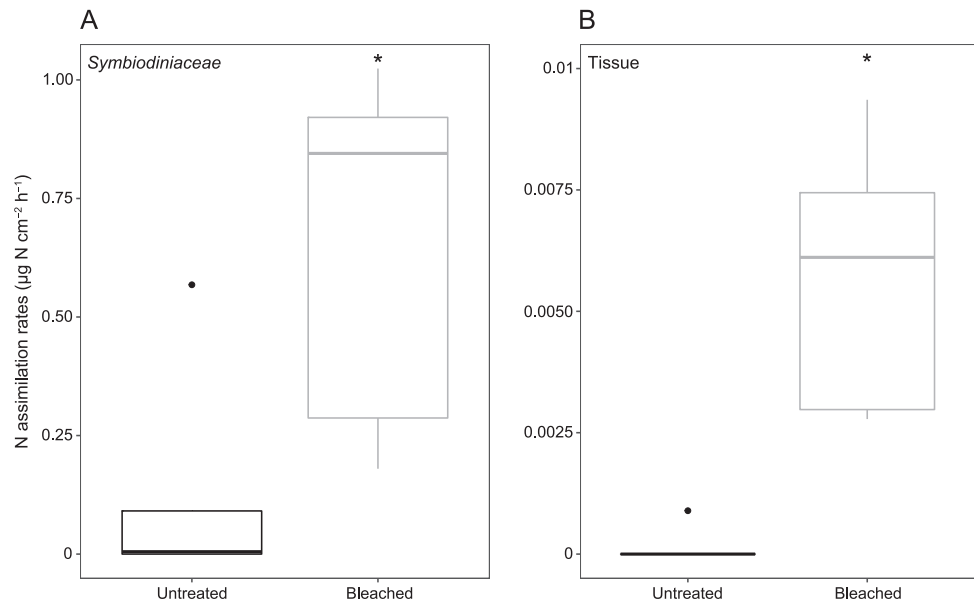
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Fig. 1 Nitrogen assimilation rates ($\mu\text{g N cm}^{-2} \text{h}^{-1}$) in *Symbiodiniaceae* (a) and coral tissue (b) in untreated and bleached corals after 12 h of exposure to $^{15}\text{N}_2$ -enriched natural plankton assemblage (mean \pm SD; $n = 5$ for each treatment). Horizontal line in each boxplot indicates the median and black dots represent the outlier samples. Asterisks indicate statistically significant differences



diazotroph-derived nitrogen transfer [14]. While some studies have demonstrated that N_2 fixation by coral symbiotic diazotroph communities increases in bleached corals [15, 16], the acquisition of nitrogen derived from planktonic diazotrophic activity has never been investigated in corals facing thermal stress.

Materials & Methods

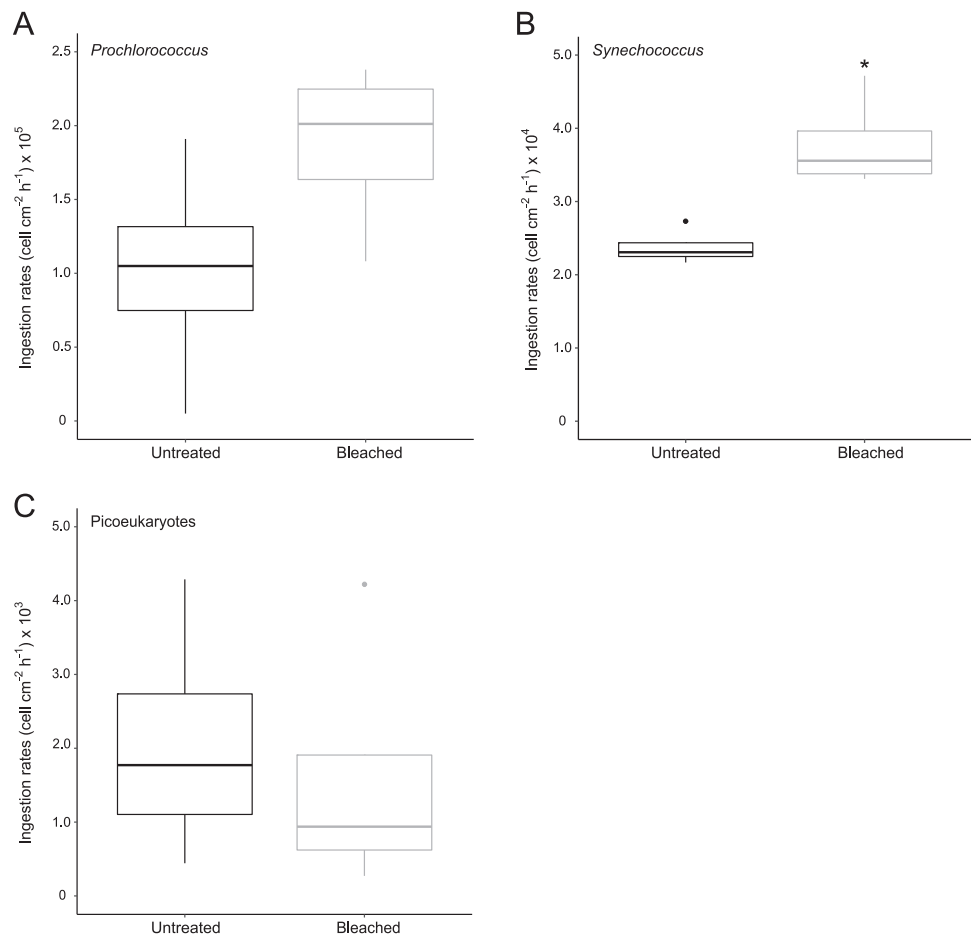
To determine if bleached corals also benefit from planktonic diazotrophs, we incubated five colonies of the branching coral *S. pistillata* with a $^{15}\text{N}_2$ -pre-labelled (24 h) natural plankton assemblage containing planktonic diazotrophs (prefiltered through a 100 μm mesh to exclude larger cells) as described in [13]. In parallel, N_2 fixation within endosymbiotic diazotrophs in colonies of the same species was measured by incubating five untreated and five bleached colonies in ^{15}N -enriched filtered seawater. Coral colonies collected in the New Caledonian lagoon were acclimated to experimental conditions for 3 weeks. They were progressively bleached over 18 days (by a gradual temperature increase up to 31 $^{\circ}\text{C}$) or left at ambient temperature (28 $^{\circ}\text{C}$) as a control (subsequently referred to as untreated corals, see supplementary information for details, Supplementary Fig. S1). The $\delta^{15}\text{N}$ isotopic values were measured in symbionts, coral tissues, and plankton before and after incubation (12 h). Nitrogen assimilation rates were calculated as previously described [17]. The contribution of endosymbiotic N_2 fixation was minor (see results in the supplementary information). Conversely, after the incubation with $^{15}\text{N}_2$ -labelled natural planktonic assemblage significant ^{15}N -enrichments

were measured in the *Symbiodiniaceae* of both untreated and bleached corals

Results & Discussion

The results suggests that *Symbiodiniaceae* used nitrogen originating from the planktonic diazotrophs [13, 15, 18]. Nitrogen assimilation rates in *Symbiodiniaceae* and tissue from bleached corals increased by 5-fold ($0.6512 \pm 0.3890 \mu\text{g N cm}^{-2} \text{h}^{-1}$; $n = 5$; Mann–Whitney–Wilcoxon test, $P < 0.05$) and 30-fold ($0.0057 \pm 0.0028 \mu\text{g N cm}^{-2} \text{h}^{-1}$; $n = 5$; Mann–Whitney–Wilcoxon test, $P < 0.01$), respectively, compared to those measured in the untreated corals (0.1330 ± 0.2465 and $0.0002 \pm 0.0004 \mu\text{g N cm}^{-2} \text{h}^{-1}$) (Fig. 1, Supplementary Table 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 bioavailable nitrogen, this increased incorporation of nitrogen derived from planktonic diazotrophs may have profound consequences for coral bleaching recovery, particularly in coral reef ecosystems characterized by high planktonic diazotroph abundance and activity. These reefs are very widespread in the Western South Pacific (e.g., New Caledonia, Papua New Guinea, and Australian Great Barrier Reef) [10, 11, 19, 20], but also in Hawaii, in the Caribbean and the Red Sea [21–23]. 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

Fig. 2 Ingestion rates ($\text{cell cm}^{-2} \text{h}^{-1}$) of *Prochlorococcus* (**a**), *Synechococcus* (**b**), and picoeukaryotes (**c**) in untreated and bleached corals (mean \pm SD; $n = 5$ for each treatment). Horizontal line in each boxplot indicates the median and black dots represent the outlier samples. The asterisk indicates statistically significant differences



several authors (e.g., [24], [13], [25], [15, 26], [16]) 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 assays to determine planktonic diazotroph abundances (UCYN-A1, UCYN-C, and *Trichodesmium*, i.e., the most important phylotypes in the lagoon [10, 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 (1) a significant abundance of diazotrophs in the incubation medium at the beginning of the experiment (UCYN-A1, UCYN-C, and *Trichodesmium* abundances were, respectively, $4.14 \pm 5.35 \cdot 10^2$, $0.97 \pm 1.26 \cdot 10^1$, and $8.63 \pm 6.03 \cdot 10^2$ *nifH* gene copies L^{-1}), and (2) 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 (Supplementary Table 2). While UCYN-A1 are $\sim 1 \mu\text{m}$ in size, their association with a picoeukaryote host [28] could increase their size to 7–10 μm and thus improve their chances of being consumed by corals. Picoeukaryotes, nanoeukaryotes, 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 confirming the ability of corals to feed on picoplankton [e.g., [9, 29; Supplementary Table 3]. One of the most notable results of this study is that the ingestion rates of *Synechococcus* were 1.6 times higher in bleached corals ($3.79 \pm 0.64 \cdot 10^4$ $\text{cell cm}^{-2} \text{h}^{-1}$) than in untreated corals ($2.38 \pm 0.24 \cdot 10^4$ $\text{cell cm}^{-2} \text{h}^{-1}$, Mann–Whitney–Wilcoxon test, $P = 0.028$; Fig. 2). Until now, studies have shown that corals can regulate their heterotrophic feeding capacities on zooplankton ($>50 \mu\text{m}$) [6] and on picoflagellates and bacteria [9] 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, but also more specifically their ingestion of a very specific taxonomic group of picoplankton: the ubiquitous marine cyanobacterium *Synechococcus*. 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 [30, 31; Supplementary Table 4] and also to benefit from nitrogen released by surrounding diazotrophs in the natural environment [12, 32]. So far, this type of selective feeding on *Synechococcus* cells has only been shown under controlled conditions in colonies of *Porites astreoides* [33]. Additional experiments are needed to determine which chemosensory cues are at the origin of this selection [34].

Without their symbionts supplying them with nutrients [3], corals thriving within an oligotrophic environment have an urgent need for nitrogen. Our results demonstrate that, unlike in a previous study [15], bleached corals do not meet this nitrogen requirement through the activity of their endosymbiotic diazotrophs but through an external source coming 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 (Supplementary Tables 4 and 5). *S. pistillata* is able to take up inorganic nitrogen (ammonium and nitrate at *in situ* concentrations) at a rate of $2 \text{ ng cm}^{-2} \text{ h}^{-1}$ [35–37] and also estimated that the uptake of organic nitrogen in the form of dissolved free amino acids was ca. $60 \text{ ng N cm}^{-2} \text{ h}^{-1}$ leading to a maximal uptake of total dissolved nitrogen of ca. $0.062 \mu\text{g N cm}^{-2} \text{ h}^{-1}$. In our study we estimate that for the bleached corals, nitrogen coming from diazotrophic plankton and *Synechococcus* ($0.658 \mu\text{g N cm}^{-2} \text{ h}^{-1}$) brings ten times more nitrogen than what corals take up in the dissolved nitrogen pool when they still contain *Symbiodiniaceae*. This specific feeding also represents a non-negligible source of carbon for corals devoid of *Symbiodiniaceae* (Supplementary Tables 4 and 5). 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 rising sea surface temperature [38, 39].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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