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► **To cite this version:**

Valentine Meunier, Sophie Bonnet, Mathieu Pernice, Mar Benavides, Anne Lorrain, et al.. Bleaching forces coral's heterotrophy on diazotrophs and Synechococcus. *The International Society of Microbiological Ecology Journal*, 2019, 13 (11), pp.2882-2886. 10.1038/s41396-019-0456-2 . hal-02458908

HAL Id: hal-02458908

<https://hal.science/hal-02458908v1>

Submitted on 29 Jan 2020

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

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Received: 9 December 2018 / Revised: 20 May 2019 / Accepted: 2 June 2019
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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/materials & methods/results and discussion

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

Supplementary information The online version of this article (<https://doi.org/10.1038/s41396-019-0456-2>) contains supplementary material, which is available to authorized users.

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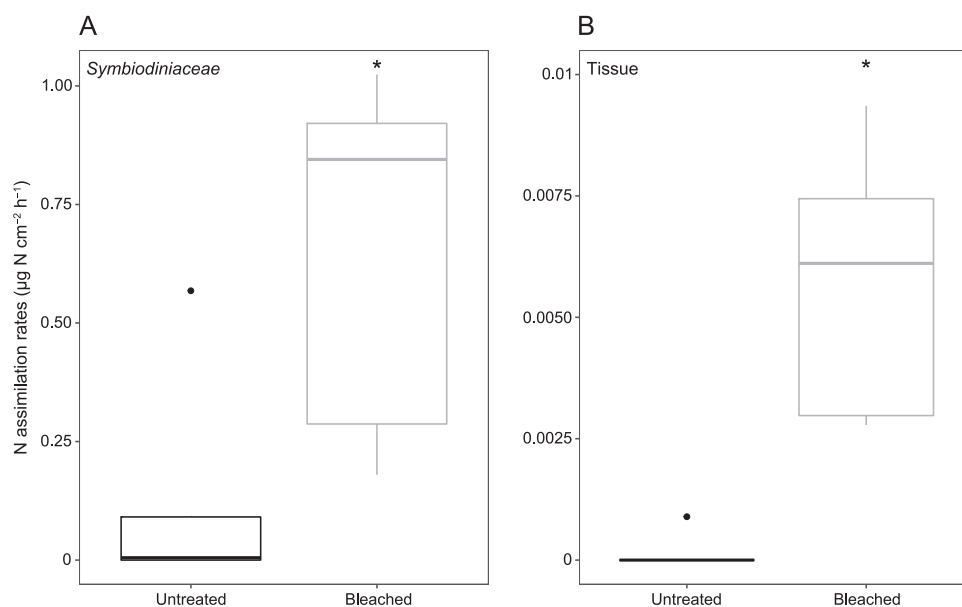
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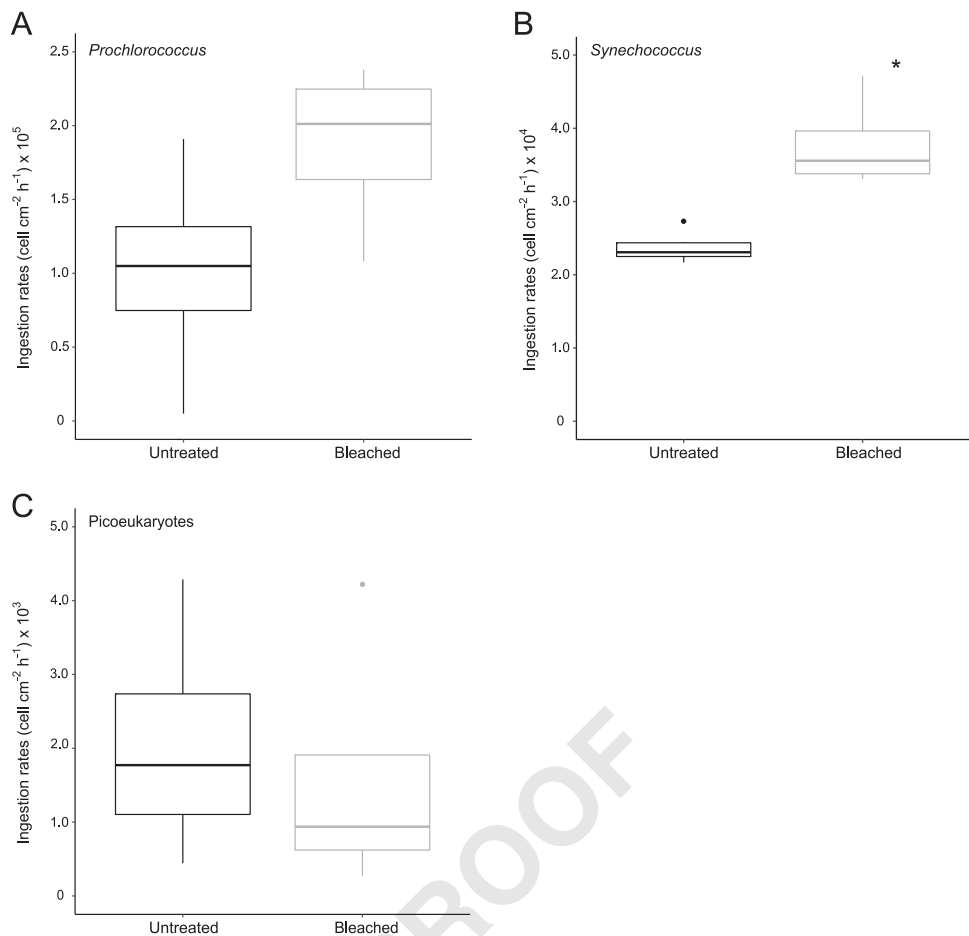
Fig. 1 Nitrogen assimilation rates ($\mu\text{g N cm}^{-2} \text{h}^{-1}$) in *Symbiodiniaceae* (a) and coral tissue (b) in untreated and bleached corals after 12 h of exposure to $^{15}\text{N}_2$ -enriched natural plankton assemblage (mean \pm SD; $n = 5$ for each treatment). Horizontal line in each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



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 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. 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 $100\ \mu\text{m}$ 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 $31\ \text{°C}$) or left at ambient temperature ($28\ \text{°C}$) 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. This suggests that *Symbiodiniaceae* used nitrogen originating from the planktonic

diazotrophs [13, 15, 18]. Nitrogen assimilation rates in *Symbiodiniaceae* 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 ($0.0057 \pm 0.0028\ \mu\text{g}^{-2} \text{h}^{-1}$; $n = 5$; 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 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

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. Asterisks indicate statistically significant differences



114 abundance of diazotrophs in the incubation medium at the
 115 beginning of the experiment (UCYN-A1, UCYN-C, and *Tri-*
 116 *chodesmium* abundances were, respectively, $4.14 \pm 5.35 \cdot 10^2$,
 117 $0.97 \pm 1.26 \cdot 10^1$, and $8.63 \pm 6.03 \cdot 10^2$ *nifH* gene copies L^{-1}),
 118 and (2) a decrease in the abundance of UCYN-A1 ($1 \mu\text{m}$) and
 119 UCYN-C ($4\text{--}8 \mu\text{m}$) in all tanks containing corals ($n = 3$)
 120 compared to the controls without corals, confirming that corals
 121 fed on these two types of preys (Supplementary Table 2).
 122 While UCYN-A1 are $\sim 1 \mu\text{m}$ in size, their association with a
 123 picoeukaryote host [28] could increase their size to $7\text{--}10 \mu\text{m}$
 124 and thus improve their chances of being consumed by corals.
 125 Picoeukaryotes, nanoeukaryotes, and bacterial abundances
 126 were further assessed by flow cytometry at the start and end of
 127 incubations to quantify their ingestion by both bleached and
 128 untreated corals. During the 12 h of incubation, *Pro-*
 129 *chlorococcus* was quantitatively the major prey ingested, fol-
 130 lowed by *Synechococcus* and picoeukaryotes in both
 131 treatments confirming the ability of corals to feed on pico-
 132 plankton [e.g., [9, 29]; Supplementary Table 3]. One of the
 133 most notable results of this study is that the ingestion rates of
 134 *Synechococcus* were 1.6 times higher in bleached corals
 135 ($3.79 \pm 0.64 \cdot 10^4$ $\text{cell cm}^{-2} \text{h}^{-1}$) than in untreated corals ($2.38 \pm$
 136 $0.24 \cdot 10^4$ $\text{cell cm}^{-2} \text{h}^{-1}$, 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 *Syne-*
chococcus 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 nat-
 ural 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

160 this nitrogen requirement through the activity of their
 161 endosymbiotic diazotrophs but through an external source
 162 coming from planktonic diazotrophs and plankton that
 163 benefited from N₂ fixation. The amount of nitrogen coming
 164 from planktonic diazotrophs and *Synechococcus* for
 165 bleached corals, compared to the other nitrogen sources can
 166 be estimated (Supplementary Tables 4 and 5). *S. pistillata* is
 167 able to take up inorganic nitrogen (ammonium and nitrate at
 168 in situ concentrations) at a rate of 2 ng cm⁻² h⁻¹ [35–37]
 169 and also estimated that the uptake of organic nitrogen
 170 in the form of dissolved free amino acids was ca. 60 ng
 171 N cm⁻² h⁻¹ leading to a maximal uptake of total dissolved
 172 nitrogen of ca. 0.062 μg N cm⁻² h⁻¹. In our study we esti-
 173 mate that for the bleached corals, nitrogen coming
 174 from diazotrophic plankton and *Synechococcus* (0.658 μg
 175 N cm⁻² h⁻¹) brings ten times more nitrogen than what
 176 corals take up in the dissolved nitrogen pool when they still
 177 contain *Symbiodiniaceae*. This specific feeding also repre-
 178 sents a nonnegligible source of carbon for corals devoid of
 179 *Symbiodiniaceae* (Supplementary Tables 4 and 5). Studying
 180 the fate of nitrogen derived from planktonic diazotrophs
 181 within coral holobionts holds great potential to improve our
 182 understanding of nutritional interactions driving coral
 183 function and resilience in the context of climate change.
 184 Benefiting from N₂ fixation could become a common
 185 strategy for coral recovery facing bleaching, as both the
 186 activity and geographical distribution of diazotrophs will
 187 likely increase with future rising sea surface temperature
 188 [38, 39].

189 **Acknowledgements** VM was the beneficiary of a PhD grant from
 190 LabEx-Coraal (MACADAM project). This work was also funded by
 191 the LabEx-Coraal FLAMENCO project and the EC2CO/BIOHEFECT
 192 program (TOUCAN project). We wish to thank the technical staff of
 193 the Aquarium des Lagons (Nouméa, New Caledonia) for their wel-
 194 come and assistance in tank maintenance. We are especially grateful to
 195 three anonymous reviewers for critical reading and valuable comments
 196 on this manuscript.

197 Compliance with ethical standards

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

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 201 jurisdictional claims in published maps and institutional affiliations.

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