The ISME Journal (2014) 8, 2272–2279
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www.nature.com/ismej

ORIGINAL ARTICLE

Climate change affects key nitrogen-fixing bacterial populations on coral reefs

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Coral reefs are at serious risk due to events associated with global climate change. Elevated ocean temperatures have unpredictable consequences for the ocean's biogeochemical cycles. The nitrogen cycle is driven by complex microbial transformations, including nitrogen fixation. This study investigated the effects of increased seawater temperature on bacteria able to fix nitrogen (diazotrophs) that live in association with the mussid coral *Mussismilia harttii*. Consistent increases in diazotroph abundances and diversities were found at increased temperatures. Moreover, gradual shifts in the dominance of particular diazotroph populations occurred as temperature increased, indicating a potential future scenario of climate change. The temperature-sensitive diazotrophs may provide useful bioindicators of the effects of thermal stress on coral reef health, allowing the impact of thermal anomalies to be monitored. In addition, our findings support the development of research on different strategies to improve the fitness of corals during events of thermal stress, such as augmentation with specific diazotrophs.

The ISME Journal (2014) **8,** 2272–2279; doi:10.1038/ismej.2014.70; published online 16 May 2014
Subject Category: Microbial ecosystem impacts
Keywords: climate change; coral reef; nitrogen cycle; *Mussismilia harttii*; diazotrophs

Introduction

Coral reefs constitute ecosystems that harbor a myriad of interwoven species. These ecosystems have key roles in the maintenance of marine biodiversity. Although coral reefs account for only 0.1% of the total ocean area, they host 25% of all marine macro-organisms that live in the oceans (Burke *et al.*, 2011). Furthermore, they have an important role in the biogeochemical cycles of carbon, nitrogen and phosphorus that occur in the sea (Wild *et al.*, 2005). Coral reefs are being threatened by direct anthropogenic impacts as well as by the effects of global climate change, which may result in a reduction in the pH of seawater and rising water temperatures (Hoegh-Guldberg *et al.*, 2007; Hoegh-Guldberg and Bruno, 2010; Burke *et al.*, 2011). Such stresses have already been linked to disease outbreaks in coral reef ecosystems (Sutherland *et al.*, 2004; Selig *et al.*, 2006).

Corals live in symbiotic relationships with a plethora of organisms, including endosymbiotic dinoflagellate algae (zooxanthellae), bacteria, archaea and fungi (Falkowski *et al.*, 1984; Rohwer *et al.*, 2002; Knowlton and Rohwer, 2003; Reshef *et al.*, 2006). The whole association has been described as a holobiont (Rosenberg *et al.*, 2007). Intricate relationships may exist between members of the microbial community that occur in the coral holobiont. An example is the production of dimethylsulfoniopropionate by symbiotic zooxanthellae (Keller *et al.*, 1989), which has been implicated in the prevention of deleterious bacterial colonization of coral. The presence of dimethylsulfoniopropionate and related sulfur compounds may provide a selective environment in which the survival of microbes is restricted (Barott and Rohwer, 2012). Dimethylsulfoniopropionate is a precursor of the volatile organic sulfur compound dimethyl sulfide, which may have a role in the formation of clouds and thus acts as a climate-cooling gas (Charlson *et al.*, 1987). On the other hand, the growth and abundance of zooxanthellae is limited by the availability of nitrogen (Falkowski

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Received 20 November 2013; revised 17 March 2014; accepted 23 March 2014; published online 16 May 2014

et al., 1993). In nitrogen-limited coral systems, the abundance of zooxanthellae may depend on the activity of diazotrophic bacteria (Lesser et al., 2007; Olson et al., 2009; Lema et al., 2012).

Recent studies have shown that increases in seawater temperature can cause shifts in the coral-associated microbial communities (for example, Bourne et al., 2008). However, the effects of temperature increase on the diazotrophic bacterial populations of corals, and the effect of this on coral health, remains unclear. The current study aimed to foster our understanding of the effect of increases in seawater temperature on the abundance and diversity of diazotrophic microbial populations associated with the coral *Mussismilia harttii.* In the present study, a comparison was made between mesocosms held at elevated temperatures and mesocosms maintained at ambient temperatures. Analysis of *nifH* genes, which encode the key dinitrogen reductase enzyme, was used to evaluate the abundance, community structure, species richness and diversity of the diazotrophic communities in individual coral specimens from mesocosms maintained at different temperatures. An enhanced understanding of coral-associated nitrogen-fixing bacterial communities on coral reefs contributes to our understanding of the resilience of coral reefs and the possible changes in the marine nitrogen cycle in the face of climate change. In addition, it aids in the establishment of potential management guidelines for future environmental protection programs.

Materials and methods

Sample collection and experimental design

M. harttii specimens were collected in 'Recife de Fora', a reef located ~3.2 km offshore near the city of Porto Seguro, Bahia, Brazil (between latitude 16°23′30″S and 16°25′06″S and longitude 38°58′30″W and 38°59′18″W). The reef area is ~17.5 km^2, and the water depth surrounding the reef is ~20 m. Seawater was sampled at ambient temperature (~27 °C). Annual temperature fluctuation ranged between 24.06 °C (min) and 29.00 °C (max), and the average water temperature was 26.74 °C (±1.01). The *M. harttii* colonies had polyps, which showed loose tissue connection with adjacent polyps. Therefore, single- or double-polyp structures were used as sampling units without harming their tissues. Physiological experiments were conducted in mesocosms established at an onshore research station, near the reef area where the samples had been collected.

Sixteen mesocosm tanks (130 l each) were supplied with seawater that was captured continuously from a fringe reef near the research station. The seawater supply rate was 8.6 l min^{-1} for each tank, providing a fourfold replacement of the mesocosm volume every hour to mimic the daily and seasonal variations that were naturally observed in the reef. The mesocosm tanks received only natural sunlight and therefore followed natural day/night cycles. To mimic the amount of incident light in the reefs, the tanks were covered with a 70% shade screen, resulting in 350 μmol photons m^{-2} s^{-1} at noon, which is consistent with the average parameters measured *in situ* at 2.5 m depth in the 'Recife de Fora' reef.

Five different polyps from four different coral outcrops all belonging to the same species, *M. harttii* (=*M. harttii* 20 polyps), were collected in the reef area of 'Recife de Fora', Brazil. A total of five treatments were evaluated (that is, pretreatment, control, +1.0, +2.0 and +4.5 °C). One polyp from each coral outcrop was used in each of the different treatments, thus generating four replicates per treatment. The pretreatment samples were frozen at −20 °C immediately after sampling. Polyps subjected to the other treatments were transferred to the experimental tanks (<12 h). Polyps in tanks were kept at natural seawater temperature for acclimation for 15 days. After this period, the tanks were subjected to the temperature treatments for a period of 21 days, as follows: ambient water temperature (tank control) and three different elevated water temperatures (+1.0, +2.0 and +4.5 °C) above the ambient temperature. One polyp from each of the four coral outcrops was used in each of the five different treatments, thus generating four replicates per treatment.

For quantitative PCR (qPCR), denaturing gradient gel electrophoresis (DGGE) analyses, and evaluation for maximum quantum yield of the Photosystem II (Fv/Fm), all treatments were sampled. For clone library analyses, samples from the following treatments were analyzed: pretreatment, control, +2.0 and +4.5 °C.

Photosystem II photochemical efficiency: pulse amplitude modulation fluorometry

Samples were evaluated for maximum quantum yield of the Photosystem II (Fv/Fm) using a submersible pulse-amplitude-modulated chlorophyll fluorometer (Diving-PAM, Walz, Effeltrich, Germany) as a proxy for the 'health' of the coral holobiont (Schreiber, 2004). The measurements were taken after sunset in order to avoid interference by diurnal photo-inhibition artifacts, after full recovery of the reaction centers. The results were analyzed using a nested analysis of variance with Statistica 7 software (StatSoft, Tulsa, OK, USA), considering the data from four replicate tanks per treatment and three polyps from three different areas of the reef per tank.

Community DNA extraction

To assess the diazotroph communities in *M. harttii*, 0.5 g of dried material from each coral sample was macerated using a mortar and pestle. Total community DNA extraction was performed using the ZR Soil Microbe DNA Kit (Zymo Research, Irvine, CA, USA) (Santos et al., 2012). The DNA quality and

concentration was evaluated using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). The amount, average molecular weight and quality of the DNA were further assessed using conventional electrophoresis in 0.8% agarose gels with 0.56x TBE buffer (45 mM Tris–Borate, 1 mM EDTA, pH 8.0).

Measurement of nifH and 16S ribosomal RNA (rRNA) gene abundances by qPCR

The abundance of the *nifH* genes (used as a proxy for the abundance of nitrogen fixers) and 16S rRNA genes (for total bacteria) was measured by qPCR. The reactions were performed in an ABI Prism 7300 Cycler (Applied Biosystems, Darmstadt, Germany) in 25 μl reactions containing 1 μl of DNA template (ca. 20 ng). For the *nifH* gene, Power SYBR green master mix was used (12.5 μl) (Applied Biosystems), and 0.25 μM each of primer FGPH19 (Simonet *et al*., 1991) and primer PolR (Poly *et al*., 2001). The amplification reactions were performed with an initial denaturation step at 95 °C for 15 min, followed by 30 cycles of 94 °C (1 min), 55 °C (27 s) and 72 °C (1 min). For 16S rRNA gene, Power SYBR green master mix was used (12.5 μl) (Applied Biosystems), supplemented with 0.5 μl 20 mg ml^{-1} of BSA and 0.25 μM of each primer (FP16S/RP16S) (Bach *et al*., 2002). The cycling protocol was 95 °C for 10 min, followed by 40 cycles of 95 °C (27 s), 62 °C (1 min) and 72 °C (30 s). The specificity of the amplifications was confirmed by melting curve analysis. Possible inhibitory effects of co-extracted compounds were assessed by spiking samples with a range of known concentrations of the plasmid. No inhibition was observed in any of the samples. Standard curves covering 6 orders of magnitude, that is, from 10^2 to 10^7 copies of template per assay, were generated using plasmids containing cloned *nifH* from *Bradyrhizobium liaoningense* and a partial 16S rRNA gene from *Serratia plymuthica*. The qPCR efficiency (E) was calculated according to the equation $E = [10^{(-1/\text{slope})} - 1]$.

Analysis of the structure of diazotrophic communities by PCR-DGGE

The total DNA extracted from all coral samples was subjected to amplification using *nifH*-specific primers, as described in the literature (Simonet *et al*., 1991; Poly *et al*., 2001) for DGGE analysis of *nifH* genes (Pereira e Silva *et al*., 2011). The amplicons obtained were checked on agarose gels before DGGE analysis. DGGE was performed in an Ingeny PhorU2 system (Ingeny, Goes, The Netherlands) using a 40–65% denaturing gradient (where 100% denaturant consisted of 7 M urea and 40% formamide) and 6.0% polyacrylamide. Electrophoresis was carried out at 60 °C at 100 V for 16 h. After electrophoresis, gels were stained with SYBR Gold at a final concentration of 0.5 mg l^{-1} (Invitrogen, Breda, The Netherlands) and photographed on a UV transilluminator. Images of the gels were obtained with Image Master VDS (Amersham Biosciences, Buckinghamshire, UK) and stored as TIFF files. DGGE patterns were compared by clustering the different lanes by Pearson's correlation implemented in GelCompar II software (Applied Maths, Sint-Martens-Latem, Belgium) using the unweighted-pair group method with arithmetic means.

Construction and analysis of nifH gene clone libraries

The DNA extracted from each sample was used to construct *nifH* gene clone libraries. One clone library was constructed for each subregion of sampling points, given the high similarity of replicates in DGGE analysis. The template DNA was subjected to amplification with the *nifH* gene primers FGPH19 (Simonet *et al*., 1991) and PolR (Poly *et al*., 2001), according to previously described methodology (Taketani *et al*., 2009). The amplicons were purified using the Wizard SV gel and PCR clean-up system (Promega, Madison, WI, USA) and cloned in plasmid pGEM-T using the pGEM-T Easy Vector system kit (Promega) according to the manufacturer's instructions. Colonies that exhibited vector inserts (white) were selected for insert detection by amplification with the M13F and M13R primers. The resulting PCR products with the inserts were sequenced at AGOWA (Berlin, Germany). Prior to sequence analyses, all chromatograms were trimmed for quality and vector removal using the Lucy algorithm (Chou and Holmes, 2001) using a threshold base quality score >20 and sequence length >100 bp. The obtained filtered sequences were aligned and inspected using MEGA 4.0 (Tempe, AZ, USA) (Tamura *et al*., 2007). Operational taxonomic unit (OTU)-based analyses were carried out using QIIME (Boulder, CO, USA) (Caporaso *et al*., 2010) by adding artificial barcodes/primer at 5′ pre-trimmed sequences. The samples in the OTU table were rarefied to the same depth (considering the lowest number of sequences in a single sample) to remove the effect of sampling effort upon analysis. Alpha-diversity measurements (that is, OTU richness (unique OTUs), and ChaoI richness and Shannon diversity indices) were determined at cutoff values of 95%, 97%, 98% and 99% of nucleotide identity. A phylogenetic tree was inferred using muscle for sequence alignment, and FastTree (http://www.microbesonline.org/fasttree/). This generated a phylogenetic tree containing representative sequences of each OTU across all samples. Non-metric multidimensional scaling plots were generated based on Bray–Curtis similarities calculated between different samples using the OTU table with OTUs clustered at 98% nucleotide identity, using PRIMER software (Clarke and Gorley, 2006).

Nucleotide sequence accession numbers

The sequences reported in this study were deposited in GenBank under the accession numbers KF656791–KF657302.

Results

This study evaluated the effects of realistic ocean water temperature increases on the photosynthetic quantum yield of the coral *M. harttii* in relation to the abundance and diversity of prokaryotic diazotrophs associated with this coral. The coral was exposed to water temperatures of +1, +2 and +4.5 °C above the ambient (baseline) temperature. Measurements were taken at three time points: (1) pretreatment (ambient); (2) after a 15-day acclimation period; and (3) 21 days after the onset of exposure. The maximum quantum yield of Photosystem II (Fv/Fm) after 21 days was not significantly different among replicates. However, differences between treatments were highly significant (analysis of variance df = 3, $F = 21\,543$, $P < 0.000001$). The Fv/Fm values declined at higher temperatures, and hence coral zooxanthella 'health' was negatively affected by higher temperature (Figure 1). Visual analysis of coral color and structure, according to the international Reef Check guidelines (and supported by pulse amplitude modulation fluorometry results), revealed that coral health indeed decreased significantly following exposure to a temperature increase of just +2 °C, and this effect increased at higher temperatures. In addition, the relative abundances of diazotrophs in the bacterial communities in the coral increased significantly ($P < 0.05$) in the +2 and +4.5 °C treatments when compared with those in the control (Figure 2). A peak in diazotroph abundance was observed in the +2 °C treatment followed by the +4.5 °C treatment. Specifically, the +2 and +4.5 °C treatments showed about 7000 and 3500 *nifH* genes per g of coral, respectively, whereas the control showed 260 *nifH* genes per g of coral. This represented around 27- and 13-fold increases in the +2 and +4.5 °C treatments over the control, respectively. By contrast, 16S rRNA gene abundance showed increases of around five and twofold in the +2 and +4.5 °C treatments, respectively.

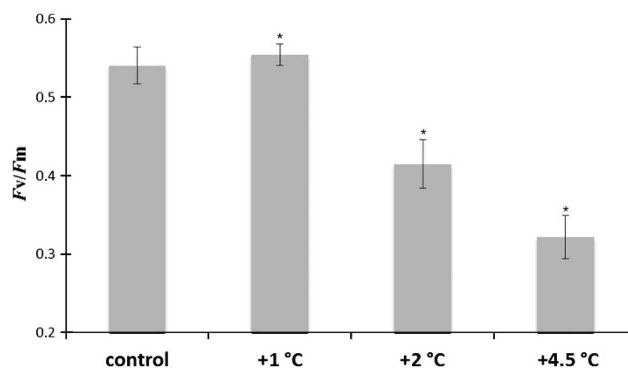

Figure 1 Dark-adapted Fv/Fm in *Mussismilia harttii* measured using a Diving-PAM chlorophyll fluorometer. *Three different elevated water temperatures (+1.0, +2.0 and +4.5 °C) above the ambient temperature.

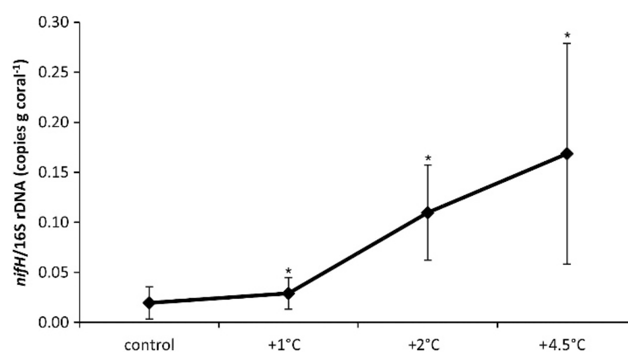

Figure 2 Relative abundance of *nifH* versus bacterial 16S rRNA gene (*nifH*/16S rRNA) copies per ng DNA determined by qPCR. R^2 (of standard curve) > 0.99. The qPCR efficiency was about 99.0%. Values: means from three replicates ($n = 3$) ± standard error of the mean. *Three different elevated water temperatures (+1.0, +2.0 and +4.5 °C) above the ambient temperature.

PCR-DGGE analysis of *nifH* genes confirmed that increased seawater temperature had an impact on the coral holobiont *nifH* gene complement. A clear tendency was found toward the formation of a single main *nifH* gene cluster in the samples with increased temperatures (Figure 3). This main cluster encompassed the *nifH* gene types found in most samples from the +1, +2 and +4.5 °C treatments, all of which clustered together within ~72% similarity. The communities from three higher-temperature samples (+1 °C IV, +2 °C I and +2 °C III) also clustered closely with this group, at ~64% of similarity. The highest similarity values were found between the communities subjected to the largest temperature increase (+2 and +4.5 °C), with an overall similarity >82%. The pretreatment and control samples did not associate closely with any of the clusters recovered from the higher-temperature treatments. Within the increased-temperature (+1, +2 and +4.5 °C) treatments, the differences between the replicates were remarkably reduced when compared with the differences between replicates from both the pretreatment and control samples. Among the increased-temperature treatments, the *nifH* gene profiles from the +1 °C treatment were most similar to those from the pretreatment and control samples.

Comparison of the diazotroph communities revealed that the pretreatment and control samples had similar OTU clusters, whereas those that had been subjected to the highest temperatures (+2 and +4.5 °C) were most distant from these (Figure 4). The diversity and richness indices were significantly higher in the higher-temperature treatments (+2 and +4.5 °C) than in pretreatment and control samples (Table 1). Specifically, these diversity and richness indices were approximately threefold higher in the increased-temperature treatments at all cutoff values of nucleotide similarity that were used in the analyses.

Taxonomic analysis of the *nifH* sequences showed dominance of different diazotroph groups across the treatments. In both pretreatment and control

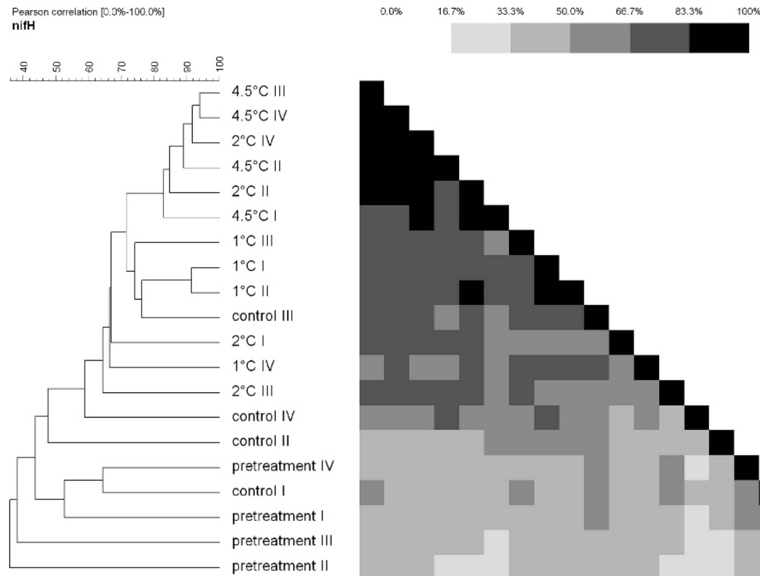

Figure 3 Dendrogram of PCR-DGGE profiles for the *nifH* gene created using UPGMA based on similarities calculated by densitometry.

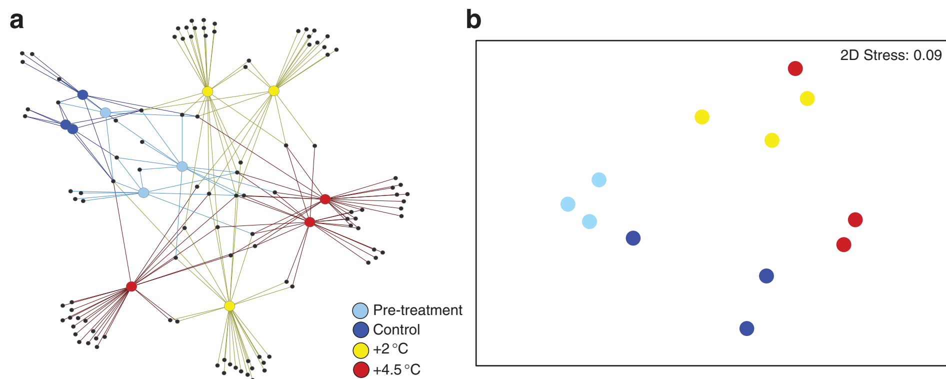

Figure 4 (**a**) Network of shared OTUs among treatment sequences, clustered at 98% cutoff (nucleotide identity). (**b**) NMDS based on Bray–Curtis similarity matrix among the 12 samples from the mesocosms.

samples, *nifH* genes were mostly affiliated with class *Gammaproteobacteria*, with *nifH* genes from class *Alphaproteobacteria* representing the second most abundant group (Figure 5). By contrast, the phylum cyanobacteria was poorly represented in these communities (1 and 2 OTUs, respectively). However, the *nifH* genes recovered from samples taken from the higher-temperature treatments showed a considerable increase in the number of *nifH* sequence types. Specifically, the *nifH* genes from *Alphaproteobacteria* were more highly represented. At lower taxonomic levels, strong increases in *Azospirillum*-, *Rhizobium*- and *Rhodobacter*-like sequences were found. Strikingly, the relative abundance of *Azospirillum*-like sequences increased 8- and 21-fold in the +2 and +4.5 °C treatments, respectively. Also, increases of 11- and 11.5-fold were found for typical *Rhizobium*-like sequences. Conversely, these shifts in dominant sequence types were reflected in decreases in the relative abundance of gammaproteobacterial *nifH* sequences. In addition, increases in the relative dominance of cyanobacterial *nifH* gene, in particular of the order *Oscillatoriales*, as well as those from *Betaproteobacteria*, were observed in the +2 and +4.5 °C treatments. Considering the betaproteobacterial *nifH* sequences, an uncontrolled tank effect, as these were not detected in pretreatment samples and appeared in both control (1 OTU) and treatment samples (2 OTUs) (Figure 5). Finally, only the +4.5 °C treatment showed the presence of *nifH* genes typical for green sulfur bacteria (phylum Chlorobi).

Discussion

The observed shifts in the abundance and diversity of diazotrophs, seen in this study, should be considered in light of the known symbiotic

Table 1 α-Diversity measurements: OTU richness (number of unique OTUs), ChaoI species richness index, Shannon diversity index, at cutoff values of 95%, 97%, 98% and 99% of nucleotide identity

	OTU richness (count of unique OTUs)				*ChaoI index*				*Shannon index*			
	99%	*98%*	*97%*	*95%*	*99%*	*98%*	*97%*	*95%*	*99%*	*98%*	*97%*	*95%*
Pretreatment	10.43 (±0.82)	6.43 (±1.69)	6 (±1.15)	6 (±1.34)	15.58 (±3.08)	7.35 (±2.54)	6.85 (±1.97)	6.65 (±1.89)	2.80 (±0.03)	2.20 (±0.17)	2.16 (±0.10)	2.17 (±0.15)
Control	12.83 (±2.32)	8.53 (±2.73)	7.96 (±2.20)	8 (±2.16)	19.61 (±5.94)	9.66 (±3.69)	8.34 (±2.46)	8.44 (±2.50)	3.06 (±0.36)	2.55 (±0.55)	2.15 (±0.50)	2.50 (±0.49)
+2 °C	26.16 (±2.87)	21.9 (±1.98)	18.6 (±2.94)	16 (±1.8)	65.70 (±10.38)	76.96 (±26.97)	42.51 (±10.68)	36.27 (±6.24)	4.50 (±0.25)	4.03 (±0.21)	3.72 (±0.31)	3.45 (±0.18)
+4.5 °C	26.53 (±2.87)	20. 96 (±2.49)	20 (±2.12)	17.56 (±1.76)	93.34 (±28.77	44.81 (±12.53)	39.17 (±6.74)	38.59 (±16.24)	4.43 (±0.29)	3.96 (±0.27)	3.91 (±0.26)	3.65 (±0.17)

Abbreviation: OTU, operational taxonomic unit.

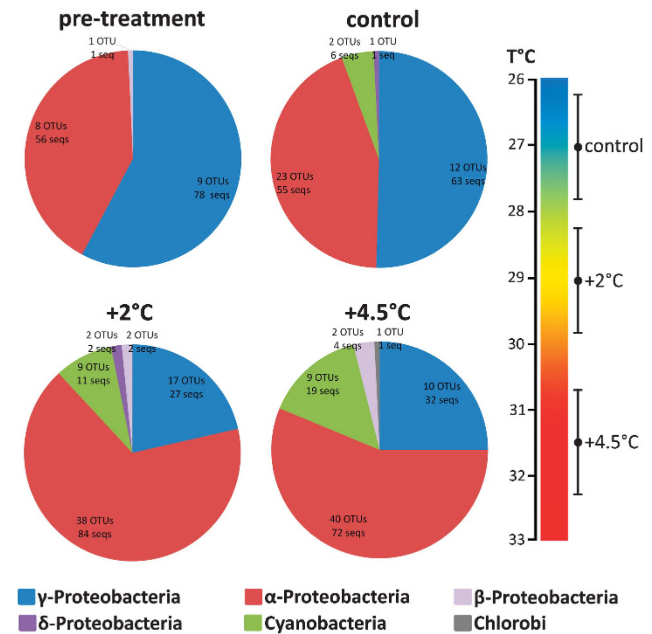

Figure 5 Taxonomic distribution of *nifH*-based OTUs among the different treatments. Sequences were clustered at a cutoff value of 98% of nucleotide similarity. Temperature averages in different treatments (control, +2 and +4.5 °C) during 21 days of experiment.

relationship between diazotrophs and zooxanthellae in corals. The growth of zooxanthellae in the coral system is probably limited by nitrogen availability (Falkowski *et al.*, 1993; Lema *et al.*, 2012). There is evidence that reduced nitrogen is often limiting in this ecosystem (O'Neil and Capone, 2008), and so the abundance and structure of diazotrophic communities become important. Indeed, coral photosynthesis rates under thermal stress have been shown to be higher in ammonium-supplemented corals than in untreated corals (Bérauda *et al.*, 2013). On the other hand, increased levels of nitrogen have been found to cause stress in corals, including reduced reproduction and growth (Koop *et al.*, 2001). The increase in the abundance of diazotrophs found in this study may be related to an increase in the numbers of zooxanthella, a response to the initial phases of thermal stress (Cunning and Baker, 2013), supporting initial survival of the holobiont organism when faced with temperature increases. Increased zooxanthella numbers may be a mechanism that explains *M. harttii* coral species resilience when confronted with thermal stress (Miranda *et al.*, 2013). Nevertheless, at later stages of prolonged thermal stress, the zooxanthella density decreased (Cunning and Baker, 2013). The decrease of Fv/Fm in the +2 and +4.5 °C treatments detected in this study was indeed indicative of photo-induced damage of the PSII machinery that leads to coral zooxanthella density decreases (Takahashi *et al.*, 2004). Concomitant with this decrease, the

production of dimethylsulfoniopropionate often decreases (Van Alstyne *et al.*, 2006), and, as such, the control of bacterial populations, some of which are potentially pathogenic, may be impaired (Slezakl *et al.*, 1994; Raina *et al.*, 2010; Barott and Rohwer, 2012). Hence, not only diazotrophs (such as measured here) but also other possibly deleterious bacterial groups may have thrived following the increase in temperature.

The increase in the abundance of Alphaproteobacterial *nifH* genes, in particular those related to *Azospirillum, Rhodobacter* and *Rhizobium* spp. (compared with Cyanobacteria), coupled with the decrease of gammaproteobacterial *nifH* genes suggests a scenario in which particular diazotrophs prevail when confronted with raised seawater temperatures. *Azospirillum*-like *nifH* genes were apparently favored in coral exposed to increased temperature. Measurement of C_2H_2 reduction activity in *Azospirillum* spp., *A. brasiliense* and *A. lipoferum* at 30 and 42 °C infers that these bacteria may be adapted to nitrogen fixation at elevated temperatures (Aggarwal and Chaudhary, 1995). This observation is consistent with the large increase in *Azospirillum*-like *nifH* sequences seen in the +4.5 °C treatment compared with that in the +2 °C treatment. Members of the genus *Azospirillum* have previously been found to be associated with another species of *Mussismilia* during bleaching stress and so they apparently thrive in stressed corals (De Castro *et al.*, 2010).

Our finding of *Rhodobacter*-like *nifH* genes in the high-temperature treatments is consistent with the finding that these organisms may be associated with deleterious processes in coral as they are observed to be abundant in diseased corals at different localities (Mouchka *et al.*, 2010). Furthermore, *Rhizobium*-like diazotrophs, such as those detected in the present study, have already been documented in many other coral species (Lema *et al.*, 2012). In terms of the Cyanobacteria-like sequences that were detected, both *Trichodesmium* and *Lyngbya* spp. have been reported to occur in blooms in coastal environments, including in corals, during the warmest seasons (O'Neil and Dennison, 2005; Paul *et al.*, 2005; Ramos *et al.*, 2005; Hewson *et al.*, 2007; O'Neil and Capone, 2008; Paerl and Huisman, 2008). The results reported here highlight the importance of certain diazotroph groups in corals and their proliferation under high-temperature stress conditions. Moreover, we propose that the densities (abundance, as measured by direct qPCR) of particular diazotroph species could be used as coral reef health bioindicators. This would provide a relatively simple tool with which to monitor the impact of thermal stress on corals. In addition, modulation of diazotroph communities should be further evaluated for potential improvement of the fitness of corals during thermal stress – for example, through the use of bioaugmentation of heat-resistant diazotroph species.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

We thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Petrobras Ambiental, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Coral Vivo Project and its sponsors (Petrobras and Arraial d'Ajuda Eco Parque) and Eduardo Fonseca for their support. The authors would also like to thank Professor Andrew Macrae (IMPPG/UFRJ) and Professor Ian Head (Newcaslte University, UK) for critical reading of the manuscript.

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