Octocoral Symbiodiniaceae, but not bacterial communities, are resistant to compositional changes from heat stress on a subtropical reef

Short title: heat stress and the octocoral holobiome

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Abstract

Microbes are essential to the holobiome of all organisms, so when organisms are impacted by environmental stressors the changes to microbial communities can inform on the health state of the host and even cause host disease. In early 2019 marine heatwaves occurred across eastern Australia, resulting in differential bleaching across Lord Howe Island reefs. Here we examined the Symbiodiniaceae and bacteria associated with bleaching susceptible *Cladiella* sp.1, and Symbiodiniaceae associated with two bleaching resistant octocoral species – *Cladiella* sp.2 and *Xenia* cf *crassa* – during and after marine heatwaves and between two reefs, Sylphs Hole (heavily impacted) and Coral Gardens (mildly impacted). While we found that *Cladiella* sp.1 Symbiodiniaceae community structure was not altered by bleaching, beta diversity dispersion of Symbiodiniaceae in unbleached colonies was higher at Sylphs Hole than Coral Gardens, suggesting dysbiosis. Unbleached colonies of all species had different Symbiodiniaceae communities between reef sites, and communities were stable through time. Bacterial communities differed between bleached and unbleached *Cladiella* sp.1, and bleached colonies' microbial communities changed after bleaching alert levels returned to "no stress", showing a delayed microbial response to bleaching. Finally, unbleached colonies from Sylphs Hole had higher bacterial alpha diversity compared to colonies at Coral Gardens, which together with possible Symbiodiniaceae dysbiosis suggests that the unbleached colonies at Sylphs Hole were impacted by heatwaves without clear physical changes in the field. So, corals and their microbial communities may be affected at heavily stressed sites regardless of bleaching status.

Keywords

Soft coral, holobiome, Lord Howe Island, Symbiodnium, Cladocopium

Introduction

Coral bleaching events are increasing in frequency across the globe, causing irreparable damage to these important marine ecosystems and disrupting their microbial associations [1, 2]. This threat is wide reaching, with coral reefs impacted in tropical, subtropical, and temperate reefs, in some cases multiple times since 2010 [3–11]. Remote and high latitude reefs were hypothesized to be bleaching refugia, but recent studies found that distance from humans and/or the equator does not protect from bleaching risk, and can actually enhance negative outcomes [3, 12, 13]. hexacoral bleaching is easily documented in the field as bleached and dead colonies remain in place, but observations are more difficult for octocorals because they often die or detach shortly after bleaching with no skeleton remaining, resulting in their heat stress responses being overlooked in the field [14–17]. Additionally, octocorals are often overlooked or lumped in with hexacorals during bleaching

surveys despite being important members of reef communities [15]. Because of this, we know little about how octocorals are affected by heat stress, particularly with respect to their Symbiodiniaceae and bacterial communities, and even less about how they respond outside of the tropics.

Coral bleaching occurs in response to stressors, including heat and light, which result in the breakdown of the symbiosis between the cnidarian animal and its endosymbiotic Symbiodiniaceae, which can lead to changes to the coral holobiome and even mortality of the coral animal [2, 7, 14, 18–23]. The coral holobiome is defined as the coral, associated Symbiodiniaceae, and all other protists, bacteria, archaea, fungi, and viruses, all of which can be impacted by ecological stressors [24–26]. The most studied components of the coral holobiome are the Symbiodiniaceae and bacterial communities which we refer to in this manuscript as the holobiome, although we note that this term is also used to encompass all microbial associations. Symbiodiniaceae provide calories to their hosts through photosynthesis, while microbes play a role in the host immune response, aid in nutrient and chemical cycling, and may even provide protection from bleaching among other functions, many of which are yet unknown [27–29].

The Symbiodiniaceae population structure of hexacorals can shift in response to heat stress events [7, 23]. The most well-studied Symbiodiniaceae genera are *Cladocopium* and *Durisdinium*, of which *Cladocopium* are susceptible to heat stress while *Durisdinium* are thermally tolerant [7, 30–32]. hexacoral colonies with high proportions of *Durusdinium* have warmer bleaching thresholds and less heat-stress-related mortality than those hosting other genera, and the Symbiodiniaceae community can become *Durusdinium* dominated during and after bleaching [33, 34]. Symbiont populations within genera can also change in response to bleaching. In the anemone *Entacmaea quadricolor,* bleaching reduced the abundance of *Cladocopium* C25 compared to *Cladocopium* C3.25 [35]. However, 20 species of octocorals from the Great Barrier Reef and two from the Florida Keys did not change symbiont type during bleaching [17, 32, 36, 37]. So, it appears that anemone, stony, and octocoral symbiont communities may respond differently to heat stress.

Bacterial alpha diversity is hypothesised to increase as opportunistic and pathogenic bacteria invade the coral animal during bleaching [38]. In *Porites compressa,* heat stress increases genes associated with invasion and increases the abundance of *Vibrio* sp., a known coral pathogen [39]. Previously, *Vibrio* spp. were implicated in several coral diseases and in declines in coral health associated with bleaching [38–41]. In the hexacoral *Acropora millepora* (Magnetic Island, Great Barrier Reef) bacterial communities were shown to shift to a *Vibrio* spp. dominated composition prior to the onset of observable bleaching [22]. While diseases were previously implicated in mortality of octocoral populations worldwide [15, 42–44], it is unclear if thermally induced bleaching and pathogenic infection are linked in octocorals as they are in hexacorals.

Bacterial communities can be indicators of unhealthy hosts or become unhealthy themselves without becoming pathogen dominated, known as dysbiosis [45, 46]. Dysbiosis is a change in the community of commensal organisms, like bacteria, relative to the community found on healthy organisms [45, 47]. The Anna Karenina hypothesis proposes microbial dysbiosis can be seen in community data as an increase in beta diversity dispersion during environmental disturbance in unhealthy individuals [38, 45, 48]. Microbial stability is so important to marine organisms that dysbiosis is both an indicator of the health of the host organism and can cause disease states [46, 49]. Because microbial communities can respond quickly to changes in the environment and dysbiosis is a strong indicator of organism health, it is suggested that dysbiosis can be an early indicator of environmental disturbance [26]. While there is evidence of community shifts and dysbiosis in hexacorals during bleaching [38, 45], dysbiosis in coral communities is only beginning to be explored, so it is unclear if octocorals exhibit microbial dysbiosis during thermal stress.

In early 2019 a series of heat waves impacted the waters off the coast of subtropical eastern Australia, causing significant bleaching and mortality at Lord Howe Island [3, 16]. This was the fourth heat-induced bleaching event recorded at this site, with previous events in 1998, 2010, and 2011 [5, 7]. Most of the octocorals within the lagoonal Lord Howe Island reef were resistant to bleaching, with only one species – *Cladiella* sp.1 – undergoing observable bleaching at one lagoonal reef site [16]. *Cladiella* sp.2 was unaffected by the heatwave, while *Xenia* cf *crassa* had higher Symbiodiniaceae and chlorophyll concentrations during the heatwave than after the event subsided [16]. Here we aimed to examine the holobiome response (Symbiodiniaceae and bacterial community structure) of bleaching-susceptible *Cladiella* sp.1, and the Symbiodiniaceae community response of bleaching-resistant *Cladiella* sp.2 and *Xenia* cf *crassa* during and after heat stress. Specifically we aimed to, 1) examine the holobiome response of bleaching susceptible *Cladiella* sp.1 by comparing bleached and unbleached colonies collected from Sylphs Hole (octocoral bleaching occurred) during the peak of the heatwave (March) and one month later (April/May); 2) examine the holobiome response of unbleached *Cladiella* sp.1 between two sites, Sylphs Hole and Coral Gardens (no observable octocoral bleaching), in March and after the heatwave had subsided (October), and 3) to examine the response of Symbiodiniaceae communities in two bleaching resistant octocorals, *Cladiella* sp.2 and *Xenia* cf *crassa* between Sylphs Hole and Coral Gardens during March and October.

Methods

Study location and design

In early 2019, Lord Howe Island coral reef lagoon was affected by three successive marine heatwaves [3, 16]. Octocorals were sampled from two sites within the northern part of the lagoon with differential bleaching

impacts – Sylphs Hole (31° 31.245' S, 159° 03.266' E) and Coral Gardens (31° 31.495' S, 159° 03.045' E). Octocorals did not bleach at Coral Gardens, while *Cladiella* sp.1 bleached at Sylphs Hole [16]. *Cladiella* sp.1 (fig. 1b,e) was examined during all three monitoring intervals (March, April/May, and October) to examine effects of bleaching and recovery, while *X.* cf *crassa* (fig. 1c) and *Cladiella* sp.2 (fig. 1d) were examined only during March and October when there was the greatest change in Symbiodiniaceae and/or chlorophyll densities [16]. The experimental design is illustrated in fig. 2.

Collection

Octocoral fragments were collected under permit number LHIMP/R/18015/12112018 using Australian Entomological Supplies PTY LTD 12.5 cm blunt-tipped surgical scissors and placed in individual zip-top bags. Five samples per health state (unbleached or bleached, figure 1b,e) of *Cladiella* sp.1 were collected at Sylphs Hole. Five samples of unbleached *Cladiella* sp.1, *Cladiella* sp.2, and *X.* cf *crassa* were collected at Coral Gardens and Sylphs Hole. Samples were transported to shore held in seawater and immediately fixed in a sterile solution of 4% formalin in 3x phosphate buffered saline (PBS) in nuclease free water and kept at 4^{o}C for 10-14 hours before being transferred to a sterile solution of 3x PBS and stored at 4^{o}C.

DNA extraction, PCR, and sequencing

DNA was extracted using a Qiagen QIAamp DNA Mini Kit following the manufacturers tissue protocol [50] with minor modifications (see supplementary material). ITS2 PCR was performed following the protocol from Hume et al. (2018) using SYM_VAR_5.8S2 - SYM_VAR_REV primers, and 16S amplified from V1-V3 PCR at the Ramaciotti Centre for Genomics, UNSW (Sydney, NSW, Aus.) using primers 27f(ACACTATGGCGAGTGAAGAGTTTGATCMTGGCTCAG) - 519r(AGTCAGTCAGGGGWATTACCGCGGCKGCTG) with Illumina overhang [52, 53] as recommended by the Earth Microbiome Project [54]. Bacterial communities were only examined in the species that bleached, *Cladiella* sp.1, as bleaching is hypothesized to disrupt bacterial communities. For the full 16s PCR protocol see the supplemental information.

Bioinformatics

ITS2 sequences were processed, quality controlled, and matched to symbiont type using SymPortal [55], which is an analytical framework for genetically resolving the algal symbionts of reef corals. Symbiodiniaceae taxa were separated into the common and rare fractions (> or <1% of total abundance, respectively) for analysis based on previous work at Lord Howe Island [7]. 16S data was processed and quality controlled using the

DADA2 pipeline in R [56]. For more information on DADA2 settings used see the supplemental information. After completing the bacterial bioinformatics pipeline, sampling depth was 294.4 ± 53.6 SD ASVs. The core microbiome was defined as ASVs identified in 90% of samples within each species [57] using the package Microbiome [58] and the resulting datasets were normalised separately using Wrench [59].

Statistical analysis

All analyses, plots, and 16S bioinformatics were conducted using R version 4.0.3 [60]. All graphs were made using the package ggplot2 [61]. Alpha diversity comparisons were run on the full bacterial datasets using a two-way ANOVA between health state and monitoring interval or site and monitoring interval, and assumptions were checked graphically. NMDS plots were generated, NMDS stress tested, PERMANOVA analyses run, and dispersion tested using the Vegan package [62]. Dispersion tested with the Betadisper function was non-significant for all datasets apart from comparisons of Symbiodiniaceae communities of unbleached *Cladiella* sp.1 between Coral Gardens and Sylphs Hole. Because the NMDS plots showed no overlap we have still interpreted compositional changes. For Symbiodiniaceae communities, analyses were performed between either health state and monitoring interval or site and monitoring interval on the entire community as well as common and rare fractions using both Bray-Curtis and Jaccard distributions. For bacterial communities, PERMANOVA analyses were performed between either health state and monitoring interval or site and monitoring interval on the full and core datasets. Bray-Curtis and Jaccard were used for the full dataset but only Bray-Curtis was used for the core microbiome as nearly all taxa were present in the core dataset, making a presence-absence analysis inappropriate. Differences in abundances of untransformed microbial core taxa at the genus level and all Symbiodiniaceae species between health state and monitoring interval or site and monitoring interval were tested using ANOVA, pairwise comparisons made using a Tukey test, and assumptions tested graphically. Alpha was adjusted using the Bonferroni-Holm method and is reported in the associated supplemental tables. Data transformations are noted in Table S7.

Comparison searches

To determine if abundant and core ASVs have previously been identified, bacterial ASVs found in greater than 1% relative abundance of the full or core datasets were searched in BLAST and the host species or habitat type where the sequence was previously found was recorded. Metadata was examined from any search result with an Expect E value of 0. If more than ten results were returned with a E score of zero on a BLAST search, only those with a percent identity of 95% or more were used. For justification of selection criteria see the supplemental information.

Results

Octocoral Symbiodiniaceae community structure

Proportional *Cladiella* sp.1 Symbiodiniaceae community structure showed no significant effects of health state or monitoring interval on composition of whole community, common, or rare taxa (Bray-Curtis or Jaccard, PERMANOVA $p > 0.05$, figure 3, table S1). When examined at the species level, no Symbiodiniaceae species were different between health states or monitoring intervals (ANOVA, $p > 0.05$, figure 3a,c,e, Table S2).

Symbiodiniaceae communities in unbleached *Cladiella* sp.1, *Cladiella* sp.2 and *Xenia cf crassa* differed between sites, but showed no effect of monitoring interval or the interaction between monitoring interval and site (PERMANOVA, $p < 0.05$, Figures 4, 5, 6 Tables S3, S4, S5). Specifically, spatial differences were observed for all, common and rare taxa in *Cladiella* sp.1 and *Xenia cf crassa*, and all and common taxa in *Cladiella* sp.2.

Fourteen *Cladiella* sp.1 *Cladocopium* spp. symbionts showed significant differences in abundance between sites (ANOVA, $p < 0.05$) but not monitoring intervals or the interaction between site and monitoring interval (ANOVA, $p > 0.05$, Figure 4a,c,e, Table S6). Of these, 1 1 had poor residuals due to the large number of zeros in the dataset, which could not be remedied through transformation or use of a GLM, so these results are interpreted cautiously.

In *Cladiella* sp.2, one *Cladocopium* sp. showed significant differences in abundance between sites (ANOVA, $p < 0.05$) but not between monitoring intervals or the interaction between monitoring interval and site (ANOVA, $p > 0.05$, Figure 5a,c,e, Table S7).

In *Xenia* cf *crassa,* seven *Cladocopium* spp. symbionts were different in abundance between sites (ANOVA, $p < 0.05$) but not between monitoring intervals or the interaction between monitoring interval and site (ANOVA, $p > 0.05$, Figure 6a,c,e, Table S8). One Symbiodiniaceae species differed between monitoring intervals only (ANOVA, $p < 0.05$).

Cladiella sp.1 bacterial community structure

87 ASVs from seven genera comprised the core microbiome of *Cladiella* sp.1: *Mycoplasma,* Candidatus

Hepatoplasma, Endozoicomonas, Pseudomonas, Serratia, and *Delftia*. When examining the effects of health state and monitoring interval on each genus in the core microbiome, *Mycoplasma* spp. abundance was affected by monitoring interval and *Pseudomonas* spp. abundance was affected by health state (ANOVA, $p < 0.05$, table S9). *Vibrio* spp. abundances were also examined as they are a known coral pathogen. *Vibrio* spp. were found in only 2 bleached samples and no unbleached samples, so we were unable to test for differences in *Vibrio* spp. abundance.

When examining the genera in the core microbiome, *Endozoicomonas* spp. and *Delftia* spp. were affected by the interaction between site and monitoring interval (ANOVA $p < 0.05$, Table S10). Specifically, during March there was higher abundance of *Endozoicomonas* spp. at Sylphs Hole than Coral Gardens; additionally, there was higher abundance of *Endozoicomonas* during March than October at Sylphs Hole (Tukey $p < 0.05$, Table S10). Pairwise comparisons of *Delftia* spp. abundance were not significant (Tukey $p > 0.05$, table S10).

Ten ASVs were each over 1% of total abundance in the untransformed whole and core bacterial community of *Cladiella* sp.1 (Table S11). Three ASVs were in the genus *Mycoplasma,* two in Candidatus *Hepatoplasma*, and five in *Endozoicomonas*. *Mycoplasma* spp. made up 51.03% of the whole and 54.82% of the core communities, Candidatus *Hepatoplasma* made up 19.04% of the whole and 20.46% of the core communities, and *Endozoicomonas* made up 10.95% of the whole and 11.77% of the core communities.

Bacterial diversity (Shannon and Simpsons) in *Cladiella* sp.1 at Sylphs hole differed with the interaction between health state and monitoring interval (ANOVA $p < 0.05$, figure 7a, table S12). Bleached corals had more diverse bacterial communities during March than April/May (Tukey $p < 0.05$). During April/May, bacterial communities in unbleached corals had higher Simpson diversity than bleached corals (Tukey $p < 0.05$, figure 7a, Table S12). Bacterial whole and core community structure differed with the interaction between health state and monitoring interval (Bray-Curtis, PERMANOVA $p < 0.05$, Figure 7b,d Table S12), while community composition differed with the main effects of health state and monitoring interval (Jaccard, PERMANOVA $p < 0.05$, Figure 7c, Table S12). Despite a significant interaction term, no whole community structure pairwise analyses were significant (Bray-Curtis, pairwise PERMANOVA $p > 0.05$, Figure 7b,d, Table S12), but dispersion was different between monitoring intervals (Betadisper, $p < 0.05$, Table S12). Patterns in core community structure were clearer, with community structure differing between monitoring intervals in bleached colonies, and between bleached and unbleached colonies in April/May.

Bacterial diversity (Shannon and Simpson) in unbleached *Cladiella* sp.1 was higher during March than October (Tukey $p < 0.05$, figure 8a, table S13). Bray-Curtis Microbiome community structure differed by site only

(PERMANOVA $p < 0.05$, figure 8c, table S13). Community composition estimated using Jaccard distance was affected by both site and monitoring interval main effects (PERMANOVA $p < 0.05$, figure 8e, table S13). Community composition dispersion was significant between monitoring intervals (Betadisper, $p < 0.05$). Bray-Curtis core microbiome community structure was affected by the interaction between site and monitoring interval (PERMANOVA $p < 0.05$, figure 8d, table S13). Specifically, community structure differed between monitoring intervals at Sylphs Hole, and between sites in October.

Discussion

Octocorals are historically understudied in coral reef ecosystems, and data is lacking on how their holobiomes respond to heat stress [15]. Here we investigated Symbiodiniaceae community structure in two species of octocoral and the holobiome community structure of a third species during and after a marine heatwave event in the Lord Howe Island lagoon. We found that *Cladiella* sp.1, the only octocoral species to bleach during the study, expelled Symbiodiniaceae proportionally. There was also evidence of dysbiosis of Symbiodiniaceae communities in unbleached *Cladiella* sp.1 at Sylphs Hole, suggesting unbleached colonies were affected by heat stress. Symbiodiniaceae communities associated with all three species were specific to each site but stable across the study period, suggesting stability through time as found in other octocorals.

Cladiella sp.1 full and core community bacterial diversity and structure differed between bleached and unbleached corals. Bacterial diversity is expected to increase in bleached corals due to pathogen invasion [38], but here bacterial diversity decreased and we found no evidence for proliferation of known pathogens. Bleached colony core community structure shifted after the onset of bleaching, suggesting that heat stress was not the cause of changes. Instead, it is likely that declining bleached colony health disrupted the core microbiome. The abundance of individual *Cladiella* sp.1 core microbial taxa are relatively resistant to change, with only one of six genera affected by bleaching and only two of six affected by monitoring interval. Overall, Symbiodiniaceae were resistant to changes induced by heat stress at Lord Howe Island, while bacterial communities were more heavily affected.

Octocoral Symbiodiniaceae community structure

Within the Lord Howe Island lagoon all octocorals examined contained only *Cladocopium* spp. Symbiodiniaceae, while Lord Howe Island hexacorals host Symbiodiniaceae in five genera [7]. Sampling technique can affect the taxa sequenced as coral Symbiodiniaceae community composition is known to vary across the hexacoral surface [63, 64], and may also vary across octocorals. Here, we sampled only the lobe tips

of *Cladiella* spp. and the area directly below the tentacles of *Xenia* cf. *crassa,* so it is possible that Symbiodiniaceae diversity is higher, or proportions are different, in other areas of these colonies, though patterns similar to what was found here were noted in other octocorals. *Cladocopium* is the dominant genera in Alcyonacean and Xeniid octocorals in the tropics [65], and the dominant genera in ~75% of all octocorals worldwide [66]. Lord Howe Island octocorals are dominated by *Cladocopium* C1, as are the majority tropical Alcyonacean octocorals (but not Xeniid octocorals, [65]) and ~18% of octocorals worldwide [66]. Previous research investigating Great Barrier Reef octocorals found associations predominantly with *Cladocopium,* with some *Symbiodinium, Durisdinium,* and *Gerakladium* [67]. Similarly, in a survey of 14 hexacorals and 2 octocorals from Lord Howe Island and Kermadec Island, NZ, all except the octocoral *Capnella* sp. contained only *Cladocopium*, while *Capnella* sp. contained *Breviolum*, though this was assessed through sequencing of bands through gel electrophoresis and could have missed rare types [68]. Overall, it appears that *Cladocopium* are dominant endosymbionts of octocorals.

Bleaching effects on Symbiodiniaceae community structure of *Cladiella* sp.1

We found that the Symbiodiniaceae community structure was stable between bleached and unbleached corals, and no Symbiodiniaceae species had differences in proportional abundance across health states despite lower density in bleached colonies [16], suggesting that *Cladiella* sp.1 expels Symbiodiniaceae species proportionally. Interestingly, hexacorals and anemones are known to shuffle their Symbiodiniaceae communities during and after bleaching, including at Lord Howe Island [7, 35], and octocorals may respond differently. During the 1998 Great Barrier Reef mass coral bleaching event, 20 species of bleached octocorals had symbiont types that were genetically indistinguishable from those in unbleached conspecifics [17]. Additionally, two octocorals from the Florida Keys had stable Symbiodiniaceae communities through bleaching, while a third had different communities before and after bleaching in the Gulf of Thailand [32, 36, 37]. Overall, it appears that many octocoral species have a mechanism for maintaining stability of their Symbiodiniaceae communities through bleaching not found in other cnidarians.

Site effects on Symbiodiniaceae community structure in three octocoral species

We found that unbleached *Cladiella* sp.1 Symbiodiniaceae beta diversity dispersion was higher at Sylphs Hole than Coral Gardens. Increased beta diversity dispersion is hypothesized to be a consequence of dysbiosis as laid out in the Anna Karenina principle [45]. Previous work also found that above-average water temperatures below the bleaching threshold can destabilize coral microbiomes [69]. So far, the Anna Karenina principle is primarily applied to bacterial communities, but here we find evidence that it may also apply to Symbiodiniaceae or other microbes. Because unbleached *Cladiella* sp.1 colonies from Sylphs Hole had lower concentrations of Symbiodiniaceae and chlorophyll than Coral Gardens during the heatwaves but recovered by October [16], it

seems unbleached *Cladiella* sp.1 colonies at Sylphs experienced thermal stress even despite no visible bleaching.

Symbiodiniaceae community site specificity is well documented and is largely attributed to differences in environmental conditions between reef sites [70, 71]. At Lord Howe Island Coral Gardens is a wave exposed site, while Sylphs Hole is sheltered and adjacent to the shoreline. We found that Symbiodiniaceae communities of all three octocoral species were specific to each site, with some Symbiodiniaceae species having different proportional abundance between sites. Similarly, Red Sea *Stylophora pistillata* hexacorals located in sheltered and exposed sites harbour different Symbiodiniaceae communities [72], suggesting that octocorals and hexacorals are similarly affected by site differences. Octocoral Symbiodiniaceae communities were also stable across monitoring intervals as was found in *Plexaura kuna* at San Blas Island, Panama, and *Briareum asbestinum* in the Florida Keys despite strong seasonal temperature changes [37, 73], which along with proportional stability through bleaching, suggests that octocorals have particularly stable Symbiodiniaceae communities.

Cladiella sp.1 bacteria community structure

Bleaching effects on bacterial community structure of bleaching susceptible *Cladiella* sp.1

In *Cladiella* sp.1, microbial responses were time-lagged compared to the onset of bleaching. This suggests that direct heat effects were not responsible for differences, instead longer-term bleaching-induced physiological changes were responsible [74]. Interestingly, in the hexacoral *Acropora millepora* bacterial communities shifted before bleaching [22], so we may have missed early microbial signs of stress. Opposed to our results, microbial alpha diversity is expected to increase during bleaching due to infiltration by opportunistic and pathogenic microbes [38, 74]. This may be because unbleached Sylphs Hole *Cladiella* sp.1 were not healthy despite being visually unbleached, evidence by lower Symbiodiniaceae concentrations at Sylphs hole than Coral Gardens [16] and evidence of dysbiosis in Symbiodiniaceae communities. Consequently, we may not see the expected patterns when comparing stressed unbleached to stressed bleached colonies. Alternatively, decreased diversity could be because we did not find pathogen infiltration/proliferation, which is the proposed mechanism by which alpha diversity increases [38].

Site effects on bacterial community structure of bleaching susceptible *Cladiella* sp.1

There was a difference in core community composition between sites during the bleaching event, and differences at Sylphs Hole during and after bleaching, so possible heatwave effects were immediate instead of

time-lagged. This is consistent with what we found in Symbiodiniaceae community structure, further suggesting that unbleached colonies at Sylphs Hole were stressed by the heatwaves. The observed patterns could also be due to site-specific seasonal responses, as was found in the microbiome of *Antillogorgia elisabethae* [75]. Similarly, experimental non-bleaching heat stress of Caribbean gorgonian octocorals found no changes to full microbiome beta diversity after exposure to elevated temperatures and/or UV radiation, but there were significant seasonal changes [76]; while in the Mediterranean microbiomes of five gorgonian octocorals were stable through seasons [44]. Overall, this suggests that octocoral microbiomes have diverse responses to site and temperature differences and clear patterns have not yet emerged. It is currently unclear whether the differences seen through time in unbleached octocoral communities at Sylphs Hole were driven by stress or seasonality, though density loss [16] and dysbiosis of Symbiodiniaceae communities suggests stress may be a factor.

Response of bacterial pathogens to heat stress

Vibrio spp. bacteria are known coral pathogens that have been implicated in heat-stress related mortality [22, 39–41, 77–79]. Although *Vibrio* sp. are often associated with corals during heat stress [22, 39, 41, 78, 79], only one *Vibrio* ASV was found in two bleached *Cladiella* sp.1 colonies, comprising 0.002% of microbial abundance. Previous studies have consistently identified *Vibrio* spp. in the microbiomes of gorgonian and sea pen octocorals, and *Vibrio* is implicated in disease and mortality of at least one Mediterranean gorgonian [44, 77]. Similarly, pathogenic *Serratia* spp. [80] abundance was not different across health state, site, or monitoring interval. *Serratia marcescens* is the cause of white pox disease in the Caribbean where it colonises the coral mucus from sewage [81], which may not be a problem on the sparsely populated Lord Howe Island. A third genus, *Ruegeria,* is associated with heatwave induced tissue necrosis in hexacorals, octocorals, and sponges [82] but was completely absent from *Cladiella* sp.1. Overall, we found no evidence of heat stress or bleaching leading to increases in disease associated bacterial species in Lord Howe Island octocorals.

Response of core and common bacteria to heat stress and reef site differences in bleaching susceptible *Cladiella* sp.1

The core microbiome of *Cladiella* sp.1 contained 87 ASVs in six genera – *Mycoplasma, Candidatus Hepatoplasma, Endozoicomonas, Pseudomonas, Serratia,* and *Delftia.* This is similar to previous octocoral microbiome studies, with *Antillogorgia elisabethae* in the Bahamas hosting 75 potential core members [75]. Microbes have a myriad of functional roles within the coral holobiome, but difficulty in culturing coral associated taxa means these roles have only recently begun to be understood and cannot be reliably determined through 16s sequencing alone [74, 83]. Despite this, several of the core microbiome genera identified in *Cladiella* sp.1 have known functions in the coral holobiome.

Mycoplasma live near the cnidocysts of corals, which allows access to nutrients from coral feeding and may aid in nutrient cycling [84, 85], therefore an increase in abundance from March to April/May suggests that *Cladiella* sp.1 changes feeding intensity between seasons. Octocorals in two genera from the GBR host a shared *Hepatoplasma* sp. operational taxonomic unit (OTU) in their core microbiomes [86], Candidatus *Hepatoplasma* spp. are seasonally stable in four Mediterranean gorgonian species [44], and in the current study, Candidatus *Hepatoplasma* abundances were stable across time, space, and health states. This suggests that *Hepatoplasma* may have a functional role in the octocoral microbiome. The Mediterranean gorgonians share another core bacterial genus with *Cladiella* sp.1 – *Endozoicomonas* spp., which is known to assist in the coral sulphur cycle, prevent mitochondrial dysfunction, and promote gluconeogenesis [87, 88]. We found that *Endozoicomonas* abundance in unbleached colonies was higher at Sylphs Hole than Coral Gardens in March, when temperatures were highest, and decreased in abundance at Coral Gardens from March to October. Higher abundances during the heatwaves may be due to their functional roles, which could mitigate some deleterious effects of heat stress [87, 88]. *Pseudomonas* spp. may also be beneficial to stressed corals, as isolates from *Sinularia polydactyla* have antibacterial activity in culture [89]. additionally, *Pseudomonas* contain a gene previously implied in the degradation of DMSP and DMS in the sulphur cycle [27]. These functions may be why *Pseudomonas* spp. abundance was higher in bleached than unbleached colonies. Interestingly, the opposite was found in *Montastrea annularis* corals in the Bahamas, where *Pseudomonas* spp. was absent from the microbiome of bleached corals [18, 90]. This suggests that *Pseudomonas* may have different roles in bleaching of hexa- and octocorals or location differences.

Conclusions and future direction

We found that octocoral Symbiodiniaceae community composition remains stable through heat-stress-induced bleaching. As all samples contained only *Cladocopium* spp. symbionts, clade differences did not affect bleaching resistance as found in hexacorals. Bleaching also did not affect the relative abundance of any species of Symbiodiniaceae, and only one of six core bacterial genera, further confirming the stability of the *Cladiella* sp.1 holobiome through bleaching. On the other hand, bacterial communities were bleaching affected, with time-lagged diversity loss and community shifts, suggesting host physiological changes after bleaching altered the microbiome. Environmental sites differences shaped both Symbiodiniaceae and bacterial communities, suggesting greater long-term site effects than short-term seasonal/heatwave effects. Similar patterns were observed in other octocoral species, suggesting that octocorals may have an unknown mechanisms for maintaining Symbiodiniaceae stability through stress [17, 36, 37]. Unfortunately, it is unclear what the long-term implications of this stability may be for octocorals, though it may partially explain why octocorals fared

better than stony corals at Lord Howe Island.

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Data Availability Statement

Data is available at Mendeley Data, DOI:10.17632/mv823ts9s4.1 [91]

Competing Interests

The authors declare no competing financial interests

References

1. Bellwood DR, Pratchett MS, Morrison TH, Gurney GG, Hughes TP, Álvarez-Romero JG, et al. Coral reef conservation in the Anthropocene: Confronting spatial mismatches and prioritizing functions. *Biol Conserv* 2019.
2. van Oppen MJH, Blackall LL. Coral microbiome dynamics, functions and design in a changing world. *Nat Rev Microbiol* 2019; **17**: 557–567.
3. Moriarty T, Leggat W, Heron SF, Steinberg RK, Ainsworth TD. Bleaching, mortality and lengthy recovery on the coral reefs of Lord Howe Island. The 2019 marine heatwave suggests an uncertain future for high-latitude ecosystems. *PLoS Clim* 2023; **2**: 1–21.
4. Sebastián CR, Sink KJ, McClanahan TR, Cowan DA. Bleaching response of corals and their Symbiodinium communities in southern Africa. *Mar Biol* 2009; **156**: 2049–2062.
5. Harrison PL, Dalton SJ, Carroll AG. Extensive coral bleaching on the world's southernmost coral reef at Lord Howe Island, Australia. *Coral Reefs* 2011; **30**: 775.
6. Thomson DP, Bearham D, Graham F. High latitude, deeper water coral bleaching at Rottnest Island, Western Australia. *Coral Reefs* 2011; **30**.
7. Boulotte NM, Dalton SJ, Carroll AG, Harrison PL, Putnam HM, Peplow LM, et al. Exploring the Symbiodinium rare biosphere provides evidence for symbiont switching in reef-building corals. *ISME J* 2016; **10**: 2693–2701.
8. Tsounis G, Edmunds PJ. Three decades of coral reef community dynamics in St. John, USVI: A contrast of scleractinians and octocorals. *Ecosphere* 2017; **8**.
9. Hughes TP, Kerry JT, Simpson T. Large-scale bleaching of corals on the Great Barrier Reef. *Ecology* 2018; **99**: 501.

10. Goyen S, Camp EF, Fujise L, Lloyd A, Nitschke MR, LaJeunensse T, et al. Mass coral bleaching of P. versipora in Sydney Harbour driven by the 2015–2016 heatwave. *Coral Reefs* 2019; **38**: 815–830.
11. Evensen NR, Fine M, Perna G, Voolstra CR, Barshis DJ. Remarkably high and consistent tolerance of a Red Sea coral to acute and chronic thermal stress exposures. *Limnol Oceanogr* 2021; **9999**: 1–12.
12. Baumann JH, Zhao LZ, Stier AC, Bruno JF. Remoteness does not enhance coral reef resilience. *Glob Chang Biol* 2022; **28**: 417–428.
13. Kim SW, Sommer B, Beger M, Pandolfi JM. Regional and global climate risks for reef corals: Incorporating species-specific vulnerability and exposure to climate hazards. *Glob Chang Biol* 2023; **29**: 4140–4151.
14. Sammarco PW, Strychar KB. Responses to High Seawater Temperatures in Zooxanthellate Octocorals. *PLoS One* 2013; **8**.
15. Steinberg RK, Dafforn KA, Ainsworth T, Johnston EL. Know Thy Anemone: A Review of Threats to Octocorals and Anemones and Opportunities for Their Restoration. *Front Mar Sci* 2020; **7**: 1–18.
16. Steinberg RK, Ainsworth TD, Moriarty T, Bednarek T, Dafforn KA, Johnston EL. Bleaching susceptibility and resistance of octocorals and anemones at the world's southern-most coral reef. *Front Physiol* 2022; **13**.
17. Goulet TL, LaJeunesse TC, Fabricius KE. Symbiont specificity and bleaching susceptibility among soft corals in the 1998 Great Barrier Reef mass coral bleaching event. *Mar Biol* 2008; **154**: 795–804.
18. Ritchie KB. Bacteria associated with bleached and nonbleached areas of Montastrea annularis. *Proceedings, 5th Symp. Nat. Hist. Bahamas.* 1994. pp 75–79.
19. Jones RJ. Changes in zooxanthellar densities and chlorophyll concentrations in corals during and after a bleaching event. *Mar Ecol Prog Ser* 1997; **158**: 51–59.
20. Saxby T, Dennison WC, Hoegh-Guldberg O. Photosynthetic responses of the coral Montipora digitata to cold temperature stress. *Mar Ecol Prog Ser* 2003; **248**: 85–97.
21. Strychar KB, Coates M, Sammarco PW, Piva TJ, Scott PT. Loss of Symbiodinium from bleached soft corals Sarcophyton ehrenbergi, Sinularia sp. and Xenia sp. *J Exp Mar Bio Ecol* 2005; **320**: 159–177.
22. Bourne D, Iida Y, Uthicke S, Smith-Keune C. Changes in coral-associated microbial communities during a bleaching event. *ISME J* 2008; **2**: 350–363.
23. Silverstein RN, Cunning R, Baker AC. Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Glob Chang Biol* 2015; **21**: 236–249.
24. Rohwer F, Seguritan V, Azam F, Knowlton N. Diversity and distribution of coral associated bacteria. *Mar Ecol Prog Ser* 2002; **243**: 1–10.
25. Thompson JR, Rivera HE, Closek CJ, Medina M. Microbes in the coral holobiont: Partners through evolution, development, and ecological interactions. *Front Cell Infect Microbiol* 2014; **4**: 1–20.
26. Glasl B, Webster NS, Bourne DG. Microbial indicators as a diagnostic tool for assessing water quality and climate stress in coral reef ecosystems. *Mar Biol* 2017; **164**: 0.
27. Raina JB, Tapiolas D, Willis BL, Bourne DG. Coral-associated bacteria and their role in the biogeochemical cycling of sulfur. *Appl Environ Microbiol* 2009; **75**: 3492–3501.
28. Van De Water JAJM, Allemand D, Ferrier-Pagès C. Host-microbe interactions in octocoral holobionts - recent advances and perspectives. *Microbiome* 2018; **6**: 1–28.
29. Doering T, Wall M, Putchim L, Rattanawongwan T, Schroeder R, Hentschel U, et al. Towards enhancing coral heat tolerance: a "microbiome transplantation" treatment using inoculations of homogenized coral tissues. *Microbiome* 2021; **9**: 1–16.
30. Fabricius KE, Mieog JC, Colin PL, Idip D, Van Oppen MJH. Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Mol Ecol* 2004; **13**: 2445–2458.
31. Berkelmans R, Van Oppen MJH. The role of zooxanthellae in the thermal tolerance of corals: A 'nugget of hope' for coral reefs in an era of climate change. *Proc R Soc B Biol Sci* 2006; **273**: 2305–2312.
32. Panithanarak T. Effects of the 2010 coral bleaching on phylogenetic clades and diversity of zooxanthellae (Symbiodinium spp.) in soft corals of the genus Sinularia. *Plankt Benthos Res* 2015; **10**: 11–17.
33. Baker AC, Starger CJ, McClanahan TR, Glynn PW. Corals' adaptive response to climate change. *Nature* 2004; **430**: 741.
34. Rowan R. Coral bleaching: Thermal adaptation in coral reef symbionts. *Nature* 2004; **430**: 742.
35. Hill R, Fernance C, Wilkinson SP, Davy SK, Scott A. Symbiont shuffling during thermal bleaching and recovery in the sea anemone Entacmaea quadricolor. *Mar Biol* 2014; **161**: 2931–2937.
36. Kirk NL, Ward JR, Coffroth MA. Stable Symbiodinium composition in the sea fan Gorgonia ventalina during temperature and disease stress. *Biol Bull* 2005; **209**: 227–234.
37. Hannes AR, Barbeitos M, Coffroth MA. Stability of symbiotic dinoflagellate type in the octocoral briareum asbestinum. *Mar Ecol Prog Ser* 2009; **391**: 65–72.

38. McDevitt-Irwin JM, Baum JK, Garren M, Vega Thurber RL. Responses of coral-associated bacterial communities to local and global stressors. *Front Mar Sci* 2017; **4**: 1–16.
39. Vega Thurber R, Willner-Hall D, Rodriguez-Mueller B, Desnues C, Edwards RA, Angly F, et al. Metagenomic analysis of stressed coral holobionts. *Environ Microbiol* 2009; **11**: 2148–2163.
40. Ben-Haim Y, Zicherman-Keren M, Rosenberg E. Temperature-regulated bleaching and lysis of the coral Pocillopora damicomis by the novel pathogen Vibrio coralliilyticus. *Appl Environ Microbiol* 2003; **69**: 4236–4242.
41. Tout J, Siboni N, Messer LF, Garren M, Stocker R, Webster NS, et al. Increased seawater temperature increases the abundance and alters the structure of natural Vibrio populations associated with the coral Pocillopora damicornis. *Front Microbiol* 2015; **6**: 1–12.
42. Calderón-Hernández A, Urbina-Villalobos A, Mora-Barboza C, Morales JA, Fernández-García C, Cortés J. Lesions in octocorals of the Costa Rican Caribbean During The 2015–2016 El Niño. *Coral Reefs* 2021; **40**: 1167–1179.
43. Tracy AM, Weil E, Burge CA. Ecological Factors Mediate Immunity and Parasitic Co-Infection in Sea Fan Octocorals. *Front Immunol* 2021; **11**: 1–14.
44. van de Water JAJM, Voolstra CR, Rottier C, Cocito S, Peirano A, Allemand D, et al. Seasonal Stability in the Microbiomes of Temperate Gorgonians and the Red Coral Corallium rubrum Across the Mediterranean Sea. *Microb Ecol* 2018; **75**: 274–288.
45. Zaneveld JR, McMinds R, Vega Thurber R. Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nat Microbiol* 2017; **2**.
46. Egan S, Gardiner M. Microbial Dysbiosis : Rethinking Disease in Marine Ecosystems. *Front Microbiol* 2016; **7**: 1–8.
47. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol* 2014; **16**: 1024–1033.
48. Giongo A, Gano KA, Crabb DB, Mukherjee N, Novelo LL, Casella G, et al. Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J* 2011; **5**: 82–91.
49. Vega Thurber R, Mydlarz LD, Brandt M, Harvell D, Weil E, Raymundo L, et al. Deciphering Coral Disease Dynamics: Integrating Host, Microbiome, and the Changing Environment. *Front Ecol Evol* 2020; **8**: 1–18.
50. QIAGEN. QIAamp DNA Mini and Blood Mini Handbook. *Qiagen* . 2016.
51. Hume BCC, Ziegler M, Poulain J, Pochon X, Romac S, Boissin E, et al. An improved primer set and amplification protocol with increased specificity and sensitivity targeting the Symbiodinium ITS2 region. *PeerJ* 2018; **6**: e4816.
52. Lane J, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR, et al. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. 1986; **83**.
53. Lane DJ. 16S/23S rRNA sequencing. *Nucleic acid techniques in bacterial systematics*. 1991. John Wiley and Sons, pp 115–175.
54. Thompson LR, Sanders JG, McDonald D, Amir A, Ladau J, Locey KJ, et al. A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* 2017; **551**: 457–463.
55. Hume BCC, Smith EG, Ziegler M, Warrington HJM, Burt JA, LaJeunesse TC, et al. SymPortal: A novel analytical framework and platform for coral algal symbiont next-generation sequencing ITS2 profiling. *Mol Ecol Resour* 2019; **19**: 1063–1080.
56. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016; **13**: 581–583.
57. Hernandez-Agreda A, Gates RD, Ainsworth TD. Defining the Core Microbiome in Corals' Microbial Soup. *Trends Microbiol* 2017; **25**: 125–140.
58. Lahti L, Shetty S. microbiome R package. 2019.
59. Muthiah SK, Corrada Bravo H. Wrench: Wrench normalization for sparse count data. 2022.
60. R Core Team. R: A language and environment for statistical computing. 2013.
61. Wickham H. ggplot2. *Wiley Interdiscip Rev Comput Stat* 2011; **3**: 180–185.
62. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan: Community Ecology Package. 2020.
63. Rowan B, Knowlton N. Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proc Natl Acad Sci U S A* 1995; **92**: 2850–2853.
64. Rowan R, Knowlton N, Baker A, Jara J. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature* 1997; **388**: 265–269.
65. Goulet TL, Simmons C, Goulet D. Worldwide biogeography of Symbiodinium in tropical octocorals. *Mar Ecol Prog Ser* 2008; **355**: 45–58.
66. Jahajeeah D, Bhoyroo V, Ranghoo-Sanmukhiya M. A review of soft corals (Octocorallia: Alcyonacea) and their symbionts: Distribution of clades and functionality. *West Indian Ocean J Mar Sci* 2020; **19**: 123–141.
67. Van Oppen MJH, Mieog JC, Sánchez CA, Fabricius KE. Diversity of algal endosymbionts (zooxanthellae) in octocorals: The roles of geography and host relationships. *Mol Ecol* 2005; **14**: 2403–2417.

68. Wicks LC, Sampayo E, Gardner JPA, Davy SK. Local endemicity and high diversity characterise high-latitude coral-Symbiodinium partnerships. *Coral Reefs* 2010; **29**: 989–1003.
69. Zaneveld JR, Burkepile DE, Shantz AA, Pritchard CE, Mcminds R, Welsh R, et al. Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. *Nat Commun* 2016; **7**: 1–12.
70. Kennedy E V., Tonk L, Foster NL, Chollett I, Ortiz JC, Dove S, et al. Symbiodinium biogeography tracks environmental patterns rather than host genetics in a key caribbean reef-builder, Orbicella annularis. *Proc R Soc B Biol Sci* 2016; **283**.
71. 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.e6.
72. Voolstra CR, Buitrago-López C, Perna G, Cárdenas A, Hume BCC, Rädecker N, et al. Standardized short-term acute heat stress assays resolve historical differences in coral thermotolerance across microhabitat reef sites. *Glob Chang Biol* 2020; **26**: 4328–4343.
73. Goulet TL, Coffroth MA. Stability of an octocoral-algal symbiosis over time and space. *Mar Ecol Prog Ser* 2003; **250**: 117–124.
74. Mouchka ME, Hewson I, Harvell CD. Coral-Associated Bacterial Assemblages: Current Knowledge and the Potential for Climate-Driven Impacts. *Integr Comp Biol* 2010; **50**: 662–674.
75. Robertson V, Haltli B, McCauley E, Overy D, Kerr R. Highly Variable Bacterial Communities Associated with the Octocoral Antillogorgia elisabethae. *Microorganisms* 2016; **4**: 23.
76. McCauley M, Jackson CR, Goulet TL. Microbiomes of Caribbean Octocorals Vary Over Time but Are Resistant to Environmental Change. *Front Microbiol* 2020; **11**: 1–16.
77. Bally M, Garrabou J. Thermodependent bacterial pathogens and mass mortalities in temperate benthic communities: A new case of emerging disease linked to climate change. *Glob Chang Biol* 2007; **13**: 2078–2088.
78. Mills E, Shechtman K, Loya Y, Rosenberg E. Bacteria appear to play important roles in both causing and preventing the bleaching of the coral Oculina patagonica. *Mar Ecol Prog Ser* 2013; **489**: 155–162.
79. Zhou J, Lin ZJ, Cai ZH, Zeng YH, Zhu JM, Du XP. Opportunistic bacteria use quorum sensing to disturb coral symbiotic communities and mediate the occurrence of coral bleaching. *Environ Microbiol* 2020; **22**: 1944–1962.
80. Krediet CJ, Ritchie KB, Alagely A, Teplitski M. Members of native coral microbiota inhibit glycosidases and thwart colonization of coral mucus by an opportunistic pathogen. *ISME J* 2013; **7**: 980–990.
81. Petersen LM, Tisa LS. Friend or foe? a review of the mechanisms that drive serratia towards diverse lifestyles. *Can J Microbiol* 2013; **59**: 627–640.
82. Rubio-Portillo E, Ramos-Esplá AA, Antón J. Shifts in marine invertebrate bacterial assemblages associated with tissue necrosis during a heat wave. *Coral Reefs* 2021; **40**: 395–404.
83. Bourne DG, Morrow KM, Webster NS. Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. *Annu Rev Microbiol* 2016; **70**: 317–340.
84. Neulinger SC, Gärtner A, Järnegren J, Ludvigsen M, Lochte K, Dullo WC. Tissue-associated 'Candidatus mycoplasma corallicola' and filamentous bacteria on the cold-water coral Lophelia pertusa (Scleractinia). *Appl Environ Microbiol* 2009; **75**: 1437–1444.
85. Gray MA, Stone RP, Mclaughlin MR, Kellogg CA. Microbial consortia of gorgonian corals from the Aleutian islands. *FEMS Microbiol Ecol* 2011; **76**: 109–120.
86. Haydon TD, Suggett DJ, Siboni N, Kahlke T, Camp EF, Seymour JR. Temporal Variation in the Microbiome of Tropical and Temperate Octocorals. *Microb Ecol* 2021.
87. Ding JY, Shiu JH, Chen WM, Chiang YR, Tang SL. Genomic insight into the host-endosymbiont relationship of Endozoicomonas montiporae CL-33T with its coral host. *Front Microbiol* 2016; **7**.
88. Tandon K, Lu CY, Chiang PW, Wada N, Yang SH, Chan YF, et al. Comparative genomics: Dominant coral-bacterium Endozoicomonas acroporae metabolizes dimethylsulfoniopropionate (DMSP). *ISME J* 2020; **14**: 1290–1303.
89. Radjasa OK, Salasia SIO, Sabdono A, Wise J, Imhoff J, Lammler C, et al. Antibacterial Activity of Marine Bacterium Pseudomonas sp. Associated with Soft Coral Sinularia polydactyla against Streptococcus equi Subsp. zooepidemicus 1. *Int J Pharmacol* 2007; **3**: 170–174.
90. Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I. The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* 2007; **5**: 355–362.
91. Steinberg RK. Lord Howe Island octocoral ITS2 and 16S data. 2023. Mendeley Data, V1.

Figure captions

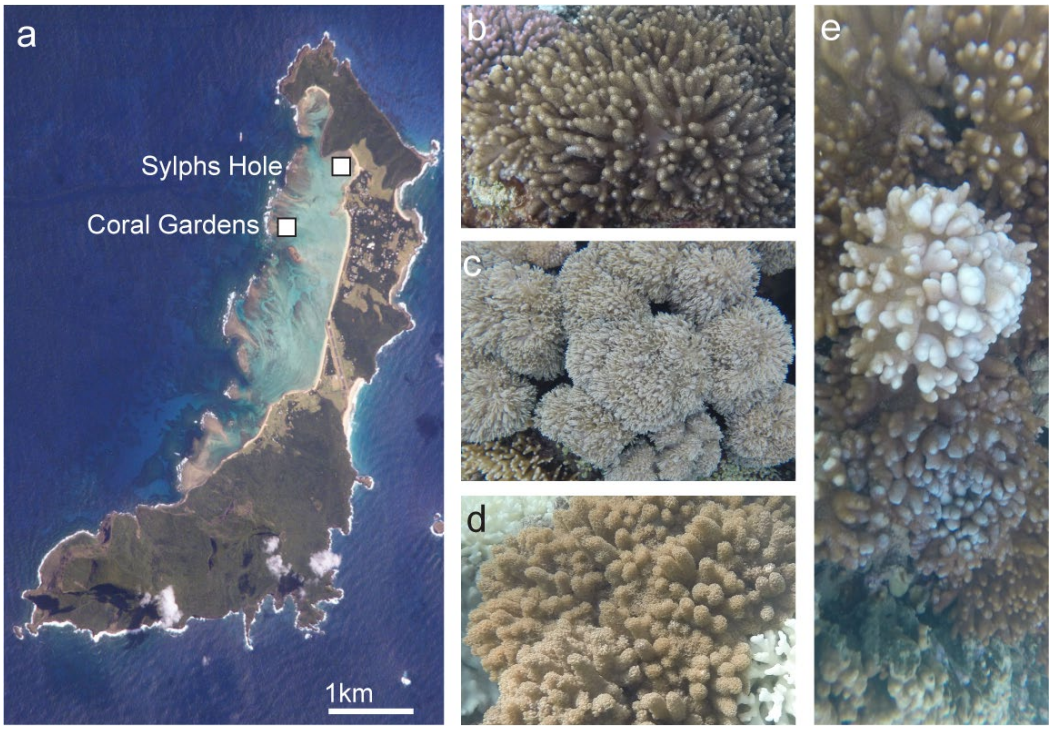

Figure 1 | Study sites and species. a) Map of Lord Howe Island with the collection sites Sylphs Hole and Coral Gardens marked, b) unbleached *Cladiella* sp.1, c) unbleached *Xenia* cf *crassa,* d) unbleached *Cladiella* sp.2, and e) bleached *Cladiella* sp.1 surrounded by unbleached conspecifics. Map courtesy of the Image Science & Analysis Laboratory, NASA Johnson Space Center. Coral photos by R.K. Steinberg.

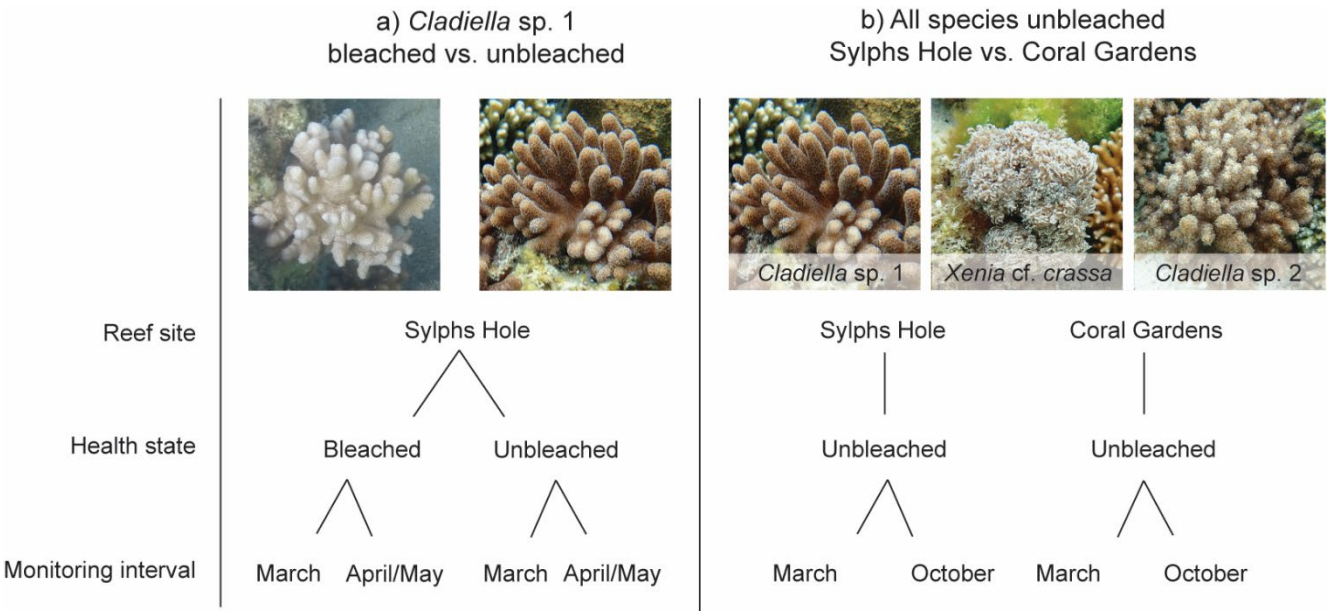

Figure 2 | Study design for a) comparing bleached and unbleached *Cladiella* sp.1 colonies and b) comparing unbleached colonies of *Cladiella* sp.1, *Xenia* cf *crassa*, and *Cladiella* sp.2 between Sylphs Hole and Coral Gardens. Both Symbiodiniaceae and bacteria were sequenced for *Cladiella* sp.1, while only Symbiodiniaceae were sequenced for *Cladiella* sp.2 and *Xenia* cf *crassa.* Five samples per condition were sequenced for Symbiodiniaceae and bacterial communities. All photos by R.K. Steinberg.

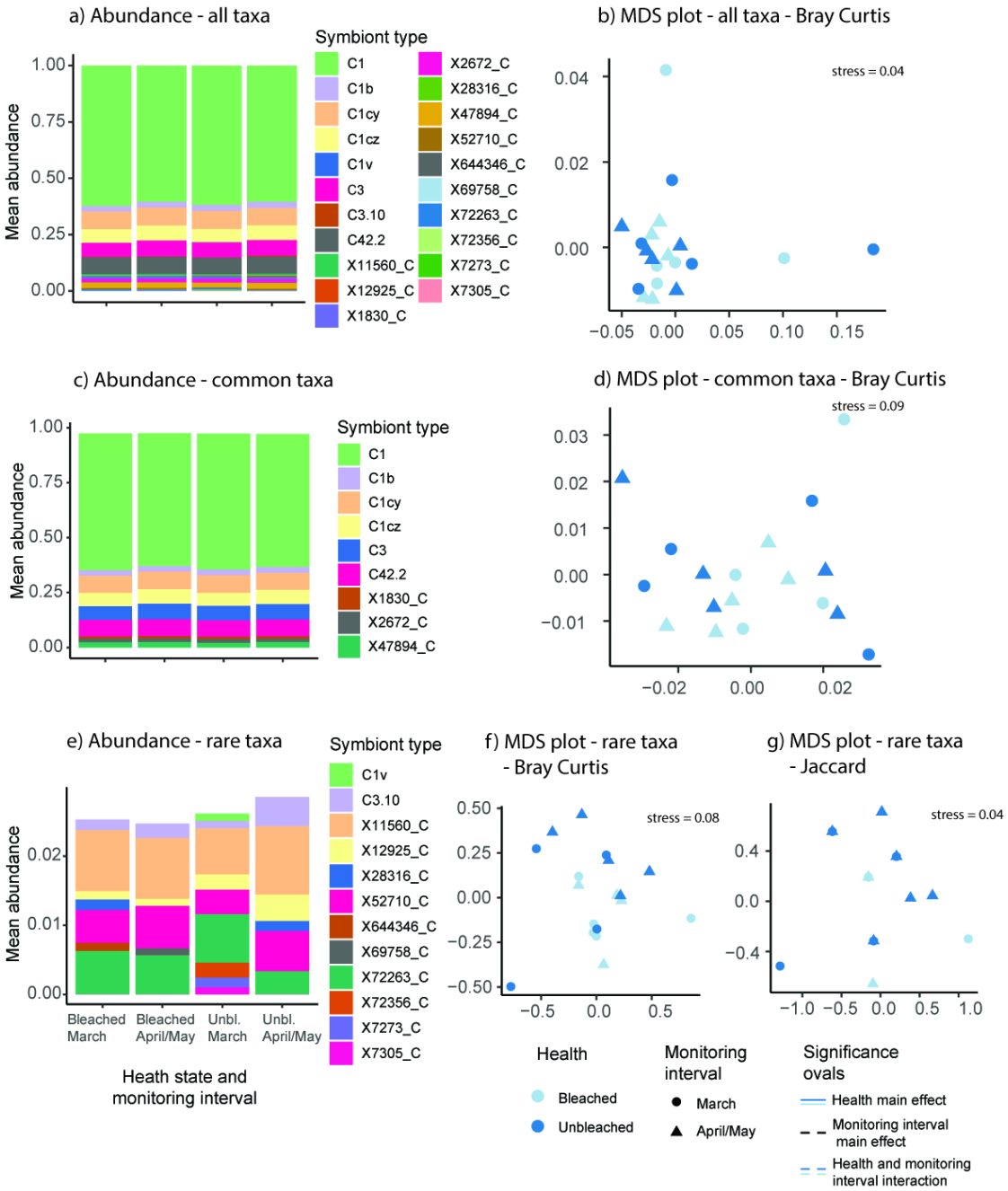

Figure 3 | Symbiodiniaceae community response of bleaching susceptible *Cladiella* sp.1 between bleached and unbleached colonies across two monitoring intervals. a) community composition of all Symbiodiniaceae taxa, b) MDS plot of Bray-Curtis beta diversity of all Symbiodiniaceae taxa, c) community composition of common Symbiodiniaceae taxa, d) MDS plot of Bray-Curtis beta diversity of common Symbiodiniaceae taxa, e) community composition of rare Symbiodiniaceae taxa, f) MDS plot of Bray-Curtis beta diversity of rare Symbiodiniaceae taxa, and g) MDS plot of Jaccard beta diversity of rare Symbiodiniaceae taxa. For all MDS plots, unbleached colonies are represented in dark blue, bleached colonies are represented in light blue, colonies collected during March are represented in circles, and colonies collected during April/May are represented in triangles. Ellipses represent 95% confidence intervals of groups involved in significant comparisons. Significant differences between health main effects are represented by solid coloured lines, significant differences in monitoring main effects are represented by black dashed lines, and significant interaction effects are represented by dashed coloured lines.

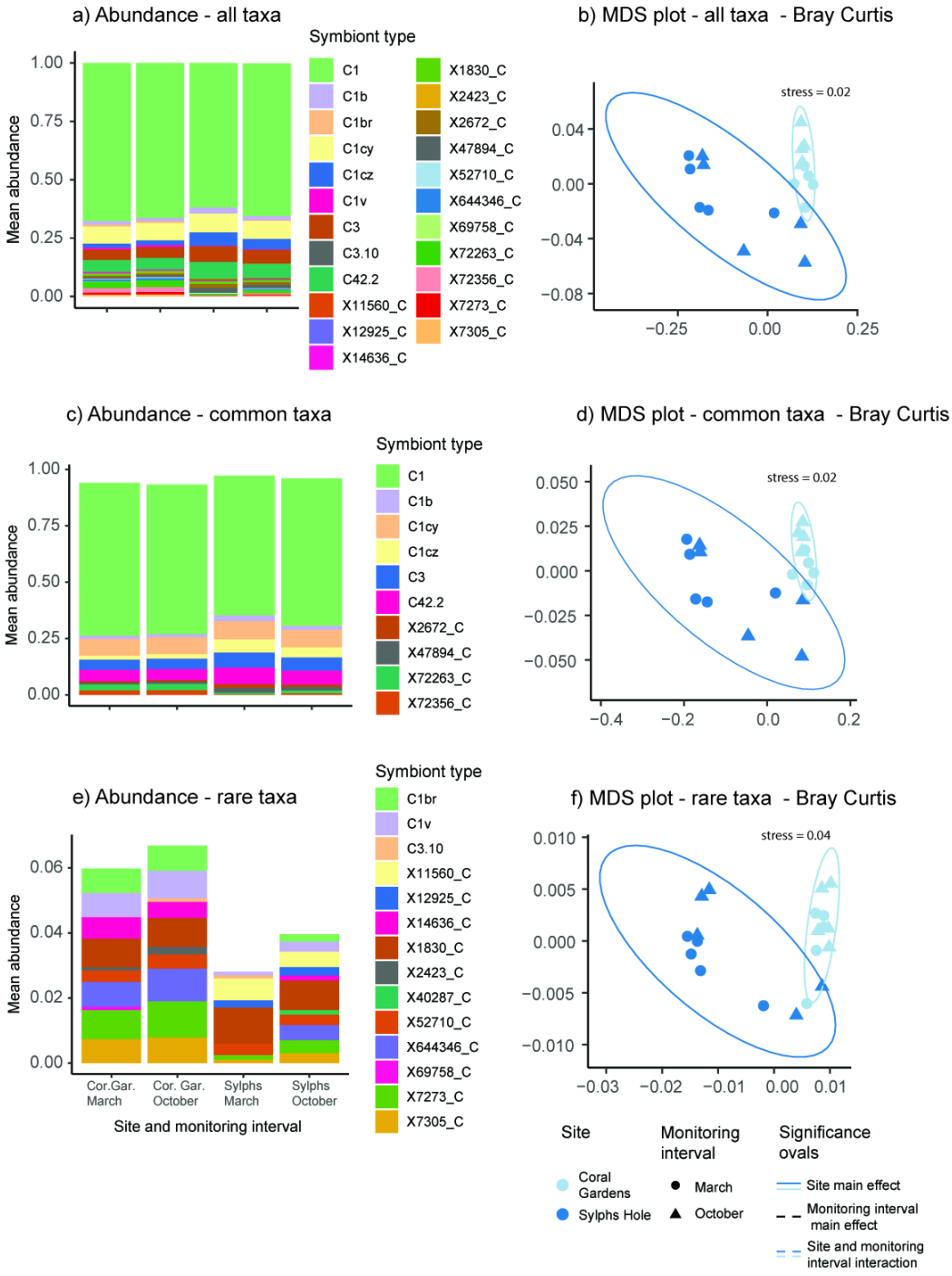

Figure 4 | Symbiodiniaceae community response of unbleached, bleaching susceptible *Cladiella* sp.1 between Sylphs Hole and Coral Gardens across two monitoring intervals. a) community composition of all Symbiodiniaceae taxa, b) MDS plot of Bray-Curtis beta diversity of all Symbiodiniaceae taxa, c) community composition of common Symbiodiniaceae taxa, d) MDS plot of Bray-Curtis beta diversity of common Symbiodiniaceae taxa, e) community composition of rare Symbiodiniaceae taxa, and f) MDS plot of Bray- Curtis beta diversity of rare Symbiodiniaceae taxa. For all MDS plots, colonies from Sylphs Hole are represented in dark blue, colonies from Coral Gardens are represented in light blue, colonies collected during March are represented in circles, and colonies collected during April/May are represented in triangles. Ellipses represent 95% confidence intervals of groups involved in significant comparisons. Significant differences between health main effects are represented by solid coloured lines, significant differences in monitoring main effects are represented by black dashed lines, and significant interaction effects are represented by dashed coloured lines.

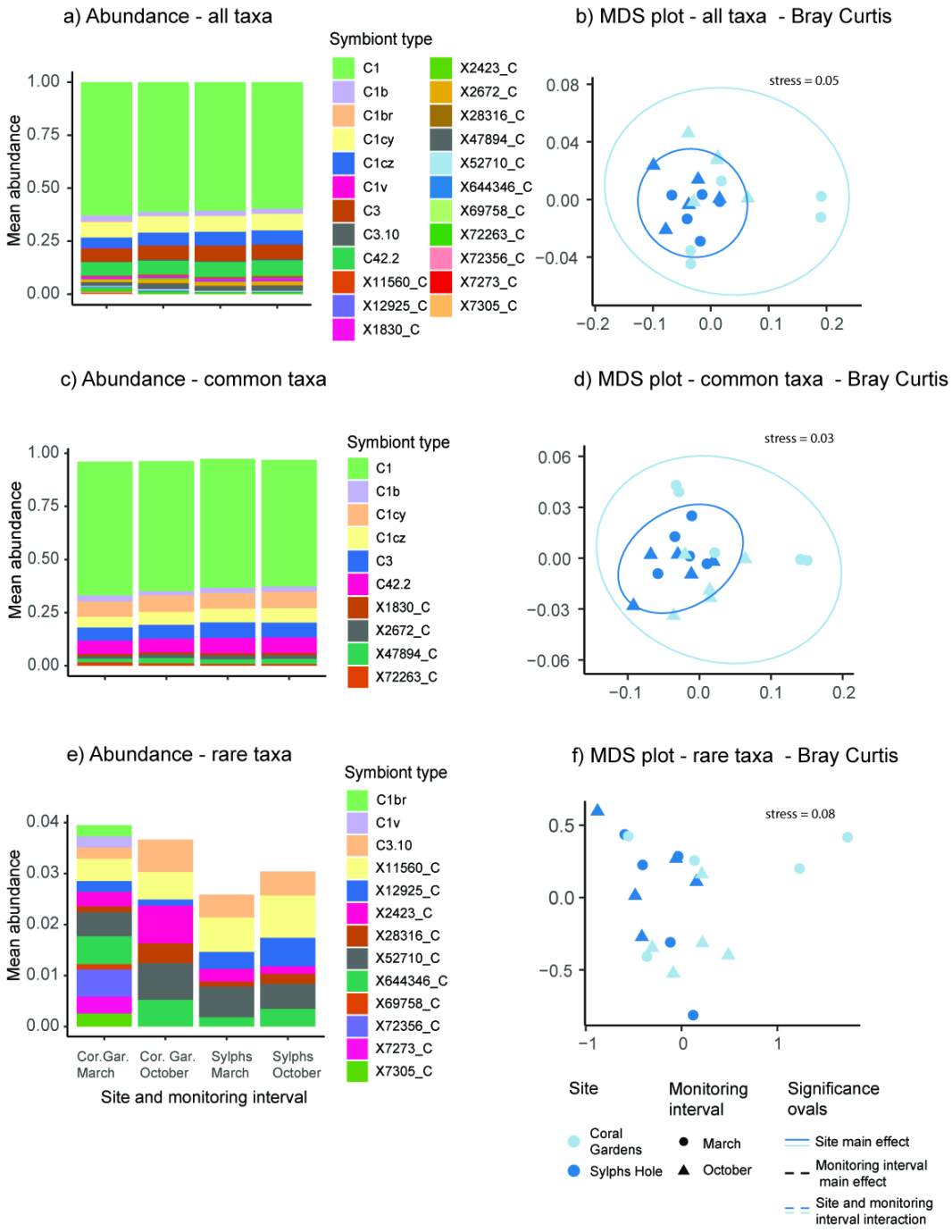

Figure 5 | Symbiodiniaceae community response of unbleached, bleaching resistant *Cladiella* sp.2 between Sylphs Hole and Coral Gardens across two monitoring intervals. a) community composition of all Symbiodiniaceae taxa, b) MDS plot of Bray-Curtis beta diversity of all Symbiodiniaceae taxa, c) community composition of common Symbiodiniaceae taxa, d) MDS plot of Bray-Curtis beta diversity of common Symbiodiniaceae taxa, e) community composition of rare Symbiodiniaceae taxa, and f) MDS plot of Bray- Curtis beta diversity of rare Symbiodiniaceae taxa. For all MDS plots, colonies from Sylphs Hole are represented in dark blue, colonies from Coral Gardens are represented in light blue, colonies collected during March are represented in circles, and colonies collected during October are represented in triangles. Ellipses represent 95% confidence intervals of groups involved in significant comparisons. Significant differences between health main effects are represented by solid coloured lines, significant differences in monitoring main effects are represented by black dashed lines, and significant interaction effects are represented by dashed coloured lines.

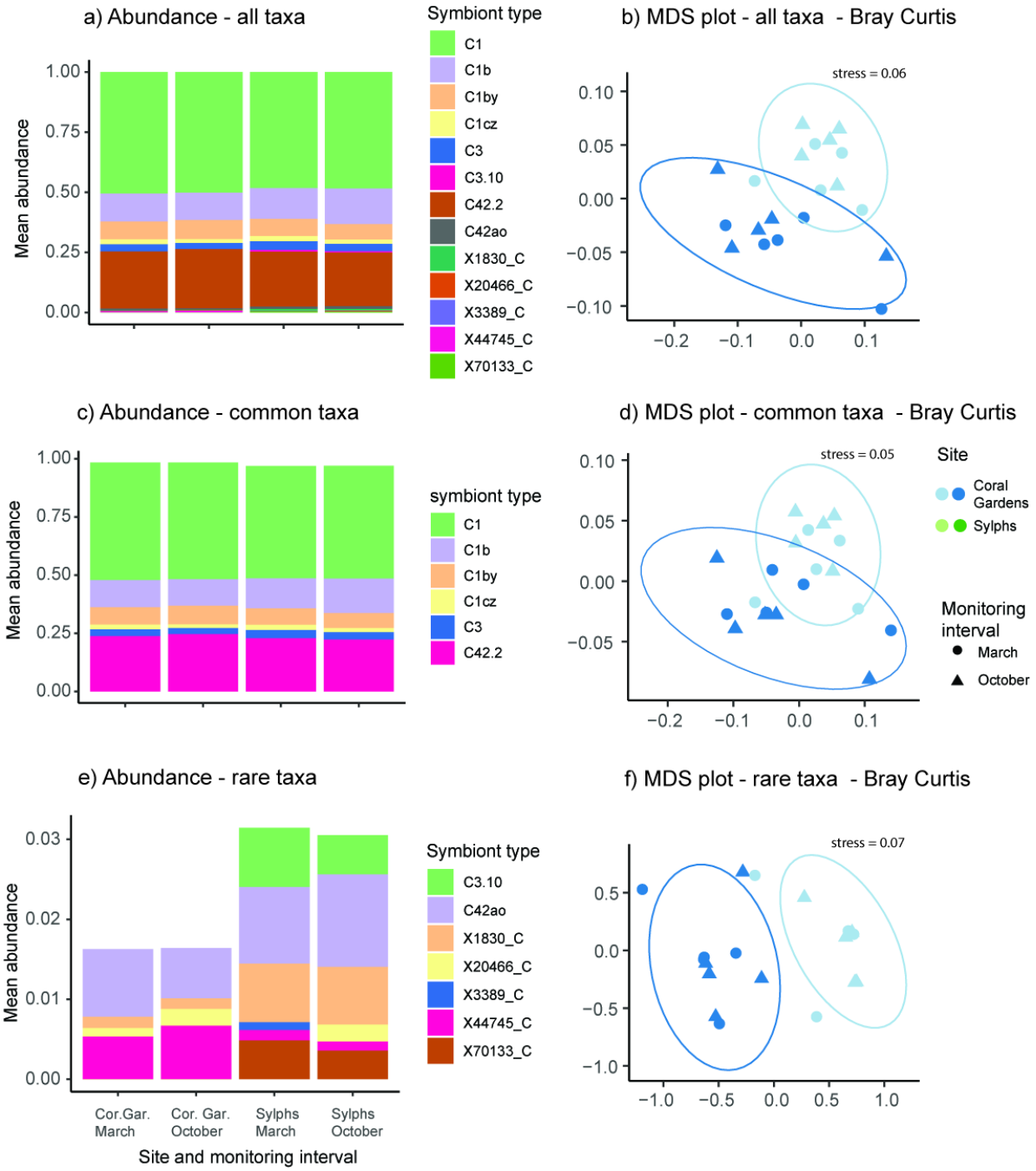

Figure 6 | Symbiodiniaceae community response of unbleached, bleaching resistant Xenia cf crassa between Sylphs Hole and Coral Gardens across two monitoring intervals. a) community composition of all Symbiodiniaceae taxa, b) MDS plot of Bray-Curtis beta diversity of all Symbiodiniaceae taxa, c) community composition of common Symbiodiniaceae taxa, d) MDS plot of Bray-Curtis beta diversity of common Symbiodiniaceae taxa, e) community composition of rare Symbiodiniaceae taxa, and f) MDS plot of Bray- Curtis beta diversity of rare Symbiodiniaceae taxa. For all MDS plots, colonies from Sylphs Hole are represented in dark blue, colonies from Coral Gardens are represented in light blue, colonies collected during March are represented in circles, and colonies collected during April/May are represented in triangles. Ellipses represent 95% confidence intervals of groups involved in significant comparisons. Significant differences between health main effects are represented by solid coloured lines, significant differences in monitoring main effects are represented by black dashed lines, and significant interaction effects are represented by dashed coloured lines.

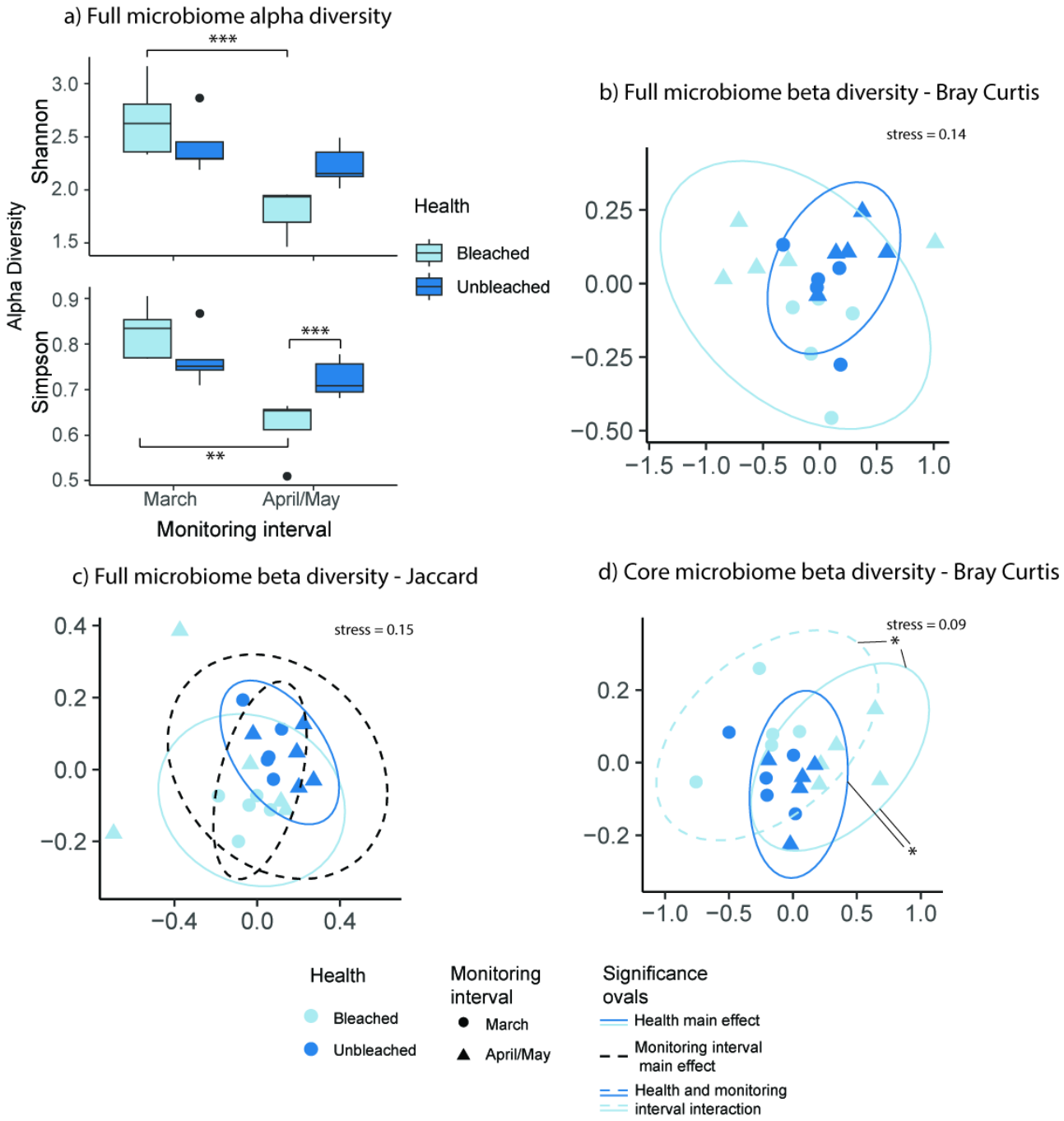

Figure 7 | Bacterial community response of bleaching susceptible *Cladiella* sp.1 between bleached and unbleached colonies across two monitoring intervals. a) alpha diversity of the entire bacterial community, b) MDS plot of Bray-Curtis beta diversity of the whole community, c) MDS plot of Jaccard beta diversity of the whole community, and d) MDS plot of Bray-Curtis beta diversity of the core community. In the alpha diversity plots, the March monitoring interval is represented in light green, while April/May is represented in dark green. In the MDS plots, bleached colonies are represented in light blue, unbleached colonies are represented in dark blue, colonies collected in March are represented by circles, and colonies collected in October are represented by triangles. Ellipses represent 95% confidence intervals of groups involved in significant comparisons. Significant differences between health main effects are represented by solid coloured lines, significant differences in monitoring main effects are represented by black dashed lines, and significant interaction effects are represented by dashed coloured lines.

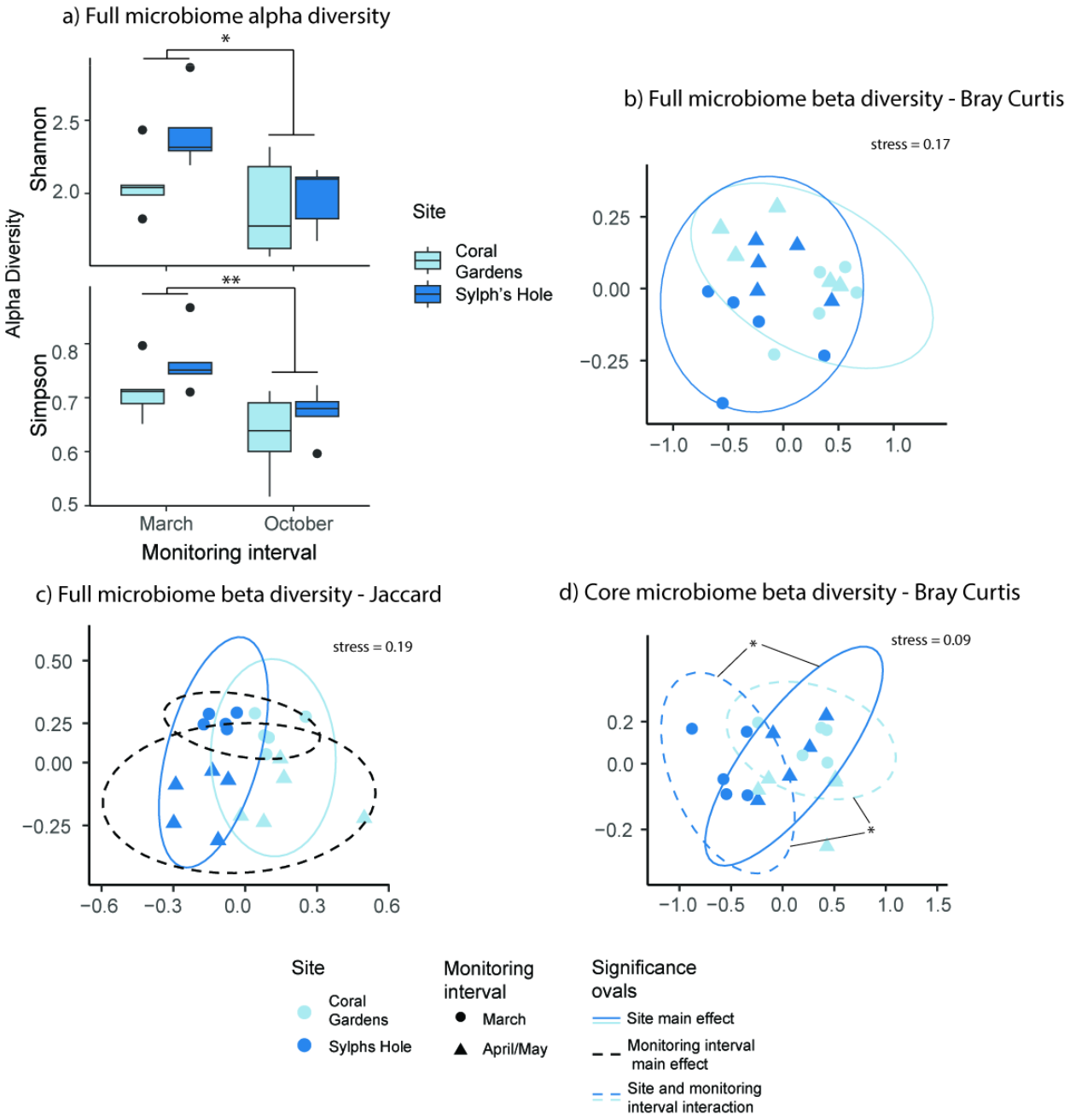

Figure 8 | Bacterial community response of bleaching susceptible *Cladiella* sp.1 between colonies from Sylphs Hole and Coral Gardens across two monitoring intervals. a) alpha diversity of the entire bacterial community, b) MDS plot of Bray-Curtis beta diversity of the whole community, c) MDS plot of Jaccard beta diversity of the whole community, and d) MDS plot of Bray-Curtis beta diversity of the core community. In the alpha diversity plots, the March monitoring interval is represented in light green, while October is represented in dark green. In the MDS plots, colonies from Coral Gardens are represented in light blue, colonies from Sylphs Hole are represented in dark blue, colonies collected in March are represented by circles, and colonies collected in October are represented by triangles. Ellipses represent 95% confidence intervals of groups involved in significant comparisons. Significant differences between site main effects are represented by solid coloured lines, significant differences in monitoring main effects are represented by black dashed lines, and significant interaction effects are represented by dashed coloured lines.