Coral Reefs
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REPORT

Reef habitats structure symbiotic microalgal assemblages in corals and contribute to differential heat stress responses

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Abstract Coral-associated microbial symbionts, particularly dinoflagellates from the family Symbiodiniaceae, play a significant role in corals' ability to survive and recover from marine heatwaves and mass bleaching events. Yet, there remain limited data on the extent to which microalgal endosymbiont associations shift in situ during and after prolonged heat stress and over small spatial scales, potentially limiting the alteration of symbiont communities as a restoration tool. In 2019, Mo'orea, French Polynesia experienced a massive thermal anomaly accompanied by widespread coral bleaching and mortality. Using ITS2 amplicon sequencing, we assessed the possibility of microalgal assemblages explaining the observed variability in *Acropora hyacinthus* thermal tolerance and recovery dynamics between reef habitats. Our data reveal flexibility in *A. hyacinthus* symbiont associations, but show that these associations were strongly linked to both heat stress response and the reef habitat in which the colony resided, indicating a prominent role of local environments in shaping in hospite symbiont communities and, consequently, holobiont thermal tolerance. Notably, bleaching resistant colonies were invariably dominated by *Symbiodinium* during and after the bleaching event. Although we did not detect temporal changes in symbiont communities during recovery from thermal stress, this novel observation of *A. hyacinthus* in association with *Symbiodinium* suggests that heat stress may have facilitated a shift. Further, we identify symbiont type profiles that are potentially diagnostic of bleaching susceptibility. Our findings illuminate the extent of symbiont flexibility for this species and location, with important implications for coral reef recovery and management in a rapidly changing ocean.

Keywords Coral bleaching · Thermal stress · *Acropora hyacinthus* · ITS2 · Symbiodiniaceae · French Polynesia

Introduction

Coral microbial symbionts play an integral role in the health, survival, and ecological functioning of their hosts (Bourne et al. 2016). Endosymbiotic dinoflagellate microalgae from the family Symbiodiniaceae are of particular importance as they facilitate a tightly regulated, mutualistic nutrient exchange that is crucial to coral holobiont metabolism and function (Rädecker et al. 2015). However, corals live close to their upper thermal physiological limits and anomalously warm seawater temperatures, or marine heatwaves (Oliver et al. 2021), resulting from anthropogenic climate change can disrupt this symbiotic relationship and lead to coral bleaching (Hoegh-Guldberg and Smith 1989). Given that marine heatwave-induced bleaching is projected to increase in the next century (Hughes et al. 2018), there is an urgent need to understand how coral–algal symbiotic relationships may be altered on the reef during or after bleaching events.

Diverse Symbiodiniaceae taxa are known to associate with coral hosts (LaJeunesse et al. 2018). These symbiont taxa exhibit varying tolerances to environmental stressors

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and, as such, modulate coral host physiology and phenotype (Rowan 2004; Berkelmans and van Oppen 2006; Cunning et al. 2015). For instance, some species within *Durusdinium* and *Cladocopium* genera confer some degree of thermal bleaching resistance to their hosts, while others are associated with holobiont thermal sensitivity (Berkelmans and van Oppen 2006; Cunning et al. 2016; Howells et al. 2020). Corals also may harbor a single Symbiodiniaceae type or multiple taxa simultaneously (Thornhill et al. 2009; Silverstein et al. 2012), and emerging evidence points to symbiont type abundance and diversity as additional important factors that influence corals' health and response to stress (Kenkel and Bay 2018; Claar et al. 2020a; Howe-Kerr et al. 2020). For example, high algal symbiont alpha diversity has been implicated in poor host performance under stressful conditions, including elevated water temperatures, carbon dioxide levels, and inorganic nutrients (Claar et al. 2020a; Howe-Kerr et al. 2020). Therefore, connections between symbiont community composition and corals' responses to changing ocean conditions should be further considered when evaluating holobiont stress tolerance, especially in the context of prolonged marine heatwaves that contribute to mass bleaching.

The modification of Symbiodiniaceae communities has been proposed as a potential mechanism to bolster corals' resilience to environmental stressors, including temperature-induced bleaching (Buddemeier and Fautin 1993; Kinzie et al. 2001; Berkelmans and van Oppen 2006; Silverstein et al. 2015; Boulotte et al. 2016; Anthony et al. 2017; Voolstra et al. 2021). According to this idea, the proportional abundance of microalgal endosymbiont taxa can shift, either through resorting of taxa already present within the coral tissues or uptake of exogenous symbiont cells, to favor types that are better suited to tolerate current levels of warming (Kinzie et al. 2001; Berkelmans and van Oppen 2006; Jones et al. 2008; Cunning et al. 2015; Boulotte et al. 2016). However, this is not a ubiquitous response. In fact, there are more examples of coral colonies not altering their dominant symbiont type in response to thermal stress, suggesting a strong role of host–symbiont genotype compatibility and calling into question how ecologically relevant alteration of Symbiodiniaceae assemblages within a colony is in situ (Goulet 2006; Sampayo et al. 2008; Stat et al. 2009; Thornhill et al. 2009; LaJeunesse et al. 2010; Smith et al. 2017). Hence, assessing in hospite microalgal assemblages and their potential to change through time is essential not only for understanding the coral holobiont's capacity to persist in the face of climate change, but also in determining the contribution of the coral–algal symbiosis to adaptation and/or acclimatization.

From December 2018 to May 2019, the island of Mo'orea, French Polynesia experienced a prolonged marine heatwave (Fig. 1b) accompanied by widespread coral bleaching and mortality (Speare et al. 2021). However, marked colony-level differences in bleaching response were observed in *Acropora hyacinthus* (Fig. 1c), a key reef-builder in the Indo-Pacific Ocean that is highly sensitive to climate change (Veron 2000; Baird and Marshall 2002), with some individuals remaining unbleached ("resistant" colonies, Supplementary Fig. S1a) and others bleaching but later recovering after the thermal stress subsided ("recovered" colonies, Supplementary Fig. S1b) (Leinbach et al. 2021). Here, we leverage this natural, thermally induced bleaching event to compare Symbiodiniaceae communities (i.e., the total assemblage of

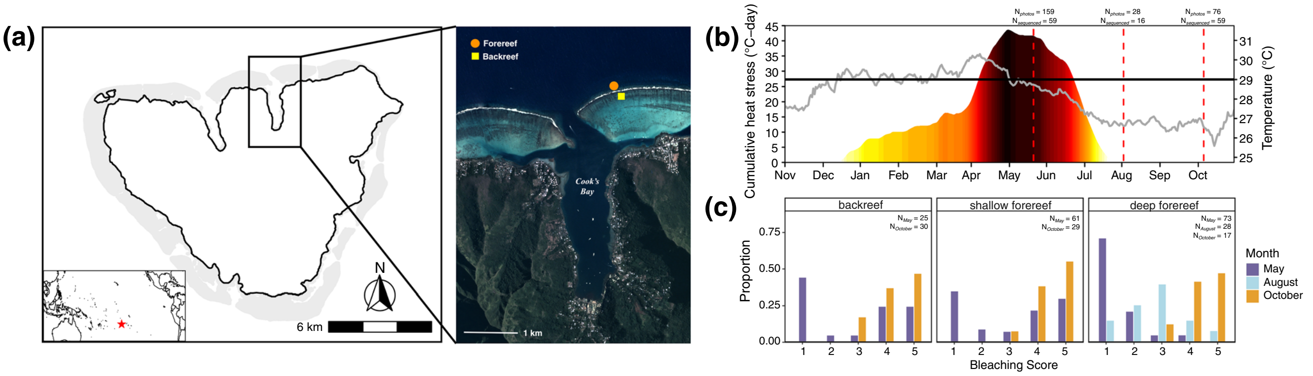

Fig. 1 Thermal stress and associated bleaching event in 2019 on the island of Mo'orea, French Polynesia. (**a**) Map of sampling locations in forereef and backreef habitats along the north shore of Mo'orea (red star, inset world map) (satellite imagery, Allen Coral Atlas 2022). (**b**) Observed sea surface temperatures from November 2018 to November 2019 (gray line) and bleaching threshold for corals in Mo'orea (black line; right axis). Vertical red dashed lines indicate sampling time points. Color gradient from yellow to black illustrates increasing cumulative heat stress (left axis) around the island. Sample sizes refer to the number of photographs taken and colonies successfully sequenced at each time point. (**c**) Bleaching severity in *Acropora hyacinthus* observed across reef habitats. A bleaching score of 1 indicates stark white bleaching and 5 indicates dark pigmentation (Supplementary Fig. S1c). Sample sizes refer to the number of colonies photographed and scored per month in each reef habitat

all symbiont types present in the coral host) between the two forenamed coral heat stress responses and across environmentally distinct reef habitats of *A. hyacinthus*. Associations between coral host and symbiont types can exhibit significant variation in response to abiotic factors and this partnership may be constrained by selective environmental variables, such as temperature and light intensity, that vary over small spatial scales across reef zones (Iglesias-Prieto et al. 2004; van Oppen et al. 2018; Dubé et al. 2021; Kriefall et al. 2022). We aimed to identify possible microalgal drivers for the observed variability in coral heat tolerance, including spatial and temporal changes in Symbiodiniaceae taxa. Addressing these questions will elucidate possible acclimatization mechanisms that coral survivors adopt when confronted with extreme heat stress.

Methods

Study site and sample collection

Mo'orea, French Polynesia is a volcanic island in the Southern Pacific Ocean surrounded by a barrier reef system (Fig. 1a). Water temperature data (Fig. 1b) were collected at one site on the north shore outer reef, approximately 1 km west of Cook's Bay, as part of the Mo'orea Coral Reef Long Term Ecological Research (MCR LTER) core time series data collection (Leichter et al. 2020). A bottom-mounted thermistor (Seabird SBE 39) attached at 10 m depth recorded water temperatures at 20-min intervals. Cumulative heat stress (in °C-days) was calculated as a 12-week running sum of average daily temperatures exceeding 29 °C, the maximum monthly mean (MMM) and a noted bleaching threshold for corals in Mo'orea (Pratchett et al. 2013), from November 1, 2018, to October 31, 2019.

In May 2019, during the height of the bleaching event, SCUBA divers surveyed one site on the north shore of Mo'orea (17.4731° S, 149.8176°W (forereef) and 14.4751° S, 149.8170° W (backreef); Fig. 1a) to identify bleached and healthy *Acropora hyacinthus* coral colonies in three reef habitats: backreef (~ 3 m depth), shallow forereef (~ 5 m depth), and deep forereef (~ 14 m depth). Individual colonies were photographed, and small branches (~ 2–4 cm length) were collected from all *A. hyacinthus* colonies encountered and preserved in 100% ethanol. Colonies from the shallow and deep forereef habitats were tagged for future sampling. On the deep forereef in May 2019, we found no *A. hyacinthus* colonies without some degree of bleaching (Leinbach et al. 2021). By August 2019, all the previously tagged bleached colonies in the deep forereef had died. Despite this high mortality, August surveys on the deep forereef (~ 14 m depth), which occurred after the period of accumulated thermal stress (Fig. 1b), identified previously bleached, untagged colonies that were observed to be visibly recovering from bleaching (Supplementary Fig. S1b). These new colonies were photographed, tagged, and sampled. Due to the high prevalence of bleaching at this site in May, we maintain that these previously untagged colonies were bleached during the bleaching event. In October, tagged colonies at both forereef depths were resampled and photographed. Untagged colonies in the backreef were also sampled and photographed. Bleaching severity and colony size for all sampled corals were determined using standardized photographs taken during the surveys. Each colony was assigned an integer score from 1 to 5 based on a visual evaluation of bleaching severity, where 1 indicates stark white bleaching and 5 indicates no bleaching with dark pigmentation (Fig. 1c, Supplementary Fig. S1c). The outline of each coral colony was traced in ImageJ to calculate planar surface area as an estimate of colony size (Schneider et al. 2012). See Supplementary Table S1 for more details on the number of colonies photographed, sampled, and sequenced from each reef habitat at each time point.

DNA extraction and ITS2 amplicon sequencing and analysis

Genomic DNA was extracted from samples (see Supplementary Tables S1 and S2 for more detailed sample sizes) using a sodium dodecyl sulfate (SDS) digestion protocol (Lundgren et al. 2013, Supplementary Methods) and cleaned with the Zymo Research Genomic DNA Clean and Concentrator-10 kit. For each sample, 900 ng of DNA was sent to the Georgia Genomics and Bioinformatics Core at the University of Georgia for sequencing. ITS2 amplicon libraries were generated using the Symbiodiniaceae-specific primers SYM_VAR_5.8S2 and SYM_VAR_REV (Hume et al. 2018) and sequenced on the Illumina MiSeq platform with 250-bp paired-end reads (Supplementary Methods).

To characterize Symbiodiniaceae taxa, raw reads from each sample were submitted directly to the analytical framework SymPortal (https://symportal.org) (Hume et al. 2019). SymPortal algorithmically searched for re-occurring sets of ITS2 sequences, called defining intragenomic variants (DIVs), and used their presence and abundance in each sample to predict ITS2 type profiles, which are representative of putative Symbiodiniaceae taxa. The final outputs from SymPortal used in downstream statistical analyses included files of ITS2 type profile sequence abundances for all coral samples and files separated by major Symbiodiniaceae genus of principal coordinate analysis (PCoA) coordinates conducted on Bray–Curtis indices (Supplementary Methods).

Statistical analyses

Statistical analyses of Symbiodiniaceae alpha and beta diversity were conducted on SymPortal outputs in R version 4.0.3. ITS2 type profile reads were normalized using trimmed mean of M-values (TMM) in the package edgeR (Robinson and Oshlack 2010) to account for differences in sequencing depth. The 21 resultant ITS2 type profiles from SymPortal were then collapsed into seven more conservative groupings based on PCoAs of their Bray–Curtis indices (Supplementary Fig. S2). We chose to further collapse the type profiles so as not to inadvertently overestimate Symbiodiniaceae diversity. The groupings were determined based on their distribution along PC1. All subsequent analyses were conducted on the collapsed ITS2 type profiles.

Alpha diversity was measured by calculating ITS2 type profile richness for each sample. Generalized linear mixed-effects models (GLMMs) were employed to examine the effects of heat stress response, reef habitat, colony health (defined as “bleached” or “healthy”), and month on richness, with a random effect of colony identity included. A linear mixed-effects model (LME) was used to analyze the relationship between richness and colony size. Poisson regressions were also utilized to investigate richness differences over time in colonies that were sampled over multiple months.

Multivariate statistics were used to examine beta diversity, specifically community structure, defined as the relative abundance of sequencing reads for each collapsed ITS2 type profile. Permutational analyses of variance (PERMANOVAs), via the *adonis* function in the package vegan (Okansen et al. 2020), were utilized to explore how coral heat stress response, reef habitat, colony health, and month impacted symbiont community structure. Additional iterations of these analyses were conducted on individual colonies that were sampled over multiple time points (referred to as “paired colonies”) to assess any changes in Symbiodiniaceae community within an individual over time. Pairwise comparisons were performed using the function *pairwiseAdonis* after any significant PERMANOVA results. Multivariate dispersion was quantified (function *betadisper*) using Bray–Curtis dissimilarities for community structure data. Differences in dispersion (i.e., if *betadisper* is significant) between samples can confound PERMANOVA results, resulting in a type I error when comparing symbiont communities. To ensure significant PERMANOVA results indicated true community differences, a bootstrapped sensitivity analysis was executed on any PERMANOVA results that showed significant heterogeneity of dispersion (Claar et al. 2020b; Supplementary Methods). All tests passed the sensitivity test.

Community structure data were visualized with non-metric multidimensional scaling (NMDS), using Bray–Curtis dissimilarities. Venn diagrams were created with the Eulerr package (Larsson 2018) to visualize shared Symbiodiniaceae ITS2 type profiles between heat stress responses, reef habitats, and colony health statuses. All model outputs and results are listed in Supplementary Table S3.

Results

Amplicon sequencing of 134 samples from 110 individual *A. hyacinthus* colonies yielded 12,491,776 sequencing reads, 8,379,219 of which passed the quality filtering in SymPortal (67.08%) (Supplementary Table S2). In total, we detected 21 ITS2 type profiles within our samples, which we further collapsed into seven more conservative type profiles (Supplementary Fig. S2). These ITS2 type profiles included representatives from three Symbiodiniaceae genera: *Symbiodinium*, *Cladocopium*, and *Durusdinium*.

Symbiont communities varied across reef habitats

Acropora hyacinthus colonies in the backreef, shallow forereef, and deep forereef were characterized by distinctive symbiont communities (PERMANOVA, $p = 0.001$; Fig. 2b; Supplementary Table S3a, b). Overall, colonies in the shallow forereef were almost exclusively associated with *Symbiodinium* A1 and colonies in the deep forereef were predominantly associated with *Cladocopium* C3ae (Fig. 2). Backreef colonies hosted highly variable symbiont associations, with many colonies (32/44, 72.72%) hosting more than one ITS2 type profile (Fig. 2a, d), often at high abundances. Furthermore, several backreef colonies (14/44, 31.82%) contained representatives from all three Symbiodiniaceae genera. A smaller proportion of colonies (9/44, 20.45%) associated with multiple ITS2 type profiles within the same genus. Backreef coral colonies exhibited significantly higher alpha diversity than both the shallow and deep forereef habitats (GLMM, $p_{\text{shallow}} = 0.0054$, $p_{\text{deep}} = 0.0014$; Fig. 2a; Supplementary Table S3l). There was no significant difference in alpha diversity between the two forereef depths (GLMM, $p = 0.84$) and the majority of forereef colonies housed only one ITS2 type profile, although mixed communities were observed (18/57, 31.58% in the shallow forereef; 7/33, 21.21% in the deep forereef) (Fig. 2a; Supplementary Table S4a). Coral colonies in the shallow forereef were dominated by *Symbiodinium* A1. However, 2/57 (3.51%) colonies were dominated by *Cladocopium* C3ae and 8/57 (14.04%) colonies contained observable levels of *Durusdinium* D1. Mixed-genera symbiont assemblages were observed in 17/57 (29.83%) samples and four of these housed multiple ITS2 type profiles from the same genera. In the deep forereef, *Cladocopium* C3ae was the predominant type profile in 30/33 (90.91%) colonies, with the remaining three samples

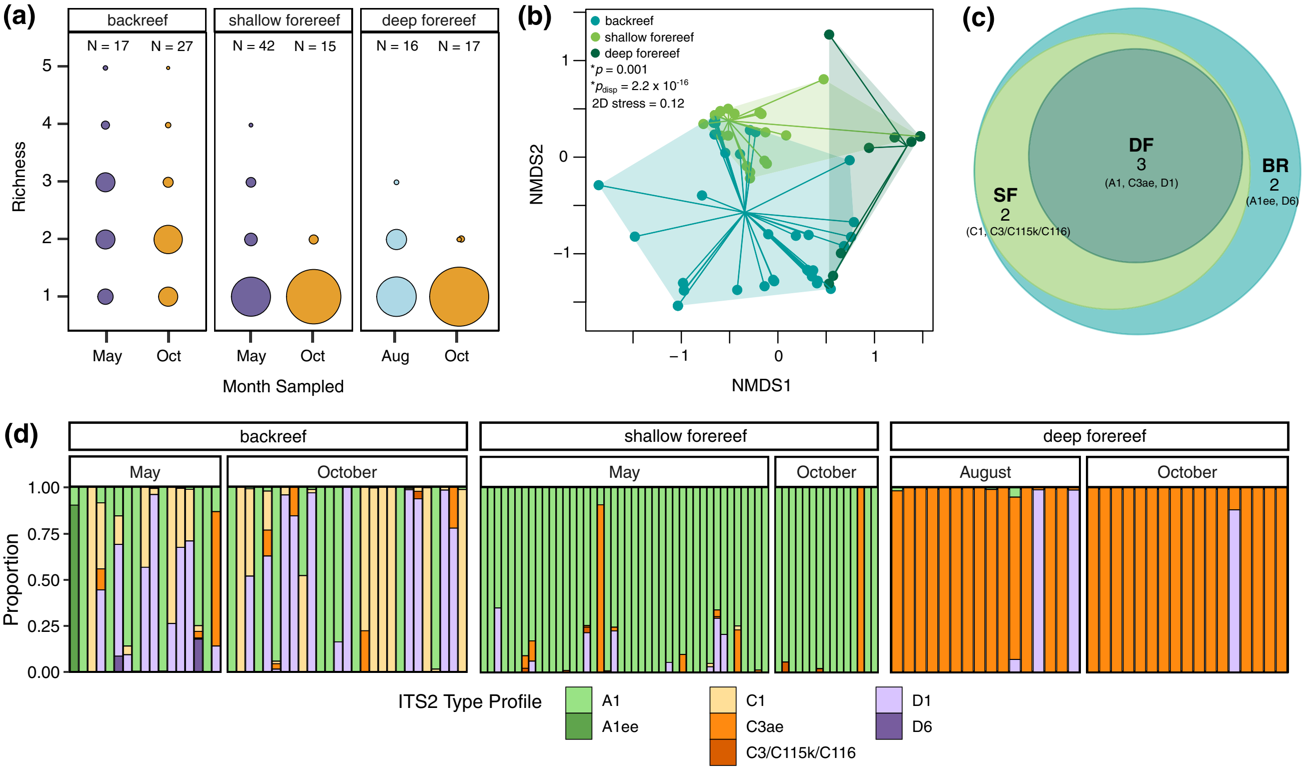

Fig. 2 Symbiodiniaceae communities across three reef habitats. (**a**) ITS2 type profile richness per colony across reef habitats and sampling time points. Size of each circle is scaled to the proportion of colonies in each reef habitat–month combination that host a given number of ITS2 type profiles. (**b**) Non-metric multidimensional scaling (nMDS) of symbiont community structure based on ITS2 type profiles. Statistical significance for PERMANOVA (*p*) and multivariate dispersion (p_{disp}) is denoted with an asterisk. (**c**) Venn diagram of the number and identity of ITS2 type profiles present in colonies found in each reef habitat. (**d**) Normalized relative proportion of ITS2 type profiles from colonies across backreef, shallow forereef, and deep forereef habitats over the sampling time points

hosting a *Durusdinium* D1 majority (Fig. 2d). Five of the *Cladocopium*-dominated colonies in August (5/16, 31.25%) supported mixed-genera symbiont assemblages and one colony housed Symbiodiniaceae from all three observed genera (Fig. 2d). Colonies in the backreef contained two unique ITS2 type profiles (A1ee, D6) that were not found in forereef colonies (Fig. 2c).

Within each reef habitat, we observed no significant shifts in Symbiodiniaceae community composition over time (PERMANOVAs, $p_{\text{backreef}} = 0.85$, $p_{\text{shallow}} = 0.52$, $p_{\text{deep}} = 0.23$; Fig. 2d; Supplementary Table S3c). Additionally, during each sampling time point, symbiont community structure in corals across reef habitats remained significantly different from each other (PERMANOVA, $p_{\text{May}} = 0.001$, Supplementary Fig. S3a; $p_{\text{October}} = 0.001$, Supplementary Fig. S3b). Although colony size differed significantly between reef habitats—on average, backreef colonies were significantly smaller than forereef colonies from both depths, and deep forereef colonies were significantly smaller than shallow forereef colonies (Supplementary Table S3o)—it did not influence ITS2 type profile richness (GLMM, $p = 0.91$; Supplementary Fig. S4).

Relationship between symbiont assemblages and coral health depended on reef habitat

Healthy and bleached coral colonies sampled at the height of the thermal anomaly in May showed no significant difference in symbiont community structure (PERMANOVA, $p = 0.18$; Fig. 3b). However, we found divergent patterns when considering the backreef and shallow forereef separately. In the backreef, healthy and bleached colonies did not support significantly different symbiont communities (PERMANOVA, $p = 0.81$; Fig. 3d; Supplementary Fig. S5b). Healthy backreef corals generally contained fewer profiles than bleached colonies, but this trend was not significant (GLMM, $p = 0.25$; Fig. 3a; Supplementary Table S4b) and the majority of colonies (13/17, 76.47%) housed more than one ITS2 type profile (Figs. 3a, d). Conversely, in the shallow forereef, healthy and bleached corals' symbiont communities were significantly different (PERMANOVA, $p = 0.001$; Fig. 3d; Supplementary Fig. S5a). Healthy shallow forereef colonies also hosted significantly fewer ITS2 type profiles compared to bleached conspecifics (GLMM, $p = 0.00085$; Fig. 3a; Supplementary Table S4b). All healthy

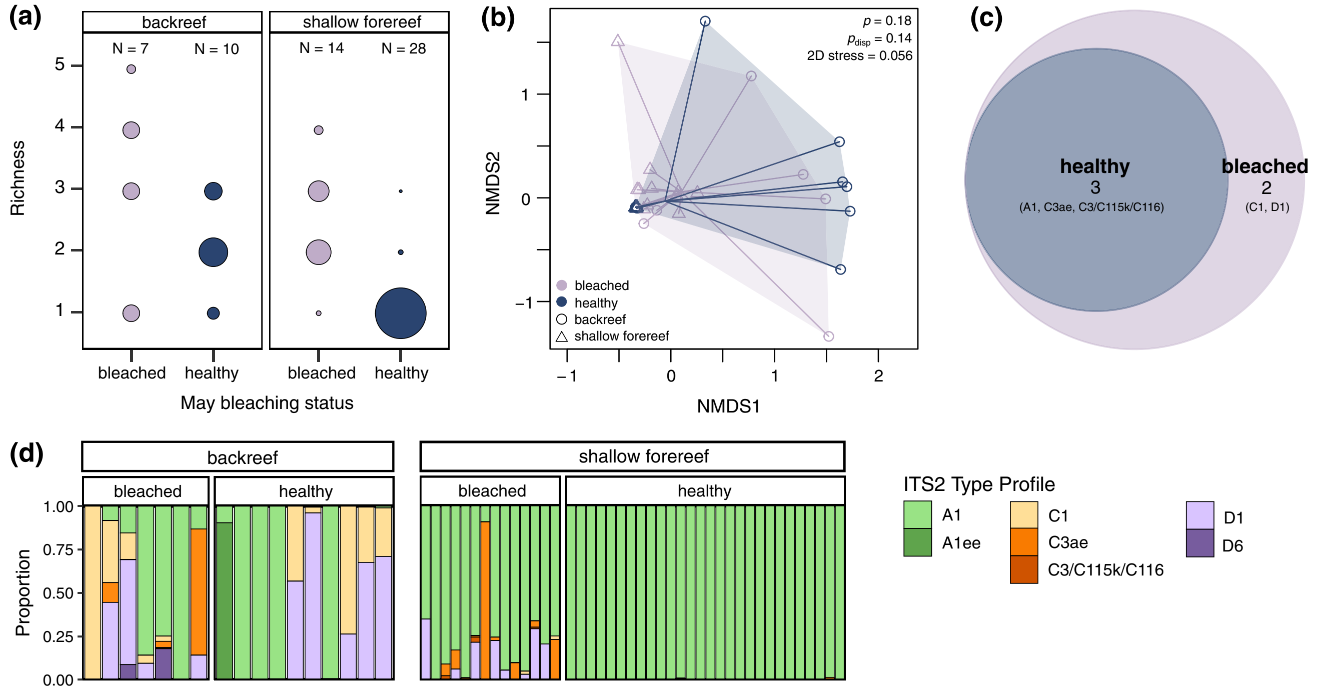

Fig. 3 Symbiodiniaceae communities in healthy and bleached colonies sampled during May in the backreef and shallow forereef. (**a**) ITS2 type profile richness per colony for healthy and bleached colonies found in two reef habitats. Size of each circle is scaled to the proportion of colonies in each reef habitat-bleaching status combination that host a given number of ITS2 type profiles. (**b**) Non-metric multidimensional scaling (nMDS) of symbiont community structure based on ITS2 type profiles. Statistical significance for PERMANOVA (*p*) and multivariate dispersion (p_{disp}) is denoted with an asterisk. (**c**) Venn diagram of the number and identity of ITS2 type profiles present in healthy and bleached colonies from the shallow forereef in May. (**d**) Normalized relative proportion of ITS2 type profiles from bleached and healthy colonies in the backreef and shallow forereef habitats

corals in the shallow forereef were dominated by *Symbiodinium* A1, whereas bleached corals tended to primarily affiliate with *Symbiodinium* A1 but also contained *Durusdinium* D1 and *Cladocopium* type profiles at lower abundance (Fig. 3d).

There was one unique type profile found only in healthy colonies (A1ee) and one unique type profile found only in bleached colonies (D6) (Supplementary Fig. S5c). Among backreef colonies, one ITS2 type profile was unique to healthy colonies (A1ee), and three were unique to bleached colonies (C3ae, C3/C115k/C116, D6) (Supplementary Fig. S5d). On the shallow forereef, there were no unique type profiles in healthy colonies and bleached colonies housed two unique ITS2 type profiles (C1, D1) (Fig. 3c).

Symbiont communities differed between coral heat stress responses

We found significant differences in symbiont community composition and structure between the two observed heat stress responses: resistance and recovery (PERMANOVA, $p=0.001$; Fig. 4b). Resistant colonies, which were found exclusively in the shallow forereef, were consistently dominated by *Symbiodinium* A1, whereas recovered corals, which we documented in both the shallow and deep forereef, displayed more flexibility in symbiont association. Recovered colonies were largely dominated by *Cladocopium* C3ae (32/36, 88.89%), but some colonies were found instead to host *Symbiodinium* A1 (1/36, 2.78%) or *Durusdinium* D1 (3/36, 8.33%) (Fig. 4d). There was no significant difference in alpha diversity between heat stress responses (GLMM, $p=0.20$; Fig. 4a; Supplementary Table S4c). Recovered colonies hosted two unique ITS2 type profiles (C1, D1) and resistant colonies hosted one unique type profile (C3/C115k/C116) (Fig. 4c).

Tagging of some resistant and recovered coral colonies allowed us to investigate if there were differences within an individual colony between sampling time points. We found no significant change in symbiont community structure within an individual throughout the study period (PERMANOVAs, $p_{\text{recovered}}=0.71$, $p_{\text{resistant}}=0.48$; Fig. 5; Supplementary Table S4d). Except for one individual (colony ID 324, Fig. 5a), all colonies were dominated by the same ITS2 type profile in both of their respective sampling time points.

Discussion

We examined Symbiodiniaceae assemblages in *Acropora hyacinthus* colonies displaying two distinct heat stress

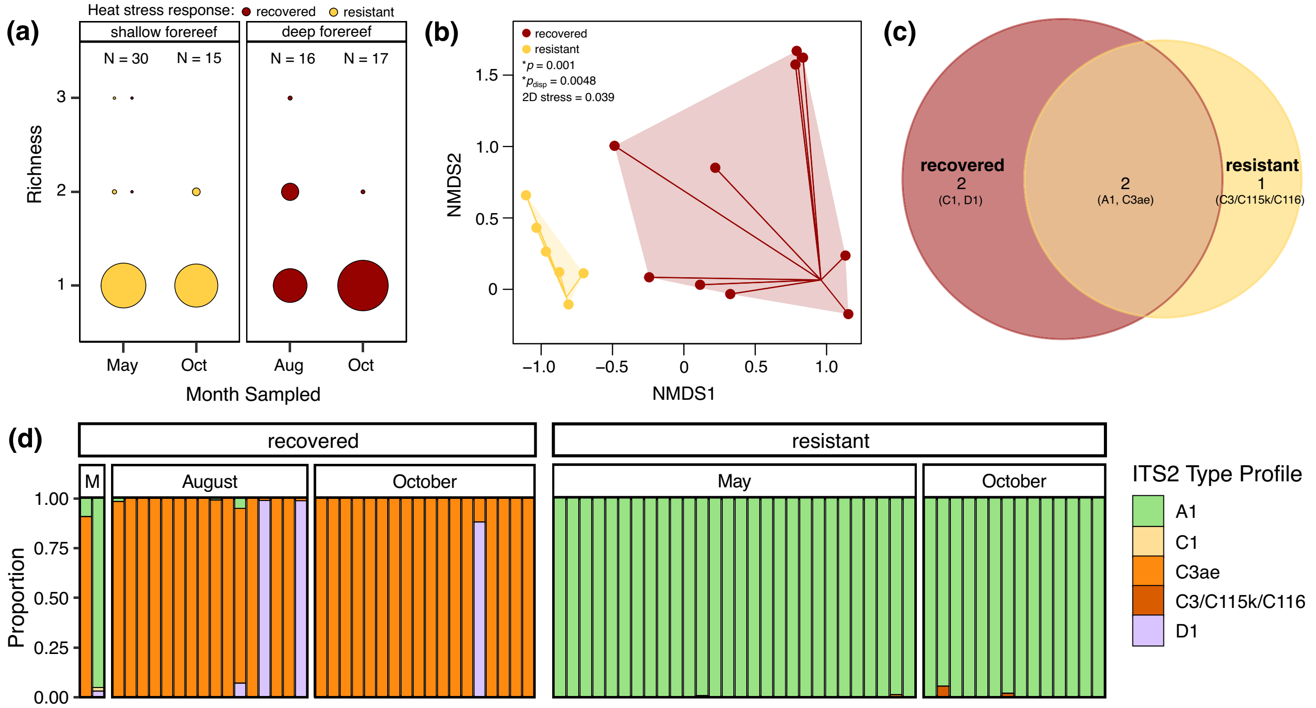

Fig. 4 Symbiodiniaceae communities in recovered and resistant colonies. (**a**) ITS2 type profile richness per colony across reef habitats and sampling time points. Size of each circle is scaled to the proportion of colonies in each reef habitat–month combination that host a given number of ITS2 type profiles. (**b**) Non-metric multidimensional scaling (nMDS) of symbiont community structure based on ITS2 type profiles. Statistical significance for PERMANOVA (p) and multivariate dispersion (p_{disp}) is denoted with an asterisk. (**c**) Venn diagram of the number and identity of ITS2 type profiles present in resistant and recovered colonies. (**d**) Normalized relative proportion of ITS2 type profiles from resistant and recovered colonies over the sampling time points. M represents May

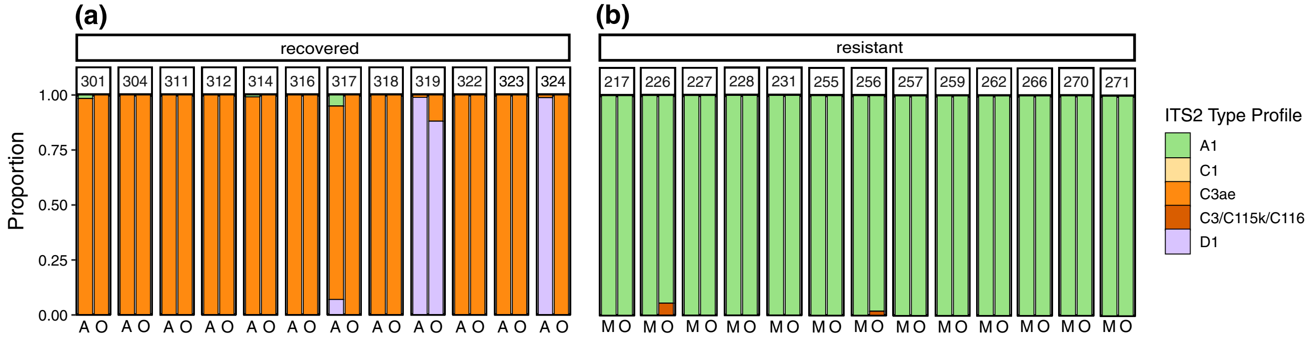

Fig. 5 Normalized relative proportion of ITS2 type profiles from paired (**a**) recovered (N = 12 pairs) and (**b**) resistant colonies (N = 13 pairs). M—May sampling time point**,** A—August sampling time point, O—October sampling time point. These resistant and recovered colonies were sampled on the shallow and deep forereef, respectively

responses and assessed the temporal stability of these relationships, including how they differ across reef habitats, in the context of a mass bleaching event. Previous studies have classified *Acropora* species as flexible with respect to Symbiodiniaceae associations (i.e., as symbiont generalists) (van Oppen et al. 2001; Putnam et al. 2012; Kriefall et al. 2022). Our results support this idea, as we found *Acropora hyacinthus* in Mo'orea harbored microalgal endosymbionts from multiple ITS2 type profiles distributed across three genera, including some colonies hosting multiple genera simultaneously. Our findings also reveal that, despite coral host–Symbiodiniaceae flexibility within a reefscape, dominant Symbiodiniaceae type profile was strongly linked to both reef habitat and holobiont propensity for thermal resistance or recovery. Further, there was covariance between these different responses to the heat anomaly and the reef habitat in which the colonies were found; resistant colonies were exclusively found in the shallow forereef, while recovered colonies were almost exclusively found in the deep forereef. Therefore, we are unable to disentangle the relative contribution of reef habitat and bleaching history to *A. hyacinthus* in hospite microalgal assemblages, but our results suggest a prominent role of local environmental conditions in structuring symbiont communities and, in turn, host response to heat stress.

Intraspecific coral holobiont responses to heat stress can vary across reef habitats and are influenced by abiotic factors including light intensity, water temperature and temperature variability, and water flow (McClanahan et al. 2005; Hoogenboom et al. 2017; Schoepf et al. 2020). We documented divergent patterns of bleaching severity and recovery in *A. hyacinthus*, with colonies residing in the deep forereef exhibiting more extensive bleaching and mortality than those in the backreef and shallow forereef (Fig. 1c). Observations of prior bleaching events in Mo'orea noted similar spatially heterogenous bleaching susceptibilities. For example, during bleaching events in 1994, 2002, and 2007, coral assemblages at shallow depths displayed less severe bleaching than those at deeper depths (Penin et al. 2007, 2013). Previous studies ascribed these results to an interplay between hydrodynamic conditions and differential acclimatization and/or adaptation of coral–algal symbioses, but did not investigate specific mechanisms (Penin et al. 2007, 2013). We extend these conclusions by demonstrating that *A. hyacinthus* colonies hosted distinct symbiont communities within each reef habitat (Fig. 2). *Acropora hyacinthus* acquires its symbionts via horizontal transmission and thus may be able to form symbioses with the endosymbionts best adapted to local environmental conditions (Buddemeier and Fautin 1993; Van Oppen et al. 2001; Quigley et al. 2017). Because Symbiodiniaceae types can confer different physiological traits to the coral host, this resultant symbiont zonation may enable coral holobionts to survive over a gradient of environmental conditions, in particular light, temperature, and nutrient availability, that can vary over small spatial scales (Rowan 2004; Frade et al. 2008; Dubé et al. 2021; Kriefall et al. 2022).

The deep forereef (~ 14 m depth) receives the lowest irradiance out of all three reef habitats we sampled (Dubé et al. 2021), and colonies located there were predominantly associated with *Cladocopium* C3ae (Fig. 2d). Some *Cladocopium* species are known to be more photosynthetically efficient than other Symbiodiniaceae and are thus frequently observed in symbiosis with coral colonies at depth, where light intensity is lower (Cooper et al. 2011; Eckert et al. 2020; Wall et al. 2020). Alternatively, in the shallow forereef (~ 5 m depth), which is characterized by a higher irradiance (Dubé et al. 2021), the vast majority of colonies were dominated by *Symbiodinium* A1. Some *Symbiodinium* species are capable of several photoprotective mechanisms, including the production of UV-adsorbing mycosporine-like amino acids (MAAs) and upregulation of alternative photosynthetic electron pathways (Banaszak et al. 2000; Reynolds et al. 2008), explaining why *Symbiodinium* is chiefly found in coral hosts in shallow waters where light levels are high (Rowan and Knowlton 1995; Reynolds et al. 2008). Backreef environments are characterized by high irradiance, temperature, temperature fluctuation, and nitrogen concentration (Kriefall et al. 2022), and as such, they may present the most stressful conditions for corals. A prior investigation of *A. hyacinthus* in Mo'orea before the 2019 thermal anomaly found that the backreef constrained Symbiodiniaceae diversity compared to the forereef (Kriefall et al. 2022), whereas we observed the opposite trend, suggesting that the heat stress and concordant bleaching event may have disrupted coral–algal associations. We found significantly higher alpha diversity in colonies on the backreef compared to both forereef depths (Fig. 2a) and highly variable symbiont community compositions (Fig. 2d). Such symbiont variation could be a form of bet-hedging, allowing corals to exploit different physiological traits of multiple symbiont taxa and thus enhance survival in the dynamic backreef environment (Loram et al. 2007; Torda et al. 2017). Because backreef colonies were significantly smaller than those on the forereef (Supplementary Fig. S4; Supplementary Table S3o), it is possible that they were younger and had not yet finished the winnowing process to establish a dominant symbiont type. Although some colonies were below the accepted size threshold for maturity in *A. hyacinthus* (~ 7 cm diameter) (Wallace 1985), we found no correlation between colony size and number of ITS2 type profiles (Supplementary Fig. S4), indicating that the increased alpha diversity of backreef colonies is linked to some other factor, such as environmental conditions, not an artifact of coral life stage.

Symbiont zonation, in addition to reflecting environmental constraints, is also related to host genetic structuring (Bongaerts et al. 2010; Brazeau et al. 2013). The reef-wide patterns of Symbiodiniaceae community diversity and composition we observed may thus reflect underlying host differentiation, which we did not address in this study. Kriefall et al., however, found high gene flow between adjacent reef habitats and no evidence for *A. hyacinthus* genetic structuring in Mo'orea (Kriefall et al. 2022). They also found no evidence for host genetic variation correlating with symbiont associations. Cryptic species of *A. hyacinthus* have also been uncovered throughout the Pacific Ocean, including in Samoa, Palau, Australia, and Japan (Ladner and Palumbi 2012; Fifer et al. 2022), but no such finding has yet come to light in Mo'orea. If there is indeed no genetic partitioning within our study system, this points to an underlying environmental driver of symbiont structuring and suggests that the distinct symbiont communities we observed between heat stress responses partitioned by depth and reef habitat may be an emergent property of reef-wide distribution of symbionts that are adapted to their environment.

At the height of the bleaching event in May 2019, all colonies within a reef habitat hypothetically experienced similar thermal stress, yet we observed significantly different Symbiodiniaceae community structures between healthy and bleached *A. hyacinthus* colonies on the shallow forereef (Fig. 3d; Supplementary S5a), suggesting that factors beyond the prevailing reef habitat conditions mediate the distribution of in hospite symbiont communities. Bleached colonies harbored higher alpha diversity compared to healthy conspecifics and housed symbiont communities that more closely resembled the variable mixed assemblages found in backreef colonies (Figs. 3a, d). This pattern could potentially be explained by the Anna Karenina principle (AKP), which posits that stressors induce stochastic changes in microbial community composition, leading to microbiomes of dysbiotic individuals exhibiting higher dispersion than those in healthy individuals (Zaneveld et al. 2017). Bleached backreef colonies also tended to host more ITS2 type profiles than healthy backreef colonies (Fig. 3a), but we did not detect significant differences in symbiont diversity or structure between them. This may be due to the decreased statistical power resulting from a smaller sample size for backreef colonies compared to shallow forereef colonies; thus, the AKP may be operating in the backreef as well as the shallow forereef and could, to some degree, explain differences in symbiont communities in our study compared to those documented by Kriefall et al. (2022). The thermal anomaly and subsequent bleaching in 2019 may have impaired host mechanisms to regulate and constrain symbiont assemblages (Moeller and Peay 2016; Zaneveld et al. 2017; Howe-Kerr et al. 2020), leading to increased variation and diversity in symbiont communities in bleached individuals. Alternatively, antagonistic interactions between diverse symbionts could have destabilized the coral–algal symbiosis and led to the observed bleaching (Miller 2007; Kenkel and Bay 2018; McIlroy et al. 2020), although our study is unable to disentangle whether the observed symbiont community variability is the result of thermal stress or the cause of differential bleaching phenotypes within a reef habitat. Because we observed both bleached and healthy individuals within a reef habitat, this points to additional factors influencing heat stress response and symbiont community composition, such as host microhabitat occupation, host genotype, or differential gene regulatory pathways involved in thermal physiology and symbiosis (Ganot et al. 2011; Barshis et al. 2013; Hoogenboom et al. 2017; Kavousi et al. 2020; Dilworth et al. 2021). Nonetheless, our results contribute to the expanding number of studies illustrating the AKP in the context of endosymbiotic dinoflagellates (Claar et al. 2020a, 2020b; Howe-Kerr et al. 2020).

Projections for the survival of future coral reefs often hinge upon colonies harboring thermally tolerant *Durusdinium* symbionts (Berkelmans and van Oppen 2006). However, our study adds to the growing body of literature demonstrating that enhanced bleaching tolerance is not universally associated with *Durusdinium* (Abrego et al. 2008; Howe-Kerr et al. 2020; Howells et al. 2020). We found that colonies resistant to bleaching in the shallow forereef were invariably dominated by *Symbiodinium* A1 (Fig. 4d). *Symbiodinium* A1 produces UV-protective MAAs and low amounts of hydrogen peroxide, a causative agent of coral bleaching, at elevated temperatures (Banaszak et al. 2000; Suggett et al. 2008), which could potentially contribute to the lack of bleaching we observed in the resistant colonies living on the shallow forereef. This is an interesting finding considering that *Symbiodinium* A1 generally enters into symbiosis with corals opportunistically, and thus resembles parasitism rather than mutualism (Stat et al. 2008). In previous experimental work that manipulated coral–algal combinations in *Acropora millepora,* colonies hosting *Symbiodinium* A1 had the lowest thermotolerance and fitness (Mieog et al. 2009). Conversely, *Symbiodinium* A1-A1v was the dominant symbiont type profile in *Acropora pulchra* thriving in a thermally extreme lagoon in New Caledonia (Camp et al. 2020), *Millepora* spp. in Belize dominated by *Symbiodinium* resisted bleaching (Schwiesow et al. 2021), and *Porites divaricata* in the Caribbean hosting *Symbiodinium* were able to swiftly acclimate to repeat bleaching (Grottoli et al. 2014). These contrasting reports suggest that the physiological costs and benefits of hosting *Symbiodinium* could be species- and/or location-specific and highlight the complexity of coral–algae symbiotic relationships, especially as they relate to thermal tolerance. We recommend that future studies further investigate the physiological ramifications of hosting *Symbiodinium* and its potential role in bleaching resistance.

Restructuring of symbiont communities or shifts in dominant symbiont type have been observed as a response to heat stress and proposed as a mechanism of host plasticity and adaptation (Buddemeier and Fautin 1993; Jones et al. 2008; Grottoli et al. 2014; Cunning et al. 2015). However, we did not observe significant temporal changes in symbiont community composition within colonies that varied in their heat stress responses or reef habitat locations during or after the thermal anomaly (Figs. 2, 4, 5). Although other studies have similarly reported temporally stable symbiont assemblages in *A. hyacinthus* after bleaching (Thomas et al. 2019), our study cannot definitively rule out the possibility that corals hosted different Symbiodiniaceae types before the bleaching event, as we did not monitor *A. hyacinthus* prior to peak thermal stress. Earlier surveys in Mo'orea conducted in 2013 found the majority of *A. hyacinthus* colonies in the backreef and forereef were dominated by *Cladocopium*, with only a very small proportion of colonies dominated by *Symbiodinium* (Kriefall et al. 2022). This is in stark contrast to our results, suggesting that the 2019 thermal anomaly, or another thermal event between 2013 and 2019, may have induced changes in symbiont communities or selected for colonies hosting *Symbiodinium*. Although we did not observe bleaching in the resistant forereef colonies, it is possible that a shift may have occurred without visible bleaching or before the sampling time point in May (Thornhill et al. 2006; LaJeunesse et al. 2009). *Symbiodinium* is commonly identified in corals recovering from bleaching or thermal stress (Toller et al. 2001), and thus, the resistant colonies we observed in the field could have potentially experienced bleaching outside the scope of our sampling regime.

Conclusions

Acropora spp. are considered to be thermally sensitive relative to other coral genera (Loya et al. 2001; Putnam et al. 2012); however, we identified individuals able to resist or recover from heat stress incurred by a severe thermal anomaly that resulted in high coral mortality (Leinbach et al. 2021; Speare et al. 2021). Intraspecific variation in coral–algal symbioses and bleaching susceptibility underpin coral adaptive potential, and surviving individuals can be harnessed for assisted evolution intervention methods used in reef restoration and management (Quigley et al. 2018; Suggett and van Oppen 2022). Understanding the spatial and temporal dynamics of coral–Symbiodiniaceae associations during and after bleaching events is crucial in identifying host–symbiont pairs that are both more tolerant to projected temperature increases and well suited for their surrounding reef habitat. Our work demonstrating flexibility of *Acropora hyacinthus* microalgal symbiont associations across a reefscape, but fidelity within a reef habitat and colony, indicates that these associations are at least partly influenced by local environmental conditions and ultimately contribute to response to heat stress. We also documented no significant temporal shifts in symbiont assemblages, including no reversion back to pre-bleaching communities (Kriefall et al. 2022), despite the cessation of thermal stress for several months, suggesting that novel coral–algal relationships may be maintained after thermal stress. At the site level, we observed a diverse pool of Symbiodiniaceae associating with *A. hyacinthus* colonies, including observations of symbioses with multiple Symbiodiniaceae taxa simultaneously. This suggests that it may be possible to enhance *A. hyacinthus* thermal tolerance, within the constraints of host–symbiont genotype compatibility, through the manipulation of symbiont types or diversity, where types could be selected based on holobiont thermal tolerance observed in each reef habitat. Moreover, we identified ITS2 type profiles that were specifically associated with resistant and recovered colonies, and healthy and bleached colonies. These symbiont associations may be diagnostic of heat stress response or bleaching susceptibility and could conceivably be developed into biomarkers for coral resilience or employed to augment thermal tolerance.

Although our study was limited to three reef habitats within one site in Mo'orea, it provides fundamental yet critical insight into natural symbiont dynamics in the field. In hospite symbiont assemblages are shaped by a complex interplay between prevailing local environmental conditions, acute and chronic stressors, symbiont physiology, and host factors. Future work should investigate symbiont flexibility and fidelity over larger spatial and temporal scales, and further characterize symbiont physiology and its relationship to thermal tolerance, especially in *Symbiodinium*, to provide a more nuanced view of host–symbiont combinations and their ability to withstand environmental perturbations, such as bleaching. The extent to which humans are able to manipulate specific symbiont types or assemblages represents a valuable path of future inquiry and action for coral reef management efforts, with the goal of promoting resistance and resilience to the anticipated impacts of anthropogenic global change.

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Data availability Raw sequence data have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA833391. All datasets and R code generated as part of this study are available on GitHub at the following link (https://github.com/sarahleinbach/hyacinthus_symbiont). Photographs available from the corresponding author upon request.

Declarations

Conflict of interests The authors declare that there is no conflict of interest.

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