Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change

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Dynamic symbioses may critically mediate impacts of climate change on diverse organisms, with repercussions for ecosystem persistence in some cases. On coral reefs, increases in heat-tolerant symbionts after thermal bleaching can reduce coral susceptibility to future stress. However, the relevance of this adaptive response is equivocal owing to conflicting reports of symbiont stability and change. We help reconcile this conflict by showing that change in symbiont community composition (symbiont shuffling) in *Orbicella faveolata* depends on the disturbance severity and recovery environment. The proportion of heat-tolerant symbionts dramatically increased following severe experimental bleaching, especially in a warmer recovery environment, but tended to decrease if bleaching was less severe. These patterns can be explained by variation in symbiont performance in the changing microenvironments created by differentially bleached host tissues. Furthermore, higher proportions of heat-tolerant symbionts linearly increased bleaching resistance but reduced photochemical efficiency, suggesting that any change in community structure oppositely impacts performance and stress tolerance. Therefore, even minor symbiont shuffling can adaptively benefit corals, although fitness effects of resulting trade-offs are difficult to predict. This work helps elucidate causes and consequences of dynamism in symbiosis, which is critical to predicting responses of multi-partner symbioses such as *O. faveolata* to environmental change.

1. Introduction

Symbiosis affects nearly every organism on the Earth [1], and may benefit organisms by expanding metabolic capabilities and realized niche space. Although high symbiont diversity may favour less mutualistic behaviour [2], the ability to associate with multiple partners may also allow hosts to access a wider array of potential benefits, exploit different partners that perform better in different environments and switch partners in response to environmental change [1,3]. This phenomenon of partner-switching may help symbiotic organisms cope with global climate change stressors and has been identified as a key mechanism by which reef corals—and the ecosystems they build—may survive in future climates [4].

Coral reefs are threatened in particular by rising temperatures, which cause mass coral bleaching events that have contributed to the loss of at least 19% of coral reefs worldwide [5,6]. However, corals’ susceptibility to thermal bleaching—the heat-induced breakdown of their symbiosis with single-celled dinoflagellate algae—is dependent on the identity of their symbiotic algal partners (different types within the genus *Symbiodinium*), which confer varying levels of heat tolerance to the symbiotic partnership [7–10]. Partner-switching following a bleaching event may occur by recovery with different symbionts that are better suited to the prevailing conditions [4,11], an idea termed the Adaptive Bleaching Hypothesis (ABH; ‘adaptive’ referring to a beneficial trait
that can be positively selected for; [12]). In particular, recovery with heat-tolerant symbionts can increase resistance to future thermal bleaching [8], but at a potential energetic cost [13–16]. Investigations of the ABH have revealed that, although corals sometimes change their symbionts [5,9,17–21], this does not always occur: sometimes corals recover with the same symbiont community they had prior to stress [22–26]. These conflicting reports have generated controversy surrounding the ABH and the potential role of partner-switching in coral responses to climate change.

Symbiont shuffling in some systems may be limited by biological factors such as host specificity for a particular type or subset of symbiont types [22], although many coral taxa are capable of associating with multiple types [27,28]. Alternatively, the drivers of (and constraints on) symbiont shuffling may operate through an ecological framework in which changing niche space, differential performance, direct or indirect competition, and succession [29] govern the interactions among symbionts that ultimately determine community composition. In this way, disturbance ecology may predict the circumstances under which communities change and to what degree. Indeed, corals need not completely replace one symbiont with another—their relative proportions may change more subtly, which may be a far more common response than wholesale community turnover.

Importantly, subtle changes in community structure may still have significant impacts on symbiosis function. Because symbiont types differ not only in heat tolerance, but also in photosynthetic performance [14,30], energetics [31] and associated coral growth rates [13,15], overall symbiosis function should be determined by the contributions of all symbionts [32]. However, the functional consequences of variation in symbiont community composition are poorly understood, in part because molecular methods to quantify mixed assemblages have only recently been developed [33,34]. Here, we apply these methods to conduct a quantitative investigation of the links between symbiont community structure and function, and the drivers of community change following disturbance.

We exploited natural landscape variation in symbiont communities within colonies of the Caribbean coral *Orbicella (=Montastraea) faveolata* [17] to experimentally investigate the effect of varying mixtures of two *Symbiodinium* types (B1 and D1a) on photophysiological performance and bleaching severity in response to low (7 days), medium (10 days) or high (14 days) thermal stress (32°C) exposure. We then monitored changes in symbiont community composition during recovery at two different temperatures (24 and 29°C) [16]. We tested the hypotheses that disturbance severity and recovery temperature determine the trajectory of symbiont community reassembly, with the prediction that more severe bleaching and a warmer recovery environment promote shuffling towards heat-tolerant clade D-dominated communities. Our overall objective was to elucidate the causes and consequences of symbiont shuffling in reef corals under global climate change, with potential applications to other multi-partner mutualisms.

2. Material and methods

(a) Coral collection and preparation

Three colonies of *O. faveolata* were collected from Emerald Reef (25°40.45' N, 80°5.92' W), near Key Biscayne (FL, USA). Replicate cores (n = 22–37 per colony) were taken from each colony using a seawater-fed drill press equipped with a 2.5 cm diameter core drill bit. Variation in symbiont identity and abundance within colonies of this species [17] creates independence among replicate cores in terms of their symbiont communities, making them well suited for this study. Cores (n = 87 total) were mounted on ceramic Reef Plugs (Boston Aqua Farms, NH, USA) using CorAffix adhesive (Two Little Fishes, FL, USA) and then maintained in experimental aquarium at 24°C for two months to recover from coring and acclimate to the experimental environment.

(b) Experimental set-up

Experimental manipulations were conducted in an indoor semi-recirculating coral culture facility consisting of four 284 l fibreglass tanks supplied with sand-filtered, UV-treated seawater from Biscayne Bay. Temperature in each tank was set and maintained within ±0.5°C using SeaChill TR-20 heater/chillers (TECO US), and light (190–280 μmol quanta m⁻² s⁻¹) was provided by two 400 W metal halide lamps (IceCap Inc., USA) over each tank on a 12 L:12 D cycle. Corals were fed throughout the experiment two to three times per week with Reef Chili (Bulk Reef Supply). These conditions were maintained throughout the experiment, except for temperature, which was manipulated as described below.

(c) Experimental treatments

To produce corals that had bleached to varying degrees, we placed corals directly into a heat stress tank maintained at 32°C (approx. 1.5°C above local maximum monthly mean temperature, VAKFI; ndbc.noaa.gov) for either 7, 10 or 14 days. Although we had a single heated tank and exposed all corals to stress, this design maximized replication within treatments and ensured the only difference among them was exposure duration. Cores from each colony were randomly, evenly divided among the three treatments, and exposures were staggered such that all corals were removed from the heated tank at the same time. Corals then recovered for three months at either 24°C or 29°C (approx. 2.5°C below and above local annual mean, respectively; VAKFI; ndbc.noaa.gov), with two replicate tanks at each temperature.

(d) Chlorophyll fluorometry

The maximum quantum yield of photosystem II (Fv/Fm) was measured for each coral core prior to stress exposure and at the end of the bleaching treatment using an imaging pulse amplitude modulated fluorometer (Walz, Effeltrich, Germany). A saturating pulse was administered at 2800 μmol photons m⁻² s⁻¹ with an LED array at 460 nm for 800 ms. To ensure that dark-adaptation, measurements were taken at approximately 6.00, approximately 1 h before lights were turned on.

(e) Symbiont identification

At each sampling interval, coral cores were sampled by excavating tissue from a single polyp using the corner of a new razor blade. This tissue biopsy was then heated to 65°C for two months to recover from coring and acclimate to the experimental environment.

(f) Symbiont quantification

Each sample was assayed using quantitative PCR (qPCR) to target specific actin loci in *O. faveolata* and *Symbiodinium* in clades B, C
and D. Clade A assays performed on a subset of samples indicated that this symbiont was not present (data not shown). The O. faveolata assay included 150 nM OfavAcT (5'-CCCTGACAGAATATCGGA AAAAAAG-3') and 100 nM OtvActR (5'-TAACTGTCAGGAG GTGCCA-3'). and actin loci were amplified in duplicate 12.5 could be compared with actin assays to estimate actin locus GAAAGAA-3'.

adjusted the results of standard curves generated following the methods of Cunning & Baker [34]. Adjusted the results of standard curves generated following the methods of StepOnePlus software package with a set fluorescence threshold CAAGATCATTGC-MGB-3'. The clade C assay included 200 nM BActF (5'-CCATGCTTGTTCACCTCAA-3'), 300 nM BActR (5'-TGTCCGGAGATTTGTCGCA-3') and 100 nM BActProbe (5'-FA M-CTGTAACCCCTGTCACT-MGB-3'). The clade C and D assays were multiplexed, using the same primers and reaction conditions as described in Cunning & Baker [34]. Target specificity consisted of an initial incubation at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 10 s and 60°C for 1 min. Cycle threshold (Ct) values were calculated by the StepOnePlus software package with a set fluorescence threshold of ∆Rn = 0.01.

Presence of target DNA was indicated by amplification of two technical replicates and no target was detected in negative control reactions. Ct values for O. faveolata and Symbiodinium clade B were reduced by 5.74 and 5.41 cycles, respectively, to normalize differences in fluorescent signal intensity among assays, based on the results of standard curves generated following the methods of Cunning & Baker [34]. Adjusted Ct values were then used to calculate symbiont to host (S/H) cell ratios [33] using the formula 2\(^{[	ext{Ct}(\text{symbiont}) - \text{Ct}(\text{host})]}\), divided by the host ploidy ratio (1/2 [37]). DNA extraction efficiency ratio (0.828 [34]) and target locus copy number ratio (see below).

(g) Symbiont copy number estimation

Symbionts were isolated from one O. faveolata core containing only clade D symbionts and one containing a mixture of B and D, and counted with a haemocytometer. DNA was extracted from six separate aliquots of 100,000 cells from each core, and template proportional to 2000 cells was then quantified by qPCR (n = 24 technical replicates). Quantities were calculated using standard curves generated from a dilution series of target standards from 10\(^{6}\) to 10\(^{0}\) copies per reaction and assuming 95.5% DNA extraction efficiency [34]. From the core containing only clade D cells, copy number for the clade D actin locus was 3.04 ± 0.28 (s.e.m.), and therefore estimated at three copies per cell. Using this value, the number of clade D cells in the mixed sample was then subtracted from the total number of cells in the reaction (2000) to determine the remaining number of clade B cells. This quantity was used to calculate a copy number of 1.18 ± 0.16 (s.e.m.) for clade B cells, which we estimate to be one copy per cell.

(h) Host copy number estimation

Primers for a single-copy marker (‘SC’; AY395789) identified in the Orbicella species complex [38] were designed using Primer Express (Applied Biosystems), and tested using a dilution series of O. faveolata DNA (OfavsCF1 (5'-TCACTTTGCAGAGC AATGC-3'); OfavsCR1 (5'-GGCAATGTTGTACCCACCAGT-3')). Amplification efficiency of 98.4% indicated that the SC assay could be compared with actin assays to estimate actin locus copy numbers. For 12 to 14 samples from each colony, both the SC and actin loci were amplified in duplicate 12.5 µl qPCR reactions using SYBR Green MasterMix and the same thermal cycling conditions as above. The SC assay contained 900 nM OfavsCF1 and OfavsCR1 primers, and the actin assay contained 240 nM OtvActF and 160 nM OtvActR. The ratio of actin : SC was calculated using the formula 2\(^{[	ext{Ct}(\text{SC}) - \text{Ct}(\text{actin})]}\). The average actin : SC ratios were 13.89 ± 0.36, 7.27 ± 0.42 and 14.43 ± 0.37 (s.e.m.) for the three colonies, and thus values of 14, 7 and 14 copies per cell were used in subsequent calculations.

(i) Statistical analysis

All statistical analysis was performed in R v. 3.1.0 [39]. Total symbiont abundance in a sample was calculated as the sum of clade D and clade B S/H cell ratios and log-transformed as a response variable. Standard linear models were used to investigate how photochemical efficiency and total symbiont abundance were impacted by parent colony, initial symbiont abundance and proportion clade D, and severity of heat stress (i.e. low, medium or high exposure). Stepwise model selection in both directions determined the best model fits by minimizing the Bayesian information criterion using the ‘R MASS’ package [40], except for Fv/Fm during early recovery, for which models were formulated to test only for interactive treatment effects and chosen by backward selection. The factors impacting the proportion of clade D after recovery were analysed using a quasi-binomial generalized linear model (GLM) with logit link, fit by backward stepwise selection. The significance of factors in each statistical model is reported by partial F-tests in table 1. In order to analyse the contributions of individual variables to the response, fitted values were computed for individual variables with other terms fixed at particular values using the ‘R effects’ package [41]. Data and full R code are available at Dryad: http://dx.doi.org/10.5061/dryad.nf568.

3. Results

(a) Initial symbiont communities

Sequencing of ITS2 rDNA identified Symbiodinium B1 and D1a as the only symbiont types present in each colony, which we refer to as clade B and clade D, respectively. qPCR assays also only detected clades B and D. The initial mean symbiont abundance, measured as the total S/H cell ratio, in all corals was 0.090, or about one symbiont cell for every 11 host cells, although mean abundance also varied by colony (ANOVA, p < 0.0001; figure 1). Cores contained a range of mixtures of clade B and clade D Symbiodinium from 1.2–90.1% clade D (except for three cores in which only clade B was detected). Photochemical efficiency (Fv/Fm) at 24°C prior to heat stress had a mean value of 0.51 ± 0.04 (s.d.) and was negatively related to the proportion of clade D (b = −0.082; p < 0.0001; table 1 and figure 2).

(b) Bleaching phase

Low, medium and high thermal stress reduced Fv/Fm to mean values of 0.356 ± 0.005 (s.e.), 0.289 ± 0.004 and 0.274 ± 0.005, respectively. Fv/Fm in bleached corals (i.e. all corals after heat stress) was positively related to the proportion of clade D (b = 0.032; p < 0.001; figure 2). There was also a significant parent colony effect on Fv/Fm in bleached corals that was independent of the symbiont community (table 1).

Exposure to more severe thermal stress elicited a more severe bleaching response (i.e. symbiont loss), with low, medium and high stress treatments resulting in mean S/H cell ratios (and % declines relative to initial mean) of 0.033 (−63.5%), 0.025 (−71.8%) and 0.016 (−82.2%), respectively (figure 1). Because thermal stress severity was correlated with symbiont loss, the term ‘bleaching severity’ hereafter refers to the levels of both treatment and response. Additional factors influencing symbiont abundance after stress were
Table 1. Results of statistical models for each response variable. Shown are the multiple $R^2$ values, significant predictor variables in each model and results of partial $F$-tests comparing the full model with reduced models lacking each factor. The absence of any significant interactions between treatment and colony indicates that all colonies responded to treatments in the same way.

<table>
<thead>
<tr>
<th>response variable</th>
<th>$R^2$</th>
<th>predictor variable</th>
<th>$F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>initial $F_v/F_m$</td>
<td>0.4205</td>
<td>initial proportion of clade D</td>
<td>61.684</td>
<td>&lt;0.0001</td>
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<tr>
<td>$F_v/F_m$ after bleaching</td>
<td>0.7497</td>
<td>stress severity</td>
<td>86.012</td>
<td>&lt;0.0001</td>
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<td>bleached proportion of clade D</td>
<td>13.986</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>colony</td>
<td>11.946</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_v/F_m$ after 5 days' recovery</td>
<td>0.8707</td>
<td>colony</td>
<td>36.076</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>stress severity × bleached proportion of clade D</td>
<td>4.420</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>recovery temperature × bleached proportion of clade D</td>
<td>4.735</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_v/F_m$ after 12 days' recovery</td>
<td>0.7795</td>
<td>stress severity × recovery temperature × bleached proportion of clade D</td>
<td>3.307</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>total symbiont abundance after bleaching</td>
<td>0.7928</td>
<td>colony</td>
<td>67.847</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>stress severity</td>
<td>8.646</td>
<td>&lt;0.001</td>
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<tr>
<td>initial symbiont abundance × initial proportion of clade D</td>
<td>7.713</td>
<td>&lt;0.01</td>
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<td>total symbiont abundance after recovery</td>
<td>0.4781</td>
<td>colony</td>
<td>31.562</td>
<td>&lt;0.0001</td>
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<tr>
<td>stress severity</td>
<td>6.192</td>
<td>&lt;0.01</td>
<td></td>
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<tr>
<td>proportion of clade D after recovery</td>
<td>0.7822</td>
<td>colony</td>
<td>11.339</td>
<td>&lt;0.0001</td>
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<td>initial proportion of clade D</td>
<td>92.923</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>stress severity × recovery temperature</td>
<td>5.258</td>
<td>&lt;0.01</td>
<td></td>
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</table>

*For GLM of proportion clade D, a pseudo-$R^2$ value was calculated as $1 - (\text{residual deviance/null deviance}).

(c) Recovery phase

During early recovery (5 and 12 days), photochemical efficiency was significantly influenced by parent colony, the proportion of clade D in bleached tissues, stress severity and recovery temperature (table 1). After 5 days, values of $F_v/F_m$ remained depressed overall (mean $0.349 \pm 0.054$ s.d.), but were higher in corals recovering from lower stress and at 29°C. Clade D-dominated communities had higher $F_v/F_m$ than B-dominated communities in corals from the high stress treatment, but the performance advantage shifted towards clade B in corals recovering from low and medium stress, especially at 24°C (figure 4a). After 12 days, $F_v/F_m$ had recovered to a mean of $0.439 \pm 0.053$ (s.d.) and was still greater in B- than in D-dominated communities in corals from low and medium stress treatments. However, D-dominated communities performed as well as B-dominated communities in corals recovering from high stress at 24°C, and much better at 29°C (figure 4b).

Total symbiotic abundance after three months of recovery was influenced by parent colony and stress severity (table 1). Mean abundance after recovery was equivalent to initial values in corals that had bleached for 7 days ($0.095; p > 0.39$) and was significantly greater in corals that had bleached for 10 and 14 days (0.176 and 0.159; $p < 0.001$; figure 3). The proportion of clade D after recovery ranged from 0 to 0.991 (figure 5). Relative to initial values, the proportion of clade D increased in some cores and decreased in others, indicating that changes in symbiont community composition varied in magnitude and direction (figures 5 and 6). The proportion of clade D after recovery depended on the initial proportion of clade D, parent colony, and an interaction between stress severity and recovery temperature (table 1). High stress resulted in decreased proportions of clade D after recovery, especially at 29°C (figure 6). Corals with less than approximately 5% clade D experienced increases in the proportion of clade D in all treatments, but when initial levels were higher than approximately 10%, low and medium severity bleaching ultimately decreased the proportion of clade D, regardless of recovery temperature (figure 6).

4. Discussion

Our investigation of the ecological drivers of symbiont shuffling helps to resolve conflict surrounding the ABH by explaining why corals sometimes change their symbionts after bleaching, and sometimes they do not. We show that symbiotic dynamism in *O. faveolata* depends on initial community composition, severity of disturbance and recovery temperature. In particular, we show that changes from *Symbiodinium* clade B- to D-dominance occur only after severe bleaching and that these transitions are promoted by warmer recovery temperature. By contrast, low and medium severity bleaching cause the proportion of clade D to decrease (except when it is initially very low, less than 5–10%), such that B-dominated corals remain B-dominated, and some D-dominated corals become B-dominated. These results indicate that the magnitude and direction of symbiont shuffling depend on the
particular conditions of disturbance and recovery, and that differences in these factors may explain variable reports of symbiont community stability or dynamism in the literature. Furthermore, these data illustrate that quantitative community metrics, rather than qualitative identification of the dominant symbiont, are necessary to evaluate symbiont shuffling responses. Indeed, the quantification of

$$F_v/F_m$$

proportion of clade D

Figure 2. Photochemical efficiency ($F_v/F_m$) as a function of symbiont community composition before and after heat stress. Values of $F_v/F_m$ and regression lines are plotted against the proportion of clade D measured at the same time point for all corals before stress (black circles, solid line) and at the end of low (dark grey circles, solid line), medium (light grey circle, dashed line) and high (white circles, dotted line) thermal stress treatments (see statistical models in table 1). Model parameters indicate that the relationship between $F_v/F_m$ and proportion of clade D is negative in unstressed corals ($b = -0.082, p < 0.0001$) but positive in stressed corals ($b = 0.032, p < 0.001$), such that communities of only clade D are 15% less efficient than only clade B when unstressed, but 11% more efficient under stress.

Figure 1. Total symbiont abundance after different stress severity treatments and after recovery. Symbiont to host ($S/H$) cell ratios (mean and 95% data range) are shown at each time point for colony 1 (squares), 2 (diamonds) and 3 (triangles). Although mean abundances differed among colonies, the lack of a significant interaction between colony and treatment indicates that all colonies responded in the same way to bleaching and recovery (table 1). Horizontal black bars and shaded grey regions indicate the mean symbiont abundance ($\pm$ s.e.) in each treatment with other predictors fixed at mean values (means that do not share a letter are significantly different (Student’s t-test, $p < 0.01$)). Relative to the initial mean, symbiont abundance declined by 63.5%, 71.8% and 82.2% after low, medium and high bleaching severity, respectively. Corals that bleached more severely had higher total symbiont abundance after recovery, which may represent greater symbiont population growth (or reduction of host cells) after bleaching [42], and/or the shift towards dominance by clade D, which tends to be maintained at higher standing stocks [26,34].

Figure 3. Remnant symbiont abundance in bleached corals after heat stress as a function of initial symbiont abundance and proportion of clade D. Response values are the modelled effect of the interaction between initial abundance and proportion of clade D on the total symbiont to host cell ratio after bleaching across the approximate data range, with other predictor variables (colony and stress severity) fixed at mean values (see statistical model in table 1).

Figure 4. Photochemical efficiency during early recovery from bleaching. The contribution of symbiont community composition in bleached tissues to overall photochemical efficiency after 5 days (a) and 12 days (b) of recovery from heat stress was computed from statistical models (table 1) for each level of bleaching severity and recovery temperature. Solid, dashed and dotted lines represent low, medium and high bleaching severity treatments, respectively, while blue and red colours indicate 24°C or 29°C recovery temperature. Shading indicates 95% confidence intervals surrounding model predictions. Symbiont communities with lower proportions of clade D (i.e. more clade B) show higher performance during early recovery from low and medium severity bleaching, while clade D-dominated communities show higher performance in the most severely bleached corals, explaining why these communities eventually shuffle towards clade B or clade D, respectively.

particular conditions of disturbance and recovery, and that differences in these factors may explain variable reports of symbiont community stability or dynamism in the literature [8,9,17–21,23–26,43]. Furthermore, these data illustrate that quantitative community metrics, rather than qualitative identification of the dominant symbiont, are necessary to evaluate symbiont shuffling responses. Indeed, the quantification of
mixed assemblages here reveals that while wholesale community turnover may be rare, changes in symbiont community composition following disturbance may be commonplace.

The role of bleaching severity and recovery temperature in driving diverse symbiont shuffling responses may be understood within the context of disturbance ecology and niche differentiation between symbiont types [29]. These particular clade B and D symbionts outperform one another in less- and more-bleached tissues, respectively (as indicated by photochemical efficiency; figures 2 and 4), which may be owing to the different microenvironments characterizing coral tissues that have been bleached to varying degrees. Healthy (or mildly bleached) tissues with higher symbiont abundances may have lower light levels owing to symbiont self-shading [44,45] and fewer nutrients owing to resource competition [46,47], which may allow clade B to outperform clade D. By contrast, higher light and nutrient levels in more severely bleached tissues (with fewer symbionts) may favour clade D. Warmer temperatures further boost the performance advantage of stress-tolerant D symbionts during recovery (figure 4). In this way, variable coral tissue microenvironments post-bleaching, mediated by the remnant symbiont population size and external conditions, define the niche space in which differential performance of symbiont types drives variable trajectories of mixed communities repopulating their hosts.

Dominance in symbiont communities may be a passive outcome of higher growth rates of the better performer [48], active host selection of the better performer or active host selection based on genetic identity [49]. The significant statistical effect of parent colony on recovered symbiont community composition provides some evidence for the role of the host in structuring these communities [50], although this may simply indicate that particular colony-specific factors (e.g. pigmentation, tissue thickness and gene expression) slightly alter the relative performance among symbionts. Regardless, the overall patterns of symbiont performance and shuffling in response to variation in bleaching severity and recovery temperature were the same in all three colonies, suggesting that they may apply generally to this symbiotic system, and by analogy to other symbioses involving multiple, ecologically differentiated symbiont taxa.

In addition to addressing the ecological drivers of symbiont shuffling in corals, our findings also address its consequences. We demonstrate continuous quantitative links between symbiont community composition, performance and stress tolerance in the *Orcibella*–B1–D1a symbiosis, indicating that any change in community structure impacts symbiosis function. First, we show that higher proportions of clade D reduce photochemical efficiency in the absence of stress (figure 2), supporting the hypothesis that clade D is a poorer performing symbiont under these conditions [14]. However, under thermal stress, more clade D was linked to higher photochemical efficiency (figure 2), illustrating a trade-off between performance and stress tolerance. Moreover, bleaching severity was proportionally reduced as the relative abundance of clade D increased (figure 3), providing the first evidence that continuous variation in symbiont community structure predicts bleaching severity in mixed assemblages. Higher total symbiont abundance also caused more severe bleaching, especially in B-dominated communities (figure 3), supporting previous reports that excess algal symbionts exacerbate bleaching, possibly owing to higher levels of oxidative stress [34].

Understanding the causes and consequences of symbiont shuffling has important ecological implications for coral reefs.

Figure 5. Scatterplot of the proportion of clade D symbionts after bleaching and recovery versus initial values for each coral. The shape of each point corresponds to parent colony (1, square; 2, diamond; 3, triangle), the colour to recovery temperature (blue, 24°C; red, 29°C) and the fill to bleaching severity (dark, low; light, medium; no fill, high). Quadrants represent regions of clade B and D dominance (more than 0.5), such that points in the upper left quadrant changed from B to D dominance, and points in the lower right changed from D to B dominance. The diagonal line represents no change in community composition, such that points above the diagonal shuffling towards clade D, and points below the diagonal shuffling towards clade B.

Figure 6. Effect of bleaching severity and recovery temperature on symbiont shuffling. Lines predict the proportion of clade D following low (solid), medium (dashed) and high (dotted) severity bleaching, and recovery at 24°C (blue) or 29°C (red), as a function of initial proportion of clade D, with other predictor variables (colony) fixed at mean values (see statistical model in table 1). Lines represent means and shading represents the 95% confidence interval for the prediction. Quadrants represent regions of clade D and B dominance (more than 0.5), so that the upper left quadrant represents transitions from B to D dominance, and the lower right quadrant from D to B dominance. The diagonal line represents no change in symbiont community composition, such that data above the line represent shuffling towards clade D, and data below the line represent shuffling towards clade B.
Here, we show that heat-tolerant symbionts are likely to become dominant after severe bleaching in the *Orcibella–B1–D1a* symbiosis, especially when recovery temperatures are warmer. These transitions will increase thermostolerance, but the reduced performance of clade D may also reduce coral growth rates in some cases, causing a trade-off whose ecological consequences are difficult to predict [51,52]. Mild bleaching, on the other hand, may reduce the proportion of clade D symbionts, thereby accelerating the reversion of community dominance to the superior performer (e.g. clade B), which may persist indefinitely if warming temperatures do not lead to subsequent disturbance [53]. Persistence of clade D dominance may therefore require sustained warmer conditions or more frequent and severe disturbance [10,54], conditions that are likely to occur under future warming scenarios, but that also may vary significantly among reef locations [55]. Repetitive disturbances that vary in severity may cause oscillations in symbiont community composition and the persistence of more evenly mixed assemblages, which may serve to balance trade-offs between performance and stress tolerance. In this way, direct links may be drawn between environmental variability and coral performance by understanding the causes and consequences of symbiont shuffling. While our data advance this effort, the establishment of such links in predictive models will require further quantitative investigation of symbiont shuffling under more sets of conditions and in other coral species, which may not be as dynamic as *O. faveolata* [53]. In addition, it will be important to understand how other aspects of environmental change, such as rising baseline temperatures, ocean acidification and eutrophication, may alter symbiont performance and symbiont shuffling.

In conclusion, this investigation helps reconcile previously equivocal findings regarding the role of ‘adaptive’ bleaching in coral responses to climate change by showing that symbiont shuffling is dependent on ecological context. We found that change in symbiotic communities following disturbance in the *Orcibella–B1–D1a* symbiosis was ubiquitous, though often subtle, and that bleaching severity and recovery temperature determined its magnitude and direction. Moreover, metrics of symbiosis performance and stress tolerance were continuous functions of symbiotic community composition. Together, these findings suggest that changes in the overall performance of symbiosis—which may have adaptive value—do not require wholesale symbiont turnover, but can result from commonly occurring, incremental shifts in community structure. Finally, these findings were only made possible through the application of quantitative molecular techniques and metrics (e.g. qPCR and S/H cell ratios), emphasizing their critical role in understanding symbiotic community dynamics in corals with potential applications to other mutualisms.

Data accessibility. All data and R code to reproduce analyses and figures are available at Dryad: http://dx.doi.org/10.5061/dryad.nzs6h.

**Authors’ contributions.** R.C., R.N.S. and A.C.B. designed the study; R.C. and R.N.S. performed experiments; R.C. analysed samples and data; R.C., R.N.S. and A.C.B. wrote the paper.

**Competing interests.** We declare we have no competing interests.

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