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Nitrogen Identity Drives Differential Impacts of Nutrients on Coral Bleaching and Mortality

Deron E. Burkepile,[1,2]* Andrew A. Shantz,[1] Thomas C. Adam,[2] Katrina S. Munsterman,[1] Kelly E. Speare,[1] Mark C. Ladd,[1] Mallory M. Rice,[1] Leïla Ezzat,[1] Shelby McIlroy,[3] Jane C. Y. Wong,[3] David M. Baker,[3] Andrew J. Brooks,[2] Russell J. Schmitt,[1,2] and Sally J. Holbrook[1,2]

[1]*Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, Santa Barbara, California 93106, USA;* [2]*Marine Science Institute, University of California, Santa Barbara, Santa Barbara, California 93106, USA;* [3]*The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Hong Kong, People's Republic of China*

Abstract

Nitrogen pollution increases the susceptibility of corals to heat-induced bleaching. However, different forms of nitrogen (nitrate vs. ammonium/urea) may have different impacts on thermal tolerance of corals. We used an 18-month field experiment on the oligotrophic fore reef of Moorea, French Polynesia, to test how different forms of nitrogen (nitrate vs. urea) impacted coral bleaching. The experiment spanned two moderate thermal stress events in 2016 and 2017. Nitrate increased bleaching prevalence in *Acropora* by up to 100% and in *Pocillopora* by up to 60% compared to control corals. Urea exposure often had intermediate effects on bleaching (not different from either control or nitrate-exposed corals) in both taxa. Importantly, nitrate prolonged bleaching in both *Acropora* and *Pocillopora* as nitrate-exposed corals remained bleached even after thermal stress ended, while control and urea-exposed corals had mostly recovered. Nitrate exposure also increased the prevalence of partial mortality in *Pocillopora* colonies and more than tripled the number of colonies that completely died. Our data are the first to show contrasting effects of different forms of nitrogen on coral bleaching and mortality in a natural reef environment, linking previous patterns from large-scale correlative studies with results from more mechanistic laboratory experiments. Most importantly, we showed that corals exposed to nitrate exhibited more frequent bleaching, bleached for longer duration, and were more likely to die than corals in low nitrogen conditions. Exposure to excess nitrogen, particularly anthropogenic nitrogen, may lower the temperature threshold at which corals bleach, triggering bleaching events on polluted reefs even when typical thermal stress thresholds have not been crossed.

Key words: nutrient pollution; climate change; coral reef; eutrophication; symbiosis; anthropocene.

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**Corresponding author; e-mail:* deron.burkepile@lifesci.ucsb.edu

Introduction

Climate change is increasing the frequency and intensity of temperature anomalies that drive coral bleaching, the dissociation of the mutualism between the coral animal and the endosymbiotic algae Symbiodiniaceae (Frieler and others 2013; Heron and others 2016). These bleaching events can cause widespread coral mortality and changes in coral species composition as species that are more sensitive to bleaching perish (Loya and others 2001; Grottoli and others 2014; Hughes and others 2017). Given that coral bleaching is becoming more frequent (Hughes and others 2018), it is critical to understand what factors can either mitigate or exacerbate the prevalence and intensity of bleaching (Cacciapaglia and Van Woesik 2015; Sully and others 2019).

Nutrient pollution is a major global change driver that has increased to such an extent over the last century that anthropogenically derived nutrients dwarf natural nutrient sources (Vitousek and others 1997; Bennett and others 2001). These nutrients dramatically alter coastal marine systems, particularly oligotrophic systems such as coral reefs. Excess anthropogenic nutrients can negatively impact coral reproduction, growth, and survivorship (D'Angelo and Wiedenmann 2014; Shantz and Burkepile 2014) and intensify coral diseases (Bruno and others 2003; Vega Thurber and others 2014). Recent experiments have shown a direct link between excess nutrients, especially excess nitrogen (N), and coral bleaching in both laboratory (Wiedenmann and others 2013) and field experiments (Vega Thurber and others 2014; Wang and others 2018). At larger scales, data from the wider Caribbean and Great Barrier Reef show positive correlations between water-column nitrogen concentrations and coral bleaching (Wooldridge and Done 2009; Wagner and others 2010).

Current hypotheses suggest that increased N availability to corals makes them more susceptible to bleaching due to a variety of mechanisms (Wooldridge 2009; Cunning and Baker 2013; Wiedenmann and others 2013). However, both field and laboratory experiments show these effects may depend on the source and type of N (Shantz and Burkepile, 2014; Ezzat and others 2015). A recent meta-analysis showed that nitrate enrichment often impeded coral growth, whereas ammonium did not (Shantz and Burkepile 2014). Based on differences in how ammonium versus nitrate enrichment affected carbon acquisition and allocation in corals, Ezzat and others (2015) hypothesized that under thermal stress, nitrate enrichment should weaken the coral–dinoflagellate symbiosis, whereas ammonium could be beneficial. The different forms of N also often have different sources: Fish excretion delivers ammonium and urea, whereas anthropogenic pollution tends to deliver more nitrate (Shantz and Burkepile 2014; Allgeier and others 2017). These different nutrient sources often have contrasting effects on coral growth with nutrient enrichment via fish excretion enhancing coral growth and human-derived nutrients suppressing coral growth (see review by Shantz and Burkepile 2014). Thus, different forms of nitrogen (nitrate vs. ammonium/urea) and different sources of nitrogen (anthropogenic vs. fish-derived) may have fundamentally different effects on coral biology and physiology, which may lead to contrasting effects on how they alter the susceptibility of corals to bleaching. Yet, it is unclear how results from laboratory experiments examining the impact of different forms of N on coral performance translate into effects under real-world conditions with natural regimes of temperature, light, and water flow.

Here, we used an 18-month field experiment to test the role of nitrogen identity on the dynamics of coral bleaching on the oligotrophic fore reef in Moorea, French Polynesia. We exposed portions of reef to either nitrate or urea and tracked the bleaching responses of $\sim$ 3000 colonies of *Pocillopora*, *Acropora*, and *Porites* spp. corals. During our study, sea surface temperatures increased above bleaching thresholds during the 2016 and 2017 Austral summers. Our experiment was a straightforward test of two ideas: (1) that N exposure can increase the prevalence, intensity, and/or duration of coral bleaching and (2) that the form in which N is delivered affects the nature of the bleaching response. We hypothesized that nitrate exposure would intensify bleaching and mortality in species that are known to be susceptible to thermal stress (that is, *Pocillopora* and *Acropora*) and induce moderate bleaching in more resistant species (that is, *Porites*). In contrast, we expected that urea exposure would not exacerbate, and possibly mitigate, coral bleaching.

Materials and Methods

Experimental Setup

Our study was conducted on the shallow fore reef on the north shore of Moorea, French Polynesia (17°30′S, 149°50′W) during 2016–2017. The fore reef in Moorea is relatively oligotrophic with low levels of dissolved inorganic nitrogen (DIN)

(0.28 ± 0.19 μM; mean ± SE) and soluble reactive phosphorus (SRP) (0.14 ± 0.05 μM; mean ± SE) (Alldredge 2019), making this an excellent setting to test the effects of increased N availability on coral bleaching. In January 2016, we established a field manipulation to test how different forms of N (nitrate vs. urea) impact coral bleaching. These forms of N are readily used as a nutrient source by corals and their symbionts (Grover and others 2003, 2006; Ezzat and others 2015). We used urea instead of ammonium, which is more commonly used in laboratory experiments (for example, Béraud and others 2013; Ezzat and others 2015), because a slow-release fertilizer containing only ammonium was not readily available when we established our experiment. However, both ammonium and urea are common forms of N that are excreted by fishes and available to corals (Crandall and Teece 2012; Allgeier and others 2017). The experiment encompassed two seasonal cycles (summer to winter) during 2016 and 2017 to capture patterns of thermal stress, bleaching, and recovery in three coral genera (*Pocillopora*, *Acropora,* and *Porites* spp.).

We exposed the areas of reef at depths of 10–12 m to either slow-release nitrate or urea fertilizers. Following our previously successful enrichment studies (for example, Vega Thurber and others 2014; Zaneveld and others 2016), nitrate-only fertilizer (polymer-coated potassium nitrate, Multicote 12-0-44, Haifa Chemicals Ltd.), urea-only fertilizer (polymer-coated urea, Apex 39-0-0, JR Simplot Company), or no fertilizer (controls) were loaded into 4 cm diameter PVC tubes that had 10–15 ~ 5 mm diameter holes drilled in them and were wrapped with window-screen (hereafter ''nutrient diffusers''). Because the potassium nitrate (12% N) and urea (39% N) had different percentages of N, we used 200 g of nitrate fertilizer and 62 g of urea fertilizer in each nutrient diffuser to standardize the amounts of N in each treatment. The nutrient diffusers were secured to the benthos with cable ties attached to either stainless steel all-thread posts or stainless steel eyebolts that had been drilled into the reef framework and epoxied in place.

The most common corals were *Acropora* spp. (primarily *Acropora retusa*, *Acropora hyacinthus*, *Acropora globiceps*), *Pocillopora* spp. (primarily *Pocillopora verrucosa*, *Pocillopora meandrina*, and *Pocillopora eydouxi*), and the *Porites lobata* complex, hereafter *Acropora*, *Pocillopora*, and *Porites,* respectively. Plots were selected haphazardly on the reef in an effort to include all three genera, when possible, within a 0.5 m radius of the nutrient diffuser. Our total replication of the treatment plots was nitrate $n = 70$, urea $n = 63$, and control $n = 67$. However, not all plots contained all of the coral taxa, making replication different for each taxon (see Table S1 for complete details of replication). *Pocillopora* was present in each plot making replication $n = 70$ for nitrate, $n = 63$ for urea, and $n = 67$ for control with an average of ~ 12 colonies per plot. For *Acropora*, replication was $n = 35$ for nitrate, $n = 32$ for urea, and $n = 40$ for control with an average of ~ 2 colonies per plot. For *Porites*, replication was $n = 59$ for nitrate, $n = 55$ for urea, and $n = 65$ for control with an average of ~ 2 colonies per plot. To test whether the presence of the PVC diffusers influenced coral bleaching, we put empty PVC diffusers on $n = 22$ control plots. All diffusers were separated by at least 1–2 m and spread over an area of ~ 11,000 m^2 of reef. Nutrient diffusers were exchanged every 10–12 weeks to ensure continuous delivery of N based on concentrations of N exiting the diffusers (Figures 2 and S1). To track temperature, we installed two thermistors at our site that measured seawater temperature at 2-min intervals from January 2016 until September 2017.

Quantifying Nitrogen Concentrations in Enrichment Treatments

To examine the dynamics of N released from the nutrient diffusers, we sampled the water from a subset ($n = 5$) of urea, nitrate, and control plots at weekly intervals over a 10-week period, beginning at the start of a fresh deployment of the fertilizer. Divers used 150-ml syringes to slowly draw water from ~ 3 cm away from nutrient diffusers. Immediately after collection, samples were placed on ice, returned to the laboratory, filtered (GF/F) into acid-washed bottles, and then used for nutrient analysis (ammonium) or frozen for later analyses (nitrate and urea). Ammonium concentrations were analyzed within 24 h using the fluorometric method following the methodologies of Schaus and others (1997) as modified by Whiles and others (2009). Frozen samples were kept at − 20°C until processing for nitrate and urea at the University of California Santa Barbara (UCSB). Nitrate concentrations were analyzed using a flow injection analyzer at the Marine Science Analytical Laboratory at UCSB. Urea concentrations were analyzed using the spectrophotometric methods in Revilla and others (2005).

Stable Isotope Analysis of Coral Nutrient Content

In early March 2017, before coral bleaching began, we collected small fragments of *Acropora retusa* and *Pocillopora verrucosa* colonies to determine the stable isotope signature of N in the host and symbiont tissues across the treatments (n = 24–30 per taxa per treatment) to examine whether corals were experiencing the N exposure and incorporating N into their tissues. Stable isotopes can serve as indicators for sources of N incorporated into biological tissues as the δ^{15}N of synthetic fertilizers tends to be much lower than that found in natural nutrient sources (Kendall and others 2007). Fragments were returned to the lab where the coral tissue was removed via an airbrush and small amounts of deionized water and frozen. Later, the thawed tissue samples were centrifuged to separate and clean the symbiont cells from the host tissues by their difference in densities. Host and symbiont fractions were then frozen, lyophilized, ground, and weighed into Sn capsules. Samples of each of the nitrate and urea fertilizers were also lyophilized, ground, and weighed into Sn capsules in order to assess the isotopic values of each fertilizer. Samples were analyzed on an EA-IRMS [Eurovector elemental analyzer—Nu Instruments Perspective IRMS] to measure δ^{15}N of each sample. All isotope analyses were conducted at SIRMS, University of Hong Kong. An in-house acetanilide standard was run between every 5–8 samples and showed an analytical precision of $< \pm 0.11‰$.

Quantifying Coral Bleaching and Mortality

We surveyed coral bleaching and tissue mortality on all of the *Acropora*, *Pocillopora*, and *Porites* colonies within a 0.5 m radius of the control plots or nutrient diffusers. For each coral, we recorded whether bleaching or tissue mortality was present, and if so, estimated the percentage of the colony surface area that was affected by each. Because corals undergo natural, seasonal variation in Symbiodiniaceae content that can affect their coloration (Fitt and others 2000), we defined bleached tissue only as tissue that had lost all pigmentation and appeared totally white. When quantifying tissue mortality, we included both recent mortality, when the exposed coral skeleton remained white and filamentous algae was just beginning to colonize the coral skeleton, as well as older mortality, when coral skeletons were heavily epiphytized and were no longer white. For consistency in bleaching estimations, the same two observers (DEB and AAS) collected all of the bleaching and mortality data.

Initial surveys were conducted in January 2016 before initiation of the treatments when bleaching and tissue mortality were virtually nonexistent across all corals (see ''Results''). We then surveyed the experiment during the bleaching event in early May 2016 and repeated the surveys in early July 2016 after seawater temperatures had cooled to document levels of bleaching, recovery, and mortality. In January 2017, we conducted bleaching surveys across a subset of the replicates to estimate levels of bleaching, which were very low, before the waters began to warm. We then surveyed bleaching during the warmest part of the year in late March 2017 and again in early July 2017 to document recovery from bleaching.

Statistical Analyses

To test whether N concentrations were different across treatments and time, we used a two-way ANOVA with treatment and time (weeks) as fixed effects followed by Tukey post hoc tests. Total N concentration was log-transformed to meet assumptions of normality. We analyzed the effect of N treatment on δ^{15}N values for each coral taxon separately using a mixed-effects model with treatment and tissue type as interacting fixed effects and included coral ID as a random effect to account for individual variation among corals.

We used generalized linear mixed-effects models to test the effects of N exposure on the prevalence (% of colonies affected per plot) of bleaching and tissue mortality in *Pocillopora* and *Acropora* corals. For each genus, we used a binomial regression with a logit link function. We included exposure treatment and survey date as interacting, fixed factors and plot as a random effect. Because there were no bleached *Acropora* in control or urea-exposed plots in July 2016, we dropped this time point from our bleaching analyses for *Acropora.* Significance of the N treatments was tested via likelihood ratio tests comparing the full model to a null model that included only survey date and the random plot effects. When there were significant differences, we conducted pairwise least-squared means post hoc tests corrected via Tukey's studentized range for multiple comparisons to assess the differing effects of N exposure on corals within time points (for example, among N treatments in May 2016). For *Porites,* the prevalence of bleaching and tissue mortality remained so low that meaningful statistical analyses were not possible (see ''Results'').

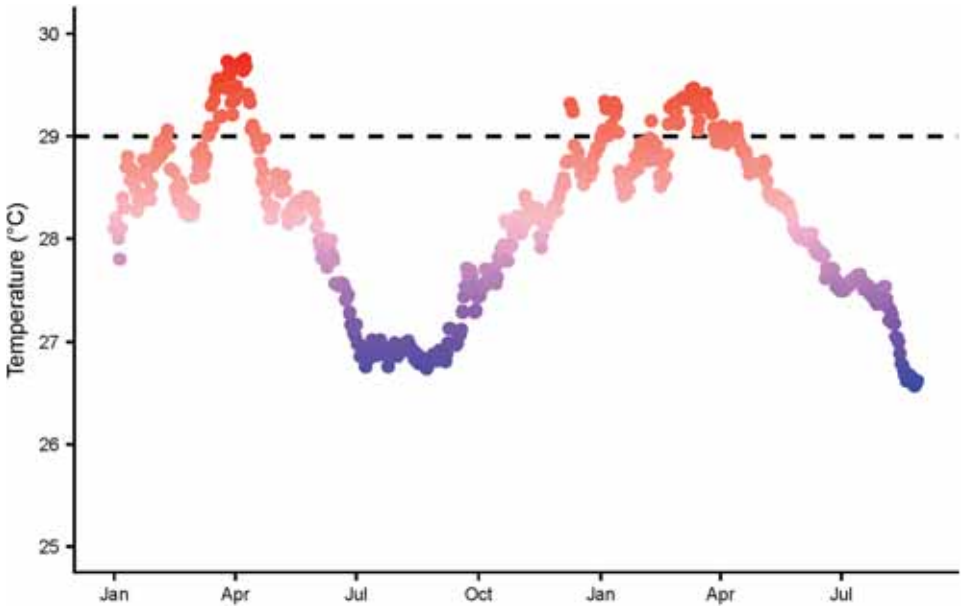

Figure 1. (Top) Plot of seawater temperature at experimental site for 2016–17. Individual data points represent average seawater temperature for each day taken from thermistor readings at 2-min intervals. Dashed line at 29°C denotes proposed temperature threshold for accumulating thermal stress for corals in Moorea (see Pratchett et al. 2013).

For corals that bleached, we tested the impact of N treatment on severity of bleaching (% of an individual colony that was bleached) using mixed-effects models. We included survey date and treatment as interacting, fixed factors and a random effect for plot. We calculated *p* values from Wald *F* tests using Satterthwaite approximate degrees of freedom to account for the unbalanced nature of the data (Luke 2017). For all of the models, we used log transformations to normalize the distribution of the residuals. Variances were equal for all models, but even after transformation, the residuals from our analyses of bleaching severity in *Pocillopora* remained non-normal. However, these analyses are relatively robust to deviations from normality.

In addition, we tested whether the proportion of *Pocillopora* colonies that completely died in a plot differed between N treatments. To do so, we divided the number of entirely dead colonies in each plot by the total number of colonies to calculate the proportion of dead colonies. We used a generalized linear mixed-effects model with a binomial distribution, as described previously, but we weighted values by the total number of colonies in each plot to test for differences in coral mortality between N treatments. There was no whole colony mortality for *Acropora* or *Porites*. All analyses were conducted in the *R* statistical environment (R Core Team 2018).

Results

Sea Surface Temperatures and Thermal Stress

During 2016, the daily average SST at our experimental site peaked in late March at 29.7°C (Figure 1). During March to May, SST was at or above 29°C, a temperature threshold that correlates well with thermal stress and coral bleaching in Moorea (Pratchett and others 2013), for a total of 45 days, including 37 consecutive days from mid-March to mid-April. By early July 2016, when we conducted our second round of bleaching surveys, SST was 27°C.

During 2017, the daily average SST peaked in mid-March at 29.5°C. SST and was at or above 29°C for a total of 76 days, including 53 consecutive days from late February to mid-April. By early July 2017 when we did our second round of bleaching surveys, SST was ~ 27.5°C. Corals in 2017 experienced more prolonged thermal stress than in 2016 with temperatures above 29°C for more total days and more consecutive days.

Patterns of Nitrogen Exposure

Both nitrate and urea diffusers increased the concentrations of N (including all sources of N—nitrate, ammonium, and urea) in the water versus the control plots over at least 10 weeks (Figure 2). Both nitrate and urea fertilizers delivered more N

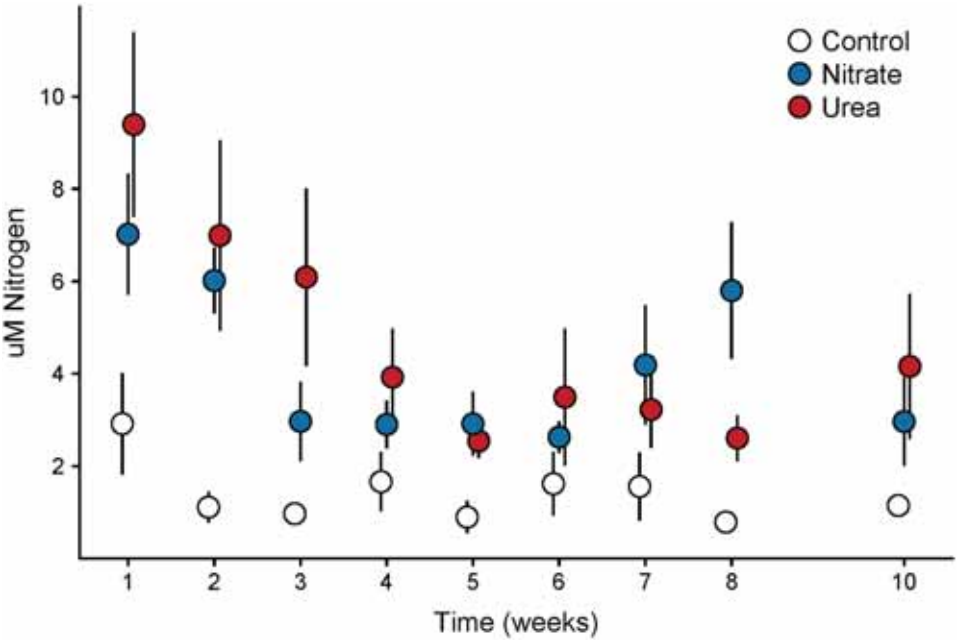

Figure 2. Total water-column nitrogen concentrations (including nitrate, ammonium, and urea) from the nitrogen enrichment treatments and controls over a 10-week period. Data are means ± SE. Note that these data are expressed as concentrations of N from all sources of N combined not as concentrations of the different types of N (that is, ammonium, nitrate, urea). N = 4–5 per treatment per sampling period. See Fig. S1 for concentrations of individual molecules (that is, ammonium, nitrate, urea) in the different treatments.

toward the beginning of their deployment, with the concentrations of N decreasing over time (time effect: $F_{8,104} = 3.56$, $p = 0.001$). There was a significant effect of treatment on N concentration (treatment effect: $F_{2,104} = 54.98$, $p < 0.001$) with control plots being lower than both nitrate and urea plots (Tukey HSD $p < 0.001$ for both comparisons) but no difference between nitrate and urea exposures (Tukey HSD $p > 0.99$). Also, there was no treatment *X* time interaction (interaction effect: $F_{16,104} = 0.94$, $p = 0.53$) suggesting that the relationship in N exposure among treatments was similar throughout diffuser deployment. As deployment progressed, the urea fertilizer appears to have been also releasing ammonium as well as a small amount of nitrate (Figure S1), which is not surprising given that urea is often rapidly converted to ammonium by microbes (Ferdie and Fourqurean 2004) and then likely oxidized to nitrate. Overall, however, the patterns of N exposure between the nitrate and urea treatments were similar during the duration of diffuser deployment.

Impact of Nitrogen Exposure on Coral Stable Isotopes

The nitrate fertilizer had a δ^{15}N isotope value of 1.6 ± 0.1‰, whereas the urea fertilizer had a value of − 1.6 ± 0.1‰. For *A. retusa,* there was a significant N fertilizer treatment effect ($\chi^2(2) = 57.47$, $p < 0.001$) and no effect of tissue type (coral vs. symbiont tissue: $\chi^2(1) = 0.01$, $p = 0.92$)) on δ^{15}N values (Figure S2). Post hoc tests showed that both nitrate ($p < 0.001$) and urea ($p < 0.001$) treatments were different than the control, whereas the nitrate and urea treatments were not different from each other ($p = 0.64$). For the δ^{15}N signature in P. *verrucosa*, we found a significant N treatment effect ($\chi^2(2) = 19.89$, $p < 0.001$) and a significant effect of tissue type ($\chi^2(1) = 153.86$, $p < 0.001$) (Figure S2). Post hoc tests revealed that the urea treatment was significantly different from control ($p = 0.002$) and nitrate ($p = 0.001$) treatments, although the nitrate treatment was not different than the control ($p = 0.96$).

Impact of Nitrogen Exposure on Prevalence of Coral Bleaching

Prior to enrichment in January 2016, there were no differences in the prevalence of bleaching among the treatments (*Pocillopora*: $\chi^2(2) = 0.626$, $p = 0.731$; neither *Acropora* nor *Porites* exhibited any bleaching before treatments began). However, nitrate exposure significantly increased bleaching prevalence (the proportion of colonies that had any

amount of bleached tissue) in both *Acropora* and *Pocillopora* (Figure 3A, B). Increased *N* did not impact *Porites*, which generally showed minimal bleaching (Figure 3C). By May 2016, there was a significant difference in bleaching prevalence among N treatments for both *Acropora* ($\chi^2(6) = 19.471$, $p = 0.003$) and *Pocillopora* ($\chi^2(8) = 131.46$, $p < 0.001$) (see Table S2 for full model results). Bleaching prevalence was almost double for nitrate-exposed *Acropora* versus controls (p = 0.043; Figure 3A, see Table S3 for post hoc results), while nitrate increased bleaching prevalence by 60% in *Pocillopora* ($p = 0.023$; Figure 3A). For both *Acropora* and *Pocillopora*, corals in the urea treatment exhibited an intermediate level of bleaching that was not different from either control or nitrate treatments. By July 2016, cooler water temperatures led to symbiont recovery in most corals; however, there were still important patterns among treatments in bleaching prevalence. For *Acropora*, only colonies in the nitrate treatment (5% of colonies) showed any bleaching. For *Pocillopora*, bleaching prevalence was 5–7 times greater in the nitrate treatment (17.1 ± 2.0% of colonies) than in either the control (3.3 ± 1.2%) or urea (2.4 ± 0.7%) treatments ($p < 0.001$ for both comparisons; Figure 3B). The PVC diffuser controls did not affect bleaching prevalence in *Pocillopora* ($\chi^2(4) = 4.233$, $p = 0.375$).

By January 2017, bleaching prevalence was again very low with ~ 1% of *Pocillopora* colonies exhibiting bleaching and no *Acropora* or *Porites* corals showing bleaching. By late March 2017, ~ 50% of all *Acropora* colonies were bleaching with no significant differences among treatments (Figure 3A). For *Pocillopora,* bleaching prevalence was significantly higher under nitrate enrichment (29.6 ± 2.1%) as compared to controls (16.6 ± 1.5%, $p < 0.001$) or urea-exposed corals (21.1 ± 2.0%, $p = 0.002$; Figure 3B). By July 2017, when water temperature had cooled significantly (Figure 1), bleaching prevalence in nitrate-exposed *Acropora* showed little change from March, remaining above 50% and almost 3 times higher

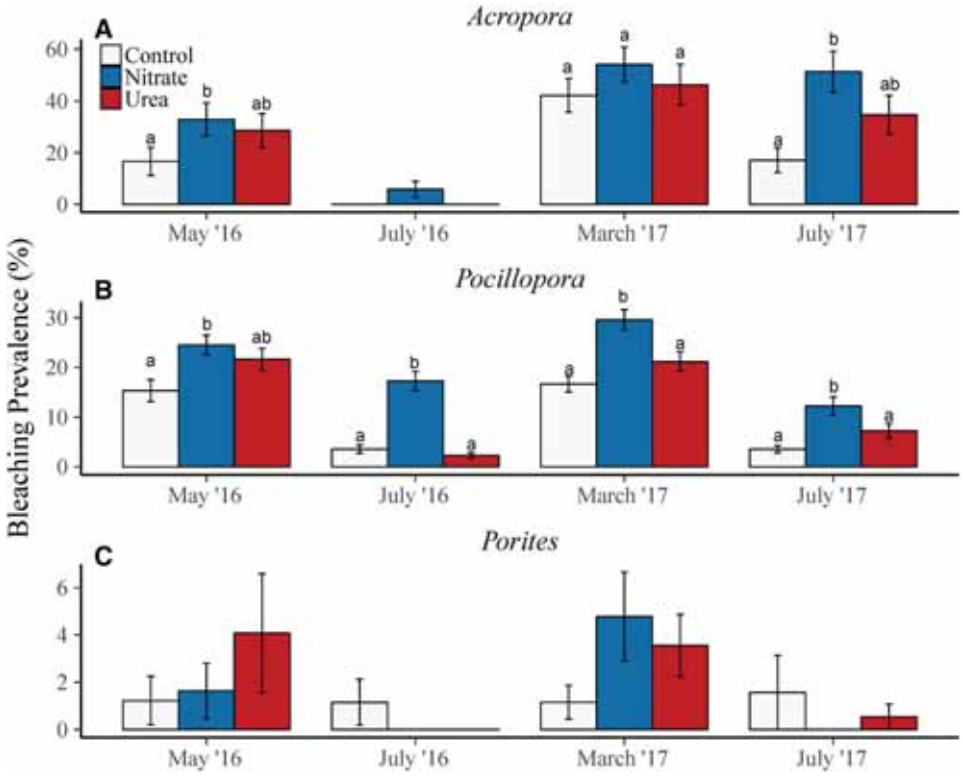

Figure 3. Prevalence of bleaching for **A** *Acropora*, **B** *Pocillopora*, or **C** *Porites* corals in the different nitrogen treatments across the different bleaching surveys in 2016–17. Prevalence was calculated as the percent of the number of individuals of each coral taxon per replicate of each nutrient treatment that exhibited any level of bleaching. Letters above bars denote significant differences among nitrogen treatments within survey periods. Statistics are from pairwise least-squared means post hoc tests corrected for multiple comparisons (see Tables S2 and S3 for statistics). No letters present indicate no differences among treatments within a survey period. Data are means ± SE. Note the differences in scale among the *Y* axes.

than in controls ($p < 0.001$) (Figure 3A). *Acropora* in the urea treatment exhibited intermediate levels of bleaching in July 2017 with no differences between either control or nitrate treatments. For nitrate-exposed *Pocillopora*, bleaching prevalence declined by over half by July 2017 but remained significantly higher than controls ($p < 0.001$) and urea-exposed corals ($p = 0.007$) (Figure 3B).

Impact of Nitrogen Exposure on Severity of Coral Bleaching

Bleaching severity (the percent of a colony that was bleached) varied across seasons with peaks during the warmest months for both *Acropora* ($\chi^2(2) = 110.32$, $p < 0.001$) and *Pocillopora* ($\chi^2(3) = 48.549$, $p < 0.001$) (see Table S4 for full model results). Yet, there was little impact of nitrogen enrichment on bleaching severity for any coral taxon. The sole exception was in *Pocillopora* in July 2016 where nitrate-exposed corals had twice the severity of bleaching than did control corals ($22.5 \pm 2.0\%$ vs. $10.4 \pm 2.1\%$ of individual colonies bleached, respectively; $p = 0.01$; see Table S5 for post hoc results) with urea-exposed corals showing an intermediate effect on bleaching.

Impact of Nitrogen Exposure on Coral Tissue Mortality

Prior to enrichment in January 2016, there was no incidence of tissue mortality on *Acropora* or *Porites* while $\sim 3.6\%$ of *Pocillopora* colonies had some partial mortality, although this did not differ across treatments ($\chi^2(2) = 2.845$, $p = 0.241$). As the experiment progressed, the impacts of N exposure on the prevalence of tissue mortality were dependent on coral species (Figure 4). For *Acropora*, the prevalence of tissue mortality gradually increased over the 2 years of the study (Figure 4A; see Table S6 for full model results and Table S7 for post hoc results) with no effect of N exposure ($\chi^2(6) = 5.99$, $p = 0.425$). Similarly, mortality in *Porites* generally increased over time, although mortality was not frequent enough for meaningful statistical analyses (Figure 4C).

In contrast, there were significant effects of both time and N exposure on the prevalence of mortality in *Pocillopora* ($\chi^2(8) = 78.332$, $p < 0.001$; Figure 4B). In May 2016, the prevalence of tissue mortality in *Pocillopora* was higher in nitrate-exposed corals than in control corals ($p < 0.001$; see Table S7 for post hoc results) and marginally higher than in urea-exposed corals ($p = 0.081$). By July 2016, around 30% of *Pocillopora* under nitrate exposure showed some tissue mortality, which was 2–3 times higher than for urea exposure ($p = 0.035$) or in controls ($p = 0.007$). By the end of the experiment, the prevalence of tissue mortality in *Pocillopora* was highest in nitrate-exposed corals and lowest in controls with urea-exposed corals being higher than control ($p = 0.039$) but lower than nitrate-exposed corals ($p = 0.009$).

Impact of Nitrogen Exposure on Pocillopora *Colony Mortality*

Complete colony mortality was observed only in *Pocillopora*, with a significant effect of N exposure on the proportion of dead corals ($\chi^2(8) = 33.458$, $p < 0.001$; Figure 5; see Table S8 for full model results). At the end of the experiment, there were significantly greater proportions of dead colonies in nitrate-exposed plots ($10.1 \pm 2.9\%$ of colonies) than in either control ($3.0 \pm 1.0\%$) or urea-exposed ($4.3 \pm 1.2\%$) plots (Figure 5; see Table S9 for post hoc results). This effect was not an artifact of how we initially set up the experiment, as the proportion of dead *Pocillopora* colonies was low in control ($2.0 \pm 0.5\%$), nitrate ($0.8 \pm 0.4\%$), and urea plots ($1.8 \pm 0.8\%$) before treatments began. The proportion of dead (relative to live) *Pocillopora* colonies appears to have decreased from July 2016 to March 2017, which may have been due to general bioerosion processes combined with a large wave event that impacted our study site in January 2017.

Discussion

Our 18-month field experiment showed how N identity affected the dynamics of coral bleaching and mortality. Nitrate exposure exacerbated bleaching prevalence and duration in both *Acropora* and *Pocillopora*. Urea exposure often resulted in intermediate effects on bleaching that were not different from either control or nitrate-exposed corals. *Acropora* corals exposed to nitrate were up to twice as likely to bleach compared to control corals in 2016. Yet, in 2017, when thermal stress was higher and bleaching was more prevalent, nitrate exposure did not affect bleaching prevalence in *Acropora* but did prolong bleaching duration. Similarly, *Pocillopora* exposed to nitrate were up to 60% more likely to bleach than control corals. Nitrate exposure also prolonged bleaching in *Pocillopora*, with over 75% of nitrate-exposed colonies continuing to show bleaching after the majority of control and urea-exposed corals had recovered. Although N exposure did not affect bleaching

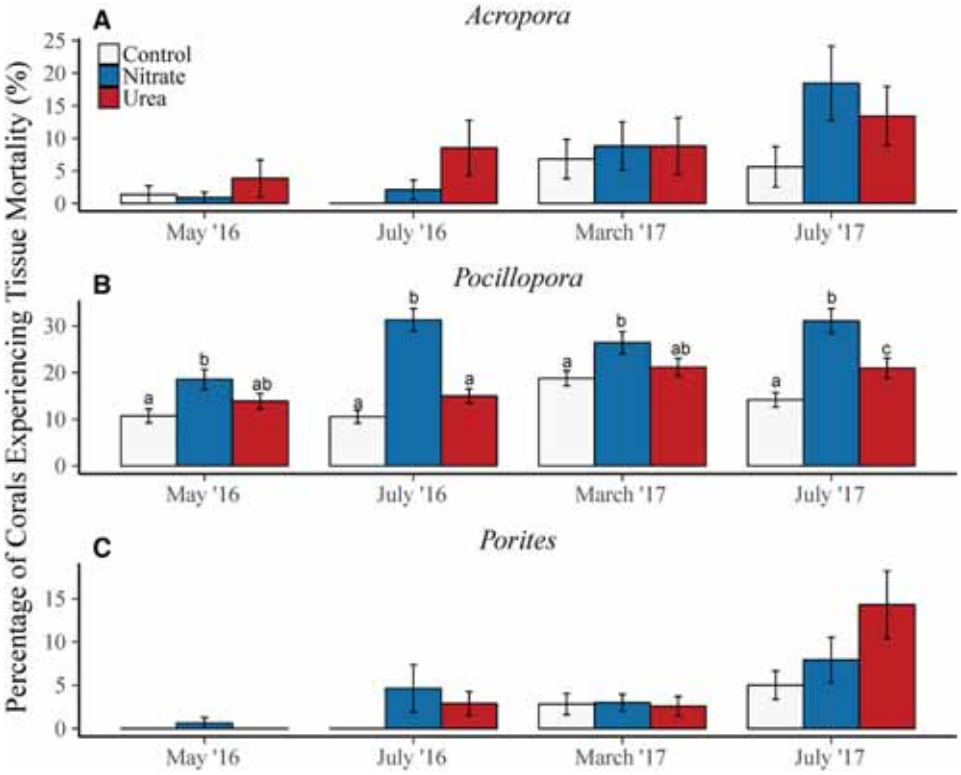

Figure 4. Prevalence of tissue mortality for **A** *Acropora*, **B** *Pocillopora*, or **C** *Porites* corals in the different nitrogen treatments across the different bleaching surveys in 2016–17. Prevalence of tissue mortality was calculated as the percent of the number of individuals of each coral taxon per replicate of each nitrogen treatment that exhibited any level of mortality. Letters above bars denote significant differences among nitrogen treatments within survey periods. Statistics are from pairwise least-squared means post hoc tests corrected for multiple comparisons (see Tables S6 and S7 for statistics). No letters present indicate no differences among treatments within a survey period. Data are means ± SE. Note the differences in scale among the *Y* axes.

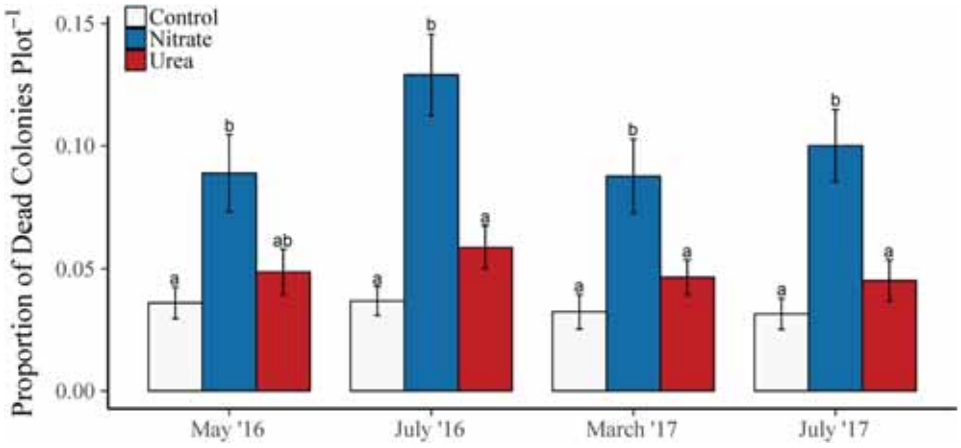

Figure 5. Proportion of completely dead colonies of *Pocillopora* per plot in the different nitrogen treatments across the different bleaching surveys in 2016–17. Letters above bars denote significant differences among nitrogen treatments within survey periods. Statistics are from pairwise least-squared means post hoc tests corrected for multiple comparisons (see Tables S8 and S9 for statistics). No letters present indicate no differences among treatments within a survey period. Data are means ± SE.

severity, nitrate exposure increased the prevalence of *Pocillopora* colonies with partial mortality and more than tripled the complete mortality of *Pocillopora* colonies. Contrary to our hypothesis, neither type of N affected bleaching in *Porites* corals, which are often bleaching resistant as compared to *Acropora* and *Pocillopora* (Loya and others 2001). Our data are the first to show contrasting effects of different forms of N exposure on coral bleaching and mortality under long-term, real-world scenarios.

Differential Effects of Nitrogen Identity on the Prevalence of Coral Bleaching

At large scales, correlations between coral bleaching and water-column nutrient concentrations suggest that excess N may exacerbate bleaching (Wooldridge and Done 2009; Wagner and others 2010), although this signal may be confounded somewhat with turbidity in some locations (MacNeil and others 2019). Recent laboratory experiments suggest that imbalanced N/P (that is, excessive nitrate concentration and P limitation) may be one of the key mechanisms underlying why nutrient enrichment facilitates coral bleaching under thermal stress (Wiedenmann and others 2013; Ezzat and others 2016a, b; Rosset and others 2017). Since water-column P is typically low at our study sites (SRP = 0.14 ± 0.05 μM; mean ± SE) (Alldredge 2019), we drastically increased the N/P ratio as the N coming out of the nutrient diffusers was up to 8× higher than background N concentrations (Figures 2 and S1). Yet, simply elevating N/P ratios did not explain patterns in coral bleaching. Nitrate exposure clearly increased the prevalence and duration of bleaching for both *Pocillopora* and *Acropora* corals versus control corals. However, corals exposed to urea frequently showed an intermediate response in bleaching, often not different from either control or nitrate-exposed corals. Thus, the type of nitrogen, in addition to the N/P ratio, influences the bleaching response of corals.

The concentrations of N near the nutrient diffusers suggests both fertilizers elevated N to similar levels as there were no differences between the two treatments throughout diffuser deployment. Thus, the intermediate effect of urea on coral bleaching does not appear to be linked to lower levels of urea exposure. However, we acknowledge that we only quantified the temporal dynamics of nitrogen enrichment at one time point during the austral winter when wave forcing is lowest at our field site. Thus, we likely did not capture seasonal variation in how nitrogen was delivered to the reef. The variability of nitrogen enrichment may have been higher in the austral summer when wave height is typically higher in Moorea. However, the potential seasonal differences in nitrogen delivery likely would not have differentially affected the two nitrogen treatments. Additionally, it is important to note that the urea fertilizer increased both urea and ammonium concentrations over the course of enrichment, with some slight increase in nitrate as compared to background levels. The increase in these other N sources was likely due to microbes transforming the urea N into these other forms, which can happen rapidly in the environment (Ferdie and Fourqurean 2004).

Stable isotope signatures also suggest that the differential effect of nitrate and urea was not due to less exposure to the urea fertilizer as both *Pocillopora* and *Acropora* were taking up urea and incorporating it into their tissues. Surprisingly, the isotopic signature of nitrate fertilizer was present in *Acropora* tissue but not in *Pocillopora* tissue, although *Pocillopora* were clearly experiencing elevated nitrate levels as there were clear negative effects of nitrate on bleaching and mortality. However, Pocilloporid corals may slow their uptake of nitrate at higher temperatures (Godinot and others 2011; Ezzat and others 2016a), suggesting that the lack of an isotopic signature from nitrate in *Pocillopora* may have been because we sampled these corals when waters were relatively warm. Regardless, the differential impacts of nitrate versus urea on bleaching were not due to differences in exposure to the two N sources but may have been due to the different physiological use of the N sources by corals and their Symbiodinaceae.

Both corals and Symbiodiniaceae assimilate and use ammonium, nitrate, and urea; however, corals may prefer urea to nitrate as a N source (Grover and others 2002, 2003, 2006). Urea is mostly incorporated into host tissues rather than symbiont tissues (Grover and others 2006), whereas the symbionts use both ammonium and nitrate rapidly (Grover and others 2002, 2003). The fact that urea is a less important N source for Symbiodiniaceae than is nitrate or ammonium could explain why we did not observe as strong of an effect of urea on coral bleaching.

At the physiological level, Ezzat and others (2015) found that, relative to controls, ammonium enrichment enhanced total C fixation in Symbiodinaceae and translocation rates in corals but nitrate enrichment reduced carbon translocation, possibly because nitrate reduction to ammonium by Symbiodiniaceae is an energy- and electron-demanding process (Dagenais-Bellefeuille and

Morse 2013). Based on differences in how ammonium versus nitrate enrichment affected carbon acquisition and allocation in corals, Ezzat and others (2015) hypothesized that under thermal stress, nitrate enrichment should weaken the coral-dinoflagellate symbiosis, whereas ammonium could be beneficial. In fact, ammonium exposure under controlled lab conditions made the coral *Turbinaria reniformis* more resistant to heat-induced bleaching (Béraud and others 2013), whereas nitrate exposure resulted in slower *T. reniformis* growth under elevated temperatures (Ezzat and others 2016b). Combined thermal stress and nitrate enrichment resulted in Symbiodiniaceae in the coral *Orbicella faveolata* becoming more ''parasitic'' and transferring less C to the coral host, resulting in a 60% reduction in coral net primary production (Baker and others 2018). Nitrate exposure could also lead to increased reactive nitrogen species that could be a potential trigger for bleaching, similar to the role of reactive oxygen species (Lesser 2006).

Although the negative effects of nitrate on coral physiology are well documented (for example, Wiedenmann and others 2013; Shantz and Burkepile 2014; Ezzat and others 2015; Baker and others 2018), much less is known about the effects of urea on coral health, especially under thermal stress. One recent paper showed that urea exposure led to rapid tissue mortality and death in corals (Pogoreutz and others 2018). However, it should be acknowledged that Pogoreutz and others (2018) used unrealistic concentrations of urea (500–800 μM) that are 40–50× ambient concentrations and over two orders of magnitude greater than our experiment (∼ 4–5 μM or about 5–10× ambient concentrations; Figure S2). The extreme exposure to urea in Pogoreutz and others (2018) makes it difficult to meaningfully compare their work with other research on urea. In our experiment, levels of urea exposure are much more similar to those of natural urea hotspots created by fish aggregations with concentrations of urea below 8 μM (Crandall and Teece 2012). Similarly, another recent study with more modest urea exposure showed that both adding urea and stimulating the urease enzyme via nickel supplementation increased coral calcification (Biscéré and others 2018), suggesting a positive role of urea in coral metabolism. In contrast, nitrate tends to suppress coral calcification (Shantz and Burkepile 2014). An important step forward will be contrasting the effects of nitrate, urea, and ammonium on both the coral and their symbionts in order to decipher the impacts of different N sources on coral physiology, especially under thermal stress.

Nitrate Exposure Prolonged Coral Bleaching and Increased Coral Mortality

Given that nitrate enrichment significantly increased bleaching prevalence, we also expected to also see an increase in bleaching severity. Yet, at the peak of bleaching in both 2016 and 2017 there was no difference in bleaching severity for either *Pocillopora* or *Acropora*. In contrast, nitrate exposure increased the duration of bleaching in both *Pocillopora* and *Acropora*, with corals showing an elevated prevalence of bleaching even after SSTs had declined. This increase in bleaching duration under nitrate exposure, or nutrient exposure in general, even after thermal stress had relaxed, has not been shown before. Nitrate exposure appears to determine, in part, whether corals bleach or not and the duration of the bleaching but may have minimal effect on bleaching severity. Yet, the ultimate measure of severity is the impact on coral mortality, and nitrate exposure dramatically increased both tissue mortality and whole colony mortality in *Pocillopora*. Corals that bleach for longer durations often have decreased energy reserves, leading to an increased likelihood of mortality (Anthony and others 2009). Thus, the increase in *Pocillopora* mortality under nitrate exposure may have been due to the increased duration of bleaching due to the compromised energy balance created by bleaching over a 2–3-month period.

One potential mechanism driving the increases in bleaching duration (in *Pocillopora* and *Acropora*) and coral mortality (in *Pocillopora*) under nitrate exposure is that Symbiodiniaceae in nitrate-exposed corals may return less fixed C to their host (Ezzat and others 2015; Baker and others 2018). The reduction in energy translocated to the coral host may help trigger corals to expel underperforming symbionts, ultimately decreasing corals' energy reserves and compromising their ability to recover from or survive bleaching events (Rodrigues and Grottoli 2007; Anthony and others 2009; Wooldridge 2016), a pattern we saw in *Pocillopora*. Differences in energy reserves among taxa may also explain why nitrate exposure did not increase bleaching in *Porites*, which are often more resistant to bleaching (Loya and others 2001) and have increased lipid reserves as compared to more bleaching-susceptible branching corals (Wooldridge 2016).

Interactions of Thermal Stress and Nitrate Exposure on Bleaching

The impact of nitrogen exposure on coral bleaching dynamics may depend on the intensity of thermal stress corals experience. Under extreme levels of thermal stress, such as those that caused the massive bleaching events on the Great Barrier Reef in 2016 (Hughes and others 2017), the overwhelming duration of thermal stress may cause bleaching regardless of the recent nutrient environment a coral has experienced. Instead, the effects of nutrients may be more relevant during moderate thermal stress events, at least for moderate levels of nitrogen exposure as in our study. For example, experimental nutrient exposure in the Florida Keys, USA, caused increased bleaching in *Agaricia* spp. corals during the early summer when SST's were well below levels where thermal stress begins to accumulate (Vega Thurber and others 2014). Larger-scale correlative patterns also support this idea, with concentrations of inorganic N in the water column positively correlated with coral bleaching prevalence in years with moderate thermal stress but uncorrelated with bleaching in years with extreme thermal stress (Wooldridge and Done 2009; Wagner and others 2010).

In our study, there were differences between 2016 and 2017 in the level of thermal stress, with 2017 having more consecutive days and more total days above 29.0°C, the proposed thermal stress and bleaching threshold in Moorea (Pratchett and others 2013). When thermal stress was lower in 2016, nitrate exposure roughly doubled the bleaching prevalence in *Acropora* as compared to controls. Yet, in 2017, more prolonged thermal stress resulted in more extensive bleaching in *Acropora* but no impact of N exposure on bleaching prevalence. However, nitrate-exposed *Acropora* remained bleached much longer in 2017 than did control corals. Thus, nitrate pollution on coral reefs could prolong bleaching in more stressful years even if the overall prevalence of bleaching is not impacted, likely increasing overall bleaching-related mortality.

Conclusions

With an increasing pace of climate change, corals are spending more time in thermally stressful conditions, a trend that will continue for the foreseeable future (van Hooidonk and others 2016; Hughes and others 2018). Climate change, along with continued changes in land use, may also result in more runoff and nutrients from terrestrial systems reaching coastal marine systems due to altered precipitation regimes (Sinha and others 2017). Both large-scale correlative studies and small-scale lab experiments suggest this combination may have dire consequences for corals as N pollution makes some corals more susceptible to thermal stress. Our long-term field experiment provides the first evidence that N pollution increases coral bleaching and mortality, linking previous large-scale correlative studies with mechanistic laboratory experiments. Most importantly, we showed that corals exposed to the nitrate form of nitrogen exhibited more frequent bleaching, bleached for longer duration, and were more likely to die than corals in low N conditions. Our data portend a concerning trend. Increased anthropogenic N pollution to the coastal oceans, which is often in the form of nitrate (Shantz and Burkepile 2014), may ultimately lower thermal tolerance of corals to bleaching, triggering bleaching events on polluted reefs even when typical thermal bleaching thresholds have not been crossed. Further, when larger thermal stress events do occur, corals subject to nitrate pollution may bleach for longer duration, which will likely lead to more coral mortality. These results suggest that mitigating sources of N pollution to coastal reefs may help increase the resistance and resilience of corals to increasing thermal stress, at least in the near term.

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