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Moderate nutrient concentrations are not detrimental to corals under future ocean conditions

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Abstract

Under predicted future ocean conditions, reefs exposed to elevated nutrients will simultaneously experience ocean acidification and elevated temperature. We evaluated if moderate nutrients mitigate, minimize, or exacerbate negative effects of predicted future ocean conditions on coral physiology. For 30 days, *Acropora millepora* and *Turbinaria reniformis* were exposed to a fully factorial experiment of eight treatments including two seawater temperatures (26.4 °C and 29.8 °C), pCO_2 levels (401 µatm pCO_2 and 760 µatm pCO_2), and nutrient concentrations (ambient: 0.40 µmol L^{-1} NO_3^- and 0.22 µmol L^{-1} PO_4^{3-}, and moderate: 3.56 µmol L^{-1} NO_3^- and 0.31 µmol L^{-1} PO_4^{3-}). Added nitrate was taken up by the algal endosymbionts and transferred to the coral hosts in both species, though to a much higher degree in *A. millepora.* When exposed to elevated temperature, elevated pCO_2, or both, effects observed for chlorophyll *a*, calcification, biomass, and energy reserves were not compounded by the moderate nutrient concentrations in either species. Moderate nutrients enabled *A. millepora* to continue to meet daily metabolic demand via photosynthesis under predicted future ocean conditions and *T. reniformis* to greatly exceed daily metabolic demand via photosynthesis and heterotrophy. Our results suggest that balanced moderate nutrients are not detrimental to corals under predicted future ocean conditions and may even provide some benefits.

Introduction

Many coral reefs are located in oligotrophic tropical waters with low dissolved inorganic nitrogen (DIN; < 2 µmol L^{-1}) and phosphorus (DIP; < 0.1 µmol L^{-1}) concentrations (e.g., Crossland 1983; d'Elia and Wiebe 1990; Tanaka et al. 2007; Erler et al. 2015). Despite being nutrient limited, corals have persisted due to the tightly coupled symbiosis between the algal endosymbionts (Symbiodiniaceae) and the coral host (Muscatine and Porter 1977; Radecker et al. 2015). Algal endosymbionts take up DIN and DIP from the water column (e.g., Muscatine 1980; Grover et al. 2003; Tanaka et al. 2006), translocate organic nutrient compounds and excess photosynthates to the host to support its metabolism (e.g., Muscatine et al. 1981; Hughes et al. 2010; Ferrier-Pages

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et al. 2016), and efficiently recycle products from host metabolism (e.g., Falkowski et al. 1984; Reynaud et al. 2009; Tanaka et al. 2018). The host acquires fixed carbon by feeding heterotrophically on zooplankton, and dissolved and particulate organic carbon (e.g., Grottoli et al. 2006; Ferrier-Pages et al. 2011; Levas et al. 2016).

While high nutrient concentrations (DIN: > 10 µmol L^{-1}, DIP: > 0.35 µmol L^{-1}; Tanaka et al. 2007), such as those found in coastal areas experiencing severe eutrophication (e.g., Stimson et al. 2001; Szmant 2002), can be beneficial by stimulating algal endosymbiont growth and chlorophyll content, they can also be detrimental by undermining calcification (e.g., Marubini and Davies 1996; Ferrier-Pages et al. 2000; Koop et al. 2001). It is hypothesized that with high nutrient concentrations, the algal-host symbiosis decouples, endosymbionts compete for and monopolize the available dissolved inorganic carbon (DIC) for photosynthesis decreasing the proportion of photosynthate translocated to the coral host and resources for calcification (e.g., Muscatine et al. 1989a; Fagoonee et al. 1999; Langdon and Atkinson 2005). In contrast, moderate nutrient concentrations (DIN: < 10 µmol L^{-1}, DIP^{-}: < 0.35 µmol L^{-1}; Tanaka et al., 2007), such as those found in upwelling regions or coastal regions moderately affected by riverine and groundwater discharge (e.g., Tanaka et al. 2007; Naumann et al. 2015; Rouze et al. 2015), can provide physiological benefits to coral health, without negatively affecting calcification in experimental studies and in situ (e.g., Bongiorni et al. 2003; Dizon and Yap 2005; Tanaka et al. 2007). However, the stoichiometry of DIN and DIP is also important; moderate nutrient concentrations only remain beneficial to coral provided they remain in beneficial ratios (Redfield ratio N:P 16:1; Wiedenmann et al. 2013; D'Angelo and Wiedenmann 2014; Ezzat et al. 2016; Rosset et al. 2017). Increases in DIN without proportionate increases in DIP have been shown to increase susceptibility of coral to light and temperature stress (Wiedenmann et al. 2013; Rosset et al. 2017; Lapointe et al. 2019). Furthermore, studies have shown DIN species to also affect coral health differently, with ammonium enrichments found to be more beneficial than nitrate enrichments (e.g., Ezzat et al. 2015; Burkepile et al. 2020; Fernandes de Barros Marangoni et al. 2020).

Coral reefs are increasingly exposed to elevated pCO_2 (i.e., ocean acidification, OA) and temperature due to anthropogenically driven climate change (e.g., Caldeira and Wickett 2003; Zeebe et al. 2008; IPCC 2014), which can cause bleaching, productivity loss, and mortality in coral (e.g., Grottoli et al. 2006; Anthony et al. 2008; Noonan and Fabricius 2016; Magel et al. 2019). Moderate nutrient concentrations appear to mitigate much of the negative effects of OA on coral growth (e.g., Marubini and Atkinson 1999; Holcomb et al. 2010; Chauvin et al. 2011) and partially or fully mitigate the negative effects of elevated temperature stress on coral photosynthesis and calcification (Ezzat et al. 2016, 2019). Yet, the effect of moderate nutrients on corals simultaneously exposed to OA and elevated temperature is only beginning to be understood (Hall et al. 2018). Our companion paper, Hoadley et al. (2016), found moderate nutrients to mitigate the dual stress effects of elevated temperature and OA on the physiology of the algal endosymbiont *Durusdinium trenchii* in the coral *Turbinaria reniformis*. However, little is known about the combined effects of moderate nutrients and these dual stressors on coral holobiont (coral host *and* algal endosymbiont) physiology.

Here, we evaluated the single and interactive effects of elevated temperature, simulated OA (elevated pCO_2), and moderate nutrient concentrations (NO_3^-: < 5 µmol L^{-1}, PO_4^{3-}: < 0.35 µmol L^{-1}; Tanaka et al., 2007) on coral energy reserves, carbon budget, and nitrogen uptake of the Pacific corals *Acropora millepora* and *T. reniformis*. We hypothesized that the physiological benefits of moderate nutrient concentrations would minimize the simultaneous negative effects of elevated temperature and simulated OA on the coral holobiont. If true, then fringing coral reefs with local anthropogenic nutrient enrichments from runoff and reefs exposed to natural upwelling resulting in balanced moderate nutrient concentrations may aid in the resilience of some corals under future ocean conditions.

Materials and methods

In April 2011, six colonies of *Acropora millepora* and *Turbinaria reniformis* were collected from 3 to 10 m depth in Fiji (17° 29′ 19″ S, 177° 23′ 39″ E) and at least 10 m apart to maximize the chance that they were genetically distinct (Baums et al. 2019). Corals were transported to the Reef Systems Coral Farm mariculture facility, New Albany, Ohio and maintained for 18 months at 26.4 °C ± 0.04 SE in 3785 L recirculating aquaria with artificial seawater (Instant Ocean Reef Crystals, Spectrum Brands) under ambient temperature, pCO_2, and nutrients in a greenhouse (max light levels on a natural light cycle: 700–1000 µmol quanta^{-1} m^{-2} s^{-1}). In January 2012, all colonies were divided into ramets ($n = 8$ per colony, one for each treatment), mounted on 5 cm PVC tiles using EcoTech coral glue, and allowed to recover. On 6 August 2012, coral ramets were transferred into indoor experimental tanks (57 L) under ambient conditions (26.4 °C, 402 µatm pCO_2, ambient nutrient concentrations), with custom-made artificial seawater (ESV Aquarium Products Inc.) designed to be of the same chemical composition and alkalinity as natural seawater, and artificial light (Tek Light T5 actinic lights, 275 µmol quanta^{-1} m^{-2} s^{-1}, 10:14 h light:dark diurnal cycle). Coral ramets were evenly distributed across the tanks and allowed to acclimate for 4 weeks. The lower light conditions are sufficient to allow corals

to reach maximum photosynthesis rates and is above the minimum light levels recommended for coral experiments (Grottoli et al. 2020). Artificial seawater is extensively used in closed systems for coral research (Berzins et al. 2008; D'Angelo and Wiedenmann 2012; Schoepf et al. 2013; Grottoli et al. 2020).

The experimental systems in this study were outlined in Hoadley et al. (2016). Briefly, from 7 to 16 September 2012, treatments were initiated (Table S1): temperature, pCO_2, and nutrients were gradually increased over the course of a week until target conditions were reached to minimize shocking any of the corals (Figure S1). Treatments consisted of a control (26.4 °C, 402 µatm pCO_2), elevated pCO_2 (26.4 °C, 760 µatm pCO_2), elevated temperature (29.8 °C, 402 µatm pCO_2), and combined elevated temperature and pCO_2 (29.8 °C, 760 µatm pCO_2), each at ambient nutrient concentrations (0.40 µmol L^{-1} NO_3^- and 0.22 µmol L^{-1} PO_4^{3-}) and moderate nutrient concentrations (3.56 µmol L^{-1} NO_3^- and 0.31 µmol L^{-1} PO_4^{3-}) (Table 1; Figure S2). Each treatment was replicated in six tanks such that one ramet of each species was in each tank (Figure S2). The control temperature (26.4 °C) represented the average annual temperature in Fiji, while the average elevated temperature (29.8 °C) was within the sea surface temperature bleaching threshold range of 29.5–30 °C in Fiji (https://coralreefwatch.noaa.gov/vs/gauges/fiji.php). Control and treatment pCO_2 levels represented present day conditions and those expected by end-of-century (2081–2100) under the RCP 6.0 scenario (720–1000 µatm pCO_2), respectively (IPCC 2014). The moderate nutrient concentration was representative of that observed during natural upwelling events or in regions which receive some anthropogenic nutrient enrichment, and are similar to average concentrations used or observed in previous studies (e.g., Marubini and Atkinson 1999; Tanaka et al. 2007; Holcomb et al. 2010). To ensure that observed effects were not due to phosphorus starvation (e.g., Wiedenmann et al. 2013; Rosset et al. 2017; Tanaka et al. 2017), NO_3^- and PO_4^{3-} concentrations were close to the Redfield ratio of 16:1 for nitrogen:phosphorus (Redfield 1958). NO_3^- was added using KNO_3 and PO_4^{3-} was added using KH_2PO_4, daily. The nitrate was isotopically enriched compared to typical coral $\delta^{15}N$ values (i.e., $\delta^{15}N_{KNO3} = 46‰$ compared to typical coral $\delta^{15}N$ of 5–8‰), so acted as an N tracer allowing the $\delta^{15}N$ of the endosymbiotic algae and coral host to be used as a proxy for inorganic nitrogen uptake. Experimental conditions (ramping plus target conditions) lasted for 30 days, and all corals were fed fresh, 2-day old *Artemia nauplii* (Carolina Biological Supply) twice each week. The seawater temperature (Figure S1), carbonate chemistry, and nutrient concentrations within each tank were monitored throughout the experiment (Table 1). pH electrodes (Thermo Scientific Orion Ross Ultra pH glass electrodes) were recalibrated to NBS standards daily. While this is not the most accurate method to determine seawater pH, it is commonly used in physiology (e.g., Venn et al. 2013; Cai et al. 2016), is the method used in our previous study with the same coral colonies (Schoepf et al. 2013; Levas et al. 2015) thus optimizing comparison between the studies, and is acceptable here given the large differences between treatments (Riebesell et al. 2011).

Assuming each colony was a different genotype, the experimental design removed genotypic variation among treatments within each species, optimizing our ability to detect treatment effects. While the six tanks within each treatment shared a single sump, technically making this a pseudo-replicated design (Hurlbert 1984; Cornwall and Hurd 2016), this disadvantage is outweighed by the advantage of being able to manipulate eight combinations of temperature, pCO_2, and nutrients simultaneously in 48 tanks.

Physiology

In the first and last 3 days of the experiment, calcification was measured using the buoyant weight technique and converted to dry weight (Jokiel et al. 1978). Calcification rates from Hoadley et al. (2016) have been recalculated as closer scrutiny of raw buoyant weight data discovered three extreme values which indicated a failure to tare the balance before weighing. During the last 3 days of the experiment, photosynthesis (P) and respiration (R) measurements were carried out on live coral incubated in sealed chambers (Hoadley et al. 2016), from which dissolved and particulate organic carbon (DOC and POC) water samples were collected (Levas et al. 2015). DOC and POC samples were not collected for *A. millepora* due to time constraints. On completion of live coral measurements, ramets were frozen and stored at − 80 °C for further analysis.

P and R were used to calculate Contribution of Zooxanthellae (Symbiodiniaceae) to Animal Respiration (CZAR) (Muscatine et al. 1981). DOC concentrations (mg L^{-1}) were determined using a Shimadzu TOC-L total organic carbon analyzer (using the 680 °C combustion catalytic oxidation method) (Levas et al. 2015). POC filters were acid fumigated (Levas et al. 2015), and combusted in an Elementar Vario EL Cube/Micro-Cube elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer at the stable isotope facility at the University of California-Davis. DOC and POC values were used to calculate Contribution of Heterotrophy to Animal Respiration from DOC ($CHAR_{DOC}$) and POC ($CHAR_{POC}$), respectively, according to methods modified from Levas et al. (2015). In brief, DOC and POC fluxes (µmol C hr^{-1} cm^{-2}) were blank corrected, and standardized to incubation time and surface area (SA) of the coral ramet using the following equation:

Table 1 Mean (± SE) carbonate chemistry parameters and nutrient concentrations for the eight treatments: control (26.4 °C, 402 µatm pCO_2), elevated pCO_2 (26.4 °C, 760 µatm pCO_2), elevated temperature (29.8 °C, 402 µatm pCO_2), and the combined treatment of elevated temperature and pCO_2 (29.8 °C, 760 µatm pCO_2), under ambient nutrients (0.40 µmol L^{-1} NO_3^- and 0.22 µmol L^{-1} PO_4^{3-}) and moderate nutrients (3.56 µmol L^{-1} NO_3^- and 0.31 µmol L^{-1} PO_4^{3-})

Treatment	Temp (°C)	pH (NBS scale)	TA (µmol/kg)	pCO_2 (µatm)	xCO_2 (ppm)	Ω_{arag}	TN (µmol N/L)	NO_3^- (µmol N/L)	NH_3 (µmol N/L)	TP (µmol P/L)	Ortho PO_4 (µmol P/L)
Control											
Ambient nutrients	26.03 ± 0.05	8.15 ± 0.06	2350.87 ± 18.12	410.63 ± 17.42	424.50 ± 18.01	3.59 ± 0.09	3.81 ± 0.5	0.39 ± 0.04	0.26 ± 0.09	0.24 ± 0.01	0.21 ± 0.00
Moderate nutrients	26.45 ± 0.02	8.18 ± 0.01	2293.55 ± 14.16	358.34 ± 6.50	370.76 ± 6.71	3.74 ± 0.06	7.41 ± 0.79	3.49 ± 0.32	0.55 ± 0.34	0.32 ± 0.01	0.32 ± 0.03
Elevated pCO_2											
Ambient nutrients	26.54 ± 0.06	8.06 ± 0.05	2333.18 ± 11.15	752.82 ± 30.49	778.95 ± 31.50	2.41 ± 0.09	5.51 ± 0.88	0.46 ± 0.08	0.21 ± 0.04	0.26 ± 0.01	0.22 ± 0.00
Moderate nutrients	26.32 ± 0.04	7.99 ± 0.01	2377.92 ± 8.78	752.10 ± 28.14	777.88 ± 29.10	2.45 ± 0.07	7.63 ± 1.33	3.68 ± 0.80	0.16 ± 0.08	0.29 ± 0.02	0.29 ± 0.03
Elevated temperature											
Ambient nutrients	29.68 ± 0.33	8.23 ± 0.06	2302.01 ± 16.34	414.56 ± 10.34	432.27 ± 10.90	3.81 ± 0.08	3.98 ± 0.72	0.35 ± 0.03	0.49 ± 0.32	0.27 ± 0.01	0.21 ± 0.01
Moderate nutrients	30.15 ± 0.25	8.24 ± 0.03	2319.94 ± 12.85	423.89 ± 10.38	442.26 ± 10.95	3.84 ± 0.06	7.10 ± 0.78	3.52 ± 0.60	0.32 ± 0.24	0.32 ± 0.04	0.32 ± 0.03
Combined treatment											
Ambient nutrients	30.28 ± 0.24	7.97 ± 0.02	2338.55 ± 12.31	741.93 ± 25.56	774.34 ± 26.90	2.74 ± 0.07	3.88 ± 0.40	0.41 ± 0.11	0.24 ± 0.06	0.25 ± 0.01	0.24 ± 0.02
Moderate nutrients	29.72 ± 0.35	8.00 ± 0.02	2365.49 ± 11.51	794.31 ± 32.51	828.10 ± 34.20	2.64 ± 0.08	7.19 ± 0.44	3.56 ± 0.48	0.19 ± 0.04	0.33 ± 0.03	0.30 ± 0.04

Adapted from Hoadley et al. (2016) in that temperature (°C) and pH have been recalculated using higher resolution data logger information; for each treatment, temperature was logged every 5 s and pH every 0.5 s from which daily average was calculated. Data were downloaded from the loggers periodically, but on some occasions, the data loggers became full and unable to log additional data

$$\text{OC flux}\left(\mu\text{mol C hr}^{-1}\text{cm}^{-2}\right) = \frac{\left(\text{OC}_{\text{coral}} - \text{OC}_{\text{blank}}\right)}{\text{incubation time} \times \text{SA}}. \quad (1)$$

CHAR for each coral ramet was then calculated according to the following equation:

$$\text{CHAR} = \left(\frac{\text{OC}_{\text{df}} \times 10}{R_T}\right) + \left(\frac{\text{OC}_{\text{nf}} \times 14}{R_T}\right) \times 100\%, \quad (2)$$

where the daytime OC flux (OC_{df}) was multiplied by the total daytime hours (10) and divided by the total µg C lost via daytime and nighttime respiration (R_T), plus the nighttime OC flux (OC_{nf}) multiplied by the total nighttime hours (14), divided by R_T, and multiplied by 100 to yield values in percentages. OC and R_T were measured in µg C and standardized to gram dry weight. $CHAR_{DOC}$ and $CHAR_{POC}$ were summed to yield CHAR of the total organic carbon ($CHAR_{TOC}$) (Levas et al. 2015). CZAR and $CHAR_{TOC}$ were summed to yield the Contribution of Total acquired fixed carbon relative to Animal Respiration (CTAR) (Grottoli et al. 2014).

Surface area of *A. millepora* was measured using the wax dipping technique (Veal et al. 2010) due to its branching morphology, and samples were prepared for downstream analyses by airbrushing or grinding (Table S2). Surface area of *T. reniformis* was measured using the aluminum foil technique (Marsh 1970) due to its plating morphology, and prepared for downstream analyses by use of a water-pik (Table S2). Chlorophyll *a* of *A. millepora* was measured using 100% acetone (Jeffrey and Humphrey 1975), while *T. reniformis* chlorophyll *a* cm^{-2} was calculated from Hoadley et al. (2016). For both species, biomass was measured to ash free dry weight (McLachlan et al. 2020), protein was measured using the bicinchoninic acid method (Smith et al. 1985) with bovine serum albumin as a standard (Pierce BCA Protein Assay Kit), total lipids were measured using chloroform:methanol (2:1, v:v) with two KCl rinses (Baumann et al. 2014), and carbohydrates were measured using the phenol–sulfuric acid spectrophotometric method with glucose standards (Dubois et al. 1956). All energy reserves were reported in Joules (Gnaiger and Bitterlich 1984).

Host and algal endosymbiont $\delta^{15}N$ isotopes

Coral host, algal endosymbiont, and whole coral (host + algal endosymbiont) $\delta^{15}N$ isotopes were measured according to methods modified from Hughes and Grottoli (2013). In brief, a subsample of coral tissue slurry was homogenized and sonicated (20% amplitude for 60 s total, 1:1 cycle) using a probe sonicator, before adding 35 mg mL^{-1} NaCl. The subsample was filtered through 20 µm nitex mesh to remove any skeletal material, the endosymbiotic algal fraction was isolated onto GF/F filters, and the host tissue filtrate dried down in 9 mm × 10 mm tin capsules (Costech Analytical Technologies Inc.) using a stream of ultra-pure nitrogen gas whilst on a 60 °C heat plate. Exposure to nitrogen gas during this step does not affect the $\delta^{15}N$ values of the samples (Sturaro et al. 2020).

Acropora millepora host and algal endosymbiont fractions were combusted in a Costech elemental analyzer stable isotope ratio mass spectrometer (EA-IRMS), and the resulting N_2 gas automatically analyzed with a Thermo Finnigan Delta IV isotope ratio mass spectrometer via a ConFlow open split interface in the Grottoli Stable Isotope Biogeochemistry Lab at The Ohio State University. Repeated measurements of internal standards had an average SD ± 0.41‰ $\delta^{15}N$. Approximately 10% of *A. millepora* samples were run in duplicate with an average SD ± 0.21‰. While *T. reniformis* algal endosymbionts were successfully separated from the host, there was insufficient sample material to produce reliable host isotopic measurements. Therefore, whole tissue slurry (host and algal endosymbiont) was freeze-dried and loaded into tin capsules for isotopic analysis. *Turbinaria reniformis* whole and algal endosymbiont fractions were combusted in an Elementar Vario EL Cube/Micro-Cube elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer at the stable isotope facility at University of California-Davis. Repeated measurements of an internal standard had an average SD ± 0.08‰ $\delta^{15}N$. The $\delta^{15}N$ values of both species are reported as the per mil deviation of the ratio of stable nitrogen isotopes ^{15}N:^{14}N relative to air.

Statistical analyses

To test the effect of treatment across physiological variables, a Euclidean distance-based resemblance matrix was constructed using normalized data of gross P, calcification, biomass, protein, and total lipids. Collinearity amongst physiological response variables was investigated using Draftsman's plots and Pearson's correlation coefficient. No variables were strongly correlated [i.e., $|r| < 0.70$ (Dormann et al. 2013)] and thus were treated as independent. Chlorophyll *a* and carbohydrates were not included, because they were not measured in all ramets due to insufficient sample material, and $\delta^{15}N$ isotopes were not included as they would have biased outcomes of the plot by acting as an N tracer. Non-metric multidimensional scaling (NMDS) plots allowed visualization of relationships between each coral ramet across all treatments, for each species. Analysis of similarities (ANOSIM) was used to evaluate the degree of dissimilarity among treatments (Clarke and Gorley 2006). All multivariate analyses were conducted using the software package Primer v6 (Clarke and Gorley 2006).

All data were tested for normality and homogeneity of variance using a Shapiro–Wilk's test plots of expected vs. residual values, respectively. *Acropora millepora* chlorophyll *a* and *T. reniformis* chlorophyll *a* and biomass were log transformed to meet the assumptions of normality. Cook's Distance identified one outlier in *T. reniformis* calcification (Cook 1977), and following its removal, these data were normally distributed. Univariate four-way analysis of variance (ANOVA) was used to test the effects of temperature, pCO_2, nutrients, and colony on each measured variable, for each species. Temperature was fixed with two levels (26.4 °C, 29.8 °C), pCO_2 fixed with two levels (401 µatm pCO_2, 760 µatm pCO_2), nutrients fixed with two levels (ambient, moderate), and colony was included as a random effect. As no single colony was systematically different from all others for a given variable, we concluded that the selected colonies represented natural variation in the population (Grottoli et al. 2014). Colony was removed and three-way ANOVAs performed (temperature, pCO_2, nutrients). Bonferroni corrections were not used (Quinn and Keough 2002; Moran 2003). The use of replicate parent colonies across all treatments reduced overall variation between treatments. Since all ramets were reared under the same conditions except for treatment, and were similarly sized, any differences between treatments and controls for any variable were assumed to be due to treatment effects alone. Finally, post hoc slice tests tested the effect of the nitrate addition within each temperature and pCO_2 combination, for each variable. All univariate parametric statistics were generated using SAS software, Version 9.3 of the SAS System for Windows. Values of $p \leq 0.05$ were considered significant.

Results

Average seawater temperature, pH_{NBS}, pCO_2, saturation state, total alkalinity, and nutrient concentrations for all eight treatments throughout the 30 days of the experiment are summarized in Table 1. On average, elevated temperature treatments were 3.6 °C higher, the elevated pCO_2 treatments had pH values that were 0.2 pH units lower (i.e., 372 ppm higher), and the moderate nutrient concentrations were 3.16 µmol L^{-1} and 0.09 µmol L^{-1} higher in nitrate and phosphate than in the ambient treatments (Table 1). Unfortunately, on 5 October 2012 a computer malfunction in the control system caused a stressful decline in pH for ~ 48 h killing the majority of control *Acropora millepora* ramets, while *Turbinaria reniformis* ramets were unaffected. Therefore, there was no true control for *A. millepora.* Despite this, data from ramets within the other treatments were still valid as each moderate nutrient treatment within elevated pCO_2, temperature, or the combination had a corresponding ambient nutrient treatment. Therefore, it was still possible to determine how the moderate nutrients affected coral response under each treatment condition.

Acropora millepora

Overall, only moderate nutrients resulted in significant changes in coral physiology (Fig. 1a, Table S3). Closer examination of each physiological variable revealed that under combined elevated temperature and pCO_2, gross photosynthesis was significantly higher in corals with moderate nutrients compared to those with ambient nutrients (Fig. 1b; Table S4). No significant differences were observed in calcification, biomass, or protein across all treatments (Fig. 1c–e, Table S4). Total lipids were significantly higher under elevated pCO_2 compared to those under ambient pCO_2, irrespective of temperature and nutrients (Fig. 1f, Table S4). In elevated temperature treatments, carbohydrates were significantly higher in corals under elevated pCO_2 compared to those under ambient pCO_2, irrespective of nutrients (Figure S3A; Table S5).

The $\delta^{15}N$ of the endosymbiotic algae ($\delta^{15}N_e$) and the coral host ($\delta^{15}N_h$) were significantly higher under ambient temperature, ambient pCO_2, and moderate nutrients (Fig. 2a; Table S6). A posteriori slice tests indicated that $\delta^{15}N_e$ was significantly enriched due to the nitrate addition (moderate nutrients) across all temperature and pCO_2 treatments compared to those without the nitrate addition (ambient nutrients) (Fig. 2a). Compared to corals under ambient nutrients, $\delta^{15}N_e$ enrichment in corals under moderate nutrients was highest under control conditions, followed by elevated pCO_2 (+4.34‰), elevated temperature (+3.83‰), and the combined treatment (+2.60‰) (Fig. 2a). A similar trend was observed for $\delta^{15}N_h$ (Fig. 2a) though the enrichment was not statistically significant in the combined treatment.

Chlorophyll *a* cm^{-2} was significantly higher in corals under moderate nutrients compared to those under ambient nutrients (Fig. 2b; Table S6). When exposed to either elevated pCO_2, elevated temperature, or the combined treatment, corals under moderate nutrients were visually darker than those under ambient nutrients (Figures S4a–h). Finally, while there were no significant differences in CZAR across all treatments, *A. millepora* were not able to meet 100% of metabolic demand through CZAR alone in either elevated temperature treatment (elevated temperature treatment and the combined treatment) under ambient nutrients but could under moderate nutrients (Fig. 2c).

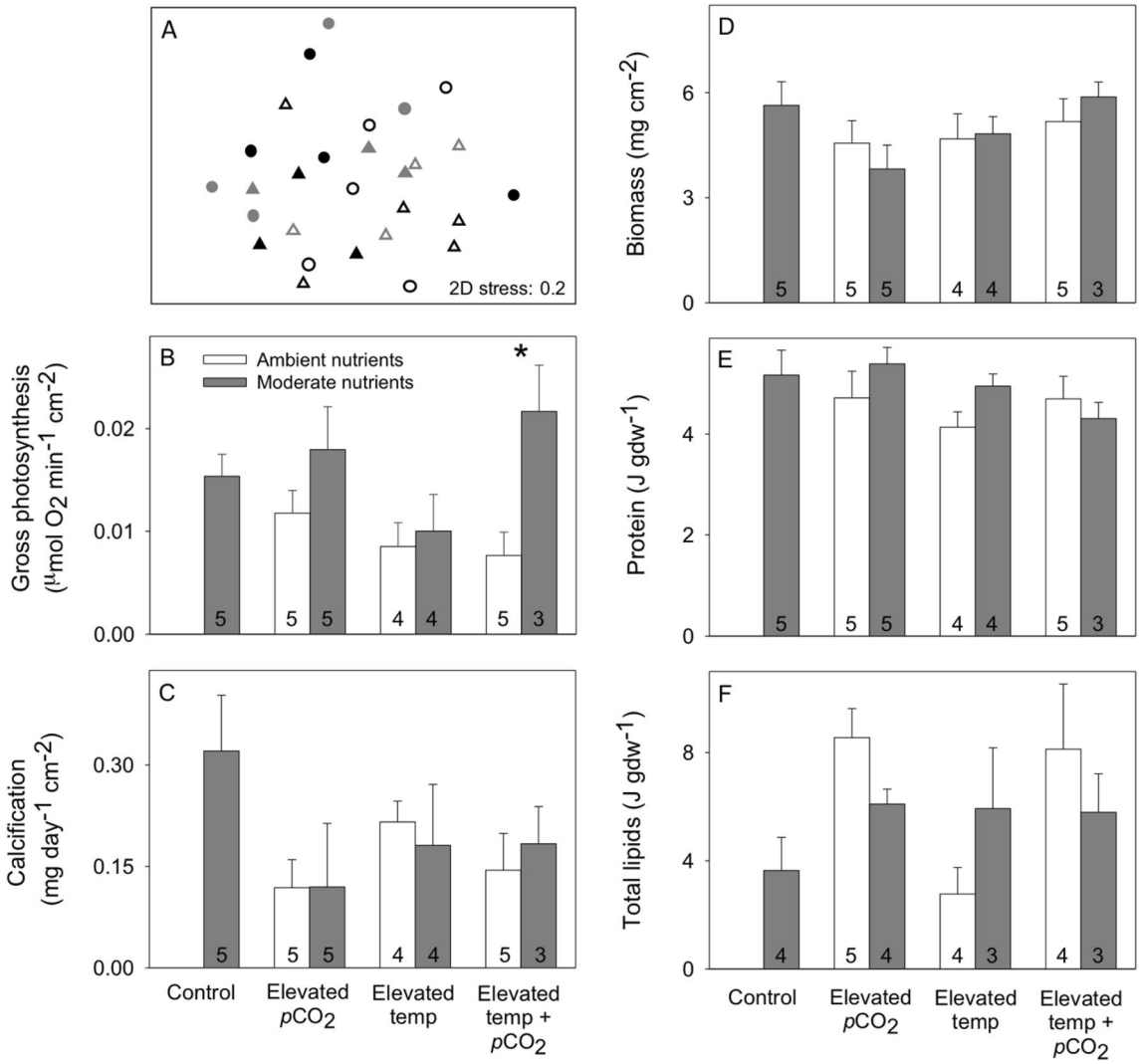

Fig. 1 *Acropora millepora* **a** non-metric multidimensional scaling (NMDS) plot with control (grey circles), elevated pCO_2 (black circles), elevated temperature (temp) (grey triangles), and elevated temperature and pCO_2 (black triangles), under ambient nutrients (open symbols) and moderate nutrient (filled symbols) treatments. Measured variables included: *A. millepora* average (±1SE) **b** gross photosynthesis, **c** calcification, **d** holobiont total biomass, **e** holobiont total protein, and **f** holobiont total lipids. Corresponding ANOSIM analyses are in Table S3. Asterisks indicate significant differences between the ambient and moderate nutrient treatments within each temperature and pCO_2 combination. Sample size is indicated within each bar. Significant effects from the corresponding ANOVAs indicated in Table S4

Turbinaria reniformis

Overall, only elevated temperature resulted in significant changes in coral physiology (Fig. 3a; Table S7). Closer examination of each physiological variable revealed no significant differences in gross photosynthesis, calcification, biomass, or total lipids across treatments (Fig. 3b–d, f; Table S8). In the control, protein declined significantly in corals with moderate nutrients compared to those with ambient nutrients (Fig. 3e; Table S8). At ambient pCO_2 (control and elevated temperature), moderate nutrients led to a decline in carbohydrates (Figure S3b; Table S9).

Moderate nutrients alone significantly affected $\delta^{15}N$ of whole holobiont tissues ($\delta^{15}N_w$), while a significant pCO_2 by nutrient interaction was observed for $\delta^{15}N_e$ (Fig. 4a; Table S10). $\delta^{15}N_w$ was enriched by an average of + 1.40‰ due to the nitrate addition (moderate nutrients) across all temperature and pCO_2 treatments compared to their counterparts without the nitrate addition (ambient nutrients) (Fig. 4a; Table S10). A posteriori slice test indicated that $\delta^{15}N_e$ of corals under moderate nutrients were more enriched at ambient pCO_2 (+ 2.28‰ and + 1.92‰) than at elevated pCO_2 (+ 1.44‰ and + 1.07‰) (Fig. 4a, Table S10).

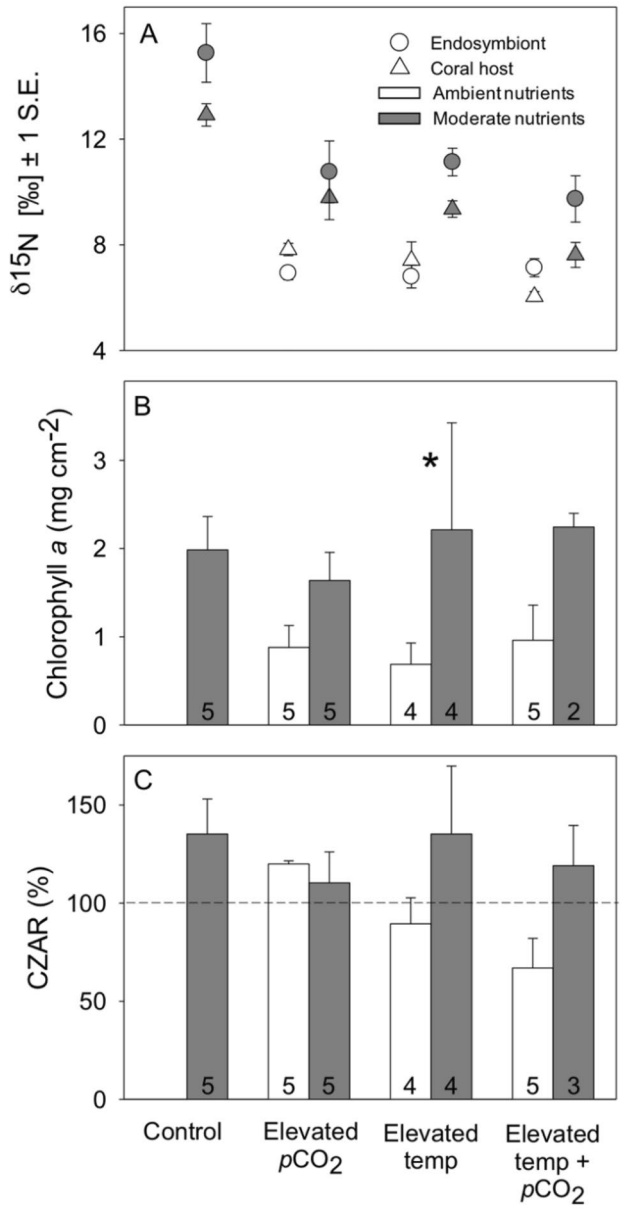

Fig. 2 *Acropora millepora* average (±1SE) **a** $\delta^{15}N$ of algal endosymbiont (circles) and coral host (triangles) under ambient nutrient treatments (open symbols) and moderate nutrient (filled symbols) treatments. Errors are sometimes smaller than the symbol and not visible. A posteriori slice tests indicated that each algal endosymbiont pair (i.e., ambient vs. moderate nutrients) within each temperature (temp) and pCO_2 combination were significantly different from each other. *A. millepora* average (±1SE) **b** chlorophyll *a,* and **c** Contribution of Zooxanthellae (Symbiodiniaceae) to Animal Respiration (CZAR), under ambient nutrients (open bars) or moderate nutrients (solid bars). The dashed line at 100% represents the threshold at which the coral is meeting 100% metabolic demand. Asterisks indicate significant differences between the ambient and moderate nutrient treatments within each temperature and pCO_2 combination. Sample size indicated within each bar. Significant effects from the corresponding ANOVAs indicated in Table S6

No significant difference was observed in chlorophyll *a* cm^{-2} across treatments (Fig. 4b; Table S11), although corals in the combined elevated temperature and pCO_2 treatment under ambient nutrients were slightly paler than those under moderate nutrients (Figures S5g, h). Similarly, there were no significant overall model effects for CZAR, $CHAR_{TOC}$, or CTAR (Fig. 4c–e; Table S11). Nevertheless, CZAR was greater than 100% of daily metabolic demand under all treatments (Fig. 4c). In all treatments except the control, coral with ambient nutrients released organic carbon into the water column (negative $CHAR_{TOC}$), while those with moderate nutrients took up organic carbon from the water column (positive $CHAR_{TOC}$) (Fig. 4d).

Discussion

This study adds to the growing body of knowledge on interactive effects of nutrients, temperature, and pCO_2 on corals. High nutrient concentrations exacerbate coral response to OA or elevated temperature (e.g., Mate ; Ferrier-Pages et al. 2000; Langdon and Atkinson 2005), while moderate nutrient concentrations can mitigate the negative effects of either of those stressors (e.g., Tanaka et al. 2007; Chauvin et al. 2011; Ezzat et al. 2016). Here, moderate nutrient concentrations were not additionally detrimental to coral physiology when simultaneously exposed to simulated OA and elevated temperature, and even provided some benefits.

Acropora millepora

Overall, *A. millepora* physiology was primarily affected by moderate nutrients (Fig. 1a). Consistent with previous findings, isotopic evidence indicated that the algal endosymbionts took up the isotopically enriched nitrate first and translocated $\delta^{15}N$-enriched compounds to the host under all treatment conditions (Fig. 2a) (e.g., Muscatine et al. 1989b; Grover et al. 2003; Tanaka et al. 2006; Kopp et al. 2013). *Acropora millepora* hosted *Cladocopium* (Hoadley et al. 2015)—a generalist algal endosymbiont able to fix and translocate inorganic carbon and nitrogen (N) efficiently under ambient conditions (Jones and Berkelmans 2010; Baker et al. 2013; Pernice et al. 2015), which is consistent with our findings as nitrate incorporation was highest under control conditions. Furthermore, the degree of $\delta^{15}N_e$ and $\delta^{15}N_h$ enrichment progressively decreased under elevated pCO_2, elevated temperature, and the combined treatment (Fig. 2a)—consistent with previous observations of diminished N and P uptake due to the cumulative stress of elevated temperature and OA (Godinot et al. 2011). However, N uptake is unaffected by elevated pCO_2 alone in *Stylophora pistillata* (Godinot et al. 2011), suggesting that N uptake regulation is coral and/or Symbiodiniaceae species-specific.

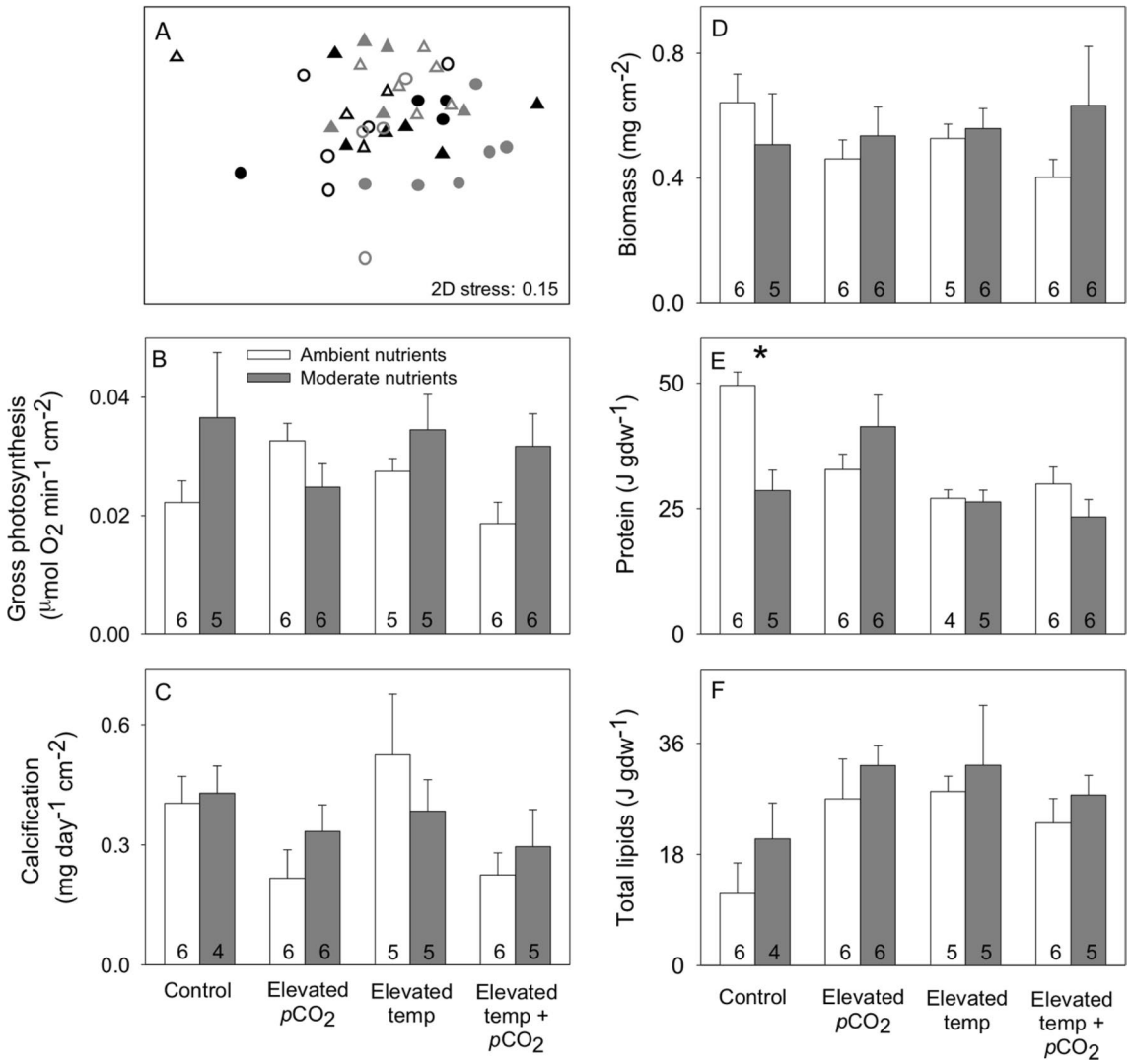

Fig. 3 *Turbinaria reniformis* **a** non-metric multidimensional scaling (NMDS) plot with control (filled circles), elevated pCO_2 (filled triangles), elevated temperature (temp) (open circles), and elevated temperature (temp) and pCO_2 (open triangles), under ambient nutrient (grey symbols) and moderate nutrient (black symbols) treatments. Measured variables included: average (±1SE) **b** gross photosynthesis, **c** calcification, **d** holobiont total biomass, **e** holobiont total protein, and **f** holobiont total lipids. Corresponding ANOSIM analyses are in Table S7. Asterisks indicate significant differences between the ambient and moderate nutrient treatments within each temperature and pCO_2 combination. Sample size indicated within each bar. Significant effects from the corresponding ANOVAs indicated in Table S8. Photosynthesis data from Hoadley et al. (2016)

Moderate nutrient concentrations have either no effect or positive effects on chlorophyll *a*, photosynthesis, and calcification in *Acropora* spp. (Tanaka et al. 2007), even when simultaneously exposed to elevated pCO_2 (Holcomb et al. 2010; Chauvin et al. 2011). Similarly, here, moderate nutrient concentrations either had no effect or had positive effects on the algal endosymbiont (i.e., gross photosynthesis and chlorophyll *a* cm^{-2}; Figs. 1b and 2b) and holobiont (i.e., calcification, biomass, protein, total lipids, CZAR, and carbohydrates; Figs. 1c–f, 2c, S3A) across treatments. The moderate nutrient concentrations stimulated gross photosynthesis (Fig. 1b) and chlorophyll *a* (Fig. 2b), presumably by the removal of N-limitation on the algal endosymbionts (e.g., Marubini and Davies 1996; Ezzat et al. 2016; Courtial et al. 2018). Enhancement of CZAR by moderate nutrients in both elevated temperature treatments provided sufficient additional photosynthetically fixed carbon for the corals to meet metabolic demand (Fig. 2c). This suggests that at elevated temperatures, moderate nutrient concentrations may provide benefits similar to that of heterotrophy (e.g., Grottoli et al. 2006, 2014; Ferrier-Pages et al. 2010), stimulating translocation of photosynthate to the coral host (Tremblay et al. 2016) and maintaining coral metabolic demand.

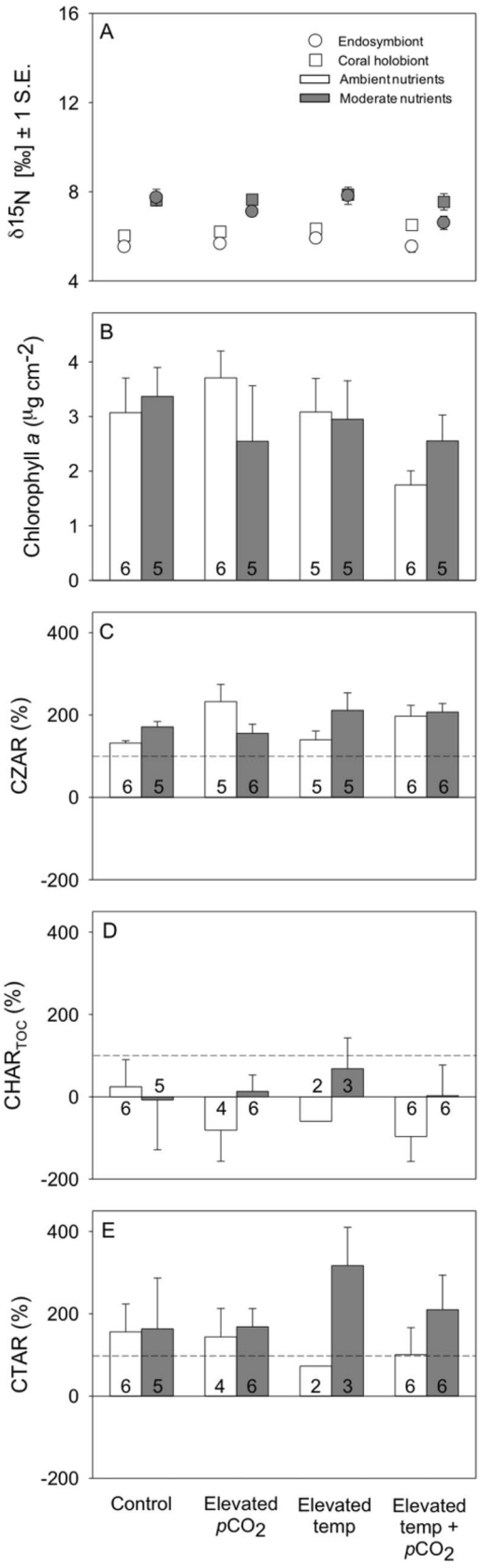

Fig. 4 *Turbinaria reniformis* average (±1SE) **a** $\delta^{15}N$ of algal endosymbiont (circles) and coral holobiont (squares) under ambient nutrient treatments (open symbols) and moderate nutrient (filled symbols) treatments. Errors are sometimes smaller than the symbol and not visible. A posteriori slice tests indicated that each algal endosymbiont pair (i.e., ambient vs moderate nutrients) and each host pair (i.e., ambient vs moderate nutrients) within each temperature and pCO_2 combination were significantly different from each other. *T. reniformis* average (±1SE) **b** chlorophyll *a*, **c** Contribution of Zooxanthellae (Symbiodiniaceae) to Animal Respiration (CZAR), **d** Contribution of Heterotrophy to Animal Respiration from Total Organic Carbon ($CHAR_{TOC}$), and **e** Contribution of Total acquired fixed carbon relative to Animal Respiration (CTAR), under ambient nutrients (open bars) or moderate nutrients (solid bars). The dashed lines at 100% represent the threshold at which the coral is meeting 100% metabolic demand. Data in **b** from Hoadley et al. (2016). Asterisks indicate significant difference between the ambient and moderate nutrient treatments within each temperature and pCO_2 combination. Sample sizes indicated within each bar. Significant effects from the corresponding ANOVAs are in Tables S10 and S11

Calcification was not inhibited by moderate nutrients when simultaneously exposed to either elevated pCO_2, elevated temperature, or both, consistent with previous studies of *Acropora* spp. (Tanaka et al. 2007; Holcomb et al. 2010; Fabricius et al. 2013). However, this contrasts with studies of other coral species which have observed declining calcification under moderate nutrient concentrations (Dizon and Yap 2005; Courtial et al. 2018; Hall et al. 2018). As with Schoepf et al. (2013), energy reserves were not metabolized to sustain calcification under elevated pCO_2 (Fig. 1e-f, S3A). Furthermore, total lipids were higher under elevated pCO_2 irrespective of temperature or nutrients (Fig. 1f), likely due to C-fertilization, simulated OA enhanced incorporation of heterotrophically derived carbon as a result of stress (Baumann et al. 2014), or some combination of the two.

Overall, moderate nutrient concentrations were not detrimental to *A. millepora* under predicted future ocean conditions. Importantly, the absence of the control data for *A. millepora* does not affect this interpretation. Our results suggest that under future ocean conditions, moderate nutrient concentrations could benefit the *A. millepora* holobiont by stimulating photosynthesis and aiding in the maintenance of daily metabolic demand. Thus, *A. millepora* may better survive future ocean conditions in locations with balanced moderate nutrient concentrations. It remains unknown, however, whether moderate nutrient concentrations would continue to provide these benefits when simultaneously exposed to elevated pCO_2 and temperature over a longer timescale, or what the upper threshold nutrient concentrations would be for these benefits to continue.

Turbinaria reniformis

The overall physiology in this species was primarily affected by elevated temperature (Fig. 3a). The similar degree of

isotopic enrichment across all moderate nutrient treatments (Fig. 4a) indicates that nitrate uptake by the algal endosymbiont and rapid recycling with the host was unaffected by temperature or pCO_2. *Turbinaria reniformis* hosted *D. trenchii* (Hoadley et al. 2016), a thermally tolerant specialist algal endosymbiont which up-regulates N-intake and metabolism when thermally stressed, presumably to maintain translocation of photosynthates to the coral host (Baker et al. 2013). Therefore, it was surprising that uptake and incorporation of the nitrate addition was not higher under elevated temperature here (Fig. 4a). However, Baker et al. (2013) added nitrate at concentrations an order of magnitude higher than those used here (i.e., 35 µmol L^{-1} NO_3^- in Baker et al. (2013), vs. 3.5 µmol L^{-1} NO_3^- in this study), and nutrient uptake is driven by external substrate concentrations (Grover et al. 2002, 2003; Bythell and Wild 2011) and nutrient history of the coral (Godinot et al. 2009). Furthermore, *D. trenchii* was not thermally stressed by the elevated temperature within this study (Hoadley et al. 2016), and therefore, perhaps did not up-regulate N-intake to the greatest extent possible.

Under predicted future ocean conditions, moderate nutrient concentrations either had no additional effect or had positive effects on the algal endosymbiont (i.e., gross photosynthesis and chlorophyll *a* cm^{-2}; Figs. 3b, 4b) and holobiont (i.e., calcification, biomass, protein, total lipids, CZAR, $CHAR_{TOC}$, and CTAR, carbohydrates; Figs. 3c–f, 4c–e, S3b) physiological variables measured. While gross photosynthesis and chlorophyll *a* did not change with elevated temperature and pCO_2 (Figs. 3b, 4b), dark acclimated maximum quantum yield of photosystem II (F_v/F_m^{MT}) decreased, likely due to down-regulation of the functional photosystem II reaction centers, rather than a sign of stress (Hoadley et al. 2016). Together, this suggests algal acclimation under future ocean conditions aided by moderate nutrient concentrations. Similarly, Beraud et al. (2013) reported that moderate nutrient concentrations increased *T. reniformis* photosynthetic and photoprotective pigment concentrations and sustained photosynthesis in response to temperature stress.

Interestingly, Ezzat et al. (2016) determined that while inorganic nutrients (2.5 µmol NO_3, 0.6 µmol PO_4) with a balanced N:P ratio maintained *T. reniformis* metabolism and calcification under thermal stress, they were enhanced when combined with heterotrophy. Here, CTAR was maintained at or above 100% of daily metabolic demand across all treatments, except at elevated temperature under ambient nutrients, although caution should be exercised here as n = 2 (Fig. 4e). This was a function of CZAR always exceeding 100% of metabolic demand being further aided by moderate nutrient enhanced $CHAR_{TOC}$ (Figs. 4c, d). As all corals were fed throughout this experiment, it is hypothesized that moderate nutrient concentrations lead to better use of photosynthates and increased incorporation of this retained carbon into coral tissues in response to elevated temperature (Beraud et al. 2013), provided N and P are available in the appropriate ratio (Tanaka et al. 2017; Morris et al. 2019). In agreement with previous studies of *T. reniformis,* calcification was not inhibited by the moderate nutrient concentrations (Fig. 3c) (Beraud et al. 2013; Ezzat et al. 2016), and energy reserves were not metabolized to sustain calcification under elevated pCO_2 (Fig. 3e–f, S3b) (Schoepf et al. 2013).

Overall, moderate nutrient concentrations were not detrimental to *T. reniformis* under predicted future ocean conditions, but instead enhanced the coral's carbon budget. Our results indicate that the *T. reniformis* holobiont will not be adversely affected under future ocean conditions and would persist in locations with balanced moderate nutrient concentrations.

Implications

Balanced moderate nutrient concentrations were not detrimental to *A. millepora* nor *T. reniformis* holobiont physiology under predicted future ocean conditions, but physiological responses were species-specific. We hypothesize that the species-specific responses observed may be due to the following factors, requiring further investigation: (1) the specific Symbiodiniaceae driving the N uptake (Suggett et al. 2017) and influencing downstream physiological responses, and/or (2) differences in coral-associated microbial community composition influencing coral resilience to environmental stressors and future ocean conditions (e.g., Ainsworth and Gates 2016; McDevitt-Irwin et al. 2017; Grottoli et al. 2018). Additional study is also required to determine if the benefits of moderate nutrient concentrations observed here would be maintained over a longer timescale.

Overall, moderate nutrient concentrations enabled *A. millepora* to meet, and *T. reniformis* to far exceed, daily metabolic demand under predicted future ocean conditions (Figs. 2c, 4e). *Acropora millepora* has previously been denoted an "ecological loser" under predicted future ocean conditions (Schoepf et al. 2013; Grottoli et al. 2018). However, coral reef environments with balanced moderate nutrient concentrations (i.e., with coastal runoff or upwelling) could reverse this conclusion and may provide refuge to the more sensitive *A. millepora*, while the more tolerant *T. reniformis* would continue to thrive.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00227-021-03901-3.

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Author contributions AGG and MEW secured the funding, and designed and coordinated the study. All authors, except for KLD participated in the fieldwork. KLD and VS carried out all laboratory analyses except for *T. reniformis* chlorophyll *a* which was conducted by KH. KLD carried out all data analyses and drafted the manuscript. All authors contributed to revising the manuscript and gave final approval for publication.

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Availability of data and materials The datasets supporting this manuscript will be deposited at https://www.bco-dmo.org/project/528004.

Declarations

Conflict of interest The authors declare no conflict of interest exists.

Ethical approval All work undertaken in this study complied with the current laws of Fiji and the United States of America for collecting and importing/exporting coral specimens.

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