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Ocean warming and acidification uncouple calcification from calcifier biomass which accelerates coral reef decline

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Global climate change will drive declines in coral reefs over coming decades. Yet, the relative role of temperature versus acidification, and the ability of resultant ecosystems to retain core services such as coastal protection, are less clear. Here, we investigate changes to the net chemical balances of calcium carbonate within complex experimental coral reefs over 18 months under conditions projected for 2100 if CO₂ emissions continue unmitigated. We reveal a decoupling of calcifier biomass and calcification under the synergistic impact of warming and acidification, that combined with increased night-time dissolution, leads to an accelerated loss of carbonate frameworks. Climate change induced degradation will limit the ability of coral reefs to keep-up with sea level rise, possibly for thousands of years. We conclude that instead of simply transitioning to alternate states that are capable of buffering coastlines, reefs are at risk of drowning leading to critical losses in ecosystem functions.

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Shallow water coral reefs provide living coastal barriers that protect people and infrastructure when the rate of accretion exceeds that of erosion^{1,2}. In the short-term, major framework builders can rapidly rebuild three-dimensional (3D) structures after acute disturbances (e.g. storms) through high rates of reproduction, growth, and calcification^{3,4}. In turn, the 3D structure reduces the motion of the water allowing for sediment infilling, such that over the long-term, high coral cover reefs stabilizing at an average porosity of 50% can vertically accrete at a maximal rate of $\sim 10 \text{ mm yr}^{-1}$ in response to sea-level rise⁵. Over the last 6000 years, modern coral reefs have been sustained under little ($< 0.1 \text{ m } 100 \text{ yr}^{-1}$) to no sea-level rise⁶ because the net chemical balances of calcium carbonate (CaCO_3) have been in equilibrium with net physical export of CaCO_3 from reefs⁷.

Recent and intensifying El Niño events have provided an acute and highly observable window into the future of coral reefs under the thermal impact of climate change. The accompanying underwater heatwaves demonstrate that there are latitude specific thermal thresholds whose exceedance initially drove observations of mass coral bleaching⁸; but, as events have become hotter for longer, large-scale coral mortality has become increasingly common⁹. The impacts of acidification on reefs, however, are not as visually evident and require microscopic examination¹⁰ or quantification via challenging chemical-physical methodologies¹¹, and lie mostly in the future. Under increasing greenhouse gas (GHG) emissions, principally carbon dioxide (CO_2), acute thermal impacts will merge with chronic changes to seawater chemistry, increasing ocean temperature and decreasing aragonite saturation (Ω_{Ar}) away from relatively stable states that have dominated for the last 420,000 years¹². The extent of the future changes and associated risks is ultimately determined by human activities. However, if GHG emissions remain unchecked over the next few decades, we risk generating an atmospheric pCO_2 anomaly that is 10– to 20-fold greater than that observed today¹³. The innumerable consequences will be mostly irreversible and imposed on humanity for hundreds to thousands of years^{13,14}.

In order to study and understand this future and its implication for coral reef ecosystems, we built experimental reefs that closely resembled those of shallow reef slopes at Heron Island on the southern Great Barrier Reef¹⁵ (Fig. 1, Supplementary Figs. 1, 2). These replicated ‘ecosystems’ included an array of functionally diverse corals plus associated infauna growing on a foundation of previously deposited CaCO_3 composed of dead coral fragments (‘live rock’)¹⁶. CaCO_3 frameworks were allowed to be further built-up by the infilling of sediments created by a range of organisms including the calcifying alga, *Halimeda*^{17,18}, and organisms such as foraminifera and *Lithothamnion* that are abundant in the sediments collected from the shallow reef slope of Heron Island¹⁹. The sediments and microbial populations therein were aerated by sea cucumbers²⁰. Gardening damselfish nurtured turf algae²¹, while blennies consumed detritus, algae and cyanobacteria²². The typical age of water parcels in shallow seaward facing reef habitat is on the order of a few hours²³. To accommodate these retention times and allow reef organisms to influence the water chemistry²⁴, CO_2 was only controlled in large (8000 L) upstream sumps with downstream tanks (300 L) containing experimental reefs turned-over once per hour.

The experimental system allowed for the direct measurement of the net chemical balances of CaCO_3 (herein referred to as NEC)²⁵. NEC is distinct from census-based carbonate balances that tend to sum percent cover of calcifiers and bioeroders by converting each component to CaCO_3 equivalents. Census-based conversions typically apply rates from the literature, as opposed to treatment or habitat-specific responses, and often focus on biological erosion rather than chemical^{26,27}. Biological agents of erosion, however, retain CaCO_3 within the reef infrastructure and

operate as new foundation for reef construction²⁸, whereas, the chemical loss of CaCO_3 from reefs directly counters the production of CaCO_3 by key reef calcifiers. CaCO_3 structures lacking living tissue protection are especially vulnerable to increased chemical erosion under acidification, but there are also many documented cases of living reef-building corals exhibiting reductions in calcification rates as a result of exposure to acidification^{10,28–30}. Ecosystem components (e.g., macroalgae) may act as a buffer against acidification, with the suggestion that algal communities may benefit from increased seawater pCO_2 , take up CO_2 and lessen the impact of ocean acidification on downstream organisms and structures²⁴. The interactive effect, however, of matched warming to acidification on corals, algae and more importantly, complex reef communities over the long-term (> 12 months) is rarely considered especially with regards to the RCP8.5 scenario³¹.

The present study involved the long-term exposure of experimental reefs to seawater environments expected by the end of the century (2100) (Fig. 1, Supplementary Figs. 1 and 2). Using this approach, we (1) mimic changes to future coral reef ecosystem dynamics; (2) identify potential ‘winners’ capable of surviving end-of-century conditions; and (3) provide functional relationships between key reef processes, such as NEC rates, across a range of abiotic and biotic explanatory variables. The study thereby serves to provide an improved basis for understanding how diverse and complex reefs of the future may respond to a rapidly changing climate.

Results and discussion

Reef biota and NEC rates over time. Treatments were consistent with a seasonally dynamic end-of-century RCP8.5 scenario (Supplementary Figs. 3 and 4). The temperature difference between present day (PD) and RCP8.5 treatments was maintained at $\sim 3.5^\circ\text{C}$, with respective means across the experimental period of 25.0°C and 28.5°C (Supplementary Fig. 3). Upstream RCP8.5 pCO_2 sumps, which reflected the acidification status of the water mass fluxing onto our experimental reef, were maintained above PD values by $\sim 550 \mu\text{atm}$ (Supplementary Fig. 3).

Temperature, pCO_2 , and time of day had an interactive effect on hourly NEC rates (Fig. 2, Supplementary Table 1). NEC rates were low and generally negative at night, increasing in the morning to a maximum and positive value around midday. The individual effect of RCP8.5 acidification had a positive effect on NEC by day, but a negative effect by night (Fig. 2). By contrast, reefs experiencing the combination of RCP8.5 temperature and acidification responded negatively at all time points relative to present day treatments (Fig. 2).

A strong seasonal trend in 24-h NEC was observed in PD treatments, with rates increasing over the austral summer and declining in winter (Fig. 3a). Experimental reefs experiencing only RCP8.5 warming began to show an increase in 24-h NEC for the final 7 months of the experiment, despite undergoing similar reductions in macro-calcifiers to those observed under RCP8.5 warming and acidification (Fig. 3a–d, Supplementary Fig. 5). Under the combined RCP8.5 effect, 24-h NEC rarely showed positive rates and existed in a state of net dissolution for the majority of the experiment (Fig. 3a).

Community composition varied by treatment with living hard coral cover maintained between 23 and 47% under PD conditions, but reduced over time to $< 10\%$ under treatments that involved RCP8.5 warming (Fig. 3b). Coral bleaching under the combination of RCP8.5 temperature and acidification occurred before the RCP8.5 temperature-only treatment (Fig. 1, Fig. 3c). However, greater sensitivity to bleaching amongst taxa was not found to be an indicator of coral thermal tolerance (e.g.,

Fig. 1 Photographs of experimental coral reefs through time. Representative communities were assembled to replicate the natural communities of the Heron Island reef slope (5 m), southern Great Barrier Reef. Black arrows highlight bleaching, recovery and bleaching in *Fungia*.

Fungia was first to bleach but amongst survivors) (Fig. 1, Supplementary Figs. 1 and 2, Supplementary Table 2). The individual effect of RCP8.5 acidification tended to promote calcifying macroalgae in summer (Fig. 3d), most notably *Halimeda* (Fig. 1), which coincided with an increase in NEC (Fig. 3a, d). The winter *Halimeda* bloom observed under the combination of RCP8.5 warming and acidification did not, however, correlate with an increase in NEC (Fig. 3a, d). The abundance of macroalgae, turf algae and/or cyanobacteria was observed to increase significantly in any treatment that increased either temperature and/or acidification over PD values (Fig. 3d–f). In RCP8.5 acidification treatments, average midday in-tank pCO_2 were significantly less than upstream sump pCO_2 values (Fig. 2).

Drivers of net ecosystem calcification (NEC). The best-fit model to explain daytime NEC rates incorporated temperature, in-tank pCO_2 , and cover of calcifying organisms (Fig. 4a, b, Supplementary Fig. 6, Supplementary Data 1, 2). The significant interactive effects of temperature and pCO_2 revealed that not only did the temperature optima for NEC decline as pCO_2 increased, but the rate of NEC at optimal temperature declined (Fig. 4a,

Supplementary Fig. 6). For the same macro-calcifier cover, the difference in mean daytime NEC between PD conditions and RCP8.5 warming or RCP8.5 warming combined with acidification were $+0.23 \text{ gCaCO}_3\text{h}^{-1}\text{m}^{-2}$ and $-0.14 \text{ gCaCO}_3\text{h}^{-1}\text{m}^{-2}$ respectively (Fig. 4a, Table 1). These estimates include observed midday mitigation of pCO_2 by reef biota (Fig. 2). For the same temperature and pCO_2 levels, a reduction from 50 to 10% or from experimental maximum 60% to <10% calcifier cover reduced daytime NEC by 0.29 and 0.88 $\text{gCaCO}_3\text{h}^{-1}\text{m}^{-2}$, respectively (Fig. 4b).

The best-fit model for night-time NEC rates incorporated temperature, in-tank Ω_{Ar} , and their interaction (Fig. 4c, Supplementary Fig. 6, Supplementary Data 1, 2). As temperature increased, increasing Ω_{Ar} had a positive effect on calcification, while decreasing Ω_{Ar} had a strong negative effect (Fig. 4c). The mean night-time NEC differences between PD conditions and RCP8.5 warming or RCP8.5 warming combined with acidification were $-0.06 \text{ gCaCO}_3\text{h}^{-1}\text{m}^{-2}$ and $-0.15 \text{ gCaCO}_3\text{h}^{-1}\text{m}^{-2}$, respectively (Fig. 4c; Table 1). RCP8.5 warming and acidification therefore had a negative interactive effect on 24 hr NEC that could be offset by increasing the cover of calcifiers by 40%

Fig. 2 Net ecosystem calcification rates by treatment and time of day. Effect of business-as-usual (RCP8.5) ocean acidification and/or warming on net ecosystem calcification rates (mean $\pm$ SE). Values above bars show mean in-tank pCO₂ concentrations (μ atm).

(equivalent to $0.29 \text{ gCaCO}_3\text{h}^{-1}\text{m}^{-2}$, Fig. 4b, Table 1). When abiotic effects are considered with biotic effects, RCP8.5 warming and RCP8.5 warming combined with acidification decreased the estimated maximal rate of reef accretion⁵ by $49 \pm 4\%$ and $77 \pm 4\%$, respectively (Table 1). Both estimates of decrease are likely conservative given that maximum calcifier cover in the present study was 60% compared to historic baseline estimates of $\sim 80\%$ or more³².

Drivers of net photosynthesis and respiration. Daytime net photosynthesis (O_2 flux) within our experimental reef systems was best described by a model that included the cover of photosynthesizing organisms, light and in-tank pCO₂, together with a significant interaction between light and pCO₂ (Supplementary Fig. 6, Supplementary Data 1, 2). The inclusion of temperature did not, however, improve the model (Supplementary Data 2). Night-time respiration was best explained by the individual effect of temperature, with increased temperature significantly increasing respiration rates (Supplementary Fig. 7, Supplementary Data 1, 2).

Future reefs under RCP8.5. Our results demonstrate that measurements of coral community abundance (e.g., percent cover) do not account for the full impact of abiotic changes on reef carbonate sustainability. Temperature and acidification had direct effects on ecosystem calcification that remained when the impact associated with the abundance of key calcifying organisms was removed. The additional decrease in NEC, associated with the interaction of warming with acidification, was not driven by external bioeroders such as excavating parrotfishes²⁷ that were absent from our experimental reefs. Currently, reefs that reach $\sim 10\%$ coral cover are considered to transition to net-erosional reefs based principally on the biological activities of such eroders²⁶. The inclusion of these bioeroders within our experimental

reefs, in combination with RCP8.5 warming and acidification, therefore has the potential to increase the accretion-erosion threshold to $\sim 50\%$ coral cover. The literature¹² and results presented here strongly suggest that the likelihood that hard corals, or even calcifying macroalgae, will flourish to this extent in the near future is small. In fact, few reefs today would meet this new target^{33,34}. At $<10\%$ coral cover, RCP8.5 reefs will not only decrease in height due to the loss of the 3D-structure provided by living hard coral, but the maximal vertical accretion of reef substrate will decrease by $\sim 7.7 \text{ mmyr}^{-1}$ (for a 50% porous reef)⁵ (Table 1). The same decrease associated with RCP8.5 temperature in the absence of acidification was only $\sim 4.9 \text{ mmyr}^{-1}$ (Table 1). Our results scale with changes to the porosity of reef substrates. Reefs constructed with greater porosity (i.e., CaCO₃ mostly distributed in branches of dead coral as opposed to infilled sediments) have greater rates of maximal vertical accretion (i.e., 20 mmyr^{-1} for a 75% porous reef)³⁵. Such reefs, however, have tended to form and subsist in sheltered locations³⁵, and therefore, would be more prone to height reductions through physical destruction associated with exposure to greater wave energies in the future³⁶.

Further and potentially catastrophic reductions in the upward extension of reefs are likely under RCP8.5 due to the increased susceptibility of previously established reef substrates to physical and chemical erosion²⁸. Once infilled and consolidated, reefs may be subsequently hollowed-out and weakened, leading to reef structures that are increasingly vulnerable to physical erosion and collapse. At maximal average vertical accretion rates of $<2.3 \text{ mmyr}^{-1}$, future reefs will be unable to keep-up with conservative estimates ($5\text{--}15 \text{ mmyr}^{-1}$) of sea-level rise³⁷ over hundreds to thousands of years¹⁴. Similarly, but excluding the synergistic effects of warming and acidification, Perry et al.³⁸ conclude that reefs would have to maintain 60–70% coral cover to avoid submergence under RCP8.5. Again, adding our 40% cover equivalence for loss of NEC due to the synergistic impacts of warming and acidification, shifts Perry et al.³⁸ submergence

Fig. 3 Influence of ocean acidification and/or warming on net ecosystem calcification and benthic cover. **a** Integrated 24-h net ecosystem calcification rates indicate strong seasonal variation. Benthic cover was determined from weekly photographs across the 18-month experimental period for: **b** Live hard coral (*Acropora*, *Montipora*, *Fungia*, *Lobophyllia*, *Porites*, *Platygyra*, *Seriatopora*, and *Stylophora*), **c** Bleached hard coral, **d** Calcifying macroalgae (crustose coralline algae, *Halimeda*), **e** Other macroalgae (i.e., *Lobophora*, *Caulerpa*), and **f** turf algae and cyanobacteria. All lines are means $\pm$ standard error. Dashed vertical line indicates the start of sampling period.

threshold to ~100% cover. Our study demonstrates how studies that only include the effect of warming significantly underestimate the potential rate of reef decline. The impact of 3D-structural loss on the reef's ability to dissipate wave-energy is expected to occur more immediately¹. However, in the long-term, the increase in the depth of the reef substrate is expected to result in catastrophic land inundation and associated economic costs³⁹. Land inundation and increased wave energy in back-reef areas is expected to increase sediment resuspension¹, reducing light penetration and the depth at which reefs “drown”¹⁷. Increases in atmospheric CO₂ under RCP8.5, therefore, have the potential to create a hiatus in reef formation, that is likely to prevail well beyond the stabilization of the planet's climate (Supplementary Fig. 8).

Not all coral genera responded equally to the experimental changes. Those that survived warming, in an average “recovery” year under RCP8.5, included *Fungia*, *Lobophyllia*, and the soft coral *Sarcophyton* (Supplementary Table 2). *Fungia*, single-polyped solitary corals, and *Lobophyllia* do not contribute to the 3D structuring of reefs. *Lobophyllia* are fleshy corals that can dispense with colonial living as they age and tend not to interlink polyp corallites with CaCO₃^{40,41}. The results are consistent with current literature that identifies fleshy corals with reduced skeletal

extension rates as more able to survive thermal stress than corals that invest less in tissue and more in skeletal extension⁴². Likewise, it is often noted that corals exposed to high pCO₂ show regular skeletal extension rates, but calcify at reduced rates leading to more porous (i.e., less dense), weaker skeletons^{10,29,30}. In exceptional cases, corals exposed to acidification can even forgo calcification and colonial living, as they convert to giant, fleshy single polyps⁴³. None of these trade-offs are promising for coral reefs in the future, and all of them are likely to significantly inhibit the ability of reefs to accrete vertically in coastal regions that are expected to be exposed to higher wave energies with sea level rise⁴⁴.

Night-time dissolution had a significant impact on the net calcification of the experimental reefs studied here. Reef sediments are sensitive to increases in organic matter deposited as organisms die because decomposition produces CO₂ that can under saturate porewater Ω_{Ar} ^{45,46}. Acidification, by decreasing the Ω_{Ar} of the overlying water mass, amplifies the impact of organic matter on sediment dissolution²⁵. Likewise, by stimulating respiration, higher temperatures can increase the pCO₂ concentration of reef waters percolating into the sediments^{46,47}. In the experimental reefs described here, temperature had no effect on seawater net O₂ flux by day, but had a highly significant

Fig. 4 Influence of environmental parameters on net ecosystem calcification. **a** Three-dimensional graphic output of the significant interaction between temperature and pCO₂ as predictors of daytime net ecosystem calcification (NEC) rates. **b** Relationship between daytime NEC and the cover of calcifying organisms for the best model at mean temperature and pCO₂, shaded zone represents 95% confidence interval. **c** Three-dimensional graphic output of the significant interaction between temperature and aragonite saturation state (Ω_{Ar}) as predictors of nighttime NEC rates. Cover of calcifying organisms includes hard coral and calcifying macroalgae.

effect by night. Based on in-field responses of sediments to acidification, Eyre et al.²⁵ recently argued that coral reefs will transition to net dissolving reefs. Another in-field study by Shamberger et al.⁴⁸ showed that 26% coral cover barely maintained positive NEC under long-term acidification conditions. Similarly, in our experimental reefs under RCP8.5 acidification, NEC was reduced relative to present day over the

first half of the experiment (~6 months), but increased significantly towards the end of the experiment. The observed increase in NEC is likely due to: (1) biotic mitigation at mid-day resulting in an observed 50% decline in in-tank pCO₂ for RCP8.5 acidification treatments; and, (2) increases in *Halimeda* biomass and calcification rates⁴⁹ that were observable due to the long-term nature of the study. These phenomena more than offset the nocturnal dissolution of substrates observed under acidification-only treatments. In the present study, the role that sediments play as contributors to the build-up of reefs and islands was only threatened when the acidification impact of an RCP8.5 scenario was matched by its thermal impact. Warming combined with acidification decoupled the relationship between calcifier cover and daytime net NEC, whilst accelerating rates of night-time dissolution.

Additional to observed night-time impacts, NEC declines relative to present day were observed under RCP8.5 acidification and warming during the day. These declines likely relate to previous observations of increased dissolution of dead coral substrates by endolithic algae and photosynthetic microbes^{50,51}, as well as potential reduction in the calcification rates of surviving calcifiers that may direct energy acquired to biomass production and maintenance, as opposed to calcification⁴². A recent study has demonstrated that following a thermal stress event, the rate of dissolution of exposed CaCO₃ framework can be significantly greater than the calcification rates of living coral⁵². Evidence for rapid structural loss following present day marine heatwaves emphasizes the potential for catastrophic loss of carbonate substrates under chronic future warming and acidification.

Some studies propose that corals will adapt to the new conditions improving the outcomes for reefs in ways that cannot be captured by short-term experiments. Against this notion, it is argued that the life cycles and generation times may be too long to facilitate this change⁵³; or that any mechanisms of rapid adaptation is only sustainable under reduced emission scenarios⁵⁴. Others argue that this can be achieved via transcription changes occurring in response to epigenetic memory⁵⁵. In such studies, bleaching susceptibility is often tied to mortality; however, in the present study and in recent observations of reefs impacted by significant underwater heatwave events, mortality was not correlated to bleaching susceptibility⁹. The focus on adaptations that prevent bleaching in the face of climate change obscure the potential costs on both primary (extension) and secondary (densification) calcification, even if they prove to facilitate coral survival. Our study argues for a decoupling of calcifier biomass and calcification rates under warming and acidification, and suggests that any adaption amongst key calcifiers would need to both increase survival potential and rates of calcification substantially above maximal rates observed in the past. The likelihood of such an outcome is poor, but reduces even further with observations regarding the ephemeral status of CaCO₃ deposition by scleractinian corals, and their survival without fossil representation through 14 million year subsequent to the Permian extinction event⁵⁶.

In summary, our results counter multiple modeling studies that argue that future acidification will play only a minor role in the future status of coral reefs^{57,58}. The results of our experiments align with earlier models⁵⁹ suggesting that acidification can seriously undermine the carbonate status of coral reef ecosystems, but do so only in combination with thermal stress. Our study suggests that reefs will erode more rapidly than under previous estimates³⁸, as they transition to alternate reef states under unmitigated climate change. Importantly, our calculations also include the potential mitigation of mid-day pCO₂ by an enriched macroalgal community²⁴, which is likely to dissipate with climate change, as the 3D structure of coral reefs and the retention of

Table 1 Net ecosystem calcification (NEC, gCaCO₃m⁻²h⁻¹) rates and percent change in vertical accretion relative to an optimal present day experimental reef.

Responses:	Abiotic predictors by time of day				Biotic predictor	Summed	
Treatment	Morning	Noon	Night	24 h relative to PD	Relative to maximum biomass*	24 h Relative to PD	Change in max vertical accretion [§]
	NEC (± SE) Temperature (°C), pCO ₂ (µatm) or Ω _{ar}						
Present day (PD)	0 (±0.1)	0.2 (±0.1)	-0.09 (±0.05)				
	25.0, 530	25.0, 320	25.0, 3.5				
RCP8.5 temperature only	0.25 (±0.1)	0.4 (±0.1)	-0.15 (±0.05)	0.14 (±0.12)	-0.88 (±0.05)	-0.74 (±0.06)	-49 ± 4%
RCP8.5 temperature and acidification	-0.23 (±0.1)	0.15 (±0.1)	-0.24 (±0.05)	-0.29 (±0.12)	-0.88 (±0.05)	-1.17 (±0.06)	-77 ± 4%
	28.5, 870	28.5, 490	28.5, 2.2				

NEC values obtained from Fig. 4. *NEC for 60 to 10% decrease in calcifier cover; [§]relative to maximal values (±SE) in⁵.

water it creates collapses²³. The conclusions strengthen the call for achieving the goals of the Paris Climate Agreement as a matter of global emergency.

Methods

Specimen collection and experimental design. The aim of the present study was to experimentally explore the impact of an average RCP8.5 future “recovery” year (i.e., non-El-Niño year) on the net ecosystem calcification (NEC) of experimental reefs reconstructed from a shallow (5 m) reef slope location at Heron Island on the southern Great Barrier Reef (23°26' S 151°52' E). As an offshore reef, the input water into an experimental system on Heron Island has very low levels of coastal pollutants that can promote algal over coral growth⁶⁰. The supply of oligotrophic water to reconstructed experimental mesocosms was important, as experimentally, we were limited to the provision of smaller motile herbivores such as gardening damselfish (*Pomacentrus wardii*), lawn mower gobies (*Salarias fasciatus*), and an array of invertebrate detritivores and herbivores. The inclusion of sediments, live rock, crabs, snails and other infauna fed by flow through water from the reef, however insured the provision of food for the organisms, inclusive of corals present in the reconstructed experimental reefs. Representative concentration pathway 8.5 (RCP8.5) was selected because the experiment was conducted between June 2014 and March 2016; that is, prior to the Paris Climate Agreement. RCP8.5, however, represents our current rate of fossil fuel emissions despite global consensus for the adoption of policies that significantly reduce emissions⁶¹. Treatment conditions were gradually achieved over the 3-month period from June–August 2014.

To monitor the individual and combined impacts of an average non-El Niño “recovery” year under RCP8.5 temperature and acidification, we selected the relatively cool year of 2013 as our present-day (PD) baseline scenario. Temporal variability was introduced by following water temperature and pCO₂ as measured at 2 hourly intervals at Harry’s Bommie over 2013 (<https://www.pmel.noaa.gov/co2/story/Heron+Island>). RCP8.5 scenarios were then off-set from this variable PD baseline by +3.5 °C and +550ppm. An orthogonal design of Temperature (PD, RCP8.5) × pCO₂ (PD, RCP8.5) was implemented with *n* = 3 downstream tanks (300 L), continuously fed at a turnover rate of 4.5 L min⁻¹ by one of four upstream sumps. Sump, as opposed to tank pCO₂, was controlled to allow organisms and their biology to influence in-tank pCO₂. The flow-through rates (>18 Lmin⁻¹), size (8000 L) and the elimination of light from the sumps limited any containment effects and hence the potential for pseudoreplication⁶². By contrast, light to tanks was mediated by filters (marine blue #131, LEE Filters, Hampshire, UK) to replicate light conditions at 5 m depth; and, reduced tank turnover to mimic bodies of water that are typically retained for a few hours within shallow seaward facing reef slope environments²³. Dove et al.¹⁵ provides further details regarding the mesocosm system and its control. Photosynthetically active radiation (Odyssey PAR sensor, Dataflow Systems Ltd, Christchurch, New Zealand) and temperature (HOBO Pendant UA-001-64, Onset, Bourne, USA) were measured continuously in each tank.

Each of the 12 experimental reefs (Fig. 1, Supplementary Figs. 1 and 2) contained sediments, algae (including *Halimeda*), live rock, soft coral (zoanthids, *Sarcophyton*, *Xenia*) and hard coral collected together with infauna from the reef slope of Harry’s Bommie, Heron Island, Great Barrier Reef at a depth of ~5 m. Sea cucumbers (*n* = 2, per tank, *Holothuria edulis*), damselfish (*n* = 1 per tank, *Pomacentrus wardii*), and blennies (*n* = 3 per tank, *Salarias fasciatus*) were collected from Heron Island reef flat. Sediments were immediately placed within each seawater filled tank to a depth of 4 cm. Hard corals, except for *Fungia*, were placed on live rock to avoid direct contact with sediment. Soft corals and *Halimeda* were collected and incorporated into the tank together with the reef structure to which they were attached. Each tank was fitted with a powerful wavemaker (JVP-202B, SunSun, Zhoushan, China) to minimize boundary layer effects that can have significant negative influence on the exchange of nutrients, CO₂ and O₂⁶³. Tanks were established over a week in June 2014 (austral winter). At the end of the

experiment, the non-coral invertebrate diversity was cataloged and found to include a broad range of organisms such as mollusks and crustaceans.

Physiological analyses. Hourly net ecosystem calcification (NEC), photosynthesis (NEP), and respiration (NER) were determined via tank incubations (1 h at 12:00, 2 h at 7:00, 18:00 and 0:00) and conducted every ~5 weeks over an 18-month experimental period. NEC was determined from changes in total alkalinity (AT). AT was determined via titration (T50 titrator, Mettler Toledo, Port Melbourne, Australia) using 0.1 M HCl and replicated 20 g seawater samples with a precision of ±3 mol kg⁻¹ or better using the Gran titration method¹⁵. Acid concentration was calibrated at the beginning of each titration session using the certified reference materials (CRM) from A.G. Dickson (Scripps Institute of Oceanography, San Diego, USA). pH electrodes (DGI101-SC, Mettler Toledo, Port Melbourne, Australia) were also calibrated at the beginning of each titration session using pH_{SW} standard buffers of 4.00 and 7.00 (Hach, Loveland, USA), with an average precision from triplicate measurements of less than 0.010 units. O₂ flux was determined with dissolved oxygen (DO) sensors (RINKO W, JFE Advantech, Hyōgo, Japan). Parameters of the carbonate system (in-tank incubation pCO₂ and Ω_{ar}) were calculated from temperature, salinity, pH (NBS) and AT using CO₂Calc⁶⁴ (Supplementary Fig. 4).

Determination of benthic community composition. Benthic cover was determined from weekly photographs (September 2014–February 2016), which were taken in the late afternoon with a high definition camera (PowerShot G12, Canon, Tokyo, Japan). Images were analyzed through automated image recognition based on machine learning using the online repository, CoralNet⁶⁵. Targeted annotations (≥500 points per category) were performed manually to establish color and texture patterns to be used as descriptors for benthic categories. Percent cover estimations were then achieved using Support Vector Machines, a machine-learning model, on 100 points per image in a 10 × 10 uniform grid. The following benthic categories were used, with the functional group displayed in brackets: *Acropora* (Live hard coral), *Montipora* (Live hard coral), *Fungia* (Live hard coral), *Lobophyllia* (Live hard coral), *Porites cylindrica* (Live hard coral), *Platygyra* (Live hard coral), *Seriatopora* (Live hard coral), *Stylophora* (Live hard coral), bleached *Acropora* (Bleached hard coral), bleached *Montipora* (Bleached hard coral), bleached *Fungia* (Bleached hard coral), bleached *Lobophyllia* (Bleached hard coral), bleached *Porites cylindrica* (Bleached hard coral), bleached *Platygyra* (Bleached hard coral), bleached *Seriatopora* (Bleached hard coral), bleached *Stylophora* (Bleached hard coral), *Halimeda* (calcifying macroalgae), crustose coralline algae (calcifying macroalgae), all other macroalgae (i.e., *Caulerpa*, *Lobophora*, *Sargassum*) and turf algae/cyanobacteria.

Statistical analyses. All statistical analyses were performed using R (version 3.5.1)⁶⁶ and graphical representations were produced using the packages *ggplot2* and *mgcv*^{67,68}.

The effects of temperature (PD, RCP8.5), pCO₂ (PD, RCP8.5) and time of day (0:00, 7:00, 12:00, 18:00) on hourly NEC rates from December 2014 to February 2016 were analyzed using a linear mixed effect model using the function *lme* within the package ‘nlme’. For all analyses, tank was included as a random effect. The interactive effect of treatment (PD temperature and pCO₂; RCP8.5 pCO₂ only; RCP8.5 temperature only; RCP8.5 temperature and pCO₂) and month (1–12) on 24-h NEC rates were determined using *lme*. The effect of treatment on the cover of live hard coral was assessed using *lme*. The effect of *Halimeda* on NEC rates under RCP8.5 pCO₂ only was assessed using *lme*. The effect of treatment on macroalgal abundance was assessed using *lme*. The effect of treatment on cover of bleached coral at the beginning of the first austral summer (November 2014) was assessed using *lme*. Significant interactive effects were followed by pairwise comparison of estimate marginal means using the *emmeans* package with Tukey’s adjusted *p* values. Data were tested for homogeneity of variance and normality of distribution

through graphical analyses of residual plots for all models. For all analyses, tanks ($n = 3$) were used as replicates.

The effect of treatment on benthic community composition was analyzed using permutational MANOVAs (PERMANOVA), with the fixed effects of temperature and pCO₂ and random effect of tank using the *adonis* function from the ‘vegan’ package⁶⁹. Resemblance matrices were obtained using Bray-Curtis dissimilarity and 9,999 permutations.

Generalized additive models (GAMs) were applied to data sets using the *gam* function from the package ‘mgcv’⁶⁷. GAMs were used to allow for any possible nonlinear effects of metrics on the response variable. Smooth terms were fit using thin plate regression splines (tp) and full tensor product interactions (te), with the number of knots restricted ($k = 5$) to produce conservative models and avoid overfitting. The simplest model within 2 AICc values of the model with the lowest AICc was selected as the best model (Supplementary Data 1, 2).

To assess the relative contribution of environmental metrics in explaining NEC, photosynthesis (NEP) and respiration (NER), we fit all possible model combinations, using the following metrics as predictor variables: (i) temperature (°C), (ii) light measured as photosynthetically active radiation (PAR in $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$), (iii) pCO₂ (μatm) or aragonite saturation state (Ω_{Ar}) and (iv) percent cover of calcifying organisms (%; sum of live hard coral and calcifying macroalgal cover, Supplementary Fig. 5), or photosynthesizing organisms (%; sum of live hard coral, calcifying macroalgae, other macroalgae, and cyanobacteria, Supplementary Fig. 5). Tank was included as a random effect in all models. The model structure was developed using a stepwise procedure, where models were compared and selected using Akaike information criterion for small sample sizes (AICc) and AICc weight (ω_i). Prior to analyses, all environmental metrics were tested for collinearity. To limit complexity, we fit models that included only up to three predictor variables and their interactions⁷⁰.

Summed errors presented in Table 1 were generated via stochastic modeling applying “Norm.Inv (Rand()), mean, SD)” in Microsoft Excel for each component over 1000 iterations (years).

Data availability

All data are available in the manuscript or the supplementary materials. Original data can be found on <https://github.com/imkristenbrown/Uncoupling-calcification-from-calcifier-biomass>.

Code availability

All R-scripts generated for this study can be found on <https://github.com/imkristenbrown/Uncoupling-calcification-from-calcifier-biomass>.

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Author contributions

S.G.D. and O.H.-G. initiated the study. S.G.D., A.H. and A.C. collected and collated the data. S.G.D. and K.T.B. analyzed the data. S.G.D., A.H. and A.C. optimized the experimental system. S.G.D., K.T.B. and O.H.-G. contributed to the writing of the paper.

Competing interests

The authors declare no competing interests.

Additional information

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