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# Water flow modulates the response of coral reef communities to ocean acidification

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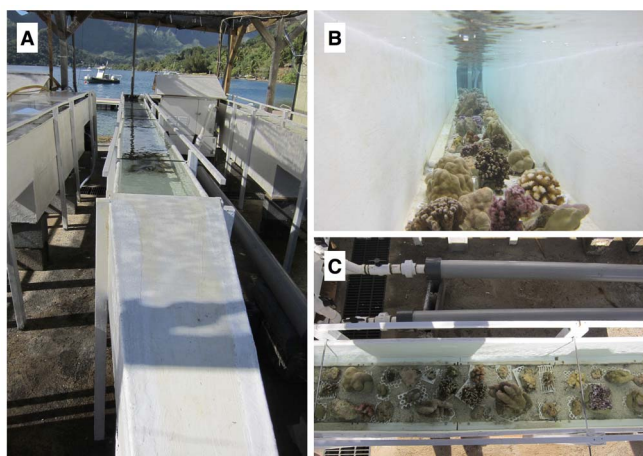
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By the end of the century coral reefs likely will be affected negatively by ocean acidification (OA), but both the effects of OA on coral communities and the crossed effects of OA with other physical environmental variables are lacking. One of the least considered physical parameters is water flow, which is surprising considering its strong role in modulating the physiology of reef organisms and communities. In the present study, the effects of flow were tested on coral reef communities maintained in outdoor flumes under ambient  $p\text{CO}_2$  and high  $p\text{CO}_2$  (1300  $\mu\text{atm}$ ). Net calcification of coral communities, including sediments, was affected by both flow and  $p\text{CO}_2$  with calcification correlated positively with flow under both  $p\text{CO}_2$  treatments. The effect of flow was less evident for sediments where dissolution exceeded precipitation of calcium carbonate under all flow speeds at high  $p\text{CO}_2$ . For corals and calcifying algae there was a strong flow effect, particularly at high  $p\text{CO}_2$  where positive net calcification was maintained at night in the high flow treatment. Our results demonstrate the importance of water flow in modulating the coral reef community response to OA and highlight the need to consider this parameter when assessing the effects of OA on coral reefs.

Ocean acidification (OA), caused by the dissolution of anthropogenic  $\text{CO}_2$  emissions in the oceans, is expected to have a strong impact on marine organisms<sup>1</sup> and particularly on tropical coral reefs<sup>2</sup>. The response of coral reef organisms to OA has received extensive attention from the scientific community<sup>3</sup>, but the burgeoning literature on this topic has revealed a diversity of experimental outcomes. Most studies have reported deleterious effects of decreasing pH on scleractinian corals and coralline algae, particularly for calcification, but responses vary among species and can include no effect<sup>4,5</sup>.

In addition to species-specific responses of coral reef calcifiers to OA, the response of reef organisms to increasing  $p\text{CO}_2$  will likely be modulated by other environmental variables such as temperature, irradiance, and water flow. The interactive effects of temperature and OA is beginning to receive more attention<sup>6,7</sup>, and recently other studies have addressed the interactive effects of irradiance and  $p\text{CO}_2$  on organism performance<sup>8–11</sup>. However studies on the interactive effects of water flow and OA are lacking. In previous studies performed under ambient  $p\text{CO}_2$  (i.e., 400  $\mu\text{atm}$ ), it has been shown that increasing water flow speed can have positive effects on the calcification of individual corals in aquaria or beakers<sup>12,13</sup>. Flow can also enhance primary production and dark respiration of corals<sup>14,15</sup> and can impact their success at particle capture<sup>16</sup>. Flow has been reported to play an important role in limiting the sensitivity of corals to bleaching under elevated temperature<sup>17,18</sup>, which likely is due to reduced oxidative stress under high flow<sup>19</sup>. Water flow can also increase photosynthesis and nitrogen fixation by coral reef algal turfs<sup>20,21</sup>. Critically, increased flow speed creates thinner boundary layers and promotes mass flux across them by turbulent eddies, which under conditions of mass transfer limitation can promote transport of metabolites and increase exchange and rates of physiological processes<sup>22</sup>. Potentially, protons could be one ion for which increasing flow favors exchange from the organism to the environment, which might enhance calcification under OA conditions<sup>23</sup>.

Previous OA studies of coral reef calcifiers mostly have focused on the response of individual organisms, but now there is growing interest in scaling these results to the response of community assemblages under similar conditions<sup>24,25</sup>. For ecological relevance, studies of coral reef communities need to be performed under physical and chemical conditions similar to those occurring in the field where the communities occur naturally. For this reason, we recently have performed incubations on tropical reef communities maintained in outdoor flumes<sup>25</sup> ( $0.3 \times 0.3 \times 5.0$  m working section) designed to test the response of assembled reef communities to OA (Fig. 1).



**Figure 1 | Photographs of the outdoor flumes and the assembled communities (photographs made by S. Comeau).** (A) The flumes consisted of a  $5.00 \times 0.30 \times 0.30$  m working section in which  $p\text{CO}_2$  and flow speeds were manipulated. (B) Communities were assembled in the flumes to match the natural communities in the back reef of Moorea in 2013. (C) Corals and calcified algae were attached to plastic supports and placed in the flumes on the epoxied bottom or on top of the sediment, which covered half of the flumes.

In addition to experiencing natural irradiance, outdoor flumes present the opportunity to test for the effects of varying flow speeds on responses to OA under controlled conditions<sup>22</sup>.

In our recent experiments conducted in flumes<sup>25</sup>, we matched the conditions in the flume to those occurring naturally in the environment, and assembled reef communities that included corals, calcified algae and sediment in proportions similar to the natural environment of the shallow (2–5 m depth) back reef of Moorea, French Polynesia, in 2013. At present (2013), the benthic community of back reef consisted of 21% coral cover, 7% macroalgal and coralline algal cover, 24% sand cover, and 48% exposed substratum and turf<sup>26,27</sup>. The working section of four replicate flumes were designed with a lower insert ( $0.3 \times 0.3 \times 2.5$  m) to their floors that accommodated boxes (30 cm deep) containing sediment transplanted from the back reef of Moorea. The sediment inserts allowed reef communities to be constructed mimicking those found in the back reef. Sediment was also included since previous studies have shown that increasing  $p\text{CO}_2$  can cause calcification of sediments to switch from net accretion to net dissolution under *in situ* conditions at  $p\text{CO}_2$  levels expected by the end of the current century<sup>28</sup>, and also in conditions naturally acidified by  $\text{CO}_2$  produced by microbial decomposition of organic matter<sup>29</sup>.

The present study investigates the effect of water flow speed on tropical coral reef communities exposed to OA conditions. Assembled communities were incubated in four outdoor flumes for 8 weeks under ambient  $p\text{CO}_2$  and an elevated  $p\text{CO}_2$  ( $\sim 1300 \mu\text{atm}$ ). Communities were maintained at flow speeds similar to Moorea back reef conditions<sup>30–32</sup> (i.e., a mean flow speed of  $10 \text{ cm s}^{-1}$ ), and calcification was measured at regular intervals dur-

**Table 2 | Mean flow characteristics in the flumes during the incubation at ambient, low and very low flow.** The dimensionless Reynolds number ( $Re$ ) was calculated for each flow speed as a function of the hydraulic diameter of the flume and the kinematic viscosity of seawater. The roughness Reynolds number ( $Re_k$ ) was calculated to take into account the mean height of the roughness elements. The friction factor ( $C_f$ ) was determined as a function mean height of the roughness elements and the hydraulic diameter of the flumes

Flow Treatment	Flow ( $\text{m s}^{-1}$ )	$Re$	$Re_k$	$C_f$
Very low	0.02	910	205	0.14
Low	0.05	2275	512	0.14
Ambient	0.1	4550	1024	0.14

ing 24-h incubations. Additionally, the flow speeds in the flumes were adjusted periodically to low ( $5 \text{ cm s}^{-1}$ ) and very low ( $2 \text{ cm s}^{-1}$ ) levels and calcification again was measured to test for the interactive effects of flow and OA. Calcification was measured during three replicate 24-h incubations at each of the three flow speeds and at three levels of biological organization: the whole community, sediments, and macro-calcifiers (i.e., corals and calcified algae as determined by subtraction of sediment only rates from the whole community rates).

## Results

Treatments were controlled successfully during the 8 weeks of incubation with  $p\text{CO}_2$  maintained at  $456 \pm 19 \mu\text{atm}$  and  $451 \pm 18 \mu\text{atm}$  in the ambient treatments, and  $1329 \pm 28 \mu\text{atm}$  and  $1306 \pm 41 \mu\text{atm}$  in the high  $p\text{CO}_2$  treatments ( $\pm \text{SE}$ ,  $n = 42$ , Table 1). There was no statistical difference in  $p\text{CO}_2$  between flumes from the same treatment ( $p = 0.852$ ), and in all flumes the aragonite saturation state remained  $>1.0$  (Table 1).

The dimensionless Reynolds numbers ( $Re$ ) based on the hydraulic diameter of the working section were 910, 2275, and 4550 at 2, 5 and  $10 \text{ cm s}^{-1}$  respectively (Table 2), indicating that seawater coming into this section was laminar at a flow speed  $2 \text{ cm s}^{-1}$ , transitionally turbulent at  $5 \text{ cm s}^{-1}$ , and fully turbulent at  $10 \text{ cm s}^{-1}$ . The roughness Reynolds numbers ( $Re_k$ ) were 205, 512, and 1024 at 2, 5, and  $10 \text{ cm s}^{-1}$  respectively. Under all tested flows,  $Re_k$  was  $>70$ , indicating that the flume bottoms were fully rough and both generated and dissipated turbulence across the assembled communities. The friction factor in the flumes ( $C_f$ ) was 0.14 (Table 2).

At the community level, calcification was highest during the day in high flow, ambient  $p\text{CO}_2$  and lowest in the low flow, high  $p\text{CO}_2$  (Fig. 2A). The community net calcification rates in the flumes in the ambient treatment during the day were similar to rates measured for reefs on the north shore of Moorea in 2012 and 2013 (R.C. Carpenter, unpublished data). During the day, there were significant effects of  $p\text{CO}_2$  ( $p = 0.019$ ) and flow ( $p = 0.040$ ), and there was no interaction between the two ( $p = 0.251$ ). At night, under three flow speeds, net calcification was positive at ambient  $p\text{CO}_2$ , but negative at high  $p\text{CO}_2$  (Fig. 2A). There was a significant interaction between

**Table 1 | Mean carbonate chemistry in the four flumes (F1–4) during the 8-week incubation.** The partial pressure of  $\text{CO}_2$  ( $p\text{CO}_2$ ), the aragonite saturation state ( $\Omega_{\text{arag}}$ ) and the calcite saturation state ( $\Omega_{\text{calc}}$ ) were calculated from  $\text{pH}_T$ , total alkalinity ( $A_T$ ), temperature and salinity using the R package seacarb. The values presented are mean  $\pm \text{SE}$  ( $n = 56$ ). SE for salinity was  $<0.1$

Flume	Treatment	$\text{pH}_T$	$A_T$ ( $\mu\text{mol. kg}^{-1}$ )	$p\text{CO}_2$ ( $\mu\text{atm}$ )	$\Omega_{\text{arag}}$	$\Omega_{\text{calc}}$	Temperature ( $^\circ\text{C}$ )	Salinity
F1	High $p\text{CO}_2$	$7.603 \pm 0.008$	$2343 \pm 1$	$1329 \pm 28$	$1.60 \pm 0.03$	$2.41 \pm 0.04$	$27.0 \pm 0.1$	35.9
F2	Ambient	$8.010 \pm 0.012$	$2339 \pm 1$	$456 \pm 19$	$3.49 \pm 0.07$	$5.26 \pm 0.11$	$26.8 \pm 0.1$	35.9
F3	High $p\text{CO}_2$	$7.617 \pm 0.014$	$2345 \pm 1$	$1306 \pm 42$	$1.68 \pm 0.05$	$2.53 \pm 0.08$	$27.1 \pm 0.1$	35.9
F4	Ambient	$8.015 \pm 0.013$	$2339 \pm 1$	$451 \pm 18$	$3.53 \pm 0.07$	$5.32 \pm 0.11$	$26.9 \pm 0.1$	35.9



pCO<sub>2</sub> and flow ( $p = 0.045$ ), which was caused by increased dissolution at low flow and high pCO<sub>2</sub>. When net calcification was integrated over 24 h, it was positive under all conditions except in the high pCO<sub>2</sub> and low flow (Fig. 2A). Net calcification integrated over 24 h was affected by pCO<sub>2</sub> ( $p = 0.003$ ) and flow ( $p = 0.013$ ), but the interaction was not significant ( $p = 0.138$ ). Net calcification increased as a function of flow under both pCO<sub>2</sub> treatments.

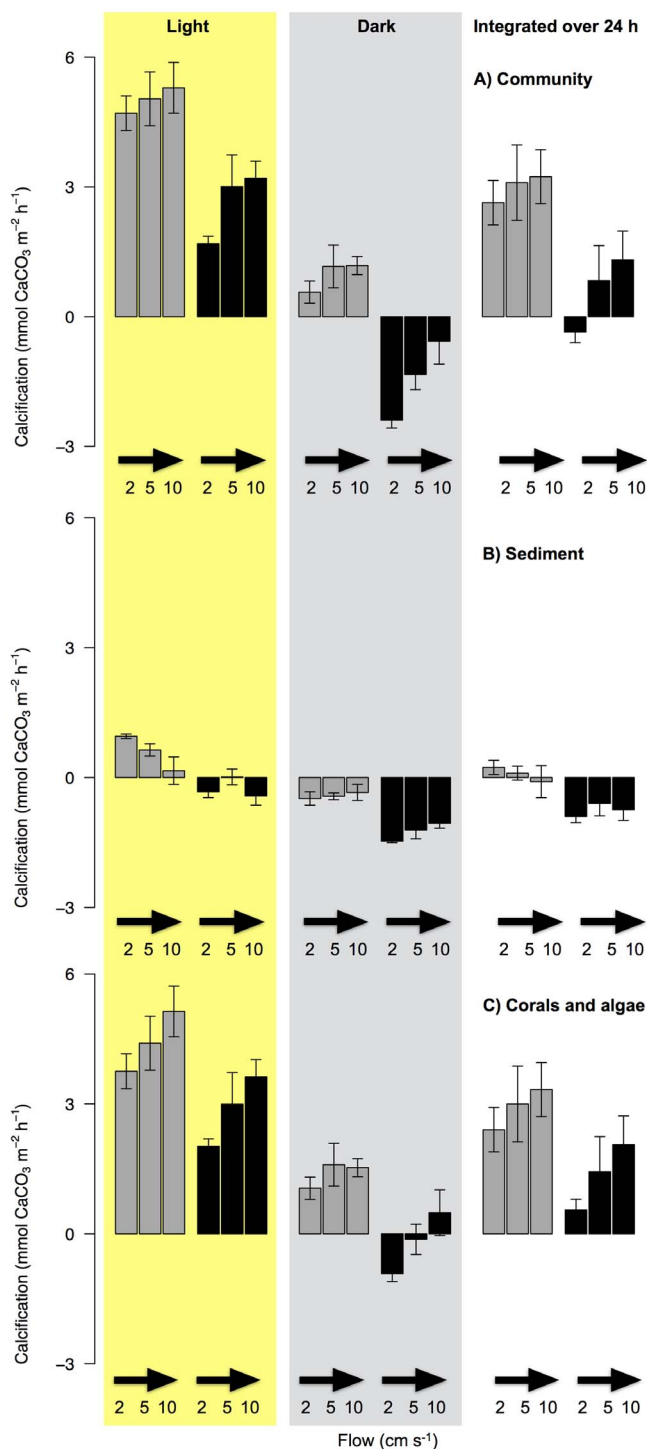
In contrast to net calcification at the community level, net calcification of the sediments was highest under ambient pCO<sub>2</sub> and low flow (Fig. 2B). For daytime calcification, there was a significant interaction between pCO<sub>2</sub> and flow ( $p = 0.005$ ), which was due to a strong effect of flow at ambient pCO<sub>2</sub>, but a limited effect at high pCO<sub>2</sub>. At night, net calcification of sediments was negative in both the ambient and high pCO<sub>2</sub> (Fig. 2B) with greater dissolution at high pCO<sub>2</sub> ( $p = 0.018$ ). However, neither flow ( $p = 0.069$ ) nor the interaction between pCO<sub>2</sub> and flow were significant ( $p = 0.324$ ). Integrated over 24 h, sediment net calcification was negative at high pCO<sub>2</sub> under all flow regimes, and positive or near zero in the ambient treatment depending on flow speed (significant interaction between pCO<sub>2</sub> and flow,  $p = 0.025$ ).

Net calcification of corals and calcifying algae, estimated by subtracting the mean calcification rates of the sediments from the total community net calcification for each treatment, was highest at ambient pCO<sub>2</sub> under high flow during the day (Fig. 2C). In contrast to the whole community and the sediments alone, the pCO<sub>2</sub> effect on daytime net calcification of corals and calcifying algae was not significant ( $p = 0.064$ ), despite a trend toward lower net calcification under high pCO<sub>2</sub>. However, estimated net calcification of corals and calcifying algae increased with flow in both pCO<sub>2</sub> treatments ( $p = 0.007$ ). At night, net calcification remained positive in the ambient pCO<sub>2</sub> treatment at all flow speeds, while at high pCO<sub>2</sub> it was negative at the 2 cm s<sup>-1</sup> and 5 cm s<sup>-1</sup> flow speeds (Fig. 2C). For the estimated nighttime net calcification of corals and calcifying algae, there was a significant interaction between pCO<sub>2</sub> and flow ( $p = 0.048$ ) with a stronger effect of flow at high pCO<sub>2</sub>. When integrated over 24 h, net calcification of corals and calcifying algae was positive for all treatments but was reduced at high pCO<sub>2</sub> at the 2 cm s<sup>-1</sup> flow speed (Fig. 2C). Both pCO<sub>2</sub> ( $p = 0.014$ ) and flow ( $p = 0.004$ ) affected net calcification significantly, but not the interaction between the two ( $p = 0.303$ ).

## Discussion

This study was designed to test the response of coral reef communities to OA as a function of seawater flow, which is an important physical parameter that has been unexplored in research on OA. To date, all studies of flow effects on coral reef organisms and communities have been performed under ambient conditions of pCO<sub>2</sub> (refs: 12–22). By assembling communities in flumes that are similar to shallow back reef communities in Moorea, and measuring net calcification at the scale of the whole community, sediments only, and macro-calcifiers, our study is the first to test the interactive effects of flow and pCO<sub>2</sub> on calcification at three functional levels of coral reef community organization.

Our results show that flow enhanced net calcification at the community level, during both day and night. The strongest effect was detected under high pCO<sub>2</sub> and very low flow (2 cm s<sup>-1</sup>), where dissolution of calcium carbonate exceeded precipitation on a daily basis. The effects of water flow on the calcification of *Porites compressa* and whole reef communities maintained in flumes under ambient conditions have been tested previously<sup>33,34</sup>, but in contrast to the present study, no effects on calcification were found. This discrepancy with the present analysis is likely due to different species assemblages, and to the lower flow speeds of 2, 5 and 10 cm s<sup>-1</sup> investigated in our study compared to previous studies<sup>34</sup>. In Langdon and Atkinson<sup>34</sup> the higher flow speeds (i.e., 20–40 cm s<sup>-1</sup>) may have reduced the magnitude of the effects of mass transfer limitation of key metabolic processes, including calcification.



**Figure 2** | Net calcification measured in the flumes at 3 flow speeds (10, 5 and 2 cm s<sup>-1</sup>) in the light (yellow background), dark (grey background), and integrated over 24 h. The grey bars represent the calcification measured in the ambient conditions and the black bars are calcification in the elevated pCO<sub>2</sub> treatment (~1300 μatm). Net calcification was determined for: (A) the community, (B) the sediment, and (C) the corals and coralline algae (by subtraction). The bars represent mean net calcification and the error bars the standard error (n = 6).

The effect of flow was less pronounced for sediments (compared to the whole community) and even reversed in the light under ambient pCO<sub>2</sub>, where net calcification was higher at reduced flow. Increased net calcification of sediments at low flow might have been caused by a reduction in the rate of exchange of carbonate ions between the water





column and the sediments. The flux of carbonate ions between the sediment and the water is driven by metabolism and chemical processes within the sediment that, in part, are fueled by organic matter remineralization, which reduces pH and favors dissolution<sup>35,36</sup>. Under low flow, dissolution was likely reduced within the sediments because of reduced advection (i.e., the flow of water through the interstices of the sediment), and limited mass transfer of carbonate ions to the water column. Previous studies have shown that dissolution of reef carbonate sediments is driven mainly by advection of pore water through the sediments and only partially by diffusion<sup>28,36</sup> of  $\text{Ca}^{2+}$  and dissolved inorganic carbon. *In situ* incubations on sediments performed at Heron Island, Australia, previously have shown a cumulative effect of advection and high  $\text{pCO}_2$  (800  $\mu\text{atm}$ ) on the dissolution of carbonate sediments, with rates of dissolution twice as fast in chambers recreating advection of seawater through the sediment versus diffusive conditions<sup>28</sup> (i.e., no water motion). Also, advection of seawater through the sediment likely was limited in our flumes since there were no waves to drive this process<sup>37</sup>.

The clearest effect of flow in the present study was measured on the corals and calcifying algae for which net calcification increased strongly as a function of increasing flow in the light. The role of flow in enhancing photosynthesis of coral reef organisms is well known, for example, for the coral *Orbicella annularis*<sup>14</sup> and algal turfs<sup>21</sup>. For corals, it has been proposed that such increases in photosynthesis under high flow are due to flow-driven enhancement of oxygen mass transfer through the diffusion boundary layer<sup>38</sup>. For algal turfs, flow may alleviate DIC limitation of photosynthesis<sup>21</sup>. Increases of net calcification in corals and coralline algae as a function of flow in the present study likely reflect mass transfer limitation of metabolites that potentially could be due to at least three nonexclusive processes. First, enhanced photosynthesis under high flow, as shown previously<sup>21</sup>, could enhance calcification by providing the metabolic energy necessary to support the costs of calcification<sup>39</sup>. In both corals and coralline algae, enhanced photosynthesis also can facilitate the maintenance of high pH at the calcification site<sup>40</sup> notably by producing hydroxyl ions that can chemically buffer  $\text{H}^+$  (refs. 41,42). Second, by reducing the thickness of boundary layers, increasing flow could favor the export of protons from the organism to the environment, which has been hypothesized to be a limiting factor for calcification<sup>23,43</sup>. Third, higher flow can favor the uptake of carbonate and bicarbonate ions, both of which are implicated in calcification of corals and algae<sup>44</sup>.

In the light, flow enhanced net calcification in both  $\text{pCO}_2$  treatments, while flow had a more striking effect on net calcification at night under high  $\text{pCO}_2$  compared to ambient  $\text{pCO}_2$ . Under high  $\text{pCO}_2$ , nocturnal net calcification of corals and coralline algae at 2 and 5  $\text{cm s}^{-1}$  was negative, with dissolution exceeding precipitation of calcium carbonate, but at 10  $\text{cm s}^{-1}$  precipitation exceeded dissolution. In view of the aforementioned hypotheses, at night the positive effect of high flow on net calcification under OA may have been due to the facilitation of uptake of carbonate and bicarbonate ions from seawater by corals and calcifying algae, and facilitation of the export of protons from the sites calcification<sup>45</sup>.

When integrated over 24 h, the difference in net calcification of corals and calcifying algae incubated under ambient versus high  $\text{pCO}_2$  was inversely proportional to flow. Differences in net calcification between high versus ambient  $\text{pCO}_2$  decreased from ~75% at 2  $\text{cm s}^{-1}$  to ~50% at 5  $\text{cm s}^{-1}$  and 35% at 10  $\text{cm s}^{-1}$ . This result highlights the possibility that corals and calcifying algae exposed to higher flow might be less susceptible to OA. However it is important to note that incubations at the lower flow speeds were performed over 24 h, which is likely to be too short to allow acclimatization to fully occur<sup>46,47</sup>. Nevertheless, short periods (<1 week) of reduced flow are not uncommon for the back reef of Moorea where water circulation mostly is wave-driven<sup>30</sup>.

Our results reveal the extent to which flow speed can affect the response of coral reefs and coral reef organisms to OA, and therefore emphasizes the importance of including flow in the design of future experiments examining the effects of OA on coral reef communities. Further studies are needed to test for the interactive effects of flow and OA on individual organisms and different reef communities that vary in species composition. For example, while our study indicates that flow partially can mitigate the effects of OA on corals and calcifying algae composing a tropical reef community, a recent study on individual organisms in a temperate environment has shown that high flow can enhance the deleterious effect of OA on the temperate coralline alga *Arthrocardia corymbosa*<sup>48</sup>. The potential mitigating effect of increasing water flow for calcifying organisms suggests that coral reefs exposed to higher flow speed may be less susceptible to the deleterious effects of OA than reefs experiencing lower flow. Nevertheless, the limited effects of flow on sediment dissolution suggests that while some corals and algae likely will maintain positive rates of net calcification under OA<sup>5</sup> (i.e., at 750  $\mu\text{atm pCO}_2$ ), the physical integrity of the reef structure will likely be adversely affected by lower pH associated with OA.

## Methods

**Collection and sample preparation.** Experiments were performed in August–October 2013 in Moorea, French Polynesia, using organisms and sediments collected from the back reef of the north shore at ~1–2 m depth. Based on data from the Moorea Coral Reef Long Term Ecological Research program, organisms were selected to match the mean cover of the back reef of Moorea in 2013 (refs. 26,27). The assembled communities were composed of four dominant taxa of coral on the back reef (massive *Porites* spp., *Porites rus*, *Montipora* spp., and *Pocillopora* spp.) that were configured to cover ~20–22% of floor of the flumes. Flumes also were populated with crustose coralline algae (*Porolithon onkodes* and *Lithophyllum flavescens*) to achieve 6% cover of these organisms. Sediment represented half of the area of the floors of the flumes, and the remaining area was exposed substratum (i.e., the epoxy-sealed bottom of the flume that became covered with algal turf). Upon collection of ~10 × 10 cm samples of corals (~12 cm height) and algae (~4 cm height), they were returned to the Richard B. Gump South Pacific Research where they were cleaned and attached to plastic supports using underwater epoxy (Z Spar A788). Subsequently, they were left to recover in a running seawater table for 3 d prior to starting the experiment.

Sediment was collected from 2-m depth in the lagoon of the north shore, ~200 m from the reef crest, using 24 custom-made boxes (0.4 × 0.3 × 0.3 m) that allowed collection and transportation with minimal disturbance to the vertical stratification of the sediment. Sediment boxes were inserted into the sediment and left for 4-d to allow reconstruction of the natural vertical stratification of the sediment. Following the 4 d, sediment boxes were returned to the laboratory and placed beneath the working sections of the flumes (6 flume<sup>-1</sup> to occupy half of the linear length of the working section).

**Flumes and flow characteristics.** The four outdoor flumes contained 700 L of seawater and consisted of a working section of 0.3 × 0.3 × 5.0 m, with half of this section (2.5 m) composed of a lower insert (0.25 m deep) designed to hold the sediment boxes. Fresh sand-filtered seawater (nominal filter size ~100  $\mu\text{m}$ ), pumped from Cook's Bay at 12-m depth, was dispensed continuously in the flumes at 5 L  $\text{min}^{-1}$ . Communities were maintained at a constant flow speed of 10  $\text{cm s}^{-1}$  using W Lim Wave II 375 Watt water pumps. At each end of the flume, seawater passed through an 88-cm transition section (rectangular to circular) that housed 20-cm (length) flow straighteners made of stacked, 3-cm diameter PVC pipe, and then into a 12.5-cm return section. Flow was measured before each of the incubations in the center of each of the four flumes using a Nortek Vectrino Acoustic Doppler Velocimeter.

To estimate the characteristics of the flow coming into the flumes, the dimensionless Reynolds number ( $Re$ ) was calculated for each flow speed.  $Re$  is defined as  $UD_h/\nu$ , where  $U$  is the flow speed,  $D_h$  the hydraulic diameter of the flume and  $\nu$  the kinematic viscosity of seawater<sup>49</sup>. To describe the bottom roughness of the flume, the roughness Reynolds number ( $Re_k$ ) defined as  $Uk'/\nu$ , where  $k'$  is the mean height of the roughness elements (corals and coralline algae), was calculated based on an estimated mean height for corals and algae of 9 cm. The friction factor ( $C_f$ ) defined as  $C_f^{-1} = (1.14 - 2.0 \log(k'/D_h))^2$ , was calculated as a function of the mean height of the roughness elements ( $k'$ ) and the hydraulic diameter of the flumes ( $D_h$ ).

**Carbonate chemistry control and measurements of physical parameters.** Two flumes were maintained at ambient  $\text{pCO}_2$  and two at high  $\text{pCO}_2$  to match the  $\text{pCO}_2$  expected by the end of the current century in a pessimistic scenario<sup>50</sup> (~1300  $\mu\text{atm}$ ).  $\text{pCO}_2$  was controlled using a pH-stat (Aquacontroller, Neptune systems) that controlled the bubbling of either pure  $\text{CO}_2$  or  $\text{CO}_2$ -free air. Natural daily variation in pH in the back reef was matched by maintaining pH at 0.1 unit lower at night (from 18:00 to 06:00 hrs) than during the day. Temperature in the flumes was maintained at 27°C, which is the mean yearly temperature in Moorea<sup>51</sup>, using JBJ 375 Watt Arctic



chillers. Flumes experienced natural lighting and were shaded to reduce the maximum light intensity to  $\sim 1500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , which is representative of the light intensity at  $\sim 2\text{-m}$  depth in the back reef of Moorea<sup>52</sup>. Photosynthetically active radiation (PAR) was measured continuously below the seawater surface using a  $4\pi$  quantum sensor LI-193 connected to a LiCor LI-1400 meter.

Discrete pH measurements were made daily using a portable pH meter (Orion 3-stars) fitted with a DG 115-SC pH probe (Mettler) calibrated every other day with 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS) buffers at a salinity of 35.0 (A. Dickson, San Diego, USA). pH also was measured spectrophotometrically using the m-cresol dye<sup>53</sup> at regular intervals and results were  $\leq 0.01$  units of those obtained with the portable pH meter. Analysis of total alkalinity ( $A_T$ ) was conducted on the day of seawater sampling by open-cell potentiometric titrations (T50, Mettler-Toledo) using 50-mL samples of seawater collected every 2–3 d<sup>53</sup>.  $A_T$  was calculated using a modified Gran function applied to pH values ranging from 3.5 to 3.0. Titrations of certified reference materials (CRM) provided by A.G. Dickson (Batch 105) yielded  $A_T$  values within  $3.8 \mu\text{mol kg}^{-1}$  of the nominal value ( $\text{SD} = 3.1 \mu\text{mol kg}^{-1}$ ;  $n = 12$ ). Parameters of the carbonate system in seawater were calculated from salinity, temperature,  $A_T$  and  $\text{pH}_T$  using the R package seacarb<sup>54</sup>.

**Net calcification measurements.** Net calcification rates were measured using the total alkalinity anomaly method<sup>55</sup>, which is based on the stoichiometric relation of two moles of  $A_T$  being removed for each mole of  $\text{CaCO}_3$  precipitated when nutrient concentrations are held constant<sup>56,57</sup>. Communities were maintained at a flow speed of  $10 \text{ cm s}^{-1}$  and net calcification was measured weekly. Net calcification also was measured at 2 and  $5 \text{ cm s}^{-1}$  at time points  $\sim 3, 5$  and 7 weeks of the incubation. To compare the effect of flow on communities exposed to  $\text{pCO}_2$  treatments for the same time period, only net calcification measurements made at time points  $\sim 3, 5$  and 7 weeks for ambient flow speed ( $10 \text{ cm s}^{-1}$ ) were considered. For each  $\text{pCO}_2$  treatment and flow, mean calcification rates were determined on six replicates (three time points for duplicate flumes for each treatment). In addition to measurements of net calcification for the whole community, net calcification also was measured for the sediments by removing macro-calcifiers (corals and coralline algae) from the flumes for 24 h. During this period, corals and coralline algae were placed in a separate tank where carbonate chemistry, light and temperature conditions were similar to those experienced in the flumes. Incubations of sediments were performed after  $\sim 4, 6$  and 8 weeks of incubation.

During calcification measurements, addition of seawater was stopped and samples for  $A_T$  were taken every 3 h during the day and every 6 h at night. Water in the flumes was refreshed every 6 h for 30 min to maintain  $A_T$  and nutrients at levels close to those on the reef. Samples for nitrate and ammonium concentrations were taken at the same time as samples for  $A_T$  during at least one 24-h incubation period for each flow speed treatment and nutrient concentrations (nitrate and ammonium) did not significantly change during incubations ( $< 2 \mu\text{mol L}^{-1}$ ). Net calcification of corals and coralline algae was calculated by subtracting the mean light and dark net calcification of the sediments from the net calcification of the intact community for day and night, respectively.

**Statistical analysis.** All analyses were performed using R software (R Foundation for Statistical Computing), and assumptions of normality and equality of variance were evaluated through graphical analyses of residuals. Net calcification rates were analyzed using a three-way repeated ANOVA in which treatment ( $\text{pCO}_2$  levels) and time (week) was a fixed effect, with two within-subjects factors (Flow and Flume).

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## Author contribution

S.C. designed and performed experiments, analyzed data and wrote the paper; C.L. performed experiments and wrote the paper; B.C. and P.E. designed experiments, analyzed data and wrote the paper.

## Additional information

**Competing financial interests:** The authors declare no competing financial interests.

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