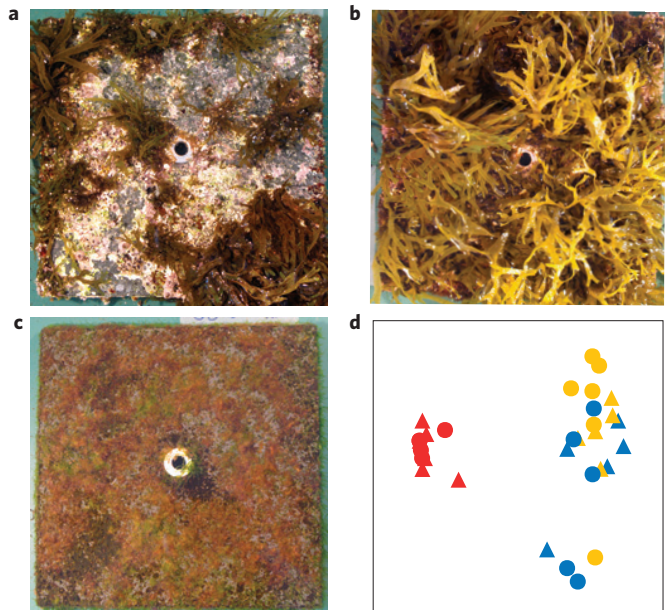


# Ocean acidification causes ecosystem shifts via altered competitive interactions

Kristy J. Kroeker<sup>1\*</sup>, Fiorenza Micheli<sup>1</sup> and Maria Cristina Gambi<sup>2</sup>

Ocean acidification represents a pervasive environmental change that is predicted to affect a wide range of species<sup>1,2</sup>, yet our understanding of the emergent ecosystem impacts is very limited. Many studies report detrimental effects of acidification on single species in lab studies, especially those with calcareous shells or skeletons<sup>3-5</sup>. Observational studies using naturally acidified ecosystems have shown profound shifts away from such calcareous species<sup>6-8</sup>, and there has been an assumption that direct impacts of acidification on sensitive species drive most ecosystem responses. We tested an alternative hypothesis that species interactions attenuate or amplify the direct effects of acidification on individual species<sup>9-12</sup>. Here, we show that altered competitive dynamics between calcareous species and fleshy seaweeds drive significant ecosystem shifts in acidified conditions. Although calcareous species recruited and grew at similar rates in ambient and low pH conditions during early successional stages, they were rapidly overgrown by fleshy seaweeds later in succession in low pH conditions. The altered competitive dynamics between calcareous species and fleshy seaweeds is probably the combined result of decreased growth rates of calcareous species, increased growth rates of fleshy seaweeds, and/or altered grazing rates<sup>13</sup>. Phase shifts towards ecosystems dominated by fleshy seaweed are common in many marine ecosystems<sup>14-16</sup>, and our results suggest that changes in the competitive balance between these groups represent a key leverage point through which the physiological responses of individual species to acidification could indirectly lead to profound ecosystem changes in an acidified ocean.

We deployed recruitment substrates in zones of extreme low, low and ambient seawater pH caused by shallow CO<sub>2</sub> vents at two replicate sites (Supplementary Fig. S1). The pH zones are caused by spatial variability in CO<sub>2</sub> venting<sup>6</sup>, resulting in decreased pH and carbonate ion concentrations and increased dissolved inorganic carbon at ambient temperature and alkalinity (Supplementary Fig. S1 and Table S1). The carbonate chemistry in the ambient pH zone is comparable to current conditions in the temperate surface ocean in the Mediterranean, whereas the low pH zones are comparable to predictions for the acidification of the near-future surface ocean (in the year 2100; ref. 17). The carbonate chemistry in the extreme low pH zones is not predicted in the near future but provides an endmember scenario for understanding acidification impacts. Previous studies have highlighted a reduction in diversity and the abundance of calcareous species in the low and extreme low pH zones<sup>6,7,18</sup>, but the processes underlying these patterns have not been investigated. Recruitment tiles were deployed at the beginning of the growing season for algae, and an independent

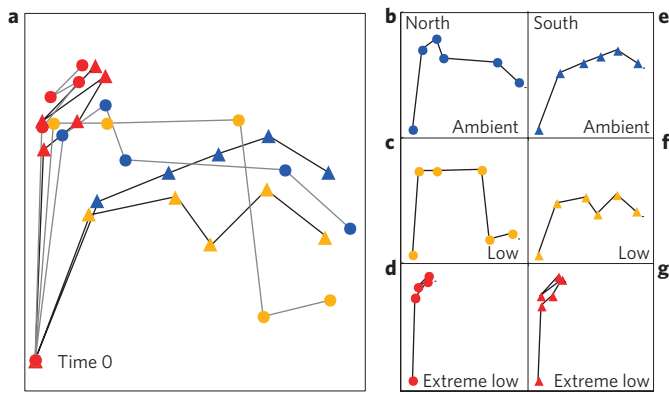


**Figure 1 | Community structure at 14 months.** **a–c**, Benthic assemblages at 14 months in **a**, ambient, **b**, low, and **c**, extreme low pH zones. Assemblages are dominated by a diversity of calcareous species, turf, and erect algae in ambient pH (**a**), whereas assemblages in low pH show a higher per cent coverage of fleshy turf forming algae (**b**). The assemblages in extreme low pH are dominated by biofilm and filamentous algae (**c**). The holes in the center of **a–c** are for attachment of the tiles to the substrate. **d**, Nonmetric Multidimensional Scaling (nMDS) plot of community structure at 14 months (red = extreme low, yellow = low, blue = ambient, circles = north, triangles = south).

subset of tiles were collected and analysed for percentage cover at 1.5, 2.5, 3.5, 6.5, and 14 months. Succession was analysed to highlight processes underlying community patterns of the natural benthos when substrate complexity and colonization history are held constant<sup>13,19,20</sup>.

After 14 months, the assemblages on the tiles were visually indistinguishable from the surrounding benthos and differed significantly among pH zones (Fig. 1 and Supplementary Fig. S2). Biofilm and filamentous algae dominated the assemblages in extreme low pH, whereas the assemblages in low and ambient pH harboured a range of calcareous invertebrates and algae (Fig. 1a–c and Supplementary Fig. S2). Whereas the composition of the assemblages (defined as the presence/absence of functional groups;

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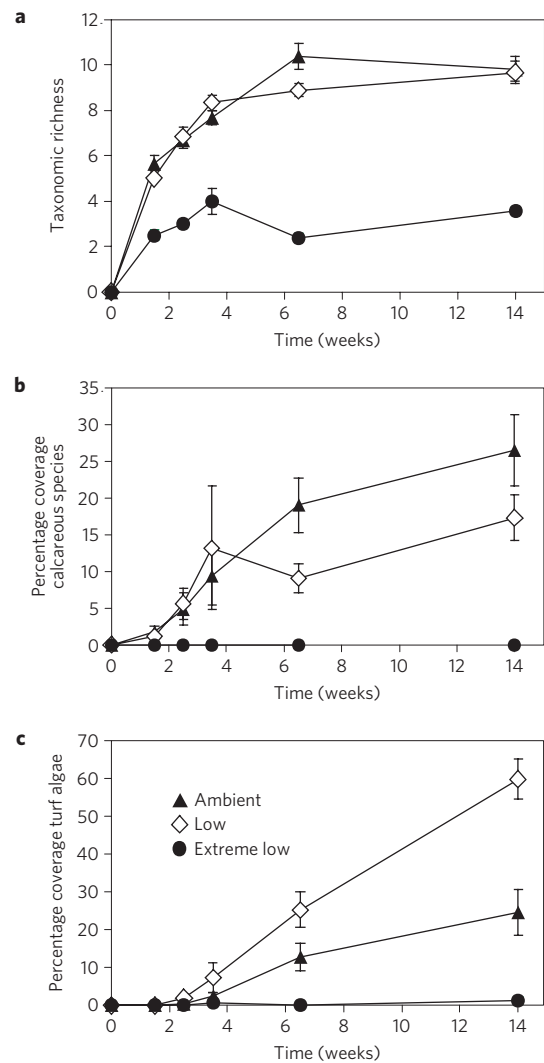


**Figure 2 | Successional trajectories defined by the mean community structure ( $N = 3-6$  tiles per time point) at 0, 1.5, 2.5, 3.5, 6, and 14 months in an nMDS plot estimated by zero-adjusted Brace-Curtis similarities between tiles.** Distance between data points in the ordination represents difference in community structure. Time zero (blank tiles) is shown in the bottom left of the plots. **a**, All successional trajectories. Trajectories at the **b-d**, northern site and **e-g**, southern site in **b,e**, ambient, **c,f**, low, and **d,g**, extreme low pH zones.

Supplementary Table S2) differed between the extreme low pH zones and the other pH zones, composition did not differ between ambient and low pH at either site after 14 months of development (Permutational Multivariate Analysis of Variance (PERMANOVA) with site and pH as fixed factors: site  $\times$  pH  $F_{2,28} = 1.78$ ,  $p = ns$ , site  $F_{1,28} = 1.84$ ,  $p = ns$ , pH  $F_{2,28} = 25.30$ ,  $p = 0.001$ ; paired  $t$ -tests: ambient, low  $t(19) = 0.66$ ,  $p = 0.94$ ; low, extreme low  $t(19) = 7.36$ ,  $p = 0.0001$ ) indicating many calcareous species may be able to persist in the conditions predicted for near-future acidification. Although the same functional groups were present in ambient and low pH (that is, no significant effect of pH on composition between these pH zones), there was a difference in the relative abundances of the functional groups (that is, community structure; Fig. 1d; PERMANOVA site  $\times$  pH  $F_{2,28} = 2.06$ ,  $p = 0.103$ ; pH  $F_{2,28} = 54.70$ ,  $p = 0.001$ ; site  $F_{1,28} = 3.77$ ,  $p = 0.043$ . Paired  $t$ -tests: ambient, low  $t(19) = 2.14$ ,  $p = 0.002$ ; low, extreme low  $t(19) = 10.42$ ,  $p = 0.001$ ; ambient, extreme low  $t(18) = 9.08$ ,  $p = 0.001$ ). Calcareous species (primarily erect and crustose coralline algae (CCA), barnacles and calcareous tubeworms) had higher percentage coverage in ambient pH, whereas non-calcareous fleshy algae were dominant in low pH (Fig. 1b,c). The assemblages on the tiles at 14 months were similar to the natural benthic communities in their respective pH zones (Supplementary Fig. S2).

The successional trajectories also differed among pH zones (PERMANOVA site  $\times$  pH  $\times$  time  $F_{8,82} = 2.58$ ,  $p = 0.004$ ; Fig. 2 and Supplementary Table S3). Biofilm and filamentous algae dominated all tiles in the early stages of succession. The assemblages in extreme low pH were locked in this species-poor early-successional stage for the entirety of the 14 months (Fig. 3a). In contrast, the assemblages in ambient and low pH continued to gain species through time, resulting in more complex successional trajectories (Fig. 2) and higher diversity (Fig. 3a). The assemblages in low pH mirrored those in the ambient pH at each site for the first 2.5 months, but diverged from the ambient pH trajectories after 3.5 months (Fig. 2).

The lack of calcareous species on the tiles in extreme low pH suggests that the physiological intolerance of early life history stages may be a bottleneck for their establishment in extremely acidified conditions. In contrast, calcareous species recruited early and grew at similar rates in low and ambient pH during the early stages of succession, suggesting their decreased abundance in low pH was not due to physiological intolerance of early life history stages (Fig. 3b and Supplementary Fig. S3 and Table S4). The



**Figure 3 | Variation in community development. a**, The taxonomic richness (based on functional groups) was significantly lower in extreme low pH zones than the other pH zones (ANOVA with site, pH, and time as fixed factors: pH  $\times$  time  $F_{8,82} = 7.56$ ,  $p = 0.001$ ). **b**, The percentage coverage of calcareous species diverged between extreme low and other pH zones by 1.5 months, and between the ambient and low pH at 6.5 months (ANOVA: pH  $\times$  time  $F_{8,82} = 2.69$ ,  $p = 0.02$ ). **c**, The percentage coverage of fleshy turf algae differed among all pH zones (ANOVA: pH  $\times$  time  $F_{8,82} = 13.24$ ,  $p = 0.001$ ). Data are means  $\pm$  s.e.m. ( $N = 11-12$ ).

coverage of calcareous species only ceased increasing in the low pH zones after 3.5 months, when turf algae overgrew the tiles (Fig. 3b,c). This trajectory was not observed at ambient pH, where calcareous species more than doubled their abundance between 3.5 and 14 months, and turf algae remained at a low percentage coverage (Fig. 3b,c). Because the trajectories documented in low and ambient pH were repeated at both sites, it is unlikely that this is a result of idiosyncratic, site-specific recruitment and growth patterns. Thus, our results suggest that even the calcareous species that are able to physiologically withstand the direct effects of acidification may suffer reductions in abundance under the predicted conditions for near-future ocean acidification if they are in competition with fleshy algae.

The altered competitive relationship between calcareous species and fleshy seaweeds could be due to slower growth of calcareous species, reduced grazing of fleshy seaweeds due to a reduction in the abundance or grazing rates of calcareous herbivores, increased

growth rates of fleshy seaweeds, or a combination of these factors. Before overgrowth by turf algae, our analyses suggest the effects of acidification on the growth rates of key taxa were minor (Supplementary Fig. S3 and Table S4). Furthermore, the total abundance and biomass of key grazers found on tiles (that is, molluscs, crustaceans and echinoderms; Supplementary Table S5) did not differ statistically between ambient and low pH (extreme low pH was excluded from the analysis; Supplementary Table S6) although there were minor differences in the relative abundances of different grazer species (Supplementary Fig. S4). This result is in agreement with previous studies that found similar mobile invertebrate assemblages in the ambient and low pH zones at this site<sup>7</sup>. Thus, we conclude that the reduction in abundance of calcareous species cannot be solely explained by either the initial growth rates of calcareous species or the abundance/biomass of grazers. There is growing evidence that some fleshy seaweeds can increase their photosynthetic and/or growth rates with the increased concentrations of CO<sub>2(aq)</sub> or HCO<sub>3</sub><sup>-</sup> associated with acidification<sup>2,12,21</sup>. Accordingly, we found the greatest biomass of fleshy algae in the low pH zone (Supplementary Fig. S5 and Table S6), and we hypothesize that the growth rates of fleshy seaweeds and/or the grazing rates of key herbivores contributed to the reduced abundance of calcareous species in low pH. Further research is needed to elucidate the relative roles of each factor in community changes.

Our results are in contrast to laboratory studies that have suggested that calcareous species are limited by recruitment at near-future levels of acidification<sup>22,23</sup>. Understanding why calcareous species were not recruitment limited in our low pH zones is important for accurate forecasts of the effects of acidification on marine ecosystems. Some of the calcareous species in this ecosystem are not calcified as larvae/spores (including CCA (ref. 24) and serpulid polychaetes) and therefore may be more tolerant than expected during early life history stages. Furthermore, closely related species can vary in their sensitivity<sup>2,25</sup>, and those found in low pH at this site may simply comprise more acidification-tolerant species. However, it is possible that the natural fluctuations in carbonate chemistry at the vents<sup>7</sup> could potentially allow even sensitive species to establish during short periods of transiently higher pH or favourable carbonate chemistry. Temporal variability in carbonate chemistry is much more pronounced in natural systems than in laboratory experiments<sup>26</sup>, and understanding if and how transiently high or low pH conditions affect community organization remains a critical area of research for forecasting the effects of ocean acidification at large. Last, if multiple generations of a species were able to stay within the low pH zone, which is possible for species with negatively buoyant spores or short dispersal ranges, then these species could have adapted to the local carbonate chemistry conditions<sup>27</sup>. Regardless of which factors underlie these patterns, our results suggest the early life history stages of some calcareous taxa may be more resilient to the direct effects of near-future acidification levels than predicted by lab experiments.

There are a few important distinctions between the vent site and the scenarios for global acidification that deserve consideration. For example, larvae and spores in this system can originate from non-acidified populations, which may not be possible in climate change scenarios. If most propagules originated from outside the vent area, our results may overestimate the effects if species are able to adapt to more gradual changes predicted in near-future acidification scenarios. In contrast, if acidification affects a species' reproductive output, then our results may underestimate the impacts. Thus, identifying the propagule/larvae source in this system and potential for evolutionary adaptation will help refine our predictions for the effect of global acidification.

Together, our results suggest competition between calcareous species and fleshy seaweeds may represent a key leverage point in

this community through which non-lethal physiological effects of ocean acidification could lead to substantial ecosystem change in the near-future. Even the calcareous species that are able to cope physiologically with acidification may be indirectly affected through altered species interactions. Competition between calcareous species and fleshy seaweeds is common to marine ecosystems<sup>12,14,15</sup>, and those ecosystems that have shifted towards algal-domination often have decreased diversity and altered ecosystem functions<sup>28</sup>, suggesting acidification could have profound effects on ecosystem function. Furthermore, acidification is one of many stressors affecting the oceans, and our results suggest that other factors that shift the competitive balance towards fleshy seaweeds in today's oceans (for example, eutrophication and overfishing<sup>29</sup>) could potentially exacerbate the future ecosystem effects of acidification.

## Methods

The volcanic CO<sub>2</sub> vents are found at 0.5–3 m depth on the north and south sides of a small islet (*Castello Aragonese*, Ischia Island) adjacent to sloping rocky reefs. The vent gas is predominantly CO<sub>2</sub> (without hydrogen sulphide) and does not elevate the temperature. We quantified the seawater carbonate chemistry in the experimental zones using custom *in situ* pH and temperature sensors that were deployed concurrently in each pH zone on the southern site from site from 12 May to 14 June 2010, and in the northern site from 13 September to 8 October 2010, coupled with discrete water samples<sup>8</sup>.

Nineteen 15 × 15 cm volcanic rock recruitment tiles were bolted to the substrate at 0.5–1.5 m depth in each of the pH zones in March 2009. Tiles were hung at a 95°–115° angle to the seafloor to approximate the orientation of the natural substrate. Three independent tiles were collected from each zone at 1.5, 2.5 and 3.5 months, four tiles at 6.5 months, and 5–6 tiles at 14 months. Tiles were placed into plastic bags underwater to prevent the loss of grazers, but we did not attempt to collect highly mobile invertebrates (primarily amphipods and tanaids). Photographs were taken of all tiles in the laboratory, which were then preserved in 4% formalin for two days and stored in 70% ethanol.

We divided the tiles into 1.5 × 3 cm subplots along the edges and 3 × 3 cm subplots on the face of the tiles for analysis. The percentage coverage of canopy algae in each subplot was estimated before removing it and estimating the percentage coverage of benthic taxa under the algal canopy. This was done 'by eye' by a single investigator (KJK). Subplot estimates were summed and pooled into functional groups (Supplementary Table S2). Grazers were collected and weighed. To compare the tiles against the natural substrate, we took photos of four 20 × 20 cm plots that had been cleaned of all benthos at the same time as the initial tile deployments, and estimated the percentage cover of the same functional groups in these plots at 14 months using photographic analysis software (Vidana; Supplementary Fig. S2).

**Statistical analyses.** Relative growth rate (RGR) of CCA was quantified by measuring the change in the area (cm<sup>2</sup>) of discrete CCA patches ( $n = 5-10$ ) or *Balanus perforatus* individuals ( $n = 2-6$ ) on photos of tiles ( $n = 3$ ) taken at 2.5 and 3.5 months (CCA) or 1.5 and 2.5 months (*B. perforatus*) using photographic analysis software (Image J). Growth rate was further divided by the initial size. We did not measure RGR at other times because the individual patches were not identifiable before 2.5 months and were covered by fleshy algae after 3.5 months.

All further statistical analyses were based on functional groups. Although some detail is lost when species are pooled into higher-ranking guilds, the patterns in abundance and community structure at finer taxonomic resolution have previously been described for this system<sup>6,7,18</sup>, and analyses of functional groups have direct implications for ecosystem function. For the final tiles, variation in community composition between pH zones and sites was analysed on a Bray–Curtis (BC) similarity matrix of presence/absence of functional groups, while the community structure was analysed on a zero-adjusted BC similarity matrix of square-root transformed total percentage coverage of functional groups. We used PERMANOVA with site and pH as fixed factors. All PERMANOVA analyses used 9,999 unrestricted permutations of the raw data and Type III SS. Post-hoc paired *t*-tests between pH zones were based on permutations of residuals under a reduced model.

The successional trajectories were estimated by taking the mean of percent coverage estimates for each group over the subset of tiles collected at each site × pH × time. Variation in successional trajectories was tested with PERMANOVA with site, pH and time as fixed factors. Because separate tiles were collected and analysed at each time point, the percentage coverage estimates are statistically independent and not subject to the issues inherent in repeated measures.

Most univariate analyses violated the assumptions of parametric statistics owing to the presence of numerous zeros in the extreme low pH zones. Thus, we used permutation-based ANOVA with site, pH, and time as fixed factors to test for variation in univariate variables ( $\alpha = 0.05$ ). However, the extreme low pH zones were excluded from grazer, CCA, and *Balanus perforatus* analyses because of their complete absence in these zones. Variation in RGR was tested by fitting



linear mixed effects (lme) models with site, pH, and the site  $\times$  pH interaction as fixed factors and tile as a random factor nested in each site  $\times$  pH zone with restricted maximum likelihood estimation in *R* (ref. 30). The assumptions were checked visually and *P*-values for fixed factors were generated with Markov chain Monte Carlo simulations.

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## Author contributions

K.J.K., F.M. and M.C.G. designed experiments, K.J.K. and M.C.G. performed field experiments, K.J.K. analysed data and wrote the paper with contributions from F.M. and M.C.G.

## Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at [www.nature.com/reprints](http://www.nature.com/reprints). Correspondence and requests for materials should be addressed to K.J.K.

## Competing financial interests

The authors declare no competing financial interests.