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Persistent natural acidification drives major distribution shifts in marine benthic ecosystems

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Ocean acidification is receiving increasing attention because of its potential to affect marine ecosystems. Rare CO₂ vents offer a unique opportunity to investigate the response of benthic ecosystems to acidification. However, the benthic habitats investigated so far are mainly found at very shallow water (less than or equal to 5 m depth) and, therefore, are not representative of the broad range of continental shelf habitats. Here, we show that a decrease from pH 8.1 to 7.9 observed in a CO₂ vent system at 40 m depth leads to a dramatic shift in highly diverse and structurally complex habitats. Forests of the kelp *Laminaria rodriguezii* usually found at larger depths (greater than 65 m) replace the otherwise dominant habitats (i.e. coralligenous outcrops and rhodolith beds), which are mainly characterized by calcifying organisms. Only the aragonite-calcifying algae are able to survive in acidified waters, while high-magnesium calcite organisms lack almost completely. Although a long-term survey of the venting area would be necessary to fully understand the effects of the variability of pH and other carbonate parameters over the structure and functioning of the investigated mesophotic habitats, our results point that in addition of significant changes at species level, moderate ocean acidification may entail major shifts in the distribution and dominance of key benthic ecosystems at regional scale, which could have broad ecological and socio-economic implications.

1. Introduction

Rising levels of CO₂ released by anthropogenic activities are driving unprecedented changes in the chemistry of the oceans [1,2]. The mean ocean surface acidity has increased by 25–30%, equivalent to a drop of 0.1 pH units, as the advent of the Industrial Revolution in the 1780s and is predicted to decline by a further 150–200% by the end of the century, representing an additional drop of 0.3 pH [3]. Changes in pH since pre-industrial times are happening at least 10 times faster than over the last 55 Myr [4]. The increasing interest in understanding how changes in pH and in the overall carbonate system will affect marine life has placed ocean acidification (OA) as one of the fastest growing research areas in marine sciences over the last few decades [5]. Most research so far has been conducted by means of laboratory experiments on a wide range of marine organisms, which have shown different responses and sensitivities [6–8]. While experiments are essential to identify species responses to OA, they fail to predict its long-term

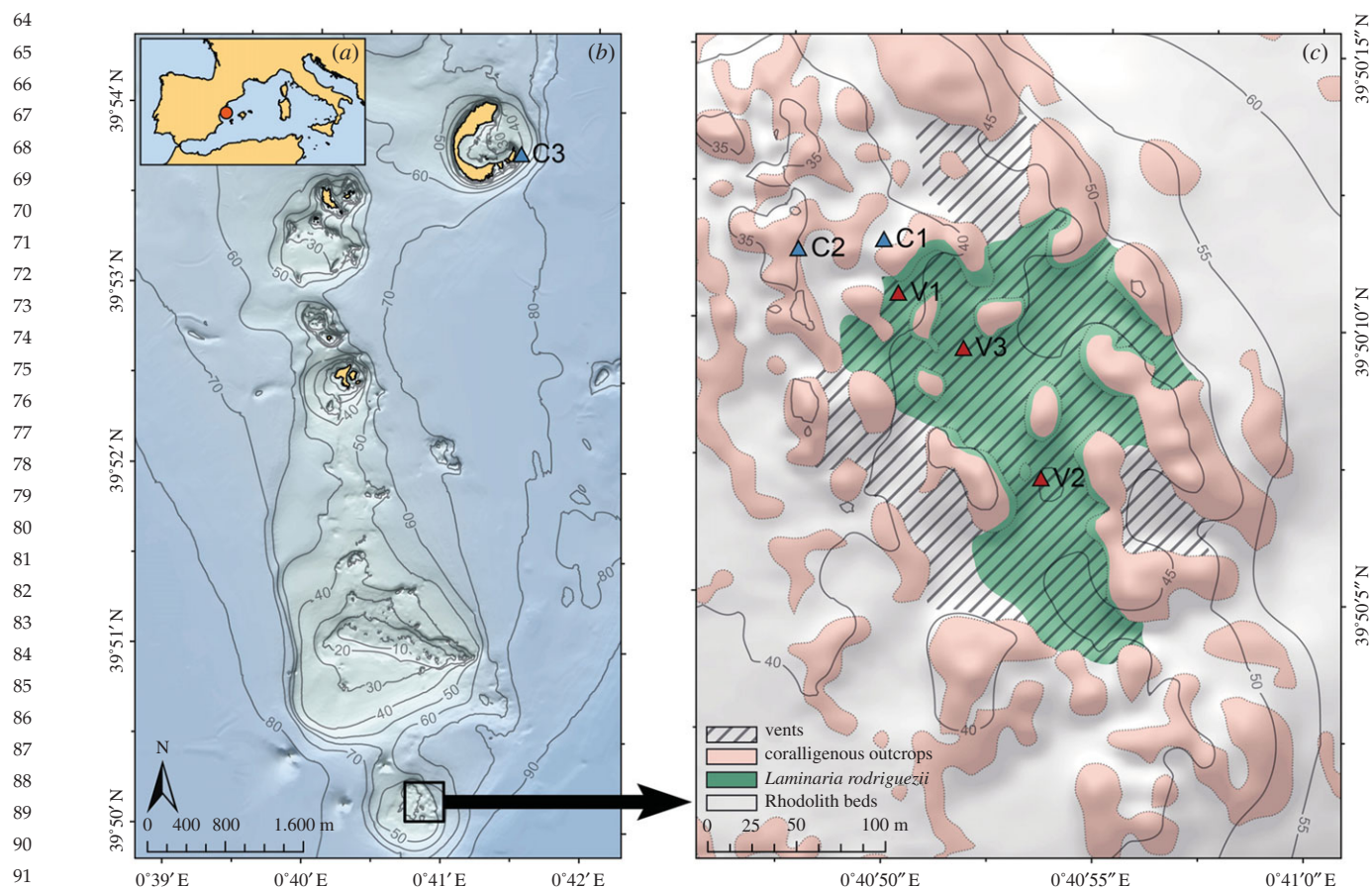


Figure 1. Map of CO_2 vents at Columbretes Islands, Spain. (a) Situation of Columbretes Islands in the NW Mediterranean Sea. (b) Location of the CO_2 vent area within the Columbretes Islands Marine Reserve (black square) and of the control station (C3) nearby the main island. (c) Detailed map of the CO_2 vent area and benthic habitats. C1, C2 and C3 are off-vent control stations, and V1, V2 and V3 are vent stations, where seawater samples and benthic community data were collected.

consequences on marine ecosystems and whether organisms will adapt to increasing seawater acidity [9].

In situ observations of naturally occurring CO_2 seafloor vents provide empirical data on marine ecosystems acclimatized to acidified waters. However, a small number of CO_2 vent systems have been investigated to date [9–13]. These systems share significant decreases in the diversity, biomass and trophic complexity of benthic marine assemblages, major declines in the number of many calcifying organisms and increased abundance of erect macroalgae, seagrasses or soft-corals [9–14]. Most of the CO_2 vent systems studied so far were placed in very shallow waters (3–5 m depth) and, therefore, are poorly representative of the broad range of habitats that occur on the continental shelf. Only very few studies investigating the biological effects of these systems at larger water depths have been performed in hydrothermal vents in the Aegean Sea [12] and in the northern Gulf of California [13], at 10–35 m and at 70–200 m, respectively.

Dominant habitats at mesophotic depths (the deepest part of the photic zone where light penetration is low, typically from 30–40 m to over 150 m) are usually characterized by a large dominance of calcifying organisms, such as endangered coralligenous outcrops and rhodolith beds in the Mediterranean Sea, which display a notable carbonate production ($170\text{--}660 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$) [15,16]. Calcareous red algae are the main framework builders in those two habitats, providing structural complexity and favouring biodiversity [16,17]. Given their wide spatial distribution and ecological roles, both habitats are among the most significant in the Mediterranean Sea [18]. Their critical ecological functions,

the long life spans and low dynamics of the dominant species as well as the high sensitivity to OA of coralline algae and other calcifying organisms dwelling in mesophotic depths [7,19] render coralligenous outcrops and rhodolith beds, especially vulnerable to a high- CO_2 ocean. In this study, we show the *in situ* effects of exposure to anomalously high natural seawater $p\text{CO}_2$ over benthic habitats dominated by calcifying algae at 40 m depth in the Columbretes Islands Marine Reserve (NW Mediterranean Sea).

2. Material and methods

(a) Study area and remotely operated vehicle survey

The Columbretes Islands emerge 30 nautical miles off the coast of Castelló (Spain, NW Mediterranean Sea; figure 1a) and are the emerged expression of a $90 \times 40 \text{ km}$, mostly buried volcanic field associated with the Neogene opening of the Valencia Trough [20]. The Columbretes form a tiny volcanic archipelago that consists of one main island, several islets and rocks and a number of shoals. The presence of CO_2 vents was examined with a *Seaeye Linx 1500* remotely operated vehicle (ROV) in September 2011 inside and outside the Columbretes Marine Reserve. The spatial distribution of these vents along this elevated rocky-bottom area was explored through five, 596–964 m long, video-transects (electronic supplementary material, figure S1).

(b) Carbonate system parameters

Vent gases and water samples were collected in June 2012. Vent gases were collected from three separate bubble streams in six replicate 12 ml glass bottles, and analysed with a gas chromatograph (TraceGC Ultra, Thermo-Finnigan). Seawater carbonate

chemistry and dissolved inorganic nutrients were measured on water samples collected by SCUBA divers in three sites (three samples per site) inside (V1, V2 and V3) and outside (C1, C2 and C3) the vents at 40 m depth using Niskin bottles (figure 1b and c). While control stations C1 and C2 were approximately located 25 m and 60 m far from the vent area, the third control station C3 was placed much farther (approx. 6.5 km far from the vent area) to check for any influence that could be attributed to vent proximity. Carbonate system parameters ($p\text{CO}_2$, CO_3^{2-} , HCO_3^- , DIC concentrations, and saturation states of calcite and aragonite) were calculated from pH (in total scale), total alkalinity (TA), temperature and salinity. Additionally, in nine stations covering the waters around the vents, seawater samples were taken at three depths (5, 20 and 40 m; eight samples per each depth) in order to study the spatial variability in pH (in total scale), TA, salinity, temperature and dissolved inorganic nutrients.

Temperature and salinity were measured with a SD204 SAIV A/S CTD. Samples for pH and TA measurements were collected in 0.5 l BOD glass bottles, poisoned with saturated mercuric chloride and stored in the dark at room temperature; samples were analysed on the same day of collection. Samples for inorganic nutrient analysis were collected on polycarbonate bottles and stored frozen until spectrophotometric analysis in an Alliance Evolution II autoanalyser following standard procedures [21]. PH_{TS} (total scale) was measured with a combined electrode aquatrode+ Pt1000 (Orion) calibrated using TRIS/HCl and 2-aminopyridine/HCl buffer solutions in synthetic seawater with a salinity of 38 PSU [21]. TA was measured by potentiometric open-cell titration using 0.1 N hydrochloric acid (Fixanal, Sigma Aldrich) and a Titrando Metrohm 888. Equivalence point was evaluated from titration points in the pH region 3.0–3.5 using a nonlinear least square procedure [22]. Carbonate system parameters were estimated using CO2SYS [23]. The pH and alkalinity of certified reference material (CRM, supplied by Dr A. G. Dickson, Scripps Institution of Oceanography, La Jolla, CA, USA) was measured for each set of measurements. From 12 measurements on the CRM, the accuracy was $3 \mu\text{mol kg}^{-1}$ for TA and 0.001 for pH. Precision, as the average of standard deviations of measurements, was $1.4 \mu\text{mol kg}^{-1}$ for TA and 0.002 for pH.

(c) Biological surveys

SCUBA diving surveys were also made in June 2012 to quantify the main species of macro-organisms present at and off the vents at 40 m depth. In the same sites where seawater samples were collected, 12 quadrats ($50 \times 50 \text{ cm}$) were placed haphazardly over the vents (four quadrats in each of the three sites; V1, V2 and V3), while six (three quadrats in two of the three sites; C1 and C2) were placed off the vents at the same depth (40 m) (figure 1b and c). Within each quadrat, we collected all the macro-organisms present in order to characterize the assemblage at each station. Specimens were identified in the laboratory, where fresh weight and cover were determined in each quadrat. When present, the number of fronds of *L. rodriguezii* was also counted inside each quadrat.

(d) Biomass of *Laminaria rodriguezii*

To characterize the assemblage at the vents, fresh weight of all specimens of *L. rodriguezii* ($n = 486$) present in each one of the 12 quadrats ($50 \times 50 \text{ cm}$) was measured in the laboratory after removing the water excess spinning the samples for 30 s in a salad spinner. Dry weight was estimated using a subsample of individuals ($n = 112$), which were weighed after 24 h at 60°C . *L. rodriguezii* biomass was not estimated outside the vents, as it was totally absent.

(e) Statistical analyses

Variation in assemblage composition inside and outside vents was analysed on a Bray–Curtis (BC) similarity matrix of log

transformed total percentage coverage of all the species identified. Significant differences between algal community inside and outside the vents were estimated through a permutational MANOVA using the function `adonis` in the `vegan` package in R [24]. Significant differences on the parameters (pH, CO_2 , CO_3^{2-} and DIC concentrations) estimated in locations inside and outside the vents were assessed using linear mixed effects models (LMM) [25]. The presence/absence of vents was treated as a fixed factor and the sampling station as the random effect. The normality of residuals and the model performance were visually inspected using residual distributions and quantile–quantile plots. The analyses were computed using `vegan` [26] and `nlme` [27] packages implemented in R.

3. Results

(a) Seawater chemistry

CO_2 vents occur between 36 and 48 m depth on the southernmost section of the Columbretes Islands Marine Reserve (figure 1a and b). The mean composition of the bubbling gas was 90.0% CO_2 , 4.8% N_2 , 1.7% O_2 and 0.2% CH_4 , subsequently resulting in water acidification (electronic supplementary material, table S1). No sulfur was detected in the gas. Field observations and sampling were performed at the vents and in off-vent nearby control stations with equal ambient seawater salinity and temperature (figure 1c; electronic supplementary material, table S1). The escaping gas had no influence on the composition of the upper water layers, as shown by samples from 5 and 20 m depth above the vents, which fell within the range of off-vent stations (electronic supplementary material, table S2).

Statistically significant differences on pH and carbonate system parameters (CO_2 , CO_3^{2-} and DIC) were found between locations inside and outside the vents ($p < 0.0001$, figure 2; electronic supplementary material, table S3). Water collected near the bottom in control stations displayed pH (total scale) values ranging from 8.04 to 8.08 and $p\text{CO}_2$ from 402 to 445 μatm , whereas at the vents pH (total scale) ranged from 7.80 to 7.95 and $p\text{CO}_2$ from 572 to 993 μatm , therefore illustrating a notable pH decrease (figure 2; electronic supplementary material, tables S1 and S3). The lowest pH (7.48) was measured at station V2, whereas the average value for the venting zone was 7.88, which is equivalent to a reduction of 0.2 units the average value found in control stations C1–C3 (electronic supplementary material, table S1). The increase in CO_2 led to an increase in the concentration of dissolved inorganic carbon (DIC), which was $86 \mu\text{mol kg}^{-1}$ higher at the vents than off-vents at relatively constant values of TA ($2561 \mu\text{mol kg}^{-1}$), salinity (38.3 PSU) and temperature (14°C) and a decrease in CO_3^{2-} concentrations (figure 2; electronic supplementary material, tables S1 and S3). The mean saturation state of seawater for the calcium carbonate minerals aragonite and calcite was 4.39 ± 0.05 – 2.82 ± 0.03 s.d. at the control stations and 3.18 ± 0.28 – 2.05 ± 0.18 s.d. at the vents, respectively (electronic supplementary material, table S1). No changes in inorganic nutrients were detected between control and vent stations (electronic supplementary material, table S2).

(b) CO_2 effects on species and habitat distribution

Field surveys showed substantial seascape changes at the venting zone in comparison to off-vent areas (figure 3),

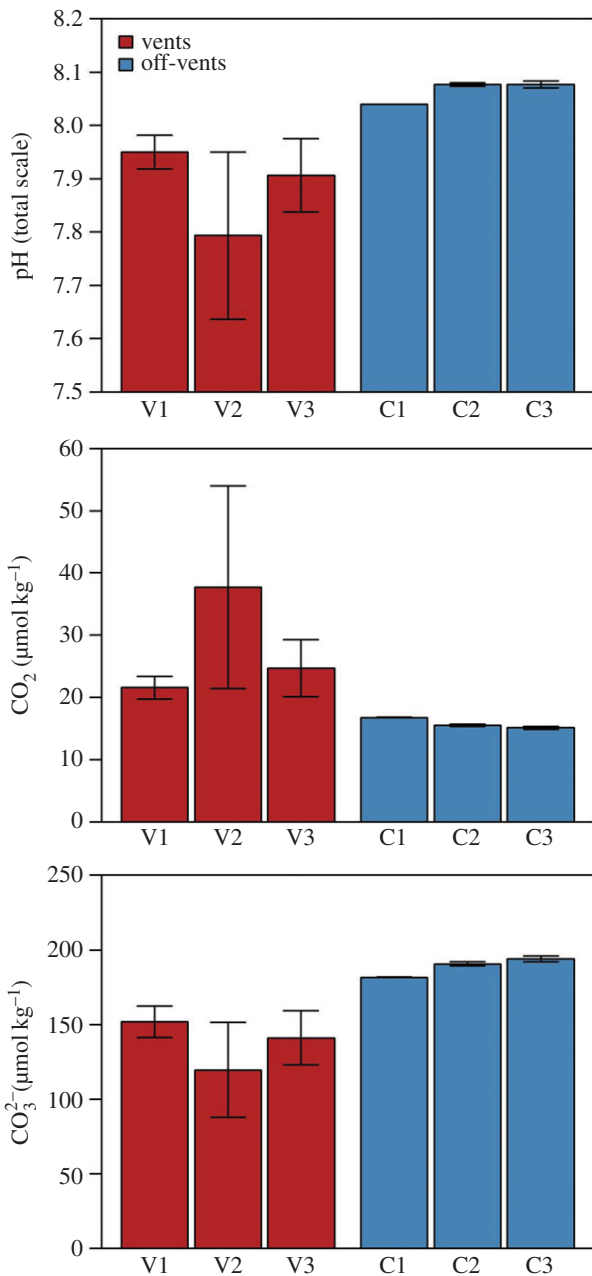


Figure 2. pH (total scale), CO₂ and CO₃²⁻ concentrations (mean ± s.e.) measured at vent and off-vent stations.

including significant differences in community composition ($p < 0.001$). While a kelp forest by the endemic *L. rodriguezii* covered the bottom directly affected by venting, coralligenous outcrops and rhodolith beds (dominated by coralline algae, gorgonians and calcareous bryozoans) were only found few metres off the vents (figures 3 and 4). At the vents, the kelp *L. rodriguezii* displayed biomass values between 22.1 and 116.5 g DW/2500 cm² with densities ranging from 55 to 210 fronds/2500 cm².

Other non-calcareous algae were found both at and off the vents (figures 3 and 4), in particular fucoids, such as the ochrophytes *Cystoseira zosteroides* and *Cystoseira spinosa*, as well as the rhodophyte *Phyllophora crispa* (electronic supplementary material, table S4). However, these species showed higher biomass off the vents (electronic supplementary material, table S4). By contrast, organisms with high-magnesium calcite skeletons such as coralline algae (*Spongites fruticulosa*, *Mesophyllum* spp., *Lithothamnion* spp., *Lithophyllum stictaeforme*) and bryozoans (*Turbicellepora avicularis*, *Reteporella* spp.) were

totally absent at the vents, whereas they were either common (bryozoans) or dominating (coralline algae) off the vents (figure 4; electronic supplementary material, table S4). Only the aragonite calcifying species *Peyssonnelia rosa-marina* was found at the vents, displaying the highest biomass and coverage (electronic supplementary material, table S4).

4. Discussion

(a) Distribution shifts in complex structurally habitats

This study revealed that moderate decreases of pH lead to dramatic shifts in highly diverse and structurally complex benthic habitats thriving at depths rarely explored in terms of OA effects. Dominant habitats at mesophotic depths, such as coralligenous outcrops and rhodolith beds, mainly characterized by a large dominance of calcifying organisms, are replaced by forests of the deep-water kelp *Laminaria rodriguezii*, which becomes dominant at depths much shallower than under normal seawater conditions. To our knowledge, this study highlights for the first time that beyond the changes at species levels reported in most of previous studies, where some species are winners and others lose out [9–11], OA can lead to significant changes at habitat level, benefiting specific habitats and compromising other habitats, also leading to vertical distribution shifts as observed in terrestrial ecosystems as a consequence of global warming [28].

The presence of kelps and other fleshy macroalgae, such as fucoids, inside the vents indicates that they are favoured by the observed changes in the carbonate system parameters. This agrees with the positive response displayed by several macroalgae and seagrasses to elevated CO₂ concentrations through the increase of photosynthesis and growth rates (Zimmerman *et al.* [29]; Palacios & Zimmerman [30]; Koch *et al.* [19]). Nevertheless, the dominance of the deep-water kelp *L. rodriguezii* observed in this study is noteworthy as this species is known to thrive between 65 and 95 m depth [31,32] and had almost never been reported before shallower than 50 m depth. These observations demonstrate that high CO₂ concentrations enhance the competitive abilities of *L. rodriguezii* at depths shallower than its common bathymetric range, thereby offsetting other existing environmental limitations (e.g. light or temperature) and allowing this species to grow at 40 m depth. The lower biomass displayed by fucoids (mainly *C. zosteroides*) inside the vents in comparison to the biomass of *L. rodriguezii* could be indicative of competition among fleshy algae. We hypothesize that *L. rodriguezii* outcompetes fucoids and other fleshy algae by inhibiting their growth due to the reduction in ambient light under the kelp canopy. Although the increase in competitive strength of seaweeds over other organisms, such as corals, as a function of CO₂ enrichment has been described previously (Díaz-Pulido *et al.* [33], Doropoulos *et al.* [34]), the impacts of OA on the interactions among algae under acidified conditions remain largely unknown. The competition between habitat-forming fleshy algae (i.e. kelps versus fucoids), as observed in this study, can result in further ecosystem shifts involving species substitution.

(b) Contrasting responses of calcifying organisms

Organisms with high-magnesium calcite skeletons such as coralline algae and bryozoans were totally completely absent at vent

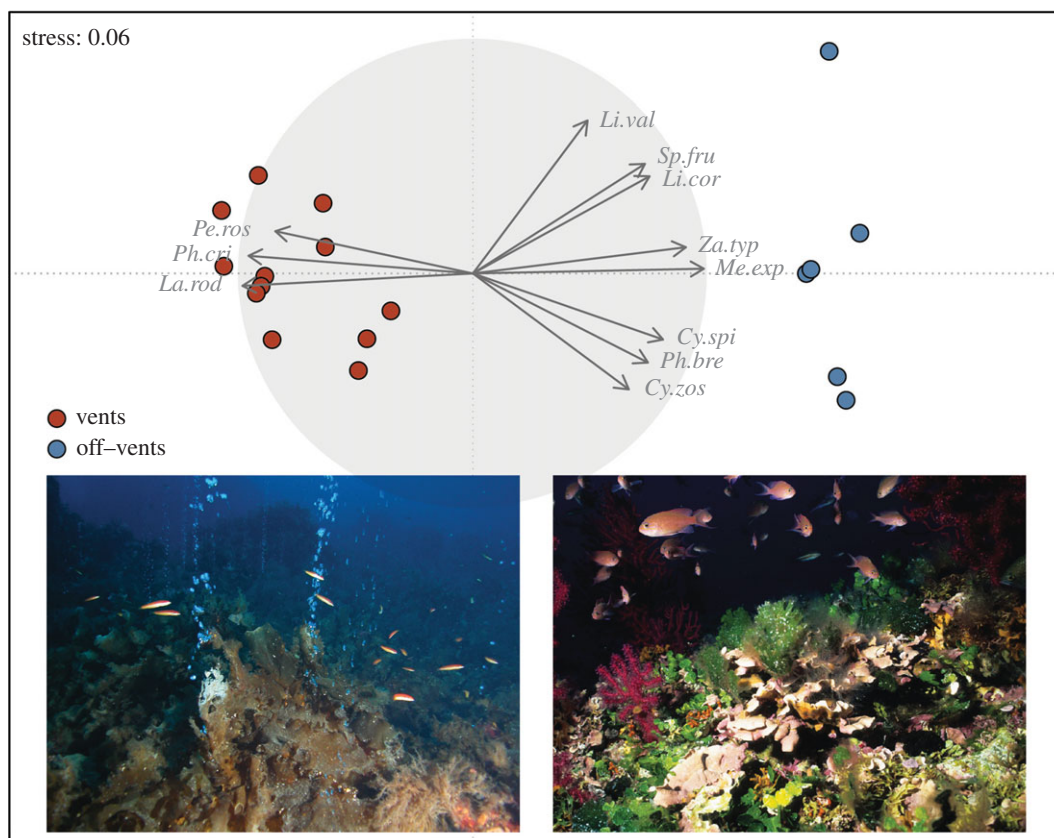


Figure 3. Non-metric multidimensional scaling (nMDS) plot based on the Bray–Curtis similarity of community structure at vent and off-vents stations. Pictures show the seascape at vent stations dominated by a kelp forest of *Laminaria rodriguezii* and the seascape off-vent stations dominated by coralligenous outcrops. Red and blue circles indicate vents and off-vent control stations. Species with a Pearson correlation greater than 0.8 are indicated as follows: *Cy.spi*, *Cystoseira spinosa*; *Cy.zos*, *Cystoseira zosteroides*; *La.rod*, *Laminaria rodriguezii*; *Li.cor*, *Lithothamnion corallioides*; *Li.val*, *Lithothamnion valens*; *Me.exp*, *Mesophyllum expansum*; *Pe.ros*, *Peyssonnelia rosa-marina*; *Ph.bre*, *Phyllariopsis brevipes*; *Ph.cri*, *Phyllophora crispa*; *Sp.fru*, *Spongites fruticulosa*; *Za.typ*, *Zanardinia typus*.

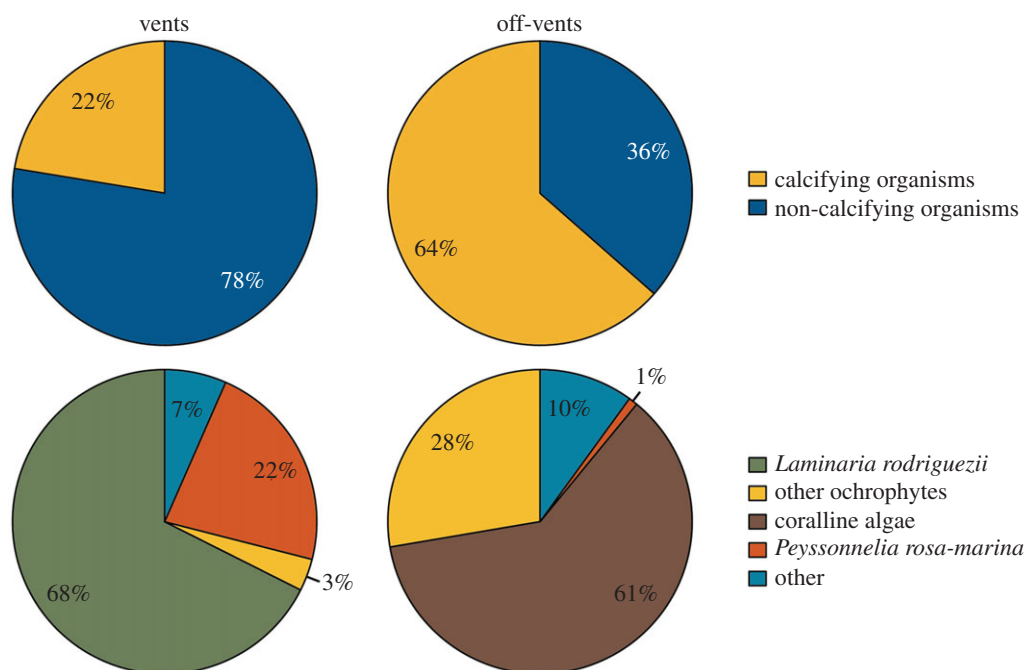


Figure 4. Pie chart of relative biomass composition by groups at vent and off-vent stations. ‘Other’ category includes chlorophytes, sponges, cnidarians, bryozoans and tunicates. ‘Other ochrophyta’ mainly include *Cystoseira zosteroides* and *C. spinosa*. See the electronic supplementary material, table S3 for further information.

sites. Only the aragonite-calcifying *Peyssonnelia rosa-marina* was able to survive in the acidified waters as previously noted in very shallow waters too [35]. Our results lend support to the idea that high-magnesium calcifying organisms are more

sensitive to OA than aragonite calcifying ones [35,36]. They also challenge the widespread concern over the global fate of calcified algae in the near future, as there are species like *P. rosa-marina* showing a moderate resilience to OA [36].

The recent discovery of calcium-magnesium carbonate (dolomite) in crustose coralline algae [37] and its lower solubility in comparison to magnesium calcite [38] suggest that some calcifying algae will not be doomed under moderate decreases in pH. This highlights the need of further research on the carbonate composition of these organisms.

(c) Long-term consequences of acidification for benthic ecosystems

The high variability in CO₂, pH and other carbonate system parameters observed in this study, as well as in other CO₂ vent studies [39], decreases the value of these sites as predictors of future ecosystem functioning face to oceanic pH levels expected to occur at the end of this century [3]. However, pH variability has been stressed to enhance acclimatization or adaptation to acidification through repeated exposure to lowered pH conditions [40]. Although a long-term survey of pH variability would be needed to fully understand its effects on the structure and functioning of the investigated system, our results indicate that regardless of variability in pH and other carbonate parameters moderate acidification can drive dramatic and persistent changes in benthic ecosystems. Moreover, expected synergies of OA with warming and other stressors are likely to occur [41,42], which can result in even more drastic changes than the ones observed in this study.

Furoids and kelps, globally recognized as key structural and functional elements of marine benthic ecosystems [43], are in decline with some local extinctions already documented [44]. Our results suggest an enhancement of the populations of these habitat-forming fleshy algae under acidified conditions, and especially of kelps at mesophotic depths. OA will negatively affect coralligenous outcrops and rhodolith beds, which are extremely threatened habitats [18]. Our results also

indicate a substitution of habitats dominated by calcifying algae by those dominated by erect fleshy algae and the replacement of high-magnesium calcite corallines by aragonite-calcifying algae (e.g. *Peyssonnelia*) as main carbonate producers. The changes observed in the highly diverse and structurally complex mesophotic habitats of the Mediterranean Sea investigated in our study may have critical ecological and social implications for coastal ecosystems at large spatial scales in an increasingly acidifying ocean.

Data accessibility. All data and supplementary material are deposited in Dryad (<http://dx.doi.org/10.5061/dryad.0f7s7>).

Authors' contributions. All authors were involved with fieldwork and sample analyses. D.K. discovered the vent site. E.A., L.N., M.V., E.B., D.A., M.C. and C.L. analysed the data. A.D. analysed the gases and E.B. identified the species. C.L. drafted the initial manuscript and wrote the paper along with M.V., M.C. and E.B. All authors discussed results and commented on the manuscript.

Competing interests. We have no competing interests.

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