**Supplementary Information for**

# Functional changes across marine habitats due to ocean acidification

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## Supplementary Text

### Supplementary Text 1: Study sites

The volcanic $CO_2$ vents are located along the coast of Ischia Island (Italy) across depths of 0.5 to 48 m depths (Figure S1). We assessed changes in the benthic community at four $CO_2$ vents, the shallow rocky vents at the Castello Aragonese, and three new $CO_2$ vents, and compared with nearby two corresponding reference ambient pH sites hosting similar habitat types but outside the influence of $CO_2$ venting. Over the last decade, studies using the shallow volcanic $CO_2$ vent system near the Castello Aragonese have generated key insights on the direct and indirect effects of ocean acidification on the surrounding ecosystems (Foo et al., 2018; Hall-Spencer et al., 2008; Kroeker et al., 2011; Teixidó et al., 2018). Briefly, water carbonate chemistry and *in situ* monitoring of seawater pH delineated a pH gradient with three carbonate chemistry zones (ambient, low and extreme low pH) caused by spatial variability in $CO_2$ venting intensity (Hall-Spencer et al 2008). For this study, we selected two pH zones at this site (ambient: pH ~8.0 and low pH: pH ~7.8 -7.5) as the other three $CO_2$ vents do not present extreme low conditions (pH ~6.6 -7.2); thereby making community surveys comparable among habitats. These new $CO_2$ vent systems locally acidify the seawater with gas comprising 92-95% $CO_2$ (no sulphur) and do not elevate temperature (see below, Table S1). These four habitats are hotspots of Mediterranean marine biodiversity and represent an exceptional natural heritage of the Mediterranean seascape (Ballesteros, 2006; Bianchi & Morri, 2000; Coll et al., 2010). Two reference sites with ambient pH (Ambient a, b) for each habitat/vent were chosen following the criteria: i) they hosted similar habitats and depths as the $CO_2$ vent sites, and ii) no venting activity was evident.

**Brief description of the $CO_2$ vent systems and reference sites with ambient pH**

*$CO_2$ vent system 1:* Shallow reef (local name *Castello Aragonese*). The volcanic $CO_2$ vents are located at 0.5 - 3 m depth on the north and south sides of the islet Castello Aragonese adjacent to sloping rocky reefs. Studies using the shallow volcanic $CO_2$ vent system near the Castello Aragonese have generated key insights on the direct and indirect effects of ocean acidification on the surrounding ecosystems (Foo et al., 2018; Hall-Spencer et al., 2008; Kroeker et al., 2011; Teixidó et al., 2018). Briefly, water carbonate chemistry and *in situ* monitoring of seawater pH delineated a pH gradient along the Castello Aragonese with three carbonate chemistry zones (ambient, low and extreme low pH) caused by spatial variability in $CO_2$ venting intensity (Hall-Spencer et al 2008). For this study, we selected two pH zones at this Shallow reef habitat: low pH zone (moderate venting activity and low pH, $pH_T = 7.8 \pm 0.31$) and Ambient pH zone (non-visible vent activity and ambient pH, $pH_T \sim 8.0 - 8.1$), and we excluded the extreme pH zone (high venting activity, and extreme low pH, $pH_T \sim 6.6 - 7.2$). The criterion was to unify the sampling design with the other three habitats, as the three new $CO_2$ vent sites present moderate venting activity and low pH conditions ($pH_T \sim 7.74$ to 7.96, Table 1 for detailed values). For the present study, we obtained species and trait diversity data for the Shallow reef habitat from Teixido et al. (2018). The low pH zone is ~ 20 m in length and separated from the ambient pH zones by at least 20 – 25 m (Ambient 1a, 1b). Benthic surveys were performed at the same depth range. *$CO_2$ vent system 2:* Cave (local name *Grotta del Mago*). The $CO_2$ vent system is located at a 5 m depth inside a semi-submerged cave of volcanic origin. The cave (total length of 110 m) consists of a main outer chamber (10 m wide x 30 m long), connected to an inner chamber by a long narrow passage. The present study was performed in the main chamber of the cave with approximately $2.3 \pm 0.8$ vents 0.25 $m^{-2}$. See Teixidó et al. (2020) for more information on this $CO_2$ vent system. The reference sites, Ambient

2a and 2b, were also semi-submerged caves with main chambers of approximately 10 m wide x 30 m long and 5 m maximum depth. Benthic surveys were performed at ~ 3 m depth. *$CO_2$ vent system* 3: Reef (local name *Chiane del Lume).* The $CO_2$ vent system is located at 11 m depth, composed of sublittoral coarse sand/gravel, and adjacent to rocky reefs. The reef facing the vents is ~50 m length. $CO_2$ vent density is estimated to be 3.1 ± 1.0 vents 0.25 $m^{-2}$. The reference sites, Ambient A3a and A3b, were also rocky habitats at the same depth. Benthic surveys were performed at ~ 10 m depth. *$CO_2$ vent system* 4: Deep reefs (local name *Madonnina*). The $CO_2$ vent system is located at the sea bottom from a rocky bank from 37 to 48 m depth. Vent density is estimated to be around 3 vents per 0.25 $m^{-2}$. This habitat is named coralligenous, which are bioconstructions resulting from the presence and growth of calcareous algae. The reference sites, Ambient A4a and A4b, were also banks with coralligenous formations at the same depths. Benthic surveys were performed at ~ 40 m. See also Figures S1 and S3.

In total, we characterized the physical and chemical parameters and changes in benthic communities in four $CO_2$ venting sites (low pH conditions with moderate venting activity, range of means from ∼ 7.7 to 7.9 $pH_T$ at total scale, ∼ 983 - 538 $pCO_2$) and 8 reference areas (two reference areas per each vent/habitat type) with ambient pH with no venting activity (range of means from ∼ 8.0 to 8.1 $pH_T$ at total scale, 375 - 567 $pCO_2$). The mean carbonate chemistry in the ambient pH zones corresponds to current average conditions, whereas the low pH zones are most comparable with values predicted for the year 2100 with a decrease of pH from -0.14 to -0.38 pH units under RCP2.6 and RCP8.5 (Kroeker et al., 2011; Kwiatkowski et al., 2020; Teixidó et al., 2018).

**Supplementary Text 2: pH sensor calibration**

Before deployment, the SeaFETs were calibrated with ambient pH water in the aquarium facilities at the Center Villa Dohrn (Ischia, Italy). Discrete water samples were collected during this acclimation period to calibrate for drift of the SeaFETs in the field. These samples, collected in borosilicate glass bottles and immediately fixed with saturated mercuric chloride, were processed for $pH_T$ (total scale) using an Ocean Optics spectrophotometer (USB2000) with 10 cm path length optical cells and with purified m-cresol purple (Fluidion) (Dickson et al., 2007; Kapsenberg et al., 2017). Purified m-cresol dye was verified for accuracy compared to tris buffer CRM $pH_T$ (± 0.002 SD for 2018 and < ± 0.001 SD for 2019 of spectrophotometer $pH_T$ measurements of tris buffer from CRM value) (#26, provided by A. Dickson, Scripps Institution of Oceanography, USA). Water samples were kept in a temperature-controlled water bath (Huber KISS K-12 Refrigerated Heating Bath) at 20 °C before analysis to minimize temperature-induced errors in absorbance measurements. The temperature of each sample was recorded immediately after analysis using a digital thermometer accurate to ± 0.05 °C (P600 Dostmann electronic Thermometer). *In situ* temperature and salinity (set as constant = 38) were used to calculate *in situ* $pH_T$ of the calibration samples. Five replicate measurements per calibration sample were analyzed to calculate the source of error for the spectrophotometric pH measurements (Figure S2) (± 0.006 SD, n= 44 mean SD of 5 replicate measurements per calibration samples). Following (Kapsenberg et al., 2017) SeaFET voltage was converted to $pH_T$ using the respective calibration samples for each sampling time with R package seacarb v3.3.2 (Gattuso et al., 2023).

## Supplementary Text 3: Carbonate Chemistry and Nutrients

Samples for total alkalinity ($A_T$) were collected using standard operating protocols in borosilicate bottles, not filtered, fixed with saturated mercuric chloride, sealed and stored in the dark at 4°C pending titration within 7-30 days after sampling. $A_T$ was determined using an autotitrator (Mettler Toledo G20S) interfaced with the data acquisition software LabX. The HCl (0.1 eq $L^{-1}$) titrant solution was calibrated against certified reference materials distributed by A.G. Dickson (CRM, Batches #153, #171, and #177). Precision of the $A_T$ measurements of CRMs was < 2.0 µmol $kg^{-1}$. Means were reported as (mean ± SD): $A_T$ = 2562.41 ± 7.8 µmol $kg^{-1}$, n = 27 in September 2018; and $A_T$ = 2543.57 ± 21.78 µmol $kg^{-1}$, n = 21 in June 2019. $A_T$ and $pH_T$ were used to determine the remaining carbonate system parameters at *in situ* temperature and depth of each sampling period, using the dissociation constants of (Dickson, 1990; Dickson & Millero, 1987) for $KHSO_4$, and (Uppström, 1974) for boric acid in the R package seacarb v3.3.2 (Gattuso et al., 2023).

Nutrient samples were filtered using pre-combusted GF/F filters (Whatman) and frozen at -20°C and transported to OGS (Trieste, Italy). Dissolved inorganic nutrients (nitrite $NO_2$, nitrate $NO_3$, ammonium $NH_4^+$, phosphate $PO_4$ and silicate $Si(OH)_4$) were determined using a colorimetric method (Hansen & Koroleff, 1999) with a QuAAtro Seal Analytical autoanalyzer. Precision of nitrite, nitrate, phosphate and silicate was 0.02 µmol $L^{-1}$ and 0.04 µmol $L^{-1}$ for ammonium (Table S3). Nutrient levels (phosphate, ammonium, nitrite, and nitrate) in the water did not differ significantly between $CO_2$ vents and reference areas.

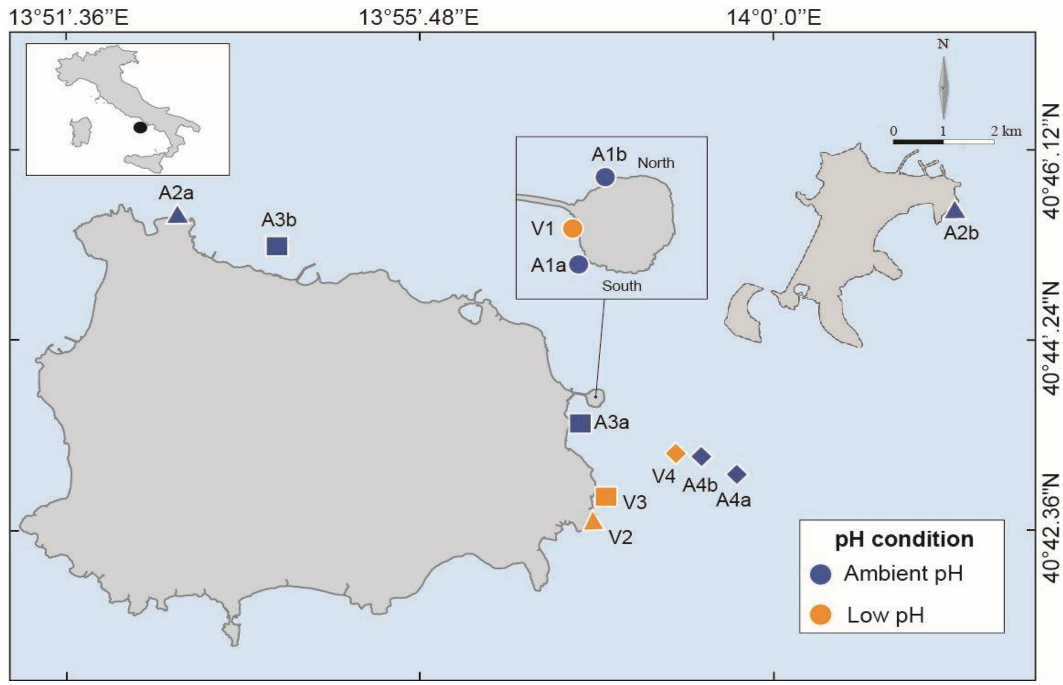


**Figure S1. Map showing the location of the study sites along the coast of Ischia Island, Italy.** V (V1, V2, V3, V4) refers to the $CO_2$ vent systems, A are off-vent reference sites with ambient pH (A1, A2, A3, and A4) for two sites (a, b). The $CO_2$ vent sites span a variety of different habitats such as shallow rocky reefs, semi-submerged caves, and deep reefs Ischia across depths from 3 to 48 m. Habitat for Vent 1: Shallow Reefs ($CO_2$ vents at 0.5- 3 m depth), $CO_2$ venting site (V1) with reference sites north and south of the islet Castello Aragonese (A1a, A1b). Benthic surveys performed at 0.5- 3 m depth. Habitat for Vent 2: Caves ($CO_2$ vents at 5 m depth), $CO_2$ venting site Grotta del Mago (V2) with reference ambient pH sites Punta Vico (A2a) and Punta Monaci (A2b). Benthic surveys performed at ~ 3 m depth. Habitat for Vent 3: Reefs ($CO_2$ vents at 11 m depth), $CO_2$ venting site Chiane del Lume (V3) with reference ambient pH sites Sant'Anna (A3a) and Santuario (A3b). Benthic surveys performed at ~ 10 m depth. Habitat for Vent 4: Deep Reefs ($CO_2$ vents at 48 m depth), $CO_2$ venting site Madonnina (V4) with reference ambient pH sites Catena (A4a) and Pertuso (A4b). Benthic surveys performed at ~ 40 m depth.

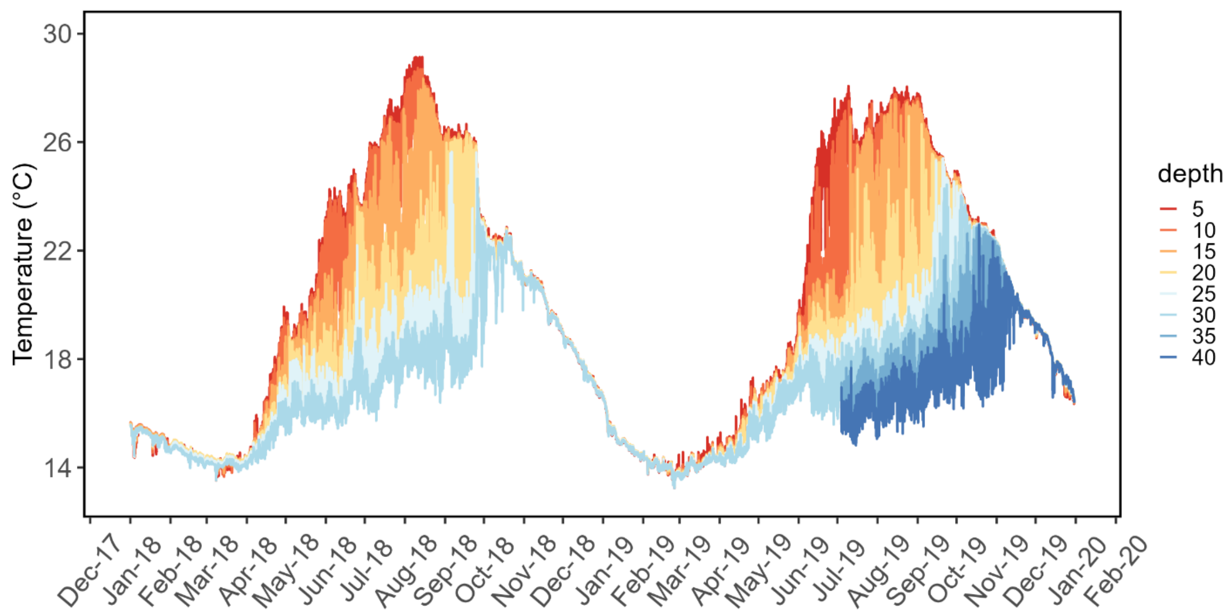


**Figure S2. Daily mean temperature across depths from 2018 to 2019.** Daily mean temperature calculated from hourly measurements taken by *in situ* HOBO sensors (HOBO Water Temp Pro V2, accuracy ±0.21°C, resolution 0.02°C) from 2018 to 2019 along a depth gradient. Temperature sensors were deployed along a rocky reef at 5 m intervals and up to 40 m depth (specifically at 5 m, 10 m, 15 m, 20 m, 25 m, 30 m, 35 m, and 40 m) in San Pancrazio on the island of Ischia (40°42'N 13°57'E). Depths and high-frequency measurements (hourly) are standardized within the T-MEDNET temperature observation network (http://www.t-mednet.org/). Temperature followed ambient seasonal fluctuations. The sites experienced similar seawater temperature conditions from late autumn to winter (marked by the breakdown of the seasonal thermocline after summer), ranging from 14.4 to 15.0 °C in winter (December 21 to March 20). However, they exhibited distinct temperature conditions in spring and summer when the thermocline developed, typically around depths of 15-25 meters. Temperature values ranged from 25.5 °C to 26.7 °C at 5 and 10 m, 20.1 °C to 23.6 °C at 15 and 20 m, and below 19 °C at depths greater than 25 m in July and August. Temperature sensors at 35 and 40 m were first deployed in 2019.

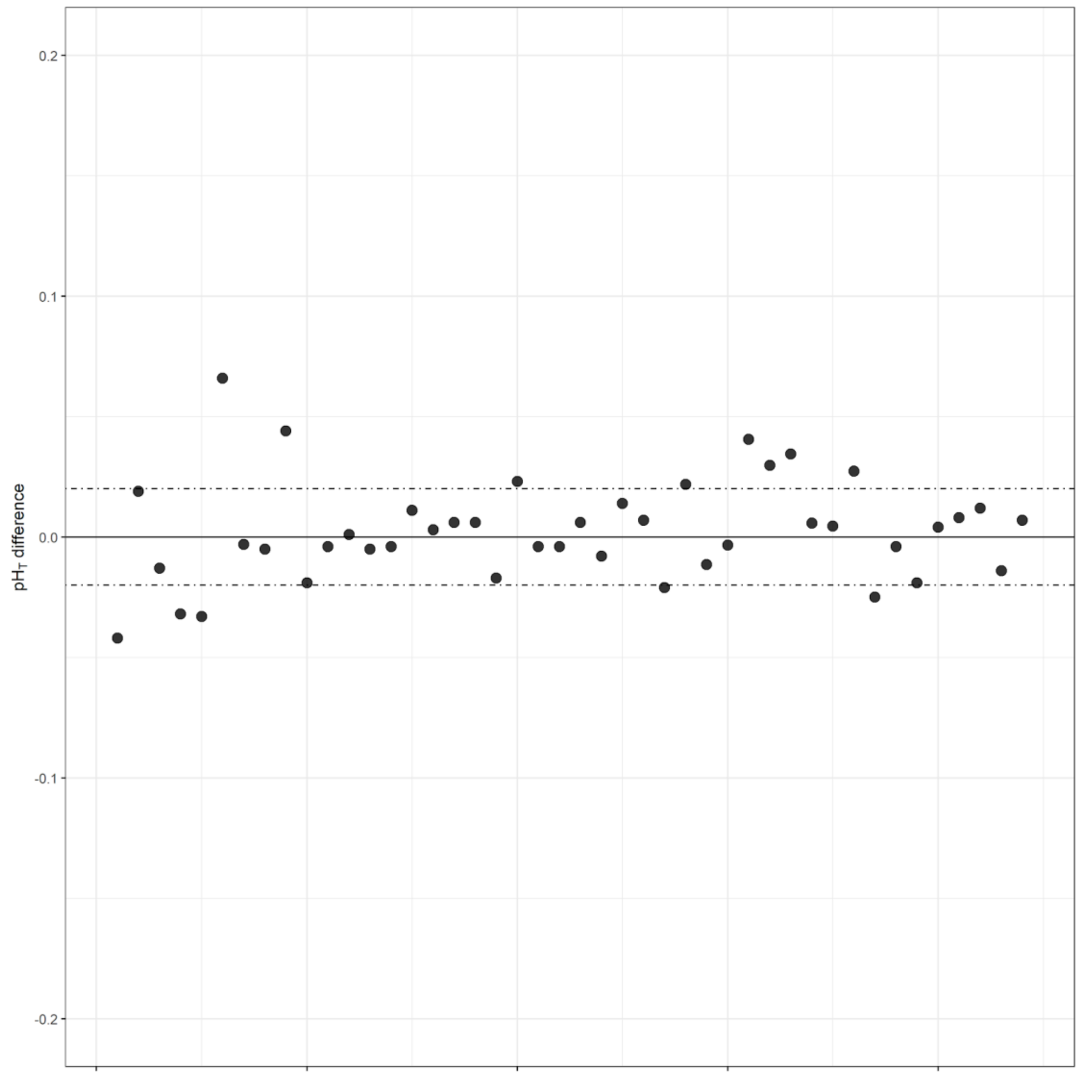


**Figure S3. Calibration sample offsets from processed pH$_T$ series (spectrophotometric pH compared to final calibrated pH SeaFET series).** Discrete water samples were collected during acclimation periods to calibrate for drift of the SeaFETs in the field. Five replicate measurements per calibration sample were analyzed to calculate the source of error for the spectrophotometric pH measurements (± 0.006 SD). N= 44 water samples.

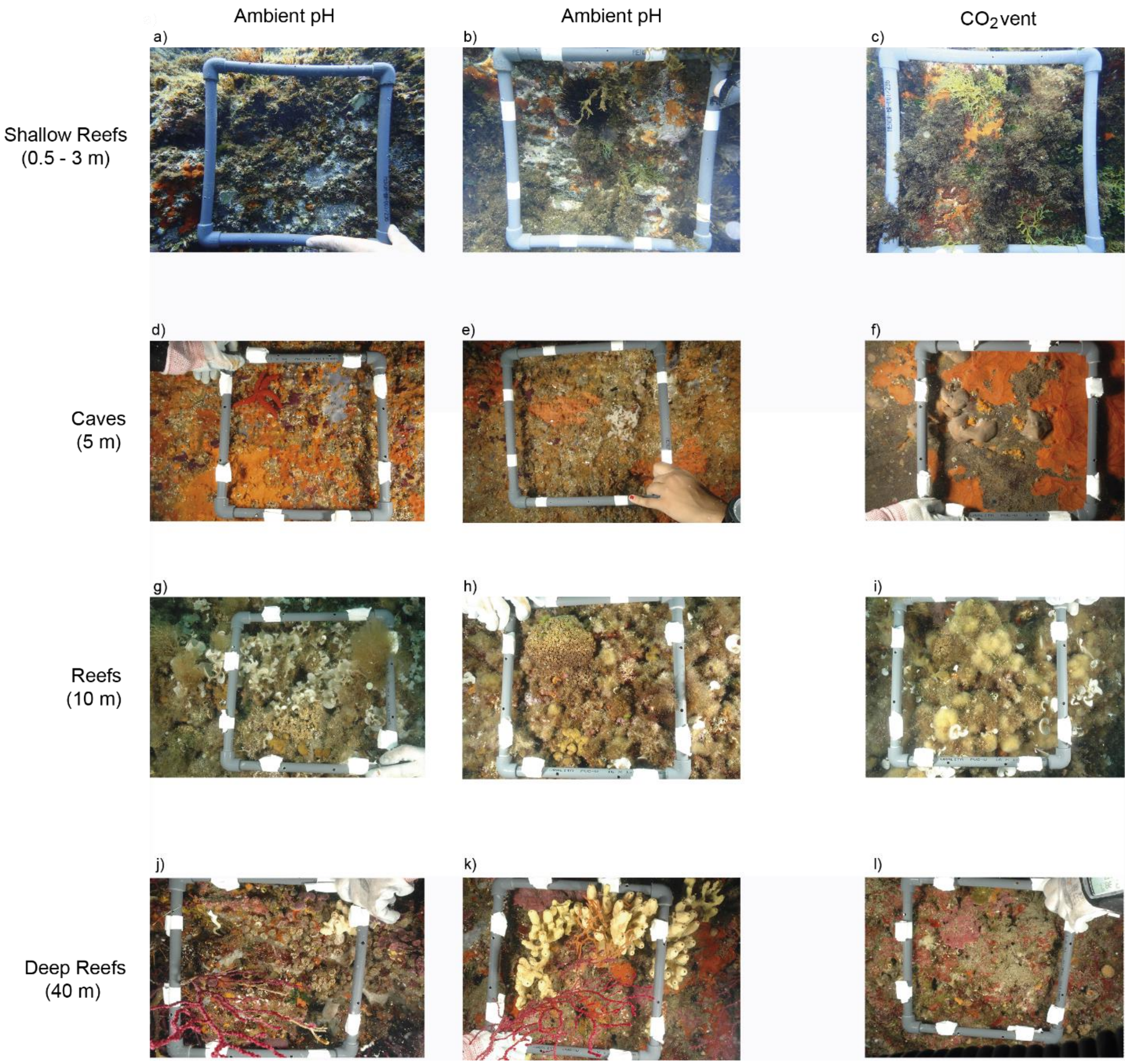


**Figure S4. Photographs taken at the ambient pH sites and $CO_2$ vents across habitats.** The ambient pH sites (a, b) of the Shallow Reef (0.5- 3 m depth) are characterized by a mosaic of calcifying and fleshy algae as well as calcifying invertebrates such as the barnacle *Perforatus perforatus* and the sea urchin *Arbacia lixula*. The low pH zone (c) shows a high percent cover of fleshy algae (e.g. *Dictyota sp.* and *Halopteris scoparia*) and the presence of encrusting sponges (e.g. the orange sponge *Crambe crambe*). The ambient pH sites (d, e) of the Cave (3 m depth) exhibit a high coverage of perennial suspension feeders, including the encrusting sponges

*Spirastrella cunctatrix*, *Phorbas tenacior*, and *Clathrina contorta* (a calcareous sponge), the scleractinian coral *Astroides calycularis*, and the encrusting bryozoan *Pentapora ottomulleriana*. The CO2 vent (f) is characterized by suspension feeders with significant reductions of calcifying species and presence of bare substrate. Species in this picture (f): the sponges *S. cunctatrix* and *Chondrosia reniformis* and the coral *A. calycularis*. The ambient pH sites (g, h) of the Reef (10 m depth) show a high coverage of fleshy (e.g. *Dictyota sp.)* and calcifying algae (*e.g. Acetabularia acetabulum, Padina pavonica, Amphiroa rigida*) with some invertebrates (e.g. the scleractinian coral *Cladocora caespitosa*). The $CO_2$ vent site presents (i) a high coverage of fleshy algae *(e.g. Sphacelaria cirrosa, Caulerpa cylindracea*-an invasive species), along with the occurrence of some calcifying algae (e.g. *P. pavonica*) and corals (e.g. *C. caespitosa*). The ambient pH sites (j, k) of the Deep reef (40 m depth) are characterized by a high coverage of encrusting coralline algae (e.g. *Mesophyllum alternans* and *Lithophyllum stictiforme*) and by a mosaic of suspension feeders with massive and tree-like forms (e.g. the red gorgonian *Paramuricea clavata*, the coral *Caryophyllia smithii*, and the bryozoan *Smittina cervicornis*). These species create habitat provision and structural complexity. The $CO_2$ vent site presents (l) relatively high coverage of the encrusting calcifying algae (e.g. *Mesophyllum alternans* and *Peyssonnelia rosa-marina*), the fleshy algae *Pseudochlorodesmis furcellata* and turf algae, and colonies of the bryozoan *Schizomavella mamillata*. Species that are important contributors of the three-dimensional habitat are lost at the Deep Reef.

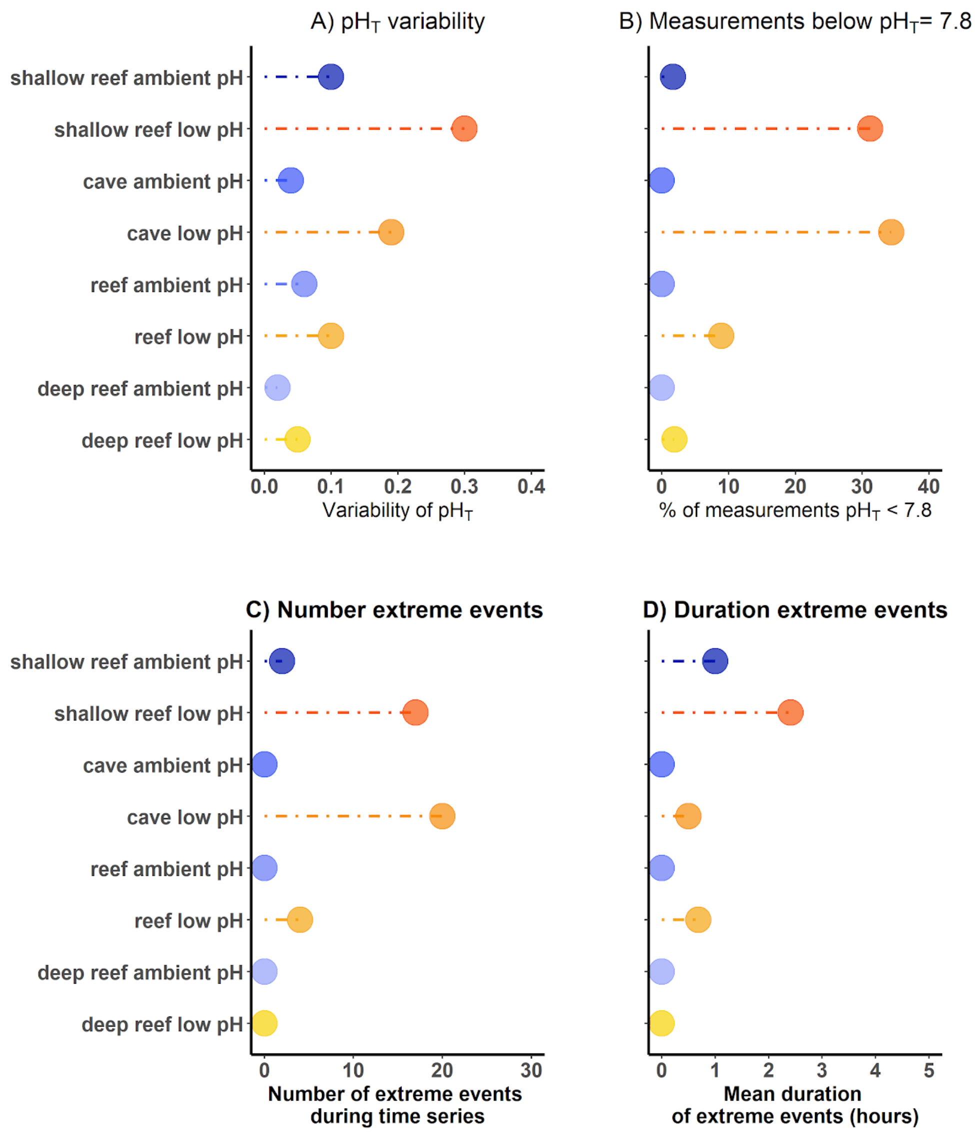


**Figure S5. Summary metrics of pH variability at the $CO_2$ vent sites with low pH (red-orange) and reference sites with ambient pH conditions (blue).** A) pH variability was calculated as the difference between 25$^{th}$ and 75$^{th}$ percentiles of measured pH$_T$; B) the percentage of measurements below pH$_T$= 7.8 (predicted average global sea surface pH for the year 2100 under the RCP8.5 scenario); C) the number of extreme events, defined as a pH value of 0.4 units less than the mean pH for each pH condition; D) mean duration of extreme events (hours).

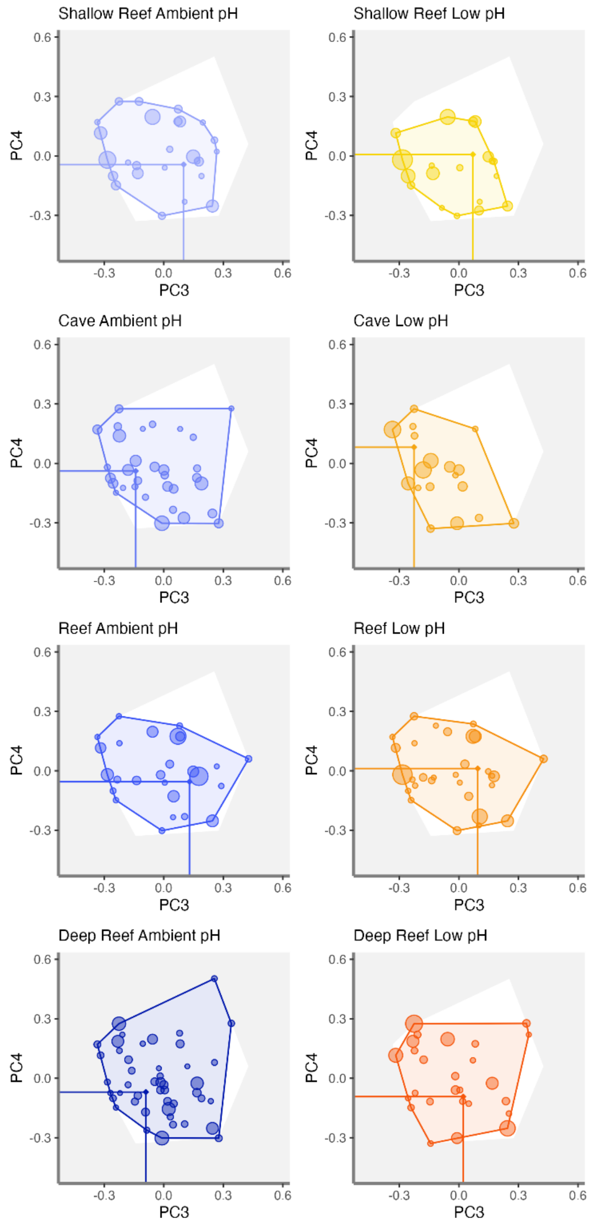


**Figure S6. Shifts in trait diversity between pH zones (low and ambient pH) and across habitats.** Trait space for the global pool of 215 benthic species and 74 functional entities (FEs) after averaging 100 randomized datasets to a unique dataset. Axes PC3-PC4 represent the last four dimensions of the 4D functional space. The global convex hull for all four habitats and pH conditions is filled in white. The size of points indicates the abundance of each FEs. Habitats: Shallow Reefs (0.5 - 3 m depth), Caves (3 m depth), Reefs (10 m depth), and Deep Reefs (40 m depth).

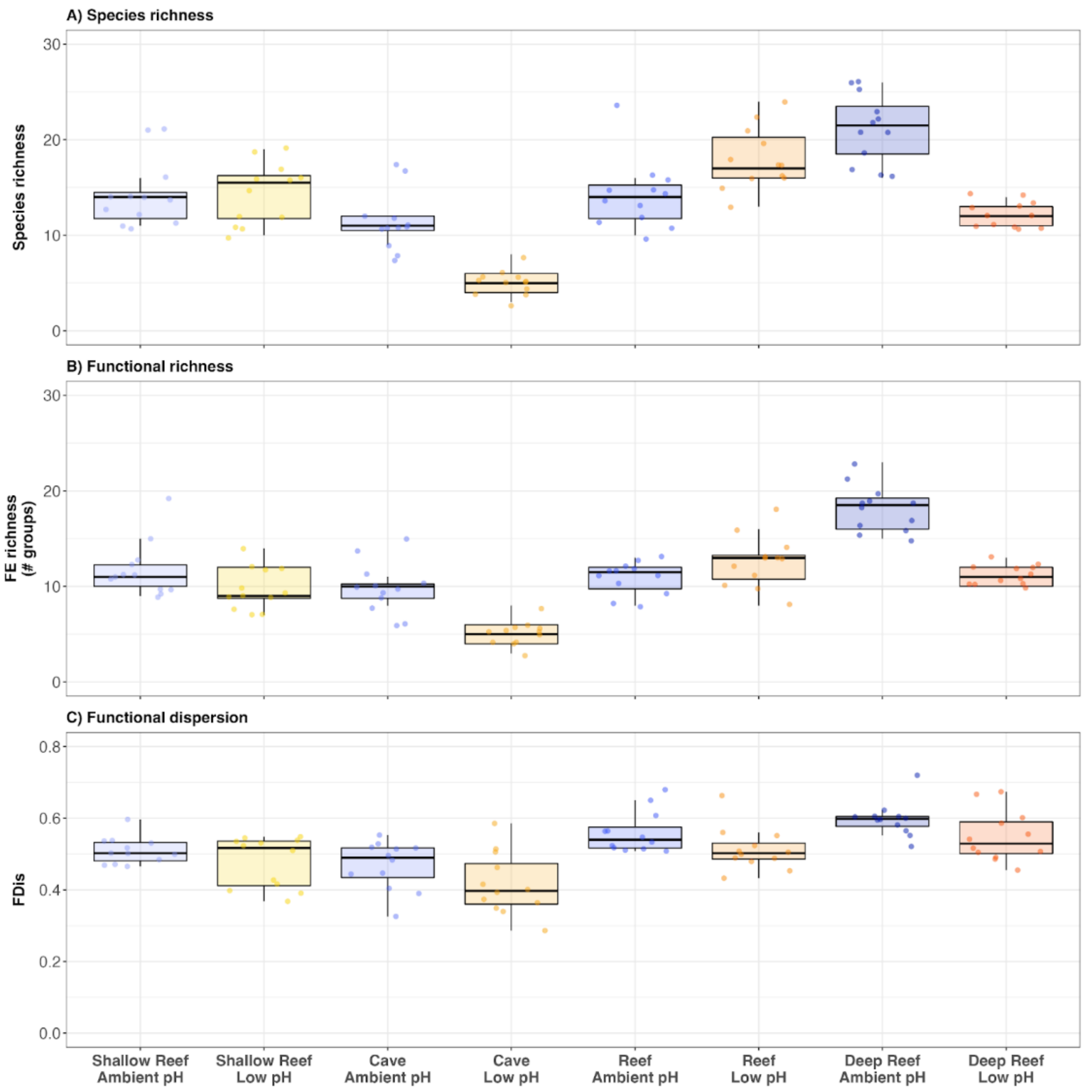


**Figure S7. Species and trait diversity indices based on trait values of FEs across habitats and among pH zones.** Dots represent the values for each habitat and pH conditions; boxes represent the mean and standard deviation. Functional dispersion (defined as the weighted deviation to center of gravity of species in the assemblage). Permanova analyses: Species richness ($F_{7,88}$=40.33, p<0.001; pairwise significance, p<0.05: Cave ambient-low pH, Reef ambient-low pH, Deep Reef ambient-low pH); FE richness ($F_{7,88}$= 31.8, p<0.001; pairwise significance, p<0.05: Shallow Reef ambient-low pH, Cave ambient-low pH, Deep Reef ambient-low pH); Functional dispersion ($F_{7,88}$= 9.9, p<0.001; pairwise significance, p<0.05: Cave Ambient-low pH). N= 12 quadrats for each habitat and pH conditions. See Table S8 for mean and standard deviation.

**Table S1. Composition of the volcanic gases at the vent sites.** Measurements were based on two separate gas samples by an Agilent 7890B gas chromatography combined using a Micro GC analyzer-INFICON, held at a constant temperature of 80 °C. The vent gas at the vent sites was predominantly $CO_2$, with undetectable levels of hydrogen sulfide (< 0.0002%), and did not elevate the temperature. Values are as mean ± SE. Depth values refer to the sea bottom depth, where gas was sampled. Data from the Shallow Reefs are originally reported in (Hall-Spencer et al., 2008).

| Vent | Habitat | Local name | Depth (m) | $CO_2$ (%) | $O_2$ (%) | $N_2$ (%) | N vents 0.25 $m^{-2}$ |
|---|---|---|---|---|---|---|---|
| Vent 1 | Shallow Reef | Castello South | 0.5-3 | 93.6 ± 0.6 | 0.44 ± 0.03 | 2.05 ± 0.2 | 5-10 vents $m^{-2}$ |
| Vent 2 | Cave | Grotta del Mago | 5 | 93.4 ± 1.0 | 0.37 ± 0.09 | 2.68 ± 0.5 | 2.3 ± 0.8 |
| Vent 3 | Reef | Chiane del Lume | 11 | 93.9 ± 0.6 | 0.11 ± 0.03 | 2.89 ± 0.2 | 3.1 ± 1.0 |
| Vent 4 | Deep Reef | Madonnina | 48 | 96.0 | | | ~ 5 vents $m^{-2}$ |

**Table S2. Seawater $pH_T$ statistics associated with $CO_2$ vents and reference areas with ambient pH.** Values are: mean, median, and 25th and 75th percentiles of measured $pH_T$. Threshold (Th.) $pH_T$ <7.8 represents the % of measurements registered below 7.8 units (projected decrease in surface pH of 0.4 units by 2100 under the RCP8.5 scenario). Measurements were taken every 15 minutes with SeaFET pH sensors. pH conditions: Vent systems (1, 2, 3, 4) and ambient pH sites. Data from the shallow reefs (0.5 - 3m) are originally reported in (Kroeker et al., 2011).

| pH condition | Habitat | Local name | Date yyyymmdd | Mean $pH_T$ | Median $pH_T$ | 25% | 75% | Th. $pH_T$<7.8 | n | Freq. |
|---|---|---|---|---|---|---|---|---|---|---|
| Vent 1 | Shallow Reef | Castello South | 20100510-20100614 | 7.833 | 7.913 | 7.735 | 8.023 | 31% | 840 | 1 hour |
| Ambient pH A1a | Shallow Reef | Castello South | 20100512-20100614 | 8.070 | 8.088 | 8.045 | 8.119 | 1.6% | 792 | 1 hour |
| Ambient pH A1b | Shallow Reef | Castello North | 20100913-20101008 | 7.955 | 7.960 | 7.920 | 7.998 | 1.9% | 607 | 1 hour |
| Vent 2 | Cave | Grotta del Mago | 20190530-20190618 | 7.742 | 7.867 | 7.741 | 7.933 | 34% | 1841 | 15 min |
| Ambient pH A2a | Cave | Punta Vico | 20190507-20190521 | 8.047 | 8.046 | 8.073 | 8.028 | - | 1331 | 15 min |
| Vent 3 | Reef | Chiane del Lume | 20190507-20190521 | 7.910 | 7.961 | 7.899 | 7.996 | 9% | 1326 | 15 min |
| Ambient pH A3a | Reef | Sant'Anna | 20190620-20190708 | 7.968 | 7.971 | 7.942 | 7.996 | - | 1691 | 15 min |
| Vent 4 | Deep Reef | Madonnina | 20190916 20191014 | 7.964 | 7.983 | 7.947 | 8.007 | - | 2692 | 15 min |
| Ambient pH A4a | Deep Reef | Catena | 20190830 20190918 | 8.004 | 8.004 | 7.993 | 8.014 | 2% | 1825 | 15 min |

**Table S3. Mean (± SD) of concentrations of inorganic nitrogen, phosphate and silicate in the seawater at the $CO_2$ vent sites and reference areas with ambient pH.** Habitat: Shallow Reefs (0.5 -3 m); Caves (3 m); Reefs (10 m); Deep reefs (40 m). pH conditions: Vent system (1, 2, 3, 4); Ambient pH (a, b for each reference site).

| pH condition | Habitat | Local name | $NO_2$(µmol $L^{-1}$) | $NO_3$(µmol $L^{-1}$) | $NO_x$($NO_2$+$NO_3$) (µmol $L^{-1}$) | $NH_4$(µmol $L^{-1}$) | $PO_4$(µmol $L^{-1}$) | $SiO_2$(µmol $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| Vent 2 | Cave | Grotta del Mago | 0.032 ± 0.004 | 0.253 ± 0.051 | 0.285 ± 0.055 | 0.184 ± 0.039 | 0.026 ± 0.001 | 1.500 ± 0.214 |
| Ambient pH, A2a | Cave | Punta Vico | 0.018 ± 0.003 | 0.118 ± 0.003 | 0.137 ± 0.001 | 0.147 ± 0.021 | 0.032 ± 0.005 | 1.175 ± 0.067 |
| Ambient pH, A2b | Cave | San Pancrazio | 0.005 ± 0.001 | 0.059 ± 0.001 | 0.064 ± 0.001 | 0.069 ± 0.013 | 0.049 ± 0.007 | 0.698 ± 0.018 |
| Vent 3 | Reef | Chiane del Lume | 0.002 ± 0.003 | 0.066 ± 0.004 | 0.068 ± 0.006 | 0.029 ± 0.009 | 0.023 ± 0.005 | 0.957 ± 0.032 |
| Ambient pH, A3a | Reef | Sant'Anna | 0.011 ± 0.009 | 0.041 ± 0.051 | 0.052 ± 0.060 | 0.068 ± 0.015 | 0.019 ± 0.007 | 0.822 ± 0.031 |
| Ambient pH, A3b | Reef | Santuario | 0.003 ± 0.003 | 0.048 ± 0.025 | 0.051 ± 0.025 | 0.101 ± 0.039 | 0.011 ± 0.003 | 0.998 ± 0.047 |
| Vent 4 | Deep reef | Madonnina | 0.033 ± 0.006 | 0.085 ± 0.041 | 0.118 ± 0.047 | 0.150 ± 0.025 | 0.020 ± 0.006 | 1.359 ± 0.270 |
| Ambient pH, A4a | Deep reef | Catena | 0.008 ± 0.009 | 0.075 ± 0.103 | 0.083 ± 0.108 | 0.089 ± 0.028 | 0.029 ± 0.009 | 1.213 ± 0.419 |
| Ambient pH, A4b | Deep reef | Pertuso | 0.007 ± 0.003 | 0.026 ± 0.036 | 0.033 ± 0.039 | 0.082 ± 0.030 | 0.018 ± 0.012 | 1.215 ± 0.196 |

**Table S4. Description of the sampling design of benthic surveys.** Percent cover of benthic species was quantified using visual census (dominance of algae) and photographic quadrats (dominance of sessile invertebrates) at the $CO_2$ vent and the two ambient pH sites for each habitat. Before analyzing the data, we performed a set of randomization analyses to unify the sampling effort between pH conditions and across habitats. We randomly selected 12 quadrats per pH conditions (low pH and ambient pH) and habitat, thus resulting in 24 quadrats per habitat and a total of 96 quadrats (12 quadrats x 4 habitats x 2 pH conditions). Quadrats = 25 x 25 cm.

| pH condition | Habitat | Local name | Lat Long | # quadrats before randomization | Type of quadrats |
|---|---|---|---|---|---|
| Vent 1 | Shallow Reef | Castello South | 40.7294708, 13.9674123 | 12 | visual census |
| Ambient pH, A1a | Shallow Reef | Castello South | 40.7300049, 13.9638274 | 12 | visual census |
| Ambient pH, A1b | Shallow Reef | Castello North | 40.7309932, 13.9677863 | 12 | visual census |
| Vent 2 | Cave | Grotta Mago | 40.7111786, 13.9640574 | 48 | photo quadrats |
| Ambient pH, A2a | Cave | Punta Vico | 40.7592784, 13.883754 | 54 | photo quadrats |
| Ambient pH, A2b | Cave | Punta Monaci | 40.7585214, 14.0324696 | 48 | photo quadrats |
| Vent 3 | Reef | Chiane del Lume | 40.7178141, 13.9666845 | 12 | visual census |
| Ambient pH, A3a | Reef | Sant Anna | 40.7253186, 13.962756 | 12 | visual census |
| Ambient pH, A3b | Reef | Santuario | 40.7518131, 13.9019176 | 12 | visual census |
| Vent 4 | Deep Reef | Madonnina | 40.72151667, 13.98108333 | 24 | photo quadrats |
| Ambient pH, A4a | Deep Reef | Catena | 40.71833333, 13.99216667 | 24 | photo quadrats |
| Ambient pH, A4b | Deep Reef | Pertuso | 40.73613889, 13.95769444 | 24 | photo quadrats |

**Table S5. Description of the 7 traits used to measure trait diversity of benthic species.** See (Teixidó et al., 2018) for a detailed description of the biological traits.  N= number of categories. Genera and species in parenthesis are exhaustive examples of benthic organisms but not all of them are necessarily found at the study sites.

| **Trait** | **Trait type** | **N** | **Categories** |
|---|---|---|---|
| 1) Morphological form | Categorical | 4 | a) Boring, encrusting, encrusting leaf-like, with blades<br>Examples: *Cliona, Palmophyllum, Mesophyllum, Crambe , Spirastrella, Peyssonnelia*.<br>b) Filaments; sheets, cylinders or blades, divided or not<br>Examples: *Ceramium, Caulerpa, Halimeda, Flabellia, Dictyota, Ulva, Padina, Gelidium, Corallina, Haliptilon, Jania*<br>c) Massive forms<br>Examples: *Leptopsammia, Caryophyllia, Chondrosia, Petrosia, Codium bursa, Colpomenia, Agelas oroides, Ircinia oros, Halocynthia*<br>d) Tree-like<br>Examples: *Sargassum, Cystoseira, Laminaria, Axinella polypoides, Paramuricea, Myriapora truncata* |
| 2) Feeding | Categorical | 5 | a) No, autotroph<br>Examples: algae<br>b) Filter feeders (active and passive filter feeders)<br>Examples: sponges, cnidarians bryozoans, sabellids, bivalves, tunicates<br>c) Herbivores/Grazers<br>Examples: *Arbacia, Paracentrotus*; *Patella*)<br>d) Carnivores<br>Examples: *Echinaster, Marthasterias, Stramonita, Hexaplex*<br>e) Detritivores<br>Examples: *Holothuria forskali*<br>f) Parasites |
| 3) Growth rates | Ordinal | 3 | 1)  Extreme low (<1 cm/year) and low (1 cm/ year) growth rates.<br>Examples: *Corallium rubrum, Cladocora, Ircina oros, Paramuricea, Lithophyllum stictaeforme*<br>2) Moderate growth rates (1-5 cm/year).<br>Examples: *Myriapora, Astroides*<br>3) High (5-10 cm/year) and very high (>10 cm/year) growth rates.<br>Examples: *Padina, Dictyota, Ericaria balearica, Ulva, Cladophora vagabunda* |

| | | | |
|---|---|---|---|
| 4) Calcification | Categorical | 2 | a) Without calcareous structures<br>Examples: *Ericaria, Sargassum*, *Ulva*, demosponges<br>b) With calcareous structures<br>Examples: *Padina, Halimeda, Haliptilon, Corallina,* calcareous sponges, gorgonians, corals, calcareous bryozoans |
| 5) Mobility | Ordinal | 2 | 1) Sessile<br>Examples: algae and most invertebrates<br>2) Vagile<br>Examples: *Paracentrotus lividus* |
| 6) Age of reproductive maturity | Ordinal | 2 | 1) < 1 year<br>Examples: *Ulva* spp., *Cystoseira compressa, Halimeda*<br>2) > 1 year<br>Examples: *Gongolaria spinosa*, *Corallium rubrum* |
| 7) Chemical defenses | Ordinal | 2 | 1) No<br>Examples: *Ulva* spp.<br>2) Yes<br>Examples: *Asparagopsis* spp., *Crambe* |

**Table S6. Selected functional trait categories of benthic species and their relation to ecosystem functions.**

| Trait | Category | Key Functions |
|---|---|---|
| 1. Feeding | 1. Autotroph | Determines primary production, trophic interactions, benthic-pelagic coupling, nutrient cycling and energy transfer in food webs (Gili & Coma, 1998; Gómez-Gras et al., 2021). |
| 1. Feeding | 2. Filter feeder | Determines trophic interactions, benthic-pelagic coupling, nutrient cycling and energy transfer in food webs (Gili & Coma, 1998; Gómez-Gras et al., 2021). |
| 1. Feeding | 3. Herbivore/Grazer | Determines the transfer of primary production to higher trophic levels and affects the physical structure and productivity (Poore et al., 2012). |
| 2. Morphological form | 4. Massive<br>5. Tree-like<br><br>*Habitat-forming species* | Determine much of the community structure and the diversity of communities (with their structural support and size) through non-trophic interactions of associated organisms (Dayton P.K, 1972; Ellison, 2019). They provide micro-habitats for the settlement and shelter of co-occurring species, and create habitat provision and structural complexity (Gómez-Gras et al., 2021).<br><br>Sustain biodiversity and influence the ability to withstand disturbance (Loya et al., 2001).<br><br>Modulate fluxes of energy and nutrient flow through the system, including water flow circulation, food and sediment retention (Baiser et al., 2013). They also play key roles in the benthic-pelagic coupling (Gili & Coma, 1998). |
| 3. Calcification | 6. Presence calcareous structures in calcifying species | Production of calcium carbonate ($CaCO_3$) by calcifying species and influences the chemistry of the ocean (Berelson et al., 2007). |

**Table S7. Differences in extreme events at the $CO_2$ vent systems and reference areas with ambient pH.** Extreme events (EV) was defined as a pH value of 0.4 units less than the mean pH for each pH condition. pH conditions: Vent system (1, 2, 3, 4); Ambient pH. Data from shallow reefs are originally reported in (Kroeker et al., 2011).

| pH condition | Habitat | Number EV | Mean duration (h) | Total hours | Longest duration (h) | Mean pH of EV | Time series (days) | N |
|---|---|---|---|---|---|---|---|---|
| Vent 1<br>mean $pH_T$ = 7.8<br>EV $pH_T$ < 7.4 | Shallow Reef | 17 | 2.4 | 41 | 10 | 7.0 | 33 | 840 |
| Ambient pH, A1a<br>mean $pH_T$ = 8.1<br>EV $pH_T$ < 7.7 | Shallow Reef | 2 | 1 | 2 | 1 | 7.3 | 33 | 792 |
| Ambient pH, A1b<br>mean $pH_T$ = 8.0<br>EV $pH_T$ < 7.6 | Shallow Reef | - | - | - | - | - | 25 | 607 |
| Vent 2<br>mean $pH_T$ = 7.7<br>EV $pH_T$ < 7.3 | Cave | 20 | 0.5 | 10 | 1.5 | 7.0 | 19 | 1841 |
| Ambient pH, A2a<br>mean $pH_T$ = 8.0<br>EV $pH_T$< 7.6 | Cave | - | - | - | - | - | 14 | 1331 |
| Vent 3<br>mean $pH_T$ = 7.9<br>EV $pH_T$< 7.5 | Reef | 4 | 0.7 | 2.8 | 2 | 7.3 | 14 | 1326 |
| Ambient pH, A3a<br>mean $pH_T$ = 7.9<br>EV $pH_T$< 7.5 | Reef | - | - | - | - | - | 18 | 1691 |
| Vent 4<br>mean $pH_T$= 7.9<br>EV $pH_T$ < 7.5 | Deep Reef | 2 | 0.25 | 0.5 | 0.25 | - | 28 | 2692 |
| Ambient pH, A4a<br>mean $pH_T$ = 8.0<br>EV $pH_T$< 7.6 | Deep Reef | - | - | - | - | - | 19 | 1825 |

**Table S8. Mean and standard deviation of species and trait diversity indices based on trait values of FEs across habitats and among pH zones.** Species richness (SR), FE richness (FEs), and Functional dispersion (FDis). N= 12 quadrats for each habitat and pH conditions.

| **Habitat and pH condition** | **SR** | **FEs** | **FDis** |
|---|---|---|---|
| Shallow Reef Ambient pH | 15 ± 0.8 | 12 ± 0.5 | 0.50 ± 0.010 |
| Shallow Reef $CO_2$ vent | 14.5 ± 0.9 | 9.8 ± 0.6 | 0.48 ± 0.021 |
| Cave Ambient pH | 12.3 ± 0.3 | 10.6 ± 0.2 | 0.52 ± 0.007 |
| Cave $CO_2$ vent | 5.5 ± 0.2 | 5.3 ± 0.2 | 0.41 ± 0.013 |
| Reef Ambient pH | 14.5 ± 0.7 | 11 ± 0.4 | 0.55 ± 0.013 |
| Reef $CO_2$ vent | 17.9 ± 0.9 | 12.6 ± 0.8 | 0.51 ± 0.017 |
| Deep Reef Ambient pH | 18 ± 0.6 | 15.5 ± 0.5 | 0.59 ± 0.006 |
| Deep Reef $CO_2$ vent | 12.6 ± 0.3 | 11.5 ± 0.3 | 0.55 ± 0.012 |

**Table S9. Percentage of change (losses or gains) of diversity (species and traits) and predicted benthic cover of some categories of key traits across habitats and between pH zones.** For change in biodiversity, data was obtained from the global pool of 215 benthic species and 74 functional entities (FEs) after averaging 100 randomized datasets to a unique dataset. Species Richness (SR), Functional Entity Richness (FEs), and Functional Dispersion (FDis). For change in cover, data was obtained from the Bayesian multinomial model.

| **Habitat and pH condition** | **Deep Reef** | **Reef** | **Cave** | **Shallow Reef** |
|---|---|---|---|---|
| **Change in biodiversity (%)** | | | | |
| SR | -53% | +28% | -59% | -24% |
| FEs | -43% | +15% | -44% | -27% |
| FDis | -5% | -10% | -10% | -5% |
| **Change in cover (%)** | | | | |
| Autotrophs | +39% | +2% | -7% | -2% |
| Filter feeders | -41% | -1% | -8% | +2% |
| Herbivores | no change | no change | no change | no change |
| Habitat-forming species | -27% | -3% | -7% | no change |
| Calcifiers | -2% | -26% | -37% | -14% |

## Supplementary References


Baiser, B., Whitaker, N., & Ellison, A. M. (2013). Modeling foundation species in food webs. *Ecosphere*, *4*(12), 146. https://doi.org/10.1890/ES13-00265.1

Ballesteros, E. (2006). Mediterranean coralligenous assemblages: A synthesis of present knowledge. In *Oceanography and Marine Biology: An Annual Review*, 44, pp. 123–195. https://doi.org/10.1201/9781420006391-7

Berelson, W. M., Balch, W. M., Najjar, R., Feely, R. A., Sabine, C., & Lee, K. (2007). Relating estimates of $CaCO_3$production, export, and dissolution in the water column to measurements of $CaCO_3$ rain into sediment traps and dissolution on the sea floor: A revised global carbonate budget. *Global Biogeochemical Cycles*, *21*(1), GB1024. https://doi.org/10.1029/2006GB002803

Bianchi, C. N., & Morri, C. (2000). Marine biodiversity of the Mediterranean Sea: Situation, problems and prospects for future research. *Marine Pollution Bulletin*, *40*(5), 367–376. https://doi.org/10.1016/S0025-326X(00)00027-8

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Lasram, F. B. R., Aguzzi, J., Ballesteros, E., Bianchi, C. N., Corbera, J., Dailianis, T., Danovaro, R., Estrada, M., Froglia, C., Galil, B. S., Gasol, J. M., Gertwagen, R., Gil, J., Guilhaumon, F., Kesner-Reyes, K., … Voultsiadou, E. (2010). The Biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. *PLOS ONE*, *5*(8), e11842. https://doi.org/10.1371/journal.pone.0011842

Dayton P.K. (1972). Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. In *Proceedings of the colloquium on conservation problems in Antarctica.* (B.C. Parker, pp. 81–96). Allen Press.

Dickson, A. G. (1990). Standard potential of the reaction: AgCl(s) + 12H2(g) = Ag(s) + HCl(aq), and and the standard acidity constant of the ion $HSO_4^-$ in synthetic sea water from 273.15 to 318.15 K. *The Journal of Chemical Thermodynamics*, *22*(2), 113–127. https://doi.org/10.1016/0021-9614(90)90074-Z

Dickson, A. G., & Millero, F. J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research Part A. Oceanographic Research Papers*, *34*(10), 1733–1743. https://doi.org/10.1016/0198-0149(87)90021-5

Dickson, A. G., Sabine, C. L., Christian, J. R., Bargeron, C. P., & North Pacific Marine Science Organization (Eds.). (2007). *Guide to best practices for ocean CO2 measurements*. North Pacific Marine Science Organization.

Ellison, A. M. (2019). Foundation species, non-trophic interactions, and the value of being common. *IScience*, *13*, 254–268. https://doi.org/10.1016/j.isci.2019.02.020

Foo, S. A., Byrne, M., & Gambi, M. C. (2018). The carbon dioxide vents of Ischia, Italy, a natural system to assess impacts of ocean acidification on marine ecosystems: An overview of research and comparisons with other vent systems. *Oceanography and Marine Biology: An Annual Review*, *56*, 237–310.

Gattuso, J.-P., Epitalon, J.-M., Lavigne, H., Orr, J., Gentili, B., Hagens, M., Hofmann, A., Mueller, J.-D., Proye, A., Rae, J., & Soetaert, K. (2023). *seacarb: Seawater Carbonate Chemistry. R package version 3.3.2* (3.3.2) [Computer software]. https://cran.r-project.org/web/packages/seacarb/index.html

Gili, J.-M., & Coma, R. (1998). Benthic suspension feeders: Their paramount role in littoral marine food webs. *Trends in Ecology & Evolution*, *13*, 316–321. https://doi.org/10.1016/S0169-5347(98)01365-2

Gómez-Gras, D., Linares, C., Dornelas, M., Madin, J. S., Brambilla, V., Ledoux, J.-B., López-Sendino, P., Bensoussan, N., & Garrabou, J. (2021). Climate change transforms the functional identity of Mediterranean coralligenous assemblages. *Ecology Letters*, *24*(5), 1038–1051. https://doi.org/10.1111/ele.13718

Hall-Spencer, J. M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Maoz Fine, Fine, M., Maoz Fine, Maoz Fine, Turner, S. M., Rowley, S. J., Tedesco, D., & Buia, M. C. (2008). Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature*, *454*(7200), 96–99. https://doi.org/10.1038/nature07051

Hansen, H. P., & Koroleff, F. (1999). Determination of nutrients. In *Methods of Seawater Analysis* (pp. 159–228). John Wiley & Sons, Ltd. https://doi.org/10.1002/9783527613984.ch10

Kapsenberg, L., Alliouane, S., Gazeau, F., Mousseau, L., & Gattuso, J.-P. (2017). Coastal ocean acidification and increasing total alkalinity in the northwestern Mediterranean Sea. *Ocean Science*, *13*(3), 411–426. https://doi.org/10.5194/os-13-411-2017

Kroeker, K. J., Micheli, F., Gambi, M. C., & Martz, T. R. (2011). Divergent ecosystem responses within a benthic marine community to ocean acidification. *Proceedings of the National Academy of Sciences*, *108*(35), 14515–14520. https://doi.org/10.1073/pnas.1107789108

Kwiatkowski, L., Torres, O., Bopp, L., Aumont, O., Chamberlain, M., Christian, J. R., Dunne, J. P., Gehlen, M., Ilyina, T., John, J. G., Lenton, A., Li, H., Lovenduski, N. S., Orr, J. C., Palmieri, J., Santana-Falcón, Y., Schwinger, J., Séférian, R., Stock, C. A., … Ziehn, T. (2020). Twenty-first century ocean warming, acidification, deoxygenation, and upper-ocean nutrient and primary production decline from CMIP6 model projections. *Biogeosciences*, *17*(13), 3439–3470. https://doi.org/10.5194/bg-17-3439-2020

Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H., & van Woesik, R. (2001). Coral bleaching: The winners and the losers. *Ecology Letters*, *4*(2), 122–131. https://doi.org/10.1046/j.1461-0248.2001.00203.x

Poore, A. G. B., Campbell, A. H., Coleman, R. A., Edgar, G. J., Jormalainen, V., Reynolds, P. L., Sotka, E. E., Stachowicz, J. J., Taylor, R. B., Vanderklift, M. A., & Duffy, J. E. (2012). Global patterns in the impact of marine herbivores on benthic primary producers. *Ecology Letters*, *15*(8), 912–922.

Teixidó, N., Caroselli, E., Alliouane, S., Ceccarelli, C., Comeau, S., Gattuso, J.-P., Fici, P., Micheli, F., Mirasole, A., Monismith, S. G., Munari, M., Palumbi, S. R., Sheets, E., Urbini, L., De Vittor, C., Goffredo, S., & Gambi, M. C. (2020). Ocean acidification causes variable trait-shifts in a coral species. *Global Change Biology*, *26*(12), 6813–6830. https://doi.org/10.1111/gcb.15372

Teixidó, N., Gambi, M. C., Parravacini, V., Kroeker, K., Micheli, F., Villéger, S., & Ballesteros, E. (2018). Functional biodiversity loss along natural $CO_2$ gradients. *Nature Communications*, *9*(1), 5149. https://doi.org/10.1038/s41467-018-07592-1

Uppström, L. R. (1974). The boron/chlorinity ratio of deep-sea water from the Pacific Ocean. *Deep Sea Research and Oceanographic Abstracts*, *21*(2), 161–162. https://doi.org/10.1016/0011-7471(74)90074-6

**Data S1.** Raw data of percent cover of benthic species obtained from field surveys. Each quadrat 25*25 cm.

**Data S2.** Species (n=215) and codes of the seven traits used to measure trait diversity.

**Data S3.** Outputs from the Bayesian model of the likelihood of benthic cover to belong to a trait category across habitats and between pH zones.