**Supplementary Information for**

High taxonomic diversity and miniaturization in benthic communities under persistent natural CO2 disturbances

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**This PDF file includes:**

Materials and Methods

Tables S1 to S7

Figure S1 and S2

Legends for Datasets S1 and S2

**Other supplementary materials for this manuscript include the following:**

Datasets S1 and S2

**Materials and Methods**

**Description of the experimental area.** The Punta de Fuencaliente CO2 vent system creates a natural pH gradient from the emission point, which is characterized by unique physico-chemical features that allow the study of OA effects, such as shallow waters and the absence of pollutants and bubbling (Gonzalez-Delgado et al., 2021).

Water samples stored in borosilicate bottles were used to measured pHNBS with a pH meter (Metrohm mobile meter with a combined electrode: Primatrode NTC IP pH electrode and temperature sensor). The pH meter was calibrated using a three-point calibration program against NIST buffer solutions (pH 4, 7 and 9 +- 0.02). Seawater total alkalinity (TA) was measured using an open cell potentiometric titration with a Metrohm Dosimat 665 titrator using 0.1 N HCl with a salinity of about 35 and following the Standard Operation Procedure 3b (Elbrecht et al., 2017). The rest of the carbonate chemistry parameters were calculated salinity, temperature, TA and pH using the package SEACARB 3.08 for R (<https://cran.r-project.org/web/packages/seacarb/>). Calculations were based on a set of constants, K1 and K2, taken from Lueker et al. (2000).

**Sampling and DNA extraction.** The six scrapes were collected by SCUBA diving at depths between 3- and 5-meters. Subsequently, the samples (a total of 24) were placed plastic containers with 96% ethanol until being processed in the laboratory. The samples were sieved into two size fractions (>1 mm and 63 µm – 1 mm). Each fraction was separately homogenized with a 600 W kitchen blender and stored at −20 °C in absolute ethanol until DNA extraction. All equipment was thoroughly washed and cleaned with diluted sodium hypochlorite between the collection of successive samples or fractions.

**Bioinformatics analyses.** To obtain a robust diversity dataset, we used the Multiple Intersection of N Tags (MINT)-all strategy, through which MOTUs that had not been detected in three PCR replicates were removed on a sample-by-sample basis. This strict strategy ensures that spurious MOTUs arising from possible cross-contamination or tag switching are removed from downstream analyses, providing a robust estimate of the diversity present in the samples.

**Table S1.** Number of Rhodophyta (R), Ochrophyta (O), Chorophyta (C), total algae (TA) and Metazoan (M) MOTUs found at different CO2 vent systems which were sampled with traditional methods; and the number of species found in the present work using metabarcoding.

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **CO2 Vents** | **Methods** | **R** | **O** | **C** | **TA** | **M** | **Sources** |
| La Palma (Spain) | COI Metabarcoding | 143 | 43 | - | 186 | 636 | Present study |
| Columbretes (Spain) | Traditional | 47 | 22 | 10 | 79 | 33 | (1)\* |
| Ischia (Italy) | Traditional | 71 | 16 | 15 | 101 | 82 | (2)\* |
| Methana (Greek) | Traditional | 7 | 9 | 2 | 18 | - | (3)\* |

\*(1) C. Linares *et al.,* Persistent natural acidification drives major distribution shifts in marine benthic ecosystems. *Proc. R. Soc. B Biol. Sci.* 282 (2015). <https://doi.org/10.1098/rspb.2015.0587>; (2) K.J. Kroeker, F. Micheli, M.C. Gambi, T.R. Martz, Divergent ecosystem responses within a benthic marine community to ocean acidification. *Proc. Natl. Acad. Sci.,* 108(35), 14515-14520 (2011); (3) C. Baggini et al., Seasonality affects macroalgal community response to increases in pCO2*. PLoS One* 9, e106520 (2014). https://doi.org/10.1371/journal.pone.0106520.

Table S2. (A) Kruskal-Wallis test for all MOTUs, algae and its subgroups and metazoans and its subgroup species richness (number of species per sample) in the natural CO2 gradient as factor ‘CO2 Gradient’ with 4 levels (‘1-Vent’, ‘2-Transition25’, ‘3-Transition75’ and ‘4-Control’) in both fraction size (small fraction: organisms with size > 1 mm; and large fraction: organisms between 1 mm and 0.64 µm). (B) Results of medians comparisons between levels of the factor ‘CO2 Gradient’ of significant Kruskal-Wallis tests.

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| --- | --- | --- |
| **(A) Kruskal-Wallis** | **Large fraction (A)** | **Small Fraction (B)** |
|  | ***df*** | ***Chi-squared*** | ***p*** | ***Chi-squared*** | ***p*** |
| **All MOTUs** | 3 | 9.190 | 0.03\* | 6.828 | 0.05\* |
| **Algae** | 3 | 6.797 | 0.08 | 5.78 | 0.12 |
| **Metazoans** | 3 | 7.565 | 0.06 | 10.853 | 0.01\* |
| **Crustose** | 3 | 5.847 | 0.12 | 11.484 | 0.01\* |
| **Epiphyte** | 3 | 10.576 | 0.01\* | 5.270 | 0.15 |
| **Mixed turf** | 3 | 4.811 | 0.19 | 5.797 | 0.12 |
| **Erected Macrophyte** | 3 | 2.837 | 0.42 | 8.133 | 0.04\* |
| **Macrofauna** | 3 | 7.661 | 0.05\* | 9.237 | 0.03\* |
| **Meiofauna** | 3 | 10.846 | 0.01\* | 9.179 | 0.03\* |
| **(B) Medians comparisons** | **1 - 2** | **1 - 3** | **1 - 4** | **2 - 3** | **2 - 4** | **3 - 4** |
|  | **All MOTUs** | **A** | **t** | 0.285 | 1.899 | 2.553 | 1.613 | 2.267 | 0.653 |
|  | **p** | 0.387 | 0.057 | 0.032\* | 0.080 | 0.035\* | 0.308 |
| **B** | **t** | -2.511 | -0.735 | -0.755 | 1.776 | 1.755 | -0.020 |
|  | **p** | 0.036\* | 0.277 | 0.337 | 0.113 | 0.079 | 0.491 |
|  | **Metazoans** | **B** | **t** | -3.184 | -0.898 | -1.143 | 2.286 | 2.041 | -0.245 |
|  | **p** | 0.004\* | 0.221 | 0.190 | 0.033\* | 0.041\* | 0.403 |
|  | **Crustose** | **A** | **t** | -2.547 | -3.065 | -1.181 | -0.517 | 1.366 | 1.884 |
|  | **p** | 0.016\* | 0.006\* | 0.142 | 0.302 | 0.128 | 0.059 |
|  | **Epiphyte** | **A** | **t** | 1.593 | 2.738 | 2.860 | 1.144 | 1.266 | 0.122 |
|  | **p** | 0.111 | 0.009\* | 0.012\* | 0.151 | 0.153 | 0.451 |
|  | **Erected Macrophytes** | **B** | **t** | -2.688 | -0.554 | -0.862 | 2.134 | 1.826 | -0.307 |
|  | **p** | 0.021\* | 0.347 | 0.291 | 0.049\* | 0.067 | 0.379 |
|  | **Macrofauna** | **A** | **t** | -1.474 | -0.143 | 1.289 | 1.330 | 2.764 | 1.433 |
|  | **p** | 0.211 | 0.443 | 0.118 | 0.137 | 0.017\* | 0.151 |
| **B** | **t** | -2.705 | -2.475 | -1.188 | 0.286 | 1.516 | 1.229 |
|  | **p** | 0.021\* | 0.023\* | 0.141 | 0.387 | 0.129 | 0.164 |
|  | **Meiofauna** | **A** | **t** | 0.266 | 2.092 | 2.728 | 1.825 | 2.461 | 0.635 |
|  | **p** | 0.394 | 0.036\* | 0.019\* | 0.051 | 0.021\* | 0.314 |
| **B** | **t** | -0.926 | 1.502 | 1.646 | 2.428 | 2.572 | 0.144 |
|  | **p** | 0.212 | 0.099 | 0.099 | 0.022\* | 0.030\* | 0.442 |

Table S3. (A) One-way permutational multivariate analyses of variance of all algae species data set in the natural CO2 gradient as factor ‘CO2 Gradient’ with 4 levels (‘1-Vent’, ‘2-Transition25’, ‘3-Transition75’ and ‘4-Control’), and separated in 4 groups of vegetation (‘crustose’ (crus), ‘epiphyte’ (Epi), ‘mixed turf’ (Mtu) and ‘erected macrophytes’ (Erec) and two size fractions (small fraction: organisms with size > 1 mm; and large fraction: organisms between 1 mm and 0.64 µm). (B) Results of pairwise comparisons for levels of the factor ‘CO2 Gradient’.

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| **(A) Source of variationFuente Variación** |  | **LARGE FRACTION** | **SMALL FRACTION** |
| **df** | **SSSS** | **MSMS** | **Pseudo-FPseudo-F** | **p (perm)p** | **SSSS** | **MSMS** | **Pseudo-FPseudo-F** | **p (perm)p** |
| **Crus** | Gradient | 3 | 2.495 | 0.832 | 8.2913 | 0.001\* | 1.7931 | 0.598 | 4.156 | 0.001\* |
|  | Residual | 2020 | 2.006 | 0.100 |  |  | 2.8766 | 0.144 |  |  |
| **Epi** | Gradient | 3 | 1.492 | 0.497 | 4.887 | 0.001\* | 1.381 | 0.460 | 4.095 | 0.001\* |
|  | Residual | 2020 | 2.036 | 0.102 |  |  | 2.248 | 0.112 |  |  |
| **Mtu** | Gradient | 3 | 1.906 | 0.635 | 10.661 | 0.001\* | 1.679 | 0.560 | 6.154 | 0.001\* |
|  | Residual | 2020 | 1.192 | 0.060 |  |  | 1.819 | 0.091 |  |  |
| **Erec** | Gradient | 3 | 0.856 | 0.285 | 6.172 | 0.001\* | 0.876 | 0.292 | 5.163 | 0.001\* |
|  | Residual | 2020 | 0.924 | 0.046 |  |  | 1.131 | 0.057 |  |  |
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| **(B)   Groups** | **1 - 2** | **1 - 3** | **1 - 4** | **2 - 3** | **2 - 4** | **3 - 4** |
| **Crus** | Large fraction | **t** | 5.097 | 4.508 | 7.535 | 0.641 | 4.499 | 2.955 |
| **p** | 0.005\* | 0.005\* | 0.005\* | 0.703 | 0.005\* | 0.011\* |
|  | Small Fraction | **t** | 2.512 | 3.477 | 3.843 | 0.452 | 1.286 | 1.394 |
| **p** | 0.061 | 0.010\* | 0.028\* | 0.908 | 0.350 | 0.331 |
| **Epi** | Large fraction | **t** | 6.133 | 4.940 | 4.310 | 3.495 | 1.837 | 2.139 |
|  | **p** | 0.004\* | 0.004\* | 0.004\* | 0.004\* | 0.058 | 0.051 |
|  | Small Fraction | **t** | 2.738 | 3.395 | 2.758 | 2.336 | 2.160 | 1.790 |
|  | **p** | 0.005\* | 0.007\* | 0.005\* | 0.009\* | 0.005\* | 0.030\* |
| **Mtu** | Large fraction | **t** | 9.442 | 14.065 | 10.154 | 4.198 | 4.292 | 2.252 |
|  | **p** | 0.005\* | 0.005\* | 0.005\* | 0.008\* | 0.005\* | 0.020\* |
|  | Small Fraction | **t** | 5.391 | 8.363 | 5.587 | 3.065 | 3.182 | 1.473 |
|  | **p** | 0.004\* | 0.004\* | 0.006\* | 0.012\* | 0.004\* | 0.092 |
| **Erec** | Large fraction | **t** | 24.133 | 9.987 | 26.938 | 1.419 | 1.124 | 1.292 |
|  | **p** | 0.007\* | 0.007\* | 0.007\* | 0.294 | 0.294 | 0.294 |
|  | Small Fraction | **t** | 8.787 | 7.430 | 13.634 | 5.117 | 2.096 | 11.558 |
|  | **p** | 0.005\* | 0.005\* | 0.005\* | 0.018\* | 0.094 | 0.006\* |

Table S4. Result of SIMPER analyses showing the differences in average transformed abundances and contribution percentage (‘Contribution %’) of algae species of (A) Crustose, (B) Epiphyte, (C) Mixed Turf and (D) Erected macrophytes, compared the ‘CO2 Gradient’ (‘1-Vent’, ‘2-Transition25’, ‘3-Transition75’ and ‘4-Control’). Large fraction are organisms with size > 1mm and small fraction are organisms between 1mm and 0.64 µm.

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| **AVERAGE ABUNDANCES** | **CONTRIBUTION %** |
| **(A) Crustose**  | **1** | **2** | **3** | **4** | **1 & 2** | **1 & 3** | **1 & 4** | **2 & 3** | **2 & 4** | **3 & 4** |
| **Large fraction** |  |  |  |  |  |  |
| *Ralfsia* sp. | 68.12 | 0.00 | 0.00 | 0.00 | 13.54 | 13.68 | 15.74 | - | - | - |
| *Phymatolithon* sp. 2 | 9.90 | 68.84 | 50.69 | 0.00 | 12.17 | 10.28 | - | - | 18.09 | 14.29 |
| *Lithophylum* sp. 1  | 0.00 | 49.54 | 55.97 | 0.00 | 10.44 | 10.97 | - | - | 13.58 | 14.18 |
| *Peyssonnelia* sp. 3 | 53.74 | 39.82 | 16.19 | 54.17 | 8.60 | 10.32 | 9.80 | - | 11.38 | 13.56 |
| *Peyssonnelia* sp. 7 | 20.17 | 45.56 | 20.38 | 28.57 | 7.87 | 5.25 | 6.96 | - | 8.36 | 6.06 |
| *Lithophylum* sp. 2 | 0.00 | 39.18 | 42.05 | 70.26 | 7.38 | 8.05 | 16.42 | - | 10.16 | 9.34 |
| *Pneophyllum* sp. | 0.00 | 35.63 | 38.90 | 49.01 | 7.00 | 7.51 | 11.57 | - | 6.36 | 5.97 |
| *Peyssonnelia* sp. 1 | 31.96 | 9.68 | 5.72 | 0.00 | 6.48 | 6.57 | 7.58 | - | - | - |
| *Peyssonnelia* sp. 4 | 28.41 | 9.52 | 30.25 | 0.00 | 5.69 | 5.81 | 6.49 | - | - | 7.37 |
| *Lithothamnion* sp. | 9.97 | 29.92 | 29.04 | 2.26 | 5.56 | 5.64 | - | - | 6.80 | 6.74 |
| *Padina gymnospora* | 13.39 | - | - | 19.11 | - | - | 6.07 |  |  | - |
| *Hildenbrandia* sp. 2 | 21.80 | - | - | 0.00 | - | - | 5.00 |  |  | - |
| **Small fraction** |  |  |  |  |  |  |
| *Phymatolithon* sp. 2 | 0.00 | 50.55 | 42.42 | 8.88 | - | 11.28 | - | - | - | - |
| *Lithophylum* sp. 2 | 0.00 | 48.82 | 49.66 | 0.00 | - | 13.29 | - | - | - | - |
| *Lithothamnion* sp. | 0.00 | 51.55 | 16.74 | 6.82 | - | 3.96 | - | - | - | - |
| *Ralfsia* sp. | 51.54 | 14.80 | 12.55 | 4.94 | - | 12.68 | 17.22 | - | - | - |
| *Peyssonnelia* sp. 3 | 69.05 | 42.84 | 38.29 | 41.38 | - | 12.27 | 17.04 | - | - | - |
| *Lithophylum* sp. 1 | 23.16 | 34.17 | 63.00 | 79.31 | - | 13.09 | 21.91 | - | - | - |
| *Pneophyllum* sp. | 0.00 | 43.27 | 56.59 | 58.12 | - | 15.06 | 18.32 | - | - | - |
| *Peyssonnelia* sp. 7 | 12.18 | - | 20.08 | 0.00 | - | 6.49 | - | - | - | - |
| *Padina gymnospora* | 6.14 | - | 0.00 | 17.05 | - | - | 5.43 | - | - | - |
| **(B) Epiphyte**  | **1** | **2** | **3** | **4** | **1 & 2** | **1 & 3** | **1 & 4** | **2 & 3** | **2 & 4** | **3 & 4** |
| **Large fraction** |  |  |  |  |  |  |
| *Ceramium virgatum* | 56.92 | 9.14 | 0.00 | 0.00 | 6.30 | 7.21 | 6.86 | - | - | - |
| *Platysiphonia* sp. | 18.69 | 50.75 | 19.75 | - | 5.31 | - | - | 7.39 | - | - |
| Kallymeniaceae sp. 2 | 8.37 | - | 63.45 | 51.62 | - | 7.14 | 5.34 | - | - | - |
| *Heterosiphonia* sp. 1 | 22.63 | 32.30 | 53.15 |  | - | 5.05 | - | 6.38 | - | - |
| *Centroceras* sp.1 | - | 63.98 | 33.52 | - | - | - | - | 5.68 | - | - |
| *Dasya* sp. 2 | - | 31.44 | 11.02 | - | - | - | - | 5.19 | - | - |
| **Small fraction** |  |  |  |  |  |  |
| *Ceramium diaphanum* | 10.50 | 59.44 | 10.13 | 22.68 | 6.45 | - | - | 7.87 | 5.38 | - |
| *Ceramium virgatum* | 44.70 | 0.00 | 2.33 | 0.00 | 5.77 | 7.90 | 5.76 | - | - | - |
| *Platysiphonia* sp. | 8.17 | 43.90 | 12.37 | 9.20 | 5.05 | - | - | 6.92 | 5.32 | - |
| Kallymeniaceae sp. 2 | 11.32 | 47.24 | 67.05 | - | 4.64 | 7.81 | - | - | - | - |
| *Heterosiphonia* sp. 1 | - | 22.66 | 38.80 | 2.36 | - | - | - | 5.54 | - | 6.82 |
| *Dasya* sp. 6 | - | - | 3.56 | 32.79 | - | - | - | - | - | 5.98 |
| *Centroceras* sp.1 | - | - | 30.09 | 48.66 | - | - | - | - | - | 5.28 |
| **(C) Mixed Turf**  | **1** | **2** | **3** | **4** | **1 & 2** | **1 & 3** | **1 & 4** | **2 & 3** | **2 & 4** | **3 & 4** |
| **Large fraction** |  |  |  |  |  |  |
| *Palisada flagellifera* | 9.29 | 59.54 | 14.12 | 0.00 | 6.85 | - | - | 8.70 | 8.21 | - |
| *Laurencia obtusa* | 85.81 | 45.29 | 34.94 | 32.39 | 5.22 | 6.29 | 6.09 | - | - | - |
| *Jania* sp. 2 | 1.01 | 42.80 | 61.54 | 55.07 | 5.21 | 6.97 | 6.14 | - | - | - |
| *Jania* sp. 4 | 2.96 | - | 61.54 | 48.84 | - | 7.34 | 5.25 | - | - | - |
| *Corallina* sp. | 0.00 | - | 57.80 | 49.86 | - | 7.15 | 5.63 | - | - | - |
| *Gelidiella* sp. 2 | 0.00 | 2.93 | 3.75 | 46.68 | - | - | 5.36 | - | 6.01 | 6.97 |
| *Palisada* sp. 2 | - | 44.44 | 15.05 | 5.88 | - | - | - | 5.93 | 5.65 | - |
| *Hypnea* sp. | - | 37.66 | 0.00 | 0.00 | - | - | - | 5.89 | 5.00 | - |
| *Jania* sp. 3  | - | 53.54 | 58.30 | 0.00 | - | - | - | - | 7.24 | 9.25 |
| *Ceratodictyon* sp. | - | - | 14.74 | 55.22 | - | - | - | - | - | 6.56 |
| **Small fraction** |  |  |  |  |  |  |
| *Jania* sp. 2 | 13.62 | 66.86 | 67.88 | 3.89 | 7.39 | 7.34 | - | - | 9.07 | - |
| *Jania* sp. 4 | 2.16 | 50.30 | 68.14 | 59.51 | 6.55 | 8.71 | 7.61 | - | - | - |
| *Jania* sp. 5 | 7.58 | 47.57 | 65.26 | 48.11 | 5.47 | 7.59 | 5.28 | - | - | - |
| *Laurencia obtusa* | 76.11 | 48.54 | 26.29 | 23.34 | - | 6.76 | 6.88 | 5.73 | - | - |
| *Palisada* sp. 2 | - | 38.56 | 8.86 | - | - | - | - | 6.48 | - | - |
| *Chondrophycus* sp. | - | 61.86 | 36.79 | 10.59 | - | - | - | 6.31 | 7.25 | - |
| *Amphiroa* sp. 2 | - | 35.59 | 11.13 | 23.09 | - | - | - | 5.00 | 5.01 | - |
| *Ceratodictyon* sp. | - | 9.53 | - | 51.40 | - | - | - | - | 6.04 | - |
| **(D) Erected macrophytes** | **1** | **2** | **3** | **4** | **1 & 2** | **1 & 3** | **1 & 4** | **2 & 3** | **2 & 4** | **3 & 4** |
| **Large fraction** |  |  |  |  |  |  |
| *Halopteris* sp. | 85.02 | 28.77 | 21.69 | 0.00 | 19.45 | 18.43 | 24.68 | - | - | - |
| *Canistrocarpus cervicornis* | 48.14 | 95.69 | 87.07 | 96.40 | 16.35 | 11.63 | 14.12 | - | - | - |
| *Dictyota fasciola* | 52.66 | 24.43 | 27.21 | 25.46 | 9.89 | 7.46 | 8.66 | - | - | - |
| *Dictyota ciliolata* | 0.00 | 22.47 | 26.66 | 22.52 | 7.41 | 7.64 | 6.29 | - | - | - |
| *Dictyota* sp .4 | 37.71 | 37.31 | 25.54 | 9.52 | 6.62 | 6.51 | 8.20 | - | - | - |
| *Taonia* sp. | 19.97 | 0.00 | 0.00 | 0.00 | 6.50 | 5.52 | 5.52 | - | - | - |
| *Asparagopsis taxiformis* | 55.67 | 40.35 | - | 40.09 | 5.86 | - | 6.56 | - | - | - |
| *Dictyota ciliolata* 2 | 31.24 | 23.01 | 36.05 | 41.90 | 5.35 | 6.99 | 5.88 | - | - | - |
| *Sargassum* sp. | 5.19 | - | 28.07 | - | - | 6.54 | - | - | - | - |
| Lobophora canariensis | 2.02 | - | 21.42 | - | - | 5.63 | - | - | - | - |
| *Taonia atomaria* | 25.03 | - | 8.70 | - | - | 5.37 | - | - | - | - |
| **Small fraction** |  |  |  |  |  |  |
| *Halopteris* sp. | 72.08 | 25.82 | 19.90 | 4.72 | 13.38 | 16.01 | 18.73 | 5.83 | - | 7.46 |
| *Canistrocarpus cervicornis* | 37.17 | 81.99 | 66.04 | 92.34 | 13.02 | 9.19 | 15.28 | 7.82 | - | 11.52 |
| *Dictyota* sp. 4 | 25.93 | 44.37 | 18.81 | 12.89 | 10.62 | 8.87 | 7.22 | 12.94 | - | 8.65 |
| *Dictyota fasciola* | 58.62 | 25.32 | 25.05 | 27.91 | 9.89 | 11.63 | 9.39 | 7.51 | - | 8.76 |
| *Dictyota ciliolata* | 2.22 | 24.24 | 17.93 | 17.57 | 6.54 | 5.08 | - | 7.89 | - | 7.42 |
| *Asparagopsis taxiformis* | 66.96 | 71.23 | 89.51 | 57.16 | 6.34 | 7.42 | 7.52 | 7.66 |  | 13.78 |
| *Taonia* sp. | 21.88 | 2.71 | 0.00 | 2.51 | 5.94 | 6.56 | 5.70 | - | - | - |
| *Taonia atomaria* | 27.14 | 22.72 | 17.67 | 7.26 | 5.76 | 6.50 | 6.71 | 5.88 | - | 6.60 |
| *Dictyota ciliolata* 2 | 21.38 | 39.59 | 26.27 | 38.60 | 5.67 | - | 5.20 | 6.51 | - | 6.32 |
| *Sargassum* sp. | 0.00 | - | 18.52 | - | - | 5.70 | - | - | - | - |
| *Lobophora littlerorum* | - | 24.20 | 5.23 | - | - | - | - | 7.85 | - | - |
| *Dictyotalean* sp. | - | 17.42 | 9.63 | - | - | - | - | 8.78 | - | - |
| *Lobophora canariensis* | - | - | 24.85 | 20.62 | - | - | - | - | - | 5.98 |

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**Figure S1.** Non-metric multidimensional scaling (nMDS) plots overlaid with a bubble plot of the selected representative algae (with their families) that made the significant differences (PERMANOVA: p<0.05, Table S3) between levels of CO2 gradient (‘1-Vent’, ‘2-Transition25’, ‘3-Transition75’, and ‘4-Control’). These species belong to one of the four layers of vegetation (‘crustose’ species (A), ‘epiphyte’ species (B), ‘mixed turf’ species (C), and ‘erected macrophytes’ species (D). The bubble size represents the percentage of relative abundances (‘reads’) of the species in each sample. The large fraction are organisms with size > 1 mm and the small fraction are organisms between 1 mm and 0.64 µm.

Table S5. (A) One-way permutational multivariate analyses of variance of all algae species data set in the natural CO2 gradient as factor ‘CO2 Gradient’ with 4 levels (‘1-Vent’, ‘2-Transition25’, ‘3-Transition75’ and ‘4-Control’) and separated in 3 groups (‘Meiofauna’ (Meio) and ‘Macrofauna’ (Macr), and two size fractions (small fraction: organisms with size > 1 mm; and large fraction: organisms between 1 mm and 0.64 µm). (B) Results of pairwise comparisons for levels of the factor ‘CO2 Gradient’.

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| **(A) Source of variationFuente Variación** |  | **LARGE FRACTION (A)** | **SMALL FRACTION (B)** |
| **df** | **SSSS** | **MSMS** | **Pseudo-FPseudo-F** | **p (perm)p** | **SSSS** | **MSMS** | **Pseudo-FPseudo-F** | **p (perm)p** |
| **Meio** | Gradient | 3 | ﻿1.741 | ﻿0.580 | ﻿ 3.708 | 0.001\* | ﻿1.903 | ﻿﻿0.634 | ﻿﻿4.683 | 0.001\* |
|  | Residual | 2020 | 3.131  |  0.157  |  |  | ﻿﻿2.710 | ﻿﻿0.135 |  |  |
| **Macr** | Gradient | 3 | 1.956 | 0.652 | 2.896 | 0.001\* | 2.152 | 0.717 | 2.931 | 0.001\* |
|  | Residual | 2020 | 4.503 | 0.225 |  |  | 4.895 | 0.245 |  |  |
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| **(B)   Groups** | **1 - 2** | **1 - 3** | **1 - 4** | **2 - 3** | **2 - 4** | **3 - 4** |
| **Meio** | Large fraction | **t** | 6.501 | 5.101 | 4.921 | 0.838 | 2.641 | 1.359 |
| **p** | 0.002\* | 0.002\* | 0.003\* | 0.621 | 0.007\* | 0.232 |
|  | Small Fraction | **t** | 6.210 | 4.850 | 4.727 | 1.014 | 5.099 | 3.516 |
| **p** | 0.002\* | 0.002\* | 0.002\* | 0.423 | 0.002\* | 0.002\* |
| **Macr** | Large fraction | **t** | 4.494 | 2.271 | 2.677 | 1.739 | 2.095 | 1.542 |
|  | **p** | 0.003\* | 0.004\* | 0.003\* | 0.046\* | 0.011\* | 0.052 |
|  | Small Fraction | **t** | 3.628 | 2.772 | 2.944 | 1.348 | 1.690 | 1.129 |
|  | **p** | 0.003\* | 0.011\* | 0.001\* | 0.027\* | 0.002\* | 0.235 |

Table S6. Result of SIMPER analyses showing the differences in average transformed abundances and contribution percentage (‘Contribution %’) of algae species of (A) Meiofauna and (B) Macrofauna, compared the ‘CO2 Gradient’ (‘1-Vent’, ‘2-Transition25’, ‘3-Transition75’ and ‘4-Control’). Large fraction are organisms with size > 1mm and small fraction are organisms between 1mm and 0.64 µm.

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| **AVERAGE ABUNDANCES** | **CONTRIBUTION %** |
| **(A) Meiofauna** | **1** | **2** | **3** | **4** | **1 & 2** | **1 & 3** | **1 & 4** | **2 & 3** | **2 & 4** | **3 & 4** |
| **Large fraction** |  |  |  |  |  |  |
| *Lumbrineris perkinsi* | 0.00 | 13.97 | - | - | 5.67 | - | - | - | - | - |
| *Chondrochelia dubia* | 31.24 | 12.81 | - | - | 2.15 | - | - | - | - | - |
| *Caprella acanthifera* | 58.39 | 23.93 | 16.03 | 43.84 | 2.11 | 2.77 | 2.14 | - | 2.72 | - |
| *Dexamine spinosa* 4 | 25.17 | 43.76 | - | 27.50 | 1.90 | - | - | - | 2.40 | - |
| *Ampithoe ramondi* | 37.81 | 0.00 | 0.00 | - | 1.66 | 2.58 |  | - | - | - |
| *Timarete* cf. *punctata* | 80.43 | 4.61 | 12.74 | 7.81 | 1.43 | 1.42 | 1.73 | - | - | - |
| *Dorvillea* sp*.* | 0.00 | 23.37 | - | 11.44 | 1.35 | - | - | - | 5.83 | - |
| *Polynoe* sp*.*  | 43.77 | 70.87 | - | 8.13 | 1.17 | - | - | - | 2.10 | - |
| *Corophium volutator* | 11.50 | - | 0.00 | - | - | 5.57 | - | - | - | - |
| *Podocerus variegatus* | 6.55 | 29.85 | 23.57 | 40.14 | - | 1.97 | 1.73 | - | 1.17 | - |
| *Capitella* cf. *capitata* | 18.65 | - | 0.00 | 0.00 | - | 1.51 | 1.61 | - | - | - |
| *Naineris* cf*. laevigata* | 46.16 | 43.10 | 31.26 | 7.03 | - | 1.04 |  | - | 2.61 | - |
| *Ototyphlonemertes (Macintoshi) macintoshi*  | 4.93 | - | - | 10.42 | - |  | 6.45 | - | - | - |
| *Dexamine spinosa* 3 | 12.54 | - | - | 3.85 | - | - | 3.46 | - | - | - |
| *Eunice gagzoi* | 24.39 | 22.28 | - | 38.67 | - | - | 3.43 | - | 3.75 | - |
| *Amphipholis* cf. *squamata* 1 | 32.54 | 40.87 | - | 3.23 | - | - | 3.24 | - | 2.64 | - |
| *Cephalothrix simula* | 36.61 | - | - | 0.00 | - | - | 1.12 | - | - | - |
| *Amphipholis cf. squamata 3* | - | 4.31 | - | 15.34 | - | - | - | - | 3.40 | - |
| *Eurythoe cf. complanata* | - | 33.19 | - | 28.82 | - | - | - | - | 1.03 | - |
| **Small fraction** |  |  |  |  |  |  |
| *Tetrastemma cf. coronatum* | 0.00 | 18.62 | - | 17.53 | 2.44 | - | 1.85 | - | - | - |
| *Leodice harassii* | 1.41 | 11.68 | - | - | 1.69 | - | - | - | - | - |
| *Desmodora sp.* | 12.66 | 14.57 | - | - | 1.36 | - | - | - | - | - |
| *Caprella acanthifera* | 68.97 | 26.62 | 3.21 | - | 1.34 | 1.07 | - | - | - | - |
| *Cephalothrix rufifrons* | 14.98 | 52.41 | 28.26 | 19.35 | - | 2.88 | - | - | 2.01 | 1.90 |
| *Eunice gagzoi* | 8.52 | - | 11.51 | 25.81 | - | 1.96 | 1.52 | - | - | - |
| *Lumbrineris perkinsi* | 0.00 | - | 10.24 | - | - | 1.85 | - | - | - | - |
| *Timarete cf. punctata* | 69.19 | - | 4.30 | 6.75 | - | 1.79 | 3.15 | - | - | - |
| *Ampithoe ramondi* | 23.95 | - | - | 0.00 | - | - | 2.70 | - | - | - |
| *Eurythoe cf. complanata* | 8.23 | - | - | 43.08 | - | - | 2.10 | - | - | - |
| *Tetrastemma sp.* | 0.00 | 4.11 | 5.05 | 50.00 | - | - | 1.49 | - | 1.69 | 1.91 |
| *Dexamine spinosa 2* | 21.72 | 20.60 | - | 0.00 | - | - | 1.17 | - | 1.12 | - |
| *Ampithoe cf. helleri* | - | 24.60 | - | 0.00 | - | - | - | - | 4.98 | - |
| *Polynoe sp.*  | - | 79.35 | 72.85 | 15.97 | - | - | - | - | 2.75 | 3.03 |
| *Baseodiscus cf. delineatus* | - | 12.20 | - | 0.00 | - | - | - | - | 2.43 | - |
| *Ototyphlonemertes (Macintoshi) macintoshi*  | - | 13.02 | - | 28.82 | - | - | - | - | 1.54 | - |
| *Dorvillea sp.* | - | - | 18.76 | 20.12 | - | - | - | - | - | 5.25 |
| *Polycirrus denticulatus* | - | - | 11.60 | 0.00 | - | - | - | - | - | 2.86 |
| *Amphipholis cf. squamata 3* | - | - | 3.13 | 16.36 | - | - | - | - | - | 1.32 |
| **(B) Macrofauna** | **1** | **2** | **3** | **4** | **1 & 2** | **1 & 3** | **1 & 4** | **2 & 3** | **2 & 4** | **3 & 4** |
| **Large fraction** |  |  |  |  |  |  |
| *Calcinus tubularis* | 14.52 | 54.69 | - | - | 2.89 | - | - | - | - | - |
| *Hippolyte cf. leptocerus* | 31.64 | 3.40 | 0.00 | 4.59 | 2.55 | 1.16 | 2.05 | - | - | - |
| *Hoploplana sp.* | 18.76 | 6.60 | - | 8.51 | 2.44 | - | 1.01 | - | - | - |
| *Mannesia sabadiega* | 4.31 | 31.22 | - | 0.00 | 1.80 | - | - | - | 2.66 | - |
| *Acanthonyx lunulatus 1* | 22.61 | 50.28 | 31.38 | 15.86 | 1.63 | 2.00 | - | 1.65 | 2.59 | - |
| *Tedania (Tedania) cf. ignis* | 22.05 | 9.79 | - | 0.00 | 1.46 | - | 1.45 | - | - | - |
| *Xantho hydrophilus* | 9.75 | 47.96 | 35.31 | - | 1.06 | - | - | 1.99 |  | - |
| *Phorbas dives* | 0.00 | 19.78 | 23.20 | 5.54 | - | 2.18 | - | - | 1.41 | - |
| *Acanthochitona crinita* | 9.73 | - | 29.00 | - | - | 1.81 | - | - | - | - |
| *Tubulipora sp. 3* | 10.51 | - | 29.49 | - | - | 1.72 | - | - | - | - |
| *Coscinasterias tenuispina* | 4.94 | - | 34.50 | - | - | 1.71 | - | - | - | - |
| *Tricolia pullus* | 20.52 | - | 0.00 | - | - | 1.37 | - | - | - | - |
| *Paracentrotus lividus* | 9.76 | 15.05 | 31.29 | 20.33 | - | 1.11 | 1.78 | 2.51 | 1.18 | - |
| *Synalpheus gambarelloides* | 9.39 | - | - | 23.81 | - | - | 2.17 | - | - | - |
| *Tubulipora sp. 1* | 0.00 | - | - | 35.81 | - | - | 1.78 | - | - | - |
| *Diplosoma listerianum 2* | 23.45 | - | - | 0.00 | - | - | 1.48 | - | - | - |
| *Alvania sp. 1* | 34.08 | - | - | 0.00 | - | - | 1.34 | - | - | - |
| *Aplysia punctata* | - | 15.33 | 0.00 | - | - | - | - | 1.63 |  | - |
| *Tritia cuvierii* | - | 18.95 | - | 0.00 | - | - | - | - | 3.14 | - |
| *Halichondria (Halichondria) melanadocia* | - | 20.99 | - | 26.67 | - | - | - | - | 1.95 | - |
| *Stelletta hispida* | - | 14.20 | - | 7.68 | - | - | - | - | 1.78 | - |
| *Pagurus cuanensis* | - | 2.08 | - | 19.38 | - | - | - | - | 1.43 | - |
| Bienmidae sp. | 0.00 | 0.00 | 0.00 | 11.11 | - | - | 1.32 | - | - | - |
| **Small fraction** |  |  |  |  |  |  |
| *Alvania sp. 1* | 58.35 | 34.59 | 27.22 | 16.10 | 3.74 | - | - | 1.18 | 1.06 | - |
| *Microcosmus squamiger* | 11.21 | 6.88 | 9.68 | 0.00 | 2.56 | 1.78 | 3.38 | - | - | - |
| *Mannesia sabadiega* | 6.15 | 24.41 | 9.30 | - | 2.32 |  | - | 1.03 | - | - |
| *Alvania sp. 2* | 8.75 | 49.05 | - | - | 1.46 | - | - | - | - | - |
| *Favorinus sp.* | 0.00 | 11.53 | - | - | 1.37 | - | - | - | - | - |
| *Cylista cf. laceratus* | 15.48 | 4.76 | - | - | 1.20 | - | - | - | - | - |
| *Xantho hydrophilus* | 0.00 | 26.15 | 23.07 | 0.00 | 1.00 | 1.93 | - | - | - | - |
| *Pisa cf. tetraodon* | 0.00 | 3.39 | 14.72 | - | - | 2.96 | - | 2.32 | - | - |
| *Dynamene magnitorata* | 0.00 | - | 20.06 | 2.70 | - | 2.85 | - | - | - | - |
| *Acanthonyx lunulatus 1* | 3.83 | - | 37.84 | 4.75 | - | 2.84 | - | - | - | - |
| *Ophlitaspongia papilla* | 12.31 | - | 0.00 | - | - | 2.65 | - | - | - | - |
| *Synalpheus gambarelloides* | 0.00 | 45.39 | 31.91 | 18.36 | - | 1.16 | - | 1.63 | 2.82 | - |
| *Tubulipora sp. 3* | 0.00 | - | 34.19 | 40.74 | - | 1.12 | 1.50 | - | - | - |
| *Paracentrotus lividus* | 0.00 | 17.60 | 32.71 | 20.79 | - | 1.09 | 1.87 | 1.92 | 1.01 | - |
| *Coscinasterias tenuispina* | 3.88 | - | 33.09 | - | - | 1.07 | - | - | - | - |
| *Stelletta hispida* | 0.00 | 13.80 | - | 10.60 | - | - | 3.59 | - | 1.06 | - |
| *Haminoea hydatis* | 2.94 | 17.64 | 0.00 | 12.28 | - | - | 1.62 | 1.11 | 1.76 | - |
| *Costasiella cf. Virescens* | 0.00 | - | - | 19.68 | - | - | 1.40 | - |  | - |
| *Jujubinus cf. vexationis* | 12.08 | - | - | 0.00 | - | - | 1.37 | - |  | - |
| *Tubulipora sp. 1* | 0.00 | 0.00 | - | 37.10 | - | - | 1.32 | - | 1.30 | - |
| *Inachus cf. dorsettensis* | - | 14.28 | 6.26 | - | - | - | - | 2.25 | - | - |
| *Halichondria (Halichondria) melanadocia* | - | 13.91 | 7.67 | 11.52 | - | - | - | 1.94 | 2.74 | - |
| *Tubulipora sp. 2* | - | 34.56 | 28.13 | - | - | - | - | 1.93 | - | - |
| *Anthelia sp.* | - | 0.00 | - | 14.84 | - | - | - | - | 2.99 | - |
| *Scopalina sp. 2* | - | 15.30 | - | 0.00 | - | - | - | - | 2.67 | - |

 **Figure S2.** Non-metric multidimensional scaling (nMDS) plots overlaid with a bubble plot of the selected representative metazoans (with their phylum) that made the significant differences (PERMANOVA: p<0.05, Table S5) between levels of CO2 gradient (‘1-Vent’, ‘2-Transition25’, ‘3-Transition75’, and ‘4-Control’). These species belong to ‘meiofauna’ (A) or ‘macrofauna’ (B). The bubble size represents the percentage of relative abundances (‘reads’) species in each sample. The large fraction are organisms with size > 1 mm and the small fraction are organisms between 1 mm and 64 µm.

Table S7. (A) One-way permutational multivariate analyses of variance of the calcifying classification in algae and metazoan, in the natural ‘CO2 Gradient’ as factor with four levels (‘1-Vent’, ‘2-Transition25’, ‘3-Transition75’, and ‘4-Control’) in both fraction size (small fraction: organisms with size > 1 mm; and large fraction: organisms between 1 mm and 0.64 µm). (B) Results of pairwise comparisons for levels of the factor ‘CO2 Gradient’ in algae.

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| **(A) Source of variationFuente Variación LARGE FRACTION (A)** | **SMALL FRACTION (B)** |
| **Algae** | **df** | **SSSS** | **MSMS** | **Pseudo-FPseudo-F** | **p (perm)p** | **SSSS** | **MSMS** | **Pseudo-FPseudo-F** | **p (perm)p** |
|  | Gradient | 3 | 0.229 | 0.076 | 0.703 | 0.001\* | 0.010 | 0.033 | 0.644 | 0.001\* |
|  | Residual | 2020 | 0.097 | 0.004 |  |  | 0.006 | 0.003 |  |  |
| **Metazoan** | **df** | **SSSS** | **MSMS** | **Pseudo-FPseudo-F** | **pp (perm)pp** | **SSSS** | **MSMS** | **Pseudo-FPseudo-F** | **p (perm)p**  |
|  | Gradient | 3 | 0.039 | 0.013 | 0.196 | 0.233 | 0.015 | 0.014 | 0.109 | 0.489 |
|  | Residual | 2020 | 0.159 | 0.008 |  |  | 0.116 | 0.006 |  |  |
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| **(B)   Groups Algae** | **1 - 2** | **1 - 3** | **1 - 4** | **2 - 3** | **2 - 4** | **3 - 4** |
| Large fraction | **t** | 26.350 | 42.647 | 2.834 | 14.625 | 0.626 | 0.493 |
| **p** | 0.002\* | 0.002\* | 0.002\* | 0.002\* | 0.730 | 0.522 |
| Small Fraction | **t** | 59.363 | 33.788 | 6.580 | 12.828 | 0.582 | 3.410 |
| **p** | 0.002\* | 0.002\* | 0.005\* | 0.005\* | 0.495 | 0.086 |

**Table S8**. Carbon system parameters obtained during low and high tides in the four zones ‘1-Vent’, ‘2-Transition25’, ‘3-Transition75’, and ‘4-Control’ in Punta de Fuencaliente (La Palma Islands, Canary Islands, see Fig. 1). TA = Total Alkalinity (µmol/kg), Ω = Saturation state, cal = Calcite, arag = Aragonite.

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| --- | --- | --- |
|  | **Low Tide** | **High tide** |
|  | **pH** | **TA** | **Ω cal** | **Ω arag** | **pH** | **TA** | **Ω cal** | **Ω arag** |
| **1-Vent** | 7.65±0.02 | 3057.69±24.5 | 2.84±0.11 | 1.85±0.07 | 8.05±0.04 | 2615.64±10.5 | 5.24±0.35 | 3.42±0.23 |
| **2-Transition25** | 7.81±0.05 | 2771.46±40 | 4.12±0.38 | 2.685±0.24 | 8.11±0.01 | 2546.63±7.5 | 5.78±0.11 | 3.77±0.07 |
| **3-Transition75** | 8.09±0.02 | 2578.52±12 | 5.64±0.08 | 3.675±0.06 | 8.14±0.01 | 2522.57±6 | 5.93±0.12 | 3.87±0.08 |
| **4-Control** | 8.14±0.02 | 2498.28±15 | 5.92±0.15 | 3.87±0.09 | - | - | - | - |

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Dataset S1 (separate file). Number of reads of each MOTU per sample. The similarity and the code of the best match in the reference table is indicated (’best\_identity’). Rank refers to the taxonomic category at which the MOTU could be assigned by the ecotag procedure, designated by ‘scientific\_name’. Taxonomic categories and ecological categories are listed. The representative sequence of each MOTU (‘sequence’) are indicate.

Dataset S2 (separate file). Algae MOTUs grouped into four different vegetation layers (‘crustose’, ‘epiphytes’, ‘mixed turf’ and ‘erect macrophytes’ *sensu* Piazzi et al. 2001) and metazoan MOTUs grouped in two layers according to their preferred habitat (‘meiofauna’ and ‘macrofauna’ *sensu* Higgins, 1988). Also, both algae and metazoans MOTUs are grouped into ‘calcifying’ and ‘non-calcifying’ systems, according to their ability to deposit or not calcium carbonate.