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1 **Biogenic habitat shifts under long-term ocean acidification show nonlinear**
2 **community responses and unbalanced functions of associated invertebrates**

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33 **Abstract**

34 Experiments have shown that increasing dissolved CO₂ concentrations (i.e. Ocean Acidification, OA) in
35 marine ecosystems may act as nutrient for primary producers (e.g. fleshy algae) or a stressor for
36 calcifying species (e.g., coralline algae, corals, molluscs). For the first time, rapid habitat dominance
37 shifts and altered competitive replacement from a reef-forming to a non-reef-forming biogenic
38 habitat were documented over one-year exposure to low pH/high CO₂ through a transplant
39 experiment off Vulcano Island CO₂ seeps (NE Sicily, Italy). Ocean acidification decreased vermetid
40 reefs complexity via a reduction in the reef-building species density, boosted canopy macroalgae and
41 led to changes in composition, structure and functional diversity of the associated benthic
42 assemblages. OA effects on invertebrate richness and abundance were nonlinear, being maximal at
43 intermediate complexity levels of vermetid reefs and canopy forming algae. Abundance of higher
44 order consumers (e.g. carnivores, suspension feeders) decreased under elevated CO₂ levels.
45 Herbivores were non-linearly related to OA conditions, with increasing competitive release only of
46 minor intertidal grazers (e.g. amphipods) under elevated CO₂ levels.
47 Our results support the dual role of CO₂ (as a stressor and as a resource) in disrupting the state of
48 rocky shore communities, and raise specific concerns about the future of intertidal reef ecosystem
49 under increasing CO₂ emissions. We contribute to inform predictions of the complex and nonlinear
50 community effects of OA on biogenic habitats, but at the same time encourage the use of multiple
51 natural CO₂ gradients in providing quantitative data on changing community responses to long-term
52 CO₂ exposure.

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54 **Keywords:** carbon dioxide, ocean acidification, phase shift, transplant, CO₂ vents, vermetid reef

55

56 **Introduction**

57 Ecological communities are constantly responding to environmental change, and the diversity among
58 functionally similar taxa broadens their range of responses (Loreau et al., 2002). Ocean acidification
59 (OA) – a global environmental perturbation in the form of seawater CO₂ enrichment and changes in
60 carbonate chemistry due to rising CO₂ emissions – is occurring at an unprecedented rate (Hönisch et
61 al., 2012) and is predicted to increase over time (Pörtner et al., 2014). Laboratory studies, often
62 carried out on single species in isolation, have documented detrimental biological consequences of
63 OA levels expected by the end of this century and beyond, with marine molluscs identified as an OA-
64 intolerant taxon, and crustaceans considered as a more tolerant group (Kroeker et al., 2013a;
65 Wittman and Portner 2013). Since difficulties occur when the results of laboratory experiments have
66 to be scaled up to assess community wide responses to increasing CO₂ (Riebesell and Gattuso 2015),
67 recently, the OA research field has moved into longer-term community experiments to ensure that
68 the effects are not just stress responses and are more ecologically realistic. In this context, marine
69 CO₂ seeps may be used as OA natural analogues to further our knowledge on effects at the
70 community level.

71 Observations along natural pH gradients off temperate and tropical CO₂ seeps have documented
72 community shifts, habitat complexity changes and biodiversity loss at high $p\text{CO}_2$ / low pH conditions
73 (Hall-Spencer et al., 2008; Fabricius et al., 2011, 2014; Inoue et al., 2013; Enochs et al., 2015).

74 Evidence from ecosystem-scale studies suggests that many different indirect OA effects might be at
75 play, ultimately affecting community emergent properties: (a) ocean acidification mediates

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76 biodiversity shifts altering competitive interactions via habitat complexity changes (e.g., coral reefs,
77 mussel beds, seagrass meadows, macroalgae) (Sunday et al., 2017); (b) the increase of primary
78 producers boosted by elevated CO₂ might counter-intuitively drive – via more food provisioning –
79 greater abundances of OA-intolerant (i.e. calcifying) grazer species (Connell et al., 2017); (c) the
80 reduced abundance of OA-sensitive species can be compensated by the increased abundance of more
81 OA-tolerant species (Fabricius et al., 2011; Kroeker et al., 2011), therefore fostering compensatory
82 dynamics and likely maintaining ecosystem function (Gonzalez and Loreau, 2009). Such processes do
83 not act in isolation and can concomitantly operate. Under these circumstances, ecological facilitation
84 (i.e., habitat and food provisioning), species interactions (i.e., changing competitive strengths) and
85 counteracting tolerant/intolerant population responses (i.e. compensatory dynamics) to CO₂
86 enrichment/pH lowering may prevent clear predictions about the impact of OA on community
87 properties and await further study (Gaylord et al., 2015; Kroeker et al., 2017).

88

89 We focus our experiment on vermetid reefs – a structurally complex intertidal biogenic habitat built
90 by gregarious vermetid snails and crustose coralline algae cementing their tubular shells – distributed
91 in many subtropical and warm-temperate regions, where they provide multiple ecological services,
92 preventing coastal erosion, regulating sediment transport and deposition, and supporting high
93 biodiversity levels (Milazzo et al., 2017; Ingrosso et al., 2018). Under undisturbed conditions the
94 vermetids *Dendropoma* spp. are the ecologically dominant species in the intertidal of the warm part
95 of the Mediterranean Sea and elsewhere (Milazzo et al., 2017), whilst macroalgae represent
96 competitive subordinates, living attached to and growing on top of the vermetid reef framework, with

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97 low cover (Di Franco et al., 2011). In contrast, under rising anthropogenic disturbance, the algae
98 increase their cover, entrap sediments, and overwhelm vermetids (Di Franco et al., 2011).
99 Under OA the recruitment of the reef-building gastropod *Dendropoma cristatum* (as *D. petraeum*) is
100 impaired and concerns were raised on the future of these biogenic reefs unless CO₂ emissions are
101 reduced (Milazzo et al., 2014). Here, using a 12-month exposure transplant experiment of whole
102 vermetid reef communities along an ocean acidification spatial gradient, we assess the effects of OA
103 on vermetid reefs and canopy macroalgae, and quantify changes in complexity along with
104 composition, structure and functional diversity of associated benthic invertebrate assemblages. We
105 hypothesize that CO₂ enrichment can reverse competitive dominance by acting as a stressor for the
106 reef building species (the vermetids) and as a resource for the subordinates (the canopy algae), hence
107 leading to competitive release. Community properties (i.e. overall associated invertebrate species
108 abundance and richness) would change as a result, and we anticipate communities to respond in a
109 complex and nonlinear way under OA. Specifically, the richness of invertebrate taxa will decrease
110 both with pH regime and indirectly via reduced vermetid reef complexity (i.e. the reef building species
111 density). At the same time, if CO₂ enrichment positively affects primary producers (i.e. boosting algal
112 productivity and their complexity), this will allow the richness and abundance of both some intolerant
113 (via more food provisioning) and tolerant invertebrate taxa (through compensatory dynamics and
114 habitat facilitation) to increase. Finally, we expect community function to be maintained despite the
115 loss or reduced abundance of taxa sensitive to OA.

116

117 **Methods**

118 **Experimental set up, study sites and carbonate chemistry analyses**

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119 On November 2010, fifteen vermetid reefs cores (13 cm diameter), having similar densities of the reef
120 building snail *Dendropoma cristatum* adults (ca. 300-350 ind. 100 cm⁻²) (Milazzo et al., 2014), were
121 collected at low tide from one location in NW Sicily (Cala Isola; 38°12.3419'N, 13°15.4909'E) using a
122 pneumatic drill (Airtec 478 SN, Italy). During collection each core was enclosed in a nylon net bag (400
123 µm mesh size), to prevent the loss of more mobile associated invertebrates, and immediately
124 transferred in isolation inside 7-L tanks filled with aerated seawater. Within 24 hours of collection, the
125 vermetid reef cores and their associated invertebrate assemblages were randomly assigned to
126 different pH treatments and transplanted at mean sea level in the intertidal rocky shore for one year.
127 Before transplants, plastic bands and four steel screws (12 cm) were used to fix bakelite plates at
128 mean sea level in five different sites, all sharing similar habitat characteristics and tidal ranges (<40
129 cm) (Milazzo et al., 2014): three sites along a pH gradient off Levante Bay on Vulcano Island (NE Sicily,
130 Italy; 38°25.1333'N, 14°57.6667'E) CO₂ seeps (Low pH, Mid pH, and High pH at increasing distance
131 from the seeps; n=3 each), one control site at >800 m from the seeps (CTL_V; n=3), and one site at the
132 original coring location (CTL_C; n=3) in the intertidal zone of Cala Isola, at ca. 80 nautical miles from
133 Vulcano island. This latter treatment was added to control the transplant effect on the vermetid reefs
134 and their associated invertebrates. The vermetid cores were fixed with epoxy to bakelite plates within
135 a 14 cm diameter PVC tube (Milazzo et al., 2014), while the nylon net bag was removed from each
136 core 6 hours after the transplantation.

137

138 Both the biogeochemistry and the seawater carbonate chemistry of the shallow waters of Levante
139 Bay on Vulcano Island have been described by a number of studies (e.g. Arnold et al., 2012; Johnson
140 et al., 2013; Boatta et al., 2013; Calosi et al., 2013; Vizzini et al., 2013; Milazzo et al., 2014; Cornwall et

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141 al., 2017; Brown et al., 2018). Here, we monitored temperature (daily averages from continuous
142 logging), salinity (n=95), pH (n=95), and total alkalinity (n=15) from November 2010 to November
143 2011 at each intertidal site. Full results on the seawater carbonate chemistry are available from
144 Milazzo et al. (2014) and data are freely accessible at
145 <https://doi.pangaea.de/10.1594/PANGAEA.836006>. In summary, the vermetid reef cores and their
146 associated invertebrate assemblages were exposed on average to ocean pH and pCO₂ levels predicted
147 for the next few decades (i.e., the High pH site, 8.03 pH units; 603 μatm pCO₂), by the end of this
148 century (Mid pH, 7.73 pH units; 1385 μatm pCO₂) and beyond (Low pH, 7.3 pH units; 3923 μatm pCO₂)
149 respectively, whilst vermetids kept at CTL_V and CTL_C sites were exposed to present-day pH and
150 pCO₂ conditions (CTL_V, 8.15 pH units; 468 μatm pCO₂; CTL_C, 8.16 pH units; 462 μatm pCO₂)
151 (Milazzo et al., 2014).

152

153 **Invertebrate sampling and laboratory analyses**

154 After 12 months, the vermetid reef cores (three for each site/treatment) resisted storms and were
155 collected at high tide using nylon net bags (400 μm mesh size) to avoid the loss of the associated
156 invertebrates. Each core was energetically rinsed with seawater to collect the invertebrate fauna and
157 the material retained by a 500 μm sieve was stored in 70% ethanol before examination under a 40×
158 binocular microscope (Leica, MZ-APO). All organisms were initially sorted by phylum and class
159 (Molluscs, Crustaceans, Polychaetes, Bryozoans, Nematodes, Anthozoans, Echinoderms, Acari,
160 Pycnogonida, Sponges, Sipunculids) and then counted, identified to genus or species level, and further
161 classified into different trophic guilds (e.g. herbivores, carnivores, omnivores and deposit/suspension
162 feeders). The top of each core was photographed to assess the %cover of the canopy-forming algae

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163 using ImageJ software (open-access, National Institutes of Health). Vermetid cores were also
164 examined under the stereomicroscope to further collect invertebrates present within crevices and
165 those strictly attached to the reef or the algae (e.g. bryozoans, sponges and serpulids), and to assess
166 the total density of *D. cristatum* adult individuals (n. of ind. >3 mm shell opening size) on each core.
167 Data of percentage cover of canopy-forming algae and density of the reef-building species of each
168 vermetid core were used as proxies of algal and reef complexity (Sunday et al., 2017; Donnarumma et
169 al., 2018), respectively.

170

171 **Statistical analyses**

172 We used generalized linear models (GLMs) to assess the effects of long-term exposure to OA
173 conditions on the competitive dominant vermetid reefs and the subordinate canopy-forming algae by
174 relating both reef density (RD) and canopy forming algae percent cover (ApC) with pH. Moreover, we
175 assessed the relationships between invertebrate community properties (i.e. overall species
176 abundance and richness), and their functional diversity (i.e. the trophic guilds) with pH and the reef
177 and algal complexities (RC and AC, respectively).

178 We further tested for potential compensatory dynamics occurring along the pH gradient. To
179 achieve this goal, we focussed our analyses on gastropods and amphipods relative abundance vs pH
180 as previous OA studies documented mechanisms of compensatory dynamics for these two taxonomic
181 groups elsewhere (Kroeker et al., 2011; Fabricius et al., 2014). The nonlinear nature of the
182 relationships was tested by introducing for each model second order (quadratic) and third order
183 (cubic) polynomial terms.

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184 GLMs were chosen since the response variables were clearly non-normal data (i.e. counts and
185 proportions). For counts (i.e. overall species richness and abundance and reef density) GLMs with
186 Poisson distribution and log link function were used. GLMs with binomial distributions were used for
187 proportional data. i.e. the relative abundance of the different trophic guilds in relation to the reef
188 complexity, both the reef (RC) and the algal complexity (AC) or pH, and the relative abundance of
189 amphipods and gastropods to assess compensatory responses in relation to pH. In instances where
190 overdispersion was detected, negative binomial GLM and a standard error correction using a quasi-
191 GLM model (quasi-binomial distribution) was used for Poisson and Binomial GLMs respectively (Zuur
192 et al., 2009). Due to the low sample size considered for each treatment, the 95% confidence intervals
193 of the high order regression parameters were also estimated by bootstrapping the cases
194 (observations) with replacement within each treatment. The randomization was carried out 100 times.
195 Following Crawley (2012), the model was fitted, and the parameters were estimated each time. The
196 95% confidence intervals were obtained from the quantiles (2.5th and 97.5th percentiles) of the
197 distribution of the high order parameter values.
198 All the analyses were run using the R software (R Core Team 2016)

199

200 **Results**

201 After a 12-month exposure off the Vulcano Island pH/CO₂ gradient, the density of the reef-
202 building vermetid was highest in cores from the control site along the seep gradient (CTL_V) as well as
203 at the procedural control at the site at which the reefs were collected (CTL_C) and decreased as pH
204 fell from 8.16 to 7.3 (i.e. on average 462 μ atm and 3923 μ atm pCO₂, respectively) as displayed by the
205 Negative Binomial (NB) GLM (deviance explained 57 %, quadratic term $\beta = -0.62$, $p=0.0002$; Fig 1,

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206 Table S1). By contrast, the percent cover of canopy-forming algae (mostly *Cystoseira compressa*)
207 displayed an opposite response to the pH/CO₂ gradient, showing higher values at ≤7.7 pH (Mid pH;
208 ≥1350 μatm pCO₂) with significant quadratic terms in the Quasi-binomial GLM (deviance explained 73
209 %; p=0.046; Fig 1, Table S1).

210 A total of 7044 individuals belonging to 228 invertebrate species were counted on vermetid cores
211 placed at four sites along the pH gradient off Vulcano Island and at one site at the original coring
212 location (Appendix S1). Overall, the most speciose taxa were molluscs (with 58 gastropod, 8 bivalve
213 and 2 chiton species), crustaceans (a total of 63 species) and polychaetes (50 species). Slightly more
214 than 75% of the total number of individuals found in the cores were crustaceans (i.e. 5375
215 individuals), with amphipods being the most abundant taxon (4164 individuals), followed by isopods
216 (670), tanaids (369), ostracods (85), decapods (78) and cumaceans (9). Crustaceans were followed in
217 abundance by molluscs (835 individuals), polychaetes (554), nematodes (112), echinoderms (48),
218 anthozoans (46), bryozoans (42 encrusting or ramified colonies), acarina (15), sipunculids (9),
219 pycnogonids (7) and sponges (1). The amphipod *Ampithoe ramondi*, the bivalve *Mytilaster minimus*
220 and the worm *Amphiglena mediterranea* were the dominant species among crustaceans, molluscs
221 and polychaetes, respectively (Appendix S1).

222 OA effects on invertebrate richness and abundance along the pH gradient were nonlinear, peaking
223 at the intermediate levels of pH from 8 to 7.7 units [cubic Poisson GLMs, deviance explained 80.5%
224 (cubic term $\beta = 0.87$, $p < 0.001$ for richness) and cubic NB GLM 78% (cubic term $\beta = 0.81$, $p < 0.0001$ for
225 abundance) respectively; Fig 2a,b, Table S2]. Invertebrate richness and abundance followed similar
226 patterns in relation to algal complexity, being maximal at intermediate complexity levels of canopy-
227 forming algae, i.e. 0.4-0.6 relative algal complexity (cubic NB GLMs), with 65% of deviance explained

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228 for richness (cubic term $\beta = 0.66$, $p=0.0012$) and 66% for abundance (cubic term $\beta = 0.77$, $p<0.05$) (Fig
229 2c,d, Table S2). Overall, associated invertebrate richness and abundance were not significantly
230 affected by different levels of vermetid reef complexity [NB GLMs, deviance explained 17% for
231 richness (linear term $\beta = 0.59$, $p=0.0867$) and 0.02% for abundance (linear term $\beta = -0.04$, $p=0.954$)
232 respectively; Fig 2c,d, Table S2].

233 Significant relationships between different trophic guilds and pH levels were found for herbivores,
234 carnivores and suspension/deposit feeders (QB GLMs, Fig 3a-d). Specifically, herbivores were non-
235 linearly related to the pH gradient, reaching their highest relative abundances on cores exposed to
236 ≤ 7.7 pH values (deviance explained 82%; Fig 3a, Table S3). By contrast, both carnivores and
237 suspension feeders linearly decreased with lowering pH (deviance explained $\sim 40\%$; Fig 3b,c, Table
238 S3). Relative abundances of omnivores did not change along the pH gradient (deviance explained
239 0.04%; Fig 3d, Table S3).

240 Carnivores and suspension feeders showed similar patterns also in relation to vermetid reef and
241 algal complexity levels (Binomial and QB GLMs, respectively; Fig 3f,g Table S3). The increase in algal
242 complexity negatively influenced these two trophic functional groups, which in turn were positively
243 affected by the increase of the vermetid reef complexity (Fig 3f,g, Table S3). An opposite pattern
244 emerged from the relative proportion of abundance of herbivores, which linearly increased with algal
245 complexity levels, whereas decreased with increasing reef complexity (Fig 3e, Table S3). Omnivores
246 were not significantly affected by changes in vermetid reef and algal complexity levels (Fig 3h, Table
247 S3).

248 Finally, a significant non-linear pattern characterized the relationship between amphipods and pH
249 (Fig 4, Table S4), with increasing relative abundances in transplanted cores exposed to 7.7 and 7.3 pH

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250 units. By contrast, gastropod relative abundance linearly decreased with the pH lowering (Fig 4, Table
251 S4).

252 In all cases, bootstrapped 95% confidence intervals confirmed our results (Tables S1-S4).

253

254 **Discussion**

255 The present study contributed to advance the understanding of the complex direct and indirect
256 effects of OA on marine ecosystems. For the first time through an *in situ* transplant experiment, rapid
257 habitat dominance shifts and altered competitive replacement from a reef-forming to a non reef-
258 forming biogenic habitat were documented over one-year exposure to low pH/elevated CO₂
259 conditions. Remarkably, our findings show that community-level changes may be nonlinear, with
260 abundance and richness of associated invertebrate species being maximal at intermediate pH/CO₂
261 conditions, as well as document changes in the relative abundances of several trophic functional
262 groups that might suggest a food web simplification under OA.

263 Transplanted reef cores experienced very large pH fluctuations particularly in Mid and Low pH
264 sites. Species living in shallow water environments may already naturally experience a mosaic of CO₂
265 conditions well above end-of-century IPCC projections (e.g. Hofmann et al., 2011, Andersson et al.,
266 2013), with large pCO₂/pH fluctuations being documented for many coastal ecosystems, where the
267 biology may daily affect the seawater carbonate chemistry (e.g. Shaw et al., 2013). However,
268 vermetids retained high densities and thus high complexity levels (Donnarumma et al., 2018) only at
269 ambient pH/CO₂ levels, whereas canopy macroalgae flourished on cores exposed to Mid and Low pH
270 conditions. Likely, the boosting effect of CO₂ enrichment on canopy-forming macroalgae – previously
271 described by Celis-Plá et al. (2015) and Cornwall et al. (2017) for the study site – counteractively drove

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272 the calcifying vermetid reefs to be further affected under low pH levels, where chronic OA conditions
273 also directly impaired the snails recruitment success and the metabolic costs of calcification,
274 dissolution and shell repairing (Milazzo et al., 2014; Garilli et al., 2015). Therefore, our results add on
275 previous observations of imbalanced OA effects on habitat-forming species, suggesting that
276 community shifts are due to a combination of positive/negative physiological effects on subordinate
277 and dominant species and, thus, to likely changes in the competition strengths between them (e.g.
278 Connell et al., 2013; Gaylord et al., 2016; Sunday et al., 2017). The role of competition in driving these
279 patterns remains to be tested in this system.

280 Remarkably, the time required for an intertidal reef habitat to transition into an alternative algal-
281 dominated state may be very short. Extensive experimental and theoretical work suggest that many
282 types of phase shifts may occur in marine ecosystems, with isolated or interacting drivers (e.g. global
283 change, overfishing, pollution) leading to abrupt changes in the structure of ecological communities
284 (e.g. Scheffer et al., 2001; Rocha et al., 2015). At present, our knowledge of ecosystem-level
285 responses to OA is rapidly advancing, with many documented shifts in temperate, warm-temperate
286 and tropical CO₂ seeps systems worldwide (e.g. Hall-Spencer et al., 2008; Fabricius et al., 2011;
287 Connell et al., 2018; Agostini et al., 2018). The type of transitions occurring under OA seems not to be
288 as deterministic as previously hypothesized (e.g. from calcifying to non-calcifying species; Hall-
289 Spencer et al., 2008) largely differing between regions. Dominance shifts from hard to soft corals or
290 macroalgae were observed in the tropical and the temperate NW Pacific Ocean (Inoue et al., 2013;
291 Enochs et al., 2015; Agostini et al., 2018). Loss of coral reef complexity and species composition
292 changes were documented in Papua New Guinea and Palau (Fabricius et al., 2014; Barkley et al.,
293 2015); whilst kelps, other canopy- or mat-forming macroalgae substitute calcifying taxa (e.g.

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294 scleractinian corals, rhodolith beds, coralline algae) off shallow and deep CO₂ seeps in the
295 Mediterranean Sea (Hall-Spencer et al., 2008; Kroeker et al., 2013b; Baggini et al., 2014; Linares et al.,
296 2015). Likely, physiological plasticity promoting species-specific resistance under elevated pCO₂
297 (Rodolfo-Metalpa et al., 2011; Inoue et al., 2013; Barkley et al., 2015, Kenkel et al., 2018), alongside
298 nutrient/food levels and other local environmental conditions may play a role in shaping species
299 resilience and the direction of community shifts (Camp et al., 2018). Experimental evidence suggests
300 that reef-building organisms can be definitely out-competed as their recovery is further suppressed –
301 e.g. with coral larvae being repelled by algal chemical cues – once ecosystem shifts towards non-reef
302 forming over a reef forming state occur in fished zones (Dixon et al., 2014). Similarly, such
303 breakdowns may likely occur in disturbed marine areas where sustained stimulation of algae converts
304 vermetid reefs into species-poor intertidal rocky shores, hence preventing vermetids recovery (Di
305 Franco et al., 2011; Milazzo et al., 2017).

306 Interestingly, the phase shift observed on transplanted cores ultimately resulted in nonlinear
307 responses of the associated benthic invertebrates. Despite potentially stressful abiotic conditions
308 under increasing CO₂ concentrations, overall species richness and abundance peaked at intermediate
309 pH/pCO₂ levels, where a mosaic of canopy-forming algae and vermetids occurred on transplanted
310 cores after 1-year exposure to CO₂ enrichment. Both the reef-building vermetid *Dendropoma*
311 *cristatum* and the canopy-forming alga *Cystoseira compressa* have a large effect on the associated
312 community by providing a complex 3-dimensional framework (e.g. Chemello and Milazzo, 2002;
313 Milazzo et al., 2017). At the same time, mechanistic studies on the physiological performance of
314 marine organisms in response to OA, as well as community-level investigations at natural analogues,
315 documented that many invertebrate taxa are unable to cope with long-term pH decrease, but those

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316 that can do are abundant (Gaylord et al., 2015). Therefore, here we suggest the responses of the
317 associated fauna to be mediated by both pH/CO₂ levels and thresholds in the complexity of canopy-
318 forming algae above which vermetids were (both directly or indirectly) negatively affected. This
319 resulted in net effects on associated tolerant-intolerant invertebrates switching from positive to
320 negative for reef-associated species or from negative to positive for algal associated ones. We argue
321 such observed, complex, and to some extent, unexpected nonlinear responses may limit predictability
322 of community responses under OA as well as posing obvious limitations to linear extrapolations
323 (Sunday et al., 2017; Kroeker et al., 2017).

324 Changes in the structure and composition of benthic assemblages can lower community
325 resilience, but if there is ecological redundancy among the compensating species, then compensation
326 could maintain ecosystem function even with the loss or reduced abundance of some vulnerable
327 species under OA. Accordingly, previous community-level studies at CO₂ seeps in Papua New Guinea
328 and the Mediterranean provided evidence of compensatory dynamics for invertebrate fauna and
329 suggested potential mechanisms of competitive release, increased refuge from predators and habitat
330 facilitation as ecological processes at play for associated benthic communities (Kroeker et al., 2011;
331 Fabricius et al., 2011; Fabricius et al., 2014). Complex canopy-forming macroalgae (e.g. *Cystoseira*
332 spp.) are considered a preferred habitat for both gastropod and amphipod species (Hay et al., 1990;
333 Chemello and Milazzo, 2002; Norderhaug, 2004). Our study highlights that CO₂ enrichment increased
334 the abundance of OA-tolerant amphipods but had an opposite effect on OA-intolerant gastropods,
335 not supporting indications of calcifying grazers thriving under OA via more food provisioning within
336 the natural complexity of their environment (Connell et al., 2017). Our findings rather support the
337 very recent evidence of the dual role of CO₂ in disrupting the state of temperate rocky shore

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338 communities by favouring turfs over kelps and inhibiting compensatory dynamics involving grazers
339 (Connell et al., 2018).

340 Interestingly, higher order consumers (e.g. carnivores and suspension feeders) failed to
341 compensate, showing lower abundances under elevated than ambient CO₂ levels. Such altered
342 functional redundancy may support the general hypothesis of CO₂-driven phase-shifts and food web
343 simplification in coral reef and temperate seagrass systems (Fabricius et al., 2014; Vizzini et al., 2017).

344 We recognize some limitations of the present study. We caution that our findings should be
345 generalized to other settings as our experiment focuses on a single CO₂ seep, and only one site (with a
346 limited sample size) was considered for each pH condition along the pH/pCO₂ spatial gradient.
347 Therefore, we encourage the use of similar transplant experiments in multiple natural CO₂ gradients
348 in temperate and tropical regions to provide quantitative data on nonlinear community responses to
349 long-term CO₂ exposures, and to anticipate drivers that accelerate or stabilise changes in ecological
350 communities under increasing CO₂ emissions.

351 In summary, since a long time is needed to reverse OA once anthropogenic CO₂ emissions are
352 reduced (Ciais et al., 2013), our experiment may raise specific concerns about the future of intertidal
353 vermetid reef ecosystem and underscores the role of OA in potentially driving cascading community
354 shifts. We documented here nonlinear changes in the abundance and the diversity of the associated
355 fauna the intertidal biogenic reefs support and added on previously observed knock-on OA effects on
356 higher trophic levels (e.g. carnivores and suspension feeders) in other marine ecosystems (Fabricius et
357 al., 2014; Vizzini et al., 2017). Intertidal reef habitats are often neglected from discussions of
358 anthropogenic impacts and conservation planning (Andrades et al., 2017). In this regard, our
359 experiment was also conducted to inform predictions of the complex community wide effects of OA

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360 on a threatened intertidal biogenic habitat, which provides essential ecological services to society
361 (Riebesell and Gattuso 2015; Sunday et al., 2017; Milazzo et al., 2017).

362

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370

371 **Authors contributions**

372 MM conducted and conceived the experiment, and wrote the manuscript in collaboration with FQ
373 and FB. CA conducted the field experiment, sorted the biological samples and identified in the lab
374 molluscs, anthozoans, sponges and other minor taxa in collaboration with RC and MM. FQ performed
375 the statistical analyses. RC contributed to the experimental setup. RD and AMV identified the
376 crustaceans. JG identified the polychaetes. SM identified the nematodes. MG identified the
377 bryozoans. All authors commented on the first draft of the manuscript.

378

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

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381 **References**

Author Generated Postprint

- 382 Agostini, A., Harvey, B.P., Wada, S., Kon, K., Milazzo, M., Inaba, K., Hall-Spencer, J.M., 2018. Ocean
383 acidification drives community shifts towards simplified non-calcified habitats in a
384 subtropical–temperate transition zone. *Sci. Rep.* 8, 11354. [https://doi.org/10.1038/s41598-018-](https://doi.org/10.1038/s41598-018-29251-7)
385 29251-7.
- 386 Andersson, A.J., Bates, N.R., Jeffries, M.A., Freeman, K., Davidson, C., Stringer, S., Betzler, E.,
387 Mackenzie, F.T., 2013. Clues from Current High CO₂ Environments on the Effects of Ocean
388 Acidification on CaCO₃ Preservation. *Aquat. Geochem.* 19, 353–369.
389 <https://doi.org/10.1007/s10498-013-9210-y>.
- 390 Andrades, R., Joyeux, J.C., Gasparini, J.L, Reis-Filho, J.A., Macieira, R.M., Giarrizzo, T., 2017. Fringe on
391 the brink: Intertidal reefs at risk. *Science* 357(6348), 261. <https://doi.org/10.1126/science.aao0403>.
- 392 Arnold, T., Mealey, C., Leahey, H., Miller, A.W., Hall-Spencer, J.M., Milazzo, M., Maers, K., 2012. Ocean
393 Acidification and the Loss of Phenolic Substances in Marine Plants. *PLoS ONE* 7(4), e35107.
394 <https://doi.org/10.1371/journal.pone.0035107>.
- 395 Baggini, C., Salomidi, M., Voutsinas, E., Bray, L., Krasakopoulou, E., Hall-Spencer, J.M., 2014.
396 Seasonality affects macroalgal community response to increases in pCO₂. *PLoS ONE* 9(9), e106520.
397 <https://doi.org/10.1371/journal.pone.0106520>.
- 398 Barkley, H. C., Cohen, A.L., Golbuu, Y., Starczak, V.R., DeCarlo, T.M., Shamberger, K.E.F., 2015.
399 Changes in coral reef communities across a natural gradient in seawater pH. *Sci. Adv.* 1(5), e1500328.
400 <https://doi.org/10.1126/sciadv.1500328>.
- 401 Boatta, F., D’Alessandro, W., Gagliano, A.L., Liotta, M., Milazzo, M., Rodolfo-Metalpa, R., Hall-Spencer,
402 J.M., Parello, F., 2013. Geochemical survey of Levante Bay, Vulcano Island (Italy), a natural laboratory
403 for the study of ocean acidification. *Mar. Pollut. Bull.* 73, 485–494.
404 <https://doi.org/10.1016/j.marpolbul.2013.01.029>.
- 405 Brown, N.E.M., Milazzo, M., Rastrick, S.P.S., Hall-Spencer, J.M., Therriault, T.W., Harley, C.G.D., 2018.
406 Natural acidification changes the timing and rate of succession, alters community structure, and
407 increases homogeneity in marine biofouling communities. *Glob. Change Biol.* 24(1), e112–e127.
408 <https://doi.org/10.1111/gcb.13856>.
- 409 Calosi, P., Rastrick, S.P.S., Graziano, M., Thomas, S.C., Baggini, C., Carter, H.A., Hall-Spencer, J.,
410 Milazzo, M., Spicer, J.I., 2013. Distribution of sea urchins living near shallow water CO₂ vents is
411 dependent upon species acid-base and ion-regulatory abilities. *Mar. Pollut. Bull.* 73, 470–484.
412 <https://doi.org/10.1016/j.marpolbul.2012.11.040>.
- 413 Camp, E.F., Schoepf, V., Mumby, P.J., Hardtke, L.A., Rodolfo-Metalpa, R., Smith, D.J., Suggett, D.J.,
414 2018. The future of coral reefs subject to rapid climate change: lessons from natural extreme
415 environments. *Front. Mar. Sci.* 5: 4. <https://doi.org/10.3389/fmars.2018.00004>.
- 416 Celis-Plá, P.S.M., Hall-Spencer, J.M., Horta, P.A., Milazzo, M., Korbee, N., Cornwall, C.E., Figueroa, F.L.,
417 2015. Macroalgal responses to ocean acidification depend on nutrient and light levels. *Front. Mar. Sci.*
418 2:26. <https://doi.org/10.3389/fmars.2015.00026>.
- 419 Chemello, R., Milazzo, M., 2002. Effect of algal architecture on associated fauna: Some evidence from
420 phytal molluscs. *Mar. Biol.* 140(5), 981–990. <https://doi.org/10.1007/s00227-002-0777-x>.

Author Generated Postprint

- 421 Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J.,
422 Heimann, M., Jones, C., Le Quéré, C., Myneni, R.B., Piao, S., Thornton, P., 2013. Carbon and Other
423 Biogeochemical Cycles, in: Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J.,
424 Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), *Climate Change 2013: The Physical Science Basis*.
425 Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on
426 Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp.
427 465–570. https://www.ipcc.ch/pdf/assessment-report/ar5/wg1/WG1AR5_Chapter06_FINAL.pdf
- 428 Connell, S.D., Kroeker, K.J., Fabricius, K.E., Kline, D.I., Russell, B.D., 2013. The other ocean acidification
429 problem: CO₂ as a resource among competitors for ecosystem dominance. *Phil. Trans. R. Soc. B* 368,
430 20120442. <https://doi.org/10.1098/rstb.2012.0442>.
- 431 Connell, S.D., Doubleday, Z.A., Foster, N.R., Hamlyn, S.B., Harley, C.D.G., Helmuth, B., Kelaher, B.P.,
432 Nagelkerken, I., Rodgers, K.L., Sarà, G., Russell, B.D., 2018. The duality of ocean acidification as a
433 resource and a stressor. *Ecology* 88, 1005–1010. <https://doi.org/10.1002/ecy.2209>.
- 434 Connell, S.D., Doubleday, Z.A., Hamlyn, S.B., Foster, N.R., Harley, C.D.G., Helmuth, B., Kelaher, B.P.,
435 Nagelkerken, I., Sarà, G., Russell, B.D., 2017. How ocean acidification can benefit calcifiers. *Curr. Biol.*
436 27, R95–R96. <https://doi.org/10.1016/j.cub.2016.12.004>.
- 437 Cornwall, C.E., Revill, A.T., Hall-Spencer, J.M., Milazzo, M., Raven, J.A., Hurd, C.L., 2017. Inorganic
438 carbon physiology underpins macroalgal responses to elevated CO₂. *Sci. Rep.* 7, 46297.
439 <https://doi.org/10.1038/srep46297>.
- 440 Crawley, M. J., 2012. *The R book*. John Wiley & Sons.
- 441 Di Franco, A., Graziano, M., Franzitta, G., Felling, S., Chemello, R., Milazzo, M., 2011. Do small marinas
442 drive habitat specific impacts? A case study from Mediterranean Sea. *Mar. Pollut. Bull.* 62(5), 926–
443 933. <https://doi.org/10.1016/j.marpolbul.2011.02.053>.
- 444 Dixon, D.L., Abrego, D., Hay, M.E., 2014. Chemically mediated behavior of recruiting corals and
445 fishes: a tipping point that may limit reef recovery. *Science* 345(6199), 892–897.
446 <https://doi.org/10.1126/science.1255057>.
- 447 **Donnarumma, L., Sandulli, R., Appolloni, L., Di Stefano, F., Russo, G.F., 2018. Morpho-structural and**
448 **ecological features of a shallow vermetid bioconstruction in the Tyrrhenian Sea (Mediterranean Sea,**
449 **Italy). *J. Sea Res.* 131, 61–68. <https://doi.org/10.1016/j.seares.2017.10.004>.**
- 450 Enochs, I.C., Manzello, D.P., Donham, E.M., Kolodziej, G., Okano, R., Johnston, L., Young, C., Iguel, J.,
451 Edwards, C.B., Fox, M.D., Valentino, L., Johnson, S., Benavente, D., Clark, S.J., Carlton, R., Burton, T.,
452 Eynaud, Y., Price, N.N., 2015. Shift from coral to macroalgae dominance on a volcanically acidified
453 reef. *Nat. Clim. Change* 5, 1083–1088. <https://doi.org/10.1038/nclimate2758>.
- 454 Fabricius, K.E., De'ath, G., Noonan, S., Uthicke, S., 2014. Ecological effects of ocean acidification and
455 habitat complexity on reef-associated macroinvertebrate communities. *Proc. R. Soc. B* 281(1775),
456 20132479. <https://doi.org/10.1098/rspb.2013.2479>.
- 457 Fabricius, K.E., Langdon, C., Uthicke, S., Humphrey, C., Noonan, S., De'ath, G., Okasaki, R.,
458 Muehllehner, N., Glas, M.S., Lough, J.M., 2011. Losers and winners in coral reefs acclimatized to
459 elevated carbon dioxide concentrations. *Nat. Clim. Change* 1, 165–169.
460 <https://doi.org/10.1038/nclimate1122>.

Author Generated Postprint

- 461 Garilli, V., Rodolfo-Metalpa, R., Scuderi, D., Brusca, L., Parrinello, D., Rastrick, S.P.S., Foggo A.,
462 Twitchett, R.J., Hall-Spencer, J.M., Milazzo, M., 2015. Physiological advantages of dwarfing in surviving
463 extinctions in high-CO₂ oceans. *Nat. Clim. Change*, 5, 678–682. <https://doi.org/10.1038/nclimate2616>.
- 464 Gaylord, B., Kroeker, K.J., Sunday, J.M., Anderson, K.M., Barry, J.P., Brown, N.E., Connell, S.D., Dupont,
465 S., Fabricius, K.E., Hall-Spencer, J.M., Klinger, T., Milazzo, M., Munday, P.L., Russell, B.D., Sanford, E.,
466 Schreiber, S.J., Thiyagarajan, V., Vaughan, M.L.H., Widdicombe, S., Harley, C.D.G., 2015. Ocean
467 acidification through the lens of ecological theory. *Ecology* 96, 3–15. <https://doi.org/10.1890/14-0802.1>.
- 469 Gonzalez, A., Loreau, M., 2009. The causes and consequences of compensatory dynamics in ecological
470 communities. *Annu. Rev. Ecol. Evol. Syst.* 40, 393–414.
471 <https://doi.org/10.1146/annurev.ecolsys.39.110707.173349>.
- 472 Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S.M., Rowley, S.J.,
473 Tedesco, D., Buia, M.-C., 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean
474 acidification. *Nature* 454, 96–99. <https://doi.org/10.1038/nature07051>.
- 475 Hay, M.E., Duffy, J.E., Fenicai, W., 1990. Host-plant specialization decreases predation on a marine
476 amphipod: an herbivore in plant's clothing. *Ecology* 71(2), 733–743.
477 <https://doi.org/10.2307/1940336>.
- 478 Hofmann, G.E., Smith, J.E., Johnson, K.S., Send, U., Levin, L.A., Micheli, F., Paytan, A., Price, N.N.,
479 Peterson, B., Takeshita, Y., Matson, P.G., Crook, E.D., Kroeker, K.J., Gambi, M.C., Rivest, E.B., Frieder,
480 C.A., Yu, P.C., Martz, T.R., 2011. High-Frequency Dynamics of Ocean pH: A Multi-Ecosystem
481 Comparison. *PLoS ONE* 6(12), e28983. <https://doi.org/10.1371/journal.pone.0028983>.
- 482 Hönisch, B., Ridgwell, A., Schmidt, D.N., Thomas, E., Gibbs, S.J., Sluijs, A., Zeebe, R., Kump, L.,
483 Martindale, R.C., Greene, S.E., Kiesslieng, W., Ries, J., Zachos, J.C., Royer, D.L., Barker, S., Marchitto Jr.,
484 T.M., Moyer, R., Pelejero, C., Ziveri, P., Foster, G.L., Williams, B., 2012. The geological record of ocean
485 acidification. *Science* 335(6072), 1058–1063. <https://doi.org/10.1126/science.1208277>.
- 486 Ingrosso, G., Abbiati, M., Badalamenti, F., Bavestrello, G., Belmonte, G., et al., 2018. Mediterranean
487 Bioconstructions Along the Italian Coast. *Advances in Marine Biology* 79, 61–136.
488 <https://doi.org/10.1016/bs.amb.2018.05.001>.
- 489 Inoue, S., Kayanne, H., Yamamoto, S., Kurihara, H., 2013. Spatial community shift from hard to soft
490 corals in acidified water. *Nat. Clim. Change* 3, 683–687. <https://doi.org/10.1038/nclimate1855>.
- 491 Johnson, R., Brownlee, C., Rickaby, R., Graziano, M., Milazzo, M., Hall-Spencer, J.M., 2013. Responses
492 of marine benthic microalgae to elevated CO₂. *Mar. Biol.* 160, 1813–1824.
493 <https://doi.org/10.1007/s00227-011-1840-2>.
- 494 Kenkel, C.D., Moya, A., Strahl, J., Humphrey, C., Bay, L.K., 2018. Functional genomic analysis of corals
495 from natural CO₂-seeps reveals core molecular responses involved in acclimatization to ocean
496 acidification. *Glob. Change Biol.*, 24(1), 158–171. <https://doi.org/10.1101/112045>.
- 497 Kroeker, K.J., Micheli, F., Gambi, M.C., Martz, T.R., 2011. Divergent ecosystem responses within a
498 benthic marine community to ocean acidification. *Proc. Natl. Acad. Sci. U.S.A.* 108, 14515–14520.
499 <https://doi.org/10.1073/pnas.1107789108>.

Author Generated Postprint

- 500 Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., Gattuso, J.-P.,
501 2013a. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction
502 with warming. *Glob. Change Biol.* 19, 1884–1896. <https://doi.org/10.1111/gcb.12179>.
- 503 Kroeker, K.J., Micheli, F., Gambi, M.-C., 2013b. Ocean acidification causes ecosystem shifts via altered
504 competitive interactions. *Nat. Clim. Change*, 3, 156–159. <https://doi.org/10.1038/nclimate1680>.
- 505 Kroeker, K.J., Kordas, R.L., Harley, C.D.G., 2017. Embracing interactions in ocean acidification research:
506 Confronting multiple stressor scenarios and context dependence. *Biol. Lett.* 13, 20160802.
507 <https://doi.org/10.1098/rsbl.2016.0802>.
- 508 Linares, C, Vidal, M., Canals, M., Kersting, D.K., Amblas, D., Aspillaga, E., Cebrián, E., Delgado-Huertas,
509 A., Díaz, D., Garrabou, J., Hereu, B., Navarro, L., Teixidó, N., Ballesteros, E., 2015. Persistent natural
510 acidification drives major distribution shifts in marine benthic ecosystems. *Proc. R. Soc. B*, 282,
511 e20150587. <https://doi.org/10.1098/rspb.2015.0587>.
- 512 Loreau, M., Naeem, S., Inchausti, P. (Eds), 2002. *Biodiversity and Ecosystem Functioning: Synthesis
513 and Perspectives*. Oxford University Press, Oxford. 294 pp.
- 514 Milazzo, M., Rodolfo-Metalpa, R., Chan, V.B.S., Fine, M., Alessi, C., Thiyagarajan, V., Hall-Spencer, J.M.,
515 Chemello, R., 2014. Ocean acidification impairs vermetid reef recruitment. *Sci. Rep.*, 4, 4189.
516 <https://doi.org/10.1038/srep04189>.
- 517 Milazzo, M., Fine, M., La Marca, E.C., Alessi, C., Chemello, R., 2017. Drawing the Line at Neglected
518 Marine Ecosystems: Ecology of Vermetid Reefs in a Changing Ocean, in: Rossi, S., Bramanti, L., Gori,
519 A., Orejas Saco del Valle, C. (Eds.), *Marine Animal Forests, the ecology of benthic biodiversity
520 hotspots*. Springer International Publishing, pp. 345–367.
- 521 Norderhaug, K.M., 2004. Use of red algae as hosts by kelp-associated amphipods. *Mar. Biol.* 144(2),
522 225–230. <https://doi.org/10.1007/s00227-003-1192-7>.
- 523 Pörtner, H.-O., Karl, D.M., Boyd, P.W., Cheung, W.W.L., Lluch-Cota, S.E., Nojiri, Y., Schmidt, D.N.,
524 Zavalov, P.O., 2014. Ocean systems, in: Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea,
525 M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N.,
526 MacCracken, S., Mastrandrea, P.R., White, L.L. (Eds.), *Climate Change 2014: Impacts, Adaptation, and
527 Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth
528 Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press,
529 New York, NY, USA, pp. 411–484. [https://www.ipcc.ch/pdf/assessment-report/ar5/wg2/WGIIAR5-
530 Chap6_FINAL.pdf](https://www.ipcc.ch/pdf/assessment-report/ar5/wg2/WGIIAR5-
530 Chap6_FINAL.pdf)
- 531 R Core Team 2016. R: A language and environment for statistical computing. R Foundation for
532 Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- 533 Riebesell, U., Gattuso, J.-P., 2015. Lessons learned from ocean acidification research. *Nat. Clim. Change*
534 5, 12–14. <https://doi.org/10.1038/nclimate2456>.
- 535 Rocha, J., Yletyinen, J., Biggs, R., Blenckner, T., Peterson, G., 2015. Marine regime shifts: drivers and
536 impacts on ecosystems services. *Phil. Trans. R. Soc. B* 370, 20130273.
537 <https://doi.org/10.1098/rstb.2013.0273>.

Author Generated Postprint

- 538 Rodolfo-Metalpa, R., Houlbrèque, F., Tambutté, É., Boisson, F., Baggini, C., Patti, F.P., Jeffree, R., Fine,
539 M., Foggo, A., Gattuso, J.-P., Hall-Spencer, J.M., 2011. Coral and mollusc resistance to ocean
540 acidification adversely affected by warming. *Nat. Clim. Change*, 1, 308–312.
541 <https://doi.org/10.1038/nclimate1200>
- 542 Scheffer, M., Carpenter, S., Foley, J.A., Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems.
543 *Nature* 413, 591–596. <https://doi.org/10.1038/35098000>.
- 544 Shaw, E.C., McNeil, B.I., Tilbrook, B., Matear, R., Bates, M.L., 2013. Anthropogenic changes to
545 seawater buffer capacity combined with natural reef metabolism induce extreme future coral reef
546 CO₂ conditions. *Glob. Change Biol.* 19(5), 1632–1641. <https://doi.org/10.1111/gcb.12154>.
- 547 Sunday, J.M., Fabricius, K.E., Kroeker, K.J., Anderson, K.M., Brown, N.E., Barry, J.P., Connell, S.D.,
548 Dupont, S., Gaylord, B., Hall-Spencer, J.M., Klinger, T., Milazzo, M., Munday, P.L., Russell, B.D.,
549 Sanford, E., Thiyagarajan, V., Vaughan, M.L.H., Widdicombe, S., Harley, C.D.G., 2017. Ocean
550 acidification can mediate biodiversity shifts by changing biogenic habitat. *Nat. Clim. Change* 7, 81–85.
551 <https://doi.org/10.1038/nclimate3161>.
- 552 Vizzini, S., Martínez-Crego, B., Andolina, C., Massa-Gallucci, A., Connell, S.D., Gambi, M.C., 2017.
553 Ocean acidification as a driver of community simplification via the collapse of higher-order and rise of
554 lower-order consumers. *Sci. Rep.* 7, 4018. <https://doi.org/10.1038/s41598-017-03802-w>.
- 555 Vizzini, S., Di Leonardo, R., Costa, V., Tramati, C.D.D., Luzzu, F., Mazzola, A., 2013. Trace element bias
556 in the use of CO₂ vents as analogues for low pH environments: Implications for contamination levels in
557 acidified oceans. *Estuar.Coast. Shelf Sci.* 134, 19–30. <https://doi.org/10.1016/j.ecss.2013.09.015>.
- 558 Wittmann, A.C., Pörtner, H.-O., 2013. Sensitivities of extant animal taxa to ocean acidification. *Nat.*
559 *Clim. Change* 3(11), 995–1001. <https://doi.org/10.1038/nclimate1982>.
- 560 Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed effects models and
561 extensions in ecology with R. *Statistics for Biology and Health*. Springer Verlag, New York, NY.
562 <https://doi.org/10.1007/978-0-387-87458-6>.

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566 **Figure legends**

567

568 **Figure 1.** Changes in vermetid reef density (RD) and algal % cover (A%C) along the Vulcano spatial
569 gradient and one external control site (i.e. from ambient to low pH levels), showing biogenic habitat
570 shifts in the intertidal after a one-year exposure to different pH/pCO₂ regimes. The fitted lines from
571 the models show the effects of pH on vermetid reefs and canopy-forming algae. Striped areas
572 represent the 95% C.I..

573

574 **Figure 2.** Observed relationships between pH/pCO₂ (a, b), complexity of vermetid/algal habitats (c,
575 d), and invertebrate community richness (a, c) and abundance (b, d) along the Vulcano spatial
576 gradient and one external control site (i.e. from ambient to low pH levels). The fitted lines from the
577 GLM models show the effects of pH and RC/AC on species richness and abundance of associated
578 invertebrates. Striped areas represent the 95% C.I..

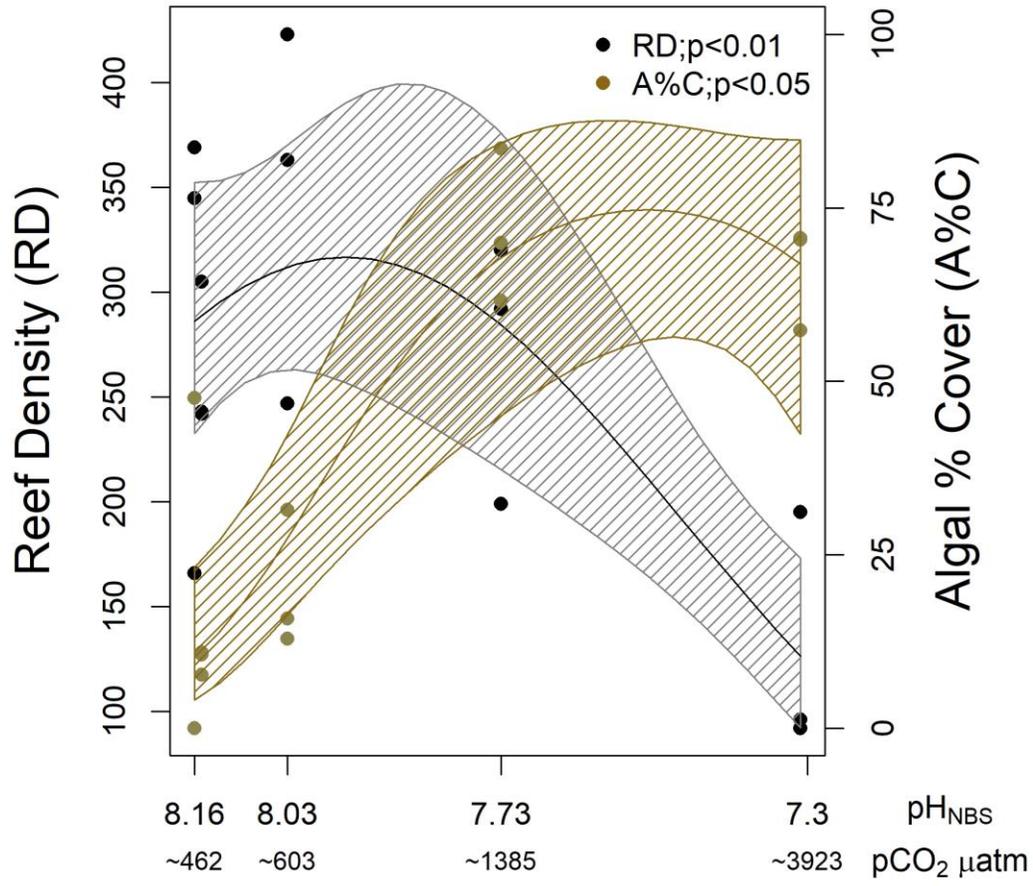
579

580 **Figure 3.** Observed relationships between pH/pCO₂ (a, b, c, d), complexity of vermetid/algal habitats
581 (e, f, g, h), and invertebrate community relative abundances by trophic functional groups along the
582 Vulcano spatial gradient and one external control site (i.e. from ambient to low pH levels). The fitted
583 lines from the GLM models show the effects of pH and RC/AC on species relative abundances of
584 associated invertebrates. Striped areas represent the 95% C.I..

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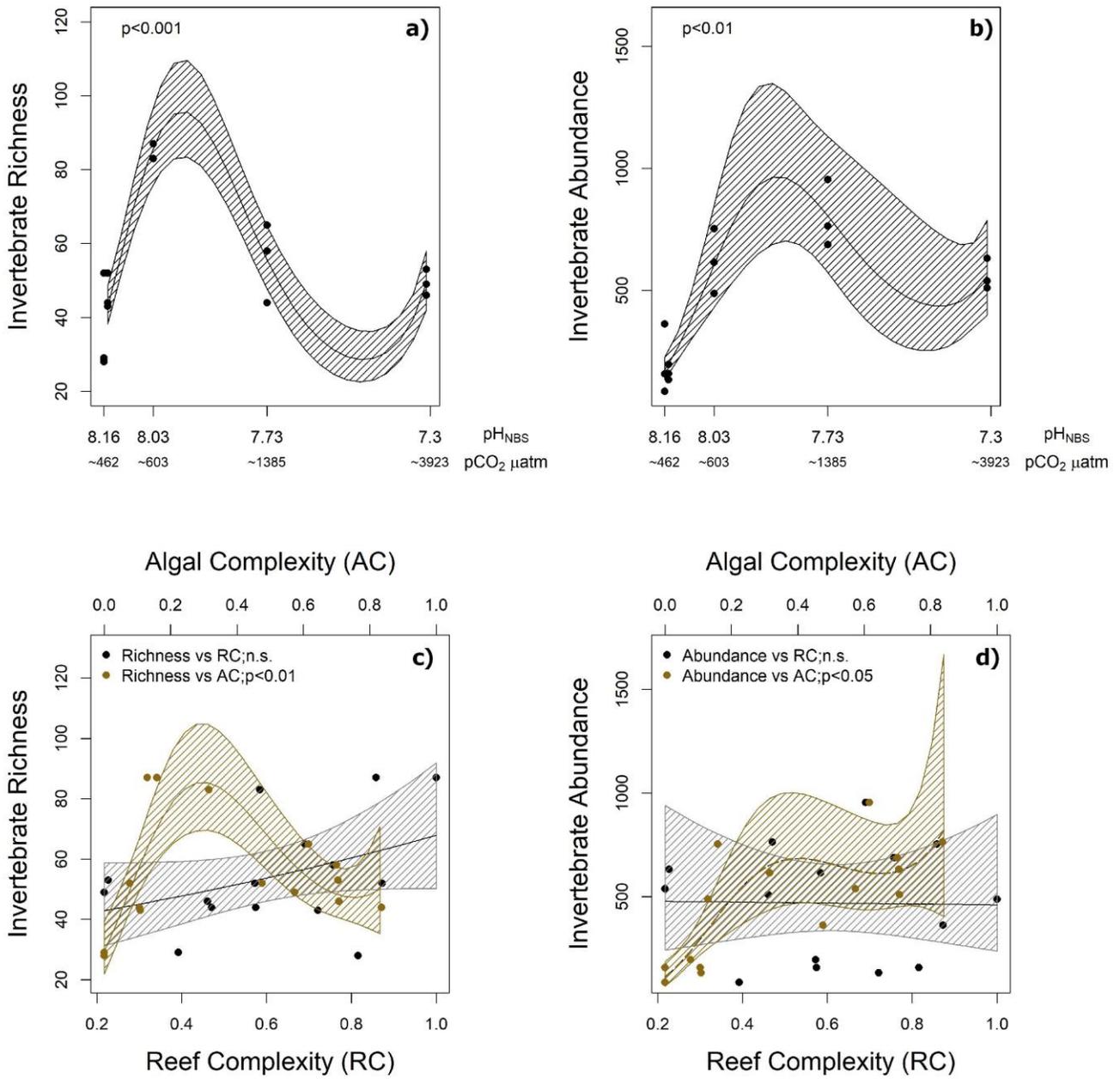
586 **Figure 4.** Observed relationships between pH/pCO₂ and the relative abundances of amphipods and
587 gastropods along the Vulcano spatial gradient and one external control site (i.e. from ambient to low
588 pH levels). The fitted lines from the GLM models show the responses of the main intertidal grazers to
589 pH. Striped areas represent the 95% C.I..

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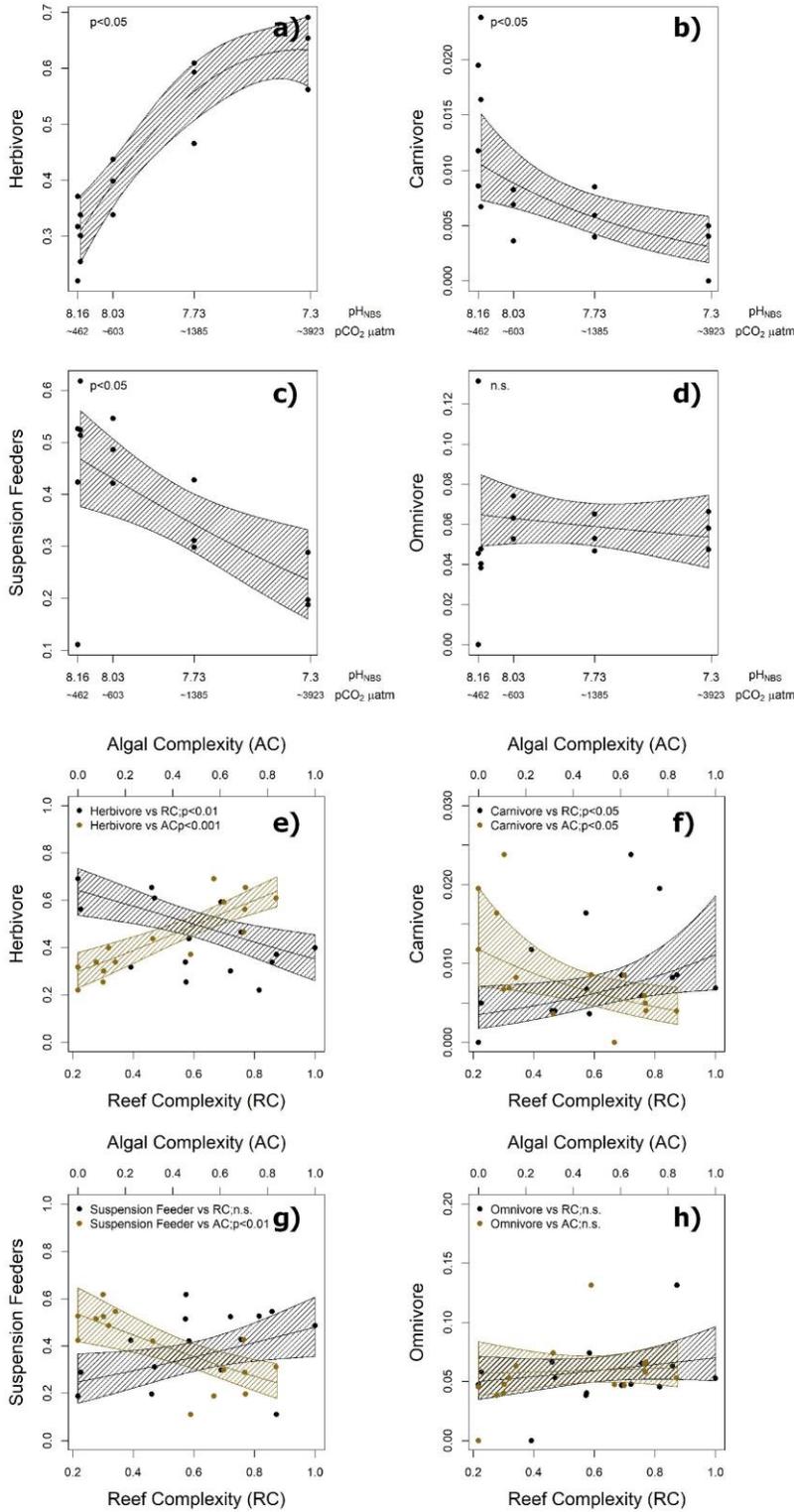
592 **Figure 1**



593

594 **Figure 2**

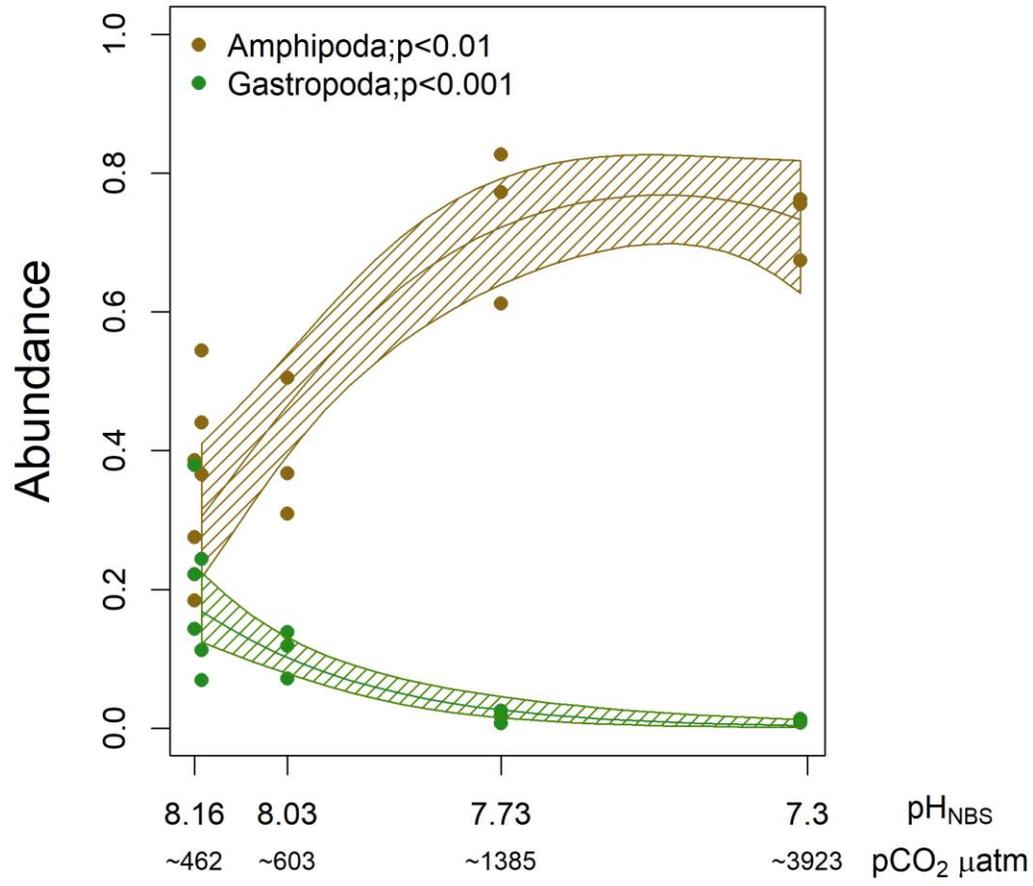
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597 **Figure 3**

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600 **Figure 4**