

2017-09-11

Natural acidification changes the timing and rate of succession, alters community structure, and increases homogeneity in marine biofouling communities

Brown, NEM

<http://hdl.handle.net/10026.1/10083>

10.1111/gcb.13856

Global Change Biology

Wiley

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

1 This is the author's accepted manuscript. The final published version of this work (the version of record)
2 is published by Wiley in Global Change Biology. The accepted manuscript was made available online on
3 the 11 September 2017 at: <http://onlinelibrary.wiley.com/doi/10.1111/gcb.13856/abstract>. This work is
4 made available online in accordance with the publisher's policies. Please refer to any applicable terms of
5 use of the publisher.

6 **i) Title:** Natural acidification changes the timing and rate of succession, alters community
7 structure, and increases homogeneity in marine biofouling communities

8 **ii) Running Head:** $p\text{CO}_2$ accelerates biofouling succession

9 **iii) List of authors:** **Norah E. M. Brown***¹, **Marco Milazzo**², **Sam P. S. Rastrick**^{3,4}, **Jason M.**
10 **Hall-Spencer**^{5,6}, **Thomas W. Therriault**⁷, and **Christopher G. D. Harley**^{1,8}

11 **iv) Institute or laboratory of origin:** ¹ Department of Zoology, University of British Columbia,
12 Vancouver, BC, Canada ² DiSTeM, CoNISMa, University of Palermo, Palermo, Italy ³ Ocean
13 and Earth Science, National Oceanography Centre Southampton, University of Southampton
14 Waterfront Campus, European Way, Southampton SO14 3ZE, UK. ⁴ Institute of Marine
15 Research, PO Box 1870 Nordness, 5870 Bergen, Norway. ⁵ Marine Biology and Ecology
16 Research Centre, University of Plymouth, UK, ⁶ Shimoda Marine Research Centre, Tsukuba
17 University, Japan ⁷ Fisheries and Oceans Canada, Pacific Biological Station, Nanaimo, BC,
18 Canada. ⁸ Institute for the Oceans and Fisheries, University of British Columbia, Vancouver, BC,
19 Canada.

20 **v) *Corresponding author:** nbrown@zoology.ubc.ca, (604) 612-4594

21 **vi) Keywords:** Ocean acidification, climate change, natural analogue, community, marine
22 biodiversity.

23 **vii) Type of paper:** Primary research article
24

25 **Abstract**

26 Rising carbon dioxide concentrations are rapidly altering the carbonate chemistry of the oceans
27 and this phenomenon, termed ocean acidification, may have far-reaching consequences for
28 marine community and ecosystem dynamics. Such impacts remain poorly understood due to the
29 difficulty of manipulating $p\text{CO}_2$ at the ecosystem level to mimic realistic fluctuations that occur
30 on a number of different timescales. It is unclear how robust ecosystems are to intermediate-
31 scale $p\text{CO}_2$ change; how quickly can communities at various stages of development respond to
32 acidification, and, if high $p\text{CO}_2$ is relieved mid-succession, do these effects persist, are the
33 effects reversed by alleviation of $p\text{CO}_2$ stress, or are the effects worsened by departures from
34 prior high $p\text{CO}_2$ conditions to which organisms had acclimatized. As nearshore CO_2 often
35 fluctuates throughout succession, a further unresolved issue is the relative importance of direct
36 acidification effects on primary colonization vs. indirect effects. Here, we used reciprocal
37 transplant experiments along a shallow water volcanic $p\text{CO}_2$ gradient to assess the importance of
38 the timing and duration of high $p\text{CO}_2$ exposure (i.e. discrete events at different stages of
39 successional development vs. continuous exposure) on patterns of colonization and succession in
40 a benthic fouling community. We monitored community development both before and after
41 reciprocal transplantation of communities at eight weeks until the end of the experiment at
42 twelve weeks. We show that succession at the acidified site was initially delayed (less
43 community change by eight weeks) but then accelerated over the next four weeks. These changes
44 in succession led to homogenization of communities maintained in or transplanted to acidified
45 conditions, and altered community structure in ways that reflected both short- and longer-term
46 acidification history. These community shifts are likely a result of interspecific variability in
47 response to increased $p\text{CO}_2$ and changes in species interactions. High $p\text{CO}_2$ altered biofilm
48 development, allowing serpulids to do best at the acidified site by the end of the experiment,

49 although early (pre-transplant), negative effects of $p\text{CO}_2$ on recruitment of these worms was still
50 detectable. The ascidians *Diplosoma* sp. and *Botryllus* sp. settled later and were more tolerant to
51 acidification. Overall, transient and persistent acidification-driven changes in the biofouling
52 community, via both current and post exposure, could have important implications for ecosystem
53 function and food web dynamics.

54

55 **Introduction**

56 Anthropogenic carbon dioxide enrichment of the atmosphere and subsequent decreases in pH in
57 the ocean are well documented (Feely *et al.*, 2004; Tans, 2009). The rate of change of ocean pH
58 is unprecedented in recent geological history (Hönisch *et al.*, 2012) and the biological
59 implications of these rapid chemical changes are being realized across a wide range of taxa
60 (Kroeker *et al.*, 2013a; Wittmann & Pörtner, 2013). In coastal marine ecosystems, changes in
61 seawater $p\text{CO}_2$ are occurring on a number of different timescales: diurnally with photosynthesis
62 and respiration, days to weeks with the lunar cycle and upwelling dynamics, seasonally due to
63 both biotic and abiotic forcing, and over many decades with anthropogenic forcing (Hofmann *et*
64 *al.*, 2011; Boyd *et al.*, 2016; Henson *et al.*, 2017). It is clear that incorporating natural
65 fluctuations in $p\text{CO}_2$ is necessary for making better predictions of the biological response to
66 ocean acidification (Shaw *et al.*, 2013; Small *et al.*, 2015; Boyd *et al.*, 2016). Although a few
67 studies have addressed this point at diurnal timescales (e.g. Clark & Gobler, 2016; Li *et al.*,
68 2016), it is unclear how marine ecosystems respond to longer term (weeks to months) $p\text{CO}_2$
69 fluctuations and whether these effects can be reversed (transient vs. persistent effects) if $p\text{CO}_2$
70 stress is relieved (but see Vaz-Pinto *et al.*, 2013).

71 In communities influenced by disturbance (i.e., most communities), the effects of fluctuating
72 $p\text{CO}_2$ will be mediated by direct effects of acidification on settlement and recruitment and by
73 indirect effects mediated by the interactions between early and later successional species. Ocean
74 acidification can influence settlement of planktonic stages and recruitment into benthic
75 populations (Cigliano *et al.* 2010; Brown *et al.*, 2016), and resident organisms influence
76 subsequent settlement (i.e. secondary colonization, Osman *et al.*, 1989). However, the
77 importance of colonization history in shaping community structure and succession in light of
78 $p\text{CO}_2$ or pH heterogeneity at a variety of temporal scales has yet to be addressed. One challenge
79 to disentangling effects of acidification on the succession and development of marine
80 communities is that, to date, our understanding of the biological effects of ocean acidification is
81 primarily informed by studies of single species in isolation. Such studies show how ocean
82 acidification might influence organisms through changes in energetic demand (Garilli *et al.*,
83 2015; Harvey *et al.*, 2016), reproduction and development (Ross *et al.*, 2011), growth rate
84 (Kroeker *et al.*, 2013a), development of defensive structures (Sanford *et al.*, 2014), and
85 behaviour (Milazzo *et al.*, 2016). However, it is not easy to extrapolate from single-species
86 studies to assess the effects of ocean acidification on community development, structure, and
87 function. To anticipate ecosystem-level changes, it is essential to understand responses of multi-
88 species assemblages to acidification. Early studies exposed pre-settled communities to
89 acidification in laboratory conditions (e.g. Hale *et al.*, 2011), but deeper understanding of
90 recruitment and settlement processes requires *in-situ* $p\text{CO}_2$ manipulation (e.g. Brown *et al.*,
91 2016) or observation.

92 Shallow water CO_2 seeps allow the study of intact communities and have been increasingly used
93 as natural laboratories, providing insights into the community- and ecosystem-level effects of

94 acidification. Changes in community composition, structure, and losses in diversity have been
95 documented along natural $p\text{CO}_2$ gradients for both macro-algal (Kroeker *et al.*, 2011; Porzio *et*
96 *al.*, 2011; Johnson *et al.*, 2012; Connell *et al.*, 2013; Baggini *et al.*, 2014, 2015; Linares *et al.*,
97 2015) and macro-invertebrate (Hall-Spencer *et al.*, 2008; Cigliano *et al.*, 2010; Fabricius *et al.*,
98 2011, 2014; Donnarumma *et al.*, 2014; Goodwin *et al.*, 2014) communities. A striking pattern of
99 community change over these pH gradients consistently is a shift away from calcifying taxa (e.g.
100 coralline algae, molluscs) towards non-calcified species (e.g. fleshy brown algae, anemones).
101 These patterns are driven by a combination of direct effects, such as the dissolution of calcareous
102 shells/skeletons combined with higher energetic costs associated with calcification (Wittmann &
103 Pörtner, 2013), and effects mediated by species interactions such as changes in competition,
104 predation and habitat structure (Connell *et al.*, 2013; Kroeker *et al.*, 2013b; Linares *et al.*, 2015;
105 Sunday *et al.*, 2017). Although community-level outcomes (and the species interactions that
106 underlie them) have been documented for a wide range of marine communities, the effects of
107 $p\text{CO}_2$ on recruitment, succession and development have been mainly investigated in algal
108 communities (Kroeker *et al.* 2011; 2012; 2013b) and similar studies are lacking for marine
109 invertebrates (although see Brown *et al.*, 2016). Furthermore, the effects of the timing and
110 duration of acidification events during succession have seldom been addressed, although the
111 response of marine communities to acidification has been shown to change with seasonality (e.g.
112 Baggini *et al.*, 2014) and with timing and length of upwelling events (Iles *et al.*, 2012).

113 At CO_2 seeps, within-seep vs. outside-seep recruitment is difficult to disentangle and life history
114 strategy may determine the extent of direct effects of acidification on species-specific
115 recruitment. If recruits are coming from within-seep source populations, which is most likely for
116 species with short pelagic larval phases, observed recruitment effects (both positive and

117 negative) could represent a culmination of both direct effects of acidification on larvae (e.g.,
118 physiological, Kurihara *et al.*, 2008; Ross *et al.*, 2011; Przeslawski *et al.*, 2015; and/or
119 behavioural Doropoulos *et al.*, 2012; Doropoulos & Diaz-Pulido, 2013; Webster *et al.*, 2013),
120 transgenerational effects of acidification on nearby adult populations (Calosi *et al.*, 2013a;
121 Harvey *et al.*, 2016; Ross *et al.*, 2016), and multigenerational adaptation to chronic acidified
122 conditions (Calosi *et al.*, 2013a). In contrast, propagules arriving from outside the seeps will not
123 experience generational effects of acidification.

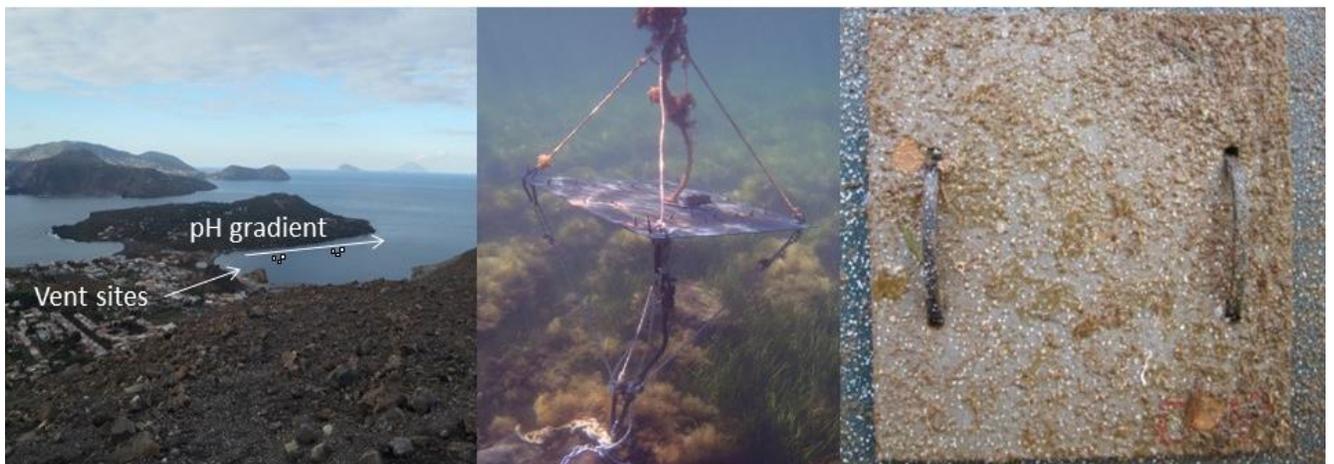
124 In this study, we observed how benthic marine invertebrate communities recruited and developed
125 along a natural $p\text{CO}_2$ gradient. We used reciprocal transplant experiments to determine if fouling
126 communities would reflect a lasting response to short but discrete exposure to elevated $p\text{CO}_2$
127 (e.g. during an upwelling event) in early succession or reflect only most recent exposure to
128 elevated $p\text{CO}_2$ regardless of prior conditions. We expected that $p\text{CO}_2$ would disrupt succession
129 by altering recruitment of primary vs. secondary colonizers and alter the interactions among
130 these taxa via inhibition or facilitation (Connell & Slatyer, 1977). We predicted that the relative
131 importance of discrete exposure early on in succession, continuous exposure throughout
132 succession or exposure later in succession on a given species would depend on life history traits
133 of species. For example, if timing of recruitment coincides with timing of acidification, a discrete
134 exposure to CO_2 that occurs early on in succession may influence primary colonizers more than
135 secondary colonizers. Other factors, like environmental tolerances, growth rate, ability to induce
136 a resting stage, and concentration of propagules may determine how a given taxa responds to
137 and/or rebounds from a short and discrete CO_2 event. At the community level, we hypothesized
138 that acidification would result in homogenized, low-diversity communities of biofouling
139 organisms, dominated by a few weed-like species with notable reductions in calcified taxa.

140 **Materials and Methods**

141 *Site description and experimental set up*

142 The study was conducted in Levante Bay on Vulcano Island (38°25'08"N, 14°57'40"E) in the
143 Aeolian archipelago in Northeastern Sicily (Italy) from March to June, 2013 (12 weeks). At this
144 site, shallow volcanic seeps emit carbon dioxide bubbles that create a gradient in seawater
145 carbonate chemistry that has been characterized by a number of recent studies (Arnold *et al.*,
146 2012; Johnson *et al.*, 2012; Lidbury *et al.*, 2012; Boatta *et al.*, 2013; Calosi *et al.*, 2013b;
147 Milazzo *et al.*, 2014). The biogeochemistry of the bay has been assessed to identify the most
148 suitable areas for ocean acidification research (see Boatta *et al.*, 2013; Vizzini *et al.*, 2013) and
149 the chosen sites were outside of any measured metal contamination. Variability in the pH
150 gradient at the site is predominantly driven by currents influenced by westerly winds, and as
151 such, the acidified water masses mostly run parallel to the northern shoreline of the bay (Boatta
152 *et al.*, 2013). When winds are high, e.g. during storms that are common in winter and early
153 spring, low pH waters are more restricted to the immediate vicinity of the seeps and do not
154 extend as far along the shoreline (Boatta *et al.* 2013). We used two sites, ~70 m apart, along the
155 $p\text{CO}_2$ gradient; the first had low mean pH (7.78), and the second had ambient mean pH (8.10)
156 (here we use high $p\text{CO}_2$ and low pH interchangeably). Our sites correspond to sites 40-60 and
157 120-130 in Boatta *et al.*, (2013) for low pH and control pH respectively. We monitored
158 temperature, salinity, and pH at each site at least once every two weeks throughout the
159 observation period but daily for the first week and the last six weeks of the experiment. Samples
160 for total alkalinity were taken every two weeks. We used these parameters to calculate
161 dissolved inorganic Carbon (DIC), $p\text{CO}_2$, HCO_3^- , and carbonate and aragonite saturation states
162 using the CO2-SYS program (Pierrot *et al.* 2006).

163 At each site, we suspended 70.5 × 70.5 cm semi-flexible PVC panels from buoys 1 m from the
164 surface and 1 m from the bottom (n=3 panels per site). We deployed the panels on similar
165 substrata at each site, which comprised of subtidal boulders and patches of seagrass. Each panel
166 was oriented horizontally (Fig 1), and 15 PVC tiles (14.5 cm x 14.5 cm) were secured to the
167 underside of each panel using cable ties (n=15 tiles nested within each of 3 panels per site,
168 therefore n=45 tiles per site). The panels were secured to the buoys and anchors using ropes to
169 avoid horizontal spinning. Storms damaged some tiles and panels, so only undamaged tiles
170 (n=20 per site, from across the three panels) were used in the analyses. After 8 weeks, we
171 reciprocally transplanted a subset of 10 tiles (selected randomly from the 20 total) from each
172 $p\text{CO}_2$ regime, while the other 10 tiles were not transplanted, to determine if $p\text{CO}_2$ effects on
173 recruitment occur early and persist or arise later during succession. At the time of this
174 transplantation, we formed new panels (2 per site) with ten tiles each, tiles from transplanted and
175 non-transplanted groups were distributed randomly to the two panels in each site.



176
177 **Fig. 1.** Photographs depicting sites (white symbols) along the pH gradient (left) and the panel
178 and tile system. Panels (centre), with downward facing PVC tiles (right) attached to the
179 underside, were suspended ~1m from both surface and bottom using a buoy and anchor.

180

181 *Species- and community- level measures*

182 Photographs (one per tile) were taken every two weeks to determine changes in community
183 composition, structure, Shannon's diversity, richness, stability over time and percent cover (point
184 count) of primary and secondary colonizing fouling species for eight weeks. After
185 transplantation, the tiles were left for one additional month before being photographed and
186 retrieved for preservation. The tiles and panels were brought to the surface for photography. We
187 conducted all photographic analyses in ImageJ (Schneider *et al.*, 2012) and identified species to
188 lowest taxonomic level possible in the laboratory. Primary colonizers were defined as those that
189 recruited in the ambient site in the first four weeks, before the community reached 100% cover.
190 Primary colonizers included two serpulid polychaete taxa (serpulidae, spirorbidae) and two algal
191 guilds, a turf-forming green filamentous alga *Cladophora* sp., and a biofilm which was
192 ubiquitous and has been described at this site as primarily a mix of diatoms and cyanobacteria,
193 the composition of which changes along gradients of $p\text{CO}_2$ (Johnson *et al.*, 2015). Secondary
194 colonizers (i.e., those that recruited only after space occupancy reached 100% at week four)
195 included bryozoans (e.g. *Schizomavella* sp. and *Patinella radiata*), ascidians (*Botryllus* sp. and
196 *Diplosoma* sp.), and *Sphacelaria* sp., which is a branched red alga. These secondary colonizers
197 may require a layer of biofilm in order to recruit, or their propagules may have only be in the
198 water column during the latter half of the experiment due to seasonal or episodic reproductive
199 patterns. Natural succession in marine communities often coincides with a disturbance regime
200 (e.g. winter storms, sedimentation) that creates space (Sousa, 1979). The disturbance regime at
201 our study site may coincide with seasonality, and we expect that the succession observed from
202 bare surfaces reflected both directional community development and response to warming
203 temperatures over time.

204 *Statistical analyses*

205 All statistical analyses were performed using open-source R (R Development Core Team, 2009).
206 We used the *adonis* and *Betadisper* functions in the *Vegan* package (Oksanen *et al.*, 2015) to
207 analyze multivariate community structure (PERMANOVA test) and homogeneity of dispersions,
208 respectively. Community structure analyses were conducted on species abundance data (percent
209 cover) of a given tile, standardized by total abundance of that species across all tiles, putting
210 abundances of ecologically different species on the same scale. This community structure metric
211 was calculated twice: pre-transplant using data from week 8, and post-transplant using data from
212 week 12. We then calculated a Bray Curtis dissimilarity matrix on the standardized data. In all
213 tests, for a given week, we used site as a fixed factor and separately tested for the effect of panel
214 within site, and if initial or final panel (nested within initial or final site, respectively) had a
215 significant effect, we included the term in the full model. To test if acidification influenced
216 community structure at week 12 we used the 10 non-transplanted replicates per site. Next, to
217 analyze if, during the reciprocal transplant, initial or final site or their interaction influenced
218 community structure, we used all 20 tiles (10 of which had been transplanted from the other site).
219 We calculated pre- and post-transplant community stability, the temporal mean over temporal
220 variability of species abundances in a given tile, using the *community.stability* function in *codyn*
221 package (Hallett *et al.*, 2016).

222 We used both linear mixed effects models (LMEs, *lme4* package, Bates *et al.*, 2015) and
223 generalized liner mixed-effects models (GLMMs, *glmmADMB* package, Fournier *et al.*, 2012;
224 Skaug *et al.*, 2013) to analyze percent cover and count data (from photographs), community
225 stability, and proportion of secondary colonizers. For the first 8 weeks (pre-transplantation), we
226 used site as a fixed effect (n=20 per site), after which (post-transplantation) we used initial site,

227 final site, and their interaction as fixed effects to incorporate tiles that were reciprocally
228 transplanted (n=10 per site) and those that were not (n=10 per site). For a given point in time, tile
229 was the level of replication and we treated both initial and final panels nested within site as
230 random factors and all models included a random intercept. Numerical response variables
231 (species richness, cumulative counts) were considered either normally distributed and analyzed
232 with LMEs or Poisson or negative binomially distributed and analyzed with GLMMs, to assess
233 effect of site, depending on distribution fit to the data. We assumed either a Beta or Binomial
234 distribution (based on fit of distribution to data) for percent cover data and analyzed the effect of
235 site with GLMMs. We used a GLMM with assumed Gamma distribution to analyze the effect of
236 acidification on Shannon's diversity.

237

238 **Results**

239

240 *Seawater parameters*

241 Seawater pH fluctuated with time of day and wind direction. However, pH was consistently
242 lower in the low pH site compared to the ambient site ($\Delta\text{pH}_{\text{NBS}} = -0.32 \pm 0.19$, mean \pm SE, daily
243 differences between sites averaged across experimental period, Table 1, LM, $F=141.7$,
244 $P<0.0001$). Total alkalinity was consistent across sites at a given time point ($\Delta\text{TA} = 3.45 \pm 1.38$,
245 Table 1, LM, $F=3.17$ $P=0.08$). During the experimental period, temperatures ranged from 14.4°C
246 to 20.8°C and salinity from 37.8 to 38.6. Differences in temperature ($\Delta\text{temperature} = 0.01^\circ\text{C} \pm$
247 0.15°C , LM, $F=0.0019$, $P=0.97$) and salinity ($\Delta\text{salinity} = 0.00 \text{ ppt} \pm 0.01 \text{ ppt}$, LM, $F=0$, $P=1$)
248 were negligible between sites during the survey.

249

250

251
252
253
254

Table 1. Carbonate chemistry of seawater from ambient and low pH sites. Temperature, salinity, pH_{NBS}, and total alkalinity were collected from March to June 2013 (mean ± SE, n = 98). Asterisks indicate calculated values in the CO₂-SYS program (Pierrot et al. 2006).

Seawater parameter	Control	Low pH	255
Temperature (°C)	18.96±0.15	18.97±0.15	256
Salinity	38.19±0.011	38.19±0.010	257
pH _{NBS}	8.10±0.13	7.78±0.24	258
Alkalinity (μmol kg ⁻¹)	2523.52±1.34	2526.97±1.41	259
pCO ₂ (μatm)*	557.69±26.48	1499.91±151.71	260
DIC (μmol kg ⁻¹)*	2271.16±6.42	2424.09±10.38	261
HCO ₃ ⁻ (μmol kg ⁻¹)*	2066.59±9.62	2270.29±10.15	262
Ω Calcite*	4.33±0.096	2.43±0.093	263
Ω Aragonite*	2.82±0.063	1.59±0.060	263

264 *Primary colonization pre- and post-transplant*

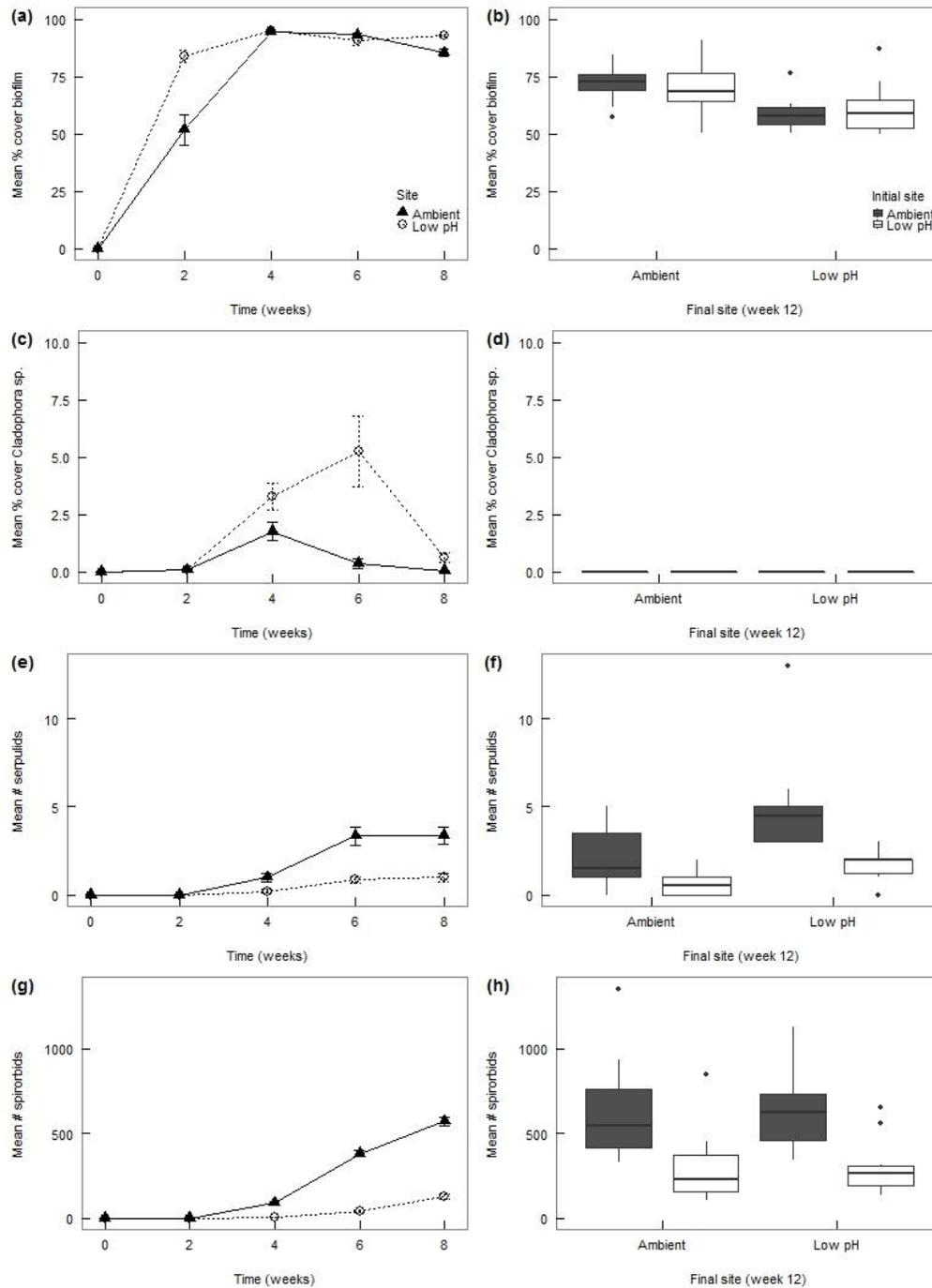
265 All tiles were colonized initially by biofilm. The biofilm grew more rapidly, and peaked and
 266 declined in cover earlier on tiles at the acidified site relative to the ambient site. After eight
 267 weeks, biofilm cover was higher on tiles at the acidified site than the ambient site (Fig. 2a;
 268 statistics are summarized in Table 2). Filamentous alga, *Cladophora* sp., had higher cover at the
 269 acidified site at its peak, although by week 8 there was no difference in cover between sites
 270 (Table 2, Fig. 2c). Calcified polychaetes – serpulid and spirorbid worms – had at least a two-
 271 week delay in recruitment at the acidified site, and, by week 8, covered significantly less space
 272 (Table 2, Fig. S1a, c) and had less than a third the density at the acidified site relative to the
 273 ambient site (Table 2, Fig. 2e, g). Both serpulids and spirorbids would be considered primary
 274 colonizers under ambient conditions but classified as secondary colonizers (arriving after 100%
 275 space occupancy had been reached) under acidified conditions.

276 **Table 2.** Statistical results from both GLMM (using z statistic) and LME (using X^2) from analysis of percent cover
 277 of a given species. ^P indicates week in which peak % cover of this species occurred.

Group	Species	Abundance measure	Week 8 (P=peak)	Mean abundance ambient site	Mean abundance low pH site	z or X^2 *	P * <0.05
	Biofilm complex	% cover	8	85.6	93.1	-4.43	<0.0001*
	<i>Cladophora</i> sp.	% cover	8	0.050	0.62	-1.44	0.15
	Serpulids	% cover	8 ^P	0.52	0.30	3.33	<0.001*
		# individuals		3.4	1.0	4.81	<0.001*
	Spirorbids	% cover	8 ^P	7.06	2.05	3.05	<0.001*
		# individuals		572.3	129.1	14.4*	<0.001*
	<i>Diplosoma</i> sp.	% cover	8	5.0	2.0	1.79	0.074
		# colonies		2.0	0.4	5.22*	0.022*
	<i>Botryllus</i> sp.	% cover	8	0.44	0.69	-1.95	0.051
		# colonies		1.9	2.8	-1.30	0.19
	Thin ramified bryozoan	% cover	8	0.69	0.07	2.57	0.01*
	<i>Patinella radiata</i>	% cover	8	0.050	0.074	-0.19	0.85
		# colonies		2.2	2.8	-0.30	0.76
	<i>Schizomavella</i> sp.	% cover	8	0.15	0.025	0.98	0.33
		# colonies		1.2	0.2	2.35	0.0019*

278 Eight weeks into the experiment, a subset of tiles was reciprocally transplanted among sites. One
 279 month after transplantation, biofilm cover was related to only the most recent exposure to $p\text{CO}_2$
 280 (i.e. tiles maintained in or transplanted to low pH) as coverage was lower on tiles that ended up
 281 at the acidified site than those in the ambient site, (GLMM, Final site $P=0.050$, Table 3, Fig.2b),
 282 regardless of origin, and there was no evidence of the pre-transplant effects of $p\text{CO}_2$ carrying
 283 over (GLMM, Initial site $P=0.69$, Table 3, Fig.2b). *Cladophora* sp. cover was reduced to zero
 284 after 8 weeks, therefore we were unable to determine if $p\text{CO}_2$ was more important in early or late
 285 succession for this taxon. Transplant results suggested that serpulid recruitment was influenced
 286 by $p\text{CO}_2$ early on and persisted, although there were also $p\text{CO}_2$ effects present during late
 287 succession. Overall, tiles that originated at the ambient site recruited more serpulid individuals

288



289

290 **Fig. 2.** Abundance of selected primary colonizers in ambient and low pH sites over time. Left-
 291 hand panels are trends through week 8 (i.e., pre-transplantation; n=20) and right-hand panels are
 292 patterns at week 12 (post-transplantation; n=10) of both transplanted and non-transplanted tiles.
 293 For the right-hand panels, shading indicates initial site and position on x-axis indicates final site.
 294 Species are: (a, b) biofilm (% cover), (c, d) *Cladophora* sp. (% cover), (e, f) serpulids (#
 295 individuals) and, (g, h) spirorbids (# individuals). Error bars indicate standard error.

296 **Table 3.** Results of GLMMs using percent cover of a given species and initial site, final site and their interaction as
 297 fixed effects (n=20).

Group	Species	Initial site		Final site		Initial site * Final site	
		<i>z</i>	<i>P</i> * <i><0.05</i>	<i>z</i>	<i>P</i> * <i><0.05</i>	<i>z</i>	<i>P</i> * <i><0.05</i>
	Biofilm complex (% cover)	-0.40	0.69	-1.96	0.050*	0.49	0.62
	<i>Cladophora</i> sp. (% cover)	0.0	1.0	0.0	1.0	0.0	1.0
	Serpulids (% cover)	-1.83	0.067	3.06	0.0022*	-0.65	0.52
	(# individuals)	-2.51	0.012*	3.19	0.0014*	0.22	0.83
	Spirorbids (% cover)	-0.79	0.43	0.81	0.42	-0.47	0.64
	(# individuals)	-2.33	0.02*	0.46	0.65	-0.17	0.87
	<i>Diplosoma</i> sp. (% cover)	0.13	0.89	0.80	0.43	1.68	0.093
	(# colonies)	-1.62	0.11	-0.80	0.42	0.82	0.41
	<i>Botryllus</i> sp. (% cover)	0.49	0.63	1.19	0.26	-0.48	0.63
	(# colonies)	-0.02	0.99	0.27	0.79	0.46	0.4
	Thin ramified bryozoan (% cover)	0.30	0.77	3.41	<0.001*	-2.21	0.027*
	<i>Patinella radiata</i> (% cover)	-0.20	0.84	1.38	0.17	-0.49	0.62
	(# colonies)	-0.04	0.97	-0.53	0.60	0.58	0.56
	<i>Schizomavella</i> sp. (% cover)	-2.59	0.0096*	-0.70	0.49	0.29	0.77
	(# colonies)	-0.34	0.73	0.20	0.84	-0.31	0.76

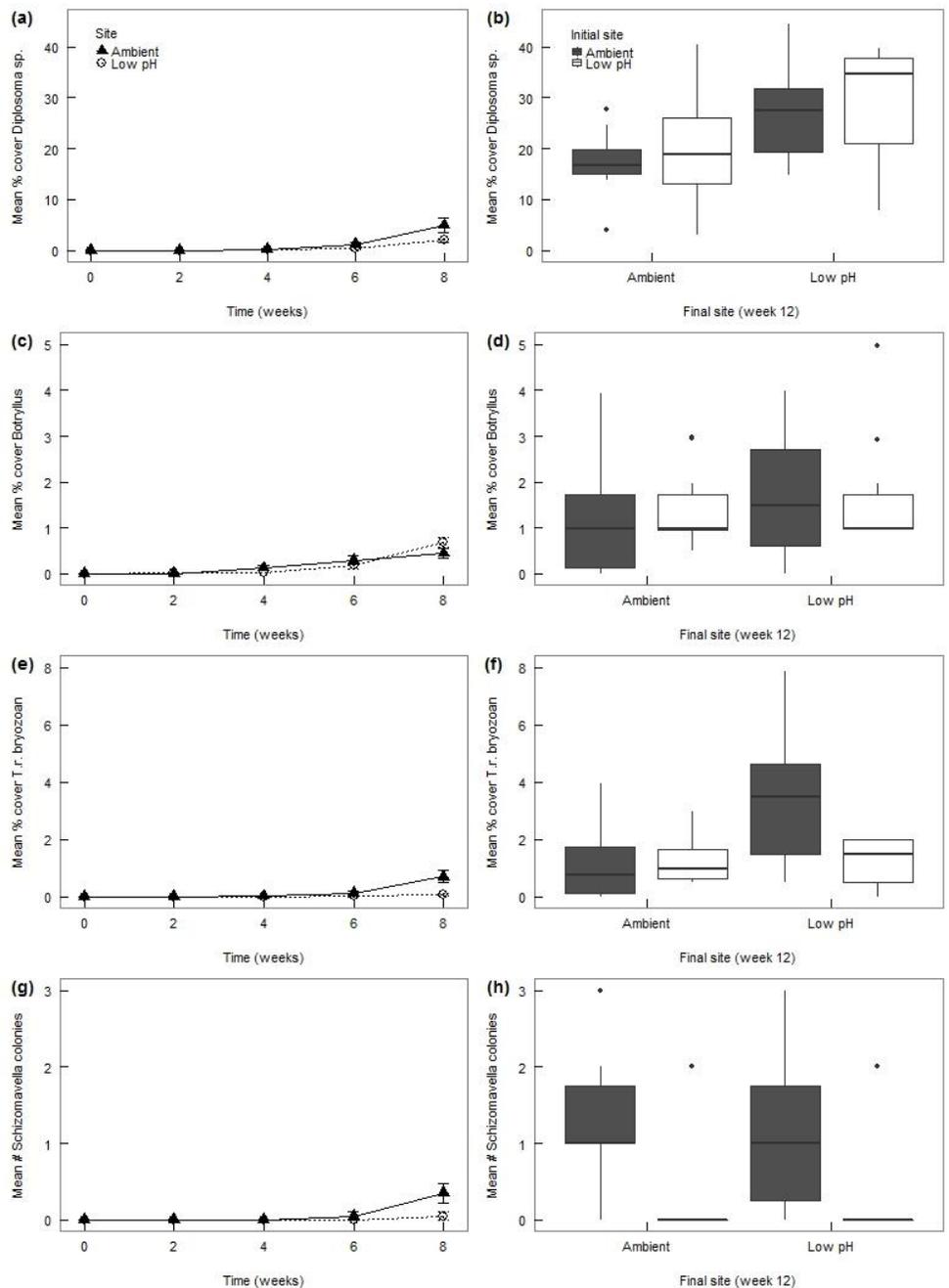
298

299 than those that originated at the acidified site, regardless of final site (GLMM, Initial site
300 $P=0.012$, Table 3, Fig. 2f) and cover of this species showed a non-significant trend in the same
301 direction (Initial site $P=0.067$, Table 3). However, by the end of the experiment, there were more
302 individuals and higher cover of serpulids on tiles that had final exposure to high $p\text{CO}_2$, rather
303 than ambient conditions (GLMM, Final site $P=0.0014$, Table 3, Fig. 2f, Fig. S1b). Initial site
304 alone influenced the number of individuals, but not cover, of spirorbids, such that tiles that
305 originated in the ambient site had more spirorbids, regardless of their final locations (GLMM,
306 Initial site $P=0.02$, Table 3, Fig. 2h, Fig. S1d).

307 *Secondary colonization pre- and post-transplant*

308 Fewer colonies of the colonial ascidian, *Diplosoma* sp., recruited by week 8 at the acidified site
309 (Table 2, Fig. S1e) but these colonies covered a similar amount of space in both sites (Table 2,
310 Fig. 3a). At the same time point, another colonial ascidian, *Botryllus* sp., had similar cover
311 between sites (Table 2, Fig. 2c) and no difference in number of recruiting colonies between sites
312 (Table 2, Fig. S1g). Bryozoans, a phylum with a broad range of morphologies, yielded mixed
313 responses to acidification. At week 8, an erect calcitic bryozoan, *Patinella radiata*, had both
314 similar cover (Table 2) and number of colonies at the acidified site compared to the ambient site
315 (Table 2, Fig. S1i). Thin ramified bryozoan had higher cover under ambient conditions while
316 essentially absent from the acidified site (Table 2, Fig. 3e), while the encrusting aragonitic
317 bryozoan, *Schizomavella* sp. recruited fewer colonies on tiles at the acidified site compared to the
318 ambient site (Table 2, Fig. 3g) but had similar cover (Table 2) between sites at week 8.

319 After the transplant experiment, soft-bodied ascidians appeared to be largely resistant to changes
320 in acidification. Neither initial site nor final site influenced cover or number of *Diplosoma* sp. or
321 *Botryllus* sp. (Fig. 3b,d, Fig. S1f,h Table 3). The bryozoan *P. radiata* remained resistant to
322 acidification after transplantation, and there was neither an effect of initial nor final site on
323 numbers of colonies and cover of this species (Table 3). Post-transplant thin ramified bryozoan
324 cover was influenced by final site, as overall cover was higher on tiles that ended up at the
325 acidified site (Final site $P < 0.001$), and this effect was especially strong for tiles transplanted
326 from the ambient site (Initial*final site $P = 0.027$, Table 3, Fig. 3f). Early successional $p\text{CO}_2$
327 effects were apparent in post-transplant recruitment of *Schizomavella* sp., such that tiles that
328 originated in the ambient site had more colonies than those from the low pH site (Initial site
329 $P = 0.0096$, Table 3, Fig. 3h).



330

331 **Fig. 3.** Abundance of selected secondary colonizers in ambient and low pH sites over time. Left-
 332 and right-hand panels as in Figure 2. Species are: (a, b) *Diplosoma* sp. (% cover), (c, d) *Botryllus*
 333 sp. (% cover), (e, f) Thin ramified bryozoan (% cover) and, (g, h) *Schizomavella* sp. (# colonies).
 334 Error bars indicate standard error.

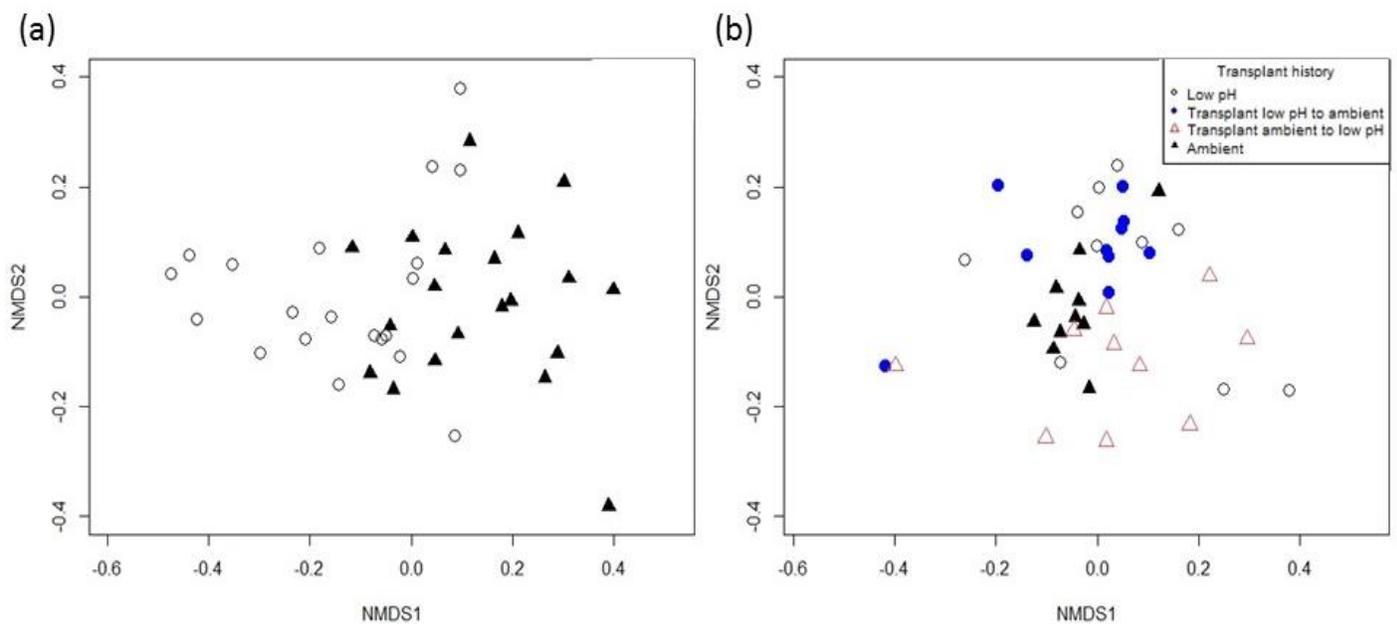
335

336

337 *Community level results pre- and post-transplant*

338 The above changes in species recruitment and succession culminated in significant shifts in
339 community structure on tiles at the acidified site at week 8 (PERMANOVA, $R^2=0.16$, $P=0.0001$,
340 Fig. 4a). Although community structure differed, there was no difference in community variance
341 between sites (Betadisper, $F=0.63$, $P=0.80$, Fig. 4a). Tiles were colonized more quickly under
342 acidified conditions than those at the ambient site but this difference was only apparent for the
343 first four weeks, and the tiles had similar cover at the 8th week (LME, $X^2=2.16$, $P=0.14$).

344



345

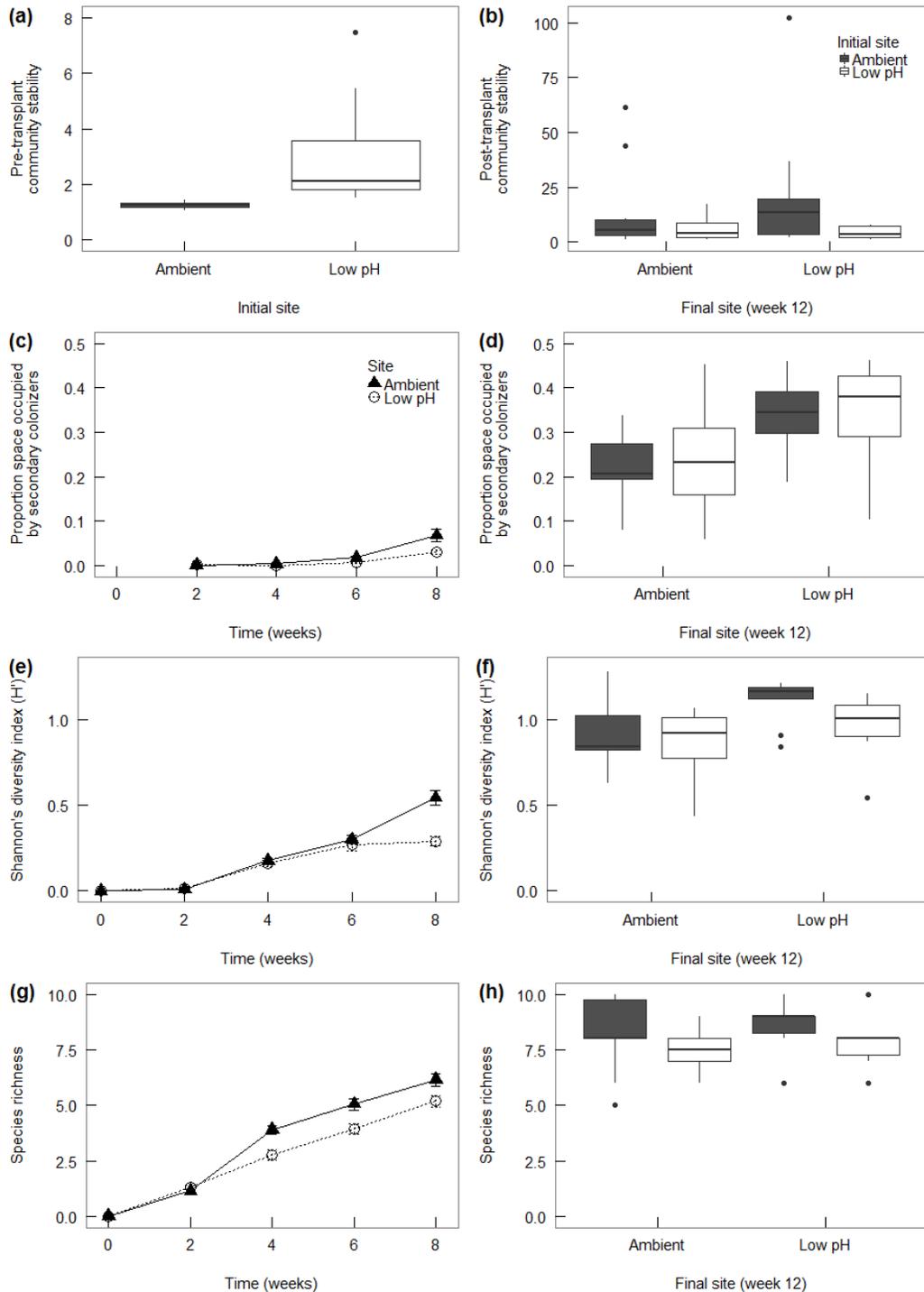
346 **Fig. 4.** nMDS ordination plot showing the relationship between communities after (a) 8 weeks on
347 tiles from low pH (open circles) vs. the ambient site (solid black triangles), $n = 20$ tiles, and (b)
348 12 week on tiles that either remained in low pH (open circles), were transplanted from low pH to
349 the ambient site (solid blue circles), were transplanted from ambient site to low pH site (red open
350 triangles), or remained in the ambient site (solid black triangles), $n = 10$ tiles.

351

352 Pre- transplant community stability was higher for tiles at the acidified site than the ambient site
353 (LME, $X^2=19.5$, $P<0.0001$, Fig. 5a), indicating that over this time period, communities at the
354 ambient site changed more than those at the acidified site. Secondary colonizers, which arrived
355 after the 4th week, initially gained cover at the same rate in both sites but by the 8th week took up
356 more space at the ambient site than at the acidified site (LME, $X^2=4.04$, $P=0.044$, Fig. 5c),
357 indicating that succession from primary to secondary species occurred earlier at the ambient site.
358 We observed negative effects of acidification on diversity (GLMM, $z=3.41$, $P=0.00065$, Fig. 5e),
359 but species richness was similar between sites (GLMM, $z=1.25$, $P=0.21$, Fig. 5g) after 8 weeks.

360 After transplantation, both initial site and final site influenced community structure, i.e.
361 communities that originated at the ambient site were different overall than those that originated
362 at the acidified site (PERMANOVA, initial site, $R^2=0.10$, $P=0.0001$, Fig 4b: triangles vs. circles)
363 and communities that ended at the acidified site differed from those that ended at the ambient site
364 (PERMANOVA, final site, $R^2=0.053$, $P=0.013$, Fig. 4b: open vs. solid symbols). There was no
365 evidence of an interaction between the effects of pCO_2 on early and late succession
366 (PERMANOVA, initial*final site, $R^2=0.027$, $P=0.26$, Fig. 4). In addition, although there was no
367 evidence that pCO_2 affected community variability early in succession (Betadisper, initial site,
368 $F=0.14$, $P=0.71$, Fig 4: triangles vs. circles), there was an influence of final site on variability –
369 as tiles that were transplanted to the high pCO_2 site were significantly less variable than those
370 that ended at the ambient site (Betadisper, final site, $F=8.04$, $P=0.0073$, Fig.4: open symbols are
371 more dispersed than solid symbols). Community stability between the 8th and 12th week was
372 similar between sites, regardless of transplantation history (LME, final site, $X^2=0.0001$, $P=0.99$,

373 initial site: $X^2=2.32$, $P=0.13$, initial*final site: $X^2=0.94$, $P=0.33$, Fig. 5b).



374

375 **Fig. 5.** Community-wide measures in ambient and low pH sites over time, left- and right-hand
 376 panels as in Figure 2. Measures are: (a, b) community stability, (c, d) secondary colonizers space
 377 occupation, (e, f) Shannon's diversity, and (g, h) species richness. Error bars indicate standard
 378 error.

379 However, the proportion of secondary colonization was higher on tiles that ended under low pH
380 conditions, regardless of origin (GLMM, final site, $z=2.63$, $P=0.20$, initial site: $z=0.33$, $P=0.75$,
381 initial*final site: $z=-0.26$, $P=0.80$, Fig. 5d). After transplantation, Shannon diversity was
382 significantly higher on tiles that ended at the elevated $p\text{CO}_2$ site (GLMM, final site: $z=2.23$,
383 $P=0.026$, Fig. 5f), while the negative effects of $p\text{CO}_2$ observed during early succession appeared
384 to have no persisting influence on diversity by the end of the experiment (GLMM, initial site:
385 $z=-0.69$, $P=0.49$, initial*final site: $z=-0.58$, $P=0.56$). Species richness appeared resistant to
386 acidification after the transplantation experiment, as neither early nor late $p\text{CO}_2$ effects
387 influenced the number of recruiting species (GLMM, initial site: $z=-0.47$, $P=0.64$, final site:
388 $z=0.31$, $P=0.76$, initial*final site: $z=-0.10$, $P=0.92$, Fig. 5h).

389

390 **Discussion**

391 Timing and abundance of species recruiting from plankton are important determinants of long-
392 term community composition and structure (Sutherland, 1974; Sams & Keough, 2012) and,
393 depending on the mechanism of succession, can determine long-term community stability
394 (Connell & Slatyer, 1977). Environmental heterogeneity and disturbance regimes during
395 recruitment can influence successional outcomes by promoting coexistence or dominance of
396 early or late recruiting species (Platt & Connell, 2003; Cifuentes *et al.*, 2010). Global change
397 impacts on communities and ecosystems may therefore stem from the particular way in which
398 environmental drivers interact with life-history trade-offs (e.g. competitive ability vs. dispersal /
399 colonization ability) of early and late successional species, and how these species inhibit or
400 promote one another. This is reasonably well understood for drivers like temperature in
401 terrestrial ecosystems (e.g. Gounand *et al.*, 2016; Lancaster *et al.*, 2016), but in marine

402 environments and for emerging drivers like ocean acidification, changes in succession – even
403 when observed (e.g. Kroeker *et al.*, 2013b) – are rarely explicitly examined in terms of the
404 underlying mechanisms and time-history contingency.

405 Here, we used transplant experiments to elucidate the relative importance of $p\text{CO}_2$ effects early
406 vs. later in succession on species abundance, diversity and composition. This is important for
407 understanding how acidification might affect dynamic communities as they progress through
408 successional and seasonal development. It is also key for determining the effects of discrete
409 acidification events on marine communities. Upwelling regions experience intermittent
410 acidification events that can span weeks (e.g. up to six week periods, Chan *et al.*, 2017) or
411 months (e.g. from early spring to late summer, Feely *et al.*, 2008) and these events are expected
412 to become less frequent but longer-lasting and stronger (Iles *et al.*, 2012). Acidification events
413 also occur in areas with high organic loading via terrestrial run-off, where discrete events can last
414 for days to weeks but multiple cumulative events can occur over several months (Guadayol *et*
415 *al.*, 2009). Eutrophication-driven acidification may intensify in the future as eutrophication will
416 likely increase with increased human development (Rabalais *et al.*, 2009).

417 On our experimental tiles, soft-bodied, weed-like taxa, algae and ascidians, had an advantage in
418 acidified conditions and outcompeted calcified taxa that were more vulnerable to the effects of
419 acidification, as has been widely reported (Wittmann & Pörtner, 2013). Developing communities
420 responded quickly to acidification (effects on some species were apparent after two weeks) and
421 some of these effects were not reversed one month after transplantation. In sum, we found that
422 succession was substantially altered by acidification, even when acidified conditions were not
423 maintained for the full duration of the experiment. Future work should carefully consider

424 temporal variation in acidification over experimental periods spanning important dynamics in
425 community succession.

426 *Recruitment and development*

427 Early successional stages in many shallow benthic habitats – including, to our slight surprise,
428 downward-facing experimental surfaces – are dominated by photosynthetic microalgae and
429 weedy macroalgae. Many photosynthetic marine taxa can take advantage of elevated $p\text{CO}_2$
430 (Connell *et al.*, 2013; Cornwall *et al.*, 2017). At Vulcano Island and other seep sites, biofilms are
431 higher in percent cover and productivity and have altered composition relative to reference sites
432 (Lidbury *et al.*, 2012; Johnson *et al.*, 2013, 2015; Baggini *et al.*, 2015). We observed the same
433 boost in biofilm under acidified conditions during the early phase of succession, although it is
434 unclear if composition changed. It is possible that the biofilm at low pH sites altered subsequent
435 invertebrate recruitment by changing settlement cues (see Doropoulos *et al.*, 2012), as succession
436 was delayed in this site, despite early biofilm abundance. Filamentous green *Cladophora* sp. had
437 a higher and slightly later peak in abundance on tiles at the acidified site. Such shifts in primary
438 producer assemblages alter biomass of resources available for grazers (Russell *et al.*, 2013) and
439 may alter settlement patterns of recruiting invertebrates (Hadfield, 2011).

440 We found that calcified primary colonizers had lasting responses to short but discrete exposure to
441 increased $p\text{CO}_2$, whereas only one of the calcified secondary colonizers exhibited this response.
442 This pattern could be caused by the differential duration of exposure experienced by primary
443 (four weeks longer) vs. secondary species or traits of the species in each of the categories (e.g.
444 primary colonizers were heavily calcified polychaetes, compared to relatively lightly calcified
445 bryozoans). The recruitment of two types of calcified tube-forming polychaetes was both
446 reduced and delayed under acidification. Delayed recruitment under acidification causes

447 individuals of these species to arrive after the community has reached 100% cover and could
448 imply that these organisms face stiffer competition for space than their counterparts that
449 recruited earlier into the ambient site. Tile site of origin influenced the recruitment of both
450 spirorbids and serpulids and these effects persisted through time, regardless of transplantation
451 into or out of the acidified site. Our results align with observations of reduced abundances of
452 both serpulids and spirorbids near CO₂ seeps off Ischia (Cigliano *et al.*, 2010; Donnarumma *et*
453 *al.*, 2014), but suggest that this effect emerges very early in the establishment of these taxa. The
454 two-week delay in recruitment could be due to a combination of direct effects on adult
455 reproduction, larval and juvenile recruitment and/or indirect effects such as inhibition by settled
456 species. Acidification has been shown to impair serpulid larval calcification and juvenile growth
457 (Lane *et al.*, 2013) and compromise tube ultrastructure (Li *et al.*, 2014) in the laboratory.
458 Spirorbid growth could have been influenced by early exposure to *p*CO₂, as there were fewer
459 spirorbids on tiles that originated in acidified sites, but similar space coverage, suggesting that
460 spirorbids under acidification were larger. This could be a consequence of accelerated growth
461 under acidification or differential mortality of smaller individuals within the population.
462 Negative effects of acidification on these polychaetes may have higher level consequences as
463 these ecosystem engineers form complex reefs that have high associated biodiversity (Smith *et*
464 *al.*, 2013; Fabricius *et al.*, 2014).

465 At various points of succession, colonial ascidians (*Diplosoma* sp. and *Botryllus* sp.) appeared
466 either to tolerate or respond positively to acidification, which may reflect increased growth rate,
467 increased facilitation, and/or reduced competition under increased *p*CO₂ conditions. It is difficult
468 to disentangle these effects as growth rate in this context is undoubtedly influenced by other
469 species, and facilitation and competition is difficult to infer without experimental manipulation.

470 These ascidians, although native to the Mediterranean, are among a suite of globally invasive
471 taxa that overgrow other filter feeders and can cause economic damage to the aquaculture
472 industry (Zhan *et al.*, 2015). Our results add to growing evidence that some ascidians respond
473 positively to both natural (Donnarumma *et al.*, 2014) and experimental (Peck *et al.*, 2015)
474 acidification (but see Fabricius *et al.* (2014) for an example of reduced ascidian cover at tropical
475 seep sites). Overall, fast-growing nuisance species like ascidians are expected to benefit from
476 future acidification (Hall-Spencer & Allen, 2015) and reduced competition with calcifying native
477 taxa might increase relative dominance of invasive ascidians in an acidified ocean.

478 We observed mixed effects of natural acidification on lightly calcified bryozoans, which may be
479 related to differences in their carbonate mineralogy. The cyclostome bryozoan *Patinella radiata*,
480 with a primarily calcitic skeleton, did not change in abundance near seep sites. Studies off Ischia
481 have shown that this species can grow and reproduce at low pH (Donnarumma *et al.*, 2014;
482 Taylor *et al.*, 2015). However, a thin ramified bryozoan appeared earlier at the ambient than the
483 acidified site and an encrusting bryozoan *Schizomavella* sp., with a mainly aragonitic or
484 bimineralic skeleton (Smith *et al.*, 2006), had reduced recruitment at the acidified site. Carbonate
485 mineralogy of bryozoans can help predict vulnerability to acidification for a given species -
486 aragonite skeletons are more soluble than those composed of mainly calcite, and calcite
487 solubility increases with proportion of Mg (Fortunato, 2015; Pickett & Andersson, 2015; Taylor
488 *et al.*, 2015). However, mineralogy is not the sole determinant of dissolution rate. Other factors
489 such as surface area, porosity, surface complexity, organic matrix material, and ambient pH at
490 the calcification surface, may also play a role in response to acidification (Ries *et al.*, 2009;
491 Smith & Garden, 2013; Taylor *et al.*, 2015). Morphological differences between related species
492 highlight the importance of examining species-specific responses to acidification. However, the

493 relevance of these morphological differences among species may be outweighed by relative
494 competitive ability if and when these bryozoans are at risk of being overgrown by other species.

495 *Species interactions and community-level results*

496 Acidification first delayed, then accelerated community succession. First, communities in
497 acidified sites developed more slowly (i.e. were more stable) than ambient communities and had
498 a smaller proportion of space used by secondary colonizers. After 8 weeks, however, the
499 acidified communities developed rapidly and rate of community development was similar
500 between acidified and ambient communities. At this point, the proportion of secondary
501 colonizers increased on tiles at the high $p\text{CO}_2$ site, independent of colonization history. This
502 mismatch in timing indicates that ocean acidification may alter species interactions between and
503 within primary and secondary colonizer guilds. For example, the biofilm trajectory, likely a
504 diatom bloom, was altered at the acidified site, resulting in reduced biofilm coverage at the
505 acidified site by the end of the experiment. This likely contributed to increased cover of serpulids
506 and bryozoans on tiles that experienced higher $p\text{CO}_2$ at the acidified site since, at ambient levels
507 of $p\text{CO}_2$, the abundant biofilm overgrew and/or pre-empted space occupation by these
508 invertebrates, compared to the acidified site where there were fewer calcified invertebrates and
509 lower biofilm cover during this time period. The benefits to the calcifying organisms
510 transplanted to the acidified site may be short lived however, as some of these species were
511 negatively affected by acidification overall.

512 Accelerated succession (despite an initial delay) and competition-mediated reductions in
513 invertebrates at the ambient site likely contributed to higher species diversity on tiles that were
514 maintained in or transferred to the acidified site. This pattern was not observed for species
515 richness however, indicating that evenness or abundance of species was driving the diversity

516 result. This unexpected result further underscores the importance of understanding shifting
517 species interactions under acidification (Gaylord *et al.*, 2015). Responses to $p\text{CO}_2$ could also be
518 modulated at seep sites by seasonal effects on both calcifying invertebrates and algal competitors
519 (Baggini *et al.*, 2014), as seawater temperature increased from 14°C to 20°C during our
520 experiment. Competition between serpulids and algae has been documented in benthic
521 communities near Ischia CO_2 seeps, although the pattern described there (Kroeker *et al.*, 2013b)
522 is opposite to what we have described here in shaded fouling communities. Thus, microhabitat
523 could play an important role in competitive outcomes under acidification, and shaded areas may
524 provide a refuge from algal competition for those calcified filter feeders that are able to recruit
525 under acidification, although see Celis-Plá *et al.* (2015) for examples of positive combined
526 effects of shade and acidification on macroalgae.

527 The observed species-level changes at the acidified site, likely driven by direct effects and
528 mediated by interspecific interactions, culminated in community-level shifts in structure. Our
529 results conformed to the general expectation that communities experiencing high $p\text{CO}_2$ may shift
530 from calcified to mainly non-calcified consumers (Christen *et al.*, 2013) likely due to energetic
531 trade-offs which result in less energy available for calcification (Gaylord *et al.*, 2015). Our
532 results compliment a growing number of studies documenting changes in community structure
533 and diversity with increased $p\text{CO}_2$ in a range of habitat types (Kroeker *et al.*, 2013c; Campbell &
534 Fourqurean, 2014; Meadows *et al.*, 2015; Raulf *et al.*, 2015; Sarmiento *et al.*, 2015; Brown *et al.*,
535 2016). The significant trend towards homogeneity among invertebrate communities under
536 acidified conditions by the end of our experiment is similar to that described for fouling
537 communities in western Canada (Brown *et al.*, 2016), and for algal communities close to CO_2
538 seeps (Porzio *et al.*, 2011; Kroeker *et al.*, 2013b).

539 *Conclusions*

540 We found that elevated $p\text{CO}_2$ conditions in the Mediterranean stimulated the initial colonization
541 of settlement panels by biofilm. Despite the promotion of biofilm, succession was delayed at the
542 acidified site and secondary colonization was lower. After eight weeks, however, subsequent
543 succession accelerated quickly, resulting in higher secondary colonization, altered community
544 structure, and a more homogeneous biofouling community than that found at ambient levels of
545 $p\text{CO}_2$. Life history strategies, such as larval dispersal ability, environmental tolerances, growth
546 rate and competitive ability, influence species-specific responses of organisms as they colonize
547 new substrata, and are important to consider, even in closely related species (Gambi *et al.*, 2016).
548 We found marked shifts in recruitment patterns which may alter routes of energy flow between
549 trophic levels; later settlers may arrive out of sync with food sources, predators, or competitors
550 (Dupont & Thorndyke, 2009; Nagelkerken & Connell, 2015). We also found that acidification
551 altered community structure and these changes were driven both by past exposure (colonization
552 history) and recent exposure to high $p\text{CO}_2$. Accelerated succession, homogenization, and
553 changes to diversity under acidification occurred independently of colonization history. These
554 processes might be driven more by proximate environmental conditions and small-scale within-
555 site recruitment. The observed community-level shifts are therefore likely a result of not only
556 persistent and transient effects of interspecific variability in response to increased $p\text{CO}_2$ but also,
557 importantly, shifting interactions between and within primary and secondary colonizer guilds
558 (Connell & Slatyer, 1977; Gaylord *et al.*, 2015). Overall, these short and longer-term
559 acidification-driven changes in community succession could have important implications for
560 ecosystem function and food web dynamics.

561

562

563 **Acknowledgements**

564 We thank Cinzia Alessi, Camilla Bertolini, Paula Celis-Plà, Ilenia Domina, Helen Graham,
565 Linnaea Meyer, Laura Newcomb, Danny Small, Joy Smith and local support for assistance in the
566 field. We further thank the INGV lab for analyses and Renato Chemello, Luigi Musco, Michele
567 Gristina, and Mariagrazia Graziano for help with invertebrate and algal identification in the lab.
568 SR was funded by UK Ocean Acidification Research Programme Added Value Award (funded
569 by NERC, Defra, and DECC; grant no. NE/H02543X/1). MM was funded by a FFR-A Unipa
570 project. This research was funded in part by the Second Canadian Aquatic Invasive Species
571 Network (CAISN II), a Michael Smith Foreign Study Supplement (MSFSS) from Natural
572 Sciences and Engineering Council of Canada (NSERC), and a Zoology Graduate Fellowship
573 from the University of British Columbia to NEMB.

574 **References**

- 575 Arnold T, Mealey C, Leahey H, Miller A, Hall-Spencer JM, Milazzo M, Maers K (2012) Ocean
576 acidification and the loss of phenolic substances in marine plants. *PLoS ONE*, **7**, e35107.
- 577 Baggini C, Salomidi M, Voutsinas E, Bray L, Krasakopoulou E, Hall-Spencer JM (2014)
578 Seasonality affects macroalgal community response to increases in $p\text{CO}_2$. *PLoS ONE*, **9**,
579 e106520.
- 580 Baggini C, Issaris Y, Salomidi M, Hall-Spencer J (2015) Herbivore diversity improves benthic
581 community resilience to ocean acidification. *Journal of Experimental Marine Biology and*
582 *Ecology*, **469**, 98–104.
- 583 Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using
584 lme4. *Journal of Statistical Software*, **67**, 1–48.
- 585 Boatta F, D'Alessandro W, Gagliano AL et al. (2013) Geochemical survey of Levante Bay,
586 Vulcano Island (Italy), a natural laboratory for the study of ocean acidification. *Marine*
587 *Pollution Bulletin*, **73**, 485–494.
- 588 Boyd PW, Cornwall CE, Davison A et al. (2016) Biological responses to environmental
589 heterogeneity under future ocean conditions. *Global Change Biology*, 1–18.
- 590 Brown NEM, Therriault TW, Harley CDG (2016) Field-based experimental acidification alters
591 fouling community structure and reduces diversity. *Journal of Animal Ecology*, **85**, 1328–
592 1339.
- 593 Calosi P, Rastrick SPS, Lombardi C et al. (2013a) Adaptation and acclimatization to ocean
594 acidification in marine ectotherms: an in situ transplant experiment with polychaetes at a
595 shallow CO_2 vent system. *Proceedings of the Royal Society B: Biological Sciences*, **368**, 1–
596 15.
- 597 Calosi P, Rastrick SPS, Graziano M et al. (2013b) Distribution of sea urchins living near shallow
598 water CO_2 vents is dependent upon species acid–base and ion-regulatory abilities. *Marine*
599 *Pollution Bulletin*, **73**, 470–484.
- 600 Campbell JE, Fourqurean JW (2014) Ocean acidification outweighs nutrient effects in
601 structuring seagrass epiphyte communities. *Journal of Ecology*, **102**, 730–737.
- 602 Celis-Plá PSM, Hall-Spencer JM, Horta PA, Milazzo M, Korbee N, Cornwall CE, Figueroa FL
603 (2015) Macroalgal responses to ocean acidification depend on nutrient and light levels.
604 *Frontiers in Marine Science*, **2**.
- 605 Chan F, Barth JA, Blanchette CA et al. (2017) Persistent spatial structuring of coastal ocean
606 acidification in the California Current System. *Scientific Reports*, **7**, 2526.
- 607 Christen N, Calosi P, McNeill C, Widdicombe S (2013) Structural and functional vulnerability to
608 elevated $p\text{CO}_2$ in marine benthic communities. *Marine Biology*, **160**, 2113–2128.
- 609 Cifuentes M, Krueger I, Dumont CP, Lenz M, Thiel M (2010) Does primary colonization or
610 community structure determine the succession of fouling communities? *Journal of*
611 *Experimental Marine Biology and Ecology*, **395**, 10–20.
- 612 Cigliano M, Gambi M-C, Rodolfo-Metalpa R, Patti FP, Hall-Spencer JM (2010) Effects of ocean

- 613 acidification on invertebrate settlement at volcanic CO₂ vents. *Marine Biology*, **157**, 2489–
614 2502.
- 615 Clark H, Gobler C (2016) Do diurnal fluctuations in CO₂ and dissolved oxygen concentrations
616 provide a refuge from hypoxia and acidification for early life stage bivalves? *Marine*
617 *Ecology Progress Series*, **558**, 1–14.
- 618 Connell JH, Slatyer R (1977) Mechanisms of succession in natural communities and their role in
619 community stability and organization. *American Society of Naturalists*, **111**, 1119–1144.
- 620 Connell SD, Kroeker KJ, Fabricius KE, Kline DI, Russell BD (2013) The other ocean
621 acidification problem: CO₂ as a resource among competitors for ecosystem dominance.
622 *Proceedings of the Royal Society B: Biological Sciences*, **368**, 1–9.
- 623 Cornwall CE, Revill AT, Hall-Spencer JM, Milazzo M, Raven JA, Hurd CL (2017) Inorganic
624 carbon physiology underpins macroalgal responses to elevated CO₂. *Scientific Reports*, **7**,
625 46297.
- 626 Donnarumma L, Lombardi C, Cocito S, Gambi MC (2014) Settlement pattern of *Posidonia*
627 *oceanica* epibionts along a gradient of ocean acidification : an approach with mimics.
628 *Mediterranean Marine Science*, **15**, 498–509.
- 629 Doropoulos C, Diaz-Pulido G (2013) High CO₂ reduces the settlement of a spawning coral on
630 three common species of crustose coralline algae. *Marine Ecology Progress Series*, **475**,
631 93–99.
- 632 Doropoulos C, Ward S, Diaz-Pulido G, Hoegh-Guldberg O, Mumby P (2012) Ocean
633 acidification reduces coral recruitment by disrupting intimate larval-algal settlement
634 interactions. *Ecology Letters*, **15**, 338–346.
- 635 Dupont S, Thorndyke MC (2009) Impact of CO₂-driven ocean acidification on invertebrates
636 early life-history – what we know, what we need to know and what we can do.
637 *Biogeosciences Discussions*, **6**, 3109–3131.
- 638 Fabricius KE, Langdon C, Uthicke S et al. (2011) Losers and winners in coral reefs acclimatized
639 to elevated carbon dioxide concentrations. *Nature Climate Change*, **1**, 165–169.
- 640 Fabricius KE, De’ath G, Noonan S, Uthicke S (2014) Ecological effects of ocean acidification
641 and habitat complexity on reef-associated macroinvertebrate communities. *Proceedings of*
642 *the Royal Society B: Biological Sciences*, **281**.
- 643 Feely RA, Sabine CL, Lee K et al. (2004) Impact of anthropogenic CO₂ on the CaCO₃ system in
644 the oceans. *Science*, **305**, 362–366.
- 645 Feely RA, Sabine CL, Hernandez-Ayon JM, Ianson D, Hales B (2008) Evidence for upwelling of
646 corrosive “acidified” water onto the continental shelf. *Science*, **320**, 1490–1492.
- 647 Fortunato H (2015) Bryozoans in climate and ocean acidification research: A reappraisal of an
648 under-used tool. *Regional Studies in Marine Science*, **2070**.
- 649 Fournier DA, Skaug HJ, Ancheta J et al. (2012) AD Model Builder: using automatic
650 differentiation for statistical inference of highly parameterized complex nonlinear models.
651 *Optimization Methods and Software*, **27**, 233–249.
- 652 Gambi MC, Musco L, Giangrande A, Badalamenti F, Micheli F, Kroeker KJ (2016) Distribution

653 and functional traits of polychaetes in a CO₂ vent system: Winners and losers among closely
654 related species. *Marine Ecology Progress Series*, **550**, 121–134.

655 Garilli V, Rodolfo-Metalpa R, Scuderi D et al. (2015) Physiological advantages of dwarfing in
656 surviving extinctions in high-CO₂ oceans. *Nature Climate Change*, **5**, 1–6.

657 Gaylord B, Kroeker KJ, Sunday JM et al. (2015) Ocean acidification through the lens of
658 ecological theory. *Ecology*, **96**, 3–15.

659 Goodwin C, Rodolfo-Metalpa R, Picton B, Hall-Spencer JM (2014) Effects of ocean
660 acidification on sponge communities. *Marine Ecology*, **35**, 41–49.

661 Gounand I, Kefi S, Mouquet N, Gravel D (2016) Trait selection during food web assembly: the
662 roles of interactions and temperature. *Theoretical Ecology*, **9**, 417–429.

663 Guadayol Ò, Peters F, Marrasé C et al. (2009) Episodic meteorological and nutrient-load events
664 as drivers of coastal planktonic ecosystem dynamics: A time-series analysis. *Marine
665 Ecology Progress Series*, **381**, 139–155.

666 Hadfield MG (2011) Biofilms and marine invertebrate larvae: what bacteria produce that larvae
667 use to choose settlement sites. *Annual Review of Marine Science*, **3**, 453–470.

668 Hale R, Calosi P, McNeill L, Mieszkowska N, Widdicombe S (2011) Predicted levels of future
669 ocean acidification and temperature rise could alter community structure and biodiversity in
670 marine benthic communities. *Oikos*, **120**, 661–674.

671 Hall-Spencer JM, Allen R (2015) The impact of CO₂ emissions on “ nuisance ” marine species.
672 *Research and Reports in Biodiversity Studies*, **4**, 33–46.

673 Hall-Spencer JM, Rodolfo-Metalpa R, Martin S et al. (2008) Volcanic carbon dioxide vents
674 show ecosystem effects of ocean acidification. *Nature*, **454**, 96–99.

675 Hallett LM, Jones SK, MacDonald AAM et al. (2016) codyn: An r package of community
676 dynamics metrics. *Methods in Ecology and Evolution*, **7**, 1146–1151.

677 Harvey BP, McKeown NJ, Rastrick SPS et al. (2016) Individual and population-level responses
678 to ocean acidification. *Scientific Reports*, **6**, 20194.

679 Henson SA, Beaulieu C, Ilyina T et al. (2017) Rapid emergence of climate change in
680 environmental drivers of marine ecosystems. *Nature Communications*, **8**, 14682.

681 Hofmann GE, Smith JE, Johnson KS et al. (2011) High-frequency dynamics of ocean pH: a
682 multi-ecosystem comparison. *PLoS ONE*, **6**, e28983.

683 Hönisch B, Ridgwell A, Schmidt DN et al. (2012) The geological record of ocean acidification.
684 *Science*, **335**, 1058–63.

685 Iles AC, Gouhier TC, Menge BA, Stewart JS, Haupt AJ, Lynch MC (2012) Climate-driven
686 trends and ecological implications of event-scale upwelling in the California Current
687 System. *Global Change Biology*, **18**, 783–796.

688 Johnson V, Russell B, Fabricius KE, Brownlee C, Hall-Spencer JM (2012) Temperate and
689 tropical brown macroalgae thrive, despite decalcification, along natural CO₂ gradients.
690 *Global Change Biology*, **18**, 2792–2803.

691 Johnson R, Brownlee C, Rickaby R, Graziano M, Milazzo M, Hall-Spencer JM (2013)

- 692 Responses of marine benthic microalgae to elevated CO₂. *Marine Biology*, **160**, 1813–1824.
- 693 Johnson V, Brownlee C, Milazzo M, Hall-Spencer J (2015) Marine microphytobenthic
694 assemblage shift along a natural shallow-water CO₂ gradient subjected to multiple
695 environmental stressors. *Journal of Marine Science and Engineering*, **3**, 1425–1447.
- 696 Kroeker KJ, Micheli F, Gambi M-C, Martz TR (2011) Divergent ecosystem responses within a
697 benthic marine community to ocean acidification. *Proceedings of the National Academy of
698 Sciences*, **108**, 14515–14520.
- 699 Kroeker KJ, Kordas RL, Crim R et al. (2013a) Impacts of ocean acidification on marine
700 organisms: quantifying sensitivities and interaction with warming. *Global Change Biology*,
701 **19**, 1884–1896.
- 702 Kroeker KJ, Micheli F, Gambi M-C (2013b) Ocean acidification causes ecosystem shifts via
703 altered competitive interactions. *Nature Climate Change*, **3**, 156–159.
- 704 Kroeker KJ, Gambi M-C, Micheli F (2013c) Community dynamics and ecosystem simplification
705 in a high-CO₂ ocean. *Proceedings of the National Academy of Sciences of the United States
706 of America*, **110**, 12721–6.
- 707 Kurihara H, Asai T, Kato S, Ishimatsu A (2008) Effects of elevated pCO₂ on early development
708 in the mussel *Mytilus galloprovincialis*. *Aquatic Biology*, **4**, 225–233.
- 709 Lancaster L, Morrison G, Fitt R (2016) Life history trade-offs, the intensity of competition, and
710 coexistence in novel and evolving communities under climate change. *Philosophical
711 Transactions of the Royal Society B*.
- 712 Lane AC, Mukherjee J, Chan VBS, Thiyagarajan V (2013) Decreased pH does not alter
713 metamorphosis but compromises juvenile calcification of the tube worm *Hydroides elegans*.
714 *Marine Biology*, **160**, 1983–1993.
- 715 Li C, Chan V, He C, Meng Y (2014) Weakening mechanisms of the serpulid tube in a high CO₂
716 world. *Environmental Science & Technology*, **48**, 14158–14167.
- 717 Li F, Wu Y, Hutchins DA, Fu F, Gao K (2016) Physiological responses of coastal and oceanic
718 diatoms to diurnal fluctuations in seawater carbonate chemistry under two CO₂
719 concentrations. *Biogeosciences*, **13**, 6247–6259.
- 720 Lidbury I, Johnson V, Hall-Spencer JM, Munn C, Cunliffe M (2012) Community-level response
721 of coastal microbial biofilms to ocean acidification in a natural carbon dioxide vent
722 ecosystem. *Marine Pollution Bulletin*, **64**, 1063–1066.
- 723 Linares C, Vidal M, Canals M et al. (2015) Persistent natural acidification drives major
724 distribution shifts in marine benthic ecosystems. *Proceedings of the Royal Society B*, **282**,
725 e20150587.
- 726 Meadows AS, Ingels J, Widdicombe S, Hale R, Rundle SD (2015) Effects of elevated CO₂ and
727 temperature on an intertidal meiobenthic community. *Journal of Experimental Marine
728 Biology and Ecology*, **469**, 44–56.
- 729 Milazzo M, Rodolfo-Metalpa R, Chan VBS et al. (2014) Ocean acidification impairs vermetid
730 reef recruitment. *Scientific Reports*, **4**, 1–7.
- 731 Milazzo M, Cattano C, Alonzo SH et al. (2016) Ocean acidification affects fish spawning but not

732 paternity at CO₂ seeps. *Proceedings of the Royal Society B*, **283**, 20161021.

733 Nagelkerken I, Connell SD (2015) Global alteration of ocean ecosystem functioning due to
734 increasing human CO₂ emissions. *Proceedings of the National Academy of Sciences*, **2015**,
735 201510856.

736 Oksanen AJ, Blanchet FG, Kindt R et al. (2015) vegan: Community Ecology package.

737 Osman R, Whitlatch R, Zajac R (1989) Effects of resident species on recruitment into a
738 community larval settlement versus post-settlement mortality in the oyster *Crassostrea*
739 *virginica*. *Marine Ecology Progress Series*, **54**, 61–73.

740 Peck LS, Clark MS, Power D, Reis J, Batista FM, Harper EM (2015) Acidification effects on
741 biofouling communities: winners and losers. *Global Change Biology*, **21**, 1907–1913.

742 Pickett M, Andersson AJ (2015) Dissolution rates of biogenic carbonates in natural seawater at
743 different pCO₂ conditions: a laboratory study. *Aquatic Geochemistry*, 459–485.

744 Platt WJ, Connell JH (2003) Natural disturbances and directional replacement of species.
745 *Ecological Monographs*, **73**, 507–522.

746 Porzio L, Buia MC, Hall-spencer JM, Cristina M (2011) Effects of ocean acidification on
747 macroalgal communities. *Journal of Experimental Marine Biology and Ecology*, **400**, 278–
748 287.

749 Przeslawski R, Byrne M, Mellin C (2015) A review and meta-analysis of the effects of multiple
750 abiotic stressors on marine embryos and larvae. *Global change biology*, **21**, 2122–2140.

751 Rabalais NN, Turner RE, Díaz RJ, Justić D (2009) Global change and eutrophication of coastal
752 waters. *Ices Journal of Marine Science*, **66**, 1528–1537.

753 R Development Core Team (2009) R: a language and environment for statistical computing.

754 Raulf FF, Fabricius K, Uthicke S, de Beer D, Abed RMM, Ramette A (2015) Changes in
755 microbial communities in coastal sediments along natural CO₂ gradients at a volcanic vent
756 in Papua New Guinea. *Environmental Microbiology*, **17**, 3678–3691.

757 Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO₂-
758 induced ocean acidification. *Geology*, **37**, 1131–1134.

759 Ross P, Parker L, O’Connor W, Bailey EA (2011) The Impact of ocean acidification on
760 reproduction, early development and settlement of marine organisms. *Water*, **3**, 1005–1030.

761 Ross PM, Parker L, Byrne M (2016) Transgenerational responses of molluscs and echinoderms
762 to changing ocean conditions. *ICES Journal of Marine Science*, **73**, 537–549.

763 Russell BD, Connell SD, Findlay HS, Tait K, Widdicombe S, Mieszkowska N (2013) Ocean
764 acidification and rising temperatures may increase biofilm primary productivity but
765 decrease grazer consumption. *Proceedings of the National Academy of Sciences of the*
766 *United States of America*, **368**.

767 Sams MA, Keough MJ (2012) Contrasting effects of variable species recruitment on marine
768 sessile communities. *Ecology*, **93**, 1153–1163.

769 Sanford E, Gaylord B, Hettinger A, Lenz EA, Meyer K, Hill TM (2014) Ocean acidification
770 increases the vulnerability of native oysters to predation by invasive snails. *Proceedings of*

- 771 *the Royal Society B: Biological Sciences*, **281**, 1–8.
- 772 Sarmiento VC, Souza TP, Esteves a. M, Santos PJP (2015) Effects of seawater acidification on a
773 coral reef meiofauna community. *Coral Reefs*, **34**, 955–966.
- 774 Schneider CA, Rasb WS, Eliceiri KW, Rasband WS (2012) NIH Image to ImageJ: 25 years of
775 image analysis. *Nature Methods*, **9**, 671–675.
- 776 Shaw E, Munday P, McNeil B (2013) The role of CO₂ variability and exposure time for
777 biological impacts of ocean acidification. *Geophysical Research Letters*, **40**, 4685–4688.
- 778 Skaug H, Fournier D, Nielsen A, Magnusson A, Bolker B (2013) glmmADMB: generalized
779 linear mixed models using AD model builder. R package version 0.7.7.4.
- 780 Small DP, Milazzo M, Bertolini C et al. (2015) Temporal fluctuations in seawater pCO₂ may be
781 as important as mean differences when determining physiological sensitivity in natural
782 systems Daniel. *Ices Journal of Marine Science*, **73**, 1–9.
- 783 Smith AM, Garden CJ (2013) Being a bimineralic bryozoan in an acidifying ocean. , Vol. 143
784 (eds Ernst A, Schafer P, Scholz J), pp. 137–153. Berlin, Heidelberg.
- 785 Smith AM, Key MM, Gordon DP (2006) Skeletal mineralogy of bryozoans: Taxonomic and
786 temporal patterns. *Earth-Science Reviews*, **78**, 287–306.
- 787 Smith AM, Riedi MA, Winter DJ (2013) Temperate reefs in a changing ocean: Skeletal
788 carbonate mineralogy of serpulids. *Marine Biology*, **160**, 2281–2294.
- 789 Sousa WP (1979) Experimental investigations of disturbance and ecological succession in a
790 rocky intertidal algal community. *Ecological Monographs*, **49**, 227–254.
- 791 Sunday JM, Fabricius KE, Kroeker KJ et al. (2017) Ocean acidification can mediate biodiversity
792 shifts by changing biogenic habitat. *Nature Climate Change*, **7**, 81–85.
- 793 Sutherland JP (1974) Multiple stable points in natural communities. *American Naturalist*, **108**,
794 859–873.
- 795 Tans P (2009) An accounting of the observed increase in oceanic and atmospheric CO₂ and the
796 outlook for the future. *Oceanography*, **22**, 26–35.
- 797 Taylor PD, Lombardi C, Cocito S (2015) Biomineralization in bryozoans: present, past and
798 future. *Biological Reviews*, **90**, 1118–1150.
- 799 Tilman D (1999) The ecological consequences of changes in biodiversity: a search for general
800 principles. *Ecology*, **80**, 1455–1474.
- 801 Vaz-Pinto F, Olabarria C, Gestoso I, Cacabelos E, Incera M, Arenas F (2013) Functional
802 diversity and climate change: effects on the invasibility of macroalgal assemblages.
803 *Biological Invasions*, **15**, 1833–1846.
- 804 Vizzini S, Di Leonardo R, Costa V, Tramati CDD, Luzzu F, Mazzola A (2013) Trace element
805 bias in the use of CO₂ vents as analogues for low pH environments: Implications for
806 contamination levels in acidified oceans. *Estuarine, Coastal and Shelf Science*, **134**, 19–30.
- 807 Webster N, Uthicke S, Botté E, Flores F, Negri A (2013) Ocean acidification reduces induction
808 of coral settlement by crustose coralline algae. *Global Change Biology*, **19**, 303–315.

- 809 Wittmann AC, Pörtner H-O (2013) Sensitivities of extant animal taxa to ocean acidification.
810 *Nature Climate Change*, **3**, 995–1001.
- 811 Zhan A, Briski E, Bock DG, Ghabooli S, Macisaac HJ (2015) Ascidians as models for studying
812 invasion success. *Marine Biology*, **162**, 2449–2470.
- 813
- 814
- 815

816 Table legends

817 **Table 1.** Carbonate chemistry of source seawater from ambient and high CO₂ sites. Temperature,
818 salinity, pH_{NBS}, and total alkalinity were collected every two weeks from March to June 2013
819 (mean +/- SE, n =). Asterisks indicate calculated values in the CO₂-SYS program (Pierrot et al.
820 2006).

821 **Table 2.** Results of GLMMs (z statistic) and LME models (using X^2) using percent cover of a
822 given species and site as fixed effects. Week 2-8 n=20, week 12 n=10. ^p indicates week in which
823 peak % cover of this species occurred.

824 **Table 3.** Results of GLMMs using percent cover of a given species and initial site, final site and
825 their interaction as fixed effects (n=20).

826 Figure Legends

827 **Fig. 1.** Photographs depicting sites and pH gradient (left) and the panel and tile system. Two
828 panels (centre), with 10 PVC tiles (right) attached to the underside of each panel, were
829 suspended ~1m from both surface and bottom using a buoy and anchor system.

830 **Fig. 2.** Abundance of selected primary colonizers in ambient and low pH sites over time, left-
831 hand panels are up to week 8 (n=20) and right-hand panels are at week 12 (n=10) of both
832 transplanted and non-transplanted tiles. For the right-hand panels, shading indicates initial site
833 and position on x-axis indicates final site. Species are: (a, b) biofilm (% cover), (c, d)
834 *Cladophora* sp. (% cover), (e, f) serpulids (# individuals) and, (g, h) spirorbids (# individuals).
835 Error bars indicate standard error.

836 **Fig. 3.** Abundance of selected secondary colonizers in ambient and low pH sites over time, left-
837 hand panels are up to week 8 (n=20) and right-hand panels are at week 12 (n=10) of both
838 transplanted and non-transplanted tiles. For the right-hand panels, shading indicates initial site
839 and position on x-axis indicates final site. Species are: (a, b) *Diplosoma* sp. (% cover), (c, d)
840 *Botryllus* sp. (% cover), (e, f) Thin ramified bryozoan (% cover) and, (g, h) *Schizomavella* sp. (#
841 colonies). Error bars indicate standard error.

842 **Fig. 4.** Fig. 4. nMDS ordination plot showing the relationship between communities after (a) 8
843 weeks on tiles from low pH (open circles) vs. the ambient site (solid black triangles), n = 20 tiles
844 and (b) 12 week on tiles that (1) remained in low pH (open circles), (2) were transplanted from
845 low pH to the ambient site (solid blue circles), (3) were transplanted from ambient site to low pH
846 site (red open triangles), and (4) remained in the ambient site (solid black triangles), n = 10 tiles.

847 **Fig. 5.** Community-wide measures in ambient and low pH sites over time, left-hand panels are
848 up to week 8 (n=20) and right hand panels are at week 12 (n=10) of both transplanted and non-
849 transplanted tiles. For the right-hand panels, shading indicates initial site and position on x-axis
850 indicates final site. Measures are: (a, b) total occupied space, (c, d) secondary colonizers space

851 occupation, (e, f) Shannon's diversity, and (g, h) species richness. Error bars indicate standard
852 error.