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

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Abstract

Rising carbon dioxide concentrations are rapidly altering the carbonate chemistry of the oceans and this phenomenon, termed ocean acidification, may have far-reaching consequences for marine community and ecosystem dynamics. Such impacts remain poorly understood due to the difficulty of manipulating pCO_2 at the ecosystem level to mimic realistic fluctuations that occur on a number of different timescales. It is unclear how robust ecosystems are to intermediate-scale pCO_2 change; how quickly can communities at various stages of development respond to acidification, and, if high pCO_2 is relieved mid-succession, do these effects persist, are the effects reversed by alleviation of pCO_2 stress, or are the effects worsened by departures from prior high pCO_2 conditions to which organisms had acclimatized. As nearshore CO_2 often fluctuates throughout succession, a further unresolved issue is the relative importance of direct acidification effects on primary colonization vs. indirect effects. Here, we used reciprocal transplant experiments along a shallow water volcanic pCO_2 gradient to assess the importance of the timing and duration of high pCO_2 exposure (i.e. discrete events at different stages of successional development vs. continuous exposure) on patterns of colonization and succession in a benthic fouling community. We monitored community development both before and after reciprocal transplantation of communities at eight weeks until the end of the experiment at twelve weeks. We show that succession at the acidified site was initially delayed (less community change by eight weeks) but then accelerated over the next four weeks. These changes in succession led to homogenization of communities maintained in or transplanted to acidified conditions, and altered community structure in ways that reflected both short- and longer-term acidification history. These community shifts are likely a result of interspecific variability in response to increased pCO_2 and changes in species interactions. High pCO_2 altered biofilm development, allowing serpulids to do best at the acidified site by the end of the experiment,

although early (pre-transplant), negative effects of pCO_2 on recruitment of these worms was still detectable. The ascidians *Diplosoma* sp. and *Botryllus* sp. settled later and were more tolerant to acidification. Overall, transient and persistent acidification-driven changes in the biofouling community, via both current and post exposure, could have important implications for ecosystem function and food web dynamics.

Introduction

Anthropogenic carbon dioxide enrichment of the atmosphere and subsequent decreases in pH in the ocean are well documented (Feely *et al.*, 2004; Tans, 2009). The rate of change of ocean pH is unprecedented in recent geological history (Hönisch *et al.*, 2012) and the biological implications of these rapid chemical changes are being realized across a wide range of taxa (Kroeker *et al.*, 2013a; Wittmann & Pörtner, 2013). In coastal marine ecosystems, changes in seawater pCO_2 are occurring on a number of different timescales: diurnally with photosynthesis and respiration, days to weeks with the lunar cycle and upwelling dynamics, seasonally due to both biotic and abiotic forcing, and over many decades with anthropogenic forcing (Hofmann *et al.*, 2011; Boyd *et al.*, 2016; Henson *et al.*, 2017). It is clear that incorporating natural fluctuations in pCO_2 is necessary for making better predictions of the biological response to ocean acidification (Shaw *et al.*, 2013; Small *et al.*, 2015; Boyd *et al.*, 2016). Although a few studies have addressed this point at diurnal timescales (e.g. Clark & Gobler, 2016; Li *et al.*, 2016), it is unclear how marine ecosystems respond to longer term (weeks to months) pCO_2 fluctuations and whether these effects can be reversed (transient vs. persistent effects) if pCO_2 stress is relieved (but see Vaz-Pinto *et al.*, 2013).

In communities influenced by disturbance (i.e., most communities), the effects of fluctuating pCO_2 will be mediated by direct effects of acidification on settlement and recruitment and by indirect effects mediated by the interactions between early and later successional species. Ocean acidification can influence settlement of planktonic stages and recruitment into benthic populations (Cigliano *et al.* 2010; Brown *et al.*, 2016), and resident organisms influence subsequent settlement (i.e. secondary colonization, Osman *et al.*, 1989). However, the importance of colonization history in shaping community structure and succession in light of pCO_2 or pH heterogeneity at a variety of temporal scales has yet to be addressed. One challenge to disentangling effects of acidification on the succession and development of marine communities is that, to date, our understanding of the biological effects of ocean acidification is primarily informed by studies of single species in isolation. Such studies show how ocean acidification might influence organisms through changes in energetic demand (Garilli *et al.*, 2015; Harvey *et al.*, 2016), reproduction and development (Ross *et al.*, 2011), growth rate (Kroeker *et al.*, 2013a), development of defensive structures (Sanford *et al.*, 2014), and behaviour (Milazzo *et al.*, 2016). However, it is not easy to extrapolate from single-species studies to assess the effects of ocean acidification on community development, structure, and function. To anticipate ecosystem-level changes, it is essential to understand responses of multi-species assemblages to acidification. Early studies exposed pre-settled communities to acidification in laboratory conditions (e.g. Hale *et al.*, 2011), but deeper understanding of recruitment and settlement processes requires *in-situ* pCO_2 manipulation (e.g. Brown *et al.*, 2016) or observation.

Shallow water CO_2 seeps allow the study of intact communities and have been increasingly used 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 pCO_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 pCO_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 pCO_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 pCO_2
127 (e.g. during an upwelling event) in early succession or reflect only most recent exposure to
128 elevated pCO_2 regardless of prior conditions. We expected that pCO_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.

Materials and Methods

Site description and experimental set up

The study was conducted in Levante Bay on Vulcano Island (38°25′08″N, 14°57′40″E) in the Aeolian archipelago in Northeastern Sicily (Italy) from March to June, 2013 (12 weeks). At this site, shallow volcanic seeps emit carbon dioxide bubbles that create a gradient in seawater carbonate chemistry that has been characterized by a number of recent studies (Arnold *et al.*, 2012; Johnson *et al.*, 2012; Lidbury *et al.*, 2012; Boatta *et al.*, 2013; Calosi *et al.*, 2013b; Milazzo *et al.*, 2014). The biogeochemistry of the bay has been assessed to identify the most suitable areas for ocean acidification research (see Boatta *et al.*, 2013; Vizzini *et al.*, 2013) and the chosen sites were outside of any measured metal contamination. Variability in the pH gradient at the site is predominantly driven by currents influenced by westerly winds, and as such, the acidified water masses mostly run parallel to the northern shoreline of the bay (Boatta *et al.*, 2013). When winds are high, e.g. during storms that are common in winter and early spring, low pH waters are more restricted to the immediate vicinity of the seeps and do not extend as far along the shoreline (Boatta et al. 2013). We used two sites, ~70 m apart, along the pCO_2 gradient; the first had low mean pH (7.78), and the second had ambient mean pH (8.10) (here we use high pCO_2 and low pH interchangeably). Our sites correspond to sites 40-60 and 120-130 in Boatta *et al.*, (2013) for low pH and control pH respectively. We monitored temperature, salinity, and pH at each site at least once every two weeks throughout the observation period but daily for the first week and the last six weeks of the experiment. Samples for total alkalinity were taken every two weeks. We used these parameters to calculated dissolved inorganic Carbon (DIC), pCO_2, HCO_3^-, and carbonate and aragonite saturation states using the CO2-SYS program (Pierrot et al. 2006).

At each site, we suspended 70.5 × 70.5 cm semi-flexible PVC panels from buoys 1 m from the surface and 1 m from the bottom (n=3 panels per site). We deployed the panels on similar substrata at each site, which comprised of subtidal boulders and patches of seagrass. Each panel was oriented horizontally (Fig 1), and 15 PVC tiles (14.5 cm x 14.5 cm) were secured to the underside of each panel using cable ties (n=15 tiles nested within each of 3 panels per site, therefore n=45 tiles per site). The panels were secured to the buoys and anchors using ropes to avoid horizontal spinning. Storms damaged some tiles and panels, so only undamaged tiles (n=20 per site, from across the three panels) were used in the analyses. After 8 weeks, we reciprocally transplanted a subset of 10 tiles (selected randomly from the 20 total) from each pCO_2 regime, while the other 10 tiles were not transplanted, to determine if pCO_2 effects on recruitment occur early and persist or arise later during succession. At the time of this transplantation, we formed new panels (2 per site) with ten tiles each, tiles from transplanted and non-transplanted groups were distributed randomly to the two panels in each site.

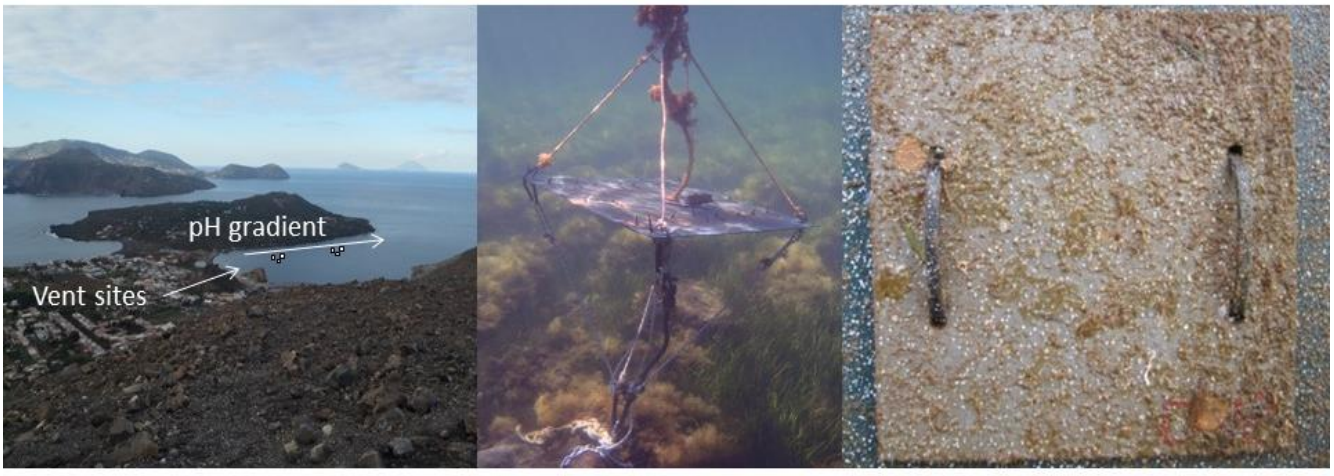


Fig. 1. Photographs depicting sites (white symbols) along the pH gradient (left) and the panel and tile system. Panels (centre), with downward facing PVC tiles (right) attached to the underside, were suspended ~1m from both surface and bottom using a buoy and anchor.

Species- and community- level measures

Photographs (one per tile) were taken every two weeks to determine changes in community composition, structure, Shannon's diversity, richness, stability over time and percent cover (point count) of primary and secondary colonizing fouling species for eight weeks. After transplantation, the tiles were left for one additional month before being photographed and retrieved for preservation. The tiles and panels were brought to the surface for photography. We conducted all photographic analyses in ImageJ (Schneider *et al.*, 2012) and identified species to lowest taxonomic level possible in the laboratory. Primary colonizers were defined as those that recruited in the ambient site in the first four weeks, before the community reached 100% cover. Primary colonizers included two serpulid polychaete taxa (serpulidae, spirorbidae) and two algal guilds, a turf-forming green filamentous alga *Cladophora* sp., and a biofilm which was ubiquitous and has been described at this site as primarily a mix of diatoms and cyanobacteria, the composition of which changes along gradients of pCO_2 (Johnson *et al.*, 2015). Secondary colonizers (i.e., those that recruited only after space occupancy reached 100% at week four) included bryozoans (e.g. *Schizomavella* sp. and *Patinella radiata*), ascidians (*Botryllus* sp. and *Diplosoma* sp.), and *Sphacelaria* sp., which is a branched red alga. These secondary colonizers may require a layer of biofilm in order to recruit, or their propagules may have only be in the water column during the latter half of the experiment due to seasonal or episodic reproductive patterns. Natural succession in marine communities often coincides with a disturbance regime (e.g. winter storms, sedimentation) that creates space (Sousa, 1979). The disturbance regime at our study site may coincide with seasonality, and we expect that the succession observed from bare surfaces reflected both directional community development and response to warming temperatures over time.

Statistical analyses

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

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

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

Results

Seawater parameters

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

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
Temperature (°C)	18.96±0.15	18.97±0.15
Salinity	38.19±0.011	38.19±0.010
pH_{NBS}	8.10±0.13	7.78±0.24
Alkalinity (μmol kg^{-1})	2523.52±1.34	2526.97±1.41
pCO_2 (μatm)*	557.69±26.48	1499.91±151.71
DIC (μmol kg^{-1})*	2271.16±6.42	2424.09±10.38
HCO_3^- (μmol kg^{-1})*	2066.59±9.62	2270.29±10.15
Ω Calcite*	4.33±0.096	2.43±0.093
Ω Aragonite*	2.82±0.063	1.59±0.060

Primary colonization pre- and post-transplant

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

Table 2. Statistical results from both GLMM (using z statistic) and LME (using X^2) from analysis of percent cover 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***
	Cladophora 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***
	Diplosoma sp.	% cover	8	5.0	2.0	1.79	0.074
		# colonies		2.0	0.4	5.22*	**0.022***
	Botryllus 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***
	Patinella radiata	% cover	8	0.050	0.074	-0.19	0.85
		# colonies		2.2	2.8	-0.30	0.76
	Schizomavella sp.	% cover	8	0.15	0.025	0.98	0.33
		# colonies		1.2	0.2	2.35	**0.0019***

Eight weeks into the experiment, a subset of tiles was reciprocally transplanted among sites. One month after transplantation, biofilm cover was related to only the most recent exposure to pCO_2 (i.e. tiles maintained in or transplanted to low pH) as coverage was lower on tiles that ended up at the acidified site than those in the ambient site, (GLMM, Final site P=0.050, Table 3, Fig.2b), regardless of origin, and there was no evidence of the pre-transplant effects of pCO_2 carrying over (GLMM, Initial site P=0.69, Table 3, Fig.2b). *Cladophora* sp. cover was reduced to zero after 8 weeks, therefore we were unable to determine if pCO_2 was more important in early or late succession for this taxon. Transplant results suggested that serpulid recruitment was influenced by pCO_2 early on and persisted, although there were also pCO_2 effects present during late succession. Overall, tiles that originated at the ambient site recruited more serpulid individuals

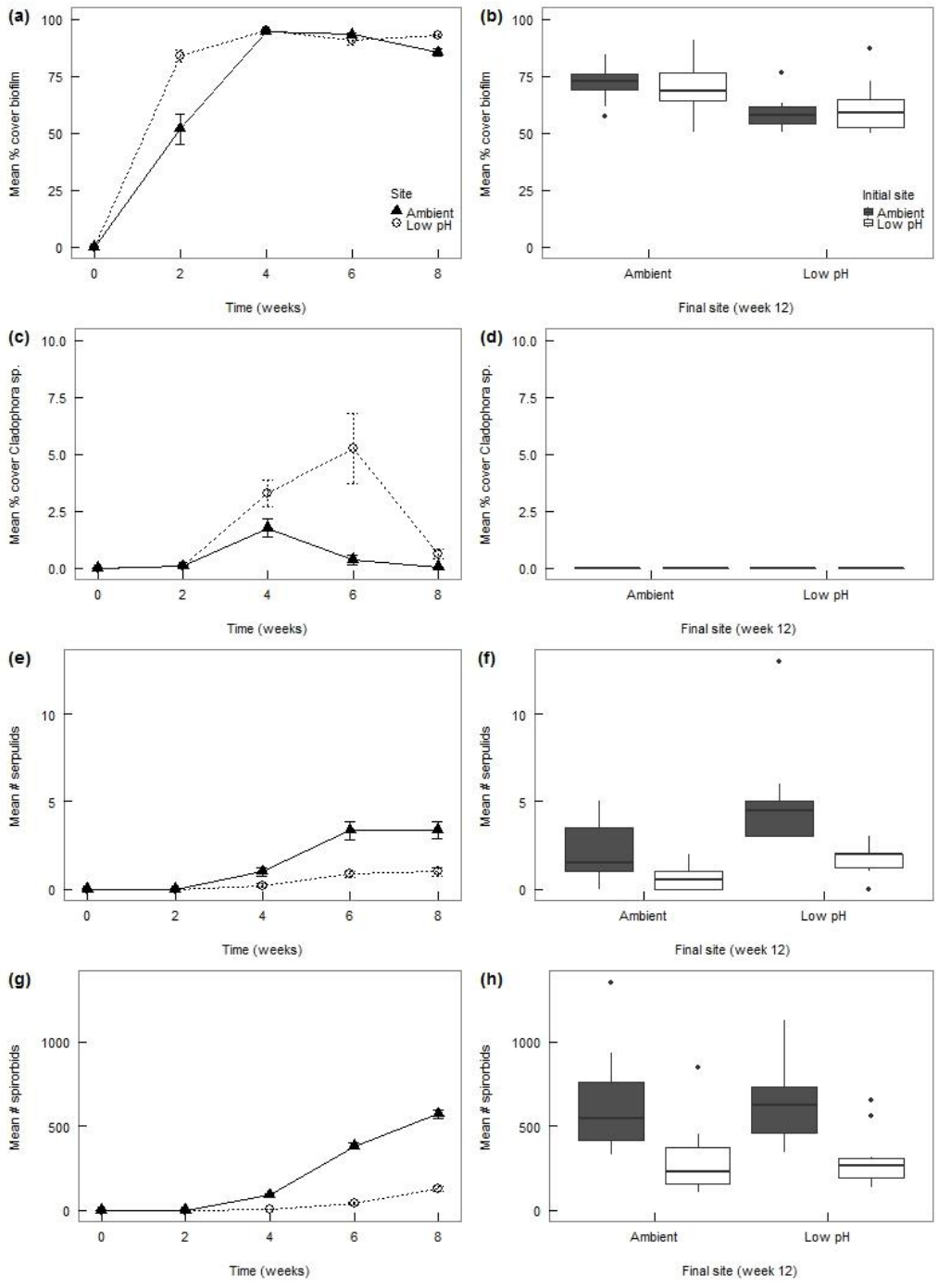


Fig. 2. Abundance of selected primary colonizers in ambient and low pH sites over time. Left-hand panels are trends through week 8 (i.e., pre-transplantation; n=20) and right-hand panels are patterns at week 12 (post-transplantation; n=10) of both transplanted and non-transplanted tiles. For the right-hand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

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

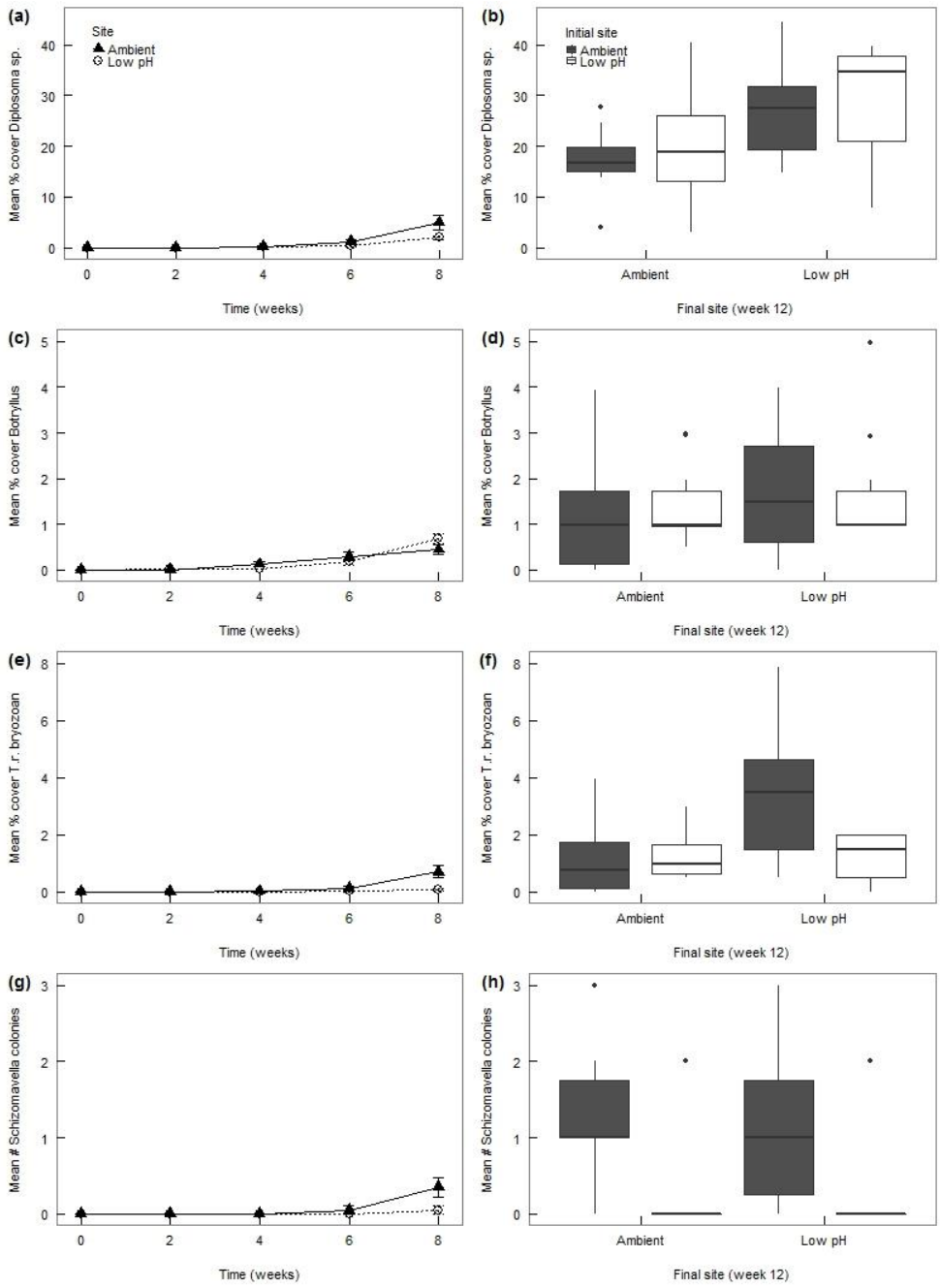
Group	Species	Initial site		Final site		Initial site * Final site	
		z	*P* **<0.05*	*z*	*P* *******<0.05*	*z*	*P* **<0.05*
	Biofilm complex (% cover)	-0.40	0.69	-1.96	**0.050***	0.49	0.62
	Cladophora sp. (% cover)	0.0	1.0	0.0	1.0	0.0	1.0
	Serpulids (% cover)	-1.83	0.067	3.06	**0.0022***	-0.65	0.52
	(# 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
	Diplosoma 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
	Botryllus 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***
	Patinella radiata (% 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
	Schizomavella 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

than those that originated at the acidified site, regardless of final site (GLMM, Initial site P=0.012, Table 3, Fig. 2f) and cover of this species showed a non-significant trend in the same direction (Initial site P=0.067, Table 3). However, by the end of the experiment, there were more individuals and higher cover of serpulids on tiles that had final exposure to high pCO_2, rather than ambient conditions (GLMM, Final site P=0.0014, Table 3, Fig. 2f, Fig. S1b). Initial site alone influenced the number of individuals, but not cover, of spirorbids, such that tiles that originated in the ambient site had more spirorbids, regardless of their final locations (GLMM, Initial site P=0.02, Table 3, Fig. 2h, Fig. S1d).

Secondary colonization pre- and post-transplant

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

After the transplant experiment, soft-bodied ascidians appeared to be largely resistant to changes in acidification. Neither initial site nor final site influenced cover or number of *Diplosoma* sp. or *Botryllus* sp. (Fig. 3b,d, Fig. S1f,h Table 3). The bryozoan *P. radiata* remained resistant to acidification after transplantation, and there was neither an effect of initial nor final site on numbers of colonies and cover of this species (Table 3). Post-transplant thin ramified bryozoan cover was influenced by final site, as overall cover was higher on tiles that ended up at the acidified site (Final site $P<0.001$), and this effect was especially strong for tiles transplanted from the ambient site (Initial*final site $P=0.027$, Table 3, Fig. 3f). Early successional pCO_2 effects were apparent in post-transplant recruitment of *Schizomavella* sp., such that tiles that originated in the ambient site had more colonies than those from the low pH site (Initial site $P=0.0096$, 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

Community level results pre- and post-transplant

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

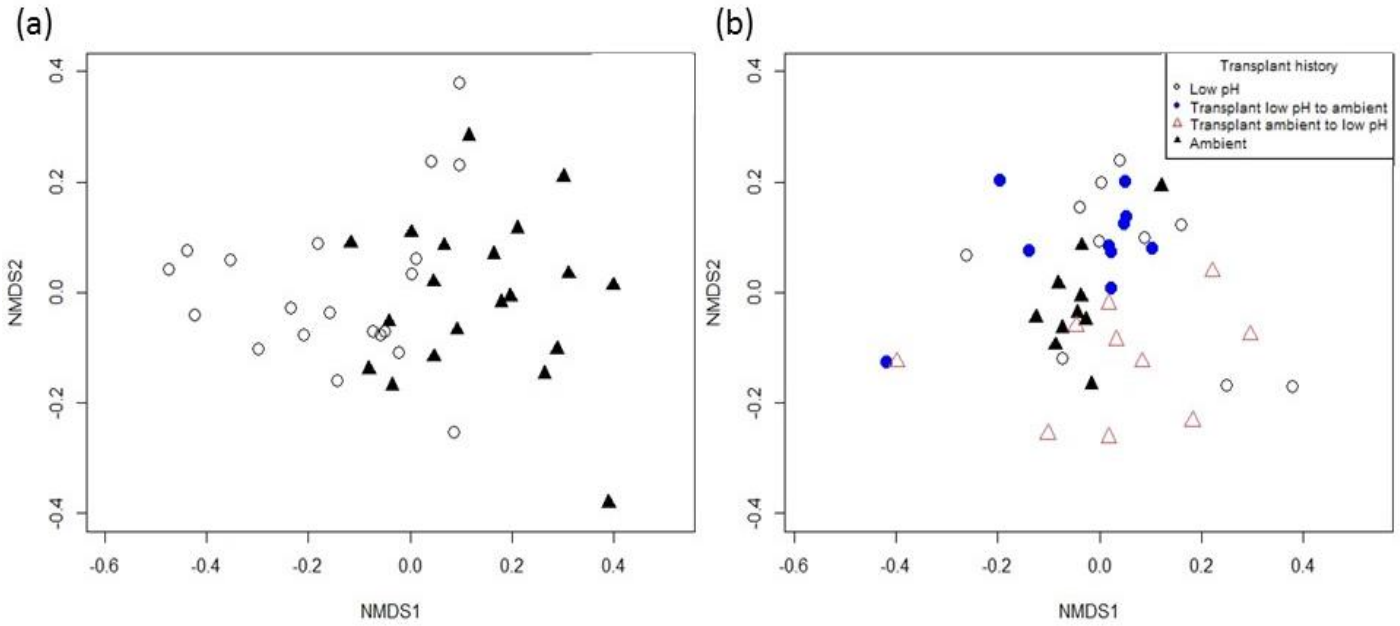


Fig. 4. nMDS ordination plot showing the relationship between communities after (a) 8 weeks on tiles from low pH (open circles) vs. the ambient site (solid black triangles), n = 20 tiles, and (b) 12 week on tiles that either remained in low pH (open circles), were transplanted from low pH to the ambient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

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

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

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

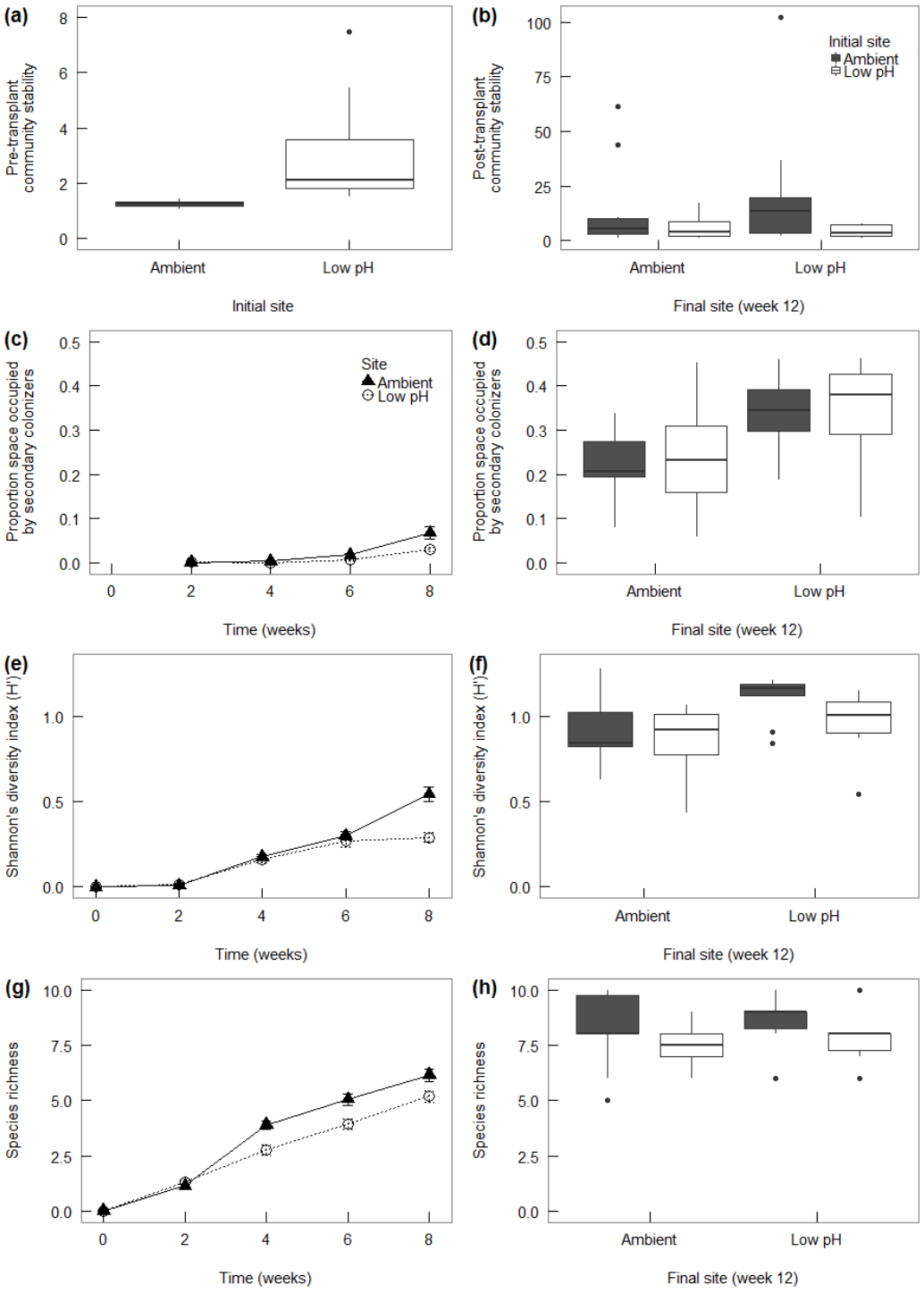


Fig. 5. Community-wide measures in ambient and low pH sites over time, left- and right-hand panels as in Figure 2. Measures are: (a, b) community stability, (c, d) secondary colonizers space occupation, (e, f) Shannon's diversity, and (g, h) species richness. Error bars indicate standard error.

However, the proportion of secondary colonization was higher on tiles that ended under low pH conditions, regardless of origin (GLMM, final site, z=2.63, *P*=0.20, initial site: z=0.33, *P*=0.75, initial*final site: z=-0.26, *P*=0.80, Fig. 5d). After transplantation, Shannon diversity was significantly higher on tiles that ended at the elevated pCO_2 site (GLMM, final site: z=2.23, *P*=0.026, Fig. 5f), while the negative effects of pCO_2 observed during early succession appeared to have no persisting influence on diversity by the end of the experiment (GLMM, initial site: z=-0.69, *P*=0.49, initial*final site: z=-0.58, *P*=0.56). Species richness appeared resistant to acidification after the transplantation experiment, as neither early nor late pCO_2 effects influenced the number of recruiting species (GLMM, initial site: z=-0.47, P=0.64, final site: z=0.31, P=0.76, initial*final site: z=-0.10, P=0.92, Fig. 5h).

Discussion

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

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

Here, we used transplant experiments to elucidate the relative importance of pCO_2 effects early vs. later in succession on species abundance, diversity and composition. This is important for understanding how acidification might affect dynamic communities as they progress through successional and seasonal development. It is also key for determining the effects of discrete acidification events on marine communities. Upwelling regions experience intermittent acidification events that can span weeks (e.g. up to six week periods, Chan *et al.*, 2017) or months (e.g. from early spring to late summer, Feely *et al.*, 2008) and these events are expected to become less frequent but longer-lasting and stronger (Iles *et al.*, 2012). Acidification events also occur in areas with high organic loading via terrestrial run-off, where discrete events can last for days to weeks but multiple cumulative events can occur over several months (Guadayol *et al.*, 2009). Eutrophication-driven acidification may intensify in the future as eutrophication will likely increase with increased human development (Rabalais *et al.*, 2009).

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

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

Recruitment and development

Early successional stages in many shallow benthic habitats – including, to our slight surprise, downward-facing experimental surfaces – are dominated by photosynthetic microalgae and weedy macroalgae. Many photosynthetic marine taxa can take advantage of elevated pCO_2 (Connell *et al.*, 2013; Cornwall *et al.*, 2017). At Vulcano Island and other seep sites, biofilms are higher in percent cover and productivity and have altered composition relative to reference sites (Lidbury *et al.*, 2012; Johnson *et al.*, 2013, 2015; Baggini *et al.*, 2015). We observed the same boost in biofilm under acidified conditions during the early phase of succession, although it is unclear if composition changed. It is possible that the biofilm at low pH sites altered subsequent invertebrate recruitment by changing settlement cues (see Doropoulos *et al.*, 2012), as succession was delayed in this site, despite early biofilm abundance. Filamentous green *Cladophora* sp. had a higher and slightly later peak in abundance on tiles at the acidified site. Such shifts in primary producer assemblages alter biomass of resources available for grazers (Russell *et al.*, 2013) and may alter settlement patterns of recruiting invertebrates (Hadfield, 2011).

We found that calcified primary colonizers had lasting responses to short but discrete exposure to increased pCO_2, whereas only one of the calcified secondary colonizers exhibited this response. This pattern could be caused by the differential duration of exposure experienced by primary (four weeks longer) vs. secondary species or traits of the species in each of the categories (e.g. primary colonizers were heavily calcified polychaetes, compared to relatively lightly calcified bryozoans). The recruitment of two types of calcified tube-forming polychaetes was both 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 CO_2 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 pCO_2, 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 pCO_2 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.

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

We observed mixed effects of natural acidification on lightly calcified bryozoans, which may be related to differences in their carbonate mineralogy. The cyclostome bryozoan *Patinella radiata*, with a primarily calcitic skeleton, did not change in abundance near seep sites. Studies off Ischia have shown that this species can grow and reproduce at low pH (Donnarumma *et al.*, 2014; Taylor *et al.*, 2015). However, a thin ramified bryozoan appeared earlier at the ambient than the acidified site and an encrusting bryozoan *Schizomavella* sp., with a mainly aragonitic or bimineralic skeleton (Smith *et al.*, 2006), had reduced recruitment at the acidified site. Carbonate mineralogy of bryozoans can help predict vulnerability to acidification for a given species - aragonite skeletons are more soluble than those composed of mainly calcite, and calcite solubility increases with proportion of Mg (Fortunato, 2015; Pickett & Andersson, 2015; Taylor *et al.*, 2015). However, mineralogy is not the sole determinant of dissolution rate. Other factors such as surface area, porosity, surface complexity, organic matrix material, and ambient pH at the calcification surface, may also play a role in response to acidification (Ries *et al.*, 2009; Smith & Garden, 2013; Taylor *et al.*, 2015). Morphological differences between related species 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 pCO_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 pCO_2 at the acidified site since, at ambient levels
507 of pCO_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

result. This unexpected result further underscores the importance of understanding shifting species interactions under acidification (Gaylord *et al.*, 2015). Responses to pCO_2 could also be modulated at seep sites by seasonal effects on both calcifying invertebrates and algal competitors (Baggini *et al.*, 2014), as seawater temperature increased from 14°C to 20°C during our experiment. Competition between serpulids and algae has been documented in benthic communities near Ischia CO_2 seeps, although the pattern described there (Kroeker *et al.*, 2013b) is opposite to what we have described here in shaded fouling communities. Thus, microhabitat could play an important role in competitive outcomes under acidification, and shaded areas may provide a refuge from algal competition for those calcified filter feeders that are able to recruit under acidification, although see Celis-Plá *et al.* (2015) for examples of positive combined effects of shade and acidification on macroalgae.

The observed species-level changes at the acidified site, likely driven by direct effects and mediated by interspecific interactions, culminated in community-level shifts in structure. Our results conformed to the general expectation that communities experiencing high pCO_2 may shift from calcified to mainly non-calcified consumers (Christen *et al.*, 2013) likely due to energetic trade-offs which result in less energy available for calcification (Gaylord *et al.*, 2015). Our results compliment a growing number of studies documenting changes in community structure and diversity with increased pCO_2 in a range of habitat types (Kroeker *et al.*, 2013c; Campbell & Fourqurean, 2014; Meadows *et al.*, 2015; Raulf *et al.*, 2015; Sarmento *et al.*, 2015; Brown *et al.*, 2016). The significant trend towards homogeneity among invertebrate communities under acidified conditions by the end of our experiment is similar to that described for fouling communities in western Canada (Brown *et al.*, 2016), and for algal communities close to CO_2 seeps (Porzio *et al.*, 2011; Kroeker *et al.*, 2013b).

539 *Conclusions*

540 We found that elevated pCO_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 pCO_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 pCO_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 pCO_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

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Table legends

Table 1. Carbonate chemistry of source seawater from ambient and high CO_2 sites. Temperature, salinity, $pH_{NBS,}$ and total alkalinity were collected every two weeks from March to June 2013 (mean +/- SE, n =). Asterisks indicate calculated values in the CO2-SYS program (Pierrot et al. 2006).

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

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

Figure Legends

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

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

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

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

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

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