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Altered epiphyte community and sea urchin diet in *Posidonia oceanica* meadows in the vicinity of volcanic CO_2 vents

Patricia Nogueira [a], Maria Cristina Gambi [b], Salvatrice Vizzini [c, d], Gianmaria Califano [e], Ana Mafalda Tavares [a], Rui Santos [a], Begoña Martínez-Crego [a, *]

[a] *Centre of Marine Sciences (CCMAR), University of Algarve-Campus de Gambelas, 8005-139 Faro, Portugal*
[b] *Stazione Zoologica Anton Dohrn, Dept of Integrative Marine Ecology, Villa Dohrn. Benthic Ecology Center (Ischia), Villa Comunale, 80121 Naples, Italy*
[c] *Department of Earth and Marine Sciences, University of Palermo, CoNISMa, Via Archirafi 18, 90123 Palermo, Italy*
[d] *CoNISMa, Piazzale Flaminio 9, 00196 Roma, Italy*
[e] *Friederich-Schiller-University Jena (FSU), Lessingstr. 8, D-07743 Jena, Germany*

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ABSTRACT

Ocean acidification (OA) predicted for 2100 is expected to shift seagrass epiphyte communities towards the dominance of more tolerant non-calcifying taxa. However, little is known about the indirect effects of such changes on food provision to key seagrass consumers. We found that epiphyte communities of the seagrass *Posidonia oceanica* in two naturally acidified sites (i.e. north and south sides of a volcanic CO_2 vent) and in a control site away from the vent at the Ischia Island (NW Mediterranean Sea) significantly differed in composition and abundance. Such differences involved a higher abundance of non-calcareous crustose brown algae and a decline of calcifying polychaetes in both acidified sites. A lower epiphytic abundance of crustose coralline algae occurred only in the south side of the vents, thus suggesting that OA may alter epiphyte assemblages in different ways due to interaction with local factors such as differential fish herbivory or hydrodynamics. The OA effects on food items (seagrass, epiphytes, and algae) indirectly propagated into food provision to the sea urchin *Paracentrotus lividus*, as reflected by a reduced *P. oceanica* exploitation (i.e. less seagrass and calcareous epiphytes in the diet) in favour of non-calcareous green algae in both vent sites. In contrast, we detected no difference close and outside the vents neither in the composition of sea urchin diet nor in the total abundance of calcareous versus non-calcareous taxa. More research, under realistic scenarios of predicted pH reduction (i.e. $\leq$ 0.32 units of pH by 2100), is still necessary to better understand cascading effects of this altered urchin exploitation of food resources under acidified conditions on ecosystem diversity and function.

1. Introduction

The concentration of carbon dioxide (CO_2) in the atmosphere is increasing mainly due to fossil fuel combustion and industrial processes. Oceans absorb approximately 30% of the anthropogenic CO_2 released to the atmosphere, which has caused a pH decrease in surface waters of 0.1 units since pre-industrial times in a process commonly known as ocean acidification (OA). An additional decrease in pH of 0.06–0.32 units is expected by the end of the century according to the different scenarios of CO_2 emissions used for projections (IPCC, 2014). Together with reduced pH, changes in the relative proportion of total dissolved inorganic carbon forms co-occur, including a reduced concentration of carbonate ions (CO_3^{2-}). These changes may negatively affect the formation of carbonate structures, shells and skeletons by calcifying organisms, as well as their metabolism (e.g. acid-base regulation), survival or abundance (Pörtner, 2008; Kroeker et al., 2011, 2013a). At the same time, increased availability of photosynthesis substrates (CO_2 and/or HCO_3^-) may enhance the photosynthesis and growth of non-calcifying primary producers such as phytoplankton, cyanobacteria, fleshy algae and seagrasses (Doney et al., 2009; Kroeker et al., 2010).

The above-mentioned responses and sensitivities of species to OA have mostly been identified by means of CO_2 enrichment experiments in laboratory or mesocosms. More recently, *in situ* observations of submarine volcanic CO_2 vents have provided data on

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* Corresponding author.
E-mail address: bmcrego@ualg.pt (B. Martínez-Crego).

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marine ecosystems long-term adapted to high CO_2/low pH levels, which integrate complex species interactions within entire communities (e.g. Fabricius et al., 2011; Kroeker et al., 2011, 2013a; Linares et al., 2015). In some of the most studied CO_2 vents around the world, seagrass meadows are exposed to pH conditions similar to future levels of ocean acidification, thus allowing to investigate long-term OA effects on such relevant ecosystems (Hall-Spencer et al., 2008; Vizzini et al., 2010; Apostolaki et al., 2014).

Seagrass meadows provide key ecosystem services such as stabilization of coastal sediments, provision of habitat and nursery for species (including several taxa of commercial interest), maintenance of biodiversity, and long-term removal of CO_2 from the atmosphere (Orth et al., 2006; Fourqurean et al., 2012). A highly diverse community of epiphytes (as defined by Steel and Wilson, 2003) colonizes seagrass leaves, which include calcareous and non-calcareous algae, hydrozoans, bryozoans, and polychaetes (Mazzella et al., 1989; Prado et al., 2007; Piazzi et al., 2016). Epiphytes are important contributors to total aboveground biomass and primary production in seagrass meadows (Champenois and Borges, 2012) and they play a major role in seagrass food webs (Mazzella et al., 1992; Williams and Heck, 2001; Vizzini, 2009). Particularly, the foraging behaviour of key herbivorous sea urchins on seagrasses has been reported to be largely driven by the occurrence of leaf epiphytes (Vergés et al., 2011; Marco-Méndez et al., 2012, 2015).

The dominance of coralline algae and other calcifying organisms and their high sensitivity to OA render the epiphyte community especially vulnerable to a high-CO_2 ocean. Previous studies, including observations in CO_2 vents as well as laboratory and mesocosm experiments, have shown a marked decline in the abundance of coralline algae and/or other calcifying taxa under high $p$$CO_2$/low pH, often associated to a proliferation of non-calcareous taxa (e.g. Hall-Spencer et al., 2008; Kuffner et al., 2008; Fabricius et al., 2011). Whilst such direct effects on single species or taxa are relevant in structuring marine communities, indirect effects of OA involving species interactions (e.g. trophic interactions) can also be of importance for the overall response of ecosystem diversity and function (Kroeker et al., 2011, 2013a; Russell et al., 2012). How OA indirectly affects key ecological meadow functions such as provision of food to herbivores has been scarcely investigated (but see Ricevuto et al., 2015; Tomas et al., 2015). Both, direct and indirect OA effects may have relevant consequences in the maintenance of the key ecological functions and ecosystem services delivered by these priority habitats.

Among herbivores inhabiting seagrass meadows, sea urchins play a major role in controlling the composition and abundance of seagrass and macroalgal communities (Palacín et al., 1998; Valentine and Heck, 1999; Poore et al., 2012). They also have a relevant commercial value around the world since sea urchin gonads are very appreciated culinary delicacies (Lawrence, 2007). Sea urchins at different development stages appear to be more resistant to near future OA than previously thought, as revealed by studies using realistic pH scenarios based on IPCC (2014) predictions for the end of the century (i.e. a decrease lower or equal than 0.32 units of pH). Reports of OA effects on early developmental stages of sea urchins are contradictory, indicating either larvae and juvenile vulnerability or resistance (reviewed by Dupont et al., 2010; see also Yu et al., 2011; Moulin et al., 2011). Furthermore, adult sea urchins appear to be more clearly resistant to OA under realistic predictions, as only highly species-specific sublethal effects have been reported by studies in which extreme pH reductions are not considered (Dupont et al., 2010; Hazan et al., 2014; Moulin et al., 2015). As a result, adult sea urchins metabolically adapted to CO_2/pH levels predicted for 2100 are expected to be sensitive to indirect effects of OA-induced changes in the availability and quality of their food. However, very few studies have investigated these indirect effects, being most of them focused on how experimentally CO_2-induced changes on seagrass traits alter urchin consumption disregarding seagrass epiphytes (Tomas et al., 2015), or on effects of monospecific macroalgal diets at different CO_2 levels on calcite structures of sea urchins (Asnaghi et al., 2013). Given the pivotal role that sea urchins play in structuring communities through grazing (Carpenter, 1986; Bulleri et al., 1999), indirect OA effects on these grazers' impacts may cause cascading effects on ecosystem functioning and diversity.

In this study, we compared the composition and abundance of the epiphyte community of the seagrass *Posidonia oceanica* in a control site and in two sites affected by high $p$$CO_2$/low pH in volcanic vents at the Ischia Island (NW Mediterranean Sea). We hypothesized that altered seawater chemistry close to the vents shifts the epiphyte community towards the dominance of more tolerant taxa (e.g. non-calcifying species), and that these shifts are propagated into indirect effects on the diet of the commercially important sea urchin *Paracentrotus lividus* that inhabits *P. oceanica* meadows.

2. Material and methods

2.1. Study area

This study was conducted in the Ischia Island, which is located in the Gulf of Naples (Italy) in the Tyrrhenian Sea (NW Mediterranean Sea). Submarine CO_2 vents of volcanic origin occur at 0.5–3 m depth in the north and south sides of a small islet (Castello Aragonese), where *Posidonia oceanica* meadows are naturally exposed to a gradient of CO_2 enrichment and pH reduction. Gas composition in this area consists of 90.1–95.3% CO_2, 3.2–6.6% N_2, 0.6–0.8% O_2, 0.2–0.8% CH_4 and 0.08–0.1 Ar, and do not contain sulphur nor involve temperature increase (Hall-Spencer et al., 2008). A more intense venting activity has been reported in the south than in the north side of the islet (Hall-Spencer et al., 2008). The south side is also more sheltered compared to the north side, with the latter exposed to north-western winds that are dominant in the area (Donnarumma et al., 2014).

We worked in two pH conditions, including a control site (Lacco-Ameno; 40° 45′26.0″ N, 13° 53′ 4.5″ E) and a low-pH area (Castello Aragonese, 40° 43′ 55.5/51.2″ N, 13° 57′ 50.5/47.6″ E) away and close to the vents, respectively. Our results revealed consistent differences between the north and south sides of the vent's area, and thus, two low-pH sites were considered separately (hereafter north- and south-low pH sites). This lowered the number of replicates, but allowed replicating CO_2 sites, which is a challenging issue in OA research given the difficulty of replicating complex ecological communities in mesocosms or laboratories. Seawater temperature and pH were measured *in situ* on 6 discrete water samples collected in the same sampling dates (October 8 and 9) and at the same depth (2.5–3 m) as for epiphyte and sea urchin samples (Multimeter 340 WTW, Weilheim, Germany; calibrated using NBS buffers). Average seawater temperature (±SE) was 23.0 ± 0.02, 22.8 ± 0.04, and 22.7 ± 0.02 °C at the control, low pH-north and low pH-south sites, respectively. Seawater pH (±SE) was 8.10 ± 0.004 in the control site and 7.78 ± 0.01 and 7.80 ± 0.02 in the low pH-north and -south sites, respectively. Total alkalinity in this season is around 2560 $\mu mol\ kg^{-1}$ in the low pH sites and 2563 $\mu mol\ kg^{-1}$ at ambient pH (measured using the titration method on discrete water samples) and salinity is 37.8 and 37.9, respectively (Kroeker et al., 2011, 2013a). These data were used to estimate the $p$$CO_2$ conditions using the CO2SYS program (Lewis and Wallace, 1998) with the constants of Mehrbach refit by Dickson and Millero (1987), which varied from 543 ± 6.0 μatm in the control and 1262 ± 33 and

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1176 ± 48 μatm in the low pH sites (north and south, respectively).

2.2. Epiphyte community

To assess differences in the composition and abundance of *P. oceanica* epiphytes in the vicinity and outside vents, the oldest leaf (outermost) of six randomly chosen shoots were collected in October 2013 at 2.5–3 m depth in the control and low pH areas (with the latter involving 3 replicates from each side of the vent system). Leaves were preserved in Kew (40% water, 40% alcohol, 10% glycerine and 10% formalin) for subsequent epiphyte identification in the laboratory. Older leaves were used because they are most commonly eaten by *P. lividus* under natural conditions (Vergés et al., 2011) and host the highest biomass and diversity of epiphytes compared to younger leaves (Casola et al., 1987; Cinelli et al., 1984; Mazzella et al., 1989). Using older leaves is also a commonly used procedure to reduce the sampling area representative of the full epiphyte community (e.g. Prado et al., 2007; Martínez-Crego et al., 2014). All sampling was performed in the shortest possible interval (2 days) to avoid the masking effect of the high seasonal variability reported elsewhere (e.g. Ballesteros, 1987; Romero, 1988; Mazzella et al., 1989) in the between-sites comparison aimed by our study.

Epiphytes (excluding unicellular organisms) were identified to the lowest possible taxon under a microscope. Relative abundance of each taxon was quantified as percentage of horizontal surface coverage after sorting using a stereomicroscope, and standardized per cm^2 of leaf area including the internal and external faces of the blade. The sampled leaves were representative of each sampling site (Martínez-Crego et al. unpublished data; Donnarumma et al., 2014), involving a mean length of 24.4 ± 0.9 cm in the control, 16.4 ± 0.9 cm in the low pH-north, and 15.3 ± 0.8 cm in the low pH-south, as well as leaves with intact or broken tips due to fish bites or other unknown causes.

2.3. Sea urchin diet

To investigate between-site differences in sea urchin diet, 10 individuals of *P. lividus* from each low pH site and 14 individuals from the control site were randomly collected within *P. oceanica* meadows avoiding rocky substrates, in the same sampling dates and at the same depth as for seawater and epiphyte samples. Adult individuals of similar size (averaging 5.1 ± 0.1 cm in diameter without spines) were collected and each specimen was dissected for extraction of the gut content. The entire intestinal track was carefully removed and food items in the gut content were identified and quantified as described above for epiphytes. One replicate of the low pH-north site was lost during sample processing and it was not further considered in the data analysis (north side $n = 9$).

2.4. Data analysis

One-way permutational multivariate analyses of variance (PERMANOVA) using 9999 unrestricted permutations of raw data were conducted to test for differences between sites in the composition (presence/absence data) and abundance (% cover data) of both, epiphyte assemblage and sea urchin diet. When significant differences were found, pairwise comparisons, as well as non-metric multidimensional scaling (nMDS) and similarity percentage (SIMPER) analyses, were performed. The nMDS analyses with Bray-Curtis distances were conducted to visually assess the pattern of differences between sites in epiphyte assemblages and sea urchin diets. The SIMPER analyses were used to identify which taxa contributed more (i.e. those with a higher % contribution) and more consistently (i.e. defined as those with a mean dissimilarity to standard deviation ratio equal or higher than 1.5, which means that mean contribution is higher than variation) to the overall dissimilarity between sites. Unidentified material represented on average < 1% and ≤4% cover in epiphyte and gut content samples, respectively, and was excluded from all analyses, except for the total epiphyte cover. Low abundant algal species identified to genus or higher taxonomic level were grouped into the same taxon prior to statistical analyses (see Appendix A).

To explore differences between sites in taxa richness and total epiphyte cover, as well as in the proportion of each main taxonomic/morphological group and in calcareous *vs.* non-calcareous taxa, we conducted one-way analyses of variance (ANOVAs) for each variable separately. Grouping details are shown in Tables A.1 (epiphytes) and A.2 (sea urchin gut contents). Prior to analysis, data were tested for normality and for homogeneity of variances. When data had a non-normal distribution even after trying several transformations, a non-parametric Kruskal-Wallis test was used, and when unequal variances were found a more restrictive significance level ($p < 0.01$) was used to minimize the possibility of type I error. When significant differences were found, ANOVA and Kruskal-Wallis tests were followed by Holm-Sidak and Dunn's post hoc comparisons, respectively, in order to determine which sites differed from each other. Unidentified material and rare groups (defined as those with an average abundance lower than 0.5%) were excluded from the univariate statistical analyses.

3. Results

3.1. Epiphyte community

A total of 34 taxa of epiphytes were identified on *P. oceanica* leaves (mostly to genus level), among which 13 were calcifying organisms (Table A.1). A taxa richness of 21 taxa was found in both the control and the low pH-north sites, whereas only 12 taxa were found in the low pH-south site. No significant differences were detected between sites in taxa richness (ANOVA $F = 2.0$, $df = 2$, $p = 0.192$). Total epiphyte cover (mean ± SE) was significantly higher in the low pH-north (85.9 ± 7.5% of the total leaf surface) than in the control (60.9 ± 5.9%) and low pH-south sites (55.5 ± 2.9%) with no significant differences between the control and the south-acidified site (post-hoc comparisons after ANOVA with $F = 5.2$, $df = 2$, $p = 0.03$).

The PERMANOVA results showed that epiphyte assemblages significantly differed between sites in both composition (Fig. 1a) and abundance (Fig. 1b). Pairwise comparisons detected significant differences between control and low pH sites, but no significant differences were detected between low pH-north and low pH-south sites. The nMDS plots represented the differences detected by PERMANOVA results with a relatively low stress value, indicating a reliable ordination pattern of the samples (Fig. 1). Control samples diverge from samples of both low pH sites, with such differences being more evident when presence/absence data were considered (Fig. 1a) compared to abundance data (Fig. 1b). Fig. 1a showed relatively low between-replicates variability in the control and low pH-north sites (i.e. replicates were close to each other), while the low pH-south site showed a high variability in epiphyte composition and abundance within the site (i.e. replicates were more disperse in the nMDS ordination space).

The SIMPER analysis conducted on epiphyte presence/absence data showed that the greatest dissimilarity (52%) occurred between control and low pH-south site, which was mainly explained by the absence of tube-building polychaetes of the subfamily Spirorbinae in the south site (Table 1). In contrast, the dissimilarity between control and low pH-north site (46%) was mostly driven by the absence of the hydrozoan *Sertularia distans* in the control site.

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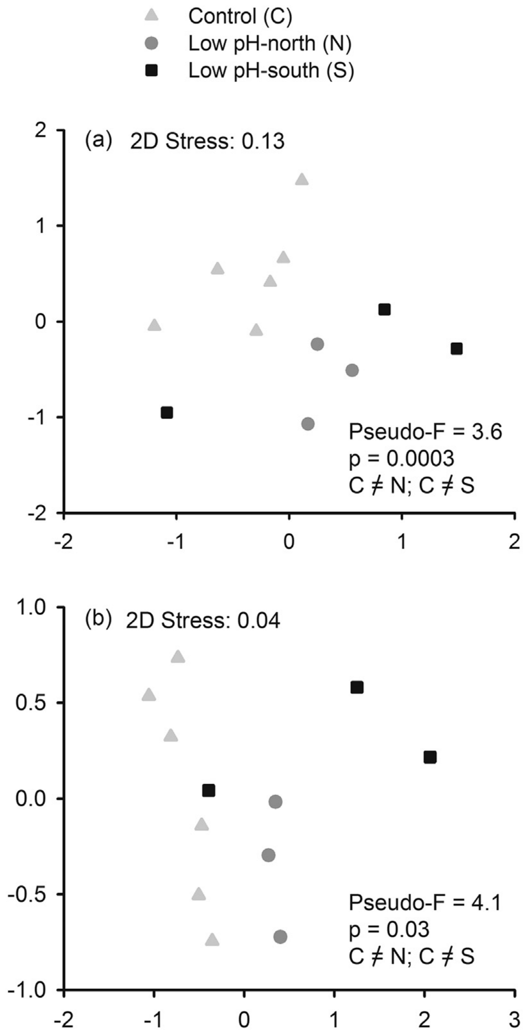

Fig. 1. Non-metric multidimensional scaling (nMDS) plot of epiphyte community samples (Bray-Curtis distances) for non-transformed presence/absence (a) and abundance (b) data. Statistics from one-way PERMANOVAs (Pseudo-F and p-value) and pairwise comparisons conducted using each dataset to examine multivariate differences between sites are shown (df = 2).

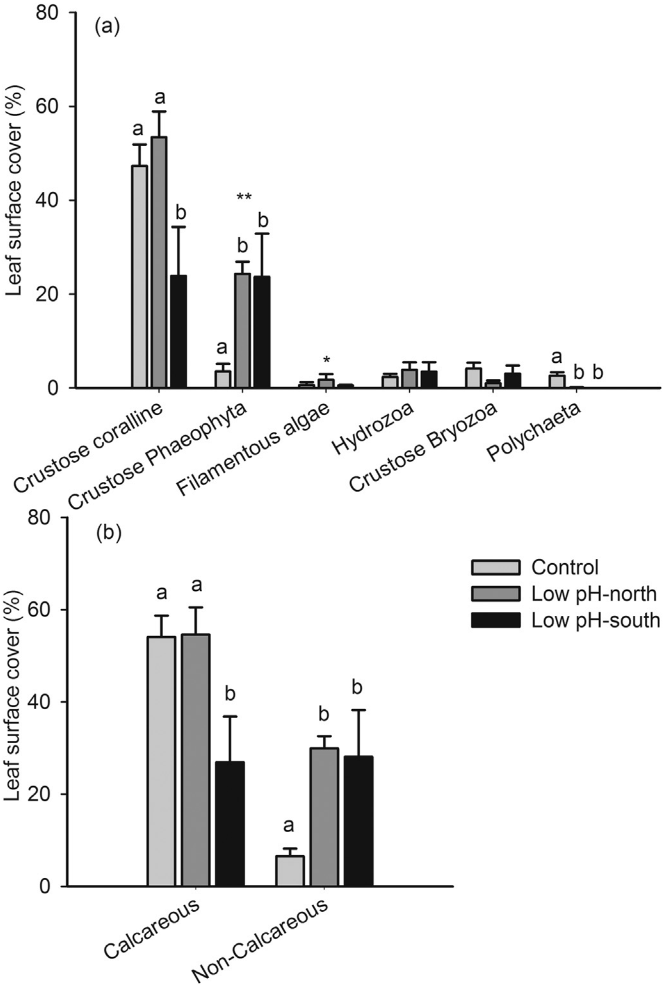

Fig. 2. Mean (±SE) proportion (%) of leaf surface covered by the different taxonomic/morphological groups (a), and calcareous *vs.* non-calcareous taxa (b) of the epiphyte community on *Posidonia oceanica* leaves. Different letters above bars within each group denote statistically significant differences between sites based on one-way ANOVA and post hoc comparisons. *Statistical analysis based on Kruskal-Wallis (non-normal data). **Reduced significance level at $p < 0.01$ (unequal variances). See Table C.1 for details on statistical results.

Dissimilarity between the two low pH sites (north and south) was high (47%), although not significant based on PERMANOVA results due to a high between-replicate variability in the low pH-south site. This dissimilarity was mainly due to the absence of calcifying taxa (Spirorbinae polychaetes and the bryozoan *Patinella* spp.) in the south site.

The SIMPER analysis based on epiphyte abundance data showed 77%, 84% and 49% similarity within replicates of each site in the control, low pH-north, and low pH-south site, respectively. The greatest dissimilarity (51%) was detected between control and low pH-south site, mostly due to a lower cover of the coralline algae *Hydrolithon*/*Pneophyllum* spp. and a higher cover of *Myrionema orbiculare* in the low pH-south site (Table 2). The absence of Spirorbinae polychaetes and a higher cover of the filamentous brown algae *Sphacelaria cirrosa* in the low pH-south site consistently contributed to this dissimilarity as well. Dissimilarity between the two low pH sites (36%) was not significant based on PERMANOVA results. Less but significant dissimilarity was observed between control and low pH-north site (33%), which was mainly driven by a higher cover (24%) of *Myrionema orbiculare* in the low pH-north site than in the control site (3.5%). A consistent but much lower contribution to this dissimilarity was due to a higher cover of the bryozoan *Patinella* spp. and the filamentous brown algae *Sphacelaria cirrosa* in low pH-north site.

When considering taxonomic/morphological groups, we found that the epiphyte community in control and low pH-north sites was dominated by crustose coralline algae of the genera *Hydrolithon*/*Pneophyllum*, which comprised 47% and 53% of the total epiphyte cover (Table 2). A significantly lower abundance of crustose coralline epiphytes was found in the low pH-south site, where it still was the most abundant taxa but showing a much lower abundance (23.9% cover) and sharing this position with crustose algae represented exclusively by the Phaeophyta *Myrionema orbiculare* (23.7% cover; Fig. 2a and Table 2). This latter species encompassed a higher percentage of cover in the two low pH sites (24%) than in the control site (3.5%). The abundance of the tube-building polychaetes (represented by Spirorbinae) was significantly lower at the low pH-north site and it was absent at the low pH-south site. No significant differences between sites were detected in the abundance of filamentous algae, hydrozoans and bryozoans. Overall, results show that the abundance of non-calcareous taxa was higher in the acidified sites than in the control. In contrast, the abundance of

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Table 1

Results of the SIMPER analysis conducted on presence/absence data showing the epiphyte taxa that mostly (highest contribution %) and more consistently (Diss/SD ≥ 1.5) contributed to the dissimilarity between sites in the community composition on *Posidonia oceanica* leaves. Complete list of taxa that contributed to 100% dissimilarity between sites in epiphyte composition is shown in Table B.1.

Dissimilarity = 46.4	Presence/Absence Control	Presence/Absence Low pH- north	Diss/SD	Contribution (%)
Sertularia distans	Absent	1.00	5.35	11.73
Dissimilarity = 51.7	Presence/Absence Control	Presence/Absence Low pH- south	Diss/SD	Contribution (%)
Spirorbinae	1.00	Absent	6.20	13.21
Dissimilarity = 46.7	Presence/Absence Low pH- north	Presence/Absence Low pH- south	Diss/SD	Contribution (%)
Spirorbinae	1.00	Absent	5.38	12.28
Patinella spp.	1.00	Absent	5.38	12.28

Table 2

Results of the SIMPER analysis conducted on abundance data showing the cover (%) of epiphyte taxa (mean ± SE) that mostly (contribution > 5%) and more consistently (Diss/SD ≥ 1.5) contributed to dissimilarity between sites in the epiphyte assemblage on *Posidonia oceanica* leaves. Complete list of taxa that contributed to 100% dissimilarity between sites is shown in Table B.2.

Dissimilarity = 32.6	Abundance Control	Abundance Low pH- north	Diss/SD	Contribution (%)
Myrionema orbiculare	3.52 ± 1.62	24.29 ± 2.58	3.55	44.62
Hydrolithon/Pneophyllum spp.	47.31 ± 4.57	53.45 ± 5.45	1.47	25.30
Sertularia distans	Absent	2.63 ± 1.26	1.40	5.92
Spirorbinae	2.61 ± 0.70	0.14 ± 0.05	1.45	5.41
Microporella spp.	2.37 ± 0.93	0.03 ± 0.03	1.04	5.07
Patinella spp.	0.09 ± 0.07	0.46 ± 0.18	1.55	0.87
Sphacelaria cirrosa	0.06 ± 0.05	0.41 ± 0.13	1.68	0.81
Dissimilarity = 51.1	Abundance Control	Abundance Low pH- south	Diss/SD	Contribution (%)
Hydrolithon/Pneophyllum spp.	47.31 ± 4.57	23.88 ± 10.41	1.72	41.65
Myrionema orbiculare	3.52 ± 1.62	23.66 ± 9.23	1.43	35.62
Spirorbinae	2.61 ± 0.70	Absent	1.52	4.59
Sphacelaria cirrosa	0.06 ± 0.05	0.44 ± 0.16	1.63	0.66
Dissimilarity = 36.5	Abundance Low pH- north	Abundance Low pH- south	Diss/SD	Contribution (%)
Hydrolithon/Pneophyllum spp.	53.45 ± 5.45	23.88 ± 10.41	1.72	57.34
Myrionema orbiculare	24.29 ± 2.58	23.66 ± 9.23	1.68	23.75
Microporella spp.	0.03 ± 0.03	2.76 ± 1.93	0.97	5.30
Patinella spp.	0.46 ± 0.18	Absent	1.67	0.90
Sphacelaria cirrosa	0.41 ± 0.13	0.44 ± 0.16	1.72	0.50
Spirorbinae	0.14 ± 0.05	Absent	1.63	0.28

calcareous taxa did not decrease consistently at both low pH sites, being only lower at the south site (Fig. 2b).

3.2. Sea urchin diet

A total of 14 food items were identified in *P. lividus* gut content, including pieces of *P. oceanica* blades and calcareous epiphytes, as well as 12 algal taxa among which only two were calcareous forms (Table A.2). Thirteen taxa were recorded in both the control site and the low pH-north site, while 12 taxa were found in the low pH-south site. No significant differences between sites were detected in the number of taxa (Kruskal-Wallis H = 3.1, df = 2, p = 0.216).

The PERMANOVA results showed significant differences between sites in sea urchin diet when considering abundance data (Fig. 3), but not when testing presence/absence data (pseudo-F = 1.9, df = 2, p = 0.08). Pairwise comparisons showed that the abundance of food items significantly varied between control and both low pH sites, but no differences were found between low pH-north and low pH-south sites. The nMDS performed on abundance data showed that control samples were grouped together, indicating that they differed from both low pH sites and showed small between-replicate variability (Fig. 3). Low pH samples from north and south sites were mixed and scattered in the ordination space, indicating no differences between sites and high between-replicate variability.

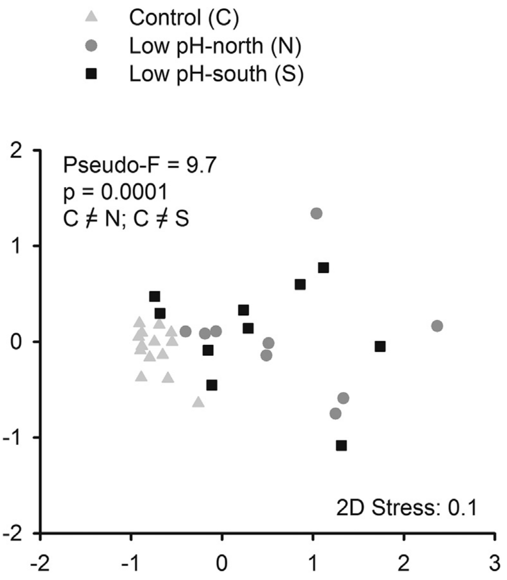

Fig. 3. Non-metric multidimensional scaling (nMDS) plot of *Paracentrotus lividus* gut content samples (Bray-Curtis distances, non-transformed abundance data). Statistics from one-way PERMANOVA (Pseudo-F and p-value) and pairwise comparisons examining multivariate differences between sites are shown (df = 2).

The SIMPER analysis based on abundance data identified 86%, 65% and 66% similarity within replicates in the assemblage of sea urchin gut contents in the control, low pH-north, and low pH-south site, respectively. The greatest dissimilarity (40.1%) occurred between control and low pH-north site, closely followed by dissimilarities between the two low pH sites (34.9%; not significant

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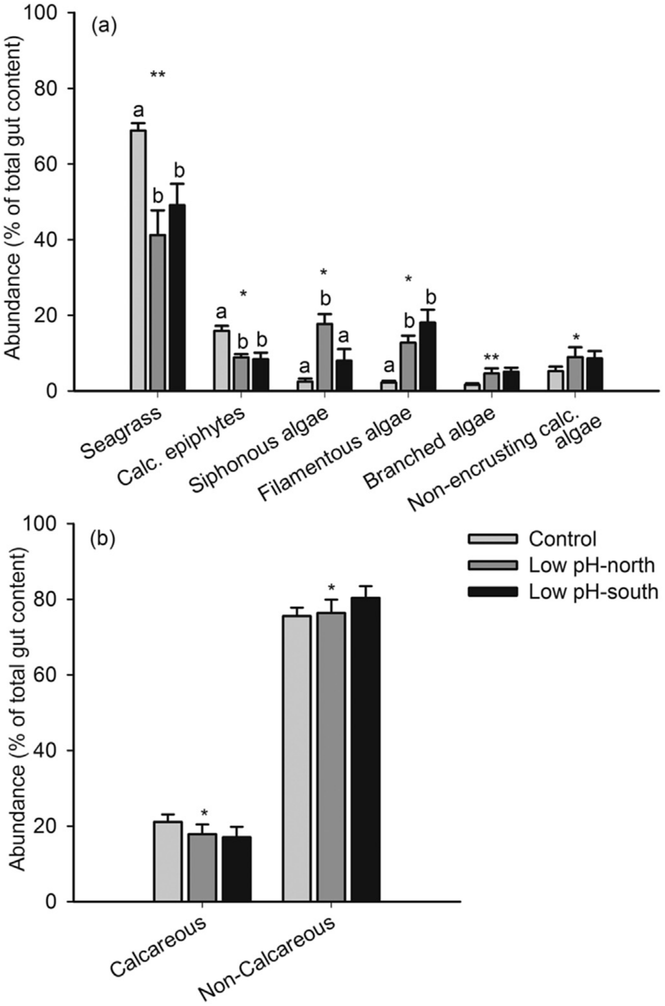

Fig. 4. Mean (±SE) proportion (%) of total gut content of the sea urchin *Paracentrotus lividus* covered by the different taxonomic/morphological groups (a) and calcareous *vs.* non-calcareous taxa (b). Different letters above bars within each group denote statistically significant differences between sites based on one-way ANOVA post hoc comparisons. *Statistical analysis based on Kruskal-Wallis (non-normal data). **Reduced significance level at $p < 0.01$ (unequal variances). Calc. = calcareous. See Table C.2 for details on statistical results.

dissimilarity based on PERMANOVA results that is not further considered) and between control and low pH-south site (34.7%). Seagrass abundance was responsible for more than 30% of all dissimilarities between sites (Table 3). Dissimilarity between control and both low pH sites was mostly due to a lower abundance of seagrass and calcareous epiphytes and a higher abundance of *Flabellia petiolata* and *Cladophora prolifera* in the gut content of sea urchins collected in acidified sites (>77% total contribution of the 4 taxa to dissimilarities). This contribution was also consistent (Diss/SD $\geq$ 1.5) to the dissimilarity between control and low pH-north sites, while only *P. oceanica* and calcareous epiphytes consistently contributed to the dissimilarity between control and low pH-south sites. A consistent but much lower contribution to dissimilarity between control and low pH-north sites was due to a higher abundance of *Peyssonnelia* spp. in the low pH site.

When considering the abundance of taxonomic/morphological groups, we found that the seagrass *P. oceanica* was the dominant food item in all sites (69%, 41% and 49% of total gut content in control, low pH-north and south sites, respectively), followed in abundance by calcareous epiphytes only in the control site (16%; Fig. 4a, Table 3). A significantly lower abundance of the dominant food item in sea urchins diet (seagrass) and calcareous epiphytes, as well as more abundant filamentous algae (mostly represented by several species of the genus *Cladophora*) were found in the two low pH sites. A tendency towards more abundant branched algae (represented by *Asparagopsis* spp., *Halopteris scoparia* and *Dictyota* spp.) was observed in the gut content of sea urchins from the two low pH sites, although it was statistically not significant ($p = 0.015$) according to the criteria used for data showing unequal variances (i.e. significance level reduced to $p < 0.01$; see Table C.2). Particularly, *C. prolifera* was the second most abundant food item in the low pH-south site (16% cover). A significantly higher abundance of siphonous green algae (represented mostly by *F. petiolata* and to a lesser extent by *Derbesia* spp.) was also observed in the low pH-north site, where it was the second dominant food item (15% cover). Overall, the abundance of all calcareous and non-calcareous food items did not significantly differ between sites (Fig. 4b), and non-calcifying taxa dominated sea urchins gut contents regardless of the studied site.

4. Discussion

Our study reveals that epiphyte communities of seagrass inside and outside the influence of CO_2 vents significantly differ in composition and abundance. A marked reduction in the abundance of the crustose coralline algae has been widely reported as a major effect of ocean acidification by manipulative experiments conducted on different seagrass species (Campbell and Fourqurean, 2014; Martínez-Crego et al., 2014; Cox et al., 2015). Our results partially support this observation, as we found a lower abundance of the coralline algae that naturally dominate epiphyte assemblages growing on shallow *P. oceanica* leaves in late summer-autumn (Ballesteros, 1987; Romero, 1988; Mazzella et al., 1989), but only in the south side of the vent system. This is coherent with a previous study conducted in the same side of the Ischia vent (Martin et al., 2008). On the contrary, the abundance of coralline algae did not differ between the north side of the vents and the control, thus suggesting that a pH reduction higher than that predicted under realistic scenarios (up to 0.32 units of pH by 2100) is necessary to critically affect the survival of coralline algae. Such extremely low pH conditions (pH $\leq$ 7.3) have been reported to dissolve coralline epiphytes of seagrasses by previous studies (Martin et al., 2008).

We found that the non-calcareous crustose brown algae *Myrionema orbiculare* together with crustose coralline algae dominated the total epiphyte cover in the south side of the vents (24% cover each taxa), whereas coralline algae dominated the assemblage in the north side (53% cover). The dominance of coralline algae in the north side of the vents and of brown algae in the south side is in agreement with epiphyte settlement on artificial leaves observed after 3 months in the same study sites by Donnarumma et al. (2014). Dominance of non-calcareous fleshy algae under ocean acidification has been proposed to result from a mixed interaction of direct (e.g. higher production due to CO_2 surplus, physiological tolerance to low pH) and indirect effects (e.g. interspecific competition such as decreased competition with coralline algae, increased leaf space available for recruitment due to the reduced coralline algae cover; Kuffner et al., 2008; Hepburn et al., 2011; Harley et al., 2012; Kroeker et al., 2013b). However, the dissimilarities in epiphyte assemblages that we observed between the north and south sides of the vent system, even when they were subjected to a similar OA level, suggests that differential factors locally interact with ocean acidification within the vent system. Spatial patterns of epiphyte assemblages in shallow meadows are often influenced by variability in local natural factors such as

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Table 3
Results of the SIMPER analysis conducted on abundance data showing the cover (%) of food items in the gut content (mean ± SE) and their contribution to the dissimilarity between sites in diet of *Paracentrotus lividus*.

Dissimilarity = 40.1	Abundance Control	Abundance Low pH- north	Diss/SD	Contribution (%)	Cumulative (%)
Posidonia oceanica	68.88 ± 1.92	41.20 ± 6.53	1.48	37.65	37.65
Flabellia petiolata	2.40 ± 0.82	14.64 ± 2.93	1.54	16.88	54.53
Cladophora prolifera	0.91 ± 0.31	11.56 ± 2.01	1.74	14.03	68.56
Calcareous epiphytes	15.90 ± 1.29	8.89 ± 0.85	1.39	9.34	77.90
Peyssonnelia spp.	3.25 ± 0.67	5.74 ± 1.37	1.46	5.67	83.57
Halopteris scoparia	1.39 ± 0.37	4.52 ± 1.35	1.29	5.36	88.93
Jania rubens	1.97 ± 0.83	3.21 ± 1.49	0.81	4.46	93.39
Derbesia spp.	0.09 ± 0.07	3.06 ± 3.02	0.37	4.02	97.41
Cladophora spp.	1.16 ± 0.32	1.19 ± 0.34	1.23	1.52	98.93
Acrodiscus cf. *vidovichii*	0.39 ± 0.39	0.03 ± 0.03	0.30	0.54	99.48
Asparagopsis spp.	0.18 ± 0.08	0.09 ± 0.03	0.81	0.26	99.73
Polysiphonia spp.	0.14 ± 0.14	Absent	0.28	0.19	99.92
Ulva spp.	Absent	0.03 ± 0.03	0.35	0.04	99.96
Dictyota spp.	0.02 ± 0.02	0.01 ± 0.01	0.40	0.04	100.00
Dissimilarity = 34.7	Abundance Control	Abundance Low pH- south	Diss/SD	Contribution (%)	Cumulative (%)
Posidonia oceanica	68.88 ± 1.92	49.15 ± 5.62	1.52	33.50	33.50
Cladophora prolifera	0.91 ± 0.31	15.97 ± 3.53	1.41	22.47	55.97
Calcareous epiphytes	15.90 ± 1.29	8.42 ± 1.66	1.62	12.79	68.75
Flabellia petiolata	2.40 ± 0.82	8.02 ± 3.05	0.84	10.69	79.45
Jania rubens	1.97 ± 0.83	4.05 ± 1.41	0.89	5.59	85.03
Peyssonnelia spp.	3.25 ± 0.67	4.56 ± 1.01	1.36	4.92	89.95
Halopteris scoparia	1.39 ± 0.37	3.62 ± 0.93	1.15	4.32	94.27
Cladophora spp.	1.16 ± 0.32	1.82 ± 0.50	1.16	2.28	96.55
Asparagopsis spp.	0.18 ± 0.08	0.94 ± 0.26	1.21	1.30	97.85
Dictyota spp.	0.02 ± 0.02	0.50 ± 0.50	0.35	0.78	98.63
Acrodiscus cf. *vidovichii*	0.39 ± 0.39	0.04 ± 0.03	0.30	0.62	99.25
Polysiphonia spp.	0.14 ± 0.14	0.30 ± 0.30	0.43	0.61	99.86
Derbesia spp.	0.09 ± 0.07	Absent	0.36	0.14	100.00
Ulva spp.	Absent	Absent	–	–	–
Dissimilarity = 34.9	Abundance Low pH- north	Abundance Low pH- south	Diss/SD	Contribution (%)	Cumulative (%)
Posidonia oceanica	41.20 ± 6.53	49.15 ± 5.62	1.38	32.33	32.33
Flabellia petiolata	14.64 ± 2.93	8.02 ± 3.05	1.47	17.64	49.96
Cladophora prolifera	11.56 ± 2.01	15.97 ± 3.53	1.30	15.31	65.27
Calcareous epiphytes	8.89 ± 0.85	8.42 ± 1.66	1.34	6.71	71.98
Jania rubens	3.21 ± 1.49	4.05 ± 1.41	0.99	6.44	78.42
Peyssonnelia spp.	5.74 ± 1.37	4.56 ± 1.01	1.43	6.25	84.67
Halopteris scoparia	4.52 ± 1.35	3.62 ± 0.93	1.41	5.92	90.59
Derbesia spp.	3.06 ± 3.02	Absent	0.36	4.52	95.11
Cladophora spp.	1.19 ± 0.34	1.82 ± 0.50	1.16	2.19	97.30
Asparagopsis spp.	0.09 ± 0.03	0.94 ± 0.26	1.19	1.33	98.63
Dictyota spp.	0.01 ± 0.01	0.50 ± 0.50	0.34	0.77	99.41
Polysiphonia spp.	Absent	0.30 ± 0.30	0.33	0.45	99.86
Acrodiscus cf. *vidovichii*	0.03 ± 0.03	0.04 ± 0.03	0.56	0.10	99.95
Ulva spp.	0.03 ± 0.03	Absent	0.35	0.05	100.00

herbivory pressure or hydrodynamics that alter seagrass leaf length (Martínez-Crego et al., 2010). For instance, intense grazing by the fish *Salpa salpa*, and subsequent shorter *P. oceanica* leaf lengths, has been observed in the most acidified areas of the studied vent system (Donnarumma et al., 2014), and also in our south study site (Garrard, 2013; and personal observations). A likely explanation for the observed within-vent differences in epiphyte assemblages could then be that the intense fish herbivory in the south side of vents is removing the aged apical parts of the blades, where crustose coralline algae (*Hydroliton*/*Pneophyllum* spp.) are more abundant (Casola et al., 1987). According to the cited study, the crustose algae *M. orbiculare* reaches a maximum cover on the basal part of older leaves, which would remain after fish grazing. Similarly, leaf shortening may affect the ability of seagrass photosynthetic activity to naturally raise pH local conditions and buffer the effects of ocean acidification (Semesi et al., 2009), since this buffer effect has been reported to be highly dependent on shoot density and leaf length (Hendriks et al., 2014). Another complementary explanation for the observed differences could be related to the higher hydrodynamics that characterize the north side of the vent system compared to the south side due to the dominant north-western winds in the area, which may influence epiphyte biomass (Lavery et al., 2007) and alter the thickness of the diffusion boundary layer and the surrounding pH and carbonate saturation (Hurd, 2015). Furthermore, other unexplored environmental factors such as differential light or nutrient availability in the north and south sides of vents may interact with ocean acidification and contributed to the within-vent differences that we observed.

We observed differences in the composition and abundance of the epiphyte assemblage that were common to both sides of the vent system compared to the control, and thus more likely related to direct effects of low pH/high pCO_2. The abundance of the crustose algae *M. orbiculare* was higher close to the vents (24%) than in the control (4%). The higher abundance of *Myrionema* contrasts with the overgrowth of more opportunistic filamentous algae observed in CO_2-enrichment experiments (Russell et al., 2009; Burnell et al., 2014; Campbell and Fourqurean, 2014). A likely explanation is that light- or nutrient-limiting conditions in our system compared with the much higher availability in these experimental systems, prevented the overgrowth of such opportunistic algae. Similarly, we

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found that the composition of the epiphyte community in the south side of the vents differed from the control mostly due to the absence of Spirorbinae serpulid worms, which form and inhabit coiled carbonate tubes. The abundance of Spirorbinae polychaetes was also significantly lower in the north side of the vents than in the control. These observations are coherent with the decline in the abundance of *Spirorbis* sp. experimentally found on *Thalassia testudinum* leaves at pH 7.88 (Campbell and Fourqurean, 2014) and with the reduced Spirorbinae abundance on mimics of *Posidonia* rhizomes found in moderately and highly acidified areas of the Ischia vent (Donnarumma et al., 2014). Lastly, overall bryozoan cover did not differ inside and outside vent influence, which is in agreement with the resistance to ocean acidification of calcifying epiphyte bryozoans showed by previous studies (Lombardi et al., 2011, 2015; Saderne and Wahl, 2013; Cox et al., 2015). However, of a total of 5 bryozoan species identified plus 5 more non-identified species, only 2 of them were found in the south side of vents (*Electra posidoniae* and *Microporella* spp.) and 6 in the north side (*Microporella* spp., *Fenestrulina joanne*, *Patinella* spp., and 2 non-identified bryozoans; Table B.2). This indicates that changes in bryozoan composition may occur without effects in the total bryozoan cover, thus suggesting inter-species differences in their resistance or sensitivity.

The OA-effects indirectly propagated into the diet of the sea urchin *Paracentrotus lividus* in the vent system. Epiphytes on *P. oceanica* leaves are the main source of calcareous epiphytes to sea urchin diet, and they were unselectively ingested by sea urchins along with seagrass leaves close to the vents. Differences in the composition and abundance of the epiphyte community that were common to both sides of the vents may, at least partially, mediate the lower *P. oceanica* exploitation by sea urchins. Other relevant changes in sea urchin diet did not directly relate to the altered epiphyte community *per se*, since urchin diet is mostly based on food items that are not included in epiphyte community such as *P. oceanica* leaves and macroalgae (Boudouresque and Verlaque, 2001; Prado et al., 2010; present study). For instance, a CO_2-induced reduction in seagrass palatability that decrease *P. lividus* feeding as observed by a previous experimental study (Tomas et al., 2015), could also partially explain the lower ingestion of seagrass and their associated calcareous epiphytes. A decreased seagrass nutritional quality (i.e. high C:N ration and/or low N content) induced by high pCO_2 levels could be behind such feeding deterrence. However, findings of experimental studies are contradictory, reporting either a CO_2-induced reduction in seagrass nutritional quality (Jiang et al., 2010) or no change (Martínez-Crego et al., 2014; Tomas et al., 2015). Furthermore, previous observations in the Ischia vents showed an increased seagrass quality in the acidified sites (Garrard, 2013; Ricevuto et al., 2015). Further investigation is needed to shed light on this controversial issue, which may have important ecological consequences due to bottom-up control of food webs by primary producers. We also found a subsequent replacement of seagrass and calcareous epiphytes in sea urchin diet close to the vents by more abundant green algae composed mostly by the filamentous *Cladophora prolifera* and the siphonous *Flabellia petiolata*. To less extent, semi-calcareous red algae of the genus *Peyssonnelia* were more abundant as well in the north side of the vents. These alternative food sources that were more abundant in sea urchin diet close to the vents are likely related to OA-induced changes in macroalgae availability, since they matched the shift observed by Porzio et al. (2011) in the adjacent rocky substrate from a community dominated by *Jania rubens* (69% cover), *Valonia utricularis* (29%), and *F. petiolata* (20%) at pH 8.14, towards dominance by *F. petiolata* (66%), *Hydrolithon cruciatum* (19%), *Jania rubens* (10%), and *Peyssonnelia* spp. (10%) at pH 7.83. This previous study also showed a high resilience of macroalgal species to lowered pH, with only a 5% reduction in the number of species at low pH. Macroalgal resilience is a likely explanation behind the lack of changes in the composition of food items that we found in sea urchin diet.

Interestingly, we found no overall differences in the vicinity and outside the vents in the abundance of calcareous and non-calcareous taxa in the sea urchin gut content, thus suggesting that the lower ingestion of calcareous epiphyte close to the vents was compensated by a higher ingestion of other calcareous macroalgae that are not epiphytic. For instance, Fig. 4a suggests a tendency albeit not statistically significant towards more abundant non-encrusting calcareous algae (represented by *Jania rubens* and *Peyssonnelia* spp.) in the urchin gut content. This compensation would be coherent with experimental studies that reported benefits, particularly under elevated CO_2 levels, of a diet based on a coralline macroalgae compared to two non-calcareous brown macroalgae for the size and/or robustness of the calcite structures of *P. lividus* such as their skeleton and grazing apparatus (Asnaghi et al., 2013, 2014). Similarly, the relevance of the red algae in supplying essential nutritional requirements that enhanced the feeding and gonad condition of *P. lividus* has also been highlighted by previous studies (Murillo-Navarro and Jiménez-Guirado, 2012).

Overall, indirect propagation of OA-effects into sea urchin diet in the vent system may result into a different functional role of sea urchins under acidified conditions, which may have cascading impacts on ecosystem diversity and function. This altered functional role would be in agreement with previous observations that revealed no variation in sea urchin abundance or biomass but lowered signs of sea urchin grazing on algae in low pH zones of the Ischia vent (Kroeker et al., 2013a). More research, conducted under realistic scenarios of pH reduction based on IPCC predictions (i.e. a decrease ≤ 0.32 units of pH by 2100), is also necessary to better understand consequences of CO_2-induced effects of diet change in sea urchin performance and population dynamics.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2017.04.002.

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