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Ocean acidification afters metodentific assemblage composition and organic matter
degradation rates in seagrass sediments, regardless of nutrient availability
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Running head: benthos responses to multiple stressors
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26 angiosperm, meiofauna, multiple stressors

27 Abstract

28 29	Seagrass meadows are an important organic matter (OM) reservoir, but are currently being lost due
30	to global and regional stressors. Yet, there is limited research investigating the cumulative impacts
31	of anthropogenic stressors on the structure and functioning of seagrass benthic assemblages, key
32	drivers of OM mineralization and burial. Here, using a 16-months field experiment, we assessed
33	how meiobenthic assemblages and extracellular enzymatic activities (as a proxy of OM
34	degradation) in Posidonia oceanica sediments responded to ocean acidification (OA) and nutrient
35	loadings, at CO2 vents. P. oceanica meadows were exposed to three nutrient levels (control,
36	moderate and high) at both ambient and low pH sites. OA altered meiobenthic assemblage
37	structure, resulting in increased abundance of annelids and crustaceans, along with a decline in
38	foraminifera. In addition, low pH enhanced OM degradation rates in seagrass sediments, by
39	enhancing extracellular enzymatic activities, potentially decreasing the sediment carbon storage
40	capacity of seagrasses. Surprisingly, nutrient enrichment had not effect on the response variables
41	analysed, suggesting that, under nutrient concentration unlikely to cause N- or P- limitation, a
42	moderate increase of dissolved nutrients in the water column had limited influence on meiobenthic
43	assemblages. These findings show that OA, regardless of nutrient availability, can significantly alter
44	meiobenthic assemblage structure and enhance OM degradation rates in seagrass sediments. Since
45	meiofauna are ubiquitous key actors in the functioning of benthic ecosystems, we postulated that
46	OA could alter the structure of benthic meiofaunal assemblages, OM degradation and organic
47	carbon sequestration over large spatial scales.

48 Introduction

49

50 Seagrass meadows are among the most productive and valued ecosystems on Earth, as they 51 sustain biodiversity and a range of ecosystem services, including enhanced water quality, coastline 52 protection from erosion and productive fisheries (Larkum et al. 2007, Barbier et al. 2011). They 53 also have a large influence on coastal biogeochemical processes, such as carbon storage and 54 nutrient regeneration, at global scale (Fourqurean et al. 2012, Duarte et al. 2013, Macreadie et al. 55 2017). These biogeochemical processes occur mostly in the belowground sediments and are driven 56 by interactions between fauna and heterotrophic prokaryotes, primary mediators of OM 57 mineralization and burial (Danovaro 1996, Snelgrove et al. 2018, Trevathan-Tackett et al. 2018a). 58 Many seagrass beds, and the ecosystem functions and services they provide, have been 59 degraded worldwide, with an estimated global decline of 7% annually since 1990 (Orth et al. 2006, 60 Waycott et al. 2009). Coastal eutrophication is one of the major drivers of seagrass loss, either 61 resulting in nitrogen toxicity for plants or reduced light availability on leaves due to epiphyte 62 overgrowth (Ralph et al. 2006, Burkholder et al. 2007, Marbà et al. 2014). More recently, global 63 scale stressors, such us seawater warming, ocean acidification and extreme events, have been shown to impair plant production and contribute to the decline and degradation of seagrasses 64 65 (Marba and Duarte 2010, Jordà et al. 2012, Ravaglioli et al. 2017, Arias-Ortiz et al. 2018, Chefaoui et al. 2018). As seagrass meadows are key for organic carbon sequestration, their decline is raising 66 67 concerns over the potential release in the atmosphere, as CO₂, of large amounts of the carbon 68 immobilized by the belowground compartment, potentially exacerbating climate changes 69 (Fourqurean et al. 2012, Pendleton et al. 2012). Nonetheless, the cumulative impacts of 70 environmental stressors on the structure and functioning of benthic assemblages associated to 71 seagrass systems, and their links to biogeochemical cycles, remain poorly understood. 72 Anthropogenic ocean acidification (OA), resulting from the global enhanced CO₂ emission, 73 is one of the greatest threats to coastal habitats (IPCC 2014). While the responses of seagrasses and

74	the associated epiphytic communities to low pH have been thoroughly assessed (Hall-Spencer et al.
75	2008, Martin et al. 2008, Campbell and Fourqurean 2014, Cox et al. 2015, Guilini et al. 2017,
76	Ravaglioli et al. 2017), there is a dearth of studies dealing with the impacts of OA on meiofauna
77	that inhabit seagrass sediments. This abundant and high diverse group of small invertebrates (<
78	1mm) plays key ecological roles in marine sediments, contributing to energy transfer to higher
79	trophic levels (Schratzberger and Ingels 2018) and increasing OM remineralisation, through the
80	stimulation of microbial activities (Nascimento et al. 2012, Bonaglia et al. 2014, Lacoste et al.
81	2018). OA can change meiobenthic assemblages, either directly, by altering metabolic processes or,
82	indirectly, by modifying interactions among species and trophic groups (e.g. predation pressure)
83	(Kurihara et al. 2004, Dashfield et al. 2008, Widdicombe and Spicer 2008, Kroeker et al. 2011,
84	Meadows et al. 2015, Mevenkamp et al. 2018). Laboratory (generally short-term) studies have
85	reported divergent responses to OA of different meiobenthic taxa, with the dominant one (typically
86	nematodes and copepods) generally remaining unaffected or even increasing in abundance, while
87	others, such as copepod naupli, gastrotrichs and foraminifera, showing opposing trends (Haynert et
88	al. 2011, Meadows et al. 2015, Lee et al. 2017, Mevenkamp et al. 2018). In contrast, long-term
89	exposure to low pH condition at submarine CO2 vents led to a severe decline in meiofaunal density,
90	suggesting limited capacities for several taxa to withstand or adapt to OA (Molari et al. 2018). The
91	alteration or loss of meiofaunal biodiversity could ultimately result in a significant decline of
92	important ecosystem functions, including prokaryote production and OM mineralization (Danovaro
93	et al. 2008, Pusceddu et al. 2014b).
94	OA could further affect OM degradation in seagrass sediments, by altering microbial
95	activities. Microbes mediate OM degradation by releasing extracellular enzymes, which catalyse the
96	degradation of complex and refractory molecules to more labile forms of OM that, in turn, can be
97	used by heterotrophs (Cunha et al. 2010). Several studies suggested that bacteria extracellular
98	activities may increase under OA scenario, likely triggered by the higher availability of organic
99	resources due to enhanced primary production (Cunha et al. 2010, Piontek et al. 2013, James et al.

100	2017). This could ultimately result in the reduction of organic carbon sequestration in seagrass
101	sediments (Trevathan-Tackett et al. 2018a).
102	In addition to the global threat of OA, the structure and functions of benthic assemblages
103	associated to seagrass system can be further affected by local changes of dissolved inorganic
104	nutrient concentrations in seawater. In seagrass sediments, a large amount of organic detritus is
105	generally refractory and not readily available for consumers (Danovaro 1996, Pusceddu et al. 2003).
106	Enhanced nutrient concentration might increase the abundance and diversity of meiofauna
107	indirectly, by enhancing the nutritional quality of food (e.g. lower C/N ratio) (Antón et al. 2011),
108	thus fostering feeding activities (Pascal et al. 2013). In contrast, excessive organic loadings, typical
109	of eutrophic waters, may strongly alter sediment characteristics (e.g. sediment biochemical
110	composition and oxygen availability) (Pusceddu et al. 2009, Pusceddu et al. 2011), which could
111	affect negatively meiofaunal assemblages (La Rosa et al. 2001, Mirto et al. 2002, Gambi et al.
112	2008).
113	Nutrient enrichment has been further shown to enhance sediment microbial biomass and
114	their enzymatic activities in seagrass sediments (López et al. 1998, La Rosa et al. 2001, Liu et al.
115	2017), potentially exacerbating the enhanced degradation rate of OM expected under OA scenario.
116	However, to date, the compounded effects of OA and nutrient enrichment on community
117	composition and OM processes associated to seagrass sediments remain largely unexplored,
118	challenging our capacity to predict alterations in ecosystem functioning and services of seagrasses
119	under future environmental conditions.
120	In this study, we investigated the effects of OA and enhanced nutrient availability on
121	meiobenthic assemblages and OM degradation rates in Posidonia oceanica sediments, at CO2 vents
122	along the coast of Ischia Island (Italy). We exposed P. oceanica meadows, at both ambient and low
123	pH, to different levels of nutrient enrichment (control, moderate and high) for 16 months. Under
124	OA scenario, food availability seems to play a critical role for marine invertebrates, by providing
125	the energy required to support physiological responses to pH stress (Thomsen et al. 2013, Queiros

126	et al. 2015, Ramajo et al. 2016). Under these circumstances, a moderate increase in nutrient
127	availability could have positive effects on meiobenthos at low pH, possibly increasing the
128	consumption of more bioavailable food (Danovaro 1996, Antón et al. 2011). By contrast, excessive
129	nutrient loadings could worsen the impacts of OA on meiobenthos, by causing severe OM
130	accumulation and lowering sediment oxygen concentration (Gambi et al. 2008). In addition, the
131	combined effects of OA and enhanced nutrient concentration were expected to significantly
132	increase OM degradation, by fostering extracellular enzymatic activities of bacteria (López et al.
133	1998, Piontek et al. 2013).
134	
135	Materials and methods
136	Study site and experimental design
137	This study was carried out between April 2014 and July 2015 in shallow P. oceanica

138 meadows at CO₂ vents off the Castello Aragonese isle (Ischia Island, 40°43'51.01"N, 139 13°57'48.07"'E; Tyrrhenian Sea, Italy). Submarine vents have been extensively used to assess the 140 effects of naturally acidified seawater on biological communities as they are characterized by the 141 emission into seawater of gases, predominantly CO2, that create gradients in pH and carbonate 142 chemistry, without confounding gradients of other environmental variables, such as temperature, 143 salinity, hydrodynamic conditions and toxic hydrogen sulphide (Hall-Spencer et al. 2008, Fabricius 144 et al. 2011, Russell et al. 2013, Milazzo et al. 2016, Doubleday et al. 2019). In particular, in the last 145 decade, previous studies carried out at Ischia Island vents have shown that areas exposed to CO₂ 146 bubbling do not differ from control areas in terms of salinity (38 ‰), temperature (seasonal 147 fluctuations of 14-25 °C), light (~7500 lx d⁻¹) and total alkalinity (2.5 mequiv. kg⁻¹), due to the fact 148 that they are just 10s of m apart, at about 2-3 m water depth (Hall-Spencer et al. 2008, Martin et al. 149 2008, Cigliano et al. 2010, Kroeker et al. 2011, Garrard et al. 2014, Scartazza et al. 2017). 150 The effects of OA (ambient and low pH) and nutrient enrichment (control, moderate and

151 high) on meiobenthic assemblages and microbial OM degradation were evaluated through a

152	manipulative experiment. We identified two pH levels in dense and continuous meadows: ambient
153	pH site and low pH site, the latter reflecting the pH value predicted by the end of the century. In
154	order to measure the relative changes in pH between sites, water samples were taken from the water
155	column using a 125 ml bottle at 11 and 10 dates, at ambient and low pH sites respectively,
156	randomly chosen between May 2014 and March 2015. Measurements were made using a Mettler
157	Toledo SG2 pH meter (accuracy \pm 0.01 pH units) equipped with an InLab 413 electrode and
158	calibrated regularly using NIST-traceable buffers. Although this approach does not measure the
159	total hydrogen ion concentration, it provides an estimate of the relative change in pH between sites.
160	The average pH (NBS scale) at ambient and low pH sites was 8.11 ± 0.007 and 7.78 ± 0.05
161	respectively (\pm SE, n=55 and n=50). In addition, in situ seawater pH measurements were recorded
162	from June to July 2015, at the low pH site, using a SeaFET pH sensor, which records pH hourly.
163	The average pH (total scale) was 7.74 ± 0.014 (±SE, n=464), with 42% of the hourly pH values
164	below 7.8 (the predicted mean seawater pH value for the year 2100), in line with the results of
165	(Kroeker et al. 2011).
166	An HOBO data logger was positioned between the two sites in order to monitor
167	continuously (every 15 minutes) seawater temperature throughout the experiment. Temperature
168	matched ambient season fluctuations, with warmest water occurring in August (26.3 \pm 0.008) and
169	coldest water in February-March (14.95 \pm 0.006). Temperature was not expected to vary between
170	sites at a depth of 2.5-3.5 m.
171	In April 2014, at each site, nine experimental plots (50 x50 cm) were established at a depth
172	of about 3 m within <i>P. oceanica</i> meadow and marked at their corners using iron rebars. Three plots
173	were then randomly assigned to each nutrient level (control, moderate and high), for a total of 18
174	replicate plots. Nutrients (Osmocote slow release fertilizer pellets, 17:11:10 N:P:K) were added in
175	three plastic net bags (1-mm mesh size) per plot, fixed by means of plastic cable ties to a iron bar
176	hammered in the middle of each plot. Nutrient bags were, thus, suspended at a distance of about 10

177 cm from the bottom, within seagrass canopy. This method has been widely used in previous

1/8	manipulative experiments to assess the impacts of elevated nutrient concentration in marine systems
179	(Worm et al. 2000, Bulleri et al. 2012, Tuya et al. 2015). The amount of fertilizer used to generate
180	the high and moderate nutrient levels were, respectively, 400 g (three bags containing 133 g each)
181	and 200 g (three bags containing 67 g each). Nutrient bags were replaced every two months,
182	ensuring their effectiveness in releasing nutrients. Fertilizer weight in each nutrient bag was
183	measured at the third decimal by means of a precision scale before deployment. Upon retrieval,
184	nutrient bags were dried in a muffle for 28 hours at 60 $^{\circ}$ C and the amount of fertilizer that had not
185	dissolved was re-weighted in order to estimate the total average nutrient released over the duration
186	of the experiment. The amount of fertilizer released was significantly higher at high than moderate
187	nutrient supply, while the amount of nutrient released did not different between pH levels
188	($F_{1,8}$ =101.32; $P < 0.001$). In addition, in order to estimate water nutrient concentration, two water
189	samples were taken from the water column in each experimental plot, using a 60 ml syringe, at
190	three random dates during the experiment (May 2014, June and July 2015). Higher concentration of
191	total dissolved inorganic nitrogen and phosphate were achieved under enhanced nutrient treatments
192	compared to control level (Fig. S1).
193	

194 Meiobenthic assemblage structure

195 At the end of the experiment (July 2015), meiobenthos (i.e. metazoan meiofauna plus 196 foraminifera) abundance and taxa diversity were assessed in two sediment samples, randomly 197 collected in each experimental plot, for a total of 36 replicates. Sediment cores were hand sampled 198 by divers, by inserting Plexiglas cores (30 mm internal diameter and 270 mm length) at least 5-10 199 cm into P. oceanica matte. Once collected, each sediment sample was transferred in net bags and 200 preserved in 70% ethanol solution until analysis. In laboratory, the meiobenthos was extracted using 201 the decantation method. The samples were sieved through a 500-µm mesh (upper limit) and 50-µm 202 mesh size (lower limit) to retain the meiobenthic organisms (Pusceddu et al. 2014b). The extraction 203 procedure was repeated five times. All animals were then counted and classified per taxon under

204	stereomicroscope. The invertebrates were classified to the taxonomic resolution varying from
205	phylum to order. Due to variations in volume and composition (sandy sediment, plant detritus and
206	gravel) among cores, sediment samples were left to dry in the laboratory for two weeks and, then,
207	the total weight of each sample and that of its sandy, gravel and plant detritus fractions were
208	measured at the third decimal by means of a precision scale. Grain size analysis was carried out by
209	dry sieving sediment through a 1-mm mesh to separate sandy sediment from gravel and plant
210	detritus fractions. Although meiofaunal abundance is typically expressed as number of individuals
211	10 cm ⁻² , due to different volume of sediment in each core, the abundance of individuals of each
212	taxon was standardized to the sediment weight (g DW) for each sample. Taxonomic diversity was
213	measured using the Shannon index.
214	Extracellular enzymatic activities in the sediment
215	Samples for extracellular enzymatic activities (aminopeptidase and ß-glucosidase) were collected at
216	the end of the experiment. Two aliquots (topmost 2 cm) of sediment were collected from each
217	experimental plot, using Plexiglas tubes, for a total of 36 replicates. For the determination of the
218	extracellular enzymatic activities, 2.5 mL of sediment subsamples were incubated at 20°C in the
219	dark for 2 h with 2.5 mL of filtered, sterile seawater containing 200 μ M L-leucine-4-
220	methylcumarinyl-7-amide and 75 μ M 4-methylumbelliferyl β -D-glucopyranoside separately for
221	aminopeptidase and β -glucosidase activities, respectively. After incubation, the slurries were
222	centrifuged and supernatants were analysed fluorometrically (at 365 nm excitation, 455 nm
223	emission for b-glucosidase, and 380 nm excitation, 440 nm emission for aminopeptidase). Data
224	were normalized to sediment dry weight (60 °C, 24 h) and reported as μmol substrate degraded g-1
225	h^{-1} (Pusceddu et al. 2003). The aminopeptidase and β -glucosidase activities were converted into C
226	degradation rates (μ grams of C per gram per hour), using 72 μ g of C per μ mole of substrate as the
227	conversion factor (Pusceddu et al. 2014a).

- 228
- 229 Statistical analyses

230	Effects of OA and nutrient enrichment on meiobenthic assemblages were tested by means of
231	a permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) performed on
232	a Bray-Curtis dissimilarity matrix of untransformed data. The model included two factors: OA
233	(fixed, with two levels: ambient and low pH) and nutrient enrichment (fixed, with three levels:
234	control, moderate and high). To visualize patterns of variations in the meiobenthic assemblages
235	between ambient and low pH and among nutrient enrichment levels an MDS plot after ordination of
236	untransformed data was obtained from Bray-Curtis dissimilarities. A SIMPER analysis was applied
237	to determine which groups were responsible for the dissimilarities among experimental treatments.
238	A principal component analysis (PCA) was used to assess differences in the sediment composition
239	(in term of sandy, gravel and plant detritus fractions) of plots under different experimental
240	conditions. A two-way analysis of variance (ANOVA), with OA and nutrient enrichment as fixed
241	orthogonal factors, was carried out on univariate data (meiobenthos abundance, taxa diversity and
242	extracellular enzymatic activities). Cochran's C-test was used to check for homogeneity of
243	variances and, when necessary, data were log- or square-root transformed. PERMANOVA, MDS,
244	SIMPER, PCA and ANOVA were performed using the software R.
245	
246	Results
247	Composition of sediment cores
248	The PCA on the sediment composition of sampling cores showed a substantial separation
249	between ambient and low pH conditions (Fig. 1). Along PC1 axis, which explains 97.42 % of the
250	total variance, there was one cluster including ambient pH on the left side of the plot and a second
251	one on the right side, represented by low pH treatment. Sandy sediment component was the most
252	negatively correlated to PC1, while gravel component contributes most to PC2 axis, which,
253	however, explains only 2.29 % of the total variance. Sandy sediment content was then included as
254	covariate in the PERMANOVA analysis assessing variations in the structure of the meiobenthic
255	assemblage.

256

257 Meiobenthic assemblage structure

258	The results of PERMANOVA showed significant differences in the structure of meiobenthic
259	assemblages between ambient and low pH, regardless of nutrient treatments, which emerged also in
260	the MDS ordination (Table 1, Fig. 2). There was no significant effect of the covariate (sandy
261	sediment component). The SIMPER analysis showed a 28.6% contribution of nematodes to the
262	dissimilarities between ambient and low pH, annelids (7%), which include polychaetes and
263	oligochaetes, foraminifera (12.4%) and crustaceans (1.9%), which include copepods, cumaceans,
264	amphipods, isopods and tanaids. All other taxa (including molluscs, ophiuroids, acarines,
265	pantopods) contributed less than 0.5% to the overall dissimilarity (Table S1). ANOVA analyses
266	were performed on meiobenthos groups mainly responsible for observed community changes. Since
267	polychaetes and oligocheates, as well as, copepods, cumaceans, amphipods, isopods, tanaids
268	responded similarly to low pH (Table S2; Fig. S2), they were collapsed into two broad taxonomic
269	groups of annelids and crustaceans, respectively.
269 270	groups of annelids and crustaceans, respectively. There were no significant differences in the abundance of nematodes under different
269 270 271	groups of annelids and crustaceans, respectively. There were no significant differences in the abundance of nematodes under different experimental conditions (Table 2, Fig. 3a). The abundance of annelids was higher at low than at
269 270 271 272	groups of annelids and crustaceans, respectively. There were no significant differences in the abundance of nematodes under different experimental conditions (Table 2, Fig. 3a). The abundance of annelids was higher at low than at ambient pH, but was unaffected by nutrient enrichment (Table 2, Fig. 3b). In contrast, foraminifera
269 270 271 272 273	groups of annelids and crustaceans, respectively. There were no significant differences in the abundance of nematodes under different experimental conditions (Table 2, Fig. 3a). The abundance of annelids was higher at low than at ambient pH, but was unaffected by nutrient enrichment (Table 2, Fig. 3b). In contrast, foraminifera significantly decreased at low pH (Table 2, Fig. 3c). Although the effects of OA were not
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- 280 The aminopeptidase and β-glucosidase activities, used as proxies of protein and
- 281 carbohydrate degradation rates, varied according to pH conditions, but were unaffected by nutrient

282 enrichments (Table 3). Both extracellular enzymatic activities were higher at low than at ambient

- 283 pH (Fig. 4 a,b).
- 284
- 285 Discussion

286 At our study site, long-term OA altered the composition of meiobenthic assemblages as well 287 as OM degradation rates in seagrass sediments. Changes in meiobenthic assemblages were mostly 288 due to an increase in the abundance of annelids and, to some extent, of crustaceans, whilst 289 foraminifera abundance significantly decreased at low pH. In addition, OA appears to stimulate the 290 microbial degradation of OM in seagrass sediments, potentially weakening the carbon storage 291 capacity of seagrass meadows. Unexpectedly, enhanced nutrient levels had no effects on 292 meiobenthic assemblages and OM degradation rates, and interactions between nutrient enrichment 293 and OA were not detected. 294 Previous studies have already shown that OA can shift meiobenthic community 295 composition, as a result of differential sensitivity of the different taxa (Hale et al. 2011, Schade et 296 al. 2016, Mevenkamp et al. 2018). In accordance with the literature, nematodes, the dominant 297 meiobenthic taxon at our study site, were unaffected by low pH. Results from previous studies, though mostly conducted under controlled laboratory settings, suggest that nematodes can be highly 298 299 tolerant to low pH, as their densities were often unaffected or even increased under the OA scenario 300 predicted for the end of this century (Dashfield et al. 2008, Widdicombe et al. 2009). Negative 301 effects on nematode survivorship have been documented only at extremely low pH levels ($\sim \leq 6$). 302 However, a recent study using a staining technique, found an increase in nematode mortality under 303 OA, while nematode density was unaffected, likely due to a reduced degradation rate of dead 304 nematode bodies at low pH (Mevenkamp et al. 2018). These results stress the importance of 305 assessing nematode mortality in OA studies, as stable or even increased densities of these animals 306 could be an artefact of reduced body decomposition, potentially hiding more severe impacts of OA 307 on this dominant group.

308	We documented an increase in the abundance of annelids at low pH, in line with reports of
309	previous studies on epibenthic fauna at submarine CO2 vents (Kroeker et al. 2011, Ricevuto et al.
310	2012, Garrard et al. 2014). In this regard, it has been reported that, around CO ₂ vents of Ischia,
311	polychaetes maintain high density along pH gradients, suggesting that some species may be tolerant
312	to OA due to their high physiological plasticity or local adaptation (Calosi et al. 2013). However,
313	responses to low pH vary among different groups of polychaetes, with filter feeder and herbivore
314	species generally favoured at expenses of deposit feeders, omnivores and carnivores (Gambi et al.
315	2016, Molari et al. 2018). Thus, a more detailed analysis on polychaete species composition or
316	functional traits could provide further insights on the sensitivity of this taxonomic group to long-
317	term low pH exposure. Furthermore, there was a tendency ($P=0.08$) in the abundance of
318	crustaceans to increase at low pH. Crustaceans are considered quite robust to OA due to their
319	internal acid-base regulation and external organic layer that protect skeleton from corrosive low pH
320	water (Melzner et al. 2009, Ries 2009). Although some studies found negative effects of low pH on
321	reproduction or larval development of copepods (Kurihara et al. 2004, Fitzer et al. 2012), studies
322	testing the effects of OA at the community level showed no changes or even an increase in
323	crustacean abundance at low pH, possibly due to decreased predation rate or increased food
324	availability (Kroeker et al. 2011, Garrard et al. 2014).
325	The abundance of foraminifera significantly decreased at low pH site. Previous studies
326	reported a substantial vulnerability of benthic foraminifera to OA (Hall-Spencer et al. 2008,
327	Fabricius et al. 2011, McIntyre-Wressnig et al. 2013, Martinez et al. 2018), likely because many of
328	them build shells of calcium carbonate. Accordingly, decreases in the diversity of foraminifera
329	community and changes in their community composition from calcifying to non-calcareous forms
330	has been reported in the Mediterranean Sea (around CO ₂ vents of Ischia; Dias et al. 2010) and
331	Pacific Ocean, around CO ₂ vents of Papua New Guinea (Fabricius et al. 2011). These unicellular
332	organisms are a key benthic component in coastal systems, since they serve as food source together
333	with the rest of meiofauna for higher trophic levels, and are important contributors of the annual

334	carbonate production and denitrification process in coastal areas (Risgaard-Petersen et al. 2006,
335	Høgslund et al. 2008, Langer 2008). Although our study cannot discern if foraminifera were alive,
336	the significant reduction in their abundance detected at low pH suggest that OA could have negative
337	cascading effects on carbon and nutrient cycles within seagrass meadows.
338	The divergent responses of taxonomic groups to low pH lead to no differences in term of
339	total meiofaunal abundance and taxa diversity. These results are in contrast with those of (Molari et
340	al. 2018), who found a decrease in the biomass and density of meiofauna in sandy areas near CO_2
341	vents. Such discrepancy could be due to the different habitat characteristics (bare sandy sediment
342	versus P. oceanica sediments in (Molari et al. 2018) and our study site, respectively). In fact,
343	seagrass sediments are generally characterized by higher supply of organic matter, derived from
344	both seagrass production and the trapping of other organic particles (Kennedy et al. 2010).
345	Although we did not measure the amount of organic matter in <i>P. oceanica</i> sediments, the supposed
346	higher food availability may have mediated the susceptibility of marine invertebrates to low pH at
347	our study site. The lack of detectable effects of low pH on taxa diversity could also be influenced by
348	the taxonomic aggregation used in this study. Thus, the use of a fine taxonomic resolution (e.g.
349	genus or species levels) could provide a deeper insight into the changes in community diversity and
350	composition under future climate scenario (Bevilacqua et al. 2012).
351	Low pH fostered extracellular enzymatic activities in seagrass sediments. Extracellular
352	enzymes play a crucial role in benthic systems as they break down high molecular weight organic
353	compounds into low molecular weight compounds that can then be readily used by heterotrophs
354	(Cunha et al. 2010). Contrary to intracellular enzymes that are buffered by the cell's cytoplasm,
355	extracellular enzymatic activities are directly impacted by external changes in pH. An increase in
356	the H ⁺ concentration, due to lower seawater pH value, may modify the three-dimensional protein
357	structure of the active site of the enzyme, thus affecting enzymatic activities (Cunha et al. 2010). At
358	the same time, changes in the meiobenthic assemblage composition at low pH, with an increase in
359	the abundance of annelids and a reduction of foraminifera, could have entailed cascading effects on

360 microbial-mediated OM degradation rate (Piot et al. 2014, Lacoste et al. 2018). For instance, 361 polychaetes are known to enhance bacterial activities, either directly, by consuming bacteria and 362 thus stimulating their growth (Montagna 1984), or, indirectly, through particle reworking and solute 363 transport due to bioturbation activity (Aller and Aller 1992). In addition, an increase in extracellular 364 enzymes under OA could also be related with enhanced availability of organic matter as a 365 consequence of higher primary productivity (Piontek et al. 2013). Regardless of the specific 366 mechanisms stimulating microbial extracellular enzymatic activities, our results suggest that long-367 term OA may lead to increased degradation of carbohydrates and proteins in seagrass surface 368 sediments. Our findings can be generalized as previous results from benthic (Molari et al. 2018) and 369 pelagic (Grossart et al. 2006, Piontek et al. 2013) systems found an increase in the extracellular 370 enzymatic activity at low pH. A further decline in pH could, however, result in a decreased rate of 371 enzymatic activity (Cunha et al. 2010). For instance, in a mesocosm experiment, (Rastelli et al. 372 2016) reported that very low pH value (< 7), associated to high CO₂ leakages, can result in a 373 significant reduction of the aminopeptidase and ß-glucosidase activities and an increase in sediment 374 protein accumulation. Finally, variable effects of OA on OM degradation rates could also depend 375 upon the different edaphic conditions (i.e. grain size and mineralogy) in different sediment 376 typologies. 377 None of the response variables analysed was affected by enhanced nutrient loading. We 378 hypothesized that a moderate nutrient enrichment would have been able to mediate meiobenthos 379 responses to low pH indirectly, by increasing food quality. However, at our study site, background 380 N P concentrations were comparable to those observed in urbanized coastal areas in the NW 381 Mediterranean (Balata et al. 2008, Balata et al. 2010), and, therefore, unlikely to be limiting for 382 benthic invertebrates. Furthermore, a previous work has documented low C/N ratio of organic 383 detritus at CO₂ vents of Ischia, suggesting no nitrogen deficiency in invertebrate diets at low pH (Ricevuto et al. 2015). In contrast, severe nutrient enrichmentmay negatively affect meiofaunal 384

385 assemblages and foster bacterial activity in seagrass sediments as a consequence of the severe

386	modifications caused to sediment chemistry (e.g. high biopolymeric carbon content and reduced O_2
387	availability) (López et al. 1998, Gambi et al. 2008, Pusceddu et al. 2011). In our experiment, the
388	simulation of heavy nutrient enrichment of the water column was not effective in generating
389	concentrations high enough to cause severe organic matter accumulation in the sediments, as
390	observed in eutrophic systems (Dell'Anno et al. 2002, Pusceddu et al. 2009). Indeed, signals of
391	seagrass meadow degradation are often reported in coastal systems characterized by dissolved
392	nutrient concentrations considerably higher than those generated in our experiment (Cardoso et al.
393	2010, Hughes et al. 2013). Also, it has been repeatedly observed that the impacts of eutrophication
394	in terms of inorganic nutrient concentration in the water column (Burkholder et al. 2007) could not
395	be automatically detected in the benthic environment (López et al. 1998, Dell'Anno et al. 2002,
396	Pusceddu et al. 2009, Pusceddu et al. 2011). Moreover, the lack of detectable effects of enhanced
397	nutrient availability in the water column on the benthos could also be explained considering that we
398	tested our hypotheses in <i>P. oceanica</i> sediments, where, because of the high background loads of
399	OM in seagrass sediments, effects of benthic eutrophication could be not clearly detected (Pusceddu
400	et al. 2007).
401	Overall, the results of our experiment show that long-term OA can significantly alter
402	meiobenthic assemblage composition and foster microbial OM degradation in P. oceanica
403	sediments, regardless of nutrient availability. To the best of our knowledge, this is the first study
404	investigating the combined effects of a global and a local stressor on meiobenthic communities and
405	ecosystem functioning in seagrass sediments. Meiofauna have been recently shown to have
406	important effects on benthic ecosystem processes, such as OM mineralization and nitrogen cycling,
407	likely by stimulating microbial activity (Nascimento et al. 2012, Bonaglia et al. 2014). Thus, further
408	studies are warranted to assess how changes in meiobenthic assemblage in response to OA could
409	entail cascading effects on microbial communities, ultimately altering ecosystem functioning.
410	Seagrass meadows are recognized hotspots of sediment organic matter sequestration
411	(Fourqurean et al. 2012), due to their high primary production and leaf ability to trap allochthonous

412	suspended particles (Kennedy et al. 2010). In addition, the low nutrient (nitrogen and phosphate)
413	content of seagrass litter and sediment hypoxic condition slow organic matter decomposition, thus
414	resulting in the immobilization of organic carbon in the belowground compartments for millennia
415	(Mateo et al. 2006, Duarte et al. 2013, Trevathan-Tackett et al. 2017). In particular, P. oceanica,
416	with its long-lived rizhomes and slow growth rate, is among the most efficient seagrasses in
417	accumulating carbon in sediments (Fourqurean et al. 2012). Nonetheless, seagrasses are declining
418	worldwide, raising concerns over a weakening of their ability to buffer climate changes through
419	carbon sequestration (Fourqurean et al. 2012, Duarte et al. 2013, Lovelock et al. 2017, Chefaoui et
420	al. 2018). Previous studies have shown how climate changes (e.g. seawater warming, heat waves)
421	and local stressors (e.g. water quality degradation, mechanical disturbance) may reduce seagrass
422	carbon storage capacity (Jordà et al. 2012, Serrano et al. 2016, Arias-Ortiz et al. 2018, Trevathan-
423	Tackett et al. 2018b). Our results indicate that OA predicted by the end of this century could trigger
424	OM degradation in seagrass sediments, reducing their carbon storage capacity and enhancing CO_2
425	release. While seagrass productivity is generally expected to increase in response to low pH, under
426	nutrient concentration unlikely to cause N-limitation (Stitt and Krapp 1999, Alexandre et al. 2012,
427	Russell et al. 2013, Sunday et al. 2016, Ravaglioli et al. 2017), our study highlights the need of
428	assessing belowground processes to understand the mechanisms underpinning the net carbon budget
429	in seagrass meadows.

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758 Figure legends

759	Fig. 1. PCA analysis conducted on the sediment components of cores (sandy sediment, gravel and
760	plant detritus) at ambient and low pH (respectively grey and black symbols) and among nutrient
761	enrichment levels (circle =control nutrient, quadrat = moderate nutrient enrichment and triangle up
762	= high nutrient enrichment).
763	
764	Fig. 2. MDS ordination on untransformed data obtained from Bray-Curtis dissimilarities showing
765	differences in meiobenthic assemblages between ambient and low pH (respectively grey and black
766	symbols) and among nutrient enrichment levels (circle control nutrient, triangle down = moderate
767	nutrient enrichment and triangle up = high nutrient enrichment).
768	
769	Fig. 3. Abundance (mean ± SE, n=6) of a) nematodes, b) annelids, c) foraminifera and d)
770	crustaceans in ambient and low pH conditions under different levels of nutrient enrichment (control,
771	moderate and high). The inserts in b) and c) indicate the mean abundance $(\pm SE)$ of annelids and
772	foraminifera at ambient and low pH level (n= 27; data pooled across nutrient treatments).
773	
774	Fig. 4. a) Aminopeptidase and b) β -glucosidase (μ mol g ⁻¹ h ⁻¹ , mean \pm SE, n=6) in ambient and low
775	pH conditions under different levels of nutrient enrichment (control, moderate and high). The
776	inserts in a) and b) indicate the concentration of aminopeptidase and ß-glucosidase, respectively,
777	under different pH conditions (data are mean ± SE, n=27, as nutrient treatments were pooled).

Commentato [F1]: segui la stessa struttura dellla Figura precedente

Table 1. PERMANOVA on the effects of OA (ambient and low pH) and nutrient enrichment

(control, moderate and high) on the meiobenthic assemblage. *P < 0.05, **P < 0.01, ***P < 0.001.

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Source of variation	df	MS	Pseudo-F
Covariate	1	0.020	0.202
OA	1	0.517	5.311**
Nutrient (Nu)	2	0.096	0.988
OA x Nu	2	0.171	1.752
Residual	11	0.097	
Total	17		

Table 2. ANOVA on the effects of OA (ambient and low pH) and nutrient enrichment (control, moderate and high) on the abundance of nematodes, annelids, foraminifera, crustaceans, total meiobenthic abundance and taxa diversity. *P < 0.05, **P < 0.01, ***P < 0.001.

		Nemat	odes	Anne	lids	Forami	nifera
Source of variation	df	MS	F	MS	F	MS	F
OA	1	3.372	2.216	3.075	32.379***	43.572	20.400***
Nutrient (Nu)	2	2.204	1.448	0.181	1.909	3.373	1.579
OA x Nu	2	2.539	1.668	0.138	1.456	2.223	1.041
Residual	12	1.522		0.095		2.136	
Transformation		Sqrt (x+1))	Log (x+1)		None	
Cochran's test		ns		ns		ns	
		Crusta	iceans	Total abu	indance	Diver	sity
Source of variation	df	MS	F	MS	F	MS	F
OA	1	0.906	3.539	1.878	1.392	0.064	1.054
Nutrient (Nu)	2	0.116	0.453	1.170	0.868	0.086	1.410
OA x Nu	2	0.015	0.058	2.512	1.862	0.140	2.210
Residual	12	0.256		1.349		0.061	
Transformation		None		Sqrt (x+1)		None	
C test		ns		ns		ns	

Table 3. ANOVA on the effects of OA (ambient and low pH) and nutrient enrichment (control,moderate and high) on aminopeptidase and β -glucosidase activities in the sediment. *P < 0.05, **P< 0.01, ***P < 0.001.

		Aminopeptidase		ß-glucosidase	
Source of variation	df	MS	F	MS	F
OA	1	389.0	6.617*	1.663	6.093*
Nutrient (Nu)	2	69.30	0.118	0.024	0.087
OA x Nu	2	140.2	0.238	0.036	0.131
Residual	12	588.8		0.273	
Transformation		None		Log (x+1)	
Cochran's test		ns		P < 0.05	



Figure 1



Figure 2



Figure 3



Figure 4