

1 **Plant and sediment properties in seagrass meadows from two Mediterranean CO<sub>2</sub> vents:**  
2 **implications for carbon storage capacity of acidified oceans**

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18 Running head: Properties of seagrass meadows in two Mediterranean CO<sub>2</sub> vents

19

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21 blue carbon

22

23 **Abstract**

24 Assessing the status of important carbon sinks such as seagrass meadows is of primary importance  
25 when dealing with potential climate change mitigation strategies. This study examined plant and  
26 sediment properties in seagrass meadows (*Cymodocea nodosa* (Ucria) Asch.) from two high  $p\text{CO}_2$ -  
27 low pH Mediterranean vent systems, located at Milos (Greece) and Vulcano (Italy) Islands,  
28 providing insights on carbon storage potential in future acidified oceans. Contrary to what has been  
29 suggested, carbon content (both inorganic and organic) and its surficial accumulation decreased at  
30 high  $p\text{CO}_2$ -low pH in comparison with controls. The decrease in inorganic carbon may result from  
31 the higher solubility of carbonates due to the more acidic conditions. At Vulcano, the seagrass  
32 properties (e.g., leaf area, biomass) appeared negatively affected by environmental conditions at  
33 high  $p\text{CO}_2$ -low pH conditions and this may have had a detrimental effect on the organic carbon  
34 content and accumulation. At Milos, organic carbon decreased at high  $p\text{CO}_2$ -low pH conditions,  
35 despite the increase in seagrass aboveground biomass, leaf length and area, probably as a  
36 consequence of site-specific features, which need further investigation and may include both biotic  
37 and abiotic factors (e.g., oligotrophic conditions, decreased sedimentation rate and input of  
38 allochthonous material). Results suggest that, in contrast to previous predictions based exclusively  
39 on the expected positive response of seagrasses to ocean acidification, carbon storage capacity of  
40 the seagrass *C. nodosa* may not increase at high  $p\text{CO}_2$ -low pH conditions. This study emphasizes  
41 the need to investigate further the potential alteration in the climate mitigation service delivered by  
42 seagrass meadows in acidified oceans.

## 43 **Introduction**

44 By tempering the atmospheric CO<sub>2</sub> rise, oceans concurrently undergo a progressive decline in  
45 seawater pH, which is termed Ocean Acidification (OA) (Doney et al. 2009), one of the most well-  
46 known climate change effects, along with global warming. Combating climate change is a high  
47 priority nowadays, especially through a range of possible mitigation strategies to remove carbon,  
48 including the protection and restoration of globally significant carbon sinks (Duarte et al. 2013).  
49 Although accounting for a very small part of the ocean, marine vegetated ecosystems such as  
50 seagrasses, mangroves and salt marshes have high potential value for carbon sequestration and  
51 mitigation of the detrimental effects of climate change (Duarte et al. 2013; Marbà et al. 2015). More  
52 specifically, the total carbon that can be buried through biosequestration within coastal oceans  
53 compares favorably with terrestrial ecosystems (Macreadie et al. 2014). Efforts to understand the  
54 mechanisms underpinning this important capacity have grown in recent years and belong to the new  
55 field of research known as “blue carbon”, which refers to the carbon that can be stored in coastal  
56 vegetated habitats (Duarte et al. 2005; Campbell et al. 2014; Fourqurean et al. 2012; Howard et al.  
57 2014; Gullström et al. 2018).

58 Recent studies have drawn attention to the capacity of seagrass beds to act as efficient and  
59 especially fast CO<sub>2</sub> sinks (e.g., McLeod et al. 2011; Nellemann et al. 2009) and have investigated  
60 the properties of both sediment and plants that drive carbon storage in these ecosystems (e.g., Dahl  
61 et al. 2016). Seagrasses form a dense stratum, composed of a mixture of sediment, roots and  
62 rhizomes, which for a few species can be several meters in depth (Nellemann et al. 2009, and  
63 references therein). Given that the storage capacity of seagrasses is very high, between 4.2 and 8.4  
64 Pg C (Fourqurean et al. 2012), and that the carbon within the sediments can be stored over  
65 millennia (see Duarte et al. 2005, and therein references), seagrasses play a major role as C sinks.  
66 In the scenario of OA, it has been suggested that seagrass biomass will most likely increase, along  
67 with seagrass cover, enhancing organic matter burial and the potential for carbon storage and  
68 sequestration (Garrard and Beaumont 2014). Accordingly, significant increases in seagrass shoot

69 density and/or biomass have been reported both at temperate (Hall-Spencer et al. 2008) and tropical  
70 CO<sub>2</sub> vents (Fabricius et al. 2011; Russell et al. 2013), which are considered analogues of future high  
71 pCO<sub>2</sub>-low pH environments, suggesting beneficial effects of OA on the capacity of seagrasses to  
72 act as carbon sinks under high pCO<sub>2</sub>-low pH conditions (Takahashi et al. 2016). Seagrasses are  
73 indeed considered as potential “winners” in future oceans: while they are carbon limited at the  
74 current pCO<sub>2</sub>, an increase in inorganic carbon, coupled with the low pH-driven reduction in  
75 encrusting calcifying epiphytes, is expected to boost photosynthetic rates and increase energy  
76 reserves (i.e., non-structural carbohydrates) (Campbell and Fourqurean, 2013; Zimmerman et al.  
77 1997). However, research findings from vents are not always consistent with this conceptual  
78 outline. The effect of high pCO<sub>2</sub>-low pH from vents on seagrass biomass, for example, is  
79 controversial, with Apostolaki et al. (2014) showing decrease in biomass of *Cymodocea nodosa* in  
80 the Vulcano vent (Mediterranean), while Russell et al. (2013) reported increased biomass of the  
81 con-generic *C. serrulata* in vents of Papua New Guinea. In this sense, a diverse response of  
82 seagrass to OA in terms of their capacity to capture carbon could be expected.

83 In this study we examined plant and sediment properties which may influence carbon storage  
84 capacity in seagrass meadows from two Mediterranean CO<sub>2</sub> vents to test whether there is a  
85 consistent response to high pCO<sub>2</sub>-low pH and to infer potential implications for carbon  
86 sequestration and storage capacity in future acidified oceans. We expect that if seagrasses benefit  
87 from high pCO<sub>2</sub>-low pH conditions, their increase in biomass and density should promote the  
88 accumulation of sedimentary carbon and hence carbon storage capacity.

89

## 90 **Materials and methods**

### 91 **Study sites**

92 This study was conducted in two Mediterranean shallow CO<sub>2</sub> vents located at: i) Milos Island  
93 (south-west Cyclades, Greece) and ii) Vulcano Island (south Italy) (Figure 1). Populations of

94 *Cymodocea nodosa* (Ucria) Asch. are found in both vents (Aliani et al. 1998; Apostolaki et al.  
95 2014; De Biasi et al. 2004; Vizzini et al. 2013).

96 The Island of Milos is characterized by submerged volcanic activity. Sampling took place at  
97 Paleohori Bay (24°33.00' E; 36°40.00' N). Intense hydrothermal activity is evident around the  
98 whole bay (Thiermann et al. 1997), where mean gas composition was 92.5% CO<sub>2</sub>, 0.13% O<sub>2</sub>, 0.67%  
99 N<sub>2</sub>, 7 ppm He, 11450 ppm H<sub>2</sub>, 0.7 ppm CO and 916 ppm CH<sub>4</sub> in 2011 (Bayraktarov et al. 2013). A  
100 specific zonation of different deposits is evident in the sediment around the emission points.  
101 Adjacent to the vent, there is a yellow-orange deposit of arsenic sulfide minerals, where pore water  
102 pH reaches values down to 4.6 and temperature values up to 95 °C (Price et al. 2013a). This region  
103 is then followed by one of white precipitates of amorphous silica and native sulfur with microbial  
104 mats present. This region is followed by a 'transition zone' of grey sediment. Lastly, a 'brown zone'  
105 of unaffected brown sediment is observed, where temperature and pH progressively reach ambient  
106 values (Bayraktarov et al. 2013). While to our knowledge no literature data are available, a  
107 preliminary survey revealed an enrichment in a few trace elements (e.g., vanadium, mercury and  
108 above all arsenic) in sediment close to the volcanic emission points (Vizzini, unpublished data).  
109 Similarly, the Island of Vulcano hosts a shallow hydrothermal system of volcanic origin, which  
110 includes several sites of gas emission. In particular, at Levante Bay (14°57.60' E; 38°25.05' N), on  
111 the north-east side of the island, there is a main venting site where gas bubbles are made up almost  
112 entirely of CO<sub>2</sub> (97-99%, for details see Capaccioni et al. 2001; Boatta et al. 2013). Previous studies  
113 reported a pH gradient occurring along the bay with values ranging from 8.1 – 8.2 to 7.4 – 7.5  
114 (Boatta et al. 2013; Horwitz et al. 2015). Due to the input of metal-rich volcanic fluids and the  
115 seawater features (low pH and Eh values) that affect the solubility and bioavailability of metals and  
116 metalloids, trace element concentration differs at a small spatial scale (Vizzini et al. 2013). Some  
117 elements (As, Ba, Hg, Mo, Ni, Pb, Zn) are more concentrated close to the primary vent, while  
118 others (Cd, Co, Cr, Cu, Fe, Mn, V) in sites at a distance of about 100-150 m upwards from the  
119 primary vent. Overall, the bay has low contamination levels with moderate potential for adverse

120 biological effects, especially in the area between about 150 and 350 m from the primary vent, where  
121 localized detrimental effects on biota may occur (Vizzini et al. 2013).

122 In each site, plant and sediment properties and carbon content in seagrass meadows were  
123 investigated at two stations: one is far from the volcanic vents (~ 1.8 km at Milos, ~500 m at  
124 Vulcano) and is characterized by normal  $p\text{CO}_2$  and pH (hereafter control) and the other is at the  
125 closest distance possible where seagrasses are found (~ 600 m at Milos, ~200 m at Vulcano) and is  
126 characterized by high  $p\text{CO}_2$  and low pH (hereafter low pH) (Table 1). Both stations have similar  
127 water depths within each site (about 7-8 m depth at Milos and 1-2 m at Vulcano) and exposure  
128 (Figure 1). Sampling was conducted in May 2013.

129

130 Field and laboratory analysis

131 Plant properties

132 *Cymodocea nodosa* shoots were collected with PVC hand-corers ( $\varnothing$  10 cm, depth 20 cm) in three  
133 random replicates. The number of shoots was counted and above- (leaf blades and sheaths) and  
134 belowground (rhizomes, roots) tissues separated, dried at 60°C and weighed. Leaf height and width  
135 were measured in a subsample of 20 shoots per replicate and the leaf area index (L.A.I.) calculated  
136 as the product of mean leaf area per shoot times the shoot density. Carbon content (%) and  $\delta^{13}\text{C}$   
137 (‰) were measured in dried seagrass tissues (3 mg), after treatment with HCl (2 N) to remove  
138 carbonates, using an elemental analyser (Thermo Flash EA 1112) interfaced to an isotope ratio mass  
139 spectrometer (Thermo Delta Plus XP). The analytical precision of the measurements based on the  
140 standard deviation of replicates of internal standards was 0.1‰.

141 Carbon stock in the living tissue component (leaf blades, leaf sheaths, rhizomes or roots) was first  
142 quantified in  $\text{g C m}^{-2}$  using the following formula:

143 
$$\text{C stock}_{\text{living tissues}} = \text{biomass (g m}^{-2}\text{)} \times \% \text{C} / 100,$$

144 and then converted into  $\text{Mg ha}^{-2}$ .

145

146 Sediment properties

147 Undisturbed surface sediment samples were collected by divers with PVC hand-corers ( $\varnothing$  4 cm) in  
148 random triplicate within the seagrass meadow. Only the top 2 cm in the case of Vulcano and 1 cm  
149 in the case of Milos were sectioned for analysis.

150 Dry bulk density (DBD) was calculated as the dry weight of the sediment (60°C for 48 h) divided  
151 by the volume of the original sediment sample. Porosity ( $\varphi$ ) was obtained through the following  
152 formula:

$$153 \quad \varphi = \beta \times \text{DBD}/100,$$

154 where  $\beta$  is percentage water content of sediment estimated as:

$$155 \quad \beta = [(\text{wet weight} - \text{dry weight}) / \text{wet weight}] \times 100.$$

156 Before grain size analysis, bulk sediment was pretreated with hydrogen peroxide and Na-  
157 hexametaphosphate to eliminate organic matter and avoid particle flocculation. Sediment was then  
158 wet sieved through a 63  $\mu\text{m}$  net, and the remaining fraction electro-mechanically sieved through a  
159 DIN ISO 3310-1 standard sieve set.

160 For chemical analysis (carbon content and  $\delta^{13}\text{C}$ ), samples were oven dried (60°C for 48 h) and  
161 ground in a ball mill (Retch MM200). Then samples were weighed (10 mg) in silver capsules and  
162 analysed using an elemental analyser (Thermo Flash EA 1112) for carbon content analysis: total  
163 carbon (TC) was analysed in sediment as it is, while, for organic carbon (OC) determination,  
164 sediment was acidified with HCl (2N) to remove carbonates. Inorganic carbon (IC) was estimated  
165 as the difference between TC and OC.  $\delta^{13}\text{C}$  was analysed in acidified (HCl, 2N) sediment weighed  
166 (10 mg) in tin capsules using an isotope ratio mass spectrometer (Thermo Delta Plus XP).

167 Organic and inorganic carbon accumulations (respectively OC and IC stock) were calculated for the  
168 top sediment layer sampled by using the following equations:

$$169 \quad \text{OC stock} = \text{DBD} (\text{g cm}^{-3}) \times \text{Slice (cm)} \times \text{OC (\%)} / 100,$$

$$170 \quad \text{IC stock} = \text{DBD} (\text{g cm}^{-3}) \times \text{Slice (cm)} \times \text{IC (\%)} / 100,$$

171 and then converted into  $\text{Mg ha}^{-2}$ . At both sites, the stocks were calculated for the first 2 cm of  
172 sediment, assuming for the second cm of Milos 's stations the same carbon content and DBD as the  
173 first cm. OC and IC stocks were summed to obtain total carbon (TC stock).

174

175 Potential sources for sedimentary organic carbon

176 Organic matter sources potentially contributing to sedimentary organic carbon were collected in  
177 triplicate. In Milos, sources were represented by i) *C. nodosa*, ii) suspended particulate organic  
178 matter (POM), iii) volcanic-derived microbial mats; no macroalgae were present. At Vulcano,  
179 organic matter sources also included macroalgal species present at both control and low pH  
180 (namely: *Cystoseira compressa*, *Dyctiota dichotoma* and unidentified filamentous algae).

181 Microbial mats were collected close to the primary vent in Vulcano, as a volcanic vent end-  
182 member, by scraping the top layer of the mat-covered rocks with a ceramic knife; these data were  
183 also used for the Milos dataset. Macroalgae and the seagrass *C. nodosa* leaves were picked by hand,  
184 epiphytes removed by gentle scraping. Surface seawater for the isotopic analysis of the suspended  
185 particulate organic matter (POM) from the primary vent, and from the control was collected using  
186 10 l bottles, and filtered through pre-combusted ( $450^{\circ}\text{C}$ , 4 h) Whatman GF/F filters ( $0.45\ \mu$ ). POM  
187 collection was not carried out in Milos Island for logistic reasons. Prior to  $\delta^{13}\text{C}$  analysis, all samples  
188 were acidified (HCl, 2 N) to remove carbonates, dried and weighed in tin capsules. Isotopic analysis  
189 was performed in an isotope ratio mass spectrometer (Thermo Delta Plus XP).

190

191 Data analysis

192 Analysis of differences in seagrass and sediment properties were performed through two-way  
193 ANOVA and factors tested were: station (2 levels: control and low pH) and site (2 levels: Milos and  
194 Vulcano). ANOVA was not run on leaf width data as no variability among replicates was detected  
195 and on silt and clay (%) data as only one replicate was collected in Milos. Linear regression was  
196 used to detect relationships between seagrass and sediment features and  $\text{TC}_{\text{stock}}$  within each site.



197 Prior to ANOVA and linear regression analyses, all data were tested for normality and  
198 homoscedasticity using, respectively, Shapiro-Wilk and Cochran's tests. When significant  
199 differences occurred, appropriate means compared using post-hoc Student–Newman–Keuls test.  
200 Bayesian mixing models (package SIAR: Stable Isotope Analysis in R; Parnell et al., 2010) were  
201 used to investigate the origin of OC present in sediments by quantifying the relative proportion of  
202 potential sources to the isotopic composition of the sediment.  $\delta^{13}\text{C}$  was used as a variable and the  
203 number of sources considered was chosen according to the stations and sites and included potential  
204 end-members that could contribute to the carbon isotopic signature of the sediment (i.e.,  
205 macrophytes, suspended particulate organic matter [POM] and volcanic-derived microbial mats, see  
206 the section above for details). As POM was collected only in Vulcano Island, data used for Milos  
207 are those available from other vents [Panarea (Vizzini unpublished data) and Vulcano (this study)  
208 Islands, Aeolian Archipelago, Italy)]. Mixing models were run separately for each station and site.  
209 Macroalgae of which the biomass was very abundant in Vulcano (*C. compressa* and *D. dichotoma*)  
210 were combined together in a group prior to the model, due to the absence of significant differences  
211 among their  $\delta^{13}\text{C}$  signature (Phillips et al. 2005) (ANOVA,  $df = 1$ , Pseudo- $F = 0.0052$ ,  $p = 0.940$ ) to  
212 reduce sources of uncertainty that may influence mixing model results when using multiple end-  
213 members (Phillips et al. 2014).

214

## 215 **Results**

### 216 Seagrass properties

217 Differences in *Cymodocea nodosa* features between stations (control and low pH) were not  
218 consistent in the two sites (Milos and Vulcano), as for most variables the interaction term “station x  
219 site” had a significant effect (Table 2). This was the case for leaf length, leaf area per shoot, leaf  
220 area index (LAI) and aboveground biomass, which were significantly lower at the control than at  
221 the low pH in Milos, whereas the opposite trend occurred in Vulcano with significant differences

222 only in two cases (leaf area and LAI). Density and belowground biomass differed only for the factor  
223 site with values lower in Milos than in Vulcano.

224 TC stock in living tissues was significantly lower at both stations in Milos than in Vulcano, and  
225 significantly higher at the low pH than at the control in Milos (Table 2).

226

227 Sediment properties

228 Sediment features did not vary between stations at both sites (Table 3). On the contrary, differences  
229 between sites were recorded for DBD, porosity and  $\delta^{13}\text{C}$ , with Milos showing higher values than  
230 Vulcano for porosity and  $\delta^{13}\text{C}$ , while a significant effect of the interaction term “station x site” was  
231 obtained for only C/N ratios with overall higher values at the control than at low pH.

232 OC stock and IC stock significantly decreased from control to low pH (2-way ANOVA: factor  
233 station  $F_{1,8}=5.88$ ,  $p=0.042$  and  $F_{1,8}=17.64$ ,  $p=0.003$  respectively) (Figure 2). Accordingly, TC stock  
234 significantly decreased at low pH ( $F_{1,8}=15.70$ ,  $p=0.004$ ) and the same also occurred summing up the  
235 C stock in the living tissues and in the sediment ( $F_{1,8}=7.07$ ,  $p=0.029$ ).

236 Linear regression analysis showed a significant positive correlation between porosity and TC in  
237 Vulcano (*adjusted R-square*=0.728,  $p=0.019$ ) and a negative correlation between aboveground  
238 biomass and TC stock only in Milos (*adjusted R-squared*=0.887,  $p=0.018$ ). Both belowground  
239 biomass and seagrass density were not correlated with TC stock in both sites ( $p>0.05$ ).

240 Organic matter sources used in the mixing models are showed in Table 4. According to the mixing  
241 model estimation, most sources showed wide and overlapping contributions (Figure 3). Organic  
242 carbon of sediment seemed to be mainly derived from POM above all at Milos (range low95%-  
243 high95%=0.65-0.89 and 0.04-0.60 at control and low pH respectively). *C. nodosa* showed similar  
244 ranges in both stations of the two sites (range low95%=0.00-0.31; range high95%=0.34-0.42). The  
245 same was also found for the contribution of volcanic-derived microbial mats (range low95%-  
246 high95%=0.05-0.56 and 0.01-0.54 at Milos and Vulcano respectively).

247

## 248 **Discussion**

249 This study focused on the seagrass *Cymodocea nodosa*, one of the four native seagrass species  
250 found in the Mediterranean Sea (Luisetti et al. 2013) and investigated both seagrass and sediment  
251 features in different  $p\text{CO}_2$ -low pH conditions occurring in two Mediterranean volcanic vents with  
252 potential implications for carbon sequestration and storage capacity in future acidified ocean.  
253 Looking at seagrass properties, no consistency was found between the two different and  
254 geographically distinct venting sites, since highly variable results between sites did not allow  
255 generalization of the observed trend. The increase in a number of seagrass features observed in  
256 Milos at low pH is consistent with previous studies in other venting sites (Hall-Spencer et al. 2008;  
257 Russell et al. 2013; Takahashi et al. 2016), but is inconsistent with those from Vulcano Island,  
258 where a different trend was in fact observed, in agreement with previous findings in the area  
259 (Apostolaki et al. 2014; Vizzini et al. 2017). In more detail, at low pH in Milos we observed a three-  
260 fold increase in the aboveground biomass and a two-fold increase in ratio of above to belowground  
261 biomass, while at Vulcano no significant variation occurred between control and low pH station.  
262 Consistent with aboveground biomass, leaf area and LAI showed the reverse pattern between the  
263 vent sites, increasing at low pH in Milos and decreasing at low pH in Vulcano compared to the  
264 corresponding controls. Experimental evidence has shown a positive correlation between LAI and  
265 pH in natural conditions, suggesting that dense meadows with big leaves have a high capacity to  
266 buffer pH (Hendriks et al. 2014). Our results, however, imply that the relationship between changes  
267 in seawater chemistry and structural variables of the meadow in naturally acidified conditions may  
268 not be straightforward.

269 It has been suggested before that the response of seagrasses to naturally increased  $\text{CO}_2$  levels is  
270 species-specific, with species showing an increase, decrease or no variation in terms of biomass  
271 (Russell et al. 2013; Takahashi et al. 2016). Here, we provide evidence that seagrass response is  
272 also site-specific, as the same species (*C. nodosa*) seems to have a different structure at the meadow  
273 level (i.e., biomass, LAI) in the low pH stations in comparison with controls in the two venting

274 sites. More effort, however, is needed to widen spatial replication within each site, in order to gain a  
275 more reliable picture.

276 The two sites were more consistent when looking at sediment properties, showing decreased carbon  
277 accumulation in surficial sediment at low pH stations. As only surficial sediment was collected, and  
278 carbon content and sediment density can change with depth (e.g., Lavery et al. 2013; Marbà et al.  
279 2015), the patterns discussed cannot straightforwardly be expanded to deeper layers of sediment and  
280 more investigation is needed to have a more comprehensive picture. So far, not much attention has  
281 been given to the capacity of *C. nodosa* to accumulate carbon both in living biomass and sediments,  
282 as no study has measured the carbon stocks in *C. nodosa* meadows (but Apostolaki et al. under  
283 revision). Only a few studies have indirectly assessed carbon sequestration by measuring the carbon  
284 metabolism of the meadows (Barrón et al. 2004; Cebrián et al. 1997; Duarte et al. 2005). As carbon  
285 storage in vegetated habitats depends, among other factors, also on the seagrass species involved  
286 (Lavery et al. 2013), the lack of other studies on *C. nodosa* prevented any comparison with the  
287 literature.

288 Here, the decrease in IC surficial stock may be related to the higher solubility of carbonates. This  
289 was an expected trend, typical of vent systems, and strictly correlated to the fact that the solubility  
290 of calcium carbonate depends strongly on pH. The intense seagrass metabolism (Apostolaki et al.  
291 2014), photosynthesis but also respiration with consequent CO<sub>2</sub> release, may additionally impede  
292 the accumulation of carbonates in seagrass sediments in these particular environments, reducing the  
293 IC stock. IC stock was low also in the controls, probably depending on the mineral composition of  
294 sediments in volcanic islands.

295 In terms of OC, we may reasonably assume that seagrass properties at Vulcano Island (such as leaf  
296 area, LAI and biomass), which appeared negatively affected (although not significantly for  
297 biomass) by environmental conditions at the low pH station, may prevent an increase in carbon  
298 accumulation in sediments. The reduced leaf area and biomass close to the vents at Vulcano may be  
299 directly related to both the higher grazing pressure of consumers on *C. nodosa* at low pH, as

300 previously hypothesized (Apostolaki et al. 2014), and/or the influence of contaminants in the area.  
301 Indeed, sediment pollution indices revealed that detrimental effects on the biota may occur in the  
302 area between about 150 and 350 m from the primary vent (Vizzini et al. 2013). Altogether, these  
303 peculiarities in the environment, sediment and seagrass features in the vents, cannot be directly  
304 related to high  $p\text{CO}_2$ , but also to the biogeochemical processes involved, such as low pH and redox  
305 potential (Eh), which enhance trace element precipitation at the sediment-water interface (Vizzini et  
306 al. 2013).

307 While at Vulcano the changes in structural features of the meadow would entail the overall  
308 reduction in carbon content and surficial accumulation, at Milos a generally less straightforward  
309 pattern is suggestive of a more complex story. In fact, the increase in seagrass aboveground  
310 biomass, leaf length, leaf area and LAI close to the vents would imply a greater potential to trap  
311 particles and to accumulate organic carbon in sediments, which however did not occur. This could  
312 be related to different factors and, firstly to the seagrass species itself. Previous studies, have  
313 highlighted a strong variability in the capacity to stock carbon among different seagrass species.  
314 Lavery et al. (2013), for example, suggested that larger seagrasses (i.e., *Posidonia australis*) can  
315 stock more carbon, efficiently trapping particles inside their beds. Accordingly, the weak capacity  
316 of *C. nodosa* to retain sediments may be related to the shortness of the leaves and low belowground  
317 biomass in comparison with other seagrass species (Russell et al. 2013), as well as to their  
318 subsequent weak entrapping and compacting role, respectively. At low density (as in the case of  
319 both control and low pH stations at Milos), *C. nodosa* is expected not to trap carbon, both  
320 autochthonous and allochthonous, in an efficient way and the carbon trapped may be easily subject  
321 to resuspension and reduction because of the impact of local hydrodynamism on low-density  
322 meadows. The belowground compartments of *C. nodosa* are generally small and the meadows not  
323 perennial; consequently, the possibility of burying large quantities of carbon could be greatly  
324 affected. The particular seagrass features may reduce the capacity to trap carbon cancelling out the  
325 potential benefit deriving from increased  $\text{CO}_2$ . Recently, Mazarrasa et al. (2018) reviewed both the

326 biotic and abiotic factors that affect long-term  $C_{org}$  sequestration in seagrass habitats, suggesting  
327 that short-leaf, low density and low canopy complexity of the meadows do not drive large  
328 accumulation of carbon in sediments, and that biotic factors combine with abiotic ones (i.e.,  
329 turbidity, nutrient availability, depth and hydrodynamics) in determining the carbon sequestration  
330 capacity of seagrasses. In the present study, control and low pH stations were selected in order to be  
331 comparable in terms of the main abiotic factors (i.e., depth, exposure and nutrient availability)  
332 within each vent to avoid any possible effect when contrasting the effect of vent conditions on  
333 carbon accumulation in sediment. Moreover, sediment features can also influence carbon  
334 accumulation potential in sediment and further prevent a high carbon burial at low pH stations. A  
335 recent study by Gullstrom et al. (2018) found out that C stocks in seagrass habitats are predicted  
336 primarily by sediment density and only to a lesser extent by seagrass features. In the present study,  
337 while density did not show any pattern, sediment porosity was correlated with TC stock in surficial  
338 sediment from Vulcano. In particular, a lower capacity to accumulate carbon corresponded to lower  
339 porosity (at low pH station), supporting the findings that abiotic factors may influence the carbon  
340 storage capacity of the seagrass (Dahl et al. 2016).

341 The Bayesian mixing model outcome provided the ranges of possible contributions of sources to  
342 sedimentary carbon, and although they were wide and often overlapped, indicating a degree of  
343 uncertainty in the models, which however is intrinsic in ecological systems (Parnell et al. 2010), the  
344 contribution of *C. nodosa* appeared not much high, also at Milos where *C. nodosa* biomass and  
345 density were lower and POM assumed a dominant role in contributing to the organic matter of the  
346 sediment. In addition, the particular origin of sedimentary C at the low pH station is corroborated by  
347 the contribution of volcanic-derived microbial mats, responsible for the labilisation of sedimentary  
348 organic matter resulting from the decreased C/N ratios, above all at Vulcano.

349 Although carbon sinks may depend strongly on the regional location of the study area (Gullstrom et  
350 al. 2018) and geographical variability occurs in seagrass capacity to stock carbon in sediments  
351 (Miyajima et al. 2015), the consistency of results from the two vents suggests that, probably

352 through different underlying mechanisms, C content and accumulation potential in *C. nodosa*  
353 sediments may not increase at high  $p\text{CO}_2$ -low pH conditions and that the prediction of positive  
354 effects of OA on seagrasses and on their capacity to stock carbon (Garrard and Beaumont 2014)  
355 requires further investigation.

356

## 357 **Conclusions**

358 Recent studies have provided evidence of increased productivity, biomass and density of seagrasses  
359 under high  $p\text{CO}_2$ -low pH conditions (Russell et al. 2013; Takahashi et al. 2016), and suggested a  
360 positive relationship between ocean acidification and carbon stock capacity of marine vegetation  
361 (Garrard and Beaumont 2014, Mazarrasa et al. 2018). The relatively simplified field observations of  
362 this study provided evidence of a non-consistent response of the seagrass *Cymodocea nodosa* in the  
363 two vents and an overall decrease in carbon surficial stocks at high  $p\text{CO}_2$ -low pH conditions.  
364 Although the assessment has been carried out only in surficial sediment, the results from two  
365 different volcanic vents contribute to the development of conceptual models of the changes  
366 expected in future high  $p\text{CO}_2$ -low pH oceans. There is also evidence that the intrinsic features of  
367 each marine site and seagrass bed should be carefully taken into account when studying carbon  
368 accumulation, and generalizing the potential effects that acidified oceans may have on the  
369 corresponding carbon stocking capacity is challenging. Moreover, while  $\text{CO}_2$  vents are used as a  
370 proxy of real long-term and chronic exposure to high  $p\text{CO}_2$ -low pH within realistic natural  
371 conditions (Fabricius et al. 2011; Takahashi et al. 2016), caution should be taken because of the  
372 limitations that are typical of field studies represented mainly by the unpredicted variability of  
373 biotic and abiotic factors, specifically by the high variability of  $p\text{CO}_2$ , and/or the bias of other  
374 variables (e.g.,  $\text{H}_2\text{S}$ , metals).

375 Scientific interest in the potential of marine vegetation as mitigation service to absorb and stock  
376 excessive carbon is growing (Russell et al. 2013). Considering this ecosystem service in relation to  
377 global climate change and, in particular, to the acidification of the seawater is highly important.

378 Further studies are required to collect similar data from different natural CO<sub>2</sub> vent systems where  
379 seagrasses thrive and to analyze deeper sediment layers and larger sample sizes in order to provide a  
380 more comprehensive analysis.

381

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392

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506

507 **Figure legends**

508

509 Figure 1. Study sites (Milos and Vulcano islands) showing the stations (control and low pH) where  
510 samples were collected (Windows Power Point 2013 drawing based on maps from ArcGIS vers.  
511 9.1).

512

513 Figure 2. Organic, inorganic and total carbon stocks (OC, IC and TC,  $\text{Mg ha}^{-2}$ , mean  $\pm$  SD) in  
514 surficial sediment (2 cm) at the stations (control and low pH) and sites (a) Milos and b) Vulcano)  
515 investigated.

516

517 Figure 3. Mixing models output showing the contribution of different organic matter sources to  
518 sediment organic carbon at the stations (control and low pH) and sites (Milos and Vulcano)  
519 investigated. Boxplots illustrate the 90%, 75% and 50% confidence intervals from light to dark and  
520 dots indicate the mode. POM: suspended particulate organic matter; Mat: microbial mats.

521

522 Table 1. Seawater carbonate chemistry at the control and low pH of both Milos and Vulcano  
523 (ranges or means  $\pm$  SD). Data of Milos are taken from Misha et al. (unpublished), Bayraktarov et al.  
524 2013, Price et al. 2013b, while those of Vulcano from Milazzo et al. 2016.

Site	Milos		Vulcano	
Station	Control	Low pH	Control	Low pH
pH	8.20 $\pm$ 0.01	7.90 $\pm$ 0.01	8.18 $\pm$ 0.01	7.83 $\pm$ 0.05
$p$ CO <sub>2</sub> ( $\mu$ atm)	402.9 $\pm$ 1.1	884.3 $\pm$ 3.1	421 $\pm$ 15	1180 $\pm$ 153
TA (mmol kg <sup>-1</sup> )	2.7-3.6	2.1-2.2	2.5	2.5

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527 Table 2. Data of *C. nodosa* properties (mean  $\pm$  SD) at the stations (control and low pH) and sites  
 528 (Milos and Vulcano) investigated and relative statistical results (2-way ANOVA, factors: station  
 529 and site, and post-hoc tests). Living tissues include leaf blades, leaf sheaths, rhizomes and roots.  
 530 Leaf width was not statistically analyzed because it was identical in each pH condition (control and  
 531 low pH) and site. M: Milos, V: Vulcano, C: control, L: low pH.

Site	Milos		Vulcano		2-way ANOVA	post-hoc Student–Newman–Keuls test
Station	Control	Low pH	Control	Low pH		
Density (shoots m <sup>-2</sup> )	122.67 $\pm$ 72.1	208.0 $\pm$ 112.0	525.0 $\pm$ 139.2	616.67 $\pm$ 202.1	site: $F_{1,8}=25.31, p=0.001$	M<V
Leaf length (cm)	10.07 $\pm$ 1.40	18.70 $\pm$ 2.90	10.12 $\pm$ 1.39	7.10 $\pm$ 0.38	station x site: $F_{1,8}=32.76, p=0.0004$	MC<ML, VL=VC, VL<ML, MC=VC
Leaf width (cm)	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	-	-
Leaf area (cm <sup>2</sup> shoot <sup>-1</sup> )	7.68 $\pm$ 1.31	13.65 $\pm$ 2.06	7.96 $\pm$ 0.59	4.94 $\pm$ 0.08	station x site: $F_{1,8}=38.46, p=0.003$	MC<ML, VL<VC, VL<ML, MC=VC
L.A.I. (m <sup>2</sup> leaves m <sup>-2</sup> )	0.094 $\pm$ 0.016	0.284 $\pm$ 0.043	0.418 $\pm$ 0.031	0.305 $\pm$ 0.005	station x site: $F_{1,8}=94.10, p=0.0001$	MC<ML, VL<VC, ML=VL, MC<VC
Aboveground biomass (g DW m <sup>-2</sup> )	3.89 $\pm$ 0.56	10.94 $\pm$ 2.00	30.33 $\pm$ 3.20	27.52 $\pm$ 1.95	station x site: $F_{1,8}=94.10, p=0.004$	MC<ML, VL=VC, ML<VL, MC<VC
Belowground biomass (g DW m <sup>-2</sup> )	12.22 $\pm$ 2.86	16.28 $\pm$ 2.25	23.06 $\pm$ 3.90	27.23 $\pm$ 8.55	site: $F_{1,8}=14.02, p=0.006$	M<V
TC stock <sub>living tissues</sub> (Mg C ha <sup>-2</sup> )	0.05 $\pm$ 0.01	0.09 $\pm$ 0.01	0.21 $\pm$ 0.01	0.21 $\pm$ 0.04	#station x site: $F_{1,8}=13.86, p=0.006$	MC<ML, VC=VL, ML<VL, MC<VC
#data Ln transformed						

532

533



534 Table 3. Data of sediment properties (mean  $\pm$  SD) at the stations (control and low pH) and sites  
 535 (Milos and Vulcano) investigated and relative statistical results (2-way ANOVA, factors: station  
 536 and site, and post-hoc tests). Statistical significance in silt and clay content could not be tested, due  
 537 to lack of replicates in Milos. M: Milos, V: Vulcano, C: control, L: low pH.

Site	Milos		Vulcano			
Station	Control	Low pH	Control	Low pH	2-way ANOVA	post-hoc Student–Newman–Keuls test
DBD (g cm <sup>-3</sup> )	1.20 $\pm$ 0.05	1.25 $\pm$ 0.04	1.55 $\pm$ 0.02	1.54 $\pm$ 0.06	site: $F_{1,8}=155.24, p=0.0001$	M<V
Porosity ( $\phi$ )	0.42 $\pm$ 0.01	0.44 $\pm$ 0.02	0.42 $\pm$ 0.03	0.38 $\pm$ 0.01	site: $F_{1,8}=7.81, p=0.023$	V<M
Silt and clay (%)	2.5	2.8	1.0 $\pm$ 0.1	1.4 $\pm$ 0.5	-	-
$\delta^{13}\text{C}$ (‰)	-18.87 $\pm$ 0.15	-18.75 $\pm$ 0.04	-21.94 $\pm$ 0.21	-22.69 $\pm$ 1.94	site: $F_{1,8}=38.32, p=0.003$	V<M
C/N	6.90 $\pm$ 0.99	5.73 $\pm$ 0.76	10.54 $\pm$ 1.72	3.66 $\pm$ 2.48	-	-

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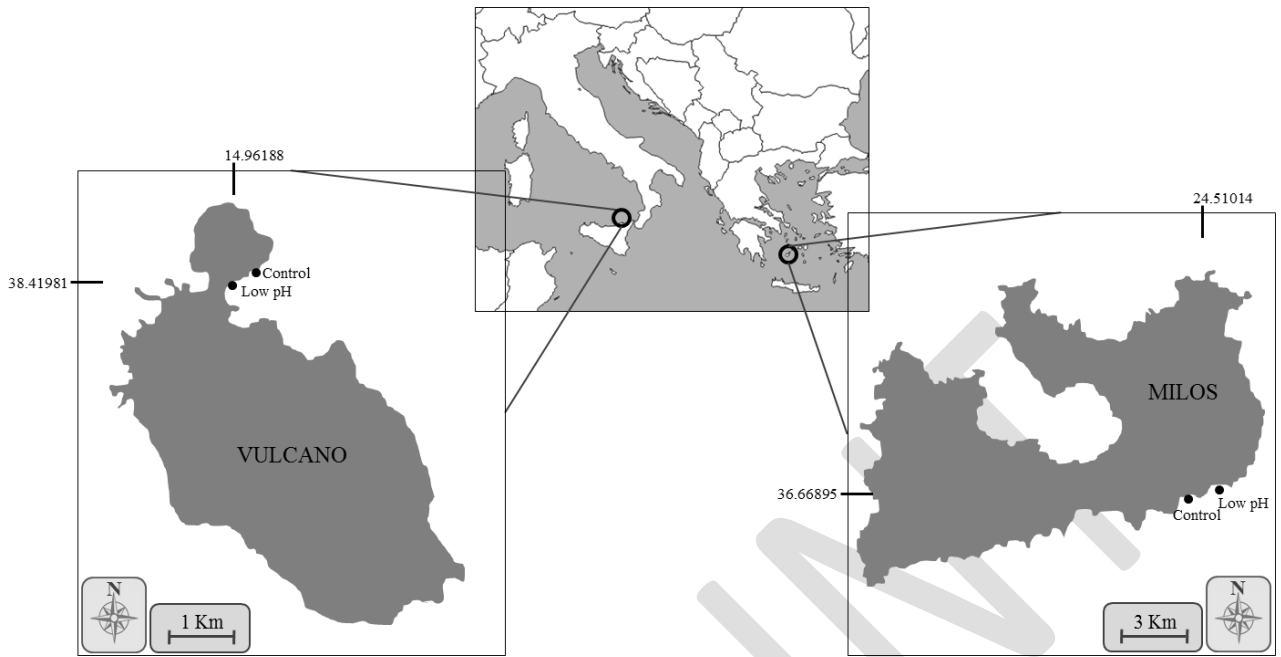
540 Table 4.  $\delta^{13}\text{C}$  data (‰; mean  $\pm$  SD) of sources potentially contributing to sedimentary carbon used  
 541 in the mixing models carried out separately for each station (control and low pH) and site (Milos  
 542 and Vulcano). POM (suspended particulate organic matter) data for Milos are averaged values  
 543 collected from other volcanic vents [Panarea (Vizzini unpublished data) and Vulcano (this study)  
 544 Islands, Aeolian Archipelago, Italy].

Site	Milos		Vulcano	
Station	Control	Low pH	Control	Low pH
<i>Cymodocea nodosa</i>	-8.82 $\pm$ 0.17	-7.56 $\pm$ 0.04	-9.19 $\pm$ 0.17	-13.12 $\pm$ 0.05
POM	-21.71 $\pm$ 0.89	-24.39 $\pm$ 0.79	-20.87 $\pm$ 1.05	-24.76 $\pm$ 0.14
Macroalgae	-	-	-	-19.75 $\pm$ 0.77
Filamentous algae	-	-	-23.44 $\pm$ 0.17	-
Microbial mats	-	-26.11 $\pm$ 0.91	-	-26.11 $\pm$ 0.91

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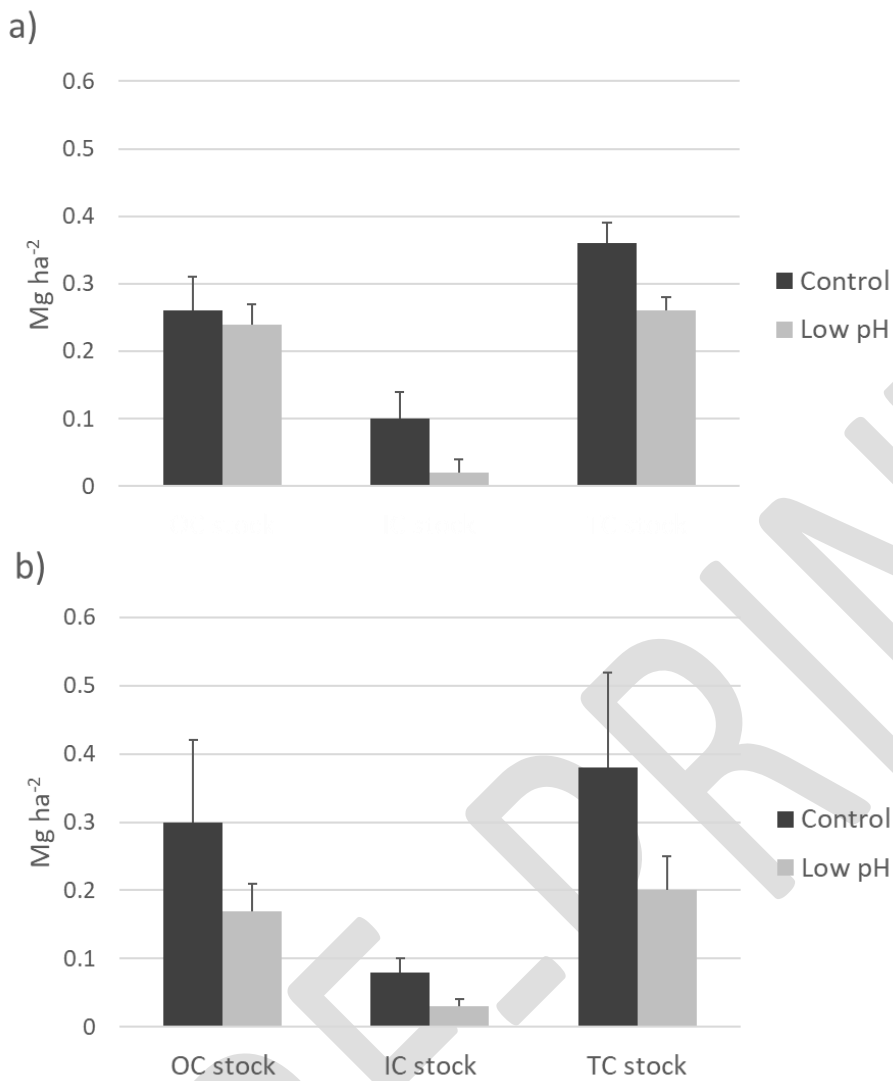
548

549 Figure 1. Study sites (Milos and Vulcano islands) showing the stations (control and low pH) where

550 samples were collected (Windows Power Point 2013 drawing based on maps from ArcGIS vers.

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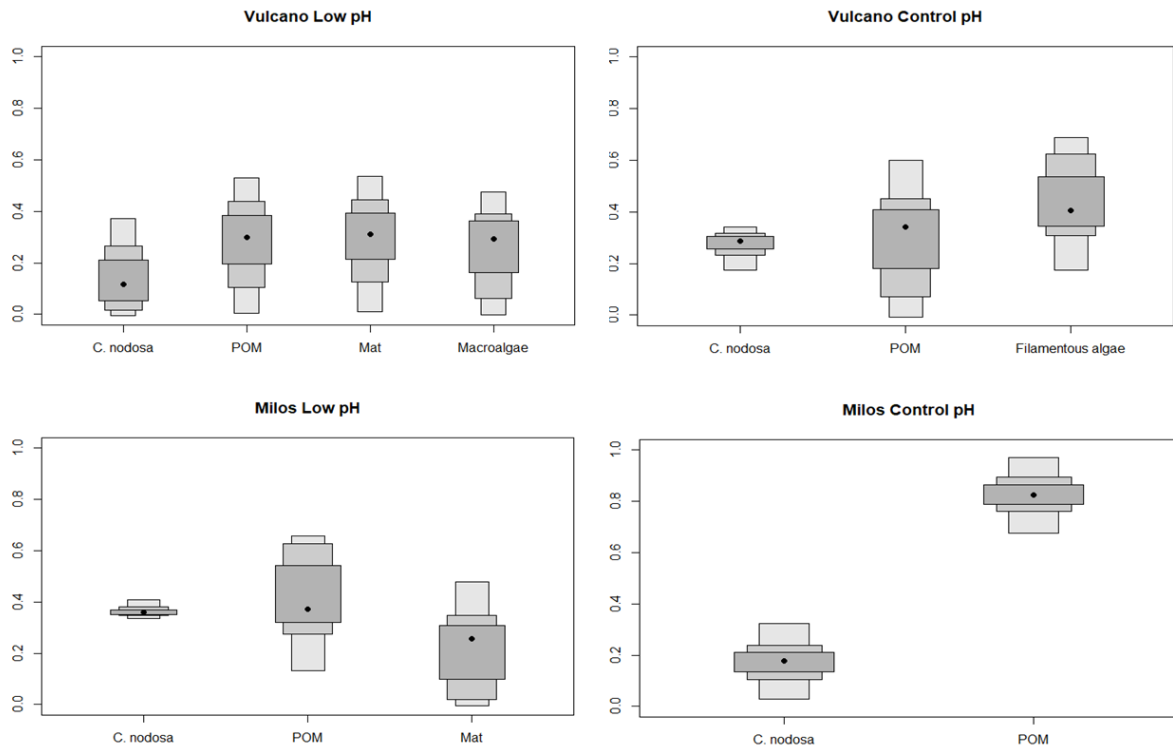
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554

555 Figure 2. Organic, inorganic and total carbon stocks (OC, IC and TC, Mg ha<sup>-2</sup>, mean  $\pm$  SD) in  
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558



560

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