1 **Long-term effects of CO₂ on the population dynamics of the seagrass *Cymodocea***

2 ***nodosa*: evidence from volcanic seeps**

3

4 [1,2]**Mishra, A.K., [1]Cabaço, S., [1]de los Santos, C. B., [3]Apostolaki, E.T., [4,5]Vizzini, S.,**

5 [1]**Santos, R.**

6

7 [1]Marine Plant Ecology research group (ALGAE), Centre of Marine Sciences (CCMAR),

8 University of Algarve, Faro, 8005-139, Portugal

9 [2] School of Biological and Marine Sciences, University of Plymouth, Plymouth, PL48A, UK

10 [3]Institute of Oceanography, Hellenic Centre for Marine Research (HCMR), PO Box 2214,

11 71003 Heraklion-Crete, Greece

12 [4]Department of Earth and Marine Sciences, University of Palermo, via Archirafi 18, 90123

13 Palermo, Italy

14 [5]CoNISMa, Piazzale Flaminio 9, 00196 Roma, Italy

15 Corresponding author: rosantos@ualg.pt /amritkumarmishra@gmail.com

16

17 **Abstract**

18 Seagrass communities are predicted to benefit from rising carbon dioxide (CO_2)

19 concentrations in the ocean, but it is not clear how CO_2 affects the different population

20 parameters and dynamics of seagrasses, as population responses are difficult to assess under

21 short-term enrichment experiments. Here we use population reconstruction techniques to

22 assess for the first time the population dynamics of a seagrass, *Cymodocea nodosa*, exposed

23 to long-term elevated CO_2 near three volcanic seeps of Greece (site1, site2) and Italy (site3)

24 and compare them with reference sites away from seeps. To account for potential

25 confounding factors of seeps, such as putative toxic effects of heavy metals and sulphide, we

26 considered only the population responses of *C. nodosa* that were common to the three

27 locations.

28 Results show that under high CO_2 the total and apical shoot density of *C. nodosa*, and the

29 vertical and horizontal rhizome production rates were higher. At the Adamas seep, where

30 CO_2 availability was the highest and nitrogen availability the lowest, total and apical shoot

31 density were highest, probably due to direct CO_2 effects on the meristems of horizontal

32 rhizomes and of induced reproductive output, respectively. On the other hand, the elongation

33 and production rates of rhizomes were highest at both the Levante seep and reference sites,

34 where the availability of nitrogen was highest. Our observations showed also that populations

35 near seeps in all three locations had higher short- and long-term recruitment, and growth rates

36 around zero, indicating that elevated CO_2 may increase the turnover of *C. nodosa* shoots.

37 This study emphasizes the advantages of using CO_2 volcanic seeps and the need to replicate

38 them to be able to capture the common responses to the environmental fluctuations among

39 seeps so that the long-term responses to the common variable of CO_2 enrichment may be

40 revealed.

41

44

45

Introduction

Seagrass photosynthesis may be limited by dissolved inorganic carbon concentration of the

water column, which has led part of the research community to suggest that seagrass could

benefit from the global increase in oceanic CO_2 levels (Koch et al., 2013; Brodie et al 2014;

Borum et al., 2016). However, several CO_2 enrichment studies (*ex-situ* and *in-situ*) on

seagrasses reveal that the response is species- specific and more complex than originally

thought. Relatively long experiments such as that carried out by Alexandre et al. (2012)

which lasted five months reported positive effects on the photosynthetic production of

Cymodocea nodosa under experimental CO_2 enrichment, but no effects on growth. Similarly,

experiments with elevated CO_2 conditions on *Zostera marina* for over one year showed no

effects on specific growth rate, size, or sugar content of leaves, but led to significantly higher

reproductive output, below-ground biomass and vegetative proliferation of new shoots

(Palacios and Zimmerman, 2007). Short-term experiments have shown increased

photosynthetic rate and shoot productivity for *Z. marina* (Pajusalu et al., 2016; Zimmerman et

al., 1997), *Thalassia hemprichii* (Jiang et al., 2010) and *Z. muelleri* (Collier et al., 2018) and

increased community production for *Z. noltei* (Mishra et al., 2018) and plant biomass for *C.*

nodosa (de los Santos et al. 2017). Contrastingly, experiments on *C. serrulata* have shown no

enhancement in productivity at higher CO_2 (Schwarz et al., 2000; Collier et al., 2018). Recent

studies on three tropical and nine temperate seagrass species showed significant increase in

net productivity with increased CO_2.

Studies on natural CO_2 seeps provide a unique opportunity to assess long-term effects of

CO_2, which are not possible under laboratory or in field-controlled conditions. Studies using

seeps suggest that seagrass species can adapt to survive and enhance metabolic processes

under elevated CO_2 conditions (Hall-Spencer et al., 2008; Fabricius et al., 2011; Russel et al.,

70 2013 and Takahashi et al., 2015). The photosynthetic activity of *C. nodosa* was stimulated

71 near natural CO_2 seeps of Vulcano, with significant increase in leaf chlorophyll-a content,

72 maximum electron transport rate and compensation irradiance (Apostolaki et al., 2014; Olivé

73 et al. 2017), but this increase in efficiency of leaf metabolic process was not translated to

74 generate higher biomass and production of the plant, probably due to nutrient limitation and

75 grazing (Apostolaki et al., 2014) or toxic effects of seeps (Olivé et al., 2017). On the other

76 hand, the biomass and net primary production of *Halophila ovalis* and *C. serrulata* were

77 higher near CO_2 seeps, whereas the abundance of species increased only for *C. serrulata*,

78 suggesting species-specific response to elevated CO_2 (Russell et al., 2013; Borum et al.,

79 2016). Even though CO_2 seep sites have been widely used to assess the long-term effects of

80 elevated CO_2 on benthic marine ecosystems and respective underlying mechanisms (Hall-

81 Spencer et al., 2008; Fabricius et al., 2011; Enochs et al., 2015), care must be taken on the

82 interpretation of results (Apostolaki et al. 2014, Vizzini et al. 2019) as other cofounding

83 factors may be present, such as the emissions of heavy metals (Dando et al., 1999, Vizzini et

84 al., 2013, Kadar et al., 2013; Mishra et al., 2019) and sulphide (Dando et al., 1999, Boatta et

85 al.,2013), which may influence the plants and population responses to elevated CO_2. These

86 environmental abiotic parameters are largely variable among seeps (Dekov and Savelli, 2004;

87 Varnavas et al., 2005), whose emissions are largely dominated by CO_2.

88   Much of the natural CO_2 seeps of Europe are concentrated in the Mediterranean Sea, in

89 particular in the shallow waters of the active volcanic arcs in Aegean Sea (Dando et al.,

90 1999). They provide future oceanic conditions (Hall-Spencer et al., 2008; Hall-Spencer and

91 Rodolfo-Metalpa, 2009) with abundant seagrass at some seeps (Hall-Spencer et al., 2008;

92 Russel et al., 2013) and sparse at others (Vizzini et al., 2013). Although these vents have

93 attracted the relative research community over the recent past, it has never been investigated

94 so far how these conditions will affect higher levels of seagrass biological organization than

95 photosynthesis and production, such as population dynamics. Seagrasses are rhizomatous

96 plants that grow by reiteration of a limited set of modules. Their past growth history can

97 therefore be reconstructed from the distinct scars left by abscised leaves on the long-lived

98 rhizomes or the seasonal signals imprinted in the frequency and size of their modules,

99 therefore allowing the detection and age determination of past growth (Duarte et al. 1994).

100 Assessing population dynamics therefore allows for an in depth understanding of the

101 response of the seagrass meadow to environmental perturbations over time.

102 The objective of this work is to assess for the first time the long -term responses of the

103 meadow structure, plant growth and production, and population age structure and dynamics

104 of the seagrass *C. nodosa* under elevated CO_2 levels near natural vents. The seagrass *C.*

105 *nodosa* is an endemic species that supports highly complex and biodiverse climax

106 communities in the Mediterranean Sea (Mazzella et al., 1986). We hypothesized that

107 increased CO_2 enhances plant growth and production, and higher shoot turnover based on

108 higher rates of shoot recruitment and mortality. To test this hypothesis, seagrass populations

109 in the vicinity and away from the influence of volcanic seeps were compared. To handle

110 possible confounding factors of the effects of CO_2 on the population dynamics of *C. nodosa*,

111 we replicated the sampling effort in three seeps, two at the island of Milos in Greece and one

112 at Vulcano island in Italy and considered the shared responses to the three seeps as effects of

113 elevated CO_2.

114

115 **Materials and Methods**

116 *Study sites*

117 Paleochori bay (36.67 N, 24.51 E) and Adamas thermal springs (36.70 N, 24.46 E) of Milos

118 island, Greece, were selected (Fig.1A), where extensive submarine CO_2 venting occurs, from

119 the intertidal to depths of more than 100 m (Dando et al.,2000). The released gases are

120 mainly composed of 92.5% CO_2, 0.13% O_2, 0.67% N_2, 11450 ppm H_2, 0.7 ppm CO and 916

121 ppm CH_4 in 2011 (Bayraktarov et al., 2013). The third selected location was Levante bay

122 (38.4 N, 15.0 E) of Vulcano island, Italy (Fig. 1B), a well-characterised area to study natural

123 processes associated to increased CO_2 levels (Boatta et al., 2013). The main underwater gas

124 seeps are located along southern and western shores of the bay at about 1 m depth (Boatta et

125 al., 2013). The underwater gas emissions are 97-98% CO_2 with 2.2% H_2S close to the seeps,

126 decreasing to less than 0.005% H_2S towards the north-eastern part of the bay, where most

127 ocean acidification research has been located (Capaccioni et al., 2001; Boatta et al., 2013;

128 Milazzo et al., 2014). There is a step gradient in carbonate chemistry with pH 5.6 at the main

129 gas seeps increasing to pH 8.1, which is typical for present day Mediterranean surface

130 seawater, (Boatta et al., 2013; Howritz et al., 2015). Subtidal *C. nodosa* beds dominate the

131 northern section of the bay (Giaccone 1969; Boatta et al. 2013).

132 In each location, two sites were established, a high CO_2 site near the seeps (called 'seep'

133 hereafter) and a reference site (called 'reference' hereafter) away from the influence of the

134 vent. All sites were established at similar depths (*ca.* < 5 m) and under similar hydrodynamic

135 conditions.

136

137 *Seawater carbonate chemistry*

138 Replicated water samples (n = 5) were collected at CO_2 seeps and reference sites in 100-mL

139 Winkler bottles, *in situ* fixed with 20 μL of mercury chloride, stored in the dark cool boxes,

140 and transported to the laboratory for total alkalinity (A_T) determination. The pH_{NBS} and

141 temperature of the water samples were measured in the field immediately after collection

142 using a pH meter (pH meter, Titrino Methron, NBS). In the laboratory, A_T was analysed in an

143 80-mL sample aliquot by titration (Lab Titrino analyser, Dickson et al. 2007b). Sterilized sea

144 water was used as reference material (CRM Batch 129, accuracy-98.7%, Dickson, 2013) for

145 A_T analysis. Temperature, pH_{NBS} and A_T data were used to calculate the CO_2 partial pressure

146 (pCO_2) and total dissolved inorganic carbon (DIC) using the CO_2SYS program developed by

147 Pierrot and Wallace (2006). Dissociation constants (K_1 and K_2) developed by Mehrbach et al.

148 (1973) and refitted by Dickson and Millero (1987) and dissociation constant for boric acid

149 (K_B) developed by Dickson et al. (2007b) were used.

150

151 *Cymodocea nodosa traits*

152 A variety of *C. nodosa* traits were analysed at each site, namely: 1) nutrient content [carbon

153 (C) and nitrogen (N) in leaves, rhizomes and roots, and the corresponding C:N ratios], 2)

154 meadow abundance (total shoot density, apical shoot density, total biomass, above and

155 below-ground biomass, and the above- to below-ground ratio), 3) rhizome growth and

156 production (vertical and horizontal), 4) population structure (age frequency distribution) and

157 5) population dynamics (long-term shoot recruitment rate, present shoot recruitment rate,

158 shoot mortality rate, and net population growth rate).

159 *C. nodosa* shoots (ten replicates) were collected from each site using a core (20-cm diameter

160 in Levante bay, and 15-cm in Adamas and Paleochori bays) into a depth of about 30 cm in

161 May 2013 (Levante bay) and May 2014 (Adamas and Paleochori bay). The sediment was

162 carefully rinsed off to prevent shoot dislodgement and to keep the rhizome mat intact as

163 required for the reconstruction techniques (Duarte et al., 1994; Fourqurean et al., 2003). Total

164 and apical shoot densities (shoots m^{-2}) were determined by counting the number of shoots per

165 sample. The number of leaves per shoot, number of rhizome scars, and rhizome internode

166 length were obtained from intact shoots in each sample for further calculations. The biomass

167 of leaves, rhizomes (vertical and horizontal) and roots were obtained by weighing the

168 separated tissues after oven-dried for 48 h at 60º C (dry weight, dw), and later used for

169 calculations of production and biomass (g dw m^{-2}). Above- to below-ground biomass ratios

170 were also calculated. Dried plant material (leaves, rhizomes and roots) was then ground and

171 analysed for C and N content (% dw) in a CHN analyser (EA 1110 Model, Elemental

172 Microanalysis Ltd, Oakhampton, Devon, UK), and C:N ratio was calculated on dw basis.

173 Reconstruction techniques, an indirect measurement of plant growth, were used to estimate

174 the rhizome growth and production, population age structure and derived population

175 dynamics (Duarte et al., 1994). The shoot age in plastochrone intervals (PI, the time required

176 between the production of two consecutive new leaves) was estimated by counting the

177 number of leaf scars in vertical rhizomes, plus the number of standing leaves in that shoot.

178 The PIs are very species-specific and remarkably constant for some species as it is the case of

179 *C. nodosa*, where it varies from 26 to 33 days (Duarte et al., 1994; Short and Duarte, 2001).

180 Here we considered a PI of 29.3 days, which was estimated by Cancemi et al. (2002) for *C.*

181 *nodosa* Mediterranean populations of Ischia, Naples Bay. Shoot ages were thus obtained by

182 multiplying the number of PIs of shoots by 29.3 days. Frequency distribution of shoot ages

183 for each population was constructed to reveal age structure and maximum longevities, and to

184 calculate the population dynamics parameters (see below).

185 The vertical rhizome elongation rate (cm yr^{-1}) for each site was obtained as the slope of the

186 linear regression between the length of vertical rhizomes (from the insertion onto the

187 horizontal rhizome to the apical meristem) and their age. Horizontal rhizome elongation rate

188 was estimated based on the linear regression of the number of horizontal internodes in

189 between two consecutive shoots against the age difference of those shoots. The slope

190 (internodes yr^{-1}) was then multiplied by the mean internode length (cm internode^{-1}) to obtain

191 the horizontal rhizome elongation in units of cm yr^{-1}. The regression analyses were based on

192 the total number of rhizomes collected at each site. As the number of rhizomes was not

193 enough to obtain significant relationships, additional *C. nodosa* plants were collected in

194 Adamas and Paleochori bay. This was not possible at Levante bay where the horizontal

195   rhizome elongation rate was estimated based on the frequency distribution of all the

196   measurements of the number of horizontal internodes produced per unit of time (age

197   differences between two consecutive shoots). The horizontal growth rate is then estimated as

198   the average of the nodes of the frequency distribution obtained for each site. With this

199   method, it was not possible to estimate the error for the horizontal rhizome elongation rate for

200   the Levante bay sites.

201   The rhizome production (g C m^{-2} yr^{-1}) at each site was calculated as the product of the

202   rhizome elongation rate (cm yr^{-1}), the number of growing rhizome apices per unit of area

203   (rhizome m^{-2}, given by non-apical and apical shoot densities for vertical and horizontal

204   productions, respectively), the specific dry weight of rhizomes (g dw cm^{-1}) and the rhizome C

205   content (g C g^{-1} dw).

206   The long-term average shoot recruitment (R) was estimated from the shoot age structure

207   using a negative decay model: $N_x = N_0\, e^{-Rx}$, where N_x is the number of shoots in age class x

208   and N_0 is the number of shoots recruited into the population. This model assumes that mean

209   mortality and recruitment are constant over the lifespan of the oldest shoots (Fourqurean et al.

210   2003, Cunha and Duarte, 2005). The recruitment for the current year of sampling, i.e. present

211   shoot recruitment rate (R_0, ln units yr^{-1}) was estimated as the difference of the total number of

212   shoots and the number of shoots older than a year in the shoot population (Duarte et al.

213   1994). The population growth rate was given by the net shoot recruitment rate (r), estimated

214   as: $r = R_0 - M$, where M is the long-term mortality rate, which equals the long-term

215   recruitment rate (R) under the assumptions of near steady state (Fourqurean et al., 2003).

216   Population was considered growing if r is positive ($R_0 > R$), shrinking if r is negative ($R_0 <$

217   R), or with the same trajectory pattern if R_0 is not significantly different from R (Fourqurean

218   et al., 2003).

219

220 *Statistical analysis*

221 Data are shown as mean ± standard error of the mean. Significant differences in seagrass

222 traits, shoot density, biomass, and rhizome production rates among sites (factor with 2 levels:

223 CO_2 vents vs reference) and locations (factor with three levels: Adamas, Paleochori and

224 Levante) were investigated using two-way ANOVA (results given by the p-value associated

225 to each fixed factor), after testing for homogeneity of variances (Fligner test) and normality

226 of distribution (Shaphiro-Wilks test). The Tukey's multiple comparison test was applied to

227 determine significant differences among factor levels. When ANOVA assumptions were not

228 verified even after root-squared or Log_{10} transformation, comparison between sites and

229 locations were performed by non-parametric Kruskal-Wallis rank sum tests (results given by

230 the p-value associated to each fixed factor) and the post-hoc Dunn's test. Differences in the

231 vertical and horizontal rhizome elongation rates between reference and CO_2 sites at each

232 location were tested by ANCOVA analysis, in which the rhizome trait (rhizome length for

233 vertical rhizome rate and number of internodes for horizontal one) was fit to rhizome age

234 using site and its interaction as fixed factor, and their slopes compared. The confidence limits

235 of the exponent coefficient from exponential decay regression model used to estimate the

236 long-term average recruitment rate (R), were used to test if it was different from the present

237 recruitment rate (R_0; Fourqurean et al. 2003). Significance levels were considered at $p < 0.05$.

238

239 **Results**

240 *Seawater carbonate chemistry*

241 Seawater carbonate chemistry varied between CO_2 seeps and reference sites in a similar way

242 for all locations, being pCO_2 2- to 6-fold higher, and pH 0.3 to 0.7 units lower at the seep

243 sites than at the reference sites (Table 1). DIC was 2-fold higher in the vents than in the

244 reference sites at Adamas and Paleochori bay but not in Levante bay, where DIC presented

245   similar values (Table 1). Much higher CO_2 concentration in the water column was observed

246   at Adamas than at the other locations.

247

248   *Cymodocea nodosa traits*

249   There were no clear effects of CO_2 seeps on the elemental contents of *C. nodosa*, i.e. that

250   were common to the three locations (Table 2). Only at Adamas, leaves of *C. nodosa* showed

251   higher C content (35.1 ± 0.2 % vs 32.1 ± 0.7 %) at the seeps than reference sites, as expected

252   due to the higher CO_2 availability (Table 1). At Paleochori bay, the rhizome N content near

253   the vent was 2.3-fold lower than at the reference site and consequently the C:N ratio was 2-

254   fold higher at the vent (Table 2). In general, the C and N contents of *C. nodosa* varied

255   significantly among locations. The C content of *C. nodosa* roots was higher at Adamas than

256   the other locations, whereas the C and N contents of leaves (and rhizome N) were higher at

257   Levante bay.

258   *C. nodosa* total and apical shoot density were higher at the CO_2 seeps than at the reference

259   sites (Fig. 2). Whereas total shoot density did not differ among locations, apical shoot density

260   did, with higher densities at Adamas (Fig. 2B). *C. nodosa* biomass was not affected by seeps

261   (Table 3). Meadows at Levante bay exhibited 4-fold higher biomass than those at the other

262   locations (Table 3). Above- to below-ground biomass ratio also differed among locations,

263   being higher at Paleochori bay (Table 3).

264   The vertical rhizome elongation rate of *C. nodosa* was significantly higher at the CO_2 seep

265   than at the reference site in all locations and ranged from 0.58 ± 0.02 cm yr^{-1} (reference site at

266   Paleochori) to 1.85 ± 0.10 cm yr^{-1} (CO_2 seep at Levante bay; Fig. 3A). The horizontal

267   rhizome elongation rate was only higher at the CO_2 seep site than at the reference at

268   Paleochori bay (Fig. 3B). *C. nodosa* populations at Adamas presented the lowest horizontal

269   elongation rates (< 5 cm yr^{-1}), whereas the highest were found at Levante bay (> 10 cm yr^{-1};

270   Fig. 3B). The vertical rhizome production rate was 2.1 to 2.7-fold higher at CO_2 seeps than at

271   the reference sites (Fig. 3C and D). The vertical and horizontal rhizome productions varied

272   significantly among the three locations, both being higher at Levante bay (Fig. 3C, D).

273   C. *nodosa* shoot longevity was higher at Levante bay (7-8 years), whereas at Adamas and

274   Paleochori bay reached maximum shoot ages of about 3 years. No differences between CO_2

275   seep and reference sites were patent (Table 4). In contrast, the present and long-term

276   recruitment rates were generally higher in populations growing at the CO_2 seeps than those at

277   the reference sites (Table 4). CO_2 seeps also affected the frequency distribution of *C. nodosa*

278   shoots, which consistently showed a higher number of younger plants (1 and 2 years old) at

279   the seeps, whereas the number of older plants (more than 3 years) were higher at the

280   reference sites in all locations (Fig. 4). The net population growth rates were generally around

281   zero, indicating stable populations, except at Paleochori reference site where the population

282   was actively growing (growth rate = 0.3, Table 4).

283

284   **Discussion**

285   Shallow water CO_2 seeps may act as natural analogues of future oceanic scenarios and the

286   presence of seagrass at the CO_2 seeps of Adamas and Paleochori of Milos island, Greece and

287   of Levante at Vulcano island, Italy, provided an opportunity to test the effects of long-term

288   CO_2 enrichment on seagrass population dynamics. Common responses of *C. nodosa*

289   populations at the three seeps indicate that under high CO_2 the total and apical shoot density,

290   and the vertical and horizontal rhizome production rates are higher. Curiously, where CO_2

291   availability was highest, at the Adamas seep, the density of shoots and of individuals were

292   highest whereas the elongation and production rates of rhizomes were highest at Levante, the

293   location with higher availability of nitrogen as indicated by the higher concentration of N in

294   *C. nodosa* tissues (about 2.5%), well above the threshold of 1.8 % of nitrogen limitation in

295   seagrasses (Duarte, 1990). The higher availability of nitrogen at Levante was not related with

296   the seep as there were no significant differences in the concentration of N between control

297   and seep *C. nodosa* tissues.

298   Higher shoot densities near seeps suggest that elevated CO_2 promotes the differentiation of

299   vertical rhizomes from the horizontal rhizome meristem. It is not clear how CO_2 may

300   influence the differentiation of vertical rhizomes, but the stimulation of meristem activity and

301   tissue-specific responses under elevated CO_2 have been well described in terrestrial grasses

302   (Kinsman et al 1997; Masie, 2000). The positive influence of CO_2 on the density of *C.*

303   *nodosa* individuals is probably related to increased reproductive output as observed by

304   Palacios and Zimmerman (2007) in *Zostera marina*.

305   Both vertical and horizontal rhizomes grew faster at Levante where more nitrogen is

306   available, as indicated by the general higher concentration of nitrogen in the plant tissues. *C.*

307   *nodosa* at this location showed also higher biomass, lower AG:BG and higher shoot

308   longevity, indicating more favourable growth conditions. At Adamas, where the N contents

309   in leaves, rhizomes and roots were lower, the highest CO_2 availability was not reflected at the

310   growth level. The N content of *C. nodosa* leaves at Adamas and Paleochori were very low,

311   below the threshold limit of 1.8% that indicates nitrogen limitation in seagrasses (Duarte.

312   1990). Nutrient limitation of seagrass growth under high CO_2 has been often invoked

313   (Alexandre et al, 2012; Apostolaki et al, 2014) even though Campbell and Fourqurean (2018)

314   failed to demonstrate that nutrient enrichment influence seagrass responses to elevated CO_2.

315   The response of seagrasses to the synergistic effects of CO_2 and nutrients are complex and

316   often mediated by epiphyte growth (Martinez-Crego et al., 2014). Negative effects of high

317   nitrogen content due to epiphyte shading has been observed for *P. oceanica* meadows at

318   Ischia CO_2 seeps (Ravagliolo et al., 2017).

319 Supporting evidence of higher density of *C. nodosa* near the Paleochori and Levante seeps

320 was provided by Vizzini et al. (2019), but not for Vulcano where no significant differences

321 were found (Apostolaki et al., 2014). In other species, significant positive effects on density

322 near the seeps were found, such as for *P. oceanica* at Ischia (Hall-Spencer et al., 2008), *C.*

323 *rotundata* at Papua New Guinea (Fabricius et al., 2011; Takahashi et al., 2015) and *C.*

324 *serratula* at Papua New Guinea (Russel et al., 2013). Even though we did not find any

325 significant site effects on *C. nodosa* biomass, as well as Apostolaki et al. (2014), positive

326 effects of seeps have been documented (Fabricius et al., 2011; Russell et al. 2013; Takahashi

327 et al., 2015). The effects of seeps are less clear in the biomass distribution between above and

328 below ground plant parts. We did not find any significant differences, contrasting with

329 Fabricius et al. (2011) and Russel et al. (2013) who found increases of below-ground biomass

330 of *C. rotundata* and *C. serratula*, respectively, near seeps. This was also observed in the one-

331 year long, experimental enrichment study of *Z. marina* (Palacios and Zimmerman, 2007).

332 The vertical and horizontal rhizome elongation rates of *C. nodosa* varied from 0.59 to 1.85

333 cm y^{-1} and 3.9 to 12.8 cm y^{-1}. The vertical elongations observed lie around the average of

334 those reported elsewhere (1.4 cm y^{-1}, Marbà and Duarte, 1998), whereas the horizontal

335 elongations are much lower than the average value reported in Marbà and Duarte (1998), 40

336 cm y^{-1}. The horizontal elongation of *C. nodosa* varies enormously, from 7 to 204 cm y^{-1},

337 being higher values typical of the "runners", i.e. the long horizontal rhizomes located in the

338 border of the meadows, which are exploring new grounds. Our observations were made in the

339 middle of the meadows and thus are consistent with the lower values of variation.

340 To our best knowledge, this is the first report of the effects of increased CO_2 on the

341 population dynamics of a seagrass. Our observations showed that populations near seeps in

342 all three locations had higher short- and long-term recruitment, and growth rates around zero,

343 indicating that the turnover of *C. nodosa* shoots under high CO_2 will be higher. This results in

344   age frequency distributions more skewed to younger individuals, as observed near seeps. On

345   the other hand, shoot longevity was more positively affected by nutrient availability (2.5-fold

346   increase) than by CO_2. The maximum shoot longevity of 8.3 years observed at Levante, was

347   higher than previously reported (7.6 at Ria Formosa lagoon, Cunha and Duarte, 2005; 4.0

348   Cabaço et al, 2010).

349   In conclusion, our findings show that under long-term CO_2 enrichment an increase of *C.*

350   *nodosa* densities, both shoot and physically independent individuals, an increase of

351   production of rhizomes, both vertical and horizontal, a faster turnover of shoots, but no

352   changes in the population biomass, are expected. Our results provide a picture of putative

353   future alterations of the population structure of *C. nodosa* meadows. We emphasize the

354   importance of using replicated CO_2 coastal seeps to be able to capture the common responses

355   to the environmental fluctuations among seeps so that the long-term responses to the common

356   variable of CO_2 enrichment may be revealed.

357

358

366

367 **Tables**

368 **Table 1.** Seawater carbonate chemistry (mean $\pm$ 1 SE, n = 5) at the reference and CO2 seeps

369 (Seep) sites in Adamas, Paleochori bay and Levante bay. pCO$_2$: CO$_2$ partial pressure, DIC:

370 dissolved inorganic carbon.

371

Variable	Adamas (Milos)		Paleochori bay (Milos)		Levante bay (Vulcano)	
	Reference	Seep	Reference	Seep	Reference	Seep
pH	8.20 ± 0.03	7.50 ± 0.04	8.17 ± 0.05	7.98 ± 0.08	8.17 ± 0.05	7.98 ± 0.08
pCO$_2$ (μatm)	406 ± 2	2458 ± 2	427 ± 68	737 ± 158	427 ± 68	737 ± 158
DIC (μmol Kg^{-1})	1407 ± 51	3474 ± 5	2244 ± 40	2377 ± 37	2244 ± 40	2377 ± 37

372 **Table 2**. *Cymodocea nodosa* carbon (C) and nitrogen (N) contents, and their ratio (mean ± SE, n = 3 to 5) in leaves, rhizome and roots at the reference

373 and CO_2 seep sites at Adamas, Paleochori bay and Levante bay locations. Summary of the 2-way ANOVA, with location and site as fixed factors, are

374 presented, where asterisks indicate the level of statistical significance (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$) and ns indicates lack of significance.

375 Different superscript letters represent significant differences revealed by post hoc Tukey pairwise groupings. dw: dry weight.

Variable	Tissue	Adamas (Milos)		Paleochori bay (Milos)		Levante bay (Vulcano)		2-way ANOVA test		
		Reference	Seep	Reference	Seep	Reference	Seep	Location	Site	Interaction
Carbon (% dw)	Leaves	32.0 ± 0.7^a	35.1 ± 0.2^b	35.2 ± 0.5^b	35.0 ± 0.8^b	41.3 ± 0.1^c	42.0 ± 0.6^c	***	*	*
	Rhizomes	34.3 ± 1.8	34.7 ± 0.2	33.1 ± 0.4	31.7 ± 0.6	33.2 ± 0.5	34.1 ± 0.3	ns	ns	ns
	Roots	34.1 ± 0.3^{ab}	35.4 ± 0.5^b	33.1 ± 0.4^{ae}	31.9 ± 0.7^{de}	28.9 ± 0.7^c	30.6 ± 0.4^{cd}	***	ns	*
Nitrogen (% dw)	Leaves	1.77 ± 0.02^a	1.87 ± 0.05^a	1.42 ± 0.08^a	1.67 ± 0.36^a	2.36 ± 0.08^b	2.55 ± 0.01^b	***	ns	ns
	Rhizomes	1.40 ± 0.13^{ab}	1.69 ± 0.26^{ab}	2.16 ± 0.31^b	0.92 ± 0.09^a	2.05 ± 0.02^b	2.22 ± 0.30^b	*	ns	**
	Roots	0.94 ± 0.11	0.94 ± 0.17	1.00 ± 0.07	0.80 ± 0.11	1.02 ± 0.12	0.90 ± 0.03	ns	ns	ns
C:N ratio (g g^{-1})	Leaves	18.1 ± 0.5^a	18.8 ± 0.5^a	25.2 ± 1.5^b	23.4 ± 3.1^b	17.6 ± 0.6^a	16.5 ± 0.3^a	**	ns	ns
	Rhizome	25.8 ± 3.4^{ab}	23.6 ± 5.2^{ab}	16.7 ± 2.5^a	35.3 ± 2.9^b	16.2 ± 0.4^a	15.9 ± 2.1^a	*	*	**
	Roots	38.4 ± 4.3	44.6 ± 9.5	33.7 ± 3.1	43.3 ± 6.7	28.9 ± 3.4	34.1 ± 1.6	ns	ns	ns

376 **Table 3**. *Cymodocea nodosa* total, above-ground (AG) and below-ground (BG) biomass, and AG:BG ratio (mean ± SE, n = 10) at the reference

377 and CO_2 seeps (Seep) sites in Adamas, Paleochori bay and Levante bay. Summary of the 2-way ANOVA (with location and site as fixed factors)

378 or Kruskal-Wallis test (for location and site, but not interaction) are presented, where asterisks indicate the level of statistical significance (***p

379 < 0.001, **p < 0.01, *p < 0.05) and ns indicates lack of significance. Different superscript letters represent significant differences revealed by

380 post hoc Tukey pairwise groupings. dw: dry weight.

Variable	Adamas (Milos)		Paleochori bay (Milos)		Levante bay (Vulcano)		2-way ANOVA or Kruskal-Wallis test		
	Reference	Seeps	Reference	Seeps	Reference	Seeps	Location	Site	Interaction
Total biomass (g dw m^{-2})	126.7 ± 8.4^a	158.7 ± 12.8^a	114.6 ± 3.3^a	167.3 ± 18.0^a	504.6 ± 43.6^b	456.2 ± 64.9^b	***	ns	ns
AG biomass (g dw m^{-2})	31.2 ± 2.9^a	48.4 ± 7.8^a	34.6 ± 4.2^a	59.3 ± 9.9^a	104.7 ± 15.5^b	97.7 ± 9.1^b	***	ns	ns
BG biomass (g dw m^{-2})	95.4 ± 6.3^a	110.3 ± 6.5^a	79.9 ± 3.2^a	108.0 ± 10.6^a	399.9 ± 36.1^b	358.5 ± 57.6^b	***	ns	ns
AG:BG ratio (g g^{-1})	0.33 ± 0.03^{ab}	0.43 ± 0.06^{ab}	0.45 ± 0.06^b	0.54 ± 0.08^b	0.27 ± 0.04^a	0.31 ± 0.04^a	**	ns	ns

18

381 **Table 4.** Maximum longevity (years) and population dynamics of *Cymodocea nodosa* shoots

382 at the reference and CO_2 seeps (Seep) sites in Adamas, Paleochori and Levante locations.

383 Long-term average recruitment rate (R) given as the exponential coefficient $\pm$ standard error

384 from the exponential decay regression. Number of shoots used for the analysis ranged from

385 126 to 336 per site.

Variable	Adamas (Milos)		Paleochori (Milos)		Levante (Vulcano)	
	Reference	**Seep**	**Reference**	**Seep**	**Reference**	**Seep**
Maximum shoot age (yr)	3.53	3.21	2.81	3.61	8.27	7.14
Present recruitment rate (yr^{-1})	0.72	0.77	0.49	1.06	0.36	0.64
Long-term recruitment rate (yr^{-1})	0.64 ± 0.09	0.70 ± 0.15	0.22 ± 0.14	0.99 ± 0.11	0.36 ± 0.05	0.63 ± 0.12
Net population growth (yr^{-1})	0.081	0.066	0.270	0.067	-0.001	0.013

386

Figure legends.

388

389 **Figure 1**. Study locations of CO_2 seeps and reference sites: A) Adamas and Paleochori bay

390 in Milos island (Greece), and B) Levante bay in Vulcano island (Italy).

391

392 **Figure 2.** *Cymodocea nodosa* total and apical shoot density (shoots m^{-2}, mean ± standard

393 error) at the reference and CO_2 seep sites at Adamas, Paleochori bay and Levante bay

394 locations. Summary of the 2-way ANOVA, showing the level of statistical significance (***p

395 < 0.001, **p < 0.01, *p < 0.05) of the location, site and their interactions. Uppercase letters

396 on bars represent post-hoc Tukey pairwise groupings that indicate differences when the effect

397 of location factor was significant.

398

399 **Figure 3.** *Cymodocea nodosa* vertical and horizontal rhizome elongation rate (cm yr^{-1}) and

400 production rates (g C m^{-2} yr^{-1}; mean ± standard error) at the reference and CO_2 seep sites at

401 locations Adamas, Paleochori bay and Levante bay. It was not possible to estimate the error

402 for the horizontal rhizome elongation rate (see Methods). Asterisks indicate the level of

403 statistical significance (***p < 0.001, **p < 0.01, *p < 0.05) of the site factor in the

404 ANCOVA analysis for the elongation rates, and of the site, location and site x location

405 interaction factor in the 2-way ANOVA for the production rates. Uppercase letters on bars

406 represent post hoc Tukey pairwise groupings that indicate differences when the effect of

407 location factor was significant.

408

409 **Figure 4.** Age frequency distribution of *Cymodocea nodosa* population along the reference

410 and CO_2 seep sites at Adamas (A, B), Paleochori bay (C, D) and Levante bay (E, F),

411 respectively. Solid line shows the fitted exponential decay line used to obtain the long-term

412 average recruitment rate (R).

413

414 **References**

415 Alexandre, A, Silva, J., Buapet, P., Bjork, R., and Santos, R., 2012. Effects of CO_2

416 enrichment on photosynthesis, growth and nitrogen metabolism of the seagrass *Zostera noltii*.

417 Eco. and Evol.10: 2620-2630

418 Apostolaki E.T., Vizzini, S., Hendriks, I.E., Olsen, Y.S., 2014. Seagrass ecosystem response

419 to long-term high CO_2 in a Mediterranean volcanic vent. Mar. Environ. Res. 99: 9–15

420 Arnold, T., Mealey, C., Leahey, H., Miller, A.W., Hall-Spencer, J.M., et al., 2012. Ocean

421 Acidification and the loss of phenolic substances in Marine Plants. PLoS ONE. 4: e35107

422 Bayraktarov, E., Price, R.E., Ferdelman, T.G., Finster, K., 2013. The pH and pCO_2

423 dependence of sulfate reduction in shallow-sea hydrothermal CO_2-venting sediments (Milos

424 Island, Greece). Frontiers in Micro. 4: 1-10.

425 Boatta, F., D'Alessandro, W., Gagliano, A.L., Liotta, M., et al., 2013. Geochemical survey of

426 Levante bay, Vulcano island (Italy), a natural laboratory for the study of ocean acidification.

427 Mar. Pollut. Bull. 73: 485-494

428 Borum, J., Pedersen, O., Kotula, L., Fraser, M.W., Statton, J., Colmer, T.D., Kendrick, G.A.,

429 2018. Photosynthetic response to globally increasing CO2 of co-occuring temperate seagrass

430 species. Plant Cell and Environ. 38: 1240-1250

431 Cabaço, S., Santos, R., Duarte, C.M., 2008. The impact of sediment burial and erosion

432 seagrasses: A review. Estu. Coast. and Shelf Sci.79: 354-366.

433 Campbell, J.E., Fourqurean, J.W., 2013. Effects of in situ CO_2 enrichment on the structural

434 and chemical characteristics of the seagrass *Thalassia testudinum*. Mar. Bio. 160: 1465-1475

435 Caldeira, K., and Wickett, M.E., 2003. Oceanography: anthropogenic carbon and ocean pH.

436 Nature. 425:365.

437 Campbell, J.E., Fourqurean, J.W., 2018. Does nutrient availability regulate seagrass response

438 to elevated CO_2? Ecosystems. 21: 1269-1282

439 Cancemi, G., Buia, M.C., Mazzella, L., 2002. Structure and growth dynamics of *Cymodocea*

440 *nodosa meadows*. Sci. Mar. 66: 365-373.

441 Capaccioni, B., Tassi, F., Vaselli, O., 2001. Organic and inorganic geochemistry of low

442 temperature gas discharges at the Baia di Levante beach, Vulcano Island, Italy. J. Volcanol.

443 Geoth. Res. 108: 173–185.

444 Collier, C. J., Prado, P., and Lavery, P. S., 2010. Carbon and nitrogen translocation in

445 response to shading of the seagrass Posidonia sinuosa. Aqu. Bot. 93: 47–54.

446 Collier, C.J., Langliois, L., Ow, Y., Johansson, C., Giammusso, M., Adams, M.P., O'Brien,

447 K.R., Uthicke, S., 2018. Losing a winnter: thermal stress and local pressures outweigh the

448 positive effects of ocean acidfication for tropical seagrass. New Phytologist. 219: 1005-1017

449 Cunha, A.H., Duarte, C.M., 2005. Population age structure and rhizome growth of

450 *Cymodocea nodosa* in the Ria Formosa (southern Portugal). Mar. Bio. 146: 841-847.

451 den Hartog, C., 1970. The Seagrasses of the World. North Holland Publication., Amsterdam,

452 275pp.

453 Dando, P. R., Stuben, D., & Varnavas, S. P., 1999. Hydrothermalism in the Mediterranean

454 Sea. Prog. in Oceano. 44: 333–367.

455 Dando, P., Aliani, S., Arab, H., Bianchi, C., Brehmer, M. et al., 2000. Hydrothermal studies

456 in the Aegean Sea. Phys. Chem. Earth B. Hydrol. Oceans Atmos. 25: 1-8.

457 Dekov, V.M., and Savelli, C., 2004. Hydrothermal activity in the SE Tyrrhenian Sea: an

458 overview of 30 years of research. Mar. Geo. 204:161-185

459   de los Santos, C.B., Godbold, J.A., Solan, M., 2017. Short-term growth and biomechanical

460   responses of the temperate seagrass *Cymodocea nodosa* to CO_2 enrichment. Mar. Ecol. Prog.

461   Ser. 572: 91-102

462   Dickson, A.G., Wolf-Gladrow, D. A., Zeebe, R.E., Klaas, C., Kortzinger, A., 2007a. Total

463   alkalinity: The explicit conservative expression and its application to biogeochemical

464   processes. Mar. Chem. 106: 287-300.

465   Dickson, A.G., Sabine, C.L., Christian, J.R., (Eds), 2007b.Guide to best practices for ocean

466   CO_2 measurements. PICES Special Publication, 3: 1-191.

467   Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for the

468   dissociation of carbonic acid in seawater media. Deep Sea Res. 34: 1733-1743.

469   Dickson, A.G., 2013. Certificate of Analysis. Reference Materials for oceanic CO_2

470   measurements. University of California, San Diego.

471   Doney, S.C., Fabry, V.J., Feely, R.A., Kleypass, J.A., 2009. Ocean acidification the other

472   CO_2 problem. Front. Mar. Sci. 5:169-194

473   Duarte, C.M., Marbà, N., Agawin, N., Cebrián, J., Enríquez, S. et al., 1994. Reconstruction of

474   seagrass dynamics: age determinations and associated tools for the seagrass ecologist. Mar.

475   Eco. Prog. Ser. 107: 195-209.

476   Duarte, C.M., Sand-Jensen, K., 1990. Seagrass colonization: biomass development and shoot

477   demography in *Cymodocea nodosa* patches. Mar. Eco. Prog. Ser. 67: 97-103.

478   Duarte, M. C., 1990. Seagrass nutrient content. Mar. Eco. Prog. Ser.   67: 201-207.

479   Enochs, I.C., Manzello, D.P., Donham, E.M., Kolodziej, G., Okano, R., et al., 2015. Shift

480   from coral to macroalgae dominance on a volcanically acidified reef. Nat. Clim. Change. 5:

481   1083–1088.

482   Fabricius, K.E., Langdon, C., Uthicke, S., Humphrey, C., Noonan, S., et al. 2011b. Losers

483   and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nat. Clim.

484   Change. 1: 165-169

485   Fabricius, K. E., Noonan, S., and Uthicke, S. 2014. Ecological effects of ocean acidification

486   and habitat complexity on reef-associated macroinvertebrate communities. Proceedings of the

487   Royal Society B: Biological Sciences, 281: 20132479

488   Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., et al., 2004. Impact of anthropogenic CO_2

489   on the $CaCO_3$ system in the oceans. Science 305:362–366

490   Fourqurean, W.J., Duarte, C.M., Marba. N., 2003. Elucidating seagrass population dynamics:

491   Theory, constraints and practice. Limnol. Oceano. 48: 2070-2074.

492   Gilhooly III, W.P., Fike, D.A., Druschel, G.K., Kafantaris, F-C., Price, R.E., Amend, J.P.,

493   2014. Sulfur and oxygen isotope insights into sulfur cycling in shallow-sea hydrothermal

494   vents, Milos, Greece. Geochemical Transactions. 15:1-19

495   Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., et al., 2008. Volcanic

496   carbon dioxide seeps show ecosystem effects of ocean acidification. Nature. 454: 96-99.

497   Hall-Spencer, J.M., Rodolfo-Metalpa, R.A.B., 2009. Using scientific diving to investigate the

498   long-term effects of ocean acidification at CO_2 seeps. Online Geosci. 22: 72-76

499   Horwitz, R., Borell, E.M., Yam, R., Shemesh, A., Fine, M., 2015. Natural high pCO2 in-

500   creases autotrophy in *Anemonia viridis* (Anthozoa) as revealed from stable isotope (C, N)

501   analysis. Sci. Rep. 5, 8779.

502   International Panel for Climate Change (IPCC). 2014. Fifth Assessment Report on Climate

503   change: Impacts, Adaptation and Vulnerability.

504   Invers, O., Zimmerman, R.C., Alberte, R.S., Perez, M., et al., 2001. Inorganic carbon sources

505   for seagrass photosynthesis: an experimental evaluation of bicarbonate use in species

506   inhabiting temperate waters. J. of Exp. Mar. Bio. Eco. 265: 203-217

507   Jiang, Z.J., Huang, X.P., Zhang, J.P., 2010. Effects of CO_2 enrichment on photosynthesis,

508   growth, and biochemical composition of seagrass *Thalassia hemprichii* (Ehrenb.) Aschers. J.

509   of Int. Plant Bio. 52: 904-913.

510   Kádár, E., Costa, V., Segonzac, M., 2007. Trophic influences of metal accumulation in

511   natural pollution laboratories at deep-sea hydrothermal seeps of the Mid- Atlantic Ridge. Sci.

512   Tot. Environ. 373: 464-472.

513   Khan, F., Alexandre, A., Ullah, H., Santos, R., 2016. Effects of elevated CO_2 and nutrients on

514   the community metabolism of a *Cymodocea nodosa* bed. Turk. J. of Bot. 40: 250-257

515   Kindeberg, T., Orberg, S.B., Rohr, M.E., Holmer, M., Krause-Jensen, D., 2018. Sediment

516   stocks of carbon, nitrogen and phosphorus in Danish eelgrass meadows. Front. Mar. Sci.

517   5:474

518   Koch, M., Bowes, G., Ross, C., Zhang, X., 2013. Climate change and ocean acidification

519   effects on seagrasses and marine macro algae. Glo. Change Bio. 19: 103-132

520   Kraemer, G.P., Mazzella, L., 1999. Nitrogen acquisition, storage, and use by the co-occurring

521   Mediterranean seagrasses *Cymodocea nodosa* and *Zostera noltii*. Mar. Eco. Prog. Ser. 183:

522   95-103.

523   Lauritano, C., Ruocco, M., Dattolo, E., Buia, M.C., et al., 2015. Response of key stress-

524   related genes of the seagrass *Posidonia oceanica* in the vicinity of submarine volcanic vents.

525   Biogeo. Discus. 12: 4947-4971

526   Lemasson, A.J., Fletcher, S., Hall-Spencer, J.M., Knights, A.M., 2017. Linking the biological

527   impacts of ocean acidification on oysters to changes in ecosystem services: A review. J. of

528   Expt. Mar. Bio. and Ecol. 492:49-62

529   Macreadie, P.I., Anton, A., Raven, J.A., Beaumont, N., Connolly, R.M., et al., 2019. The

530   future of Blue carbon science. Nature Communications. 10:1-13

531   Marbà, N., Duarte, C.M., 1998. Rhizome elongation and seagrass clonal growth. Mar. Eco.

532   Prog. Ser. 174: 269-280.

533   Mazzella, L. and Alberte, R.S.,1986. Light adaptation and the role of autotrophic epiphytes in

534   primary production of the temperate seagrass, *Zostera marina* (L). J. of Exp. Mar. Bio. Eco.

535   100: 165-180.

536   Mazarrasa, I., Samper-Villarreal, J., Serrano, O., Lavery, P. S., Lovelock, C. E., Marbà, N., et

537   al., 2018. Habitat characteristics provide insights of carbon storage in seagrass meadows.

538   Mar. Pollut. Bull. 134, 106–117. doi: 10.1016/j. marpolbul.2018.01.059

539   Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicz, R.M., 1973. Measurements of the

540   apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol.

541   Oceanogr. 18: 897-907.

542   Milazzo, M., Rodolfo-Metalpa, R., Chan, V.B.S., Fine, M. et al., 2014. Ocean acidification

543   impairs vermetid reef recruitment. Scienti. Rep. 4 (4189).

544   Milazzo M., Alessia C., Quattrocchi F., Chemello R., et al., 2019. Biogenic habitat shifts

545   under long-term ocean acidification show nonlinear community responses and unbalanced

546   functions of associated invertebrates. Sci. of the Total Env. 667: 41-48.

547   Mishra, A.K., Silva, J., Santos, R., 2018. Short term CO_2 enrichment increases carbon

548   sequestration of air-exposed intertidal communities of a coastal lagoon. Front. Mar. Sci.

549   4:439

550   Mishra, A.K., Santos, R., Hall-Spencer, J., 2019. Elevated trace elements in sediments and

551   seagrass at CO_2 seeps. Mar. Environ. Res. https://doi.org/10.1016/j.marenvres.2019.104810

552   Nogueira, P., Gambi, M.C., Vizzini, S., Califano, G., et al., 2017. Altered epiphyte

553   community and sea urchin diet in *Posidonia oceanica* meadows in the vicinity of submarine

554   volcanic CO_2 vents. Mar. Environ. Res. 127:102-111.

555   NOAA,2018. http://www.esrl.noaa.gov/gmd/ccgg/trends/global.html; Available from:

556   https://www.esrl.noaa.gov. Accessed August 29, 2018.

557   Olivé, I., Silva, J., Lauritano, C., Costa, M.M., et al., 2017. Short term responses of

558   seagrasses exposed to CO_2 in volcanic vents. Scientific Rep.7:42278

559   Ow. Y.X., Collier. C.J., Uthicke. S., 2015. Response of three tropical seagrass species to CO_2

560   enrichment. Mar. Bio. 162: 1005-1017.

561   Pajusalu, L., Martin, G., Põllumäe, A., Paalme, T., 2016. The influence of CO_2 enrichment on

562   Net Photosynthesis of seagrass *Zostera marina* in a brackish water environment. Front. Mar.

563   Sci.3:239

564   Palacios, S.L., Zimmerman, R.C., 2007. Response of eelgrass *Zostera marina* to CO_2

565   enrichment: possible impacts of climate change and potential for remediation of coastal

566   habitats. Mar. Eco. Prog. Ser. 344: 1-13

567   Pierrot, D. E. L., and Wallace, D. W. R., 2006. MS Excel Program Developed for CO_2

568   System Calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Centre,

569   Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN.

570   Price, R.E., Lesniewski, R., Nitzsche, K.S., Meyerdierks, A., Saltikov, C., Pichler, T.,

571   Amend, J.P., 2013. Archaeal and bacterial diversity in an arsenic-rich shallow-sea

572   hydrothermal system undergoing phase separation. Front. Microbiol. 4, 158.

573   Ravaglioli, C., Lauritano, C., Buia, M.C., Balestria, E., et al., 2017. Nutrient loading fosters

574   seagrass productivity under ocean acidification. Sci. Rep. 7: 13732

575   Romero, J., Lee, K.-S., Pérez, M., Mateo, M. A., and Alcoverro, T. (2006). "Nutrient

576   dynamics in seagrass ecosystems," *in Seagrasses: Biology, Ecology and Conservation,* eds A.

577   W. D. Larkum, R. J. Orth, and C. M. Duarte (Dordrecht: Springer), 227–254.

578   Russell, B.D., Connell, S.D., Uthicke, S., Muehllehner, N., et al., 2013. Future seagrass beds:

579   can increased productivity lead to increased carbon storage? Mar. Pollut. Bull. 73: 463-469

580   Schwarz, A.M., Bjork, M., Buluda, T., Mtolera, H., et al., 2000. Photosynthetic utilisation of

581   carbon and light by two tropical seagrass species as measured in situ. Mar. Biol. 137: 755–

582   761

583   Short, F.T., Coles, R.C., 2001. Global Seagrass Research Methods. Elsevier Science, B.V.,

584   Amsterdam, 473 pp.

585   Solomon S, Qin D, Manning, M, et al., 2007. Technical summary. In: Solomon S, Qin D,

586   Manning M, et al. (Eds). Climate change: the physical science basis. Contribution of

587   Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on

588   Climate Change. Cambridge, UK, and New York, NY: Cambridge University Press

589   Sunday, J.M., Fabricius, K.E., Kroeker, K.J., Anderson, K.M. et al., 2016. Ocean

590   acidification can mediate biodiversity shifts by changing biogenic habitat. Nat. Clim. Change.

591   7: 81-85

592   Takahashi. M., Noonan. S. H. C., Fabricius. K. E., Collier. C. J., 2015. The effects of long-

593   term in situ CO_2 enrichment on tropical seagrass communities at volcanic seeps. ICES J. of

594   Mar. Sci. 73: 876-886.

595   Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., et al., 1997. Macroalgal blooms in

596   shallow estuaries: References and Ecophysiological Ecosystem Consequences. Limnol. and

597   Oceano. 42: 1105-1118

598   Varnavas, S.P., Cronan, D.S., 2005. Submarine hydrothermal activity off Santorini and Milos

599   in the Central Hellenic Volcanic Arc: a synthesis. Chem. Geol. 224: 40-54.

600   Vizzini, S., Tomasello, A., Maida, G.D., Pirrotta, M., et al., 2010. Effects of shallow

601   hydrothermal seeps on the δ 13 C and growth performance in the seagrass *Posidonia*

602   *oceanica*. J. of Eco. 98: 1284-1291.

603 Vizzini et al. 2013 Trace element bias in the use of CO2 vents as analogues for low pH

604 environments: Implications for contamination levels in acidified oceans. Estuarine, Coastal

605 and Shelf Science 134 (2013) 19-30

606 Vizzini, S., Apostolaki, E.T., Ricevuto, E., Polymenakou, P., Mazzola, A., 2019. Plant and

607 sediment properties in seagrass meadows from two Mediterranean CO_2 vents: Implication for

608 carbon storage capacity of acidified oceans. Mar. Environ. Res. 146: 101-108

609 Welsh, D., Bourgues, S., de Wit, R., Auby, I., 1997. Effect of plant photosynthesis, carbon

610 sources and ammonium availability on nitrogen fixation rates in the rhizosphere of *Zostera*

611 *noltii*. Aq. Micro. Eco. 12:285-290

612 Zimmerman. R. C., Kohrs. D.G., Steller. D.L., Alberte. R.S., 1997. Impacts of CO_2

613 enrichment on Productivity and Light requirements of Eelgrass. Plant Physio. 115: 599-607.