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3 **Meiofauna communities, nematode diversity and C degradation rates in seagrass**
4 **(*Posidonia oceanica* L.) and unvegetated sediments invaded by the algae *Caulerpa***
5 ***cylindracea* (Sonder)**

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44 **Highlights**

- 45 • Effects of *Caulerpa cylindracea* were tested in seagrass and bare sediments.
- 46 • *C. cylindracea* significantly affected sedimentary organic C contents and turnover.
- 47 • Meiofauna varied significantly among invaded and not invaded bare sediments.
- 48 • Nematode α -diversity was not affected by the invasive algae.
- 49 • Nematode β -diversity was variably affected by the presence of the invasive algae.

50

27 **Abstract**

28 We investigated meiofauna and sedimentary C cycling in seagrass (*Posidonia oceanica*) and
29 unvegetated sediments invaded and not invaded by the non-indigenous tropical algae *Caulerpa*
30 *cylindracea*. In both habitats, invaded sediments were characterized by higher organic matter
31 contents. No effect was observed for prokaryotes and C degradation rates. In seagrass
32 sediments, C turnover in invaded beds was about half that in not invaded ones. Meiofaunal
33 communities varied significantly among invaded and not invaded grounds only in bare
34 sediments. In both habitats, nematode species richness and assemblage composition were not
35 affected by the algae. The effect of *C. cylindracea* on the turnover and nestedness components
36 of the Jaccard dissimilarity varied between the two habitats. We show that the presence of *C.*
37 *cylindracea* gives rise to variable consequences on meiofauna biodiversity and C cycling in
38 different habitats. We conclude that further studies across different habitats and ecological
39 components are needed to ultimately understand and predict the consequences of *C.*
40 *cylindracea* invasion in shallow Mediterranean ecosystems.

41

42 **Key words:** Non-indigenous invasive species, *Caulerpa cylindracea*, seagrass, meiofauna,
43 nematodes, marine biodiversity

44 **1. Introduction**

45 Biological invasions can have major effects on diversity, structure and functioning of marine
46 ecosystems (Stachowicz and Byrnes, 2006; Galil, 2007) and, consequently, on their ability to
47 provide goods and services to the humans (Pimentel et al., 2000; Bulleri et al., 2008; Thomsen
48 et al., 2011). However, results from both field and experimental studies on the role of invasions
49 on marine biodiversity are still contradictory (Fridley et al., 2007; Stachowicz et al., 2007
50 Tamburello et al., 2015). Some reviews have highlighted the occurrence of both positive and
51 negative interactions between invasive species and native communities (Wallentinus and
52 Nyberg, 2007; Rilov and Crooks, 2009). Other studies have reported either negligible or non-
53 negative effects of biological invasions on native marine biodiversity (Lonsdale, 1999;
54 Mckinney and Lockwood, 1999; Byrnes et al., 2007). The overall emerging picture is thus that
55 the effects of alien species on native marine assemblages might vary among recipient
56 assemblages characterized by different biotic and abiotic conditions (Levine and D'Antonio,
57 1999; Beisner et al., 2006; Ceccherelli and Sechi, 2002; Grosholz, 2002; Kennedy et al., 2002;
58 Arenas et al., 2006; Piazzzi and Balata, 2009; Bulleri et al., 2008). A conceivably common
59 conclusion of most studies is that the effects of biological invasions on marine biodiversity can
60 be context dependent, varying at different spatial and temporal scales (Ceccherelli and Campo,
61 2002; Bulleri et al., 2010; Heiman and Micheli, 2010). As a consequence, very few
62 generalizations can be reliably made and a better understanding of the effects of invasive
63 species is therefore urgently needed (Pyšek and Hulme, 2009).

64 The effects of algal species invasion on marine ecosystems have been so far assessed mostly
65 on macroalgae (e.g., Piazzzi et al., 2001; Thomsen et al., 2009; Piazzzi and Ceccherelli, 2006) or
66 macrofauna (e.g., McKinnon et al., 2009; Taylor et al., 2010; Lorenti et al., 2011; Gallucci et
67 al., 2012; Maggi et al., 2015), whereas information about the effects of invasive macroalgae on
68 smaller-size benthic organisms (i.e., meiofauna) is still rather limited (Yuhás et al., 2005; Reise

69 et al., 2006; Usio et al., 2006; Zhu et al., 2006; Chen et al., 2007; Olenin et al., 2010; Hänfling
70 et al., 2011; Cordell et al. 2013). Moreover, despite the fact that several studies have addressed
71 the effects of macroalgal invasions on the structure of native marine communities (Piazzi and
72 Balata, 2008, Piazzi and Balata, 2009), only very few have documented the effect of new
73 invaders on aquatic ecosystem functions (Reise et al., 2006; Usio et al. 2006; Zhu et al., 2006
74 and literature therein; Olenin et al., 2010; Eyre et al., 2011; Hänfling et al., 2011).

75 The Mediterranean Sea, hosting both temperate and subtropical species, is characterized
76 by very high biodiversity (Coll et al., 2010). In the last decades, local changes of climate
77 conditions (Rivetti et al., 2014) and increasing maritime transportations in the Mediterranean
78 Sea have facilitated the invasion by approximately 1000 non indigenous (alien) species (Mack
79 et al., 2000; Galil, 2007; Zenetos et al., 2012). Biological invasions in the Mediterranean, and
80 particularly the Lesspsian migrations, will further increase due to the doubling of the Suez
81 channel (Galil et al., 2014).

82 The introduced tropical algae *Caulerpa cylindracea* (Sonder) (Belton et al., 2014),
83 previously known as *C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman and
84 Boudouresque (Verlaque et al., 2003) is now widely distributed in the Mediterranean Sea
85 (Verlaque et al., 2000; Verlaque et al., 2004; Klein and Verlaque, 2008). The presence of this
86 species can alter abundance and diversity of several benthic assemblages and poses serious
87 threats to the status of the seagrass *Posidonia oceanica* and of rocky sub-tidal grounds
88 (Argyrou et al., 1999; Ceccherelli et al., 2002; Piazzi et al., 2001; Dumay et al., 2002; Cavas et
89 al., 2006; Raniello et al., 2007; Claudet and Fraschetti, 2010; Bulleri et al., 2011). Habitats
90 invaded by *C. cylindracea* can undergo a “biotic homogenization” (Piazzi and Balata, 2008;
91 Pacciardi et al., 2011). Human impacts in sub-tidal habitats can promote the distribution of this
92 invasive species (Piazzi et al., 2007; Bulleri et al., 2011), altering sedimentation processes
93 (Piazzi et al., 2007; Casu et al., 2009; Holmer et al., 2009), the structure of the benthic food

94 webs (Deudero et al., 2011) and the composition of the benthic assemblages (Pandolfo and
95 Chemello, 1995; Argyrou et al., 1999; Sandulli et al., 2004; Vazquez-Luis et al., 2009a, 2009b;
96 Box et al., 2010; Lorenti et al., 2011).

97 We hypothesized that the presence of *C. cylindracea* alters the patterns of meiofaunal and
98 nematode abundance, biomass and diversity differently across different habitats as a result of
99 the changes in benthic trophic conditions due to the additional inputs of primary organic matter
100 and detritus that this alga produces. To test such hypothesis, we investigated organic matter
101 quantity, biochemical composition and degradation rates, meiofaunal community composition
102 and free-living nematodes biodiversity (in terms of both species richness and equitability)
103 across two very common Mediterranean coastal habitats, the seagrass *P. oceanica* and soft-
104 bottom sediments, both invaded by and free of *C. cylindracea*. These two habitats have been
105 selected due to their different primary productivity, trophic conditions (Pusceddu et al., 1999;
106 Pusceddu et al., 2007).

107 We focused our investigation on nematodes, which are an abundant component of the
108 benthic assemblages, characterized by high species richness (Danovaro et al., 2001, Danovaro
109 et al., 2008). They play also a key ecological role in marine benthos by linking the detrital food
110 chain with the higher trophic levels, covering different food levels, from detritivorous to
111 grazers, from microbial feeders to omnivores, to predators; meiofauna are also food for
112 macrofauna and fish juveniles (Watzin, 1983; De Morais and Bodiou, 1984; Montagna, 1984;
113 Heip et al., 1985; Danovaro et al. 1995) and can increase bacterial denitrification (Bonaglia et
114 al., 2014).

115

116 **2. Methods**

117 *2.1. The non-indigenous species *Caulerpa cylindracea**

118 *Caulerpa cylindracea* (Sonder) (Belton et al., 2014) has been reported for the first time in the
119 Mediterranean Sea in 1926 and progressively spread over the Mediterranean Sea at water
120 depths from 0 to ca 70 m (Piazzi et al., 2005a) at a much faster rate than the congeneric
121 *Caulerpa taxifolia* (Verlaque et al., 2003). This difference is likely due to fact that *C.*
122 *cylindracea*, besides a remarkable capability of adjustment to the ecological factors such as
123 temperature, substratum and depth (light) (Verlaque et al., 2000), is supported also by sexual
124 reproduction (Panayotidis and Žuljevič, 2001), whereas *C. taxifolia* reproduces mostly or only
125 by fragmentation (Meinesz and Hesse, 1991; Meinesz et al., 1998; Klein and Verlaque, 2008).

126 The complex structure of the *C. cylindracea* thalli increases spatial heterogeneity
127 (Vázquez-Luis et al. 2008), so that cascading effects on abundance, biomass, and biodiversity
128 of small metazoan (meiofauna and particularly nematodes) and C degradation rates could be
129 expected.

130

131 2.2. Study area and sampling strategy

132 Sampling was carried out in September 2005 within the Marine Protected Area of Torre
133 Guaceto (MPA, Southern Adriatic Sea, Italy, 40°42'N; 17°48'E, Fig. 1). This MPA, established
134 in 2000, covers a surface of about 2.207 ha and extends off shore till 50 m depth. The “no-
135 entry no-take” zone of the MPA covers an area of 183 ha and is characterized by the presence
136 of both sandy and rocky substrata. The seagrass *P. oceanica* covers about 20% of the total
137 surface of the protected area and is interspersed among sandy patches and dead “matte” (i.e.,
138 seagrass dead rhizomes and roots, including interstitial sediment) covering the seafloor up to
139 17 m depth (Fraschetti et al., 2005).

140 Two, invaded and not invaded by *C. cylindracea*, putatively different habitats were
141 identified: *P. oceanica* seagrass meadow without *C. cylindracea*, *P. oceanica* with *C.*

142 *cylindracea*, unvegetated soft bottom sediments with and without *C. cylindracea*. For each of
143 the two habitats, presence and absence of *C. cylindracea* were selected. For each invaded and
144 not invaded habitats, two areas were randomly chosen at a distance of tens of meters and
145 within each area, two plots at a distance of few meters were randomly selected. Sampling was
146 carried out during the vegetative growth phase of *C. cylindracea* at ca. 6-9 m depth in two
147 different coastal habitats characterized by the presence (always over the 60% cover of the
148 plots) or the absence of the algae. From each plot (25x25 cm), six replicated sediment cores
149 were collected by scuba divers using plexiglass corers (4.7 cm internal diameter), of which
150 three dedicated to the analysis of sediment organic matter and prokaryotic variables, and three
151 to the analysis of meiofauna. For the determination of sediment grain size, samples were
152 collected with 25 cm-diameter stainless steel cores and stored at -20°C until analysis.

153 Samples were immediately brought to the laboratory for preservation and/or processing. The
154 cores were sliced into different sediment layers (0-1, 1-3, 3-5, 5-10 cm), preserved with
155 buffered 4% formaldehyde solution and stained with Rose Bengal (0.5 g L⁻¹) for meiofaunal
156 counts. For prokaryotic counts and the biochemical composition of organic matter the top 1 cm
157 layer of the replicate sediment cores were immediately frozen and stored at -20°C until
158 analysis.

159

160 2.3. Sediment characteristics

161 Grain size analyses were carried out by dry sieving of sediments through a 0.0625 mm mesh,
162 to distinguish between the sandy and the silt-clay fractions. Fraction retained on the filter
163 (sand) were additionally sieved through a 25 mm mesh to distinguish between medium (>0.25
164 mm) and fine (<0.25 and >0.0625 mm) sandy fractions. The sediment water content was

165 calculated as the difference between the wet and dry weights and expressed as percentages
166 (Dell'Anno et al. 2002).

167 Chlorophyll a and phaeopigments were analyzed fluorometrically after extraction with 90%
168 acetone (24 h in the dark at 4°C) and their sum defined as total phytopigments (Pusceddu et al.,
169 2009a). Phytopigment were converted into C equivalent using 40 as a conversion factor
170 (Pusceddu et al., 2009a). Protein, carbohydrate and lipid contents were determined
171 photometrically and expressed as bovine serum albumin, glucose and tripalmitine equivalents,
172 respectively (Pusceddu et al., 2009a). For each biochemical assay, blanks were obtained using
173 pre-combusted sediments (450°C for 4 h). All analyses were performed in three replicates on
174 about 1 g of wet sediment. Carbohydrate, protein, and lipid concentrations were converted into
175 carbon equivalents (using the conversion factors 0.40, 0.49, and 0.75 mg C mg⁻¹, respectively),
176 normalized to sediment dry weight (60°C, 24 h) and their sum reported as biopolymeric carbon
177 (BPC, Pusceddu et al., 2009a). The algal fraction of biopolymeric C (BPC) is defined as the
178 percentage ratio of phytopigment (once converted into C equivalents) and biopolymeric C
179 contents (Pusceddu et al., 2009a).

180

181 *2.4. Meiofaunal abundance and biomass*

182 Each sediment sample (and slice) was fixed with 4% buffered formaldehyde (in filtered
183 seawater solution) and, once in the laboratory, was sieved through 1000 µm (to retain
184 macrobenthos and macroalgae) and 32 µm (to retain smaller meiofauna) sieves. The sample
185 fraction retained by a 32 µm mesh net was added to Ludox HS 40 (density arranged to 1.18 g
186 cm⁻³), for density centrifugation extraction (10', 800 × g, for 3 times) from the sediment (Heip
187 et al., 1985). All metazoan animals, after staining with Rose Bengal (0.5 g L⁻¹), were counted
188 and classified per taxon under a stereomicroscope. Nematode abundance was calculated

189 integrating values from each of the sediment horizon down to 10-cm depth in the sediment, and
190 normalised to a surface of 10 cm². Nematode biomass was calculated from the biovolume,
191 which was estimated from all of the specimens using the Andrassy (1956) formula ($V = L \times$
192 $W^2 \times 0.063 \times 10^{-5}$, in which body length, L, and width, W, were expressed in μm). The
193 biovolume of all of the specimens of other taxa encountered were calculated using the formula
194 $V = L \times W^2 \times C$, where L and W were expressed in mm, and C is the approximate conversion
195 factor as reported for by Feller and Warwick (1988) for ostracods, kinorinchs, turbellarians,
196 gastrotrichs, tardigrades, polychaetes, oligochaetes, acarins, tanaidaceans, isopods, and
197 copepods. For all other taxa, we adopted the conversion factor of the taxon with the body shape
198 most similar to that of the unknown one. The total biovolume was multiplied by a mean density
199 of 1.13 g cm⁻³, to estimate the wet weight. The dry weight was calculated as 25% of the wet
200 weight (Wieser, 1960) and converted into carbon content, which was assumed to be 40% of the
201 dry weight (Feller and Warwick, 1988).

202

203 *2.5. Nematode biodiversity*

204 Nematodes were sorted and identified to genus or species using the pictorial keys of Platt and
205 Warwick (1983, 1988), and Warwick et al. (1998), implemented according to NeMys
206 (<http://nemys.ugent.be>). Species identity was not considered in this study but, for the purposes
207 of estimating species richness and other diversity indexes, the different morphotypes belonging
208 to each genus were indicated as sp1, sp2, sp3 and considered as separate species (De Mesel et
209 al., 2006). Species richness (SR) was calculated as the total number of species collected in each
210 replicate using the routine DIVERSE included in the PRIMER 6+ software (Plymouth Marine
211 Laboratory; Clarke, 1993).

212

213 2.6. *Prokaryotic biomass and production*

214 Prokaryote counts were carried out using epifluorescence microscopy after staining with SYBR
215 green I (Zeiss Axioplan; magnification, $\times 1000$) (Danovaro et al., 2010). A minimum of 10 to
216 20 microscope fields and at least 400 prokaryote cells were examined for each filter.
217 Prokaryote biovolume (as maximal length and width) was estimated using a micrometer ocular
218 assigning prokaryote cells to different size classes and then converted into carbon content
219 assuming $310 \text{ fg C } \mu\text{m}^{-3}$ (Palumbo et al., 1984).

220 Prokaryotic heterotrophic (C) production was measured by ^3H -leucine incorporation (Van
221 Duyl and Kop, 1994). Sediment sub-samples (200 μl), added to an aqueous solution of ^3H -
222 leucine (6- μCi final concentration per sample), were incubated for 1 h in the dark at *in situ*
223 temperature. After incubation, prokaryotic C incorporation was stopped with 1.7 ml of 80%
224 ethanol before scintillation counting. Sediment blanks were made adding ethanol immediately
225 after ^3H -leucine addition. Data were normalized to sediment dry weight after desiccation (60°,
226 24 h).

227

228 2.7. *Extracellular enzymatic activities, C degradation rates and turnover*

229 Extracellular enzymatic activities (aminopeptidase and β -glucosidase) in sediments were
230 determined by cleavage of artificial fluorogenic substrates (L-leucine-4-methylcoumarinyl- 7-
231 amide, Leu-MCA; 4-methylumbelliferone- β -D-glucopyranoside, Glu-MUF, respectively;
232 SIGMA) (Hoppe, 1993) at saturating concentrations (200 μM for Glu-MUF and Leu-MCA).
233 Incubations were performed in the dark at *in situ* temperature for 1 h (enzymatic activities
234 increased linearly with time up to 3 h). After incubation, supernatants were analyzed
235 fluorometrically (at 380 nm excitation, 440 nm emission for Leu-MCA and 365 nm excitation,
236 455 nm emission for Glu-MUF). Fluorescence was converted into nmol of hydrolyzed

237 substrate using calibration curves obtained from standard solutions of 7-amino-4-
238 methylcoumarin for Leu-MCA and of 4-methylumbelliferone for Glu-MUF. The amount of
239 hydrolyzed substrates was normalized to the incubation time and the sediment dry weight
240 (60°C, 24 h). Activities are hence reported as nmol of substrate released $\text{g}^{-1} \text{h}^{-1}$.
241 Aminopeptidase and β -glucosidase activities were converted into equivalents of C mobilized
242 assuming that 1 nmol of substrate hydrolyzed enzymatically corresponds to 72 ng of mobilized
243 C, and their sum reported as C degradation rates (Pusceddu et al., 2009b). The turnover of
244 biopolymeric C was calculated as the ratio of the hourly C degradation rates (per day once
245 multiplied by 24) and the biopolymeric C contents in the sediment (Pusceddu et al., 2014).

246

247 *2.8. Statistical analyses*

248 The experimental design consisted of four factors: 1) Habitat (Ha, fixed factor with 2 levels:
249 seagrass beds vs. bare sediments); 2) Caulerpa (Ca, fixed factor with two levels – present and
250 absent – orthogonal to Habitat); 3) Area (A, random factor with 2 levels nested in Ha and Ca);
251 and 4) Plot (P, random factor nested in Ha, Ca and A), with $n=3$ for each combination of
252 factors.

253 Variations in sediment grain size, chlorophyll-a, phaeopigment, protein, carbohydrate,
254 lipid and biopolymeric C sedimentary contents, prokaryote abundance and biomass;
255 heterotrophic C production and extracellular enzymatic activities were assessed by means of
256 univariate ANOVA, using the GMAV software (Underwood and Chapman, 1997).

257 The differences across the four conditions in the composition of meiofauna as a whole, of
258 nematode assemblages, and OM biochemical composition were investigated using
259 PERMANOVA (Anderson, 2001; McArdle and Anderson, 2001). The PERMANOVA
260 analyses were based on Euclidean distances of previously normalized data (OM) and Bray-

261 Curtis similarity triangular matrixes (faunal data), using 999 random permutations of the
262 appropriate units (Anderson and ter Braak, 2003). The pseudo-multivariate variance
263 components for each term in the model were calculated using direct multivariate analogues to
264 the uni-variate ANOVA estimators (e.g., Searle et al., 1992). For those PERMANOVA tests
265 providing significant differences for the different sets of variables, pairwise tests for the
266 relevant factors were also carried out, followed by Multidimensional Scaling (MDS) plots,
267 drawn using the same matrixes as for the PERMANOVA tests (see supplementary figures).
268 Moreover, CAP (canonical analysis of principal coordinates) plots were also obtained to
269 ascertain the allocation of experimental groups to those established *a priori*.

270 Since PERMANOVA is sensitive to differences in multivariate dispersion among groups,
271 we used also a test of homogeneity of dispersion (PERMDISP) to test the null hypothesis of
272 equal dispersions among groups. Since the PERMDISP analysis reveals no significant
273 differences in the multivariate dispersion of both meiofaunal and nematode assemblages across
274 scales, results are not reported.

275 PERMANOVA (followed by pairwise comparisons when appropriate), MDS, CAP, and
276 PERMDISP tests were carried out using Primer6+ software (Anderson et al., 2008).

277

278 2.9. Beta diversity

279 Beta diversity is generally defined as the ratio between gamma and mean alpha diversities
280 (Tuomisto, 2010). Since gamma diversity can differ from mean alpha diversity if, and only if,
281 local sites differ in species composition, this ratio represents the degree to which species
282 composition changes from site to site, i.e., beta diversity (Anderson et al., 2011). Differences in
283 species composition between two sites can be produced by: 1) the replacement of some species
284 by others from site to site (i.e. spatial turnover; Gaston and Blackburn, 2000); 2) nestedness, a

285 pattern characterized by the poorest site being a strict subset of the richest one (Baselga, 2010).
286 Beta diversity can be partitioned into two components: dissimilarity due to species replacement
287 and dissimilarity due to nestedness (nestedness-resultant dissimilarity). Nestedness occurs
288 when the biotas of sites with smaller numbers of species are subsets of the biotas at richer sites
289 (Ulrich and Gotelli, 2007), therefore reflecting a non-random process of species loss as a
290 consequence of any factor that promotes the disaggregation of assemblages (Gaston and
291 Blackburn, 2000).

292 In this study, beta diversity was assessed by Jaccard dissimilarity (Jaccard, 1912; Koleff et
293 al., 2003), and then decomposed into the turnover and nestedness resultant components
294 according to Baselga (2012), using the following equations:

295 1) Jaccard dissimilarity:

$$296 \quad \beta_{\text{jac}} = \frac{b+c}{a+b+c}$$

297 2) turnover component of the Jaccard dissimilarity:

$$298 \quad \beta_{\text{itu}} = \frac{2 \min(b,c)}{a+2 \min(b,c)}$$

299 3) nestedness resultant component of the Jaccard dissimilarity:

$$300 \quad \beta_{\text{jne}} = \frac{\max(b,c) - \min(b,c)}{a+b+c} \times \frac{a}{a+2 \min(b,c)}$$

301 where, a is the number of species present in both sites or sampling units, b is the number of
302 species present in the first site but not in the second, and c is the number of species present in
303 the second site but not in the first.

304

305 **3. Results**

306 *3.1. Sediment characteristics*

307 Data on sediment grain size and organic matter contents in the sediments of the investigated
308 habitats are reported in Table S1.

309 The sediments of the two investigated, invaded and not invaded by *C. cylindracea*,
310 habitats are characterized by a highly variable fine sand fraction varying from 29 ± 14 %
311 (seagrass beds without *C. cylindracea*, on average of all plots) to 69 ± 9 % (bare sediments
312 without *C. cylindracea*, on average of all plots).

313 ANOVA does not reveal significant differences in the percentage of fine sands among the
314 two habitats, nor any effect of the presence of the algae, but significant variations only among
315 plots within each area, presence/absence of the algae and habitat (Table 1). In both bare and
316 seagrass sediments, protein, carbohydrate and biopolymeric C contents were affected by the
317 presence of *C. cylindracea* (Table 1), with highest values consistently observed in invaded
318 sediments (Fig. 2a-c), whereas lipid contents varied among the two habitats, but were not
319 affected by the presence of the algae (Table 1). In both habitats, the presence of *C. cylindracea*
320 resulted in values of the algal fraction of biopolymeric carbon in the sediments significantly
321 higher than those in not invaded grounds (Fig. 2d).

322 The biochemical composition of sedimentary organic matter was significantly affected
323 by the presence of the algae (Table 1; Fig. 3; Fig. S1), with a large variability also explained at
324 the scale of plot. The pairwise comparisons reveal the presence of a significant effect of the
325 invading algae on the biochemical composition of sediment organic matter in both habitats
326 (bare sediments: $t = 4.157$, $p < 0.001$; seagrass sediments: $t = 3.427$, $p < 0.001$).

327

328 *3.2. Prokaryotic abundance, biomass, production, enzymatic activities and C turnover*

329 Data on prokaryotic abundance, biomass and C production and extracellular enzymatic
330 activities are reported in Table S2.

331 The results of the ANOVAs reveal that the presence of the algae does not alter
332 significantly the microbial variables, but significant differences in prokaryotic C production,
333 aminopeptidase activity and C degradation rates were observed among the two habitats (Table
334 2), with values in seagrass sediments significantly higher than those in bare grounds (Fig. 4a-
335 c). In seagrass sediments, biopolymeric C daily turnover mediated by extracellular enzymatic
336 activities was about half than in not invaded beds, whereas in bare sediments this effect was
337 much less evident (Fig. 4d).

338

339 3.3. Meiofaunal abundance, biomass, and community composition

340 Abundance data for each meiofaunal taxa, and for total meiofaunal abundance, biomass and
341 richness of taxa are reported in Table S3 and Table S4, respectively. Total meiofaunal
342 abundance and biomass did not vary between habitats nor between invaded and not invaded
343 sediments of both habitats (data not shown).

344 Overall, meiofauna was dominated by Nematoda representing the 55-97% of the total
345 abundance (Fig. 5a), followed by Copepoda (2-33%), Polychaeta (0.2-13%), Tardigrada (0.1-
346 4.4%) and thirteen other taxa (cumulatively 1-10%) (Fig. 5b).

347 The results of the PERMANOVA reveal a significant effect of the Habitat \times *Caulerpa*
348 interaction on the composition of the whole meiofaunal communities (Table 3a). A-posteriori
349 pairwise comparisons, corroborated by the MDS plot (Fig. S2), indicate that the effect of the
350 invasive algae on the composition of meiofaunal communities is significant only in bare
351 sediments (PERMANOVA $t=2.073$; $p<0.05$), where the presence of the algae is characterized
352 by an increased contribution of oligochaetes (Fig. 6).

353

354 3.4. Nematode biodiversity

355 The results of univariate ANOVAs reveal that nematode species richness varies significantly
356 only among plots in each area, presence/absence of the algae and habitat (Table S5).

357 The PERMANOVA results, corroborated by the MDS plot (Fig. S3), reveal that the
358 composition of the nematode assemblages does not vary between habitats, presence of
359 *Caulerpa* and areas within each habitat, but are highly variable among plots (Table 3b).

360 The highest values of the Jaccard dissimilarity are observed among plots (on average 0.62,
361 range 0.59-0.65), and the lowest between invaded and not invaded sediments of both habitats
362 (0.52 and 0.48 in bare and seagrass sediments, respectively). In both habitats the turnover
363 component (species replacement) of Jaccard dissimilarity between plots in invaded sediments
364 is higher than that in not invaded grounds, whereas the presence of *C. cylindracea* is associated
365 with lower values of turnover diversity among areas (Fig. 7a-b). The turnover diversity
366 between invaded and not invaded bare sediments is higher than that in seagrass sediments (Fig.
367 7c). The nestedness component of the Jaccard dissimilarity is much lower than the turnover
368 one at all the investigated scales, with highest values between invaded and not invaded
369 sediments of both habitats (0.06 and 0.11 in bare and seagrass sediments respectively), and
370 lowest values between areas of not invaded sediments of both habitats (0.006 and 0.004 in bare
371 and seagrass sediments, respectively). In both habitats, the nestedness component of the
372 Jaccard dissimilarity between plots of invaded sediments is lower than that in not invaded
373 sediments (Fig. 7d), whereas nestedness between areas is higher in invaded sediments (Fig.
374 7e). The nestedness component of Jaccard dissimilarity between invaded and not invaded
375 sediments in seagrass sediments is higher than that in bare sediments (Fig. 7f).

376

377 **4. Discussion**

378 Our results show that the presence of *C. cylindracea* in the Marine Protected Area “Torre
379 Guaceto” was associated with a significant accumulation of organic matter in surface
380 sediments of both seagrass and bare (unvegetated) sediments. Such accumulation, although
381 significant, did not result in the eutrophication of the investigated habitat (i.e., biopolymeric C
382 contents always $<2.5 \text{ mg C g}^{-1}$; *sensu* Pusceddu et al., 2009a). In all investigated sediments the
383 fresh micro-algal contribution (determined as chlorophyll-a content of the sediments) to the
384 biopolymeric C pool was typically $<5\%$. This finding, along with the relatively high
385 concentrations of phaeopigments, suggests the prevalence of detrital (i.e., non-living) and
386 heterotrophic (i.e., non-algal) composition of the sedimentary organic pools in the investigated
387 sediments. However, both habitats, when invaded by *C. cylindracea*, displayed a higher micro-
388 algal (total phytopigment) contribution to the sedimentary C pools (Fig. 2d), indicating the
389 presence of a larger fraction of organic matter bioavailable for benthic consumers. We would
390 conclude that the presence of *C. cylindracea* in both seagrass and unvegetated sediments
391 resulted in an increased availability of fresh primary organic substrates available for benthic
392 heterotrophic metabolism, with potentially positive effects for the benthos inhabiting sediments
393 invaded by this macroalgae. However, seagrass sediments colonized by *C. cylindracea* were
394 characterized by significantly lower C turnover rates than those observed in sediments without
395 the macroalgae. Our results show that the increased availability of organic substrates in
396 sediments invaded by *C. cylindracea* does not stimulate microbial abundance and activity,
397 conversely to what expected. Since in both habitats organic loads in invaded sediments are
398 much higher than those in grounds free of the algae, C turnover rates are lower in presence of
399 *C. cylindracea*. This result indicates that the biogeochemical processes are affected by the
400 presence of this invasive algae. This effect is more evident in seagrass than in bare sediments,

401 likely as a result of their more complex architectural structure, which makes these systems
402 more vulnerable to the effects of *C. cylindracea* than bare sediments.

403 Taken as a whole, the abundance and biomass of meiofauna in sediments of both habitats
404 invaded by *C. cylindracea* did not change significantly from those without the macroalgae.
405 These results are in contrast with those reporting a positive effect of the presence of *C.*
406 *cylindracea* on total meiofaunal abundance (Carriglio et al., 2003), and let us to hypothesize
407 that the organic pools derived from *C. cylindracea* biomass could be not easily exploited as a
408 primary resource by meiofauna. However, the presence of *C. cylindracea* in unvegetated
409 sediments was associated with a meiofaunal community significantly different from that
410 observed in vegetated sediments invaded by *C. cylindracea*, in particular because of an
411 increased abundance of oligochaetes (Fig. 6), and the appearance of isopods, gastrotrichs and
412 kinorinchs, otherwise absent in not invaded sediments (Table S3). These taxa, once pooled
413 together, represented on average less than 1.0% of the total meiofaunal abundance. In
414 accordance with Vazquez-Luis et al. (2009a) we also observed that *C. cylindracea* stands
415 supported a higher abundance of amphipods (Table S3). Even though these results have to be
416 substantiated with additional information collected in larger space and longer time, it can be
417 inferred that this invasive macroalgae can influence in particular rare meiofaunal taxa (*sensu*
418 Bianchelli et al., 2010), possibly as a result of changes in the (spatial/trophic) resources offered
419 by the macroalgae itself.

420 Our initial hypothesis was that the presence of *C. cylindracea* is able to modify the local
421 structural complexity, having measurable effects on nematode biodiversity. Contrarily to
422 previous studies dealing with other (macro)benthic components (e.g., Argyrou et al., 1999), we
423 found that in both habitats *C. cylindracea* had not effects on nematodes α -diversity (i.e.,
424 nematode species richness). In addition, *C. cylindracea* did not have any effect on the levels of
425 multivariate dispersion of meiofaunal and nematode communities in both habitats. This result,

426 corroborated for the nematodes by values of total Jaccard dissimilarity between invaded and
427 not invaded sediments, suggests that this algae can have only a minor, if any, role on the spatial
428 organization of meiofaunal and nematode communities. However, when the Jaccard
429 dissimilarity in nematode assemblages is disentangled into the turnover (species replacement)
430 and nestedness resultant (species loss) components, our results show (Fig. 7) that, in both
431 habitats, in presence of *C. cylindracea* the compositional turnover between plots is higher than
432 in not invaded sediments. On the other hand, in both habitats the values of the nestedness
433 resultant component of Jaccard dissimilarity between areas in invaded sediments are higher
434 than in not invaded areas, whereas the opposite is observed when contrasting plots. These
435 results suggest that the effects of this algae on species replacement is evident at the smallest
436 spatial scale, whereas its effects on species loss is better identified at the largest spatial scale
437 (i.e. area).

438 When comparing the two habitats, irrespectively of variations at the scales of area and
439 plot, the presence of *C. cylindracea* determines and increased compositional heterogeneity,
440 more specifically determining a larger species replacement between invaded and not invaded
441 bare sediments, but a higher rate of species loss between invaded and not invaded seagrass
442 sediments.

443 Overall, our results could be due to the fact that the habitat complexity provided by the
444 seagrass *Posidonia oceanica*, that is a habitat former, could interact with the effects of the
445 macroalgae invasion on the meiofauna. In this regard, previous investigations have shown that
446 the spreading of *C. cylindracea* within *P. oceanica* meadows is favored at the edge of the
447 meadow where the density of the seagrass shoots is low and sand grounds are sufficiently vast
448 (Ceccherelli et al., 2000), indicating that seagrass meadows could, to a certain extent,
449 counteract the potential consequences of the invasion by this macroalgae. Most recently, it has
450 been also demonstrated that *C. cylindracea* growth within seagrass beds can be limited by the

451 shading determined by the meadow (Marín-Guirao et al., 2015). However, although our results
452 overall suggest variable effects of the presence of *C. cylindracea* on meiofauna and nematode
453 assemblages, the compositional turnover between invaded and not invaded grounds in seagrass
454 sediments are relatively larger than those in unvegetated sediments, indicating that the former
455 habitat is potentially more vulnerable than the latter one.

456 Our results thus confirm that the effects of invasive species on local assemblages might
457 vary idiosyncratically among different benthic components, and, wherever documented, could
458 be context dependent, varying also at different spatial scales (Ceccherelli and Campo, 2002;
459 Ceccherelli and Sechi, 2002; Grosholz, 2002; Kennedy et al., 2002; Pyšek and Hulme, 2009;
460 Bulleri et al., 2010; Piazzini et al., 2005b; Arenas et al., 2006; Beisner et al., 2006; Bulleri et al.,
461 2008; Heiman and Micheli, 2010). Results presented here indicate that the presence of *C.*
462 *cylindracea* could exert consistent effects on most of the investigated variables, with exception
463 of C turnover and nematode species turnover and loss rates (Table 4), and suggest that the so-
464 far documented negative effects of this invasive algae on Mediterranean macroalgal
465 communities could be context dependent on heterotrophic communities.

466

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472

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801 **Figures' caption**

802 **Fig. 1** Study area.

803 **Fig. 2** Protein (a), carbohydrate (b), biopolymeric C (c) sedimentary contents and values of the
804 algal fraction of BPC (d) in bare (unvegetated) and seagrass sediments invaded and not
805 invaded by *C. cylindracea*. Error bars indicate standard errors.

806 **Fig. 3** CAP plot illustrating differences in the biochemical composition of sedimentary organic
807 matter between unvegetated and seagrass sediments invaded and not invaded by *C.*
808 *cylindracea*.

809 **Fig. 4** Prokaryotic C production (a), aminopeptidase activity (b) and C degradation rates (c), in
810 bare and seagrass sediments, and C turnover rates in bare (unvegetated) and seagrass
811 sediments invaded and not invaded by *C. cylindracea*. Error bars indicate standard
812 errors.

813 **Fig. 5** Composition of meiofaunal communities in in bare (unvegetated) and seagrass
814 sediments invaded and not invaded by *C. cylindracea*: a) all meiofaunal taxa, b) all
815 taxa but Nematoda and Copepoda.

816 **Fig. 6** CAP plot illustrating differences in the composition of meiofaunal communities in bare
817 (unvegetated) and seagrass sediments invaded and not invaded by *C. cylindracea*.

818 **Fig. 7.** Turnover (a, b, c) and nestedness resultant (d, e, f) components of Jaccard dissimilarity
819 of nematode assemblages between plots, areas and invaded and not invaded sediments.

830 **Table 1** Results of the univariate and multivariate permutational ANOVAs ascertaining
 831 variations in the quantity and composition of sedimentary organic matter among levels of the
 832 experimental factors: Ha = habitat (seagrass vs. unvegetated sediments); Ca = Caulerpa (present
 833 vs. absent); Ar = area (nested in Ha and Ca); Pl = plot (nested in Ha, Ca and Ar). df = degrees
 834 of freedom; MS = mean square; F = statistic F. P= probability level; ns = not significant; * =
 835 $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Reported are also the results of the Cochran's test and the
 836 eventual data transformation.
 837

Source	Df	Find sands (%)			Chlorophyll-a			Phaeopigments		
		MS	F	P	MS	F	P	MS	F	P
Ha	1	6503.27	1.9	ns	5.62	13.32	*	4.46	5.58	ns
Ca	1	315	0.09	ns	11.17	26.47	**	13.41	16.75	*
Ar(HaXCa)	4	3423.21	1.9	ns	0.42	0.4	ns	0.8	1.17	ns
Pl(HaXCaXAr)	8	1804.26	55.12	***	1.06	8.51	***	0.69	12.35	***
HaXCa	1	3309.06	0.97	ns	0.1	0.25	ns	0.2	0.25	ns
Residual	32	32.73			0.12			0.06		
Cochran's test		0.18			0.3			0.2		
Transformation		None			ln(x)			ln(x)		

Source	df	Proteins			Carbohydrates			Lipids		
		MS	F	P	MS	F	P	MS	F	P
Ha	1	0	0.15	ns	0.02	2.34	ns	0.07	46.55	**
Ca	1	0.11	22.26	**	0.15	18.63	*	0.01	7.45	ns
Ar(HaXCa)	4	0.01	0.65	ns	0.01	1.51	ns	0	0.31	ns
Pl(HaXCaXAr)	8	0.01	3.93	**	0.01	4.6	***	0	25.03	***
HaXCa	1	0	0.02	ns	0.02	2.96	ns	0	0.09	ns
Residual	32	0			0			0		
Cochran's test		0.23			0.3			0.3		
Transformation		None			ln(x+1)			None		

Source	df	Biopolymeric C			Biochemical composition		
		MS	F	P	MS	F	P
Ha	1	0.22	15.98	*	36.96	5.27	*
Ca	1	0.83	60.47	**	69.98	9.98	*
Ar(HaXCa)	4	0.01	0.31	ns	7.57	1.08	ns
Pl(HaXCaXAr)	8	0.04	6.89	***	7.01	0.851	ns
HaXCa	1	0.05	3.48	ns	8.24	9.94	***
Residual	32	0.01			0.829		
Cochran's test		0.25					
Transformation		None					

838
839

840 **Table 2.** Results of the univariate ANOVAs ascertaining variations in prokaryotic abundance, biomass and production, extracellular enzymatic
 841 activities, and C degradation rates among levels of the experimental factors: Ha = habitat (seagrass vs. unvegetated sediments); Ca = Caulerpa (present
 842 vs. absent); Ar = area (nested in Ha and Ca); Pl = plot (nested in Ha, Ca and Ar). df = degrees of freedom; MS = mean square; F = statistic F. P=
 843 probability level; ns = not significant; * = p<0.05; *** = p<0.001. Reported are also the results of the Cochran's C test and the eventual data
 844 transformation.
 845

Prokaryotic abundance						Prokaryotic biomass				Prokaryotic C production			
Source	df	MS	F	P	F versus	MS	F	P	F versus	MS	F	P	F versus
Ha	1	9.35	6.19	ns	Ar(HaXCa)	10.57	6.22	ns	Ar(HaXCa)	65255.45	10.99	*	Ar(HaXCa)
Ca	1	1.17	0.78	ns	Ar(HaXCa)	1.51	0.89	ns	Ar(HaXCa)	7289.07	1.23	ns	Ar(HaXCa)
Ar(HaXCa)	4	1.51	0.86	ns	Pl(HaXCaxAr)	1.7	0.98	ns	Pl(HaXCaxAr)	5935.86	0.56	ns	Pl(HaXCaxAr)
Pl(HaXCaxAr)	8	1.77	25.4	***	RES	1.74	34.33	***	RES	10587.78	5.01	***	RES
HaXCa	1	0.27	0.18	ns	Ar(HaXCa)	0.63	0.37	ns	Ar(HaXCa)	34239.48	5.77	ns	Ar(HaXCa)
Residual	32	0.07				0.05				2112.35			
Cochran's test	0.30					0.14				0.29			
Transformation	None					ln(x)				None			

Beta-glucosidase						Aminopeptidase				C degradation rate			
Source	df	MS	F	P	F versus	MS	F	P	F versus	MS	F	P	F versus
Ha	1	1.6	11.18	*	Ar(HaXCa)	612.77	2.59	ns	Ar(HaXCa)	1.47	9.14	*	Ar(HaXCa)
Ca	1	0.21	1.44	ns	Ar(HaXCa)	170.74	0.72	ns	Ar(HaXCa)	0.12	0.75	ns	Ar(HaXCa)
Ar(HaXCa)	4	0.14	0.18	ns	Pl(HaXCaxAr)	236.67	1.19	ns	Pl(HaXCaxAr)	0.16	0.32	ns	Pl(HaXCaxAr)
Pl(HaXCaxAr)	8	0.78	7.92	***	RES	198.92	7.53	***	RES	0.5	6.34	***	RES
HaXCa	1	2.85	19.94	*	Ar(HaXCa)	31.8	0.13	ns	Ar(HaXCa)	2.24	13.91	*	Ar(HaXCa)
Residual	32	0.1				26.41				0.08			
Cochran's test	0.23	0.27				0.33				0.19			
Transformation	None	ln(x)				None				ln(x)			

847 **Table 3.** Results of the PERMANOVAs ascertaining variations in the composition of the whole
 848 meiofaunal communities (a) and nematode assemblages (b) among levels of the experimental
 849 factors: Ha = habitat (seagrass vs. unvegetated sediments); Ca = Caulerpa (present vs. absent);
 850 Ar = area (nested in Ha and Ca); Pl = plot (nested in Ha, Ca and Ar). df = degrees of freedom;
 851 MS = mean square; F = statistic F. P(perm)= probability level; ns = not significant; * = $p < 0.05$;
 852 *** = $p < 0.001$.

853

a) Whole meiofaunal community					
Source	df	MS	Pseudo-F	P(MC)	% of explained variance
Ha	1	2924	6.791	**	21
Ca	1	1479	3.434	**	9
HaxCa	1	1360	3.159	**	16
Ar(HaxCa)	4	431	0.679	ns	0
Pl(Ar(HaxCa))	8	635	7.812	***	38
Residual	32	81			17

b) Nematode assemblages					
Source	df	MS	Pseudo-F	P(MC)	% of explained variance
Ha	1	9886	3.040	**	12
Ca	1	4414	1.357	ns	2
HaxCa	1	3537	1.088	ns	1
Ar(HaxCa)	4	3252	1.199	ns	4
Pl(Ar(HaxCa))	8	2712	2.044	***	21
Residual	32	1327			60

854

849 **Table 4.** Synopsis of the effects of the invasive algae *Caulerpa cylindracea* on sedimentary
 850 features, prokaryote, meiofauna and nematode variables in seagrass and bare sediments. + =
 851 positive effect on univariate variables (increase); - = negative effect on univariate variables
 852 (decrease); o = significant effect on multivariate sets of variables, ns = not significant effects.

853

Typology	Variable	Seagrass sediments	Bare sediments
Sediments	Sediment grain size	ns	ns
	Total phytopigment	+	+
	Protein	+	+
	Carbohydrate	+	+
	Lipid	ns	ns
	Biopolymeric C	+	+
	Algal fraction of organic C	+	+
	Biochemical composition	o	o
Prokaryotes	Abundance	ns	ns
	Biomass	ns	ns
	C production	ns	ns
	Enzymatic activities	ns	ns
	C turnover	-	ns
Meiofauna	Abundance	ns	ns
	Biomass	ns	ns
	Community composition	ns	o
Nematodes	Species richness	ns	ns
	Assemblage composition	ns	ns
	Species replacement between plots	+	+
	Species replacement between areas	-	-
	Species loss between plots	-	-
	Species loss between areas	+	+

854

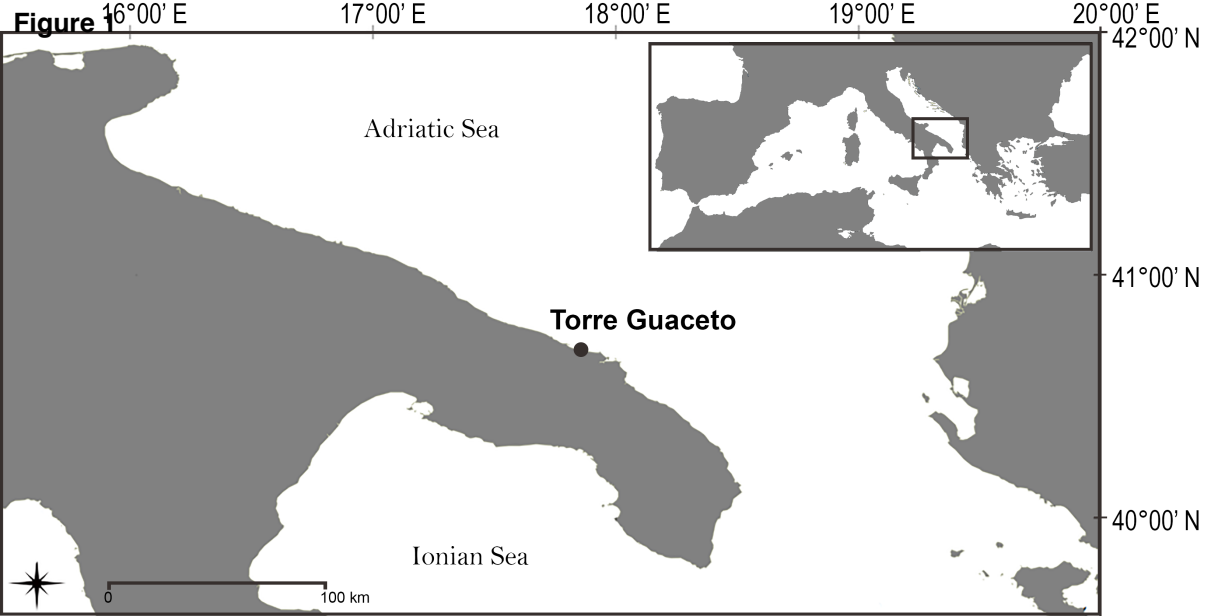


Figure 2

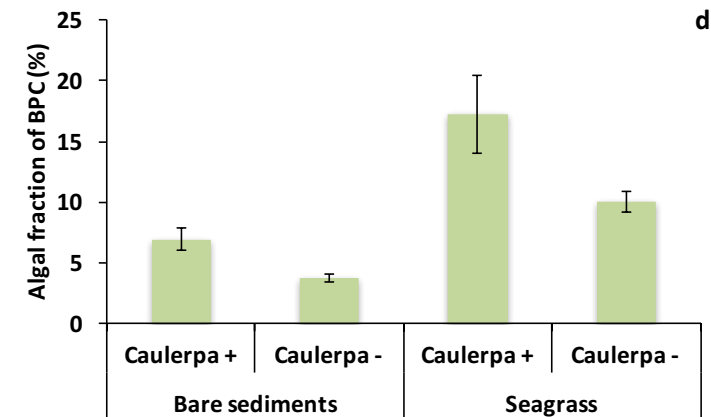
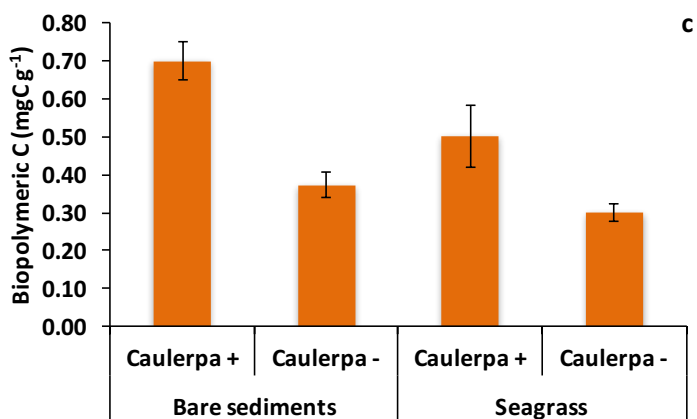
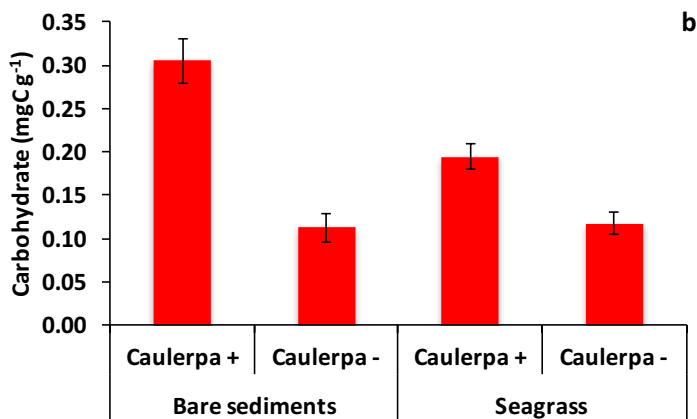
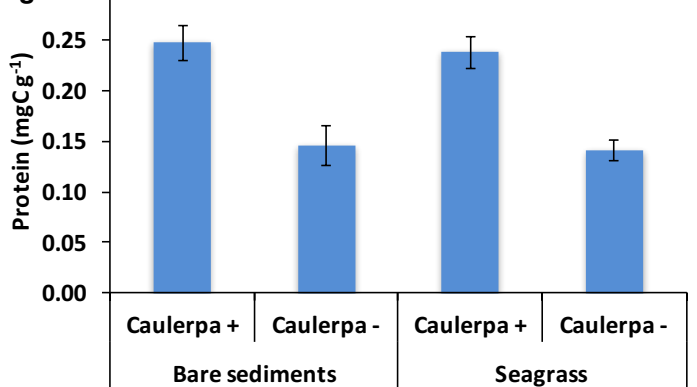
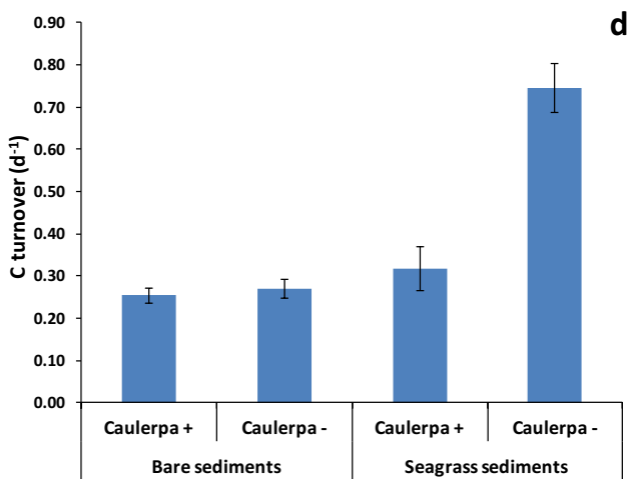
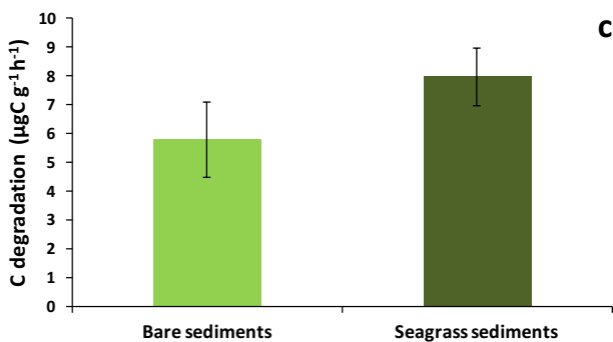
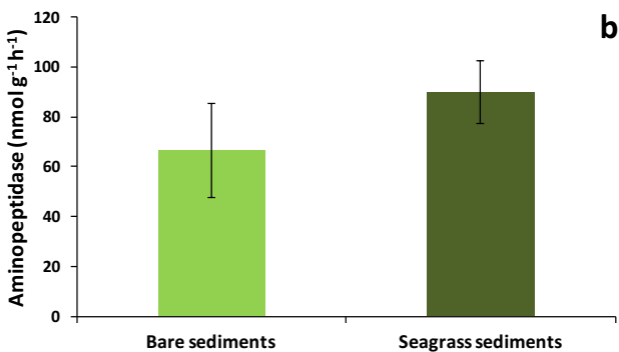
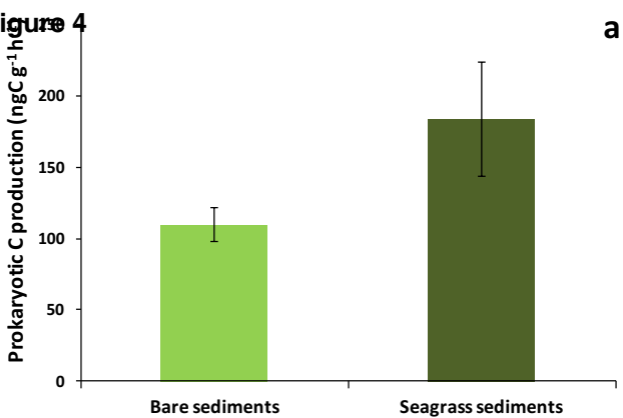
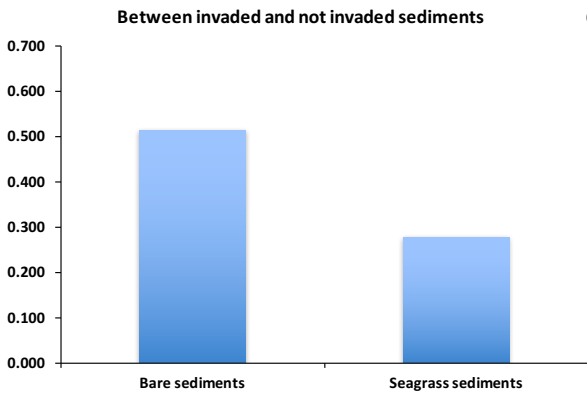
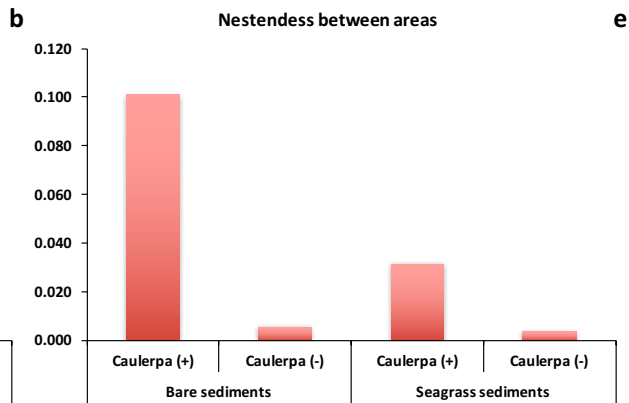
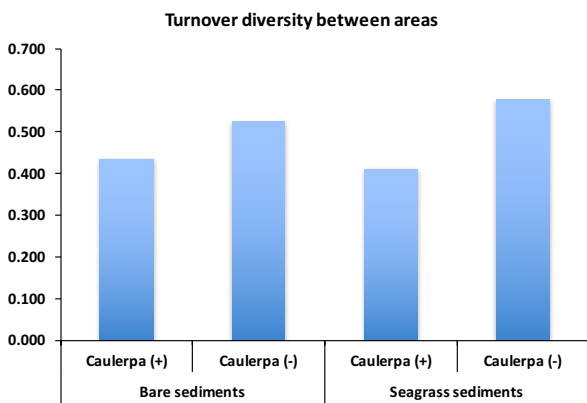
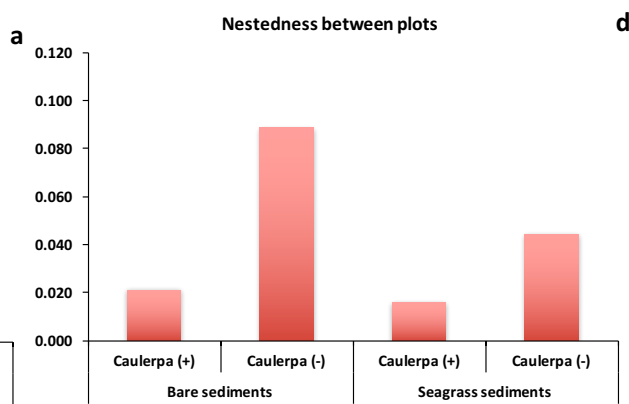
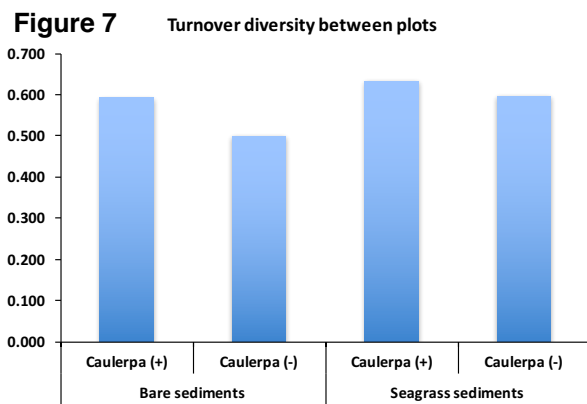


Figure 4



Pusceddu et al. - Meiofauna communities, nematode diversity and C degradation rates in seagrass (*Posidonia oceanica* L.) and unvegetated sediments invaded by the algae *Caulerpa cylindracea* (Sonder)

1 **Table S1.** Fine sand fraction (%) and organic matter content in the investigated sediments.

Habitat	Area	Plot	Fine sand %	Chlorophyll-a $\mu\text{g g}^{-1}$	Phaeopigment $\mu\text{g g}^{-1}$	Protein mgC g^{-1}	Carbohydrate mgC g^{-1}	Lipid mgC g^{-1}	Biopolymeric C mgC g^{-1}
Bare sediments with <i>Caulerpa</i>	1	1	52 ± 4	1.94 ± 0.01	22.25 ± 8.82	0.30 ± 0.07	0.23 ± 0.06	0.11 ± 0.00	0.65 ± 0.14
		2	82 ± 9	1.07 ± 0.18	13.50 ± 3.48	0.22 ± 0.02	0.31 ± 0.07	0.15 ± 0.03	0.68 ± 0.12
	2	1	13 ± 1	3.30 ± 0.91	16.00 ± 6.27	0.28 ± 0.02	0.40 ± 0.11	0.23 ± 0.03	0.91 ± 0.16
		2	43 ± 5	1.02 ± 0.22	4.28 ± 0.36	0.18 ± 0.03	0.27 ± 0.04	0.10 ± 0.02	0.55 ± 0.04
Bare sediments without <i>Caulerpa</i>	1	1	77 ± 10	0.59 ± 0.23	3.08 ± 0.08	0.21 ± 0.08	0.09 ± 0.01	0.13 ± 0.00	0.43 ± 0.09
		2	83 ± 9	0.81 ± 0.38	4.89 ± 0.50	0.14 ± 0.02	0.07 ± 0.01	0.12 ± 0.01	0.32 ± 0.04
	2	1	43 ± 3	1.42 ± 0.81	3.17 ± 0.61	0.17 ± 0.06	0.19 ± 0.01	0.12 ± 0.00	0.48 ± 0.07
		2	74 ± 5	0.49 ± 0.10	3.31 ± 0.57	0.07 ± 0.02	0.11 ± 0.05	0.08 ± 0.02	0.26 ± 0.09
Seagrass with <i>Caulerpa</i>	1	1	90 ± 9	2.58 ± 1.49	14.34 ± 1.80	0.26 ± 0.03	0.17 ± 0.01	0.04 ± 0.00	0.47 ± 0.04
		2	14 ± 1	11.35 ± 0.89	51.56 ± 15.71	0.27 ± 0.09	0.21 ± 0.05	0.10 ± 0.00	0.57 ± 0.04
	2	1	37 ± 4	2.45 ± 0.56	13.22 ± 3.34	0.18 ± 0.05	0.15 ± 0.02	0.08 ± 0.02	0.41 ± 0.09
		2	22 ± 3	2.64 ± 0.84	13.88 ± 4.09	0.24 ± 0.02	0.26 ± 0.01	0.06 ± 0.00	0.55 ± 0.03
Seagrass without <i>Caulerpa</i>	1	1	37 ± 5	1.13 ± 0.04	7.28 ± 1.65	0.14 ± 0.04	0.15 ± 0.01	0.05 ± 0.00	0.34 ± 0.05
		2	66 ± 8	1.32 ± 0.08	8.71 ± 0.63	0.17 ± 0.02	0.16 ± 0.03	0.04 ± 0.00	0.37 ± 0.06
	2	1	4 ± 1	1.26 ± 0.06	7.61 ± 0.35	0.10 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.19 ± 0.01
		2	10 ± 1	1.16 ± 1.16	6.44 ± 1.31	0.16 ± 0.02	0.10 ± 0.02	0.04 ± 0.00	0.30 ± 0.04

2

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3 **Table S2.** Prokaryotic abundance, biomass and C production and extracellular enzymatic activities in the investigated sediments.

Habitat	Area	Plot	Abundance	Biomass	C Production	Aminopeptidase	β -glucosidase
			N. cells 10^7 g ⁻¹	μ gC g ⁻¹	ngC g ⁻¹ h ⁻¹	nmol g ⁻¹ h ⁻¹	nmol g ⁻¹ h ⁻¹
Bare sediments with <i>Caulerpa</i>	1	1	2.25 ± 0.24	1.49 ± 0.15	10.9 ± 2.3	31.8 ± 7.3	21.3 ± 6.0
		2	3.44 ± 0.34	2.19 ± 0.15	174.0 ± 38.9	139.4 ± 10.2	8.9 ± 5.3
	2	1	0.25 ± 0.07	0.14 ± 0.05	132.2 ± 15.8	69.7 ± 18.9	14.7 ± 3.9
		2	3.80 ± 1.95	2.09 ± 0.67	64.6 ± 10.4	118.5 ± 33.4	6.6 ± 2.0
Bare sediments without <i>Caulerpa</i>	1	1	2.26 ± 0.18	1.60 ± 0.02	152.2 ± 19.3	44.9 ± 13.7	32.3 ± 3.5
		2	4.18 ± 0.64	2.69 ± 0.56	71.8 ± 12.1	39.1 ± 9.9	12.7 ± 0.3
	2	1	2.19 ± 0.49	1.48 ± 0.39	120.4 ± 23.5	57.1 ± 17.7	10.2 ± 2.1
		2	2.03 ± 0.42	1.55 ± 0.35	152.3 ± 13.1	31.3 ± 1.0	4.9 ± 2.5
Seagrass with <i>Caulerpa</i>	1	1	6.01 ± 1.73	3.89 ± 0.65	118.6 ± 135.3	47.1 ± 7.5	13.0 ± 3.4
		2	5.16 ± 1.25	3.61 ± 0.58	263.0 ± 84.3	74.3 ± 27.2	16.7 ± 5.5
	2	1	6.92 ± 1.32	4.95 ± 0.45	208.7 ± 66.5	104.7 ± 6.2	17.3 ± 4.8
		2	2.01 ± 0.50	1.44 ± 0.30	234.0 ± 41.9	68.5 ± 38.8	26.7 ± 4.4
Seagrass without <i>Caulerpa</i>	1	1	9.39 ± 2.08	6.02 ± 1.78	220.0 ± 99.5	100.3 ± 13.1	18.1 ± 7.4
		2	6.28 ± 2.44	4.65 ± 1.39	154.4 ± 47.9	92.9 ± 5.1	34.0 ± 11.8
	2	1	3.34 ± 0.06	2.14 ± 0.17	60.9 ± 13.1	66.5 ± 31.2	17.3 ± 3.1
		2	4.27 ± 1.22	2.93 ± 0.88	142.8 ± 20.8	163.1 ± 67.6	25.9 ± 5.2

4

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5

Table S3. Abundance of meiofaunal taxa in the investigated habitats. sd = standard deviation among n=3 replicates.

Habitat	Area	Plot	Nematode		Copepod		Polychaete		Bivalve		Ostracod		Kynorhinc	
			n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	sd
Bare sediments with <i>Caulerpa</i>	1	1	783.4	518.3	40.6	31.4	13.1	5.6	2.1	0.8	4.9	2.6	0.0	0.0
		2	1279.1	790.0	67.5	29.1	20.3	15.6	3.5	1.2	1.6	1.1	0.0	0.0
	2	1	764.7	349.1	133.7	52.4	77.7	12.5	0.6	0.3	1.6	1.5	0.6	0.3
		2	1649.0	751.7	26.9	10.6	35.4	17.8	1.8	0.9	1.0	1.0	0.6	0.3
Bare sediments without <i>Caulerpa</i>	1	1	2380.7	1167.9	38.7	20.0	3.9	1.0	1.0	1.0	11.1	7.5	0.0	0.0
		2	1261.1	202.1	58.6	49.9	5.9	4.3	1.0	1.0	17.0	4.0	0.0	0.0
	2	1	1342.4	875.9	38.7	23.9	23.9	18.3	0.6	0.3	9.7	3.8	0.0	0.0
		2	1751.9	717.1	31.8	20.0	8.8	2.9	1.8	0.9	25.2	15.4	0.0	0.0
Seagrass with <i>Caulerpa</i>	1	1	1632.0	624.0	38.0	18.5	16.4	3.5	6.8	2.5	5.1	2.1	0.0	0.0
		2	482.9	307.2	247.4	89.5	14.7	8.4	3.1	1.4	2.0	2.0	0.0	0.0
	2	1	1134.3	863.6	177.0	106.6	40.0	37.2	3.9	1.0	5.2	4.6	0.0	0.0
		2	713.0	516.1	82.7	32.3	21.6	19.7	4.1	1.5	4.6	4.0	0.6	0.3
Seagrass without <i>Caulerpa</i>	1	1	2019.6	950.0	45.9	25.3	9.8	5.9	4.6	3.2	3.3	1.1	0.0	0.0
		2	530.5	67.9	21.0	10.8	10.5	7.2	1.3	1.1	2.6	2.0	0.0	0.0
	2	1	440.7	247.9	68.5	19.7	16.7	12.1	0.0	0.0	3.3	1.3	0.6	0.3
		2	809.9	786.6	29.5	9.2	26.6	10.5	0.7	0.6	1.6	0.6	0.0	0.0
Habitat	Area	Plot	Turbellarian		Oligochaeta		Tardigrade		Gastrotrich		Cumacean		Amphipod	
			n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	Sd
Bare sediments with <i>Caulerpa</i>	1	1	0.0	0.0	0.6	0.3	0.0	0.0	1.6	1.5	1.3	0.6	1.6	0.6
		2	0.0	0.0	2.1	0.8	0.0	0.0	0.0	0.0	2.4	1.1	12.0	5.1
	2	1	0.0	0.0	3.3	1.3	11.5	4.3	0.0	0.0	1.6	1.5	4.9	2.3
		2	0.0	0.0	1.8	0.9	0.0	0.0	1.0	1.0	1.2	0.6	4.7	1.8
Bare sediments without <i>Caulerpa</i>	1	1	0.0	0.0	0.0	0.0	0.6	0.3	0.0	0.0	0.6	0.3	2.0	2.0
		2	0.0	0.0	0.0	0.0	0.6	0.3	0.0	0.0	0.6	0.3	2.0	2.0
	2	1	0.0	0.0	2.4	1.1	4.5	1.9	0.0	0.0	2.4	1.1	0.7	0.6
		2	0.0	0.0	0.0	0.0	0.6	0.3	0.0	0.0	1.2	0.6	1.8	0.9
Seagrass with <i>Caulerpa</i>	1	1	0.0	0.0	2.1	0.8	0.0	0.0	0.0	0.0	0.6	0.3	2.6	1.1
		2	0.0	0.0	1.0	1.0	33.2	15.2	0.6	0.3	0.0	0.0	2.1	0.8
	2	1	0.6	0.3	1.3	0.6	8.6	3.3	0.6	0.3	0.0	0.0	1.0	1.0
		2	0.0	0.0	2.0	1.0	6.6	5.8	0.0	0.0	0.0	0.0	1.0	1.0

Seagrass without <i>Caulerpa</i>														
	1	1	0.0	0.0	0.6	0.3	0.0	0.0	0.0	0.0	0.6	0.3	2.1	0.8
		2	0.0	0.0	2.7	1.0	2.0	2.0	2.1	0.8	0.0	0.0	0.0	0.0
	2	1	0.0	0.0	4.5	1.9	8.1	2.9	1.0	1.0	0.0	0.0	0.6	0.3
		2	0.0	0.0	1.0	1.0	0.6	0.3	0.6	0.3	0.6	0.3	0.6	0.3
<hr/>														
Habitat	Area	Plot	Isopod		Tanaidacean		Acarin		Decapod larvae		Others			
			n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	sd	n. ind. 10cm ⁻²	Sd		
Bare sediments with <i>Caulerpa</i>														
	1	1	0.0	0.0	14.7	10.3	0.6	0.3	2.0	2.0	0.0	0.0		
		2	0.0	0.0	7.5	7.1	0.0	0.0	5.6	1.5	0.0	0.0		
	2	1	0.0	0.0	3.6	2.0	1.2	0.6	2.0	0.0	0.0	0.0		
		2	2.4	1.1	11.6	4.7	0.0	0.0	1.0	1.0	0.0	0.0		
Bare sediments without <i>Caulerpa</i>														
	1	1	0.0	0.0	2.9	2.0	0.0	0.0	7.9	7.8	0.0	0.0		
		2	0.0	0.0	3.3	2.5	0.6	0.3	8.5	3.0	0.0	0.0		
	2	1	0.0	0.0	2.0	1.7	0.0	0.0	3.6	3.0	0.0	0.0		
		2	0.0	0.0	2.9	1.7	0.0	0.0	3.3	2.5	0.0	0.0		
<hr/>														
Seagrass with <i>Caulerpa</i>														
	1	1	0.0	0.0	3.9	1.6	0.7	0.6	1.2	0.6	0.6	0.3		
		2	0.0	0.0	1.2	0.6	25.1	11.6	4.3	2.0	1.2	0.6		
	2	1	0.0	0.0	2.7	1.0	14.2	5.3	2.3	1.5	0.6	0.3		
		2	0.0	0.0	0.0	0.0	1.2	0.6	6.1	2.1	0.6	0.3		
Seagrass without <i>Caulerpa</i>														
	1	1	0.0	0.0	0.0	0.0	0.6	0.3	1.6	0.6	1.6	1.5		
		2	0.0	0.0	0.0	0.0	0.7	0.6	0.6	0.3	0.0	0.0		
	2	1	0.0	0.0	0.6	0.3	2.9	1.7	1.2	0.6	1.2	0.6		
		2	1.8	0.9	0.0	0.0	0.0	0.0	0.6	0.3	0.0	0.0		

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8 **Table S4.** Meiofaunal abundance, biomass and richness of taxa in the two habitats invaded and
9 not invaded by *C. cylindracea*.

Habitat	Area	Plot	Abundance n. ind. 10 cm ⁻²	Biomass µgC 10 cm ⁻²	N. taxa
Bare sediments with <i>C. cylindracea</i>	1	1	867 ± 573	538 ± 659	10 ± 1
		2	1402 ± 849	132 ± 57	9 ± 1
	2	1	1007 ± 422	182 ± 48	9 ± 2
		2	1739 ± 788	145 ± 36	8 ± 3
Bare sediments without <i>C. cylindracea</i>	1	1	2449 ± 1209	291 ± 189	8 ± 1
		2	1359 ± 268	112 ± 15	8 ± 2
	2	1	1431 ± 927	133 ± 20	8 ± 1
		2	1830 ± 761	114 ± 52	7 ± 2
Seagrass with <i>C. cylindracea</i>	1	1	1710 ± 652	217 ± 104	8 ± 1
		2	819 ± 394	78 ± 36	8 ± 5
	2	1	1392 ± 1020	318 ± 305	10 ± 2
		2	844 ± 567	108 ± 10	9 ± 2
Seagrass without <i>C. cylindracea</i>	1	1	2090 ± 988	145 ± 70	7 ± 1
		2	574 ± 93	70 ± 34	7 ± 1
	2	1	550 ± 286	90 ± 6	7 ± 1
		2	874 ± 804	232 ± 330	7 ± 2

10

11

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12 **Table S5.** Results of ANOVAs ascertaining variations in nematode species richness (SR),
 13 among levels of the experimental factors: Ha = habitat (seagrass vs. unvegetated sediments); Ca
 14 = *Caulerpa* (present vs. absent); Ar = area (nested in Ha and Ca); Pl = plot (nested in Ha, Ca and
 15 Ar). df = degrees of freedom; MS = mean square; F = statistic F. P= probability level; ns = not
 16 significant; ** = $p < 0.01$.

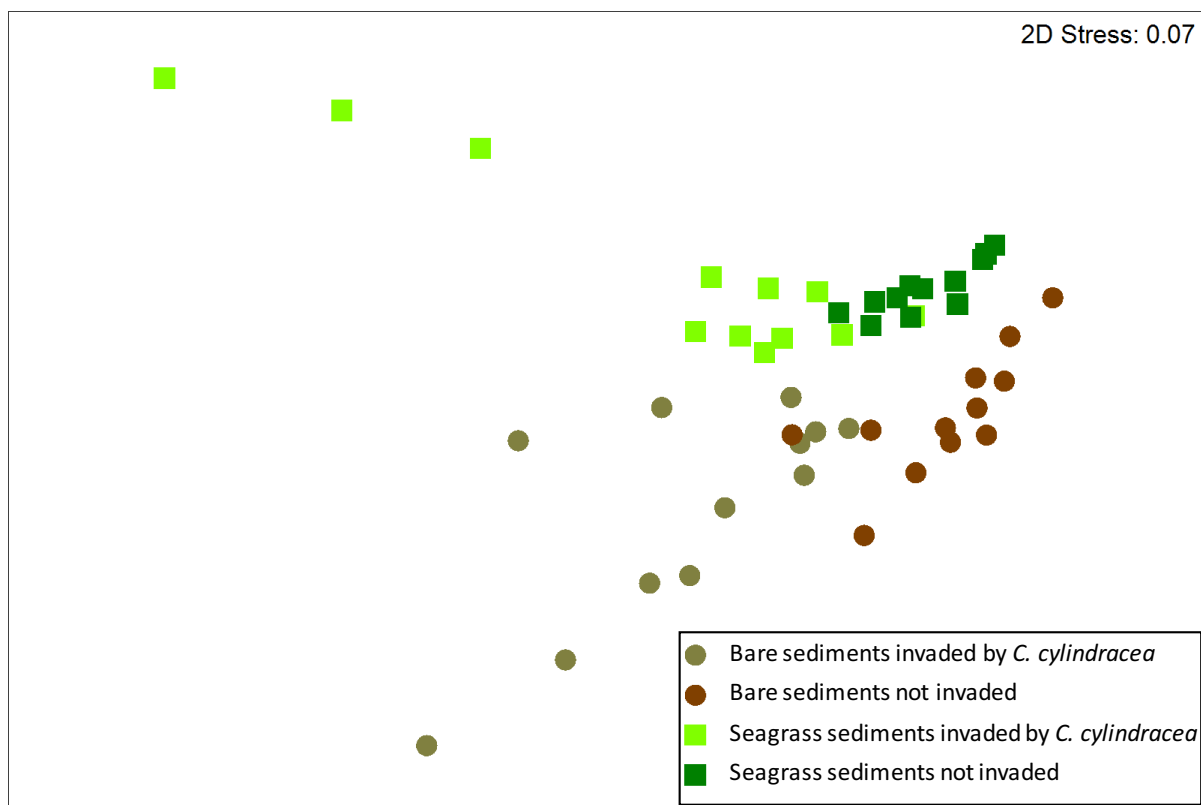
17

Index	Source	df	MS	F	P
Species Richness	Ha	1	184.080	4.90	ns
	Ca	1	5.333	0.14	ns
	HaxCa	1	192.000	5.11	ns
	Ar(HaxCa)	4	37.542	0.66	ns
	Pl(Ar(HaxCa))	8	56.625	2.35	**
	Residual	32	24.083		

18

19 **Figure S1.** MDS plot illustrating differences in the biochemical composition of organic matter
20 between in bare and seagrass sediments invaded and not invaded by *C. cylindracea*.

21

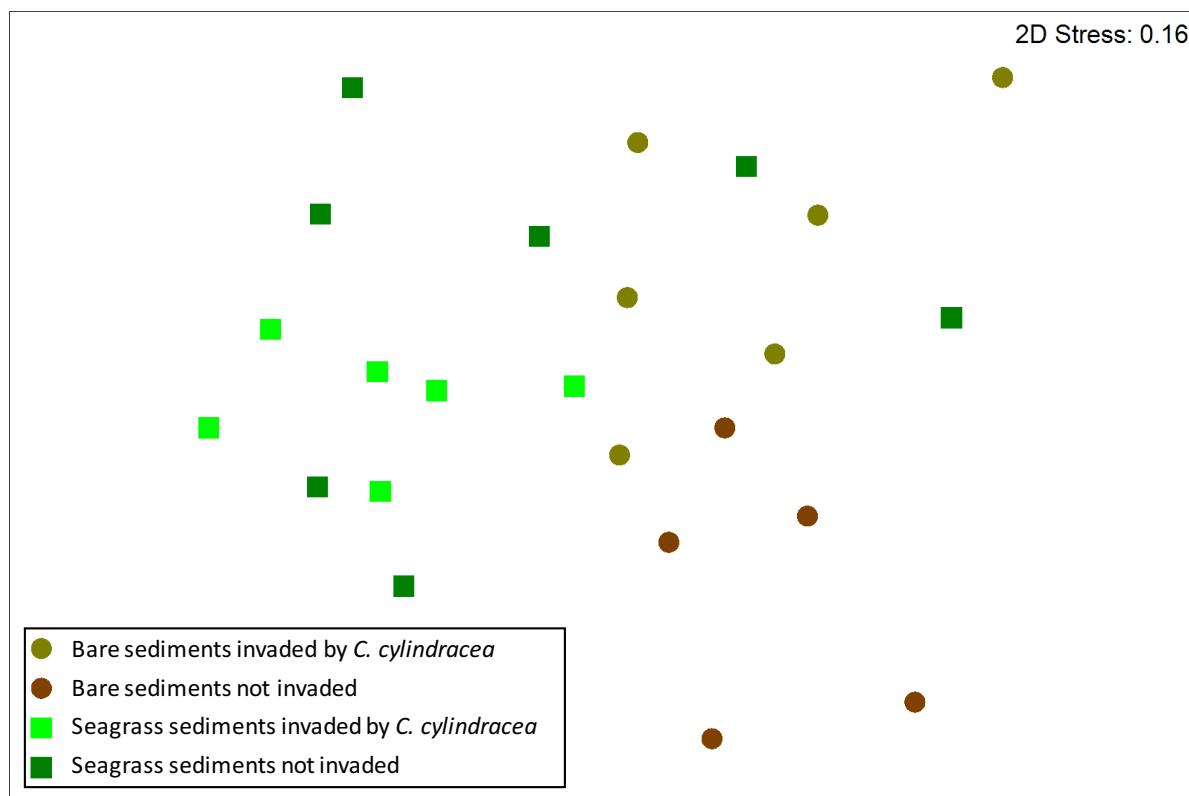


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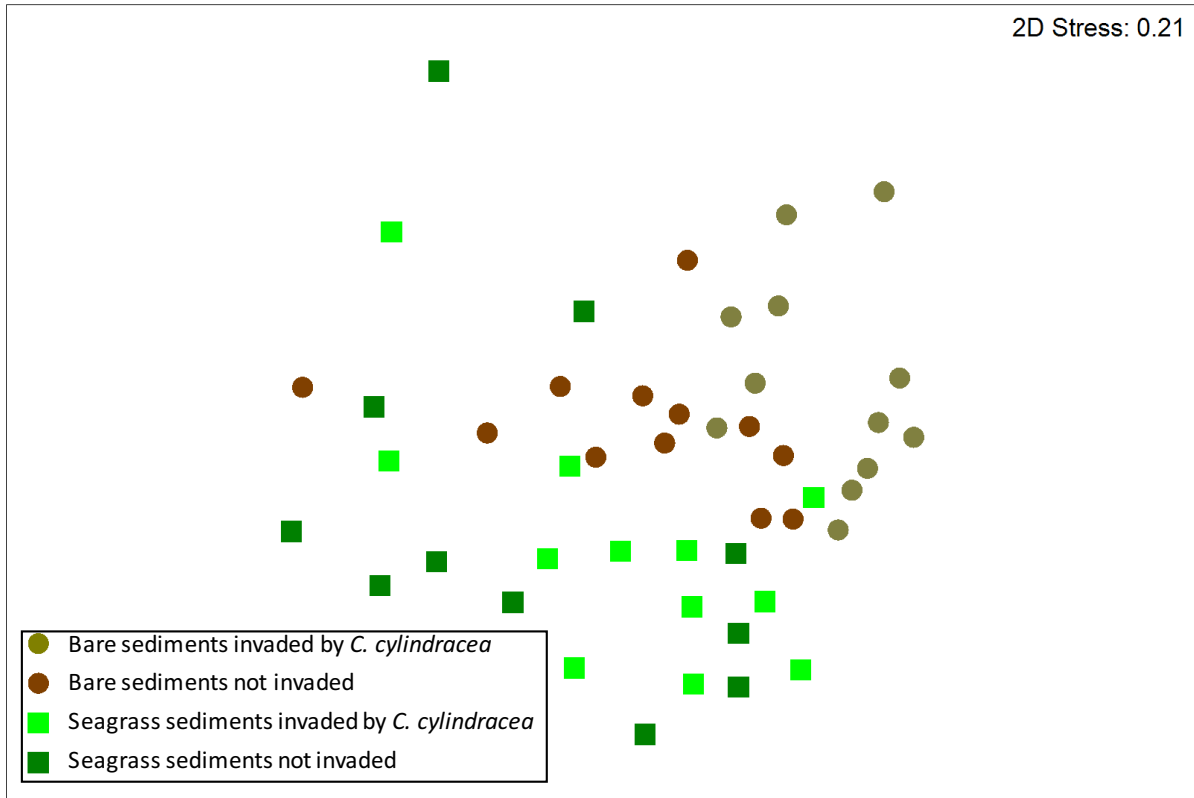
24 **Figure S2.** MDS plot illustrating differences in the composition of meiofaunal communities
25 between bare and seagrass sediments invaded and not invaded by *C. cylindracea*.

26



29 **Figure S3.** MDS plot illustrating differences in the composition of nematode assemblages
30 between unvegetated and seagrass sediments invaded and not invaded by *C. cylindracea*.

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