The interactive effect of herbivory, nutrient enrichment and mucilage on shallow rocky macroalgal communities

Sarah Caronni[1][*], Chiara Calabretti[1], Sandra Citterio[1], Maria Anna Delaria[2], Rodolfo Gentili[1], Giovanni Macri[3], Chiara Montagnani[1], Augusto Navone[4], Pieraugusto Panzalis[4], Giulia Piazza[1], Giulia Ceccherelli[2]

[1]Department of Earth and Environmental Sciences, University of Milan-Bicocca. Piazza della Scienza 1, I-20126 Milan. Italy.
[2]Department of Science for Nature and Environmental Resources, Via Piandanna 4, University of Sassari, I-07100 Sassari. Italy.
[3] Mac Pro e GIS, Via C. Goldoni 7, I-27100 Pavia. Italy.
[4]Marine Protected Area Tavolara Punta Coda Cavallo. Via Dante1, I-07026 Olbia (OT). Italy.

*Corresponding author
Telephone number: +39 0382 984875
Fax number: + 39 0382 986801
E-mail address: sarah.caronni@unipv.it

41 **Abstract**

42   This paper focuses on the interactive short and long-term effect of three different stressors on a

43 macroalgal assemblage. Three stressors are considered: herbivory, nutrients and mucilage. The

44 experiment was conducted in Tavolara Punta Coda Cavallo Marine Protected Area

45 (Mediterranean Sea) during a bloom of the benthic mucilage-producing microalga

46 *Chrysophaeum taylorii* (*Pelagophyceae*); this microalga is recently spreading in the

47 Mediterranean Sea. On a rocky substratum, 36 plots 20x20 cm in size were prepared. Factorial

48 combinations of 3 experimental treatments were applied in triplicate, including three grazing

49 levels crossed with two nutrient enrichment and two mucilage removal treatments. Significant

50 differences were observed among treatments 8 weeks later, at the end of summer. In particular,

51 dark filamentous algae were more abundant in all enriched plots, especially where mucilage and

52 macroalgae had been removed; a higher percent cover of crustose coralline algae was instead

53 observed where nutrients had been increased and no grazing pressure acted. Furthermore, the

54 abundance of *Dictyota* spp. and *Laurencia* spp. was significantly higher in enriched mucilage-

55 free plots where the grazing pressure was null or low. However, the effects of the treatments on

56 the overall assemblage of the macroalgal community were not long persistent (36 weeks later).

57 These results illustrate the capacity of a shallow-water macroalgal community to quickly recover

58 from the simultaneous impacts of herbivory, nutrient enrichment, and mucilage.

59

60

61

62

63

1 Introduction

Marine ecosystems and especially near-shore coastal areas are typically subjected to several natural and anthropogenic abiotic and biotic stressors, which can seriously affect the structure of habitats and produce nearly irreversible shifts, leading to a significant reduction of ecosystem resistance and resilience (Adams, 2005; Claudet and Fraschetti, 2010; Guarnieri et al., 2014;). A substantial research effort has therefore been done to investigate the effects of the most widespread threats to marine environment (Crain et al., 2009); anyway, understanding the complex effects of multiple stressors on marine communities still represents one of the major challenges in marine ecology (Sala et al., 2000. Zeidberg & Robison 2007, Guarnieri et al., 2014). Moreover, whether stressors are more harmful in combination than alone is still widely unanswered (Zeidberg & Robison 2007). Interactions among multiple stressors, where often the ecological effect of one stressor depends on the magnitude of another, are very common across ecosystems (Jackson et al., 2016) and several scenarios can occur when they simultaneously act: their effects can be cumulative, synergistic or antagonistic (Vinebrooke at al., 2004). Two or more stressors are defined as cumulative if their result is the mere sum of the effects of each of them (combined effect: stressor a + stressor b) while they are synergistic or antagonistic when their combined effect is respectively larger or smaller than the one expected by each of them (synergistic= combined effects are larger than adding stressor a + stressor b; antagonistic= combined effects are lower than adding stressor a + stressor b) (Crain et al., 2008). For example, pH usually can have an antagonistic effect on heavy metal availability in water environments, as the heavy metal solubility normally increases when pH decreases (Millero et al., 2009). On the contrary, a synergistic effect has been described for *Daphnia*: Folt et al. (1999) observed that the reproduction rate of *the crustacean* increased when high temperatures and food levels were

87   simultaneously present, while the positive effect of temperature on the population growth rate

88   was smallest at limiting food levels (Folt et al. 1999).

89   In coastal ecosystems, canopy macroalgae play critical ecological roles (Koch et al., 2013),

90   contributing significantly to total primary production and deeply affecting higher trophic levels

91   (Graham, 2004). In fact, they provide the three-dimensional structure of marine faunal habitats

92   and facilitate larval settlement of marine invertebrates (Rodríguez. et al., 1993). In the last

93   decades, shallow temperate reefs are experiencing a dramatic reduction and loss of macroalgal

94   habitats and their replacement by persistent barren grounds are of increasing concern (Guidetti et

95   al., 2003; Sala et al., 2012). Several studies highlighted the importance of herbivore pressure in

96   defining the composition and the abundance of macroalgal communities (i.e. Karez et al., 2004;

97   Arévalo et al., 2007). Particularly, the abundance of herbivores (mainly sea-urchins and limpets)

98   increases in relation to over-exploitation by large-sized predators, producing a rapid shift in

99   dominance from canopy forming to crustose macroalgae with the creation of persistent barrens

100 worldwide (Filbee-Dexter & Scheibling, 2014; Piazzi et al., 2016). However, not only herbivore

101 pressure but also nutrient enrichment can alter the structure of benthic communities (Smith et al.,

102 2010) and it can determine macroalgal abundance, even if its importance is extensively debated

103 (i.e. McGlathery, 2001; Armitage et al., 2005). Recently, it has been suggested that nutrient

104 enrichment may mitigate over-grazing by enhancing algal growth (Boada et al., 2017).

105   The relationship between nutrient enrichment and microalgal abundance has been

106 extensively described in literature (e.g. Hecky & Kilham, 1988; Anderson et al., 2002), as

107 nutrient enrichment is an important driver of microalgal proliferation (Smith & Schindler, 2009).

108 There are numerous examples worldwide of harmful algal blooms linked to an increased nutrient

109 loading and there is a growing consensus that degraded water quality caused by nutrient

110   pollution, in particular by N compounds, contributes to the development and persistence of

111   microalgal proliferations and, consequently, of mucilage abundance (Obernosterer & Herndl,

112   1995; Rinaldi et al., 1995). The presence of the mucilage corresponds to the appearance of a

113   gelatinous material suspended in marine waters (pelagic aggregates) or covering large portions of

114   the substratum (benthic aggregates); it is primarily produced by planktonic or benthic microalgae

115   respectively (Mingazzini & Thake, 1995). Mucilage is a problem during blooms when

116   microalgae proliferate rapidly, reaching very high-density, and covering large portions of

117   substrata, as described by Schiaparelli et al (2007) or Lugliè et al (2008).

118   Detrimental effects due to mucilage proliferation can affect macroalgae and other benthic

119   organisms and should therefore be considered an emerging threat to coastal ecosystems (Claudet

120   & Fraschetti, 2010). In fact Devescovi & Iveša (2007) observed that primary branches of

121   macroalgae usually show signs of necrosis after blooms (Devescovi & Iveša, 2007) and Misic et

122   al. (2011) assert that the persistence of mucilage on hard substrata can overgrow algae and cause

123   their depletion. Finally, thick mucilage carpet that usually covers the substratum during blooms

124   can be gradually torn off by the continuous twisting action of currents, engulfing and

125   mechanically detaching macroalgae (Schiaparelli et al., 2007). Nevertheless, only a few papers

126   describe the detailed effects of mucilage on macroalgal communities and currently no

127   information about effects of the interaction among nutrient enrichment, herbivory and mucilage

128   on the above-mentioned communities are available in literature.

129   In this paper results of a manipulative experiment to evaluate the interactive short- and

130   long-term effect of the three above mentioned stressors (herbivory, nutrient enrichment and

131   mucilage) on macroalgal assemblages are presented. The experiment was conducted during a

132   bloom of the benthic mucilage-producing microalga *Chrysophaeum taylorii* Lewis and Bryan

133 (*Pelagophyceae*), recently spreading in the Mediterranean Sea (Caronni et al. 2014 and 2015).

134 *C. taylorii* can exude large amounts of mucilaginous material macroscopically visible when its

135 cells density reaches values over 1000 cells ml^{-1} (Caronni et al., 2014). Although it is considered

136 a public nuisance also in its native range (Atlantic and Pacific Ocean), its blooms are infrequent

137 there (at least in the Great Barrier Reef) and their effects are not so detrimental as those of other

138 microalgae (e.g. Schaffelke et al., 2004). Conversely, in the Mediterranean Sea, large portions of

139 hard rocky and sandy substrata as well as seagrasses and macroalgae can be covered by *C.*

140 *taylorii* mucilage during summer (Luglié et al., 2008; Caronni et al., 2011 and 2015). For this

141 reason, an investigation on the effects of *C. taylorii* mucilage on native benthic communities

142 exposed to other common stressors is strictly required.

143 In this paper, following a full-factorial design, nutrients were added to simulate eutrophication,

144 macroalgae (both erect and crustose) were removed to simulate barrens produced by grazers and

145 mucilage was manually removed to simulate mucilage-free conditions. We predict that the

146 presence of mucilage would buffer the effect of nutrient enrichment on macroalgal abundance

147 (antagonistic effect). On the contrary, mucilage would reasonably worsen the effects of

148 herbivory, inhibiting macroalgal recovery after massive grazing events (total macroalgal

149 removal) and enhancing the development of permanent barrens (synergistic effect). Therefore,

150 the effect of mucilage was expected to be higher in non-enriched conditions especially when

151 herbivores where present (Burkpile & Hay, 2006; Guarnieri et al., 2014).

152
153 **2 Materials and methods**
154
155 *Study site and experimental design*
156
157   The manipulative field experiment was conducted in Tavolara Punta Coda Cavallo Marine

158 Protected Area (hereafter TPCC MPA, North-East Sardinia, Western Mediterranean), during the

159   summer of 2014. Punta Don Diego Bay (40°52'34.62'' N; 9°39'21.19" E), located in a partially

160   protected zone of the MPA (traditional economic activities as fishing and recreation are

161   allowed), was chosen for the experiment as *C. taylorii* blooms have been recurrently abundant

162   there in the recent years (Caronni et al., 2014) (Fig. 1). The study area is characterized by

163   oligotrophic waters (P: $0.006\pm0.0003\mu M$; N: $0.3\pm0.008\mu M$) and a well-developed and

164   diversified macroalgal community is present. The main grazers in the area are sea-urchins

165   (*Paracentrotus lividus*; Lamarck, 1816), whose grazing may cause important changes in the

166   distribution patterns of benthic communities, exerting a paramount role in the transition from

167   macroalgal beds to barrens where only a few coralline algae are present (Boudouresque and

168   Verlaque, 2001, Hereu et al., 2004). Urchin density in the study area is of about 3.5 ind m^{-2}

169   (Panzalis P., personal communication).

170   Two rocky areas of about 10 m^2 (20 m apart), comparable in water motion, topography, and

171   inclination of the rocky substrate, were randomly chosen in the bay at 1.5 m of depth (highest *C.*

172   *taylorii* cell density depth, Caronni et al., 2015). In each area 18 plots 20x20 cm in size were

173   prepared and randomly assigned to one treatment.

174   The experimental design consisted of three factors: three grazing levels crossed with two

175   nutrient enrichment and two mucilage removal treatments, following a factorial design, and

176   three replicates for each combination of treatments were considered (N=36). To obtain such

177   treatments, plots were differently manipulated at the beginning of July: 1. the mucilaginous

178   aggregates were manually removed from half of the plots (M-), while mucilage was maintained

179   in the other half of them (M+); 2. the substratum was scraped at three levels: total (G100%: all

180   the surface was fully scraped), partial (G50%: the 50% of the surface was fully scraped) and no

181   (G0%) removal of macroalgae (both erect and crustose) was conducted, using an iron brush to

182   simulate the effect of grazers such as *P. lividus*, responsible for the formation of extended sea

183   urchin barrens (Hereu, 2005); 3. nutrient enrichment was obtained in half of the plots, all in the

184   same area, to avoid nutrient enrichment of control plots (E+ *vs* E- refereed to enriched and non-

185   enriched) (N=36). For nutrient enrichment, small-mesh nylon bags (2 mm mesh size) filled with

186   slow-release fertilizer pellets (Osmocote®; 18:9:10, N:P:K) were used, following Bulleri et al.,

187   (2012) and Guarnieri et al. (2014). The bags were fixed to a brick and positioned on the rocky

188   bottom at the edge of each unit. Overall, 40 g of fertilizer were added in each plot, placing two

189   bags with 20 g of pellet at two opposite sides of the plot. The amount of fertilizer in each bag

190   was decided according to previous studies (Worm & Sommer 2000). To ensure enriched

191   conditions throughout the experiment, nutrient bags were monthly replaced.

192   The concentration of nutrients (N and P) in the water was estimated two times from July to

193   September 2014; 10 water samples (125 ml) in each area were randomly taken in July (S_1) and 4

194   weeks later, in August (S_2). Samples were taken at approximately 10 cm from the bottom and at

195   least 50 cm apart from nutrient bags. After collection, water samples were shaken, filtered (0.45-

196   μm mesh size filter) and frozen, as suggested by Balata et al. (2010). They were transported to

197   the University of Milan Bicocca, where concentrations of inorganic N and P (ammonia, nitrate,

198   nitrite and phosphate) were estimated using a Spectrophotometer Lambda EZ201 Perkin Elmer

199   (precision=0.3 nm). Chemical analyses of water samples confirmed that for both nutrients

200   differences due to the enrichment were significant and consistent before and 4 weeks after the

201   manipulation (Table 1).

202   After an initial sampling, performed at the beginning of July, before the manipulation, in

203   each plot benthic assemblages were sampled on 2 times; 8 weeks later (at the beginning of

204 September 2014,) and 36 weeks later (in March 2015), to evaluate the short and long-term

205 effects of the three stressors, respectively.

206 The assemblages in each plot were sampled photographically using a Nikon Coolpix AW130

207 underwater camera (16 Megapixel). The camera was held at a consistent distance from the

208 substrate (approximately 50 cm) as suggested by Jonker et al. (2008), paying attention to frame

209 the entire experimental plot. Applying image analysis tools, the percent cover (%) of each

210 macroalgal taxon was assessed, superimposing a grid of twenty-five sub-quadrats onto each

211 image, scoring each sub-quadrat from 0 to 4% and adding the 25 resulting values to obtain the

212 total cover (Dethier et al., 1993).

213

214 *Statistical analyses*

215 A distance-based permutational multivariate analysis of variance (PERMANOVA)

216 (Anderson, 2001) was performed using the software PERMANOVA (Anderson, 2005), to

217 analyse the response of the macroalgal assemblage to experimental conditions across time. The

218 analyses were based on Bray–Curtis dissimilarities calculated on non-transformed data. Each

219 term in the analysis was tested using 9,999 random permutations. To test for short and long-term

220 effects of treatments and to analyse independent data (Underwood, 1997), two PERMANOVAs

221 were performed on data collected 8 and 36 weeks after the manipulation), respectively. The

222 experimental design consisted of three factors: nutrient enrichment (two levels, fixed), grazing

223 exclusion (three levels, fixed and orthogonal) and mucilage removal (two levels, fixed and

224 orthogonal). Significant terms relevant to the hypotheses were investigated through post hoc

225 pair-wise comparisons and a SIMPER test was also run (Primer v6) to point out the relative

226 contribution of each taxon to the dissimilarities observed among treatments (Clarke, 1993).

227   Finally a non-metric multidimensional scaling (nMDS) was used for the graphical ordination of

228   data.

229   To investigate the differences among treatments evidenced by the SIMPER test for the main

230   macroalgal taxa, a three-way ANOVA was also run for each taxon abundance using the software

231   GMAV5 (University of Sydney, Australia). Cochran's test was run prior to each ANOVA to test

232   for homogeneity of variances and normality was assured by Kolmogorov-Smirnov test. Student–

233   Newman–Keuls (SNK) tests were used for *a posteriori* comparison of means (Underwood,

234   1997).

235

236   **3 Results**

237   **3.1 Short-term macroalgal response to disturbance**

238   At the end of the experiment, 8 weeks after the manipulation, eight macroalgal

239   taxa/morphological groups were found: *Acetabularia acetabulum* (L.) Silva., Dark filamentous

240   algae (DFA), *Dasycladus vermicularis* (Scopoli) Krasser, Crustose coralline algae (CCA),

241   *Dictyota* spp. *Laurencia* spp., *Liagora* spp., and *Padina pavonica* (L.) Thivy. The combination of

242   three analyzed stressors affected the short-term recovery of assemblages (PERMANOVA

243   significant E×G×M interaction, Table 2). Pair-wise comparisons showed statistically significant

244   differences between enriched and non-enriched plots only when the other two stressors were

245   absent or when their effect was poor (M-G0% and M-G50%). Furthermore, in non-enriched

246   conditions statistically significant differences due to mucilage were recorded, especially in plots

247   where the grazing pressure was high (E-M+G100%; ≠E-M-G100%). On the contrary, the same

248   effect was not observed in enriched plots. Additionally, the effect of mucilage was not detected

249   where the grazing pressure was null or low, in both enriched and non-enriched plots (E+G0%

250   and E-G0%), as clearly depicted in the nMDS graphs (Fig. 2).

251   Differences in the composition of macroalgal assemblages were found to be related to all three

252   considered stressors and the Simper test evidenced that four taxa remarkably contributed to the

253   observed dissimilarities: dark filamentous algae, crustose coralline algae, *Dictyota* spp. and

254   *Laurencia* spp.

255   Finally, all the ANOVAs performed on these taxa (DFA, CCA, *Dictyota* spp. and *Laurencia*

256   spp.) detected differences for the E×G×M interaction term (Table 3 and 4; Fig. 3). Particularly,

257   dark filamentous algae seemed to be more abundant in enriched plots, especially where mucilage

258   and macroalgae had been removed (E+M-G100%), while a higher percent cover of crustose

259   coralline algae was observed where nutrients had been increased especially if the grazing

260   pressure was null, independently from mucilage presence (E+M-G0% and E+M+G0%). Finally,

261   both the erect species (*Dictyota* spp. and *Laurencia* spp.) were more abundant in enriched plots,

262   where the grazing pressure was null or low and mucilage had been removed (E+M-G0% and

263   E+M-G50%) (Table 3 and 4; Fig. 3).

264   **3.2 Long-term macroalgal response to disturbance**

265   36 weeks after the manipulation only six macroalgal taxa/groups were overall found and the

266   effect of treatments (nutrient enrichment, grazing exclusion and mucilage removal) was neither

267   highlighted on the most abundant algae (DFA, CCA, *Dictyota* spp., *Laurencia* spp. and *P.*

268   *pavonica*) nor on the whole structure of the macroalgal assemblages, as graphically evidenced

269   also by the nMDS ordination (Fig. 2).

270

271

4 Discussion

An interactive effect of the three stressors on the short-term recovery (8 weeks after the manipulation) of the considered macroalgal assemblages was highlighted in the study (Fig. 2 and 3; Table 3 and 4). Significant differences in the composition and abundance of macroalgae were observed depending on enrichment, but only when mucilage and grazing were absent (Fig. 2 and 3; Table 3 and 4). These results are in accordance with those of other studies investigating the role of nutrient enrichment in determining macroalgal abundance (*e.g.* McGlathery, 2001; Teichberg et al., 2008; Sotka & Hay, 2009) and confirm that, when nutrient enrichment is the only stressor, it leads to a remarkable increase of total macroalgal biomass. More in detail, a positive response to nutrient enrichment of the different macroalgal taxa present in the study area was observed, with an enhancing effect on both turf-forming and erect macroalgae. Our evidence supports the results obtained by Bulleri et al., (2012) who found a positive effect of enrichment on all macroalgae, contrarily to Pedersen & Borum (1996), who observed an enhancement of turf-forming algae, in particular of DFA.

Instead, no relevant differences were observed in the composition of macroalgal assemblages exposed to nutrient enrichment when one of other two stressors was present (Fig. 2 and 3; Table 3 and 4). In particular, a buffering effect of mucilage on nutrient enrichment was recorded. In plots where mucilage was present, no significant differences in macroalgal assemblages' abundance and composition were noticed between enriched and non-enriched treatments (Fig. 2 and 3; Table 3 and 4). Results can be explained considering that microalgae take up nutrients faster than macroalgae and consequently mucilage might be responsible for the sequestration of large amounts of nutrients from the water column, elements then used by microalgae embedded in aggregates to survive and proliferate (Reynolds, 2007). As a matter of fact, aggregates are

295   biota-rich environments where the concentration of nutrients can be dramatically higher than in

296   the surrounding seawater (Del Negro et al., 2005). For this reason, it is plausible that, in plots

297   with mucilage, only a small amount of total nutrients released in the water was available for

298   macroalgae as a conspicuous portion of them was sequestrated by mucilage and used by.

299   Furthermore, even if Huang and Boney (1983) observed that, in laboratory conditions, the

300   growth of some species of green and brown algae was enhanced by diatoms mucilage,

301   mucilaginous aggregates are generally known to overgrow macroalgae causing their mechanical

302   suffocation and rapid biomass depletion (Misic et al., 2011). Moreover, Müller et al. (1998)

303   assumed that all benthic organisms are seriously damaged by mucilage aggregates, even if their

304   cover is thin because they usually release toxins directly affecting cell metabolism. Therefore,

305   when mucilage and nutrient enrichment acted simultaneously, the expected increase in

306   macroalgal abundance due to nutrient enrichment could have been counterbalanced by the

307   presence of mucilage on the substratum.

308   The positive effect of nutrient enrichment did not seem buffered by mucilage; only considering

309   crustose coralline algae, their abundance was equal in all enriched plots, independently from the

310   presence of mucilage (Fig. 2 and 3; Table 3 and 4). Schiaparelli et al. (2007) and Figueiredo &

311   Steneck (2000) suggested that coralline algae could be seriously damaged by mucilage

312   (especially when its presence on the substratum lasted for a long time), but our results mostly

313   agree with Bulleri (2006) who evidenced their ability to survive for long periods if overgrown

314   by other species.

315   As for mucilage, also the effect of grazing seemed to buffer that of nutrient enrichment (Fig. 2

316   and 3; Table 3 and 4). About this, Guarnieri et al. (2014) observed a relatively constant

317   macroalgal cover, also in nutrient enriched conditions, when a high grazing pressure acted. Our

318   results highlight that the presence of herbivores can strongly affect the proliferation of

319   macroalgae, lowering the positive effect of enrichment. Therefore, even if results of several

320   previous experiments suggested that both increased nutrient loading and reduced grazer densities

321   favour an intense macroalgal growth (*e.g.* Geertz-Hansen et al., 1993; Hauxwell et al., 1998;

322   Lotze & Worm, 2000), the reduction of herbivory often seems to be the main factor triggering

323   the restoration process of macroalgal assemblages after disturbance (Scheffer et al., 2001), and

324   the role of nutrient enrichment appears to be only secondary. Nevertheless, herbivory does not

325   represent the exclusive process in structuring macroalgal assemblages, as our results highlight its

326   interactive effect with nutrient availability in mediating the outcomes of grazing pressure,

327   similarly to Burkepile & Hay (2006). In this interaction, the mucilage plays a relevant role that

328   should not be neglected. In fact, where the grazing pressure was null or low the presence of

329   mucilage did not influence the abundance of the main taxa (Fig. 2 and 3; Table 3 and 4) except

330   for where enrichment was done. This suggests a primary role of herbivores and nutrients in

331   regulating the abundance of macroalgae (Lawrence, 1975; Underwood, 1980; Scheibling, 1986;

332   Geertz-Hansen et al., 2003). However, mucilage effect is corroborated by the effect of

333   enrichment on *Laurencia* spp. and *Dictyotales*, at no grazing pressure, only where the mucilage

334   was removed. This would support the hypothesis that the effect of enhancement produced by

335   nutrient enrichment can only be conspicuous where the negative effect of mucilage was nullified.

336   Therefore, the effect of mucilage seems to be significantly detrimental only where communities

337   are stressed by high densities of herbivores and nutrients are not so abundant to remarkably

338   increase macroalgal biomass. In such conditions, indeed, damages caused by both mucilage

339   suffocation and mechanical detachment of macroalgae (especially of erect and frondose species)

340   (Schiaparelli et al., 2007; Lugliè et al., 2008), are not worsened by grazing pressure and balanced

341   by nutrient enrichment. Also, the lower abundance of erect species such as Dictyotales and

342   *Laurencia* spp. confirms that the erect species are more damaged by mucilage than the turf-

343   forming ones. Furthermore, turf-forming species appeared to be more abundant also where

344   macroalgae were removed because, in such conditions, a wider free space was available.

345   Furthermore, in this last case, lower competition favoured the development of opportunistic

346   species such as turf-forming macroalgae (Bulleri et al., 2012).

347   Finally, the lack of effects of the three considered stressors observed after about eight months of

348   study deserves a deeper insight. Whether the change through time of effects was biased by a

349   seasonal effect (because of the variable phenology of algal taxa) it will remain unknown because

350   unfortunately no data are available at present. Therefore, very hard disentangling mechanisms

351   (seasonal effect vs lack of complete recover) are responsible for the lack of persistent differences

352   through time. However, even if the variability of effects through the year could occur depending

353   on the composing species seasonal performance, there are reasons to hypothesize that the

354   macroalgal assemblage would cope with the considered stressors assisted by cyclical responses.

355

356   **5 Conclusions**

357   The short-term effect (8 weeks) of the considered multiple stressors on the macroalgal

358   community suggest the mucilage and grazing to be antagonistic to nutrient enrichment, while

359   mucilage presence and herbivore pressure seem to act synergistically. Particularly, dark

360   filamentous algae were more abundant in enriched plots, especially where mucilage and

361   macroalgae had been removed; conversely, a higher cover of crustose coralline algae was

362   observed where nutrients had been increased and no grazing pressure acted. Furthermore, the

363 abundance of *Dictyota* spp. and *Laurencia* spp. was significantly higher in enriched mucilage-

364 free plots where the grazing pressure was null or low.

365 However, effects of treatments on the overall assemblage of the macroalgal community were not

366 persistent on the longer-term (36 weeks after the manipulation) and this indicate the capacity of a

367 shallow-water macroalgal community to quickly recover from these simultaneous impacts.

368 Therefore, even if these results substantially confirm the predictions on the interactive stressors

369 (mucilage would have buffered the effect of nutrient enrichment, worsened the one of herbivory

370 and that a greater effect of mucilage would have been observed in non-enriched conditions

371 especially when herbivores were present), the rapid fading of their effects fade in few months

372 makes not strictly necessary or urgent the laying out of guidelines for managers.

373 At the same time, results of this study can also help to forecast areas where nutrient pollution

374 will have more detrimental effects on macroalgal assemblages, basing on the grazers density and

375 the frequency of mucilaginous blooms. Furthermore, some interesting ecological lessons can also

376 be gained, as relevant information has been provided about the resilience of macroalgal

377 communities to mucilage effects at non-pristine conditions, which concerns the nutrient water

378 status and the herbivores control by predators. In such conditions indeed the effects of

379 disturbances, even when they are due by multiple stressors acting together, may be more

380 efficiently buffered within the system (consistently to Agardy 1994 and to Jentoft et al., 2007),

381 highlighting that stability of the state would be provided by other feed-back mechanisms.

382

383

384 **Acknowledgements**

385

386   We thank the two anonymous referees and the editor Craig Nelson for providing useful

387   suggestions to improve the ms. Furthermore, we thank Luisa Polastro, Sidney Freedman and

388   Nadia Carlier for the English revision, Luigi Piazzi for the fundamental help in data analysis and

389   Matteo Grechi and Gianluca Cavagna for the precious help during image analysis.

390   **References**

391   Adams SM. 2005. Assessing cause and effect of multiple stressors on marine systems. *Marine*

392   *Pollution Bulletin*, 51:649-657.

393   Agardy MT. 1994. Advances in marine conservation: the role of marine protected areas. *Trends*

394   *in Ecology & Evolution*, 9:267-270. DOI:10.1016/0169-5347(94)90297-6.

395   Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral*

396   *Ecology*, 26:32-46.

397   Anderson DM, Glibert PM, Burkholder JM. 2002. Harmful algal blooms and eutrophication:

398   nutrient sources, composition and consequences. *Estuaries*, 25:562-584.

399   Anderson MJ. 2005. Permutation ANOVA: A FORTRAN Computer Program for Permutational

400   Multivariate Analysis of Variance. *New Zealand, Auckland: Department of Statistics, University*

401   *of Auckland*, 24.

402   Arévalo R, Pinedo S, Ballesteros E. 2007. Changes in the composition and structure of

403   Mediterranean rocky-shore communities following a gradient of nutrient enrichment: Descriptive

404   study and test of proposed methods to assess water quality regarding macroalgae. *Marine*

405   *Pollution Bulletin*, 55:104-113. DOI:10.1016/j.marpolbul.2006.08.023.

406   Armitage AR, Frankovich TA, HeckJames KL, Fourqurean W. 2005. Experimental nutrient

407   enrichment causes complex changes in seagrass, microalgae, and macroalgae community

408   structure in Florida Bay. *Estuaries and Coasts*, 28:422-438.

409 Balata D, Piazzi L, Nesti U, Bulleri F, Bertocci I. 2010. Effects of enhanced loads of nutrients on

410 epiphytes on leaves and rhizomes of *Posidonia oceanica*. *Journal of Sea Research*, 63:173-179.

411 Boada J, Arthur R, Alonso D, Pagès JF, Pessarrodona A, Oliva S, Ceccherelli G, Piazzi L,

412 Romero J, Alcoverro T. 2017. Immanent conditions determine imminent collapses: nutrient

413 regimes define the resilience of macroalgal communities. *Proceedings of the Royal Society B*,

414 284:20162814. DOI:10.1098/rspb.2016.2814.

415 Boudouresque, CF, Verlaque M. 2001. Ecology of *Paracentrotus lividus*. *Developments in*

416 *aquaculture and fisheries science,* 32:177-216.

417 Bulleri F. 2006. Duration of overgrowth affects survival of encrusting coralline algae. *Marine*

418 *Ecology Progress Series*, 321:79-85.

419 Bulleri F, Russell BD, Connell SD. 2012. Context-dependency in the effects of nutrient loading

420 and consumers on the availability of space in marine rocky environments. *PLoS ONE*, 7:e33825.

421 Burkepile DE, Hay ME. 2006. Herbivore vs. nutrient control of marine primary producers:

422 context-dependent   effects.   *Ecology*,   87:3128-3139.   DOI:10.1890/0012-

423 9658(2006)87[3128:HVNCOM]2.0.CO;2.

424 Caronni S, Ceccherelli G, Navone A, Panzalis P, Pinna S, Sechi N. 2011. I popolamenti

425 bentonici nell'Area Marina Protetta Tavolara Punta Coda Cavallo (Sardegna nord-orientale)

426 dopo una fioritura della microalga *Chrysophaeum taylorii* Lewis & Brian. *Studi Trentini Sci*

427 *Naturali Acta Geologica*, 89:107-110.

428 Caronni S, Delaria MA, Navone A, Panzalis P, Sechi N, Ceccherelli G. 2014. Relevant scales of

429 variability of the benthic allochthonous microalga *Chrysophaeum taylorii*. *Marine Biology*,

430 161:1787-1798.

431 Caronni S, Bresciani A, Delaria MA, Meloni F, Navone A, Panzalis P, Heimann K, Ceccherelli

432 G. 2015. Ecology of the benthic mucilage-forming microalga *Chrysophaeum taylorii* in the W

433 Mediterranean Sea: substratum and depth preferences. *Estuarine, Coastal and Shelf Science*,

434 161:38-45.

435 Clarke KR. 1993. Non-parametric multivariate analyses of changes in community structure.

436 *Australian Journal of Ecology*, 18:117-143.

437 Claudet J, Fraschetti S. 2010. Human-driven impacts on marine habitats: a regional meta-

438 analysis in the Mediterranean Sea. *Biological Conservation*, 143:2195-2206.

439 Crain CM, Kroeker K, Halpern BS. 2008. Interactive and cumulative effects of multiple human

440 stressors in marine systems. *Ecology Letters*, 11:1304-1315.

441 Crain CM, Halpern BS, Beck MW, Kappel CV. 2009. Understanding and Managing Human

442 Threats to the Coastal Marine Environment. *Ecology and Conservation Biology*, 1162: 39-62.

443 DOI: 10.1111/j.1749-6632.2009.04496.x.

444 Del Negro P, Crevatin E, Larato C, Ferrari C, Totti C, Pompei M, Giani M, Berto D, Fonda

445 Umani S. 2005. Mucilage microcosms. *Science of the Total Environment*, 353:258-269.

446 Dethier MN, Graham ES, Cohen S, Tera LM. 1993. Visual versus random-point percent cover

447 estimations: objective is not always better. Marine Ecology Progress Series, 96:93-100.

448 Devescovi M, Iveša L. 2007. Short term impact of planktonic mucilage aggregates on

449 macrobenthos along the Istrian rocky coast (Northern Adriatic, Croatia). *Marine Pollution

450 Bulletin*, 54:887-893. DOI:10.1016/j.marpolbul.2007.03.009.

451 Figueiredo MA de O, Steneck RS. 2000. Floristic and ecological studies of crustose coralline

452 algae on Brazil's Abrolhos reefs. *Bali: Proceedings 9th International Coral Reef Symposium*, 1.

453   Filbee-Dexter K, Scheibling RE. 2014. Sea urchin barrens as alternative stable states of collapsed

454   kelp ecosystems. *Marine Ecology*, 495:1-25. DOI:10.3354/meps10573.

455   Folt, CL, Chen CY, Moore MV, Burnaford J. 1999. Synergism and antagonism among multiple

456   stressors. *Limnology and oceanography*, 4:864-877.

457   Geertz-Hansen O, Sand-Jensen K, Hansen DF, Christiansen A. 1993. Growth and grazing

458   control of abundance of the marine macroalga, *Ulva lactuca* L. in a eutrophic Danish estuary.

459   *Aquatic Botany*, 46:101-109. DOI:10.1016/0304-3770(93)90039-Y.

460   Graham MH. 2004. Effects of Local Deforestation on the Diversity and Structure of Southern

461   California Giant Kelp Forest Food Webs. *Ecosystem*, 7:341-357.

462   Guarnieri G, Bevilacqua S, Vignes F, Fraschetti S. 2014. Grazer removal and nutrient

463   enrichment as recovery enhancers for overexploited rocky subtidal habitats. *Oecologia*, 175:959-

464   970.

465   Guidetti P, Fraschetti S, Terlizzi A, Boero F. 2003. Distribution patterns of sea urchins and

466   barrens in shallow Mediterranean rocky reefs impacted by the illegal fishery of the rock-boring

467   mollusc *Lithophaga lithophaga*. *Marine Biology*, 143:1135-1142. DOI: 10.1007/s00227-003-

468   1163-z.

469   Hauxwell J, Mcclelland J, Behr PJ, Valiela I. 1998. Relative Biomass Importance of Grazing and

470   Nutrient Controls of Macroalgal in Three Temperate Shallow Estuaries. *Estuaries*, 21:347-360.

471   Hecky RE, Kilham P. 1988. Nutrient limitation of phytoplankton in freshwater and marine

472   environments: a review of recent evidence on the effects of enrichment. *Limnology and*

473   *Oceanographhy*, 33:796-822.

474   Hereu, B, Zabala M, Linares C, Sala E. 2004. Temporal and spatial variability in settlement of

475   the sea urchin *Paracentrotus lividus* in the NW Mediterranean. *Marine Biology*, 144:1011-1018.

476 Hereu, B. 2005. Movement patterns of the sea urchin *Paracentrotus lividus* in a marine reserve

477 and an unprotected area in the NW Mediterranean. *Marine Ecology*, 26:54-62.

478 Huang, R, Boney AD. 1983. Effects of diatom mucilage on the growth and morphology of

479 marine algae. *Journal of Experimental Marine Biology and Ecology*, 67:79-89.

480 Jackson MC, Loewen CJG, Vinebrooke RD, Chimimba CT. 2016. Net effects of multiple

481 stressors in freshwater ecosystems: a meta-analysis. *Global Change Biology*, 22:180-189. DOI:

482 10.1111/gcb.13028.

483 Jentoft S, van Son TC, Bjørkan M. 2007. Marine Protected Areas: A Governance System

484 Analysis. *Human Ecology*, 35:611-622.

485 Jonker M M., Johns KK, Osborne KK 2008. Surveys of benthic reef communities using

486 underwater digital photography and counts of juvenile corals. Long-term Monitoring of the Great

487 Barrier Reef. *Standard Operational Procedure*.

488 Karez R, Engelbert S, Kraufvelin P, Pedersen MF, Sommer U. 2004. Biomass response and

489 changes in composition of ephemeral macroalgal assemblages along an experimental gradient of

490 nutrient enrichment. *Aquatic Botany*, 78:103-117. DOI:10.1016/j.aquabot.2003.09.008.

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

492 seagrasses and marine macroalgae. *Global Change Biology*, 19:103-132. DOI:10.1111/j.1365-

493 2486.2012.02791.x.

494 Kroeker KJ, Kordas RL, Harley CDG. 2017. Embracing interactions in ocean acidification

495 research: confronting multiple stressor scenarios and context dependence. *Biology Letters*.

496 DOI:10.1098/rsbl.2016.0802.

497 Lawrence JM. 1975. On the relationships between marine plants and sea urchins. *Oceanography*

498 *and Marine Biology, An Annual Review*, 13:213-286.

499   Lotze HK, Worm B. 2000. Variable and complementary effects of herbivores on different life

500   stages of bloom-forming macroalgae. *Marine Ecology-Progress Series*, 200:167-175.

501   Lugliè A, Satta C, Padedda B, Pulina S, Sechi N. 2008. What is *Chrysophaeum taylorii* Lewis &

502   Bryan doing in Sardinia (Tyrrhenian Sea, Mediterranean)? *Harmful Algae News*, 36:4-5.

503   McGlathery KJ. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-

504   enriched coastal waters. *Journal of Phycology*, 37:453-456. DOI:10.1046/j.1529-

505   8817.2001.037004453.x.

506   Millero, FJ, Woosley R, Ditrolio B, Waters J. 2009. Effect of ocean acidification on the

507   speciation of metals in seawater. *Oceanography,* 22:72– 85.

508   Mingazzini M, Thake B. 1995. Summary and conclusions of the workshop on marine mucilages

509   in the Adriatic Sea and elsewhere. Science of Total Environment, 165:9-14.

510   Misic C, Schiaparelli S, Covazzi Harriague A. 2011. Organic matter recycling during a mucilage

511   event and its influence on the surrounding environment (Ligurian Sea, NW Mediterranean).

512   *Continental Shelf Research*, 31:631-634. DOI:10.1016/j.csr.2010.12.016.

513   Müller WEG, Riemer S, Kurelec B, Smodlaka N, Puskaric S, Jagic B, Müller-Niklas G, Queric

514   NV 1998. Chemosensitizers of the multixenobiotic resistance in amorphous aggregates (marine

515   snow): etiology of mass killing on the benthos in the Northern Adriatic? *Environmental*

516   *Toxicology and Pharmacology*, 6:229-238.

517   Obernosterer I, Herndl GJ. 1995. Phytoplankton extracellular release and bacterial growth:

518   dependence on the inorganic N: P ratio. *Marine Ecology Progress Series*, 116:247-257.

519   Pedersen MF, Borum J. 1996. Nutrient control of algal growth in estuarine waters. Nutrient

520   limitation and the importance of nitrogen requirements and nitrogen storage among

521   phytoplankton and species of macroalgae. *Marine Ecology Progress Series*, 142:261-272.

522   Piazzi L, Ceccherelli G, Cinelli F. 2001. Threat to macroalgal diversity: effects of the introduced

523   green alga *Caulerpa racemosa* in the Mediterranean. *Marine Ecology Progress Series*, 210:149-

524   159.

525   Piazzi L, Bulleri F, Ceccherelli G. 2016. Limpets compensate sea urchin decline and enhance the

526   stability of rocky subtidal barrens. *Marine Environmental Research*, 115:49-55.

527   Reynolds SC. 2007. Variability in the provision and function of mucilage in phytoplankton:

528   facultative responses to the environment. *Hydrobiologia*, 578:37-45.

529   Rinaldi A, Vollenweidera RA, Montanari G, Ferrari CR, Ghetti A. 1995. Mucilages in Italian

530   seas: the Adriatic and Tyrrhenian Seas, 1988–1991. *Science of The Total Environment*, 165:165-

531   183. DOI: 10.1016/0048-9697(95)04550-K.

532   Ritson-William R, Arnold SN, Fogarty ND, Steneck RS, Vermeij MJA, Paul VJ. 2009. New

533   Perspectives on Ecological Mechanisms Affecting Coral Recruitment on Reefs. *Marine and*

534   *Environmental Science*, 38:437-457. DOI: 10.5479/si.01960768.38.437.

535   Rodríguez SR, Ojeda FP, Inestrosa NC. 1993. Settlement of benthic marine invertebrates.

536   *Marine Ecology-Progress Series*, 97:193-207.

537   Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke

538   LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NLR,

539   Sykes MT, Walker BH, Walker M, Wall DH. 2000. Global biodiversity scenarios for the year

540   2100. *Science*, 287:1770-1774.

541   Sala E, Ballesteros E, Dendrinos P, Di Franco A, Ferretti F, Foley D, Fraschetti S, Friedlander A,

542   Garrabou J, Güçlüsoy H, Guidetti P, Halpern BS, Hereu B, Karamanlidis AA, Kizilkaya Z,

543   Macpherson E, Mangialajo L, Mariani S, Micheli F, Pais A, Riser K, Rosenberg AA, Sales M,

544   Selkoe KM, Starr R, Tomas F, Zabala M. 2012. The Structure of Mediterranean Rocky Reef

545   Ecosystems across Environmental and Human Gradients, and Conservation Implications. *PloS*

546   *ONE*, 7:e32742. DOI:10.1371/journal.pone.0032742.

547   Schaffelke B, Heimann K, Marshall PA, Ayling AM. 2004. Blooms of *Chrysocystis fragilis* on

548   the Great Barrier Reef. *Coral Reefs*, 23:514-514.

549   Scheffer M, Carpenter S, Foley JA, Folke C, Walker B. 2001. Catastrophic shifts in ecosystems.

550   *Nature*, 413:591-596. DOI:10.1038/35098000.

551   Scheibling R. 1986. Increased macroalgal abundance following mass mortalities of sea urchins

552   (*Strongylocentrotus droebachiensis*) along the Atlantic coast of Nova Scotia. *Oecologia*, 68:186-

553   198.

554   Schiaparelli S, Castellano M, Povero P, Sartoni G, Cattaneo-Vietti R. 2007. A benthic mucilage

555   event in North-Western Mediterranean Sea and its possible relationships with the summer 2003

556   European heatwave: short term effects on littoral rocky assemblages. *Marine Ecology*, 28:341-

557   353. DOI:10.1111/j.1439-0485.2007.00155.x.

558   Smith JE, Smith CM. 2001. An experimental analysis of the effects of herbivory and nutrient

559   enrichment on benthic community dynamics on a Hawaiian reef. *Coral Reefs*, 19:332-342. DOI:

560   10.1007/s003380000124.

561   Smith VH, Schindler DW. 2009. Eutrophication science: where do we go from here?. *Trends in*

562   *Ecology & Evolution*, 24:201-207.

563   Smith JE, Hunter CL, Smith CM. 2010. The effects of top-down versus bottom-up control on

564   benthic coral reef community structure. *Oecologia*. DOI 10.1007/s00442-009-1546-z.

565   Sokolova IM. 2013. Energy-Limited Tolerance to Stress as a Conceptual Framework to Integrate

566   the Effects of Multiple Stressors. *Integrative & Comparative Biology*, 57:597-608.

567   DOI:10.1093/icb/ict028.

568 Sotka EE, Hay ME. 2009. Effects of herbivores, nutrient enrichment, and their interactions on

569 macroalgal proliferation and coral growth. *Coral Reefs*, 28:555-568. DOI: 10.1007/s00338-009-

570 0529-1.

571 Teichberg M, Fox SE, Aguila C, Olsen YS, Valiela I. 2008. Macroalgal responses to

572 experimental nutrient enrichment in shallow coastal waters: growth, internal nutrient pools, and

573 isotopic signatures. *Marine Ecology Progress Series*, 368:117-126. DOI: 10.3354/meps07564.

574 Underwood AJ. 1997. Experiments in ecology: their logical design and interpretation using

575 analysis of variance. *Cambridge University Press*, Cambridge.

576 Underwood AJ. 1980. The effects of grazing by gastropods and physical factors on the upper

577 limits of distribution of intertidal macroalgae. *Oecologia*, 46:201-213.

578 Vinebrooke RD, Cottingham KL, Norberg MS, Dodson SI, Maberly SC, Sommer U. 2004.

579 Impacts of multiple stressors on biodiversity and ecosystem functioning: The role of species

580 co-tolerance. *Oikos,* 104:451-457.

581 Worm B, Sommer U. 2000. Rapid direct and indirect effects of a single nutrient pulse in a

582 seaweed-epiphyte-grazer system. *Marine Ecology Progress Series*, 202:283-288.

583 Zeidberg LD, Robinson BH. 2007. Invasive range expansion by the Humboldt squid, *Dosidicus*

584 *gigas*, in the eastern North Pacific. *Proceedings of the National Academy of Sciences*,

585 104:12948-12950.

586

587
588
589
590
591
592
593
594
595

596
597
598
599

Figure 1

Localization of the study area

Localization of the study area in Tavolara Punta Coda Cavallo Marine Protected Area. The differently protected zones of the MPA are also indicated: light grey indicates C zones; middle grey B zones and dark grey A zones.

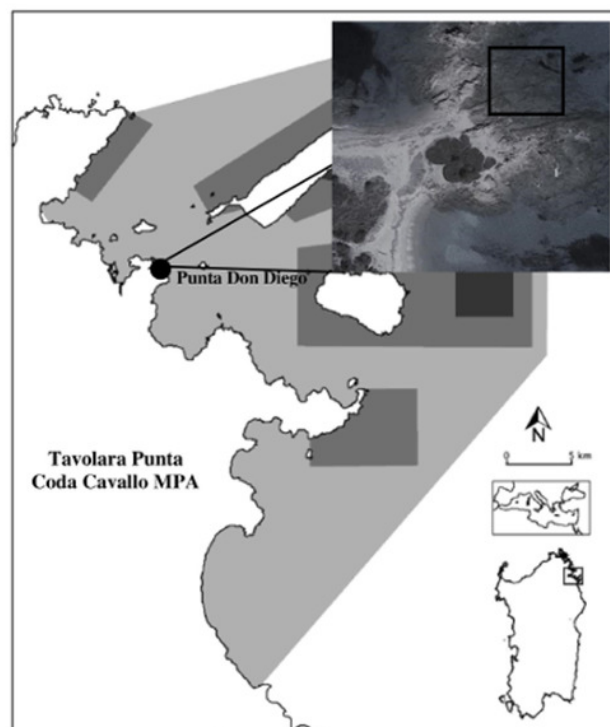



Figure 2

Experimental design and nMDS

In the experimental design (A) 3 grazing levels were considered (G100%, G50% and G0% referred to total, partial and no removal of macroalgae; for nutrient enrichment 2 levels were considered (E+ vs E- referred to enriched and non-enriched) as well as for mucilage (M+ vs M- referred to plots with and without mucilage). nMDS was performed on short- (8th week, B) and long-term (36th week, C) data. nMDS ordination was not significant on short-term.

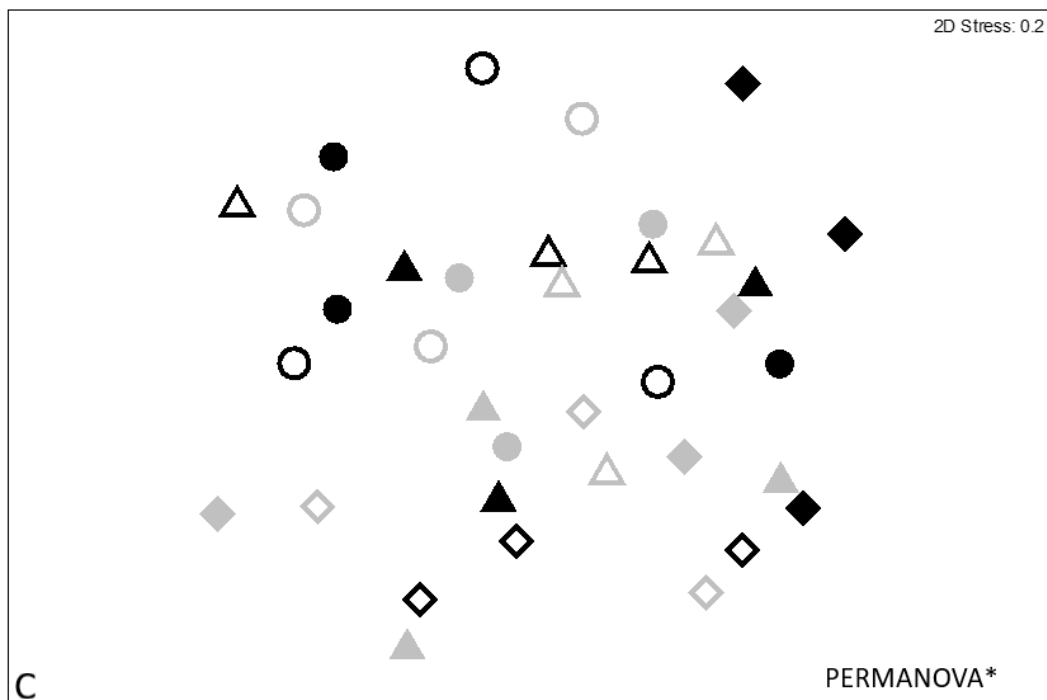
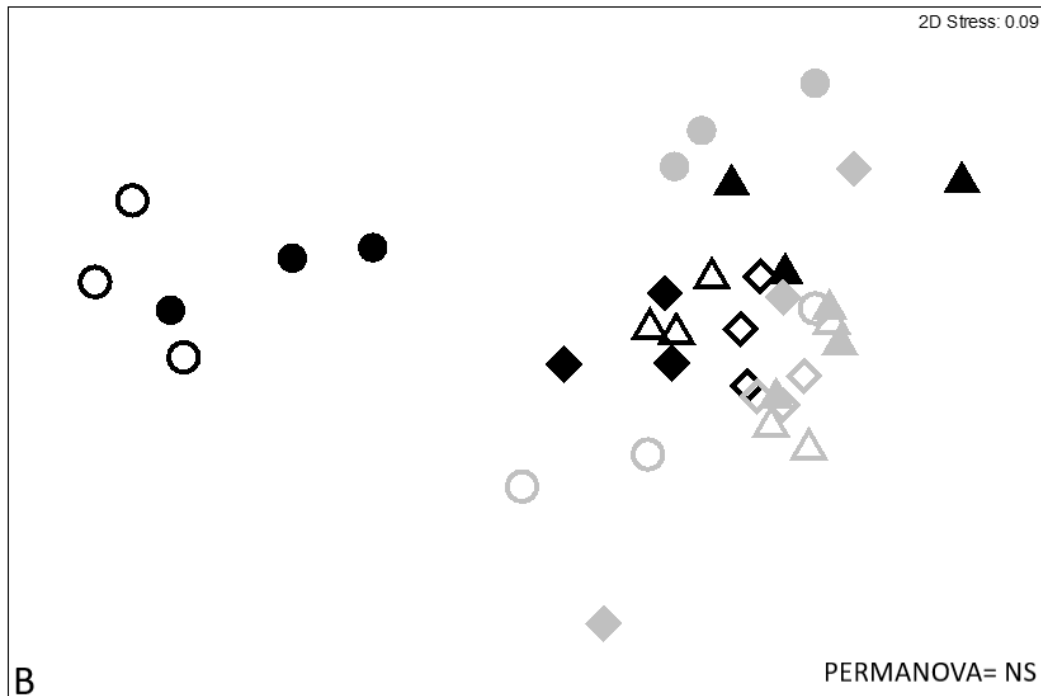
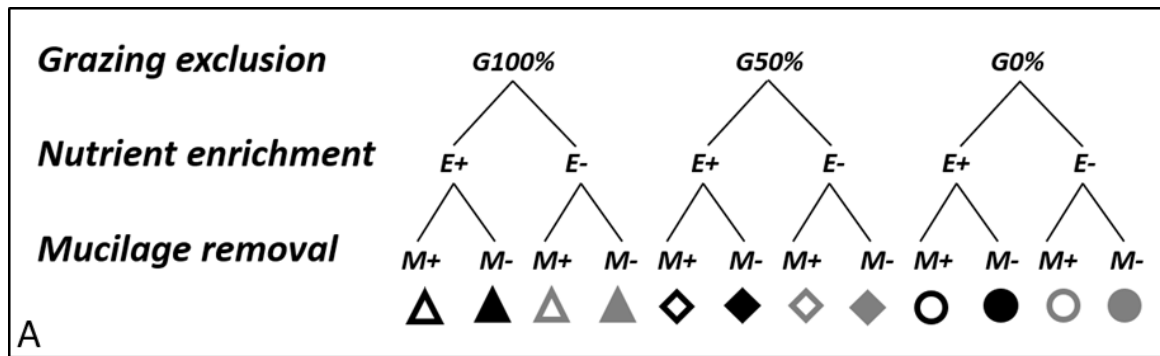
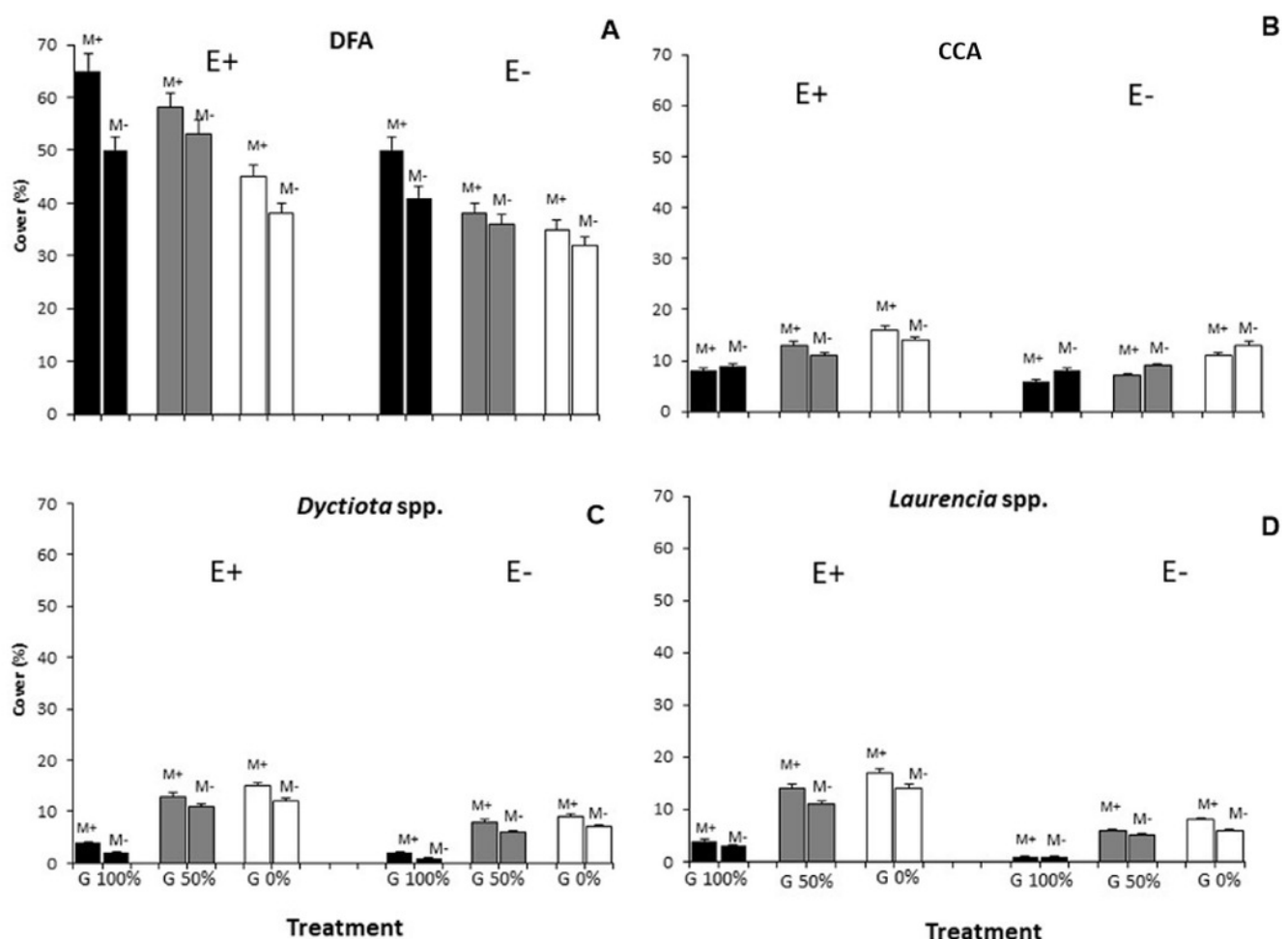



Figure 3

Main taxa percent cover

Percent cover (mean % ± SE, n = 20) of the 4 main taxa (dark filamentous algae (DFA), crustose coralline algae (CCA), *Dictyota* spp. and *Laurencia* spp.) for each combination of treatments: nutrient enrichment (E+ and E-); grazing (100%, 50% and 0% of macroalgal removal); mucilage (M+ and M-)).

8 weeks after the manipulation

Nutrient enrichment effectiveness

Nutrient enrichment effectiveness. Mean nutrient (inorganic N and P) concentration between nutrient addition and control plots (E+ and E-) on each sampling time.

Time	Treatment	Inorganic N (µM)			Inorganic P (µM)		
		Mean (n=10)	Stan. dev.	t-test (P)	Mean (n=10)	Stan. dev.	t-test (P)
8th week	E+	0.720	0.00039	0.0001	0.170	0.00027	0.0003
8th week	E-	0.290	0.00035		0.001	0.00013	
36th week	E+	0.780	0.00034	0.0002	0.190	0.00031	0.0001
36th week	E-	0.320	0.00021		0.010	0.00021	

PERMANOVA results at short-term (8 weeks)

Results of permutational multivariate analyses of variance (PERMANOVA) testing the effect of nutrient enrichment (*E*), grazing exclusion (*G*) and mucilage removal (*M*) on the structure of macroalgal assemblages at short-term (8 weeks). Analyses were based on Bray-Curtis dissimilarities and each test was performed using 9.999 permutations of appropriate units. Significant P-values are given in bold.

Source of variation	df	SS	MS	F	P (perm.)
Nutrient enrichment (E)	1	1538.0381	1538.0381	23.3065	**0.0001**
Grazing exclusion (G)	2	3080.7599	1540.3799	23.3420	**0.0001**
Mucilage removal (M)	1	246.5692	246.5692	3.7364	**0.0348**
$E \times G$	2	1428.6083	714.3041	10.8241	**0.0001**
$E \times M$	1	192.8727	192.8727	2.9227	0.0656
$G \times M$	2	292.5294	146.2647	2.2164	0.0824
$E \times G \times M$	2	286.0645	143.0322	2.1674	**0.0472**
Residual	24	1583.8026	65.9918		
Total	35	8649.2446			

ANOVA results

Results of ANOVAs on the effect of each treatment (nutrient enrichment (E), grazing exclusion (G) and mucilage removal (M) on the percent cover of DFA, CCA, *Dictyota* spp. and *Laurencia* spp. at short-term (8 weeks). Significant P-values are given in bold.

Source of variation	df	DFA		CCA		Dictyotales		*Laurencia spp.*	
		F	P	F	P	F	P	F	P
Nutrient enrichment (*E*)	1	5.68	**0.0254**	69.84	**0.0000**	0.52	**0.0471**	67.83	**0.0000**
Grazing exclusion (*G*)	2	2.24	**0.0388**	32.29	**0.0000**	37.10	**0.0000**	48.23	**0.0000**
Mucilage removal (*M*)	1	7.36	**0.0122**	1.92	**0.0383**	**0.12**	**0.0252**	0.25	**0.0213**
E x *G*	2	9.06	**0.0012**	8.52	**0.0016**	9.60	**0.0009**	22.80	**0.0000**
E x *M*	1	9.06	**0.0012**	0.32	0.5792	0.01	0.9102	8.75	**0.0069**
G x *M*	2	3.47	**0.0473**	3.62	**0.0424**	0.45	0.6451	0.54	0.5918
E x *G* x *M*	2	1.88	**0.0487**	2.71	**0.0469**	0.12	**0.0490**	1.37	**0.0373**
Residual	24								
Total	35								
Cochran's test (*C*)		0.3556 (NS)		0.3542 (NS)		0.3453 (NS)		0.3996 (NS)	

SNK results

Results of SNK test on ExGxM interaction for the 4 main taxa for: nutrient enrichment (E+ vs E-), grazing exclusion (G100%, G50% and G0%) and mucilage removal (M+ vs M-).

DFA	Mucilage removal	Nutrient enrichment
Grazing exclusion **M+** **E+** G100%>G50%=G0% **E-** G100%=G50%=G0% **M-** **E+** G100%>G50%>G0% **E-** G100%=G50%>G0%	**G100%** **E+** M+<M- **E-** M+=M- **G50%** **E+** M+=M- **E-** M+=M- **G0%** **E+** M+=M- **E-** M+=M-	**G100%** **M+** E+=E- **M-** E+>E- **G50%** **M+** E+=E- **M-** E+>E- **G0%** **M+** E+=E- **M-** E+=E-
CCA	Mucilage removal	Nutrient enrichment
Grazing exclusion **M+** **E+** G100%<G50%<G0% **E-** G100%=G50%=G0% **M-** **E+** G100%<G50%<G0% **E-** G100%=G50%=G0%	**G100%** **E+** M+=M- **E-** M+=M- **G50%** **E+** M+=M- **E-** M+=M- **G0%** **E+** M+<M- **E-** M+=M-	**G100%** **M+** E+=E- **M-** E+=E- **G50%** **M+** E+=E- **M-** E+=E- **G0%** **M+** E+>E- **M-** E+>E-
Dictyotales	Mucilage removal	Nutrient enrichment
Grazing exclusion **M+** **E+** G100%=G50%=G0% **E-** G100%=G50%=G0% **M-** **E+** G100%<G50%=G0% **E-** G100%=G50%=G0%	**G100%** **E+** M+=M- **E-** M+=M- **G50%** **E+** M+<M- **E-** M+=M- **G0%** **E+** M+<M- **E-** M+=M-	**G100%** **M+** E+=E- **M-** E+=E- **G50%** **M+** E+=E- **M-** E+>E- **G0%** **M+** E+=E- **M-** E+>E-
Laurencia **spp.**	Mucilage removal	Nutrient enrichment
Grazing exclusion **M+** **E+** G100%=G50%=G0% **E-** G100%=G50%=G0% **M-** **E+** G100%<G50%=G0% **E-** G100%=G50%=G0%	**G100%** **E+** M+=M- **E-** M+=M- **G50%** **E+** M+<M- **E-** M+=M- **G0%** **E+** M+<M- **E-** M+=M-	**G100%** **M+** E+=E- **M-** E+=E- **G50%** **M+** E+=E- **M-** E+>E- **G0%** **M+** E+=E- **M-** E+>E-