

A peer-reviewed version of this preprint was published in PeerJ on 13 May 2019.

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Caronni S, Calabretti C, Citterio S, Delaria MA, Gentili R, Macri G, Montagnani C, Navone A, Panzalis P, Piazza G, Ceccherelli G. 2019. The interactive effect of herbivory, nutrient enrichment and mucilage on shallow rocky macroalgal communities. PeerJ 7:e6908
<https://doi.org/10.7717/peerj.6908>

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

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In this paper the results of a manipulative experiment aimed to evaluate the interactive short- and long-term effect of three different stressors, herbivory, nutrient and mucilage, on a macroalgal assemblage are presented. The experiment was conducted in Tavolara Punta Coda Cavallo Marine Protected Area during a bloom of the benthic mucilage-producing microalga *Chrysophaeum taylorii* Lewis and Bryan (*Pelagophyceae*), recently spreading in the Mediterranean Sea. On a rocky substratum, 18 plots 20x20 cm in size were prepared and, according to different treatments, nutrients were added in some of them to simulate eutrophication, macroalgae were removed to simulate clearings produced by grazers and mucilage was manually removed to simulate mucilage-free conditions. Differences in the composition of macroalgal assemblages were found when considering the short term effect of the considered stressors, and also the response of the most abundant taxa (DFA, ECA, Dictyotales, *Laurencia* spp. and *Padina pavonica*) varied among treatments, proving that a combined effect of such stressors on the recovery of macroalgae was present. On the contrary, the effect of treatments was neither highlighted on the most abundant algae nor on the whole structure of the macroalgal assemblage.

1 **The interactive effect of herbivory, nutrient enrichment**
2 **and mucilage on shallow rocky macroalgal communities**
3

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42 Abstract

43 In this paper the results of a manipulative experiment aimed to evaluate the interactive short- and
44 long-term effect of three different stressors, herbivory, nutrient and mucilage, on a macroalgal
45 assemblage are presented. The experiment was conducted in Tavolara Punta Coda Cavallo
46 Marine Protected Area during a bloom of the benthic mucilage-producing microalga
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48 Mediterranean Sea. On a rocky substratum, 18 plots 20x20 cm in size were prepared and,
49 according to different treatments, nutrients were added in some of them to simulate
50 eutrophication, macroalgae were removed to simulate clearings produced by grazers and
51 mucilage was manually removed to simulate mucilage-free conditions.

52 Differences in the composition of macroalgal assemblages were found when considering the
53 short term effect of the considered stressors, and also the response of the most abundant taxa
54 (DFA, ECA, Dictyotales, *Laurencia* spp. and *Padina pavonica*) varied among treatments,
55 proving that a combined effect of such stressors on the recovery of macroalgae was present. On
56 the contrary, the effect of treatments was neither highlighted on the most abundant algae nor on
57 the whole structure of the macroalgal assemblage.

58

59 **Keywords:** interactive effects, multiple stressors, macroalgae, mucilage, Mediterranean Sea.

60

61 1 Introduction

62 Marine ecosystems and especially near-shore coastal areas are typically subjected to several
63 abiotic and biotic stressors, both natural and anthropogenic, which can seriously affect the
64 structure of habitats and often lead to regime shifts nearly irreversible (Guarnieri et al., 2014;
65 Adams, 2005). A substantial research effort has been therefore done to investigate the effects of
66 the most widespread threats in marine environment (Crain et al., 2009) but understanding the
67 complex effects of multiple stressors on marine communities still represents one of the major
68 current challenges in marine ecology (Sala et al., 2000. Zeidberg & Robison 2007, Guarnieri et
69 al., 2014), and whether stressors are more harmful in combination than alone is still widely
70 unanswered.

71 In fact, several scenarios can occur when multiple stressors act simultaneously, as their
72 interactive effects can be merely cumulative, synergistic or antagonistic (Vinebrooke at al.,
73 2004). Two or more stressors are defined as cumulative if their result is the mere additive effect
74 of each of them while they are synergistic or antagonistic when their combined effect is
75 respectively larger or smaller than the one expected evaluating each single stressor (Crain,
76 Kroeker & Halpern, 2008).

77 In coastal ecosystems, canopy macroalgae play critical ecological roles (Koch et al., 2013),
78 contributing significantly to total primary production and deeply affecting higher trophic levels
79 (Peckol & Rivers, 1996). In fact, they provide the three-dimensional structure of marine faunal
80 habitats and facilitate larval settlement of marine invertebrates (Ritson-Williams et al., 2009). In
81 the last decades, shallow temperate reefs are experiencing a dramatic reduction and loss of
82 macroalgal habitats and their replacement by persistent barren grounds are of increasing concern
83 (Guidetti et al. 2003; Sala et al. 2012). Several studies proved the importance of both herbivory
84 pressure and nutrient enrichment in defining the composition and the abundance of macroalgal

85 communities (i.e. Karez et al., 2004; Arévalo, Pinedo & Ballesteros, 2007). Particularly,
86 herbivores (mainly sea-urchins and limpets) increase related to the over-exploitation of large-
87 sized predators, and produce a rapid shift in dominance from canopy forming to encrusting
88 macroalgae with the creation of persistent barrens worldwide (Filbee-Dexter & Scheibling, 2014;
89 Piazzzi, Bulleri & Ceccherelli, 2016). Furthermore, the role of nutrient enrichment in determining
90 macroalgal abundance has been extensively debated in the last two decades (i.e. McGlathery,
91 2001; Armitage et al., 2005) and recently it has been highlighted that nutrients mediate
92 herbivores grazing and algal growth (i.e. eutrophication sustain higher grazing pressure),
93 modifying thresholds of regime shifts (Boada et al., 2017).

94 Mucilage has to be included among the threats that currently concur to affect a suite of
95 response variables in coastal water ecosystems (Claudet & Fraschetti, 2010) and its effect can be
96 detrimental especially on benthic organisms and, in particular, on macroalgae. Although no
97 deleterious effects of mucilage have been ascertained on macroalgal populations yet, primary
98 branches usually show signs of necrosis after blooms (Devescovi & Iveša, 2007). Moreover,
99 Mistic, Schiaparelli & Covazzi Harriague (2011) assert that the persistence of mucilage on hard
100 substrata can overgrow algae and cause macroalgal depletion. Finally, large portions of the thick
101 mucilage carpet that usually covers the substratum during blooms can be strengthened by the
102 continuous twisting action of currents, engulfing and mechanically detaching macroalgae,
103 especially those with an erect and frondose habitus (Schiaparelli et al., 2007).

104 Several studies have investigated the interactive effects of nutrient enrichment and
105 herbivory on macroalgal communities (i.e. Lapointe, Barile & Matzie, 2004; Guarneri et al.,
106 2014) and nowadays there is a growing evidence that they interact detrimentally (Worm et al.,

107 2002). No information on the effects of the interaction among nutrient enrichment, herbivory and
108 mucilage on macroalgal communities are instead available yet.

109 In this paper the results of a manipulative experiment aimed to evaluate the interactive
110 short- and long-term effect of the three stressors (herbivory, nutrient and mucilage) on
111 macroalgal assemblages are presented. The experiment was conducted during a bloom of the
112 benthic mucilage-producing microalga *Chrysothrix taylorii* Lewis and Bryan
113 (*Pelagophyceae*), recently spreading in the Mediterranean Sea (Caronni et al. 2014; Caronni et
114 al., 2015). Following a complete full-factorial design, nutrients were added to simulate
115 eutrophication, macroalgae were removed to simulate clearings produced by grazers and
116 mucilage was manually removed to simulate mucilage-free conditions. We predict that the
117 mucilage would buffer the effect of nutrient enrichment on macroalgal abundance (antagonistic
118 effect). On the contrary, mucilage would reasonably worsen the effects of herbivory, inhibiting
119 macroalgal recovery after massive grazing events and enhancing the development of permanent
120 barrens (synergistic effect). Considering that, nutrient enrichment seems to act especially when
121 herbivores are absent (Burkpile & Hay, 2006; Guarnieri et al., 2014), the effect of mucilage was
122 expected to be greater in non-enriched conditions when herbivores were present.

123

124

125 **2 Materials and methods**

126

127 *Study site and experimental design*

128

129 The manipulative field experiment was done in Tavolara Punta Coda Cavallo Marine
130 Protected Area (hereafter TPCC MPA, North-East Sardinia, Western Mediterranean), during
131 summer 2014. Punta Don Diego Bay (40°52'34.62'' N; 9°39'21.19'' E), located in a C zone of

132 the MPA, was chosen for the experiment as *C. taylorii* blooms have been recurrently abundant
133 there in the recent years (Caronni et al. 2014).

134 Two rocky areas of about 10 m² (20 m apart) were randomly chosen in the bay at 1.5 m of
135 depth (highest *C. taylorii* cell density depth, Caronni et al. 2015). In each area 18 plots 20x20 cm
136 in size were prepared. Three treatments (each corresponding to a stressor) were randomly
137 assigned to the plots following a completely orthogonal design (n = 3). To the purpose, with
138 regard to mucilage, the mucilaginous aggregates were manually removed from half of the plots
139 (M-), while mucilage was not manipulated in the other half of them (M+). The effect of grazing
140 by herbivores was obtained by scraping off the substratum using an iron brush at three levels:
141 total (G100%), partial (G50%) and no (G0%) removal of macroalgae. Finally, nutrient addition
142 was obtained in half of the plots (E+ vs E- refereed to enriched and non-enriched) using small-
143 mesh nylon bags (2 mm mesh size) filled with slow-release fertilizer pellets (Osmocote®;
144 18:9:10, N:P:K) following Bulleri, Russell & Connell (2012) and Guarnieri et al. (2014). The
145 bags were fixed to a brick and positioned on the rocky bottom at the edge of each unit. Overall,
146 40 g of fertilizer were added in each plot, placing two bags with 20 g of pellet at two opposite
147 sides of the plot. The amount of fertilizer in each bag was decided according to previous studies
148 (Worm & Sommer 2000). In order to ensure enriched conditions throughout the experiment,
149 nutrient bags were monthly replaced. Furthermore, to avoid nutrient enrichment of control plots,
150 nutrient addition was assigned to plots segregated within the same area.

151 The concentration of nutrients (N and P) in the water was monitored two times from July to
152 September 2014; 10 water samples (125 ml) in each area were randomly taken in July (T₁) and
153 August (T₂). Samples were taken at approximately 10 cm from the bottom and at least 50 cm
154 apart from nutrient bags. After collection, water samples were shaken, filtered (0.45-µm mesh

155 size filter) and frozen, as suggested by Balata et al. (2010). They were then transported to the
156 University of Pavia, where the concentrations of inorganic N and P (ammonia, nitrate, nitrite and
157 phosphate) were estimated. Chemical analyses of water samples confirmed that for both nutrients
158 differences due to the enrichment were significant and consistent during the whole experiment
159 (ANOVA_{TxE} $F_{1,36} = 1272.5$, $P < 0.01$ for N, $F_{1,36} = 1557.4$, $P < 0.01$ for P) (Fig. 1).

160 In each plot the benthic assemblages were sampled on 3 times, the beginning of July (T_0)
161 and the beginning of September (T_1) 2014, to evaluate the short term effects, and in March 2015
162 (T_2) to investigate the long-term effects of the three stressors. The assemblages in each plot were
163 sampled photographically using a Nikon Coolpix AW130 underwater camera (16 Megapixel).
164 The percent cover (%) of each macroalgal taxon was assessed on a computer screen,
165 superimposing a grid of twenty-five sub-quadrats onto each image, scoring each sub-quadrat
166 from 0 to 4% and adding the 25 resulting values to obtain the total cover.

167 *Statistical analyses*

168 A distance-based permutational multivariate analysis of variance (PERMANOVA)
169 (Anderson, 2001) was performed (Anderson, 2005) to analyse the response of the macroalgal
170 assemblage to experimental conditions across time. The analyses were based on Bray–Curtis
171 dissimilarities calculated on non-transformed data. Each term in the analysis was tested using
172 9,999 random permutations. To test for short and long term effects of treatments and to analyse
173 independent data (Underwood, 1997), two PERMANOVAs on data collected at T_1 and T_2 ,
174 respectively, were performed. The experimental design consisted of three factors: nutrient
175 enrichment (two levels, fixed), grazing (three levels, fixed and orthogonal) and mucilage (two
176 levels, fixed and orthogonal). Significant terms relevant to the hypotheses were investigated
177 through post hoc pair-wise comparisons. Furthermore, a SIMPER test was run (Primer v6) to

178 point out the relative contribution of each taxon to the dissimilarities among treatments (Clarke,
179 1993); finally, a multidimensional scaling (MDS) was used to graphical ordination of data.

180 To investigate the effect of treatments on the taxa evidenced by SIMPER, a three-way
181 ANOVA was also run for each taxon abundance. Cochran's test was run prior to each ANOVA
182 to test for homogeneity of variances and Student–Newman–Keuls (SNK) tests were used for *a*
183 *posteriori* comparisons of means (Underwood, 1997).

184

185 **3 Results**

186 **3.1 Short-term macroalgal response to disturbance**

187 At the end of the experiment, in September (T_1), 8 macroalgal taxa/morphological groups were
188 on the whole found: *Acetabularia acetabulum* (L.) Silva. dark filamentous algae (DFA),
189 *Dasycladus* spp., Encrusting coralline algae (ECA), Dictyotales, *Laurencia* spp., *Liagora* spp.,
190 and *Padina pavonica* (L.) Thivy. The combination of the three stressors affected on the short-
191 term the recovery of disturbed assemblages (PERMANOVA significant $E \times G \times M$ interaction,
192 Tab. 1). Pair-wise comparisons showed significant differences between enriched and non-
193 enriched plots only when the other two stressors were not present or when their effect was low
194 (M-G0% and M-G50%). Furthermore, in non-enriched conditions significant differences due to
195 the mucilage were recorded, especially in plots where the grazing pressure was high (E-
196 M+G100%; \neq E-M-G100%). On the contrary, the same effect was not observed in enriched plots.
197 Additionally, the effect of mucilage was not detected where the grazing pressure was null or low,
198 in both enriched and non-enriched plots (E+G0% and E-G0%), as MDS ordination clearly
199 depicted (Fig. 2).

200 Differences in the composition of macroalgal assemblages were found due to the nutrient and
201 mucilage manipulation (SIMPER test) that were mainly due to the cover of dark filamentous
202 algae and encrusting coralline algae. Conversely, with regard to grazing also the abundance of
203 two erect macroalgae, Dictyotales and *Laurencia* spp., remarkably contributed to the
204 dissimilarities. Finally, the ANOVAs performed on these taxa (DFA, ECA, Dictyotales and
205 *Laurencia* spp.) all detected differences for the E×G×M interaction term (Table 2; Fig. 3).
206 Particularly, the Dark filamentous algae seemed more abundant in enriched plots, especially
207 where mucilage and macroalgae had been removed (E+M-G100%), while a higher percent cover
208 of encrusting coralline algae was observed where nutrients had been increased and the grazing
209 pressure was null, independently from mucilage presence (E+M-G0% and E+M+G0%). Finally,
210 both the two erect species (Dictyotales and *Laurencia* spp.) were more abundant in enriched
211 plots, where the grazing pressure was null or low and mucilage had been removed (E+M-G0%
212 and E+M-G50%) (Tab. 2; Fig. 3).

213 **3.2 Long-term macroalgal response to disturbance**

214 On the last sampling effort, in March 2015 (T₂), only 6 macroalgal taxa/groups were on the
215 whole found and the effect of treatments (nutrient enrichment, grazing and mucilage) was neither
216 highlighted on the most abundant algae (DFA, ECA, Dictyotales, *Laurencia* spp. and *P.*
217 *pavonica*) nor on the whole structure of the macroalgal assemblage (Tab. 2 and 3).

218

219 **4 Discussion**

220 A combined effect of the three considered stressors on the short-term recovery of the considered
221 macroalgal assemblages was underlined by the results of the study. First of all, significant
222 differences in the composition and abundance of macroalgal assemblages between enriched and

223 non-enriched plots were observed but only when the effect of the other two stressors (mucilage
224 and grazing) was low or null.

225 These results are in accordance with those of other studies investigating the role of nutrient
226 enrichment in determining macroalgal abundance (*e.g.* McGlathery, 2001; Teichberg et al., 2008;
227 Sotka & Hay, 2009) and confirm that, when nutrient enrichment is the only stressor, it leads to a
228 remarkable increase of total macroalgal biomass, facilitating the occurrence of blooms.
229 Analysing in detail the response to nutrient addition of the different macroalgal taxa present in
230 the study area, an enhancing effect of the above mentioned factor was observed for both turf-
231 forming and erect macroalgae. In enriched plots where no other stressors acted, indeed, the
232 assemblages were more abundant, in terms of percent cover, of both turf-forming and erect
233 species suggesting that a positive effect of enrichment also on the abundance erect macroalgae
234 was present, as already suggested by Bulleri, Russell & Connell (2012), contrarily to what
235 asserted by Fujita, (1985) and Pedersen & Borum (1996), who observed an enhancement of turf-
236 forming algae, and in particular of DFA only.

237 No relevant differences in the composition of macroalgal assemblages exposed to nutrient
238 enrichment were instead observed when one of the other two considered stressors acted
239 simultaneously.

240 In particular, a buffering effect of mucilage on nutrient enrichment was noticed as where
241 mucilage was present no significant differences in macroalgal assemblages were recorded
242 between enriched and non-enriched plots. These results can be explained considering that
243 aggregates are biota-rich environments where the concentration of nutrients can be orders of
244 magnitude higher than in the surrounding seawater (Del Negro et al., 2005). Moreover, mucilage
245 might be responsible for the sequestration from the water column of large amount of nutrients

246 necessary to microalgae embedded inside the aggregates to survive and proliferate (Reynolds,
247 2007). For this reason it is plausible to assert that in plots with mucilage only a small amount of
248 the nutrients released in the water was available for macroalgae proliferation, as a conspicuous
249 portion of them was instead sequestered by mucilage and used by microalgae. Furthermore,
250 even if Huang and Boney observed that, in laboratory conditions, the growths of some species of
251 green and brown algae were enhanced by diatoms mucilage, mucilaginous aggregates are
252 generally known to overgrow macroalgae causing their mechanical suffocation and rapidly
253 leading to their biomass depletion (Misic, Schiaparelli & Covazzi Harriague, 2011). Moreover,
254 Müller et al. (1998) assumed that all benthic organisms are seriously damaged by mucilage even
255 if they are only lightly covered by aggregates because they contain toxins directly affecting vital
256 mechanisms of cell metabolism. Therefore, where mucilage and nutrient enrichment acted
257 simultaneously, the expected increase in macroalgal abundance due to nutrient enrichment could
258 have been significantly lowered or even nullify by the presence of mucilage covering the
259 substratum.

260 The positive effect of nutrient addition seemed not to be buffered by mucilage only for
261 encrusting coralline algae, the abundance of which was equal in all enriched plots, both with and
262 without mucilage. Even if Schiaparelli et al. (2007) and Figueiredo & Steneck (2000) suggested
263 that coralline algae could be seriously damaged by mucilage (especially when its presence on the
264 substratum lasted for a long time), the obtained results can be explained considering that Bulleri
265 (2006) proved their ability to survive to long periods of overgrowing by other species.

266 As for mucilage, also the effect of grazing seemed to buffer that of nutrient addition. About this,
267 Guarnieri et al. (2014) observed a relatively constant macroalgal cover, also in nutrient addition
268 conditions, when a high grazing pressure acted, thus proving that the presence of herbivores can

269 strongly lower the proliferation of macroalgae, even if nutrients were added, buffering the
270 positive effect of enrichment. Therefore, even if the results of several previous experiments
271 conducted on this topic suggested that both increased nutrient loading and reduced grazer
272 densities favour an intense macroalgal growth (*e.g.* Geertz-Hansen et al., 1993; Hauxwell et al.,
273 1998; Lotze & Worm, 2000), the reduction of herbivory seems to be the main factor triggering
274 the restoration process of macroalgal assemblages after disturbance (Scheffer et al., 2001). The
275 role of nutrient enrichment instead appear to be only secondary. Nevertheless, herbivory does not
276 represent the exclusive process in structuring macroalgal assemblages, as the obtained results
277 prove the role of the nutrient background in mediating the outcomes of grazing pressure, as
278 stated by Burkepile & Hay (2006).

279 Also the absence of significant differences between plots with and without mucilage recorded
280 where the grazing pressure was null or low prove the primary role of herbivores in regulating the
281 abundance of macroalgae (Lawrence, 1975; Underwood, 1980; Scheibling, 1986; Geertz-Hansen
282 et al., 2003) and demonstrate that the effect of mucilage can be considered as secondary too. This
283 last hypothesis is supported also by the significant differences in macroalgal assemblages with
284 and without mucilage recorded in non-enriched conditions but not in enriched ones, suggesting
285 that the enhancement produced by nutrient addition can be so conspicuous to nullify the negative
286 effect of mucilage.

287 Therefore, the effect of mucilage seems to be really detrimental only where communities are
288 stressed by high densities of herbivores and where nutrients are not so abundant to remarkably
289 increase macroalgal biomass because in such conditions, damages caused by both mucilage
290 suffocation and mechanical detachment of macroalgae, and especially of erect and frondose
291 species (Schiapparelli et al., 2007; Lugliè et al., 2008), are not worsened by grazing pressure and

292 balanced by nutrient addition. Also the lower abundance of erect species such as Dictyotales and
293 *Laurencia* spp. confirm that erect species are more damaged by mucilage than turf-forming ones.
294 Furthermore, turf-forming species appeared to be more abundant also where macroalgae were
295 removed because in such conditions a great availability of free space was present and the lower
296 rates of competition favored the development of opportunistic species such as turf-forming
297 macroalgae (Bulleri, Russell & Connell, 2012).

298

299

300

301 **5 Conclusions**

302 The results obtained considering the short-term response of macroalgal communities to the
303 considered multiple stressors suggest that the effect of both mucilage and grazing appear to be
304 antagonistic to nutrient enrichment while mucilage presence and herbivore pressure seem to act
305 synergistically on them.

306 Finally, quite different results were, instead, obtained considering the long term response to
307 disturbance of macroalgal communities as no effect of the three considered stressors was
308 observed after about eight months from the beginning of the study. In March 2015, indeed, only 6
309 taxa were observed on the substratum but this reduction is ascribable to the season, as the other
310 taxa observed in summer are typical of hot seasons (Piazzi, Ceccherelli & Cinelli, 2001). These
311 results therefore prove the high resilience and the good restoration ability of well conserved and
312 healthy communities, such as those of marine protected areas where there the enforcement of
313 protection is effective (even in the C zones). In such conditions indeed the effects of

314 disturbances, even when they are produced by different stressors acting together, appear not to be
315 so destructive, as already observed by Agardy (1994) and by Jentoft, van Son & Bjørkan (2007).

316 **Acknowledgements**

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318 We thank Luisa Polastro for the English revision and Matteo Grechi and Gianluca Cavagna
319 for the precious help during image analysis.

320 This work was supported by L'Oréal-Unesco which funded Sarah Caronni with a L'Oréal-
321 Unesco for Women in Science Fellowship Grant.

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474 **Figure legends**

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476 Figure 1 - Nutrient enrichment effectiveness. Mean nutrient (inorganic N and P) concentration (+
477 SE) between nutrient addition and control plots (E+ and E-) on each sampling time.

478 Figure 2 - Multidimensional scaling on the basis of Bray–Curtis dissimilarities of each

479 combination of treatments (n=3). Triangles: nutrients enriched (E+), inverted triangles:

480 nutrients non-enriched (E-), black symbols: mucilage removal (M-), white symbols:

481 mucilage maintenance (M+); rectangle: total macroalgal removal (G100%); oval: partial

482 macroalgal removal (G50%); no symbol around triangles: macroalgal maintenance (G0%).

483 Figure 3 – Percent cover (mean % \pm SE, n = 20) of the 4 taxa that mainly contributed to the
484 differences observed in the composition of assemblages (dark filamentous algae (DFA);
485 encrusting coralline algae (ECA), Dictyotales and *Laurencia* spp.) for each combination of
486 treatments: nutrient enrichment (E+ and E-); grazing (100%, 50% and 0% of macroalgal
487 removal); mucilage (M+ and M-)).

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Table 1 (on next page)

Results of PERMANOVA on the structure of macroalgal assemblages at T₁.

1 Table 1. Results of permutational multivariate analyses of variance (PERMANOVA) testing the
 2 effect of nutrient enrichment (*E*) grazing (*G*) and mucilage (*M*) on the structure of macroalgal
 3 assemblages at T₁. Analyses were based on Bray–Curtis dissimilarities and each test was
 4 performed using 9.999 permutations of appropriate units. Significant P-values are given in bold.

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

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Table 2 (on next page)

Results of statistical analysis on the percent cover of DFA, ECA, Dictyotales and Laurencia spp.

1 Table 2. Results of ANOVAs on the effect of each treatment (Nutrient enrichment (*E*), grazing (*G*)
 2 and mucilage (*M*)) on the percent cover of DFA, ECA, Dictyotales and *Laurencia* spp (dark
 3 filamentous algae (DFA); encrusting coralline algae (ECA), Dictyotales and *Laurencia* spp.).
 4 Significant P-values are given in bold. Also the results of SNK test on ExGxM interaction for the
 5 4 taxa that mainly contributed to the observed differences in the composition of assemblages are
 6 given for: nutrient enrichment (E+ vs E-), grazing (G100%, G50% and G0%) and mucilage (M+

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

7 vs M-).

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<p style="text-align: center;">DFA</p> <p style="text-align: center;">Grazing</p> <p>M+ E+ G100%>G50%=G0% E- G100%=G50%=G0%</p> <p>M- E+ G100%>G50%>G0% E- G100%=G50%>G0%</p>	<p style="text-align: center;">Mucilage</p> <p>G100% E+ M+<M- E- M+=M-</p> <p>G50% E+ M+=M- E- M+=M-</p> <p>G0% E+ M+=M- E- M+=M-</p>	<p style="text-align: center;">Nutrient enrichment</p> <p>G100% M+ E+=E- M- E+>E-</p> <p>G50% M+ E+=E- M- E+>E-</p> <p>G0% M+ E+=E- M- E+=E-</p>
<p style="text-align: center;">ECA</p> <p style="text-align: center;">Grazing</p> <p>M+ E+ G100%<G50%<G0% E- G100%=G50%=G0%</p> <p>M- E+ G100%<G50%<G0% E- G100%=G50%=G0%</p>	<p style="text-align: center;">Mucilage</p> <p>G100% E+ M+=M- E- M+=M-</p> <p>G50% E+ M+=M- E- M+=M-</p> <p>G0% E+ M+<M- E- M+=M-</p>	<p style="text-align: center;">Nutrient enrichment</p> <p>G100% M+ E+=E- M- E+=E-</p> <p>G50% M+ E+=E- M- E+=E-</p> <p>G0% M+ E+>E- M- E+>E-</p>
<p style="text-align: center;">Dictyotales</p> <p style="text-align: center;">Grazing</p> <p>M+ E+ G100%=G50%=G0% E- G100%=G50%=G0%</p> <p>M- E+ G100%<G50%=G0% E- G100%=G50%=G0%</p>	<p style="text-align: center;">Mucilage</p> <p>G100% E+ M+=M- E- M+=M-</p> <p>G50% E+ M+<M- E- M+=M-</p> <p>G0% E+ M+<M- E- M+=M-</p>	<p style="text-align: center;">Nutrient enrichment</p> <p>G100% M+ E+=E- M- E+=E-</p> <p>G50% M+ E+=E- M- E+>E-</p> <p>G0% M+ E+=E- M- E+>E-</p>
<p style="text-align: center;">Laurencia spp.</p> <p style="text-align: center;">Grazing</p> <p>M+ E+ G100%=G50%=G0% E- G100%=G50%=G0%</p> <p>M- E+ G100%<G50%=G0% E- G100%=G50%=G0%</p>	<p style="text-align: center;">Mucilage</p> <p>G100% E+ M+=M- E- M+=M-</p> <p>G50% E+ M+<M- E- M+=M-</p> <p>G0% E+ M+<M- E- M+=M-</p>	<p style="text-align: center;">Nutrient enrichment</p> <p>G100% M+ E+=E- M- E+=E-</p> <p>G50% M+ E+=E- M- E+>E-</p> <p>G0% M+ E+=E- M- E+>E-</p>

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Table 3 (on next page)

Results of PERMANOVA on the structure of macroalgal assemblages at T₂.

1 Table 3. Results of PERMANOVA testing the effect of nutrient enrichment (*E*) grazing (*G*) and
 2 mucilage (*M*) on the structure of macroalgal assemblages at T₂. Analyses were based on Bray–
 3 Curtis dissimilarities and each test was performed using 9,999 permutations of appropriate units.
 4 Significant P-values are given in bold.

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Source of variation	<i>df</i>	SS	MS	<i>F</i>	<i>P</i> (perm.)
Nutrient enrichment (<i>E</i>)	1	0.5567	0.5567	0.1393	0.8834
Grazing (<i>G</i>)	2	14.8763	7.4382	1.8610	0.1012
Mucilage (<i>M</i>)	1	4.9494	4.9494	1.2383	0.3307
<i>E</i> x <i>G</i>	2	3.3504	1.6752	0.4191	0.8501
<i>E</i> x <i>M</i>	1	3.4060	3.4060	0.8522	0.4989
<i>G</i> x <i>M</i>	2	8.8034	4.4017	1.1013	0.3973
<i>E</i> x <i>G</i> x <i>M</i>	2	1.4009	0.7004	0.1753	0.9544
Residual	24	95.9234	3.9968		
Total	35	133.2665			

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Figure 1 (on next page)

Nutrient enrichment effectiveness.

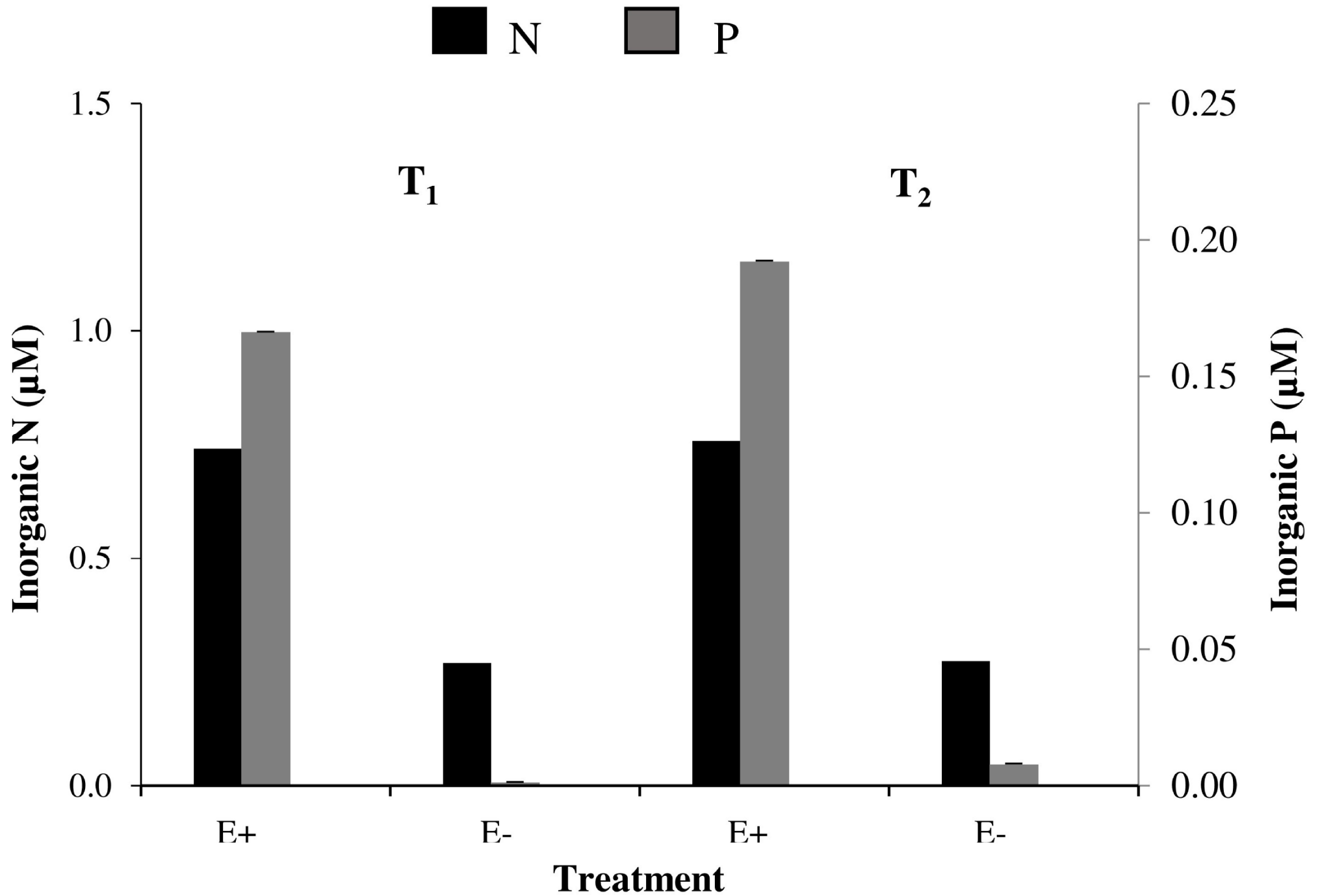


Figure 2 (on next page)

Multidimensional scaling on the basis of Bray- Curtis dissimilarities of each combination of treatments.

2D Stress: 0.09

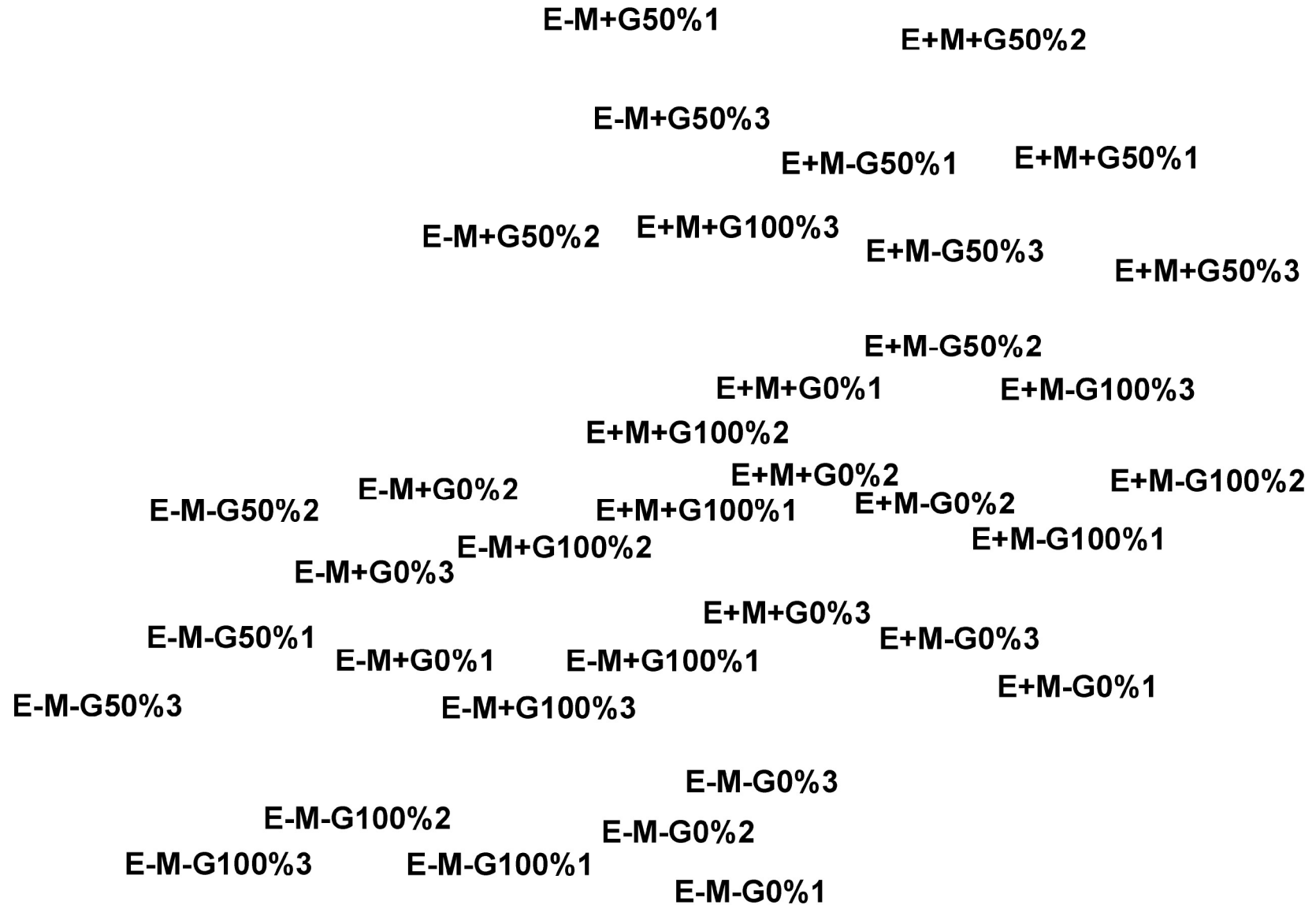


Figure 3 (on next page)

Percent cover of the 4 taxa that mainly contributed to the differences in the composition of assemblages.

