# Cumulative effects of an invasive species and nutrient enrichment on rock pool communities

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1    **Title**: Cumulative effects of an invasive species and nutrient enrichment on rock pool

2    communities

3    **Running head:** Cumulative effects of multiple stressors

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16  **Abstract**

17  Most ecosystems are affected by multiple anthropogenic stressors simultaneously, however,

18  there is a lack of information describing the cumulative effects of many common stressor

19  pairs. Consequently, we have but a rudimentary understanding of the roles that stressor

20  characteristics and environmental context play in determining interactions among

21  stressors. Nutrient enrichment often affects coastal ecosystems that may have already been

22  affected by invasive species. To identify the effects of nutrient enrichment on communities

23  under different invasion scenarios, the presence of the invasive fucoid

24  algae, *Sargassum muticum*, and nutrient conditions were manipulated in the field to test for

25  their independent and cumulative effects. Their combined effects on the diversity and

26  functioning of rock pool communities were quantified. Rock pools with *S. muticum* contained

27  fewer species, lower macroalgal and microalgal biomass, and their overall benthic

28  assemblage structure differed from pools without *S. muticum*. Both the presence of *S.*

29  *muticum* and nutrient enrichment affected different functional groups of algae differently.

30  Their cumulative effects, however, did not differ with increasing intensity of nutrient

31  enrichment. Furthermore, invaded communities from which *S. muticum* had been removed

32  manually, tended towards greater species richness following removal than pools where *S.*

33  *muticum* remained present, indicating a potential for recovery. These findings highlight the

34  importance of identifying the cumulative effects of multiple stressors on the responses of

35  individual functional groups, alongside effects on overall assemblage structure, in order to

36  fully understand the consequences for ecosystems.

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38  **Keywords:** *Sargassum muticum*, eutrophication, community ecology, biodiversity, multiple

39  stressors, coastal ecology

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## Introduction

An emerging field of research aims to disentangle the impacts of multiple anthropogenic stressors and to better predict their cumulative effects on ecosystems (Crain et al. 2008, Darling & Côté 2008, Russell & Connell 2012, Boyd & Hutchins 2012). Among the most common anthropogenic stressors in marine ecosystems are invasive species (Crain et al. 2008). Invasive species often occur in coastal and estuarine environments that are already affected by one or more anthropogenic stressors, which may affect invasion success and impact (Vitousek et al. 1997, Lotze et al. 2006). Predicting the impacts of invasive species under multiple stressor scenarios is limited by our lack of understanding of how environmental or experimental context determines their effects in natural communities and similarly, how invasive species alter the impacts of other stressors. The introduction of a secondary stressor, such as nutrient enrichment, to a system that is already under stress from an invasive species, may lead to stress-induced tolerance or stress-induced sensitivity of the community (Vinebrooke et al. 2004). Stress-induced sensitivity may occur when the effects of a stressor leads to a community that is less resilient towards the second stressor and the cumulative impact of the stressors is synergistic or greater than the sum of the individual effects. Alternatively, stress-induced tolerance may occur when the effects of the initial stressor drives the community to be more resilient to the effects of the second stressor and thus, the second stressor has a reduced impact and the cumulative impact of the two stressors is antagonistic (Folt et al. 1999, Vinebrooke et al. 2004). Where the combined effects of multiple stressors do not interact, their cumulative effect is additive, or equal to the sum of the individual effects (e.g. Vye et al. 2017).

Coastal ecosystems that are influenced by invasive species are often exposed to both press and pulse nutrient enrichment from a range of sources, such as land run-off and sewage outfalls (Lotze & Worm 2002, Lapointe et al. 2004). Nutrient enrichment may influence the

65    invasion process in some invasive seaweeds (Sanchez & Fernandez 2006, Vaz-Pinto et al.

66    2013, Uyà et al. 2017, Vieira et al. 2017). For example, nutrient enrichment has been shown

67    to increase the invasion success of *Sargassum muticum*, a common invasive macroalga in

68    Europe (Vieira et al. 2017), suggesting not only could nutrient enrichment affect the native

69    community independently, but it could also increase the impacts of the invader on the native

70    community structure and functioning. Therefore, the cumulative effect of these stressors

71    would be synergistic. Alternatively, *S. muticum* could reduce the impacts of nutrient

72    enrichment itself on the native community by exploiting the excess resource in nutrient

73    enriched ecosystems, as outlined in the fluctuating resources theory presented by Davis et al.

74    (2000). In this scenario, there may be effects of increased invasion success of the invader on

75    the community, however, the interactive effect may be antagonistic as the effect of nutrient

76    enrichment on community structure and functioning would be lessened.

77    The majority of experimental studies to date have focused primarily on the introduction of

78    both nutrient enrichment and invasive species simultaneously (Vye et al. 2015, Vieira et al.

79    2017). Nutrient enrichment events, however, often occur in systems where invasive species

80    are already established (Lotze et al. 2006). Testing how an established invasive species alters

81    the impacts of a nutrient enrichment event on the native community is important to enable us

82    to understand the cumulative effects of the stressors in a realistic scenario (Strayer 2012).

83    Furthermore, a common management approach to invasive species is to undertake manual

84    removal of individuals either for eradication or population control (Thresher & Kuris 2004).

85    If the invasive species is modulating the impacts of another stressor, such as nutrient

86    enrichment, indirect effects of removing the invader from the system can be complicated and

87    unpredictable. As such, there is a need for experimental studies that allow a better

88    understanding of the future consequences of invasive species management in coastal

89    ecosystems influenced by multiple stressors (Zavaleta et al. 2001).

90    In addition to the presence or absence of stressors, other stressor characteristics, such as

91    intensity or temporal variability, may also have a role in determining the cumulative effects

92    of multiple stressors (Benedetti-Cecchi et al. 2006, Molinos & Donohue 2010, O'Connor et

93    al. 2015). Stressor intensity has been shown previously to determine the cumulative impacts

94    of invasive species and other stressors (Vye et al. 2015). Specifically, a recent study

95    identified an antagonistic cumulative effect of the presence of the invasive fucoid, *S.*

96    *muticum*, and nutrient enrichment on total algal biomass accumulation in an assembled rock

97    pool community, but only at certain levels of nutrient enrichment (Vye et al. 2015). Such

98    shifts in algal biomass production are an important proxy for energy flow and although not

99    analogous with primary productivity *per se*, are indicative of an implicit change in ecosystem

100   functioning (O'Connor & Crowe 2005, Masterson et al. 2008).


101   The aim of this study, therefore, was to identify and characterise the effects of nutrient

102   enrichment on communities in rock pools that have been invaded by *S. muticum* and compare

103   them with rock pool communities that have not been invaded by *S. muticum.* We also

104   removed *S. muticum* manually from pools and compared these communities to those with and

105   without *S. muticum*. This removal treatment was an essential control for the potentially

106   confounding influences of factors that may covary with the presence of *S. muticum*. It also

107   allowed us to assess the potential impacts of invader management by means of targeted

108   removal over the peak growth season. The hypotheses tested were that: 1) the presence of an

109   invasive species will modulate the effects of nutrient enrichment on benthic assemblage

110   structure and diversity; 2) the presence of invasive species and nutrient enrichment will have

111   different cumulative effects on different algal functional group biomass; and 3) the level, or

112   intensity, of nutrient enrichment will determine the cumulative impacts of the presence of an

113   invader and nutrient enrichment on benthic assemblage structure, diversity and functional

114   group biomass.

## Materials and methods

*Experimental site*

The experiment was conducted in intertidal rock pools on an exposed rocky shore at
Muighinis, Co. Galway, Ireland (53°17'39.46"N, 9°51'2.87"W) between April and June 2014.
The shore is comprised of exposed granite bedrock and has a tidal range of approximately
four metres (Firth & Crowe 2010). These rock pool assemblages were comprised of many
different morphological forms of algae including fucoids, such as *Fucus serratus* and
*Halidrys siliquosa,* ephemeral green algae, including *Ulva* spp. and *Cladophora rupestris* and
many species of red algae, both encrusting (e.g. *Lithothamnium* sp. and *Mesophyllum*
*lichenoides*) and branched (e.g. *Gelidium* spp. and *Polysiphonia* spp.). Invertebrate
communities in the pools included gastropod grazers, such as the limpet, *Patella*
*ulyssiponensis,* the topshell, *Gibbula umbilicalis* and the periwinkle, *Littorina littorea*
(O'Connor & Crowe 2005). Within the past two decades, a large proportion of the rock pools
have also been colonised by the invasive fucoid *Sargassum muticum* (Baer & Stengel 2010),
which increases in percentage cover during summer (February to July locally; Baer & Stengel
2010).

*Experimental design*

A field experiment was designed to test for the individual and cumulative effects of the
presence of an invasive species and nutrient enrichment on rock pool assemblage structure
and functioning. Based on an orthogonal experimental design, the experiment allowed
mensurative (i.e. natural presence vs. natural absence of invader) and manipulated
comparisons (i.e. natural presence vs. manipulated removal of invader, Hurlbert 1984). The
factorial experiment had two fixed factors: presence of an invader (three levels: present,
absent and removed) and nutrient enrichment (three levels: ambient, intermediate, high), and

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139     tested for all interactions among treatments. All nine treatments were replicated five times,

140     each in separate rock pools with a minimum distance of two metres apart, yielding 45

141     experimental units. Fifteen rock pools without *Sargassum muticum* and thirty rock pools with

142     *S. muticum* present were selected randomly on the mid shore. *Sargassum muticum* was

143     removed carefully (using a flat chisel to ensure the whole of the holdfast was removed,

144     preventing plant regrowth) from half of the rock pools with *S. muticum* present (15 pools).

145     This removal treatment was important to test for and assess any potentially confounding

146     variables that may have co-varied with the presence of *S. muticum* (O'Connor et al. 2006,

147     Underwood 2009) and to identify nutrient enrichment effects on recovering communities

148     after invader removal. All rock pools were similar in size (mean $\pm$ S.E.: length = $118.6 \pm 4.5$

149     cm; width = $66.4 \pm 3.1$ cm; and depth = $14.4 \pm 0.8$ cm), within the same shore height and

150     exposure. The initial percentage cover of *S. muticum* in all invaded pools was $7.3 \pm 1.7$ %

151     (mean $\pm$ S.E.), increasing to $18.9 \pm 6.4$ % cover by the end of the experiment (peak growth

152     period).

153     Rock pools were assigned randomly to treatments ($n = 5$). To manipulate nutrient

154     concentrations in nutrient enriched pools, Everris Osmocote® Exact (Geldermalsen,

155     Netherlands) slow release fertiliser pellets (11N: 11P: 18K) were used (Worm et al. 2000,

156     Atalah & Crowe 2010, O'Connor et al. 2015). Fertiliser was contained within mesh cases

157     attached to the base of each pool. Ambient treatments contained an empty mesh bag to

158     control for potential artefact effects of the presence of the bag. Intermediate and high nutrient

159     enrichment treatments contained 1 g l$^{-1}$ and 3 g l$^{-1}$ of fertiliser pellets respectively. At the end

160     of the experimental period, water samples were taken immediately after the emersion of the

161     pools at low tide and were analysed using an autoanalyzer for dissolved inorganic nitrogen

162     (DIN) and phosphate to confirm the efficacy of the nutrient enrichment treatments. Ambient

163     treatments contained $1.00 \pm 0.12$ μm l$^{-1}$ DIN and $0.17 \pm 0.01$ μm l$^{-1}$ phosphate. Intermediate

164    nutrient enrichment treatments contained $19.22 \pm 3.67$ µm l$^{-1}$ DIN and $3.11 \pm 0.62$ µm l$^{-1}$

165    phosphate, and high nutrient enrichment treatments contained $29.99 \pm 5.64$ µm l$^{-1}$ DIN and

166    $4.25 \pm 1.01$ µm l$^{-1}$ phosphate (DIN: MS = 38.12, $F_{2,40}$ = 20.15, $P < 0.05$, SNK post-hoc: A <

167    N+ < N++; phosphate: MS = 6.64, $F_{2,40}$ = 7.60, $P < 0.05$, SNK post-hoc: A < N+ < N++). As

168    the nutrient treatment represented a pulse of nutrient enrichment similar to that of land based

169    run-off into the intertidal zone (Sharp 1983, O'Connor et al. 2015), a further subset of

170    samples were taken 5 hours after initial emersion to identify how much nutrient flux occurred

171    in the pools during the emersion period. In intermediate nutrient enrichment treatments, DIN

172    ($40.09 \pm 12.76$ µm l$^{-1}$) approximately doubled and phosphate concentrations ($35.58 \pm 11.23$

173    µm l$^{-1}$) increased by a magnitude of ten over five hours. In high nutrient enrichment

174    treatments DIN ($111.66 \pm 57.74$ µm l$^{-1}$) approximately quadrupled and phosphate

175    concentrations ($78.24 \pm 28.27$ µm l$^{-1}$) increased by a magnitude of twenty. These

176    concentrations are unlikely to limit macroalgal growth (Gordillo et al. 2002) and are similar

177    to levels achieved in previous nutrient enrichment studies in intertidal systems (Atalah &

178    Crowe 2010, O'Connor et al. 2015).

179    The experiment ran between April and June 2014 to focus on the peak growth period of the

180    invasive species, *S. muticum* (Baer & Stengel 2010), which tends to die back in the winter

181    months. This duration also reflects the length of similar multiple stressor experiments in

182    artificial systems to allow for broad comparisons of findings (Boyer et al. 2009, Vye et al.

183    2015).

184    *Response variables*

185    Benthic assemblage structure (percentage cover of macroalgae and abundance of slow

186    moving or sessile invertebrates >1cm) was quantified using a 25 cm x 25 cm quadrat with 64

187    intersections prior to the application of nutrient enrichment treatments and before *S. muticum*

188    was removed from the removal treatments, to test for any initial differences between invaded

189    and non-invaded communities. One quadrat for each pool may have reduced statistical power,

190    however, we have based our analyses on means of each pool (replicate) for each treatment.

191    This size was chosen as it allowed a standard random sample to be taken in all pool shapes

192    including the narrowest pools in the range. Quantification of benthic assemblage structure

193    was repeated at the end of the experiment, similarly using a randomly placed 25 cm x 25 cm

194    quadrat with 64 intersections in each rock pool. All species present in the quadrat, but not

195    beneath an intersection, were recorded as 0.5 % cover. Algal taxa were identified to the

196    lowest practicable taxonomic level using taxonomic keys (e.g. Dixon & Irwine 1977, Hiscock

197    1986) and epiphytes, which are common in this system, were not distinguished from other

198    epilithic algae for analyses. Abundance of slow moving and sessile invertebrates (>1cm) was

199    also estimated at the same time. There were no initial differences in benthic assemblage

200    structure between pools assigned to each treatment, including invaded and non-invaded

201    communities (PERMANOVA: MS = 0.1869, pseudo-$F_{2, 36}$ = 1.383, $P$ > 0.05) based on an

202    extensive survey prior to treatment allocation at the start of the experiment and before the

203    anticipated *S. muticum* seasonal growth period.

204    At the conclusion of the experiment, species richness (N), Shannon-Wiener diversity (*H'*) and

205    Pielou's evenness (*J*) indices were also estimated for each pool based on percentage cover

206    and abundance data. Furthermore, all benthic taxa were classified into functional groups and

207    analysed to test for more general trends based on potential functional traits (Jänes et al. 2017,

208    Table S1 in Supplementary Material). Algal taxa were assigned to functional groups based on

209    their expected response to treatments and functional role in the rock pool community (Arenas

210    et al. 2006). These included turf-forming algae (taxa typically < 5 cm vertical height) and

211    sub-canopy space-holding algae, which were hypothesised to be affected by shading by *S.*

212    *muticum* (Britton-Simmons 2004, Olabarria et al. 2009). Canopy algae were hypothesised to

213    be affected by competition for resources, such as space, by *S. muticum* (Viejo 1997). Green

214    ephemerals and coralline algae were also classified into different functional groups (distinct

215    from turf-forming taxa) because these groups have been shown to have different responses to

216    nutrient enrichment (Hawkins et al. 1994, Delgado & Lapointe 1994, Karez et al. 2004,

217    O'Connor 2013, Vieira et al. 2017). Invertebrate functional groups included grazing

218    gastropods (e.g. winkles, *Littorina littorea*), suspension feeders (e.g. beadlet anemone, *Actina*

219    *equina*) and mobile predators (e.g. dog whelk, *Nucella lapillus;* Little et al. 2009).


220    At the end of the experiment, destructive samples of algae were taken to quantify the effects

221    of the presence of *S. muticum* and nutrient enrichment on total algal biomass and the biomass

222    of each algal functional group (canopy, sub-canopy, turf, coralline, and green ephemerals,

223    Table S1 in Supplementary Material). Algae were collected from within each quadrat, sorted

224    into taxa, except for encrusting coralline algae (e.g. *Lithothamnium* sp.) and dried to a

225    constant mass (at 60°C). Up to 80% of coralline algae consists of calcium carbonate and thus

226    dry biomass of coralline algae was adjusted by a conversion factor of 0.2 (Griffin et al. 2010,

227    Mrowicki & O'Connor 2015). Microalgal biomass was also estimated *in situ* by

228    quantification of chlorophyll *a* concentration of biofilm in the rock pools (Murphy et al.

229    2005, Carpentier et al. 2013, Kahlert & McKie 2014, Mrowicki et al. 2014). Three

230    measurements (1 cm$^2$ each) were taken randomly from the base of the pools using a benthic

231    fluorometer (BenthoTorch, bbe Moldaenke[©]). The mean of these measurements was used in

232    analyses to incorporate potential effects of small-scale variability (Sandulli & Pinckney 1999,

233    Murphy et al. 2005). Three pools that had *S. muticum* removed and one pool from the

234    treatment where it was thought *S. mutium* was absent were excluded from the analysis

235    because the presence of the invader was noted (>0.5 % cover) at the end of the experiment,

236    suggesting the experimental treatment was not effective in these pools.

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237   Permutational multivariate analysis of variance (PERMANOVA; Anderson 2001, McArdle &

238   Anderson 2001) was used to test hypotheses relating to benthic assemblage structure

239   (percentage cover of macroalgae and abundance of slow moving or sessile invertebrates

240   >1cm) at the end of the study, with a similar factorial experimental design (two fixed factors:

241   the presence of *S. muticum* [three levels]; and nutrient concentration [three levels]). Non-

242   metric multi-dimensional scaling (nMDS) was used to visualise differences among

243   assemblages based on dissimilarities of their assemblage structure. Similarities of percentages

244   (SIMPER) analyses were used to identify which taxa contributed most to differences in

245   assemblage structure among treatments (Clarke & Warwick 2001). All multivariate analyses

246   were conducted on Bray-Curtis dissimilarity matrices with 9,999 permutations of residuals

247   under the reduced model and tested for all possible interactions among treatments. Data were

248   square-root transformed to reduce the influence of the highly abundant canopy species

249   (Clarke & Warwick 2001). The percentage cover estimates of *S. muticum* were not included

250   in analyses of assemblage structures to prevent confounding independent (manipulated) and

251   dependent (response) variables (Huston 1997) and to identify the impact of *S. muticum* on the

252   rest of the assemblage (Thomsen et al. 2016). Post-hoc pairwise *t*-tests were used to identify

253   differences between levels of significant terms. Post-hoc PERMDISP routines were

254   conducted on significant terms, after inspection of the nMDS plots, to detect differences in

255   the assemblages caused by treatment effects on the variation in assemblage structure rather

256   than shifts in assemblage structure. Percentage cover and abundance of taxa were classified

257   into functional groups and reanalysed to test for effects of the presence of *S. muticum* and

258   nutrient enrichment on functional diversity (Table S1 in Supplementary Material).

259   Analysis of variance (ANOVA) was used to test hypotheses relating to species richness,

260   Shannon-Wiener diversity and Pielou's evenness using a similar design with two fixed

261   factors: *S. muticum* (three levels) and nutrient concentration (three levels). *Sargassum*

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262 *muticum* was not included in this analysis in order to identify effects on native diversity. In

263 addition to the multivariate tests on functional group abundance data, a univariate approach

264 was also undertaken to identify the biomass response of individual algal functional groups to

265 *S. muticum* and nutrient concentration. This approach was considered prudent because algal

266 biomass may be more indicative of different algal functional group contributions to energy

267 flow and productivity than percent cover estimates (Masterson et al. 2008, Atalah & Crowe

268 2010, Crowe et al. 2011, White & Shurin 2011). To further disentangle the effect of the

269 invader, biomass results for total macroalgae and canopy algae were analysed with and

270 without *S. muticum* biomass (Thomsen et al. 2016). Data were first tested for normality and

271 homogeneity of variances using Shapiro-Wilk and Levene's tests and were transformed

272 where assumptions were not met. Shannon-Wiener diversity, green ephemeral, turf, coralline

273 and canopy algal biomass were square-root transformed, microalgal biomass data were

274 natural-log transformed and Pielou's evenness data were arcsine transformed. Student-

275 Newman-Keuls post-hoc procedures were used to make comparisons among levels of

276 significant terms. Post-hoc test results presented are with *S. muticum* biomass included. All

277 analyses were undertaken in R 3.1.0 (R Development Core Team 2011) and PRIMER v6

278 (Clarke & Gorley 2006, Anderson et al. 2008).

279 **Results**

280 At the end of the experiment, there was an effect of the presence of *S. muticum* on benthic

281 assemblage structure (MS = 4056.5, pseudo-$F_{2, 32}$ = 2.397, $P = 0.006$), however, there was no

282 effect of nutrient enrichment (MS = 771.9, pseudo-$F_{2, 32}$ = 0.456, $P > 0.05$). Post-hoc tests

283 showed that assemblage structure in rock pools without *S. muticum* differed from those where

284 it was present (Absent ≠ Present, $t = 1.793$, $P = 0.008$, Fig. 1) and from pools where it was

285 removed experimentally (Absent ≠ Removed, $t = 1.731$, $P = 0.004$, Fig. 1). When all taxa

286 were classified into functional groups, there remained an effect of the presence of *S. muticum*

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287  on benthic assemblage structure (MS = 1748.4, pseudo-$F_{2, 32}$ = 2.320, $P$ = 0.042) and no

288  effect of nutrient enrichment (MS = 275.61, pseudo-$F_{2, 32}$ = 0.366, $P$ > 0.05). Post-hoc tests

289  again identified differences in assemblages without *S. muticum* compared to rock pools where

290  *S. muticum* was present (Absent ≠ Present, $t$ = 1.689, $P$ = 0.046) or had been removed

291  (Absent ≠ Removed, $t$ = 2.240, $P$ = 0.007). SIMPER analyses showed that these differences

292  in benthic assemblage structure were driven primarily by algal taxa, which were dominant in

293  both richness and abundance, rather than faunal taxa, and therefore, algal taxa were focused

294  on for the rest of the analysis. There was a greater proportion of the canopy alga, *Fucus*

295  *serratus*, the turf and sub-canopy algae (*Ceramium* spp. and *Chondrus crispus)*, and all

296  ephemeral green algae (*Chaetomorpha* sp., *Ulva* spp. and *Cladophora rupestris)* in

297  treatments without *S. muticum* compared to treatments where it was present or had been

298  removed (Table S2 in Supplementary Material). In addition, there was an effect of the

299  presence of *S. muticum* on variation in benthic assemblage structure (PERMDISP: $F_{2, 38}$ =

300  4.886, $P$ = 0.016, Fig. 1), however, post-hoc tests could not identify conclusively where

301  differences among treatments lay because pools with and without *S. muticum* had similar

302  variation in assemblage structure to rock pools where *S. muticum* had been removed

303  experimentally. There was also an effect of the presence of *S. muticum* on species richness

304  (MS = 34.15, $F_{2, 32}$ = 3.4165, $P$ = 0.045, Fig. 2), however, post-hoc tests could not identify

305  where differences lay because although species richness differed, i.e. was lower when *S.*

306  *muticum* was present than when it was absent, both these treatments were similar to the rock

307  pools where *S. muticum* was removed experimentally (Fig. 2). Nutrient enrichment did not

308  affect species richness (MS = 8.45, $F_{2, 32}$ = 0.848, $P$ > 0.05). Also, there was no effect of the

309  presence of *S. muticum* (MS = 0.052, $F_{2, 32}$ = 2.267, $P$ > 0.05), nor nutrient enrichment (MS

310  = 0.006, $F_{2, 32}$ = 0.264, $P$ > 0.05) on Shannon-Wiener diversity, nor were there any effects of

311  the presence of *S. muticum* (MS = 0.001, $F_{2, 32}$ = 0.447, $P > 0.05$) or nutrient enrichment (MS

312  = 0.001, $F_{2, 32}$ = 0.341, $P > 0.05$) on Pielou's evenness.

313  There was an effect of the presence of *S. muticum* on total macroalgal biomass (Table 1a, Fig.

314  3A), however post-hoc tests were not fully conclusive because, although algal biomass was

315  greater in the pools where *S. muticum* was present compared to pools where it was absent,

316  both these treatments were similar to the treatment from which *S. muticum* was removed

317  experimentally. There was no effect of nutrient enrichment on total macroalgal biomass

318  (Table 1a, Fig. 3A). In terms of responses of individual functional groups of algae, pools

319  where *S. muticum* was absent had a greater biomass of canopy algae than pools with *S.*

320  *muticum* and pools where *S. muticum* had been removed, but there was no effect of nutrient

321  enrichment (Table 1b, Fig. 3B). Furthermore, the impacts of *S. muticum* on total and canopy

322  algal biomass did not differ depending on whether *S. muticum* biomass was included in the

323  analysis or not (Table 1a & b, Fig. 3A & B). The effect of the presence of *S. muticum* on the

324  biomass of sub-canopy algal species differed with nutrient enrichment (as indicated by the

325  significant interaction between presence of *S. muticum* and nutrient enrichment, Table 1c),

326  however, post-hoc tests were unable to identify where differences among treatments lay (Fig.

327  3C). The effects of the presence of *S. muticum* on the biomass of green ephemeral algae also

328  differed with nutrient enrichment, however, post-hoc tests could not determine where

329  precisely differences among treatments lay (Table 1f, Fig. 3F). There was no effect of the

330  presence of *S. muticum* or nutrient enrichment on coralline or turf algal biomass (Table 1d &

331  e, Fig. 3D & E). Microalgal biomass was lower in rock pools where *S. muticum* was present

332  or removed compared to pools without *S. muticum* (Table 1g, Fig. 3G) and there was no

333  effect of nutrient enrichment on microalgal chlorophyll *a* concentration (Table 1g, Fig. 3G).

334  **Discussion**

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335 This study found that pools where *Sargassum muticum* was present had different benthic

336 assemblage structure from those without *S. muticum*, where the former tended to have a lower

337 abundance of canopy algae and reduced benthic species richness. Surprisingly, none of the

338 effects on assemblage structure were modulated by nutrient enrichment. Our findings did

339 show, however, that nutrient enrichment had variable interactive cumulative effects with the

340 presence of *S. muticum* on the biomass of some algal functional groups. There was no clear

341 effect of intensity of nutrient enrichment, which suggests that, contrary to our hypotheses, the

342 intensity of this stressor was not important in determining the cumulative effects in this rock

343 pool system.

344 The invasion-driven shift in benthic assemblage structure coincided with the peak algal

345 growth period. Unlike many native algal species, *S. muticum* undergoes senescence during

346 the winter months, where it loses a large proportion of biomass and percentage cover, re-

347 growing during spring and reaching peak biomass during summer months (Wernberg et al.

348 2000, Baer & Stengel 2010). When taxa were classified into functional groups and re-

349 analysed, the overall assemblage structure still differed where *S. muticum* was present, which

350 shows that the effect of *S. muticum* was apparent on whole functional groups of algae not

351 based on individual species responses. The increase in percentage cover of *S. muticum* during

352 the experiment may have reduced light penetration to the understory species (Britton-

353 Simmons 2004), and reduced space available for other canopy species, such as *F. serratus*

354 (Viejo 1997, White & Shurin 2011). Furthermore, increased light intensity, daylight duration

355 and temperature during the summer stimulates the growth of many native sub-canopy algal

356 species, such as *Chondrus crispus* and *Furcellaria lumbricalis* (Bird et al. 1979), which may

357 increase competition for primary resources with *S. muticum* (Britton-Simmons 2004). Thus,

358 seasonal macroalgal growth, in combination with increases in percentage cover of *S.*

359  *muticum,* may have led to seasonal differences in benthic assemblages between the invaded

360  and non-invaded communities (Thomsen et al. 2005).

361  The differences among benthic assemblages were characterised by a tendency towards

362  increased variability of assemblage structure and towards decreased species richness in the

363  presence of *S. muticum.* High spatial variability has been suggested to be a symptom of

364  stressed communities when exposed to anthropogenic disturbances (Warwick & Clarke 1993,

365  Cottingham et al. 2001, Ives & Carpenter 2007, Donohue et al. 2013). Although the findings

366  of this study are contrary to others that identified an invasive species-driven homogenisation

367  of communities (Olden & Rooney 2006, Baiser et al. 2012), increased variability may be

368  expected owing to the non-linearity and context-dependency of invasion impacts (Parker et

369  al. 1999, Thomsen et al. 2011, Vaz-Pinto et al. 2014). Our invaded assemblages also tended

370  to be less species rich, which may contribute to increased variability within invaded rock

371  pools (Loreau et al. 2001, Campbell et al. 2011). Increases in community variability driven

372  by invasion could decrease the predictability of the response of communities to biological

373  invasions. This could lead to 'ecological surprises' becoming more common and

374  complicating management decisions (Paine et al. 1998).

375  Benthic assemblage structure was less variable and tended towards greater species richness

376  and total algal biomass in assemblages where *S. muticum* had been removed, compared to

377  assemblages where *S. muticum* remained present. Although some of these responses were not

378  conclusive, this does indicate some potential for recovery. This finding provides some initial

379  evidence that the removal or management of *S. muticum* could allow intertidal communities

380  to begin to recover within a relatively short time period. However, there was no evidence of

381  the recovery of canopy algal biomass, which may be owing to the slow growth rates

382  associated with the primary native canopy species, *Fucus serratus* (Knight & Parke 1950).

383  The temporal duration of this study, designed to look at impacts during the main growth

384   season of *S. muticum,* may not have been long enough for these slow growing species to

385   show detectable signs of recovery. Other invasive macroalgal removal studies have found a

386   lag of five or more months before some species or functional groups of algae recover from

387   the impacts of an invasive macroalgae, which can depend on other perturbations in the

388   system as well as the original biomass of the invader (Piazzi & Ceccherelli 2006, Gribben et

389   al. 2013). Although this study occurred over the main period of *S. muticum* growth, and

390   therefore the main period of expected impact, further work should follow the trajectory of

391   macroalgal communities over a longer duration to assess the full potential for community

392   recovery after *S. muticum* removal.

393   Previous studies have identified antagonistic cumulative effects of the presence of *S. muticum*

394   and nutrient enrichment on total algal biomass in artificial assemblages (Vye et al. 2015). The

395   current study however, showed that the effects of *S. muticum* on total macroalgal and

396   microalgal biomass were not determined by nutrient enrichment. Here, the presence of *S.*

397   *muticum* was characterised by less total algal biomass regardless of whether *S. muticum* was

398   included in the analysis, indicating that the presence of *S. muticum* drove decreases in the

399   biomass of native species, such as the native canopy alga, *F. serratus*. Furthermore, there was

400   also less total microalgal biomass in treatments where *S. muticum* had been removed

401   suggesting that effects on total microalgal biomass were persistent even after removal of the

402   invasive species. This could be due to a number of mechanisms, for example, the increase in

403   algal biomass could indicate increased competition for resources with microalgae, such as

404   light and space, inhibiting recovery (Williams et al. 2000). Natural communities, such as the

405   rock pools in this study, are more complex and provide greater potential for species

406   interactions than the artificially assembled communities used in mesocosm experiments

407   (Bracken et al. 2008, Stachowicz et al. 2008, Crowe et al. 2012, Vye et al. 2015). Such

408   species interactions may moderate the cumulative impacts of stressors and reduce the

17

409 sensitivity of communities to changes in stressor characteristics, such as intensity (Petersen &

410 Hastings 2001). Furthermore, our previous mesocosm-based study, introduced both stressors

411 simultaneously (Vye et al. 2015), whereas this field study introduced nutrient enrichment to

412 pools where *S. muticum* was already established. The introduction of a second stressor to an

413 already stressed or destabilised system may determine the interactive effect of the stressors

414 (Mrowicki et al. 2016, Donohue et al. 2016). Our findings highlight the importance of testing

415 multiple stressor hypotheses in different of environmental contexts, i.e. field as well as

416 mesocosm experiments, in order to better understand the cumulative impacts of different

417 communities and assemblages. This study has an obvious limitation in that it was only

418 conducted on a single shore. Replicating multiple stressor studies at multiple sites would

419 incorporate spatial variation in environment context which could then be tested explicitly

420 (Bustamante & Branch 1996, Mrowicki et al. 2014).

421 Functional diversity has been shown previously to be an important determinant of the

422 invasibility of a community (Britton-Simmons 2006, Arenas et al. 2006). Our findings

423 suggest that species that use primary resources, such as light and space, differently may also

424 respond differently to the cumulative effects of invasion and nutrient enrichment. The

425 cumulative effects identified in this study were not consistent across all algal functional

426 groups present, because nutrient enrichment affected invasion effects on green and sub-

427 canopy algae, but not on canopy algae or turf algae. Mechanisms behind this interactive

428 effect are unclear, however, it is possible that *S. muticum* may have limited light penetration

429 to other algae (Britton-Simmons 2004), possibly offsetting nutrient enrichment effects on

430 growth. Differences in the cumulative effects among functional groups may lead to further

431 consequences for communities, including decreased resistance and resilience to other

432 invasive species and anthropogenic stressors (Elmqvist et al. 2003, Folke et al. 2004). To

433 conclude, this study shows complex cumulative effects of nutrient enrichment on

434    communities under different invasion scenarios, including the removal of the invader from

435    the system. These findings highlight the importance of identifying the cumulative effects of

436    multiple stressors on the responses of individual functional groups, alongside effects on

437    overall assemblage structure, in order to fully understand the consequences for ecosystems.

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661

662 **Table 1.** ANOVA of effects of the presence of *S. muticum* and nutrient enrichment treatments

663 on the biomass of algae: a) total algal biomass (values in brackets represent algal biomass

664 with *Sargassum muticum* included); b) canopy algal biomass (values in brackets represent

665 algal biomass with *Sargassum muticum* included); c) sub-canopy algal biomass; d) turf algal

666 biomass; e) coralline algal biomass; f) green ephemeral biomass g) microalgal biomass.

667 Significant values are in bold ($P < 0.05$).

668 | | | a) Total algal biomass | | |

| Source | df | MS | *F* | *P* |
|---|---|---|---|---|
| 669 | | | | |
| 670 Invasion (=Inv.) | 2 | 2970 (2198) | 4.491 (3.504) | **0.019** (**0.042**) |
| 671 Nutrient (=Nut.) | 2 | 50 (103) | 0.075 (0.165) | 0.928 (0.849) |
| 672 Inv. x Nut. | 4 | 166 (154) | 0.251 (0.246) | 0.907 (0.910) |
| 673 Residual | 32 | 661 (627) | | |
| 674 | | b) Canopy algal biomass | | |
| 675 Invasion (=Inv.) | 2 | 44.505 (30.340) | 5.151 (4.099) | **0.012** (**0.026**) |
| 676 Nutrient (=Nut.) | 2 | 5.780 (7.235) | 0.669 (0.977) | 0.519 (0.387) |
| 677 Inv. x Nut. | 4 | 10.918 (11.560) | 1.264 (1.562) | 0.305 (0.208) |
| 678 Residual | 32 | 8.639 (7.400) | | |
| 679 | | c) Sub-canopy algal biomass | | |
| 680 Invasion (=Inv.) | 2 | 2.350 | 0.094 | 0.911 |
| 681 Nutrient (=Nut.) | 2 | 1.160 | 0.046 | 0.955 |
| 682 Inv. x Nut. | 4 | 78.740 | 3.154 | **0.027** |
| 683 Residual | 32 | 24.960 | | |
| 684 | | d) Turf algal biomass | | |
| 685 Invasion (=Inv.) | 2 | 0.164 | 0.465 | 0.632 |
| 686 Nutrient (=Nut.) | 2 | 0.065 | 0.185 | 0.832 |
| 687 Inv. x Nut. | 4 | 0.106 | 0.302 | 0.874 |
| 688 Residual | 32 | 0.351 | | |
| 689 | | e) Coralline algal biomass | | |
| 690 Invasion (=Inv.) | 2 | 2.127 | 2.875 | 0.071 |
| 691 Nutrient (=Nut.) | 2 | 1.872 | 2.530 | 0.095 |

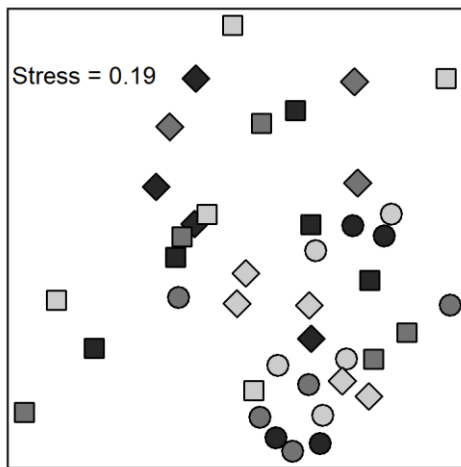| 692 | Inv. x Nut. | 4 | 19.688 | 1.663 | 0.183 |
| 693 | Residual | 32 | 0.740 | | |
| 694 | | f) Green ephemeral | | | |
| 695 | Invasion (=Inv.) | 2 | 7.333 | 4.369 | **0.021** |
| 696 | Nutrient (=Nut.) | 2 | 1.725 | 1.028 | 0.369 |
| 697 | Inv. x Nut. | 4 | 4.526 | 2.696 | **0.048** |
| 698 | Residual | 32 | 1.679 | | |
| 699 | | g) Microalgal biomass | | | |
| 700 | Invasion (=Inv.) | 2 | 2.791 | 9.099 | **0.001** |
| 701 | Nutrient (=Nut.) | 2 | 0.291 | 0.949 | 0.398 |
| 702 | Inv. x Nut. | 4 | 0.303 | 0.988 | 0.428 |
| 703 | Residual | 32 | 0.307 | | |

**Figure titles**



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Figure 1. Non-metric multidimensional scaling plot (nMDS) of rock pool assemblages
(percentage cover of macroalgae and abundance of slow moving or sessile invertebrates
>1cm) in experimental treatments at the end of the experiment based on a Bray-Curtis
similarity matrix (data are square-root transformed). $\diamond$ = *S. muticum* removed; $\bigcirc$ = *S. muticum* absent; $\square$ = *S. muticum* present. Light grey symbols = ambient nutrient
concentrations, mid grey symbols = intermediate nutrient concentrations and dark grey
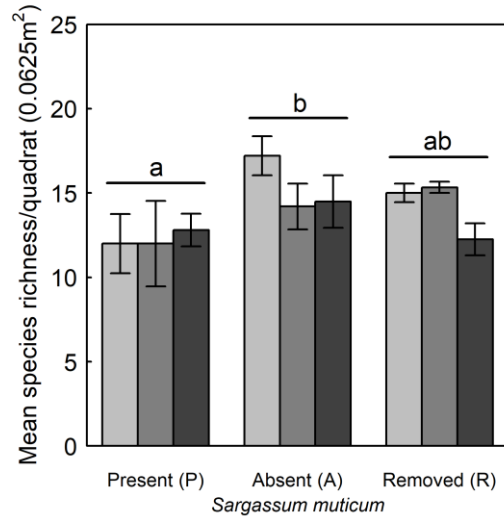symbols = high nutrient concentrations.

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Figure 2. Mean species richness ($\pm$ S.E.) of rock pools in experimental treatments. Light grey

bars = ambient nutrient enrichment, mid grey bars = intermediate nutrient enrichment and

dark grey bars = high nutrient enrichment ($n = 3$: invader removed, intermediate nutrient

enrichment; $n = 4$: invader removed, high nutrient enrichment and invader absent, high

nutrient enrichment; $n = 5$: all other treatments). Lower case letters indicate significant

differences among treatment levels or groups or groups of treatments ($P < 0.05$).
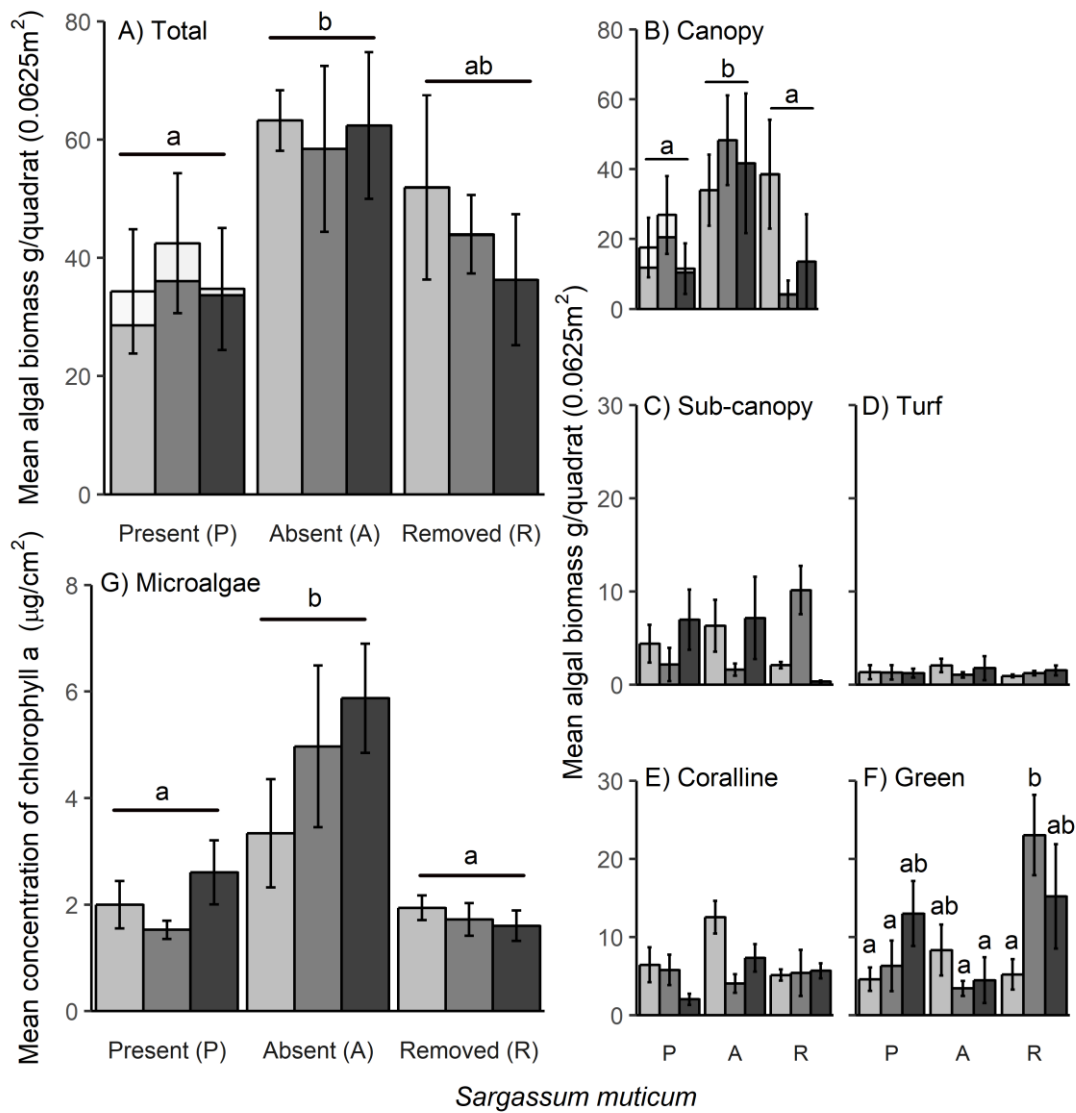
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Figure 3. Mean biomass (± S.E.) of: A) total macroalgal biomass; B) canopy algal biomass;
C) sub-canopy algal biomass; D) turf algal biomass; E) coralline algal biomass, F) ephemeral
green algal biomass; and G) total microalgal chlorophyll *a* concentration (± S.E.), in rock
pools where *S. muticum* was present, absent or removed. Light bars = ambient nutrient
enrichment, mid grey bars = intermediate nutrient enrichment and dark grey bars = high
nutrient enrichment, open section of bars = *S. muticum* biomass. Lower case letters indicate
significant differences among treatment levels or groups or groups of treatments ($P < 0.05$).