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Role of top-down and bottom-up forces on the invasibility of intertidal macroalgal assemblages

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ABSTRACT

Despite the available information regarding the negative effects of non-indigenous species (NIS) on ecosystem structure and functioning, the mechanisms controlling NIS invasion remain poorly understood. Here, we investigated the relative roles of top-down and bottom-up control on the invasion of intertidal macroalgal assemblages by the macroalga *Sargassum muticum* (Yendo) Fensholt. Using a factorial experiment, nutrient availability and intensity of herbivory were manipulated along an intertidal rocky shore. We found that early recruitment of *S. muticum* was enhanced by low nutrient enrichment but no effect of grazers was observed. In contrast, at the end of the experiment (9-months after invasion) top-down control, together with the number of NIS and the percentage cover of ephemerals, was a significant predictor for the invasion success of *S. muticum*. In addition, both top-down and bottom-up forces played a significant role in structuring macroalgal assemblages, which indirectly could have influenced invasion success. Hence, by shaping community structure, main and interactive effects of bottom-up and top-down forces may indirectly act on invasion. Our study highlights the importance of the recipient community structure on the invasion process and emphasizes the specific regulation of top-down and bottom-up forces in different stages of *S. muticum* invasion.

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1. Introduction

Invasions by non-indigenous species (NIS), one of the main human-mediated impacts on ecosystems, can directly and indirectly induce major effects on the structure and functioning of ecosystems (Grosholz, 2002). Understanding which factors influence the susceptibility of communities to invasion has become a central concern in ecology.

Although several factors are thought to play a role in the susceptibility of an ecosystem to invasion (Stachowicz and Byrnes, 2006), the composition and diversity of native species have been considered as some of the primary causes (e.g. Arenas et al., 2006; Britton-Simmons, 2006; Elton, 1958). Commonly, reduced resource availability is the most cited mechanism driving community invasibility (e.g. Britton-Simmons, 2006; Stachowicz et al., 1999). Increased diversity of species and functional groups is expected to increase the efficiency of resource use, generating a negative relationship between native diversity and invasion success (Stachowicz and Byrnes, 2006). Despite some controversy, the relationship between native diversity and invasibility is well reported and widely accepted (Davis et al., 2000; Stachowicz et al., 2007). In this context, the

Fluctuating Resources Availability Theory (FRAT) suggests that the invasibility of a community may change as the amount of unused resources fluctuates (Davis et al., 2000). Accordingly, the susceptibility of a community to invasion would increase whenever the availability of a limiting resource is increased.

In coastal systems, resource supplies and consumers play a crucial role in the regulation of intertidal habitats, i.e. bottom-up versus top-down control (Menge, 2000; Worm et al., 2000). Specifically, it has been suggested that bottom-up forces, e.g. nutrient levels, are of great importance in regulating species composition while top-down regulation, e.g. herbivory, exert stronger effects over macroalgal biomass (Burkepile and Hay, 2006). Increased availability of resources in an environment can be induced either by introducing resources at a faster rate than the community can sequester them (e.g. nutrient addition) or by reducing resource uptake by the community (e.g. decrease of species biomass) (e.g., Davis et al., 2000; Grime, 1977). Furthermore, complex interactions among herbivory and nutrients levels are also frequent (Hillebrand, 2003; Masterson et al., 2008; Worm et al., 1999, 2002).

Although a large number of studies have addressed the influence of top-down and bottom-up forces in structuring benthic communities (e.g. Bulleri et al., 2012; Burkepile and Hay, 2006; Worm et al., 2002), few experiments have been conducted to address the relative roles of nutrient supply and herbivore processes on invasion success of macroalgae (but see Vermeij et al., 2009). Roughly 20% of the marine

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invasive species in the world are macroalgae. These invaders can cause important ecological and economic damage by altering the structure and functioning of recipient ecosystems (Schaffelke et al., 2006). Understanding the mechanisms and processes that allow marine invasive macroalgae to become abundant is crucial for prevention and management of marine invasions in the future.

In the marine environment, macroalgal assemblages depend on resources such as space, nutrients and light (Carpenter, 1990). Thus, the susceptibility to invasion by a macroalgal community is expected to be influenced by the availability of those limiting resources (Arenas et al., 2006). For example, space availability and nutrient enrichment facilitated the establishment and spread of the brown invasive alga *Sargassum muticum* on an intertidal shore (Sánchez and Fernández, 2006). In addition, it has been suggested that susceptibility to invasion should be the highest when disturbance is accompanied by nutrient enrichment (Burke and Grime, 1996). The presence of grazers in a habitat can, however, induce disturbance of different kinds either by increasing space availability or by reducing algal biomass. Hence, disturbance by grazers may reduce resource use by decreasing the biomass of algal assemblages (Davis et al., 2000). This phenomenon would increase the amount of resources available and create a favorable time period for new recruitment. Nonetheless, experimental studies addressing the relative roles of herbivory and nutrients in the recruitment of algae indicate complex and possible interactive effects of both factors. For instance, a study in the Great Barrier Reef that simultaneously manipulated intensity of nutrients and herbivory, found that herbivory effects on algal recruitment (density and size) were not consistent among species and were stronger within nutrient-enriched treatments (Diaz-Pulido and McCook, 2003).

The invasion process is characterized by different transitional stages of invasion (Williamson, 2006) and survivorship through each stage is the key to success of invasive NIS. Here, we used a field factorial experiment using intertidal assemblages as a model system to test the individual, combined and potential interactive effects of nutrient enrichment and intensity of herbivory on the invasibility of intertidal macroalgal assemblages by the invasive macroalga *S. muticum* (Yendo) Fensholt. Previous studies on *S. muticum*, both from the intertidal and low subtidal, suggested that invasibility of assemblages was mediated by different resources at different stages of invasion (Britton-Simmons, 2006; Sánchez and Fernández, 2006). Thus, experiments conducted from early life stages throughout adult stages are of great importance in macroalgal invasion ecology (White and Shurin, 2007).

S. muticum Yendo (Fensholt) is a brown alga native to East Asia, and it is considered a highly invasive species (Critchley et al., 1983; Norton, 1976). Attached individuals were first recorded in Europe in the British Isles in 1973 (Critchley et al., 1983). The invasive seaweed *S. muticum* arrived at the rocky shores of the northern Spanish coast in the 1980s (Fernández et al., 1990) and is now present from Portugal in the south to Norway in the North. Characteristics such as being a fast growing species, monoecious, self-fertile and pseudo-perennial, among others, have been cited as responsible for the success of this species as an invader (Norton, 1976). A fertile individual of *S. muticum* bears thousands of receptacles (reproductive structure). Fertilized eggs are retained on the external surface of the receptacles until the development of tiny germings with adhesive rhizoids (Norton, 1981). Embryos are then liberated, they sink and generally reattach to the substratum within a few meters of the parent plant (Deysner and Norton, 1982). Adult plants stay attached to the substratum by a perennial holdfast. Seasonal patterns of growth and reproduction have been reported for this species (Arenas and Fernández, 1998; Deysner, 1984). In the study area, *S. muticum* has a spring-summer reproductive period while a senescence period is observed at the end of the summer (Arenas and Fernández, 1998; Fernández, 1999). Biomass shows a marked seasonality with a period of rapid growth starting around January and

a period of decrease in biomass at the end of the reproductive period when fronds detach themselves from the thallus.

By experimental manipulation of nutrients and herbivory levels and by assessing success of the invader after an artificial invasion, we tested the hypothesis that a greater availability of resources, through an increase in herbivory or due to nutrient enrichment, would affect the invasion of macroalgal assemblages. Additionally, we expected an interactive effect between nutrient concentrations and intensity of herbivory, where the magnitude of top-down forces of herbivory should increase with the bottom-up forces of nutrient availability.

2. Material and methods

2.1. Study site

The field study was conducted from February 2010 to March 2011 along an intertidal rocky shore in Cabo Estai (42°11' N, 8°48' W), Ria de Vigo (Galicia, Spain). Seawater temperature for the experimental period was about 15 °C (± 0.84 , mean \pm SE), while salinity remained stable at around 34.5‰ (www.meteovigo.es).

The site can be described as a gently sloping granitic rocky shore, with a wide variability of fauna and flora. Natural assemblages at low intertidal were characterized by a range of macroalgae with differing morphologies such as the leathery alga *Bifurcaria bifurcata* Ross, articulated calcareous algae such as *Corallina* sp. Linnaeus and *Jania rubens* (Linnaeus) Lamouroux, the corticated alga *Stypocaulon scoparium* Linnaeus, and green filamentous and foliose algae (e.g. *Ulva* spp.). NIS such as *S. muticum* (Yendo) Fensholt, *Asparagopsis armata* Harvey, *Grateloupia turuturu* Yamada and *Undaria pinnatifida* (Harvey) Suringar were also present at the site. Invertebrate assemblages were dominated by gastropod grazers, including several topshells from the genera *Gibbula*, the limpet *Patella* spp. and the common topshell *Monodonta lineata* Da Costa.

2.2. Experimental design and sampling procedure

Forty-five plots (35 × 35 cm) of natural assemblages were manipulated in the low intertidal (0.4–0.8 m above the lowest astronomical tide) following a two-way factorial design including the intensity of Nutrients and Herbivory as orthogonal fixed factors. All replicate plots were distributed randomly within the study site along 200 m of sea shore, separated by at least 2 m. The corners of the experimental plots were marked with epoxy putty to allow repeated sampling.

To study the effect of increased nutrient concentration, three levels were included in factor Nutrients: “Control”, corresponding to no nutrient addition and mesh bags control; “Low nutrients”, corresponding to an addition of 1 kg of nutrient pellets, N+; and “High nutrients”, corresponding to an addition of 2 kg of nutrient pellets, N++. Concentrations of nutrients were manipulated using slow controlled-release fertilizer pellets (Multicote®) in small mesh bags with 1 mm mesh size. Fertilizer pellets consisted of 15% N (8% NH₄⁺, 7% NH₃), 7% P (PO₂), 15% K (K₂O) and 2% MgO. Each experimental plot had 2 mesh bags (500 g each or 1 kg each, for low and high nutrients, respectively) on opposite sides anchored to the substrate with cable ties fasten to two steel screw eyes. Control mesh bags were filled with a plastic bag containing sand. Mesh bags were replaced every 3 months, or before if lost due to rough sea conditions, to ensure continuous delivery of nutrients. Herbivory intensity also had three levels: Natural herbivory, “H+”, where herbivores had free access to experimental plots; Low herbivory, “H-”, where antifouling paint was used to avoid herbivore access to experimental plots; and Procedural control, “PC”, to test for possible artifacts due to the use of antifouling paint, where antifouling paint was only used on two opposite sides of the plots. There were 5 replicates per treatment combination.

Experimental manipulation of nutrients and herbivory was maintained for three months prior to invasion. Prior to the invasion procedure, individuals of *S. muticum* were removed from the experimental plots and immediate surroundings. To assure a similar propagule pressure by *S. muticum* in all experimental plots, we invaded the plots artificially. Artificial invasion was set on 13th of May, 1 day before the new moon, due to the fact that *S. muticum* has a semilunar periodicity of egg expulsion coinciding to 24–48 h around new or full moons (Monteiro et al., 2009; Norton, 1981). Fertile individuals of *S. muticum* (≈ 30 cm long) were collected from an intertidal rocky-shore nearby. Approximately 500 g wet weight of algae was fixed to opposite sides in each plot using cable ties and 1 steel screw eye and were left for 1 week.

Algal percentage cover and density of herbivores were recorded immediately before treatment manipulation (at the beginning of the experiment, February 2010) and every 2–3 months thereafter until March 2011. Plots were monitored using a 35×35 cm quadrat divided into 100 sub-quadrats of 3.5×3.5 cm each. Primary and secondary algal covers were estimated by summing up 1% cover of each taxon individually and adding up the total. By joining the primary and secondary algal covers, total cover may greatly exceed 100% within each plot. In addition, percentage cover of sessile invertebrates and bare rock were also assessed adding up to the assemblage structure of the experimental plot. Macroalgae were identified to the most detailed level of taxonomic resolution achievable in the field. Grazers were individually counted and were left in or taken out of the plot depending on the herbivory treatment. Macroalgal species were assigned to different functional groups following Steneck and Dethier (1994). Nutrient enrichment is known to often increase the biomass of opportunistic fast-growing ephemeral species (Kraufvelin et al., 2010). Thus, in order to examine the potential indirect effect of the presence of these particular species, we chose to incorporate together species known as being highly opportunistic into a single category, Ephemerals. We incorporated together annual filamentous and foliose algae belonging to the genera *Ulva*, *Ceramium*, *Pterosiphonia* and *Polysiphonia* (Supplementary data, Table A.1). Additionally, known NIS were identified and included as a single variable named “NIS” (excluding *S. muticum*). Overall, the macroalgal categories used were: corticated, foliose, leathery macrophytes, crustose, articulated calcareous, ephemerals and NIS.

S. muticum individuals were first visible in experimental plots in December 2010. At the end of the experiment (March 2011), recruits of *S. muticum* were scraped from each experimental plot, taken to the laboratory and oven-dried at 60 °C for 48 h to estimate biomass (grams of dry weight). Several variables such as number of recruits, length and biomass were used as estimates of invasion success (Arenas et al., 2006; Britton-Simmons and Abbott, 2008).

2.3. Statistical analysis

Due to the specific amount of nutrient release in each plot, we assumed dependent repeated measures sampling. In this context, effectiveness of nutrient enrichment was examined using a Repeated measures ANOVA (rmANOVA) with Nutrients as a fixed factor and Month as within-subjects variable. The rm-ANOVA assumption of sphericity was evaluated using Mauchly's criterion. Bonferroni post-hoc comparisons were performed between pairs of variables. Furthermore, effectiveness of herbivory treatment was analyzed with a two-way ANOVA with Herbivory intensity (3 levels) as a fixed factor and Month (3 levels) as a random factor ($n = 15$).

Firstly, we tested for differences in assemblage structure between patches assigned to each experimental plot, to assure that any differences found later on were due to experimental treatments. Structure of assemblages (measured as percentage cover of algae, bare rock and sessile invertebrates) was compared through multivariate analysis of variance based on permutations (PERMANOVA). Based on a root-transformed Bray–Curtis similarity matrix, PERMANOVA analysis included 2 fully crossed fixed factors, Nutrients (3 levels) and

Herbivory intensity (3 levels) with 5 replicates per factorial combination of treatments. Additionally, PERMANOVA analyses were also performed at the end of the experimental period to compare structure of assemblages at the beginning and at the end of the experiment. For the three-way permanova analysis, we included Nutrients (3 levels), Herbivory intensity (3 levels) and Time (2 levels) as fixed factors. SIMPER analysis was used to identify taxa contributing most to multivariate differences between experimental assemblages.

To identify the main variables that influenced early recruitment and invasion success of *S. muticum* we used generalized linear models (GLMs). For early recruitment, count data expressed as number of recruits was analyzed assuming a quasipoisson distribution to account for overdispersion. For final recruitment data we assumed a negative binomial distribution to account for high overdispersion using the `glm.nb` function from the MASS library (Crawley, 2009) in R. We assumed a Gaussian distribution for biomass and length data and used `glm()` function with an identity-link. Predictive variables for both set of analyses were the factors Nutrients and Herbivory intensity, and the variables number of native taxa, number of non-indigenous taxa, percentage cover of morpho-functional groups (crustose, leathery, corticated, articulated calcareous) and percentage cover of ephemeral species.

Generalized Estimating Equations (GEEs), an extension of GLMs for repeated measures data analysis (Liang and Zeger, 1986), were used to assess how the structure of assemblages within each experimental plot varied over the experimental period due to nutrient enrichment and herbivory intensity. The specific response of assemblages was analyzed through changes in percentage cover of functional groups, ephemerals and species richness. We used the `geepack` package (Halekoh et al., 2006) and the `geeglm()` function in the R-program. For the analysis, a link function and an error structure for the residuals were specified as in GLM models described above. Hence, for count data we assumed a Poisson distribution and for continuous percentage cover data we assumed a Gaussian distribution. We also specified a first order autoregressive model, using the `corAR1` function, in the whole analysis assuming time dependence for each experimental plot unit.

All univariate analyses were carried out using the R-program 2.14.1 (R Development Core Team, 2011). Multivariate analyses were performed with PERMANOVA + for PRIMER v.6 (Anderson et al., 2008; Clarke and Gorley, 2006).

2.4. Effectiveness of treatments

Effectiveness of the nutrient enrichment was assessed by measuring nutrient concentrations in experimental plots in May, August and December 2010. Water samples were collected following low tide, with water 10–15 cm above experimental plots, using acid cleaned 50-ml plastic syringes with microfiber filters (Fisherbrand® MF 300). Immediately after collection, samples were placed on ice, returned to the laboratory, and frozen at -20 °C until analysis (less than three months after). Analyses of NO_3^- and PO_4^{3+} were carried out using a continuous-segmented flow autoanalyzer (Bran + Luebbe AA3). Analysis revealed marginally significant differences in nitrate availability over the different treatments (rm-ANOVA, $F_{2,42} = 3.055$, $P = 0.058$). Within treatment levels, the Control differed from the High nutrient treatments while the Low nutrient treatment showed intermediate values, not significantly different from the Control or High nutrient treatments. Phosphate concentration was not significantly affected by nutrient enrichment (rm-ANOVA, $F_{2,42} = 1.443$, $P = 0.248$).

To evaluate the effectiveness of the herbivory treatments, the intensity of herbivory in each experimental plot was estimated using wax-disks, as described by Thompson et al. (1997). Three wax disks (1 cm in diameter) were used per experimental plot. Small holes slightly larger than the disks were made with a small drill and filled with epoxy putty where disks were deployed for periods of 10 days every four months. Disks were examined under a dissecting

microscope. The percentage area of each disk scraped by radulae was estimated using an acetate grid with twenty evenly spaced holes. Percentage cover of grazing marks was estimated by assessing the number of radular marks observed under these holes. The herbivory treatment was effective because percentage cover of grazing marks varied significantly among treatments, consistently over time (ANOVA, $F_{2,4} = 7.63$, $P = 0.043$). Specifically, High intensity treatments (H^+) had a larger number of grazers than Low intensity treatments (H^-). No significant differences were observed between PC and the other treatments (Student Newman Keuls test, $P > 0.05$).

3. Results

Results indicated that assemblages from natural patches assigned to each experimental plot did not differ at the beginning of the experimental study (PERMANOVA, $P > 0.05$ for both studied factors).

3.1. Early recruitment of *S. muticum*

S. muticum recruits were not visible during sampling in early September (3 months after induced invasion) as expected. Recruits were first monitored in December 2010, the following sampling date. Number of recruits ranged from 0 to 26 individuals per plot and was significantly affected by nutrients (GLM, $P = 0.038$) with larger number of recruits in low nutrient enriched plots (Fig. 1). In contrast, herbivory intensity had no significant effect on early recruitment of *S. muticum*. Length of early recruits was also not related to any of the predicted variables analyzed.

3.2. Invasibility of macroalgal assemblages

Invasion success of *S. muticum*, measured as the length, biomass and number of recruits at the end of the experimental period (March 2011), varied in their response to the predictive variables studied. In general, experimental manipulation of nutrients showed no significant effect on the final invasion success of the invader (Table 1). Moreover, length of *S. muticum* recruits was not significantly related to any other predictive variable (Table 1a). In contrast, for both the biomass and number of recruits, the number of non-indigenous species (NIS) present at the recipient assemblage was a significant predictor for the observed patterns (Table 1b, c). Both invasion metrics decreased in relation to the larger number of NIS present in the experimental plots (Figs. 2, 3c). In addition, the number of recruits was also significantly affected by herbivory ($P = 0.046$) and marginally affected by ephemeral algae (Table 1c). Consistently, higher intensity of herbivory and greater percentage

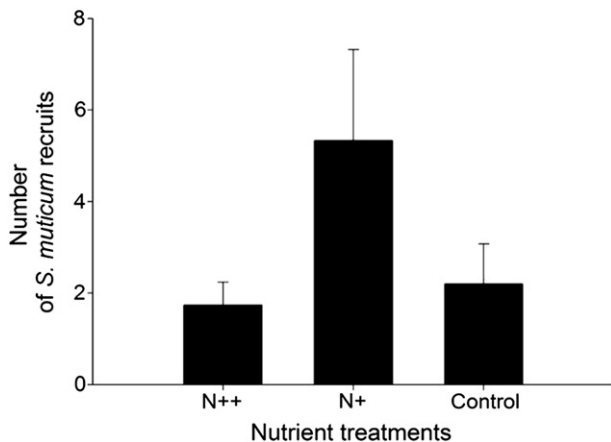


Fig. 1. Number of *Sargassum muticum* recruits ($n = 15$) 6 months after invasion (December 2010) over the different nutrient treatments. N^{++} , high nutrient enrichment; N^+ , low nutrient enrichment; Control, natural nutrient levels.

Table 1

Results from generalized linear models (GLMs) for a) the length, b) the biomass and c) the number of *S. muticum* recruits at the end of the experimental period. Model fitted to the explanatory variables Nutrients, Herbivory intensity, Number of native taxa (native), Number of non-indigenous species (NIS), Percentage cover of crustose algae (Crustose), Percentage cover of leathery algae (Leathery), Percentage cover of corticated algae (Corticated), Percentage cover of articulated calcareous algae (Artcalcareous), Percentage cover of filamentous and foliose algae (Ephemeral) and biologically meaningful interactions. The NULL (intercept only) model is $y \sim 1$, reflecting no dependence of y on the explanatory variables. Akaike information criterion (AIC). ^{1, 2, 3}, indicate the family of distribution applied to the full model.

Variables	Df	Deviance	Residual df	Residual deviance	P	AIC
a) Length of recruits¹						
NULL			44	3325.8		
NIS	1	251.2	43	3074.6	0.067	
Minimum adequate model						323.80
Full model						360.9
b) Biomass of recruits²						
NULL			44	3.290		
NIS	1	0.31	43	2.978	0.039	
Minimum adequate model						11.5
Full model						35.32
c) Number of recruits³						
NULL			44	63.517		
Herbivory	1	3.97	43	59.544	0.046	
NIS	1	5.07	42	54.470	0.024	
Ephemeral	1	3.61	41	50.858	0.057	
Minimum adequate model						268.34
Full model						291.97
Nutrients + Herbivory + Nutrients:						
Herbivory + native + NIS + Crustose + Leathery + Leathery:						
Nutrients + Leathery:Herbivory + Corticated + Corticated:						
Nutrients + Corticated:						
Herbivory + Artcalcareous + Ephemeral + Ephemeral:						
Nutrients + Ephemeral:Herbivory,						
^{1,2} family = Gaussian						
³ family = negative binomial						

cover of ephemeral algae species led to the smaller number of recruits at the end of the experiment (Fig. 3a, b).

3.3. Top-down and bottom-up regulation on macroalgal assemblage structure

Significant differences between initial and final assemblage structure were evident over the experimental period (Permanova, $Pseudo-F_{Time} =$

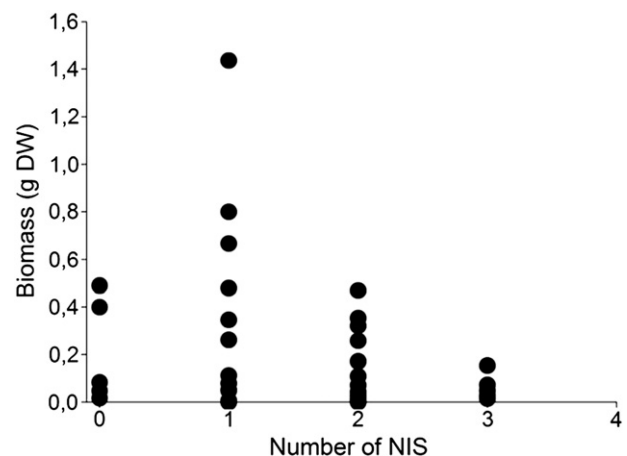


Fig. 2. Relationship between final biomass of *Sargassum muticum* recruits and the number of non-indigenous species (NIS).

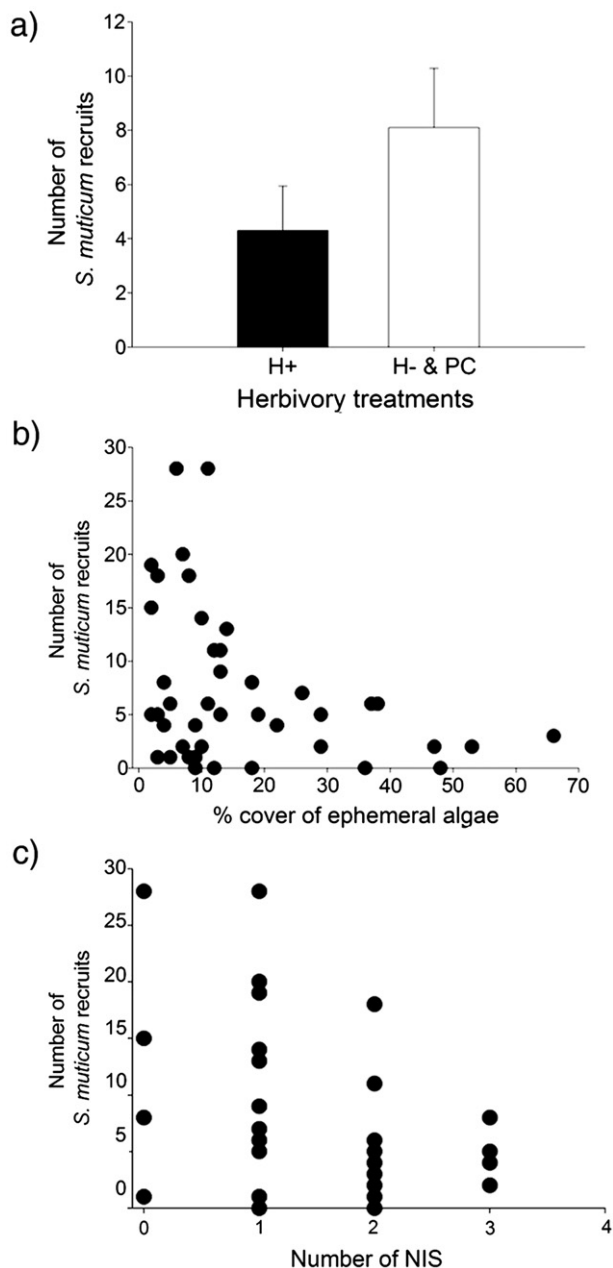


Fig. 3. Invasion success of *Sargassum muticum*. Effect of a) herbivory intensity, b) percentage cover of ephemeral algae and c) number of non-indigenous species (NIS) on the final number of recruits (9 months after invasion). H⁺, natural herbivory; H⁻, herbivory exclusion; PC, procedural control. Data in a) are mean values (+SE, n = 15 per herbivory treatment).

15.112, $P = 0.0001$). SIMPER analysis revealed that the average dissimilarity value between initial (pre-treatment) and final (1-year treatment) assemblage structure was 58.97%. These changes were mainly due to a general reduction in dominant species (e.g. *Corallina elongata*, *B. bifurcata* and *S. scoparium*) and further replacement by species such as *Ulva* spp., *Dictyota dichotoma* and *Sabellaria* sp. (Table 2).

Over the experimental period, both top-down and bottom-up regulation had varying influence on the number of native and NIS taxa and the cover of individual morpho-functional groups (Table 3). Herbivore exclusion significantly increased the number of native taxa, whereas nutrient enrichment resulted in their decrease (Fig. 4a). Similar to native taxa, the number of NIS decreased with nutrient enrichment, although no effect of herbivory was observed (Fig. 4c). The percentage cover of large leathery species showed similar variation to the one

Table 2

Summary of similarity percentage (SIMPER) analysis. Differences in average percentage cover of species contributing to dissimilarities between initial and final sampling dates (February 2010 and March 2011, respectively). A cut-off of a cumulative percentage dissimilarity of 60 was applied.

Species	Average abundance		Dissimilarity	% contribution
	Feb 2010	March 2011		
<i>Corallina officinalis</i>	6.03	4.03	5.79	9.81
<i>Bifurcaria bifurcata</i>	3.14	2.26	4.06	6.88
<i>Stypocaulon scoparium</i>	3.06	2.22	4.00	6.79
<i>Lithophyllum incrustans</i>	2.73	1.18	3.64	6.17
<i>Ralfsia verrucosa</i>	1.73	0.52	3.13	5.31
<i>Ulva</i> spp.	0.90	2.51	3.04	5.15
<i>Dictyota dichotoma</i>	1.60	2.15	3.02	5.11
Bare rock	1.56	1.13	2.92	4.95
<i>Sabellaria</i> sp.	0.00	1.62	2.90	4.91

observed over the number of native species (Fig. 4b). In addition, there was a significant interactive effect of grazing and nutrients on the cover of crustose, corticated, articulated calcareous and ephemeral algal species (Table 3). In particular, crustose algae showed opposite patterns at natural herbivore densities, depending on the nutrient treatment (Fig. 5a). Percentage cover of both corticated and articulated calcareous algae decreased with high nutrient enrichment at natural herbivore densities. Nonetheless, in herbivore exclusion treatments, both morpho-functional groups showed contrasting patterns, depending on the nutrient experimental treatments (Fig. 5b, c). Finally, ephemeral species showed a substantial increase in percentage cover at low nutrient enrichment and natural herbivore densities. Additionally, exclusion of herbivores resulted in a significant increase in percentage cover under nutrient enrichment conditions, i.e., low and high enrichment (Fig. 5d).

4. Discussion

By maintaining similar propagule pressure and disturbance, this study gave evidence of the importance of inherent features of the invasive species (invasiveness) and recipient communities (invasibility) to invasion success. A key finding from our study was that invasion success of *S. muticum* was governed by several variables and the magnitude of each effect depended on the invasion metrics used (i.e. abundance, growth and survivorship of recruits). Additionally, top-down and bottom-up forces induced specific regulation in different stages of the invasion.

Over the initial stage of the invasion, early recruitment of *S. muticum* was positively influenced by low nutrient enrichment whereas high nutrient enrichment and grazers had limited effects. Two justifications could explain the lack of response to high nutrient enrichment during *S. muticum* recruitment. Used to low summer nutrient concentration, *S. muticum* may use nutrients more efficiently at low concentrations than at high concentrations. This fact could explain their greater survival at low nutrient environments. This first explanation is, however, not in agreement with previous manipulative experiments with *S. muticum* in which higher nutrient enrichment played an important role in controlling community invasibility (Sánchez and Fernández, 2006). Another possible explanation is based on the community structure at the time of the invasion. Over the artificial invasion period a substantial increase in the percentage cover of fast-growing ephemeral species can be observed (Supplementary data, Fig. B.1). The observed bloom of ephemerals could have prevented the settlement and/or early survival of *S. muticum* by reducing available nutrient resources or simply by settling over the germlings preventing their survival. A study along the rocky shores of New England suggested that *Enteromorpha* could have outcompeted the long-lived *Chondrus crispus* by settling on top of its thallus which became bleached and then disappeared (Lubchenco, 1978).

Table 3
Analysis of Wald statistic table from Generalized Estimating Equations (GEE) examining the main and interactive effects of Nutrient and Herbivory intensity on the number of native species, number of non-indigenous species (NIS), percentage cover of crustose algae, percentage cover of leathery algae, percentage cover of corticated algae, percentage cover of articulated calcareous algae, and percentage cover of ephemeral filamentous and foliose algae (Ephemeral). Data from February 2010 to March 2011.

	Df	Number of native species		Number of NIS		Crustose algae		Leathery algae		Corticated algae		Articulated calcareous		Ephemeral	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Nutrients (N)	2	34.4	<0.0001	14.45	0.001	5.76	0.056	16.44	0.0003	3.63	0.163	2.1	0.350	14.08	0.001
Herbivory (H)	2	6.5	0.039	2.01	0.365	5.81	0.055	8.73	0.013	14.37	0.001	2.2	0.330	2.17	0.338
N×H	4	2.6	0.635	7.10	0.132	23.63	<0.0001	8.25	0.083	14.12	0.007	48.4	<0.0001	9.84	0.043

A recent study in northern Portugal (Monteiro et al., 2012) described a varying effect of grazers' exclusion depending on shore-height, suggesting differences in grazing pressure at mid- and low intertidal. Low intertidal habitats showed no effect of grazer exclusion (Monteiro et al., 2012), which could explain our results over the recruitment period. Differences in the assemblage and abundance of herbivores between habitats could be responsible for the patterns found (Monteiro et al., 2012). In contrast, at the end of the experiment the presence of grazers together with specific traits of the recipient assemblage influenced the invasion success of the invader. Here, top-down control, together with number of non-indigenous species and percentage cover of ephemerals, was a significant predictor for the number of *S. muticum* recruits, a proxy of post-settlement survival. Additionally, nutrient

enrichment might have promoted invasion success of *S. muticum*, though indirectly. Nutrient availability may modify food preference of grazers (Russell and Connell, 2005) and so bottom-up factors may be linked to top-down forces (Menge, 1992). The fast-growing strategy of *S. muticum* linked to its possible rapid nutrient uptake might induce grazing preference over later *S. muticum* recruits. The mechanisms promoting *S. muticum* recruitment are suggested to be the same behind the expansion of native and other non-indigenous species (Davis et al., 2000). Hence, competition for resources may explain our results if we assume NIS in general to be better competitors than natives. Moreover, ephemeral species could act as a barrier to invasion. Accordingly, our results indicated that the cover of ephemerals negatively affected the number of recruits, reinforcing this idea. Furthermore, no significant

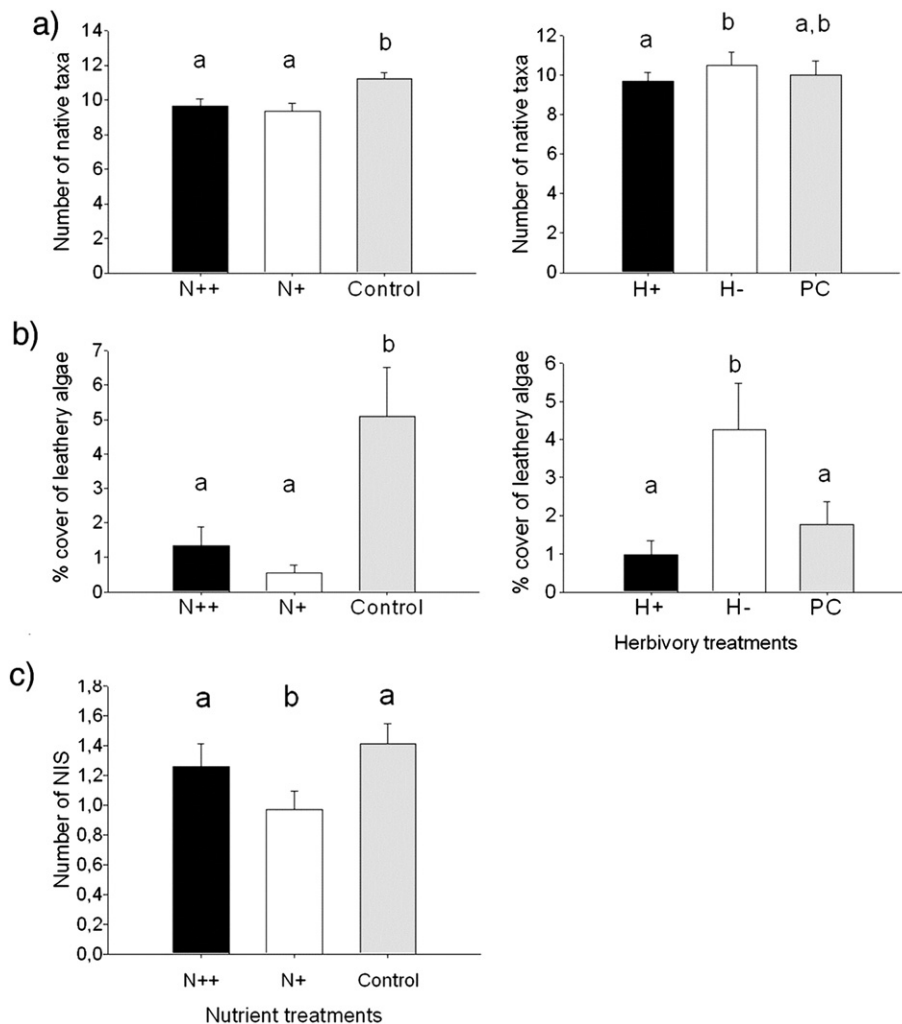


Fig. 4. Structure of assemblages throughout the experimental period (mean \pm SE, $n = 90$). a) Number of native taxa, b) percentage cover of leathery algae across Nutrient and Herbivory intensity treatments and c) non-indigenous species (NIS) across nutrient treatments. Abbreviations as in Figs. 1 and 3. Means are calculated by averaging data from each plot unit (15) over time (6). Means with a common letter do not differ from each other based on SNK tests at $P = 0.05$ level.

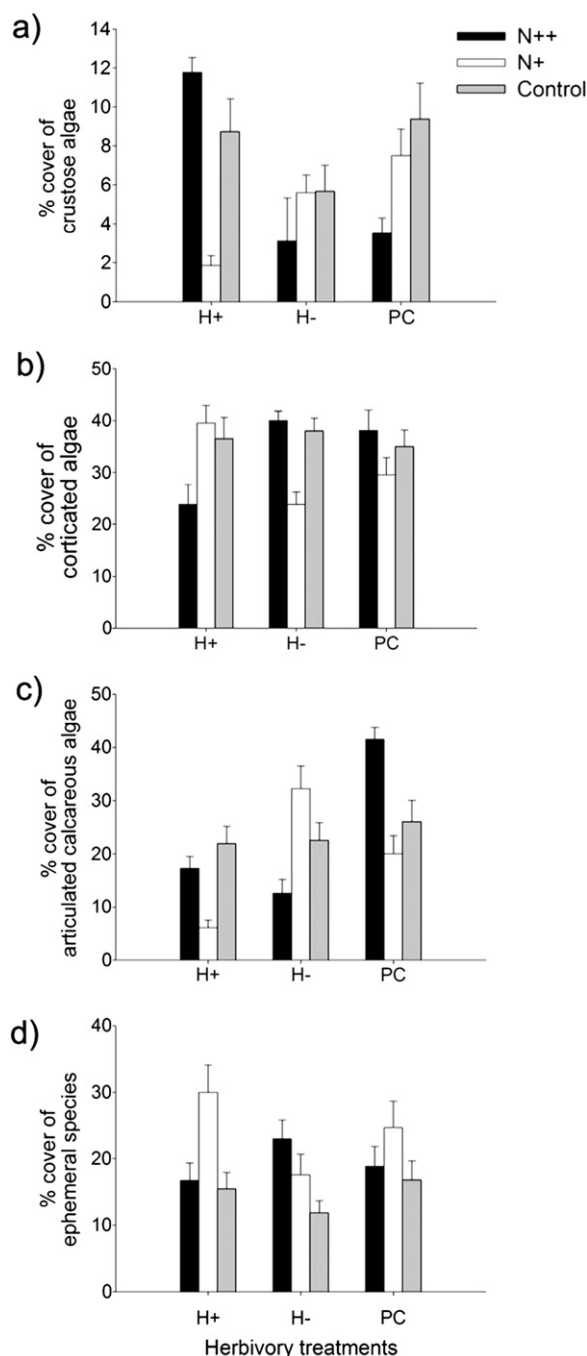


Fig. 5. Mean (\pm SE, $n = 30$) percentage cover of crustose, corticated, articulated calcareous and ephemeral species across Nutrient and Herbivory intensity treatments over 6 sampling dates. Means calculated by averaging data from each plot unit (5) over time (6). Abbreviations as in Figs. 1 and 3.

growth response of *S. muticum* to herbivory was observed in our study. The fact that there was no effect of herbivory on growth when it negatively affected the abundance of recruits could suggest that predation only influences *S. muticum* directly in the germling stage and decreases with growth and maturation. Several studies have indicated that phenolic compounds can act as a chemical defense in marine brown algae (Steinberg, 1985) and suggested that those defensive compounds increase with growth and maturation (Van Alstyne et al., 2001). Another possible explanation could be that the effect of grazers involves consumption of the entire germling, thus affecting abundance but not growth of the remaining individuals (Diaz-Pulido and McCook, 2003). We should also mention that the method used to exclude grazers was

effective excluding those grazers that crawl and creep on the rock but was probably not efficient with those species that swim or “jump” from individual to individual.

Strong top-down control in marine habitats may influence species production or biomass (Bracken and Stachowicz, 2007; Jenkins et al., 2008). This control was not evident in our study, where the number of NIS in the recipient community was the key predictor of the overall final *S. muticum* biomass. The presence of NIS has been suggested to create facilitative interactions among invaders, i.e. an “invasion meltdown” process. Invasion meltdown describes positive interactions among invaders with consequent increased probability of survival and/or ecological impact at the community level (Simberloff and Von Holle, 1999). In the present study, however, not only was no facilitation apparent but a negative impact on the final biomass of the invader was also found, linked to the presence of NIS on the recipient assemblage. Consistently, the majority of described interactions between NIS are those in which individuals of one species are benefited and those of the other are negatively affected (Simberloff and Von Holle, 1999). Here, the presence of one NIS species seems to be an advantage to *S. muticum* biomass production, whereas the presence of more than one species had a negative impact. These results suggested mutual interference or competition between NIS, giving evidence of a “biotic resistance” process. The mechanisms supporting such interference or competition were not, however, addressed by the present experimental design. Quantification of population-level impacts of NIS on one another is of great interest and should be addressed in future studies (Parker et al., 1999).

Species coexistence in natural communities is driven by resource partitioning (Chesson, 2000; Schoener, 1974) which is dependent on species biological traits. Over the experimental period leathery algae responded to both manipulated factors independently, whereas all other functional groups showed interactive effects. Surprisingly, there was a substantial increase in leathery cover with herbivorous exclusion. This functional group is expected to be less palatable and more defended than other functional groups such as turf algae (Littler et al., 1983) and so significant top-down effects were not expected. Negative grazing effects on leathery species could also be related to selective herbivory. A recent study suggested that by grazing on specialized tissues, herbivores may reduce N uptake and thus have a greater effect on communities than expected (Bracken and Stachowicz, 2007). Conversely, it has been suggested that by grazing filamentous/turf algae to low biomass levels, herbivores may then be forced to feed on less palatable algae (Burkpile and Hay, 2006). This phenomenon may explain the observed effect of herbivores on leathery cover. In this experiment, there was significantly more grazing (reduction in percentage cover) over corticated algae exposed to elevated nutrients than ambient nutrient concentration. In contrast to corticated algae, crustose algae increased in percentage cover when elevated nutrients and high grazing intensity were present in combination. These results are in agreement with previous experiments in which crustose coralline algae were more abundant under high herbivore pressure (Belliveau and Paul, 2002). Although calcareous macroalgae may be less nutrient limited than fleshy species (Delgado and Lapointe, 1994), the presence of herbivores resulted in higher percent cover probably due to higher consumption of fleshy algae. By limiting algal biomass, grazing promotes patches of bare rock which are then quickly occupied by crustose coralline algae (Wai and Williams, 2005).

In this study, both top-down and bottom-up forces played a significant role in structuring assemblages. Our results suggested that the susceptibility of a community to invasion might be affected by changing specific functional traits of the recipient community, due to potential indirect effects of bottom-up and top-down forces (Leibold et al., 1997). These results may, however, be context-dependent as indicated by a recent study across regions of contrasting productivity in Australia (Bulleri et al., 2012). Additionally, a study in New Zealand’s perennial short tussock grasslands suggested that the characteristics of the resident community were more critical in determining invasion success

than fluctuating resource availability (Walker et al., 2005). Although it may appear contradictory, our results agree and reinforce this perspective. The dynamics and structure of the recipient community are key components of an invasion success. Hence, by shaping community structure, main and interactive effects of bottom-up and top down forces may be considered as indirect factors acting on invasion.

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