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Functional diversity and climate change: effects on the invasibility of macroalgal assemblages

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Abstract Climate-driven and biodiversity effects on the structure and functioning of ecosystems are increasingly studied as multiple stressors, which subsequently may influence species invasions. We used a mesocosm experiment to test how increases in temperature and CO_2 partial pressure (pCO_2) interact with functional diversity of resident macroalgal assemblages and affect the invasion success of the non-indigenous macroalga *Sargassum muticum*. Early settlement of *S. muticum* germlings was assessed in the laboratory under common environmental conditions across three monocultures and a polyculture of functional groups of native macroalgae, which had previously grown for 3 weeks under crossed treatments of temperature and pCO_2. Functional diversity was a key driver shaping early settlement of the invader, with significant identity and richness effects: higher settlement occurred in low-diversity and low-stature assemblages, even after accounting for treatment biomass. Overall, early survivorship of settled germlings responded to an interaction of temperature and pCO_2 treatments, with survivorship enhanced in one treatment (high pCO_2 at ambient Temperature) after 3 days, and reduced in another (ambient pCO_2 at high Temperature) after 10 days, although size was enhanced in this same treatment. After 6 months in the field, legacy effects of laboratory treatments remained, with *S. muticum* reaching higher cover in most assemblages previously subjected to ambient pCO_2, but ephemeral green algae appearing disproportionately after elevated-pCO_2 treatment. These results caution that invasion outcomes may change at multiple points in the life cycle under higher-CO_2, higher-temperature conditions, in addition to supporting a role for intact, functionally diverse assemblages in limiting invader colonization.

Keywords Multiple stressors · Invasibility · Functional diversity · *Sargassum muticum* · pCO_2 · Temperature

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Introduction

It is widely accepted that human activities are causing global environmental changes with a high ecological impact on natural systems (Sala et al. 2000; Halpern et al. 2008). Increased anthropogenic CO_2 emissions over the past 200 years have led to greater CO_2 uptake by the oceans (Feely et al. 2004). This ongoing process of atmospheric CO_2 uptake is changing seawater carbon chemistry and is expected to substantially decrease oceanic pH (i.e. pH reduction by 0.7 units based on IPCC scenario for 2000–2100, Caldeira and Wickett 2003), a phenomenon known as "ocean acidification" (Feely et al. 2004). In addition to ocean acidification, the increase in atmospheric CO_2 also has been reflected in increased global temperatures, with consequent increase of the mean sea-surface temperature of the oceans (IPCC 2007). Similarly, the introduction of non-indigenous species (NIS) is also recognized as a significant component of global change (Sala et al. 2000; Williams and Smith 2007), and their impact on native populations, communities and ecosystems have been widely recognized for decades (Elton 1958; Lodge 1993).

Currently, ecologists concentrate on investigations examining the effects of single global change agents, yet research on the interactive effects of multiple agents is mostly lacking. Approximately 60 % of climate-related research publications in the marine realm are focused on independent effects of temperature (Harley et al. 2006). More recently, several studies examined the independent effect of ocean acidification (e.g. Feely et al. 2004; Porzio et al. 2011), whereas very few studies have examined the interaction of both factors (but see Martin and Gattuso 2009; Connell and Russell 2010). Yet, global change agents will not act in isolation and their combination could produce unexpected results (Harley et al. 2006; Darling and Côté 2008). For example, although ocean acidification has negative effects on calcifying algae (Martin and Gattuso 2009; Hofmann et al. 2012), there is growing evidence that the combined effect of pCO_2 with other global-change drivers may also have positive effects on non-calcareous algae (nutrients or temperature, Russell et al. 2009; Connell and Russell 2010).

Similarly, studies examining the interactions between global change drivers and invasions have been largely ignored. Most experimental work on invasion ecology done to date supports Elton's (1958) ideas in demonstrating greater resistance to invasion in diverse communities (Tilman 1997; Stachowicz et al. 1999), although patterns are scale-dependent (Fridley et al. 2007), and the mechanism driving such relationship remains unclear. Invasive species can modify ecosystems by displacing native species and/or altering habitat characteristics (Crooks 2002). Hence, there is increasing concern that multiple global change drivers of ecological change could interact synergistically to accelerate biodiversity loss. Several experimental studies found a positive relationship between biodiversity and invasion resistance (Stachowicz et al. 1999, 2002a), although others suggested a more important role of species identity over species richness (Crawley et al. 1999; Arenas et al. 2006). Diversity also plays a role in the stability of marine assemblages by reducing the magnitude of changes and increasing resilience of assemblages to environmental stressors (Allison 2004; Bertocci et al. 2010). Nevertheless, species identity can be even more important than diversity, particularly when species play strong roles in structuring assemblages (Kroeker et al. 2010). Acting simultaneously, ocean warming and acidification are promoting shifts in marine ecosystem through changes in species survival (Fabry et al. 2008) and assemblage composition (Widdicombe and Spicer 2008). Consequently, by altering disturbance regimes and resource dynamics global-change components can interact with biological invasions (Dukes and Mooney 1999). In marine systems, NIS are an increasing concern, especially in coastal environments. In particular, macroalgae are a significant component of invasions, representing up to 40 % of the total number of introduced NIS (Schaffelke et al. 2006) in a given area. Although the ecological effects of introduced macroalgae have been studied in only 6 % of the species (Williams and Smith 2007), results show mostly negative impacts or changes on the recipient communities (see review in Schaffelke and Hewitt 2007; Thomsen et al. 2009). The spread of NIS and climate change are indeed pointed as some of the main threats to marine coastal systems (Stachowicz et al. 2002b; Hoegh-Guldberg and Bruno 2010) that will simultaneously impact marine communities in a cumulative way over time and space. Climate change may interact with NIS and influence colonization, distribution and the main effect of marine invaders (Ruiz et al. 1999). For example, climate-change

conditions might increase the invasion success of NIS, in particular by increasing their competitive ability and survival rate (Byers 2002; Sorte et al. 2010).

There is evidence that the effects of climate change on communities might occur in two ways: (1) Via direct impact on the diversity and abundance of native species and (2) via indirect impact by increasing invasions by NIS (Sorte et al. 2010). To improve our knowledge on the role of functional diversity and global change drivers on the invasibility of marine communities, we conducted an experiment in mesocosm using synthetic assemblages of varying functional diversity that resembled macroalgal assemblages from intertidal rock pools characteristic of the western Atlantic coast of the Iberian Peninsula. There is no single driver of coastal biodiversity impacts, rather a combination of stressors. Rocky intertidal habitats are particularly vulnerable to those stressors as many of the global change agents act at the terrestrial-marine interface (IPCC 2007). For example, the foraging behavior of the keystone predator *Pisaster ochraceus* was related to water temperature (Sanford 1999) while bivalve species such as *Mytilus* spp. may be affected by both air and water temperature (Harley and Helmuth 2003). Due to the constantly changing nature of intertidal rock pools, organisms from those systems need to be adapted to deal with those changes. The adaptation of intertidal organisms to short-term changes in their habitat makes them convenient model species to test the effects of climate change over sudden changes in environmental factors. The brown alga *Sargassum muticum* (Yendo) Fensholt 1955, considered one of the most invasive alga in Europe and North America (Norton 1977), is a good model species for such experimental studies. *S. muticum* inhabits diverse types of habitats from intertidal channels to shallow subtidal being also present throughout the intertidal in rock pools. Native to Southeast Asia (Yendo 1907), it was first recorded in Europe in 1973 (Critchley et al. 1983) and on the Galician coast in 1986 (see Pérez-Cirera et al. 1989) where its distribution is widespread (Incera et al. 2011). This species is now present from Portugal in the south to Norway in the north.

Ecologists aim to understand whether ecosystems will became more (or less) susceptible to invasion due to global change and its direct consequences on the impacts of invaders (Dukes and Mooney 1999). In order to assess whether global-change drivers will induce higher susceptibility of marine communities to invasions, it first needs to be demonstrated that survivorship at early stages of invasion will be positively/negatively affected by those drivers. The goal of this study was to examine the combined effects of two climate change-related factors, temperature and CO_2 partial pressure, and diversity erosion in shaping the invasion success of the invasive brown macroalga *S. muticum*. For that, we first subjected macroalgal assemblages of varying levels of functional diversity to combined experimental conditions of increased CO_2 partial pressure and temperature for 3 weeks. After this 3-week period, assemblages were seeded with propagules of the invader *S. muticum* at ambient conditions and early settlement of *S. muticum* was evaluated. The invasion of the assemblages was performed under ambient conditions due to the uncertainty of the effects of climate change on the process of propagule release. Survivorship of settled germlings was quantified under the same combined experimental conditions for a period of 2 weeks. Then assemblages were placed in intertidal rock-pools where *S. muticum* recruitment success and legacy effects of climate change disturbance were evaluated after 6 months.

Methods

Laboratory mesocosm system

Our mesocosm system was set in 350-L PVC independent tanks supplied with 1 μm sand-filtered seawater from the Ria de Vigo (42°13′20″N; 8°49′28″W, Galician coast, NW Spain), renewed once a week. Each tank contained two submersible pumps (3,000 L h^{-1}) to insure water movement and a set of 8 cool white fluorescent lamps, (F18 W/840; water surface irradiance 140–150 μE m^{-2} s^{-1}) with 12:12 photoperiod (light: dark, h) to resemble natural fluctuations. A nutrient solution (1 mL each nutrient solution per liter; 42.50 g L^{-1} $NaNO_3$ and 10.75 g L^{-1} Na_2HPO_4) was added to each tank once a week.

Climate change scenario was achieved by manipulating two climate-change factors, temperature and partial pressure of carbon dioxide (pCO_2). The experimental set-up included two temperature levels (low: average ambient temperature of 15 °C and high: increased temperature of 20 °C) and two pCO_2 levels

(ambient 390 ppmv and increased 1,000 ppmv) following a 2 × 2 orthogonal design (Fig. 1). Average temperature of 15 °C corresponds to the average temperature values recorded at the surface of Ria de Vigo for April and May between 2006 and 2010 (14.93 ± 0.38 °C, www.meteogalicia.es). IPCC climate change scenario for 2100 (IPCC 2007) shows a possible average increase in the global temperature of 1.4–5.8 °C and atmospheric pCO_2 up to 984 ppmv.

Temperature in each tank was controlled with one titanium aquarium chiller with UV sterilizer (TECO-TC15). During the experiment, ambient air from outdoors (ambient pCO_2 treatments) and CO_2-rich air (high pCO_2 treatments) were continuously bubbled to the appropriate tanks. Tanks were covered with plastic caps to prevent gas exchange between them. Thus, by bubbling air with ≈1,000 ppmv, our experimental design manipulated an increase of 600 ppmv in ambient atmospheric pCO_2.

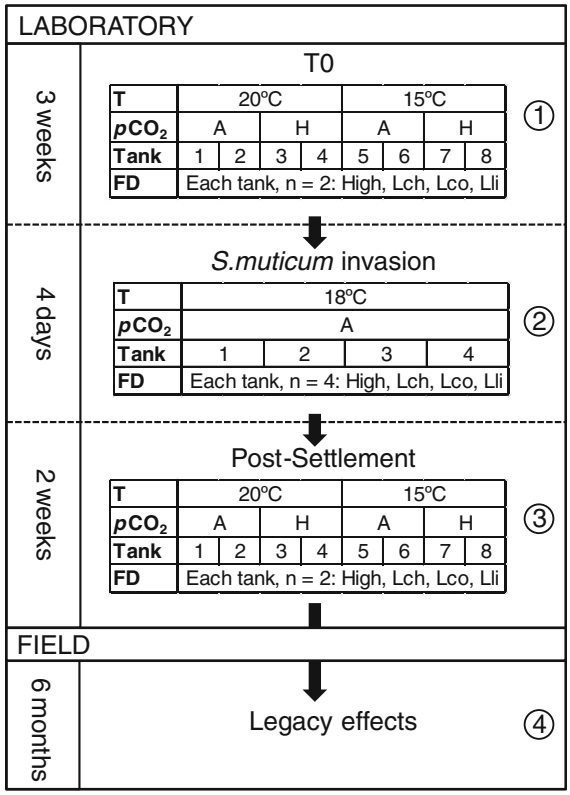

Fig. 1 Diagram of experimental design. T0 points out the beginning of the experiment, May 2010, under laboratory conditions, and a time sequence is represented afterwards ending on December 2010 under field conditions. *T* temperature, *A* ambient pCO_2, *H* high pCO_2, *FD* functional diversity, *High* high functional diversity assemblages, *Lch* monospecific assemblages of subcanopy species, *Lco* monospecific assemblages of turf-forming species, *Lli* monospecific assemblages of encrusting species

Environmental parameters in tanks

Temperature was continuously monitored during the experiment using StowAway Tidbit Data Loggers (Onset Computer Corporation, Pocasset, MA, USA). Salinity and pH were measured in each tank every day around midday with a glass electrode (Cond 340i and pH 340i, respectively, WTW, a Nova Analytics Company, Germany). Total dissolved inorganic carbon (TDIC) was assessed several times a week by filtering water samples through glass microfiber filters (Whatman International Ltdl, Maidstone, UK) into serum vials that were capped without head space. Samples were analysed the same day using an Infrared Gas Analyzer (LiCOR 7000) and TDIC was then partitioned into pCO_2, bicarbonate, carbonate and total alkalinity using the program csys.m (Zeebe and Wolf-Gladrow 2001).

Synthetic assemblages

Synthetic assemblages were created in order to simulate functional variability/gradients in natural macroalgal assemblages. By relating similarities in morphology and resources use (Arenas et al. 2006), three morpho-functional groups were selected a priori (modified from Steneck and Dethier 1994): (a) encrusting coralline species, *Lithophyllum incrustans* (hereafter 'Lli'); (b) turf-forming species from the genus *Corallina* (hereafter 'Lco'); and (c) a mixture of the two morphologically similar subcanopy species, *Chondrus crispus* and *Mastocarpus stellatus* (hereafter 'Lch'). Experimental assemblages consisted of 20 × 20 × 1 cm PVC plates with 14 cubes of rock holding the above referred morpho-functional groups and 2 PVC cubes used to hold two recruitment discs (5.11 cm^2) to evaluate the early settlement of germlings. The rock and PVC cubes were framed by 4 strips of PVC. To build the experimental assemblages, small boulders bearing the selected morpho-functional groups were collected in intertidal rock-pools. Using a commercial tile cutter, rock pieces were cut into cubes (2 × 2 × 2 cm) which were held in position in the plate using fast setting underwater cement and

screws. In total, we constructed 64 synthetic assemblages: 48 with one functional group (16 replicates per functional group, i.e. low diverse assemblages) and 16 with three functional groups (hereafter mentioned as high diverse assemblages, 'High'). Synthetic assemblages were randomly arranged in 8 experimental tanks with the treatment conditions orthogonally applied and were maintained in those conditions for 3 weeks (Fig. 1, sequence 1).

Invasion of assemblages

Initial biomass of assemblages was different because of the identity of the species included and changed after 3 weeks of exposition to the environmental factors. To incorporate these differences in biomass as predictor in our statistical analyses of invasion resistance, we estimated biomass before the inoculation of assemblages. Biomass of turf-forming and subcanopy species was estimated following Åberg (1990), from length of primary lateral and perimeter of each alga inside the cubes. We used data of dry weight and size from 60 individuals previously collected from each functional group to construct a regression model that allowed our estimations. In the case of the encrusting species, we selected 2 × 2 cm rock pieces colonized with encrusting coralline species, oven-dried for 48 h at 50 °C and weighted for dry-weights. We then placed it in hydrochloric acid (0.5 M HCl) for 48 h to remove the calcium carbonate. Rock pieces were then rinsed with freshwater, oven-dried for 24 h and then re-weighted. The difference from the dry-weights, i.e. before and after the HCl treatment, was used to obtain the biomass of the algae at the square and the average of 40 squares allowed us to estimate the biomass in our plates.

Fertile individuals of *S. muticum* were collected from an intertidal area in the Ria de Vigo. Then, algae were rinsed with freshwater to eliminate epiphytes. Before invasion, assemblages were returned to unmanipulated conditions resembling field environment (18 °C and ambient pCO_2), and randomly distributed in 4 tanks (Fig. 1, sequence 2). We took the decision to perform the invasion procedure at 18 °C for 2 reasons: (1) it was an average temperature between 15° and 20° and (2) reproduction and embryo liberation is trigged by temperature in *S. muticum* (Deysher 1984; Arenas and Fernández 1998). We aimed for the embryos liberation by adult *S. muticum* to be as intense as possible and thus 18 °C was the temperature selected. On 26 May, assemblages were experimentally invaded by suspending fertile *S. muticum* individuals over the 4 tanks for 4 days (average ± SD biomass of 5,836.90 g FW ± 30.57). Experimental invasion occurred around the full moon because *S. muticum* has a semilunar periodicity of egg expulsion around new or full moon (Monteiro et al. 2009). For estimating propagule pressure in each tank, 7 additional recruitment discs (5.11 cm^2) were randomly placed at the bottom of the tanks and the number of *S. muticum* germlings was counted using a stereo microscope with additional dimmed light. Propagule pressure, calculated as the number of germlings per 0.5 cm^2, differed significantly among tanks with a lower propagule pressure in tank 3 (ANOVA, $F_{3,116} = 20.69$, $P < 0.001$; SNK-tests, $P < 0.05$). Invasibility of assemblages was evaluated by counting the number of *S. muticum* germlings in the two recruitment discs installed in each assemblage.

After experimental invasion, assemblages were placed back to the 8 tanks where the experimental conditions were restored for another 2 weeks (Fig. 1, sequence 3). Initial success of the invasion was measured as the initial percentage of survivorship and length of settled germlings. Percentage of survivorship was calculated 3 and 10 days after experimental conditions of temperature and pCO_2 were restored and length of settlers (mm) was measured at day 10. Percentage of survivorship was calculated as N_t/N_0*100, where N_t is the number of surviving settlers at day 3 and 10, for $t = 3$ and $t = 10$, respectively, and N_0 is the number of settled germlings just before experimental conditions were restored.

Development of assemblages in the field

At the end of June 2010, assemblages were placed in a rocky shore in the Ria de Vigo and were kept until December 2010 (Fig. 1, sequence 4). Development of assemblages and recruitment of *S. muticum* were monitored in the field by measuring primary and secondary algal cover (cover of algae on the substratum and overstory canopy, respectively) and number and length (cm) of individuals of *S. muticum*. Primary algal cover was estimated by visually dividing each rock quadrat in 4 and attributing a score from 0 to 4 to the functional groups present, and adding up the 16 estimates (Dethier et al. 1993). Secondary algal percentage cover was determined with a sampling

frame (8 × 8 cm) divided by monofilament line into 2 × 2 cm quadrats.

Statistical analyses

Differences in pCO_2 levels among treatments were examined using a Repeated measures ANOVA (rmANOVA) with Temperature and pCO_2 as fixed factors, Tank as a random factor and Time as within-subjects variable. The rm-ANOVA assumption of sphericity was evaluated using Mauchly's criterion.

The number of germlings of *S. muticum* settled in each assemblage at time 0, i.e. immediately after invasion under unmanipulated conditions, was analysed by a two-way ANCOVA with Functional diversity as a fixed factor, Tank as a random factor and Biomass of assemblage as a covariate. First we tested for homogeneity among slopes by including the interaction term 'covariate × main factors' in the model. With a non-significant interaction term, homogeneity of slopes can be assumed and the model can be re-run, excluding the interaction (McDonald 2009). Post-hoc pair-wise comparisons were performed with Tukey's HSD test.

After analysing early settlement, assemblages were returned to experimental climate change conditions (Fig. 1, sequence 3). In order to check if the plates that were placed in each treatment combination in sequence 3 differed in the initial number of settled germlings and thus produce biased results, we performed a 4-way analyses of variance (n = 2) with Temperature, pCO_2 and Functional diversity as fixed factors and Tank as random factor nested within Temperature and pCO_2 (as in Fig. 1, sequence 3). This a priori analysis revealed no significant differences between assemblages placed at different temperature (ANOVA, $F_{1,4} = 1.63$, $P = 0.271$) and pCO_2 (ANOVA, $F_{1,4} = 1.34$, $P = 0.312$) treatments.

Then, percentage of survivorship at 3 and 10 days and length of *S. muticum* settled germlings were analysed using a 3-way analyses of variance (n = 4). Temperature (low and high), pCO_2 (ambient and high) and Functional diversity (High, Lch, Lco, Lli) were fixed factors. Previously we had performed a 4-way analyses of variance incorporating Tank as an additional random factor (2 levels), nested within interaction Temperature × pCO_2 (n = 2). There was no tank effect, thus we pooled data from tanks with the same treatment. Post-hoc pair-wise comparisons were performed with Student–Newman–Keuls (SNK) tests.

Recruitment data from field conditions were analysed with a 3-way analysis of variance with Temperature, pCO_2, and Functional diversity (3 levels: High, Lch, Lco) as main fixed factors (n = 4). We used 3 levels of functional diversity because many monospecific plates of encrusting species were lost in the field. In 8 occasions there was one replicate missing, so the design was balanced by adding the averaged values of the other three replicates to the model and recalculating the *F* values (see Underwood 1997).

When significant, Functional diversity SS from the ANOVA was partitioned into two orthogonal components in order to separate the effects of species richness and species identity (see McDonald 2009 for details). Prior to all analyses, the homogeneity of variances was examined using Cochran's *C* test. Data were transformed when necessary and in those cases in which transformation did not remove heterogeneity, the level of significance applied was $P = 0.01$.

Changes in the structure of assemblages due to experimental treatments were tested with a permutational multivariate analysis of variance (PERMANOVA, Anderson 2001a), with Temperature, pCO_2, and Functional diversity (High, Lch, Lco, Lli) as main fixed factors (n = 4). Due to the fact that PERMANOVA can be applied to unbalanced datasets resulting from missing data (PERMANOVA unbalanced designs, Anderson et al. 2008) we were able to re-incorporate Lli data in our analysis. Pair-wise comparisons were done when significant differences were detected ($P < 0.05$). SIMPER analyses were done to identify the species that contributed most to dissimilarities between treatments.

The effect of treatments on the cover of the two species that contributed most to dissimilarities, i.e. *Ulva* spp. and *S. muticum*, was examined using PERMANOVA in an approach similar to parametric ANOVA. Univariate PERMANOVA tests were run on Euclidean distances matrices with 9,999 permutations (Anderson 2001b) using Temperature, pCO_2 and Functional Diversity as fixed factors. PERMANOVA was chosen for univariate analyses because it allows for unbalance datasets and does not assume a normal distribution of errors.

Multivariate and univariate PERMANOVA analyses were conducted with PRIMER v6 (Clarke and Gorley 2006) with the PERMANOVA extension. Univariate analyses were performed with SPSS

(PASW 18) statistical software and WinGMAV (http://sydney.edu.au/science/bio/eicc).

Results

Environmental parameters in tanks

Mean pH and salinity values in tanks over the post-invasion experimental period were 8.20 (±0.01, all values presented as mean ± SE) and 35.34 ‰ (±0.09), respectively. Temperature was maintained constant with average values of 19.87 (±0.06, n = 32) and 15.16 °C (±0.30, n = 32) for high and low treatments, respectively. Partial pressure of CO_2 differed between treatments although such differences were marginally non-significant ($F_{1,3} = 6.17$, $P = 0.069$). Marginal non-significance detected might be due to high, somewhat predictable, variability in pCO_2 concentrations due to interactions between physic-chemical and biological processes (see Morris and Taylor 1983). Values of pCO_2 were 392.79 (±7.76, n = 44) and 423.96 (±6.86, n = 44) ppmv for ambient and high pCO_2 levels, respectively. Associated pH values across treatments were 8.19 (±0.008, n = 32) and 8.21 (±0.007, n = 32) for ambient and high pCO_2 levels, respectively.

Resistance to invasion and invasiveness of *S. muticum*

As expected, biomass of assemblages significantly affected the settlement of *S. muticum* germlings (ANCOVA, $P = 0.032$; Table 1). The number of germlings also differed significantly depending on functional diversity (ANCOVA, $P = 0.018$; Table 1). Both richness and identity effects contributed significantly for this result (Table 1). The number of *S. muticum* germlings settled was smaller on high diverse assemblages compared to low diverse assemblages (richness effect; Fig. 2a), mostly driven by a significantly larger number of germlings in monospecific assemblages of encrusting species (identity effect; Fig. 2b).

Survivorship of *S. muticum* settlers after 3 days was affected interactively by temperature and pCO_2 (ANOVA, $P < 0.05$; Table 2a). An increase in temperature led to a decrease in the survivorship of *S. muticum* settlers at elevated pCO_2, with no significant differences observed at ambient pCO_2 (SNK-tests, $P < 0.05$; Fig. 3a). At low temperature an increase in pCO_2 led to a significant increase in survivorship (SNK-tests, $P < 0.01$; Fig. 3a). Survivorship 10 days after settlement was still affected interactively by

Table 1 Two-way analysis of covariance (ANCOVA) for the number of *S. muticum* germlings

Source	*df*	Adj MS	*F*	*P*
Biomass	1	2,059.1	4.87	0.032
Functional diversity (FD)	3	2,696.0	5.10	0.018
Richness effect	1	2,343.4	5.54	0.023
Identity effect	2	2,872.25	6.80	0.002
Tank	3	9,570.0	17.69	<0.0001
FD × tank	9	546.1	1.29	0.267
Residual	47	422.7		

Functional diversity was a fixed factor and tank was a random factor, biomass was a covariate (n = 4). Homogeneous variances

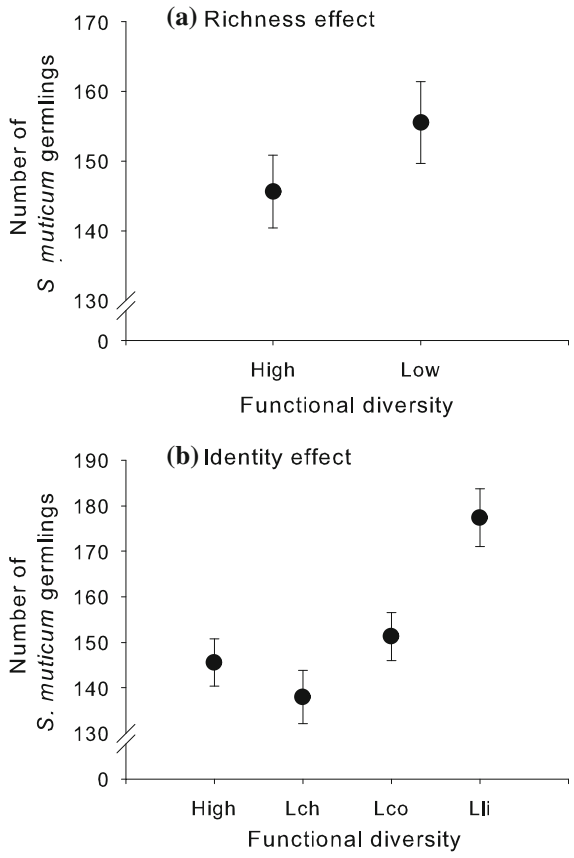

Fig. 2 *Sargassum muticum* settled germlings (per 0.5 cm^2). **a** Richness effect; adjusted mean (±SE, n = 16 and n = 48, for high and low functional diversity, respectively), **b** identity effect; adjusted mean (±SE, n = 16). See Fig. 1 for functional diversity abbreviations

temperature and pCO_2 and there was an additional influence of functional diversity (ANOVA, $P = 0.007$ and $P = 0.033$, respectively; Table 2b). Contrasting with previous results from survivorship after 3 days, an increase in temperature led to a decrease in survivorship at ambient pCO_2 whereas at high temperature treatments survivorship of *S. muticum* germlings was greater at high pCO_2 (Fig. 3b). Survivorship after 10 days was influenced by richness effects rather than identity effects (ANOVA, $P = 0.006$; Table 2b). The highest survivorship of settlers was observed in assemblages of high functional diversity (High FD > Lch = Lco with 65.91 ± 3.09 %, 58.02 ± 3.45 % and 56.53 ± 2.71 %, respectively, while Lli with 60.17 ± 2.73 % showed similar survivorship with all assemblages).

The length of *S. muticum* settlers was influenced at different degree by temperature and pCO_2 (ANOVA, $P = 0.003$; Table 3). Under ambient pCO_2, temperature had a positive effect on the length of *S. muticum* germlings. Settlers were longer at ambient pCO_2 and high temperature and shorter at ambient pCO_2 and low temperature (SNK-tests, $P < 0.05$; Fig. 4). In contrast, there was no effect of temperature under high pCO_2 (SNK-tests, $P < 0.05$; Fig. 4).

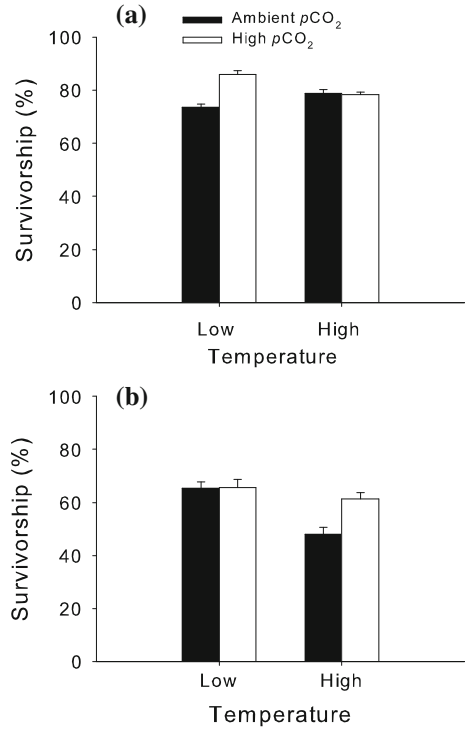

Fig. 3 Mean (+SE, n = 16) percentage survivorship of *S. muticum* settled germlings. Interaction term Temperature × pCO_2 at **a** 3 days after experimental conditions were imposed, **b** 10 days after experimental conditions were imposed

Development of assemblages in the field

Six months after the experimental invasion, the structure of assemblages (i.e. identity and abundance of species) in field conditions was interactively affected by previous experimental conditions (PERMANOVA Temperature × pCO_2, $Pseudo\text{-}F_{1,34} = 3.70$; $P = 0.010$). At ambient pCO_2 there were significant differences (pair-wise tests, $P = 0.018$) in

Table 2 Four-way ANOVA for survivorship of *S. muticum* settlers at (a) 3 days and (b) 10 days after experimental conditions were imposed (n = 4)

Source	df	(a) 3 days			(b) 10 days		
		MS	*F*	*P*	MS	*F*	*P*
Temperature (T)	1	25.547	0.26	0.615	1,860.519	21.75	<0.0001
pCO_2	1	554.865	5.55	0.023	740.368	8.65	0.005
Functional diversity (FD)	3	70.429	0.70	0.554	271.142	3.17	0.033
Richness effect	1	–	–	–	706.315	8.26	0.006
Identity effect	2	–	–	–	53.555	0.63	0.539
T × pCO_2	1	677.201	6.78	0.012	688.389	8.05	0.007
T × FD	3	223.188	2.23	0.096	56.226	0.66	0.582
pCO_2 × FD	3	201.558	2.02	0.124	224.321	2.62	0.061
T × pCO_2 × FD	3	163.206	1.63	0.195	153.403	1.79	0.161
Residual	48	99.922			85.546		

Temperature, pCO_2 and functional diversity were fixed factors and tank was a random factor. Homogeneous variances

Table 3 Four-way analysis of variance (ANOVA) for length (mm) of *S. muticum* settlers (n = 4)

Source	*df*	MS	*F*	*P*
Temperature (T)	1	0.331	95.00	<0.0001
pCO_2	1	0.176	51.29	<0.0001
Functional diversity (FD)	3	0.006	1.89	0.144
T × pCO_2	1	0.286	82.24	<0.0001
T × FD	3	0.010	3.00	0.039
pCO_2 × FD	3	0.010	2.83	0.048
T × pCO_2 × FD	3	0.004	1.07	0.370
Residual	48	0.003		

Temperature, pCO_2 and functional diversity were fixed factors and tank was a random factor. Variances heterogeneous (Cochran's test, $C = 0.2382$, $P < 0.01$). Significance level at $P = 0.01$

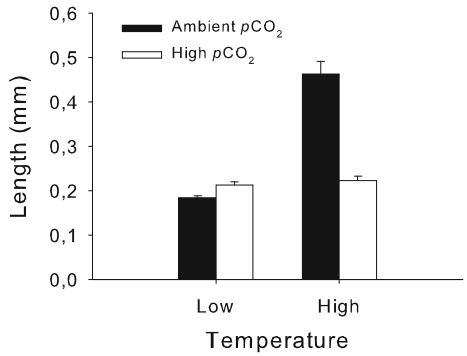

Fig. 4 Mean (+SE, n = 16) length (mm) of *S. muticum* settled germlings. Interaction term Temperature × pCO_2

the structure of assemblages between temperature treatments, not observed at high pCO_2 (pair-wise tests, $P = 0.071$). In addition, assemblages previously exposed to both low and high temperature treatments showed significant differences in the structure of assemblages (pair-wise tests, $P = 0.0001$). Functional diversity also shaped the structure of assemblages, but interactively with pCO_2 (PERMANOVA pCO_2 × Functional diversity, *Pseudo*-$F_{3,34} = 2.22$; $P = 0.015$). Differences between assemblages previously exposed to ambient and high pCO_2 were evident for high functional diversity, Lch and Lco assemblage types (pair-wise tests, $P < 0.02$) and marginally significant for Lli assemblages (pair-wise tests, $P = 0.051$). Also, the structure of assemblages previously exposed to high pCO_2 was similar between functional diversity treatments, with the exception of significant dissimilarities between Lch and Lco (pair-wise tests, $P = 0.028$). In contrast, at ambient pCO_2 the structure of assemblages differed among functional diversity treatments, with the exception of high diverse assemblages and Lli (pair-wise tests, $P = 0.101$). The observed differences were mainly due to *Ulva* spp. and/or *S. muticum*. In fact, *S. muticum* together with the green alga *Ulva* spp. accounted for more than 60 % of observed dissimilarity among assemblages of different functional diversity.

In the field, ephemeral green algae became more abundant on assemblages that had experienced elevated pCO_2 in the laboratory (PERMANOVA, *Pseudo*-$F_{1,34} = 18.20$, $P = 0.0003$; Fig. 5). Reciprocally, *S. muticum* reached higher cover on assemblages previously exposed to ambient pCO_2 (PERMANOVA, *Pseudo*-$F_{1,34} = 15.84$, $P = 0.001$; Fig. 5), with the exception of Lco assemblages, which showed no legacy effects of prior pCO_2 exposure. In terms of numbers of recruits, *S. muticum* showed legacy effects in response to temperature and pCO_2 conditions experienced in the laboratory (ANOVA Temperature × pCO_2, $F_{1,28} = 13.52$; $P = 0.001$). Highest recruitment occurred on assemblages that had grown at elevated temperature but ambient pCO_2, and recruitment under elevated laboratory pCO_2 was lower and not related to temperature (Fig. 6). No individuals or cover of *S. muticum* were recorded in high-diversity assemblages that experienced high-pCO_2 laboratory conditions (Fig. 5). Laboratory pCO_2 also affected the length of *S. muticum* recruits in the field, with longer recruits when previously exposed

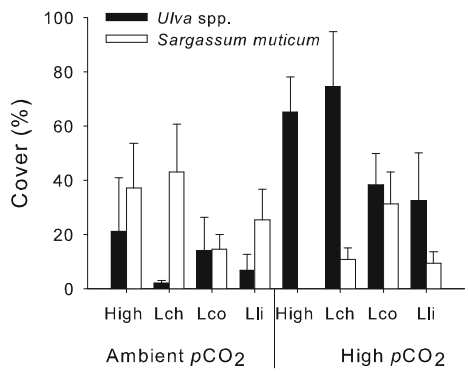

Fig. 5 Mean (+SE, n = 8) percentage cover of *Ulva* spp. and *S. muticum* within functional diversity in each pCO_2 experimental treatments. See Fig. 1 for functional diversity abbreviations

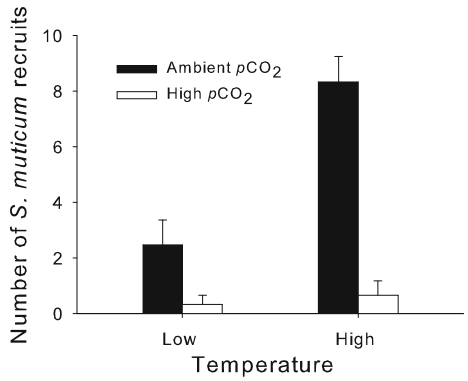

Fig. 6 Mean (+SE, n = 16) number of *S. muticum* recruits (64 cm^2) at each temperature and pCO_2 treatment conditions

to ambient (3.76 ± 0.42 cm, n = 24) than elevated pCO_2 (0.31 ± 0.23 cm, n = 24).

Discussion

Whether or not interactions among climate-related factors will enhance biological invasions is still rather unknown. To our knowledge, the present study is the first to experimentally attempt to identify the interactive effects of enhanced temperature and pCO_2 on invasibility of marine macroalgal assemblages of different functional diversity. The present study suggested that functional diversity, both richness and identity, was a key driver in the settlement success of *S. muticum* germlings. Additionally, results showed that survival and growth of the invader were conditioned by environmental conditions. Early survivorship of settled germlings responded to an interaction of temperature and pCO_2 treatments, with survivorship enhanced in one treatment (high pCO_2 at ambient Temperature) after 3 days, and reduced in another (ambient pCO_2 at high Temperature) after 10 days. After 6 months in the field, legacy effects of laboratory treatments remained, given the highest recruitment in assemblages previously exposed to ambient pCO_2 and elevated temperature, moderate recruitment at ambient pCO_2 and low temperature, and the lowest recruitment in treatments of elevated laboratory pCO_2.

Our results indicated higher resistance to invader settlement by high diverse assemblages (richness effect), in accordance with Elton′s theory. However, individual functional groups also performed differently, suggesting that idiosyncratic traits of each group played an important role over settlement (identity effect). Resistance to invasion by high diverse assemblages was not higher than the best resistant monospecific assemblages (i.e. subcanopy species), suggesting that sampling effect was the mechanism behind the richness effect (Cardinale et al. 2006). Recent experimental studies have also revealed the key role of species identity of macroalgal assemblages in the process of invasion (Crawley et al. 1999; Arenas et al. 2006) and settlement of fucoids (Schiel and Lilley 2011). In this study, the largest number of *S. muticum* settled germlings was found in monospecific assemblages of encrusting species, a suitable substrate for settlement of new colonizers (Arenas et al. 2006) as well as adult individuals which is able to tolerate overgrowth (Airoldi 2000). The establishment and spread of *S. muticum* is indeed controlled by space availability (Deysher and Norton 1982; Fernández 1999), thus the smallest number of settled germlings in monospecific assemblages of subcanopy species could be explained by the presence of subcanopy fronds, a physical barrier between propagules and hard substrate (Deysher and Norton 1982).

The relationship between diversity and invasibility varied throughout the invasion process. Functional diversity had a negative effect on early invasibility, no effect on the survivorship of *S. muticum* at 3 days and described a positive effect on the survivorship of *S. muticum* at 10 days. These findings are related to those found by White and Shurin (2007). Using synthetic assemblages and *S. muticum* as the invader species, these authors found a positive effect of diversity on the initial cover of *Sargassum* juveniles 47 days after the invasion. Sequential mortality was also greater in high diverse assemblages (White and Shurin 2007). Functional diversity of macroalgal assemblages has been found to affect availability of key resources, i.e., space and light (see Arenas et al. 2006), which are related to survivorship and growth of fucoid germlings (Kendrick 1994). Here, invasion success remained higher in lower diversity assemblages, even though survivorship in high diverse assemblages was highest, suggesting a possible shift in the effects of diversity with development stage (White and Shurin 2007).

Survivorship in early post-settlement phases is critical to the establishment success of macroalgal populations (Vadas et al. 1992), as germlings are more susceptible to biological and environmental stress than adults (Schiel and Foster 2006). In this study, the

interactive effects of temperature and pCO_2 on the survivorship of settlers varied with time. Increased pCO_2 was beneficial to *S. muticum* short-time survivorship, particularly under low temperature. After 3 days, a positive effect of temperature was observed under ambient pCO_2 while 10 days after experimental conditions were imposed survivorship was the lowest under that same treatment. These results suggest possible ontogenic-specific differences in susceptibility to mortality (Vadas et al. 1992). Also, observed lower survivorship after 10 days under high temperature and ambient pCO_2 could be related to higher growth under those conditions. Density-dependent survivorship and growth have been reported for *S. muticum* recruits (Kendrick 1994), which could explain our results. It has been suggested that whether interactions between germlings describe competition or facilitation depends on the environmental conditions (Steen and Scrosati 2004). Studies with fucoid germlings have demonstrated stronger intraspecific competition at high temperatures under nutrient enrichment conditions (Steen 2003; Steen and Scrosati 2004). Overall, temperature has been positively related to *S. muticum* performance (Norton 1977; Deysher 1984). Nevertheless, our results suggest that the magnitude of the temperature effects on the length of *S. muticum* settlers would be considerably lower under high pCO_2 levels, highlighting interactive effects between manipulated global change drivers.

Additionally, the structure of assemblages in the field latter on was influenced by environmental conditions experienced in laboratory, invasion success and functional diversity of assemblages. Response of macroalgal assemblages to environmental stressors depends strongly on the type and magnitude of the stressor as well as composition of assemblages (Allison 2004). Assemblages from high pCO_2 were more homogeneous than those maintained under ambient pCO_2 6 months after experiencing environmental stress in the laboratory. The persistent shifts in macroalgal assemblages observed 6 months after the source of disturbance disappeared suggests legacy effects of disturbance by global change drivers. The observed differences were mainly attributed to the dominance of the invader, *S. muticum*, and green ephemerals. *S. muticum* was the most abundant species in assemblages under ambient pCO_2 whereas *Ulva* spp. was the dominant species in assemblages that experienced high pCO_2 in laboratory. Green opportunistic algae colonized those assemblages that experienced more stress, i.e., combined increase of temperature and pCO_2, and were not successfully colonized by the invader in the laboratory. As many ephemeral species, the opportunistic *Ulva* species require availability of resources (i.e. space and light) to be competitively superior to perennial or pseudo-perennial species (Sousa 1979). The opportunistic nature in combination with the perennial persistence of *S. muticum* is an unusual feature in canopy forming alga (Rueness 1989). It is thus very likely that survival and growth of the invader set the stage for posterior competitive interactions (Steen and Scrosati 2004) and mediated the response of assemblages (Byers 2002; Sorte et al. 2011). For example, a recent study has described a synergistic positive interaction between future CO_2 and temperature levels on the abundance of non-calcareous algal turfs enhancing the probability of phase shifts in kelp forests (Connell and Russell 2010). It would be, however, interestingly to investigate for how long legacy effects of disturbance by global change drivers would persist after disturbance and its impacts. For instance, legacy effects of canopy disturbance have been suggested to still affect macroalgal community structure and primary productivity 8 years after disturbance (Schiel and Lilley 2011; Tait and Schiel 2011).

Evidence suggests that contrary to calcified algae (e.g. Martin and Gattuso 2009) non-calcareous algae can increase in growth and abundance with CO_2 enrichment (Hall-Spencer et al. 2008; Porzio et al. 2011; Johnson et al. 2012). Previously cited research has been conducted over natural pH gradients, i.e., volcanic vents, not taking into consideration effects over reproductive life cycle of species that recruit into the acidified areas from nearby populations (Porzio et al. 2011). In this context, studies on the microscopic stages of the reproductive life cycle have been mostly overlooked (but see Roleda et al. 2012). The present study highlighted the negative effect of ocean acidification, alone and in combination with temperature, in the recruitment success of the brown canopy macroalga *S. muticum*. In addition, many species interactions are under strong abiotic control (O'Connor 2009), thus it would be interesting to assess the effects of changing conditions on trophic interactions.

In conclusion, we addressed the potential for climate change to facilitate invasions and precipitate shifts in structure of marine macroalgal assemblages

by testing effects of increasing temperature and pCO_2. This study revealed interactive effects of temperature and pCO_2 and highlighted the fact that results differ across life stages. The lowest germling survival rates were obtained at elevated temperatures while legacy effects of earlier exposure to experimental treatments revealed increased survival and growth of 6-months recruits of *S. muticum* from elevated temperature, but only when in combination with current pCO_2 levels. High CO_2 conditions seemed to reduce the final invasion success of *S. muticum*. Thus, the effect of global environmental change will vary depending on the relative intensity of change of the different environmental factors involved. Our results highlighted the need to consider multiple stressors in combination when addressing the impact of invasions in a climate change scenario.

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