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Response of macroalgal assemblages from rockpools to climate change: effects of persistent increase in temperature and CO_2

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Anthropogenically induced global climate change has important implications for marine ecosystems with unprecedented ecological and economic consequences. Climate change will include the simultaneous increase of temperature and CO_2 concentration in oceans. However, experimental manipulations of these factors at the community scale are rare. In this study, we used an experimental approach in mesocosms to analyse the combined effects of elevated CO_2 and temperature on macroalgal assemblages from intertidal rock pools. Our model systems were synthetic assemblages of varying diversity and understory component and canopy species identity. We used assemblages invaded by the non-indigenous canopy forming alga *Sargassum muticum* and assemblages with the native canopy species *Cystoseira tamariscifolia*. We examined the effects of both climate change factors on several ecosystem functioning variables (i.e. photosynthetic efficiency, productivity, respiration and biomass) and how these effects could be shaped by the diversity and species identity of assemblages. CO_2 alone or in combination with temperature affected the performance of macroalgae at both individual and assemblage level. In particular, high CO_2 and high temperature (20°C) drastically reduced the biomass of macroalgal assemblages and affected their productivity and respiration rates. The identity of canopy species also played an important role in shaping assemblage responses, whereas species richness did not seem to affect such responses. Species belonging to the same functional effect group responded differently to the same environmental conditions. Data suggested that assemblages invaded with *S. muticum* might be more resistant in a future scenario of climate change. Thus, in a future scenario of increasing temperature and CO_2 concentration, macroalgal assemblages invaded with canopy-forming species sharing response traits similar to those of *S. muticum* could be favoured.

The increase of greenhouse gases emissions and other human activities are already causing rapid change in the Earth's climate system (IPCC 2007). Although the ecological effects of anthropogenic climate change are already being experienced (Sanderson et al. 2002), the full impacts for ecosystems remain poorly understood.

Marine systems are among the most ecologically and socio-economically important on the planet providing numerous goods and services, especially coastal areas (Barbier et al. 2011). They are currently threatened by anthropogenic global climate change (Hoegh-Guldberg and Bruno 2010) that is affecting biodiversity and ecosystem services at multiple scales (Schröter et al. 2005). In the past century, atmospheric CO_2 concentrations and Earth's surface temperatures have risen by approximately 120 ppmv and 0.8°C, respectively. By the end of this century, CO_2 concentrations are expected to double or triple, and global average surface temperatures are projected to increase between 1.4 and 5.8°C (IPCC 2007). Thus, more CO_2 will dissolve into the ocean increasing acidification levels and global average sea surface temperatures.

Increasing temperature and CO_2 concentration may affect the physiology and performance of organisms, alter marine biodiversity and lead to changes in species composition and biological interactions among species (Harley et al. 2006, Kroeker et al. 2010, Johnson et al. 2012). For example, species with higher temperature tolerance will be better able to cope with global warming (Calosi et al. 2008), while warm-adapted species might be more vulnerable because they live closer to their absolute tolerance limits (Harley et al. 2006). Acidification can lead to lower growth rates in marine calcifiers (Guinotte and Fabry 2008, Martin and Gattuso 2009), causing community shifts towards non-calcifying organisms (Kuffner et al. 2008, Connell and Russell 2010) and a subsequent loss of habitat for many other species. Some seagrasses and brown macroalgae

(i.e. early life-history stages or adults) are, for example, resilient to naturally or induced high CO_2 concentrations, either maintaining or accelerating their physiological processes (Hall-Spencer et al. 2008, Porzio et al. 2011, Roleda et al. 2012). Furthermore, the combined effect of increasing CO_2 concentrations and temperature on organisms and ecosystems may be greater than the impact of CO_2 or temperature alone (Anthony et al. 2008, Martin and Gattuso 2009, Connell and Russell 2010, Rodolfo-Metalpa et al. 2011). For example, a recent transplant experiment along CO_2 vents in the Mediterranean Sea showed that some calcifying species were more vulnerable to the effects of ocean acidification at warmer seawater temperatures (Rodolfo-Metalpa et al. 2011).

Until now, most research has focused on the effects of climate change on organisms in isolation, rather than on whole communities. While this approach is useful for understanding species-specific mechanisms behind the effects of environmental changes, it ignores species interactions which may buffer or amplify individual responses thereby altering predicted assemblage-level responses (Kroeker et al. 2010). For instance, increasing CO_2 can cause seagrass photosynthetic rates to increase by 50%, which may deplete the surrounding CO_2 pool, maintain an elevated pH and thus, protect associated calcifying organisms (Kroeker et al. 2010). Diversity may also reduce the magnitude of changes and increase community resilience to environmental stressors (Allison 2004). Nevertheless, species identity can have an even greater effect on marine communities than diversity, particularly when species play disproportionately strong roles in community structure and function (Eriksson et al. 2007, Kroeker et al. 2010). For instance, habitat-forming species, such as dominating canopy-forming macroalgae, may increase the resistance of marine communities to nutrient enrichment (Eriksson et al. 2007) or protect understory encrusting species from bleaching (Hawkins and Harkin 1985).

Non-indigenous species are also impacting marine communities around the world at an unprecedented rate (Thomsen et al. 2011). These species are often ecosystem engineers (e.g. brown canopy algae) that can replace native species and their functional role in the ecosystem, or modify habitat characteristics and food sources for consumers (Britton-Simmons 2004). Established non-indigenous species may alter community response to climate (Hall-Spencer et al. 2008, Walther et al. 2009). We currently have no information on how invaded communities will react to climate change compared to non-invaded communities. Recent studies, however, indicate that non-indigenous species usually have the same general traits as those exhibited by most successful species, regardless of their native or invasive character, or traits of their life-history or body plan which determine how they respond to a changing world scenario (Thompson and Davis 2011). Such 'response' traits may differ among the species that play a similar role in the ecosystem, i.e. those sharing 'effect' traits (Lavorel and Garnier 2002). For example, macroalgae response to increases in temperature and CO_2 varies greatly, even among species belonging to the same morpho-functional group (Martin and Gattuso 2009, Connell and Russell 2010, Porzio et al. 2011).

Little is known about how natural systems will respond to future environmental conditions, where the combination of multiple climate stressors may produce complex and unforeseen effects. Even less is known about how the loss of native diversity and changes in natural assemblages derived from the introduction of non-indigenous species will affect the ability of systems to deal with stressors. Thus, models and experiments that simultaneously incorporate climate stressors and new diversity scenarios will help to understand future impacts of these changes in ecosystems.

In this study, we used an experimental approach in mesocosms to analyse the combined effects of elevated CO_2 concentration and temperature on macroalgal assemblages from mid-intertidal rockpools. Macroalgal assemblages from marine intertidal rock pools are very suitable systems for studying climate-driven changes. Although these assemblages are exposed to great daily and seasonal variations in environmental conditions (Morris and Taylor 1983, Metaxas et al. 1994), their response to more persistent increases in temperature and CO_2 and decreases in pH are unknown. We used synthetic assemblages of varying composition and diversity of species resembling those characteristic of the western Atlantic coast of the Iberian Peninsula (Incera et al. 2011). These assemblages were composed of primary space holders like turf-forming algae (e.g. *Corallina* spp., *Chondracanthus* spp., *Gelidium* spp.), coexisting with encrusting coralline algae (e.g. *Lithophyllum incrustans*) and subcanopy species like *Mastocarpus stellatus* or *Chondrus crispus*, and were dominated by two canopy-forming species, the native *Cystoseira tamariscifolia* and the invasive *Sargassum muticum*. Although the latter two species can be included in the same functional effect group, they differ in biological and ecological traits (Arenas et al. 1995, Engelen and Santos 2009) and may respond differently to climate change. *Sargassum muticum* exhibits faster growth and metabolism than *C. tamariscifolia* and produces more germlings with higher dispersal abilities. Such differences could affect the performance of invaded and non-invaded assemblages in a climate change scenario. For example, evidence from terrestrial systems suggests that slow-growing plant species may be favoured by increasing CO_2 concentrations (Lloyd and Farquhar 1996).

We specifically examined the effects of these two climate-change agents on several ecosystem functioning variables at the individual and assemblage level, and analysed how these effects could be shaped by the diversity and species identity of assemblages. We hypothesised that 1) elevated temperature and CO_2 concentration would affect macroalgae performance, especially in calcareous species (Martin and Gattuso 2009), 2) elevated temperature and CO_2 would act synergistically, 3) the response of assemblages dominated by native *C. tamariscifolia* would differ from that of assemblages dominated by invasive *S. muticum*, and 4) diversity would shape assemblage response.

Material and methods

Synthetic assemblages

We selected species from four morpho-functional groups (modified from Steneck and Dethier 1994): a) encrusting

calcareous species, mostly *Lithophyllum incrustans*; b) turf-forming species, a group of primary space-holders represented by the species *Corallina* spp.; c) subcanopy space-holder species, including a mixture of *Mastocarpus stellatus* (the erect gametophytic phase) and *Chondrus crispus* that form a secondary cover in many tide pools and d) canopy species, native *Cystoseira tamariscifolia* and invasive *Sargassum muticum* that form a true shade-forming canopy. The species included in groups a), b) and c) were considered the understory component of assemblages.

We used these groups to assemble communities of varying diversity (two and four species) and identity of understory and canopy species. Experimental assemblages consisted of PVC plates (20 × 20 cm) containing an array of 14 pieces of slate rock and two pieces of PVC (2 × 2 cm). Each rock piece was fully covered by one of the selected species/groups, and each PVC piece held one individual from the canopy species group (*C. tamariscifolia* or *S. muticum*) (Supplementary material Appendix 1 Fig. A1).

To construct the synthetic assemblages, pieces of boulders bearing the selected species/group were chipped off from mid-shore rock pools, transported to the laboratory and cut into 2 × 2 cm quadrats using a commercial tile cutter. The quadrats were then randomly arranged on the PVC plate and held in position using quick-drying cement and screws. All assemblage plates contained different combinations of one or three understory groups (low and high diversity, respectively) and either *C. tamariscifolia* or *S. muticum* as canopy species. A total of 128 plates were constructed: 96 plates combining one single understory morpho-functional group and the canopy species (three single understory groups and two canopy species, 16 replicates per combination) and 32 plates combining all three understory groups (i.e. high diversity) with each canopy species (16 replicates per combination).

The construction of assemblages and plates was carried out between February and March 2010. Upon completion, the plates were placed in mid-intertidal rock pools in the Ria de Vigo, northwestern Spain (42°13′26″N; 8°46′18″W) and attached to the substratum until the start of the mesocosm experiment in April 2010.

Experimental set-up in mesocosms

Synthetic assemblages were thoroughly cleaned of epiphytic organisms and randomly assigned to 350-l PVC tanks receiving light from above provided by eight cool white fluorescent lamps (F18W/840) under a 12/12 h light/dark photoperiod. Irradiance at the air-water interface was 140–150 µmol photons m^{-2} s^{-1}, which is well above the compensation point of the two canopy species and close to the light saturation point (Rico and Fernández 1997). Similar rock pool assemblages had a compensation point intensity of 17.8 ± 1 µmol photons m^{-2} s^{-1} (mean ± SE, n = 40) (Arenas et al. 2009).

CO_2 levels and temperature were manipulated following a 2 × 2 orthogonal, full cross factorial experimental design in four treatment combinations: 1) ambient CO_2 concentration (ca 380 ppmv) and 15°C (low temperature); 2) ambient CO_2 concentration and 20°C (high temperature); 3) high CO_2 concentration (ca 1000 ppmv) and 15°C; and 4) high CO_2 concentration and 20°C. Each treatment combination was replicated in two randomly-positioned tanks, and two random replicates of synthetic assemblages with different levels of understory diversity and canopy identity were placed in each tank. Plates were randomly placed and separated 20 cm from each other. Each tank setting, including the lamp and the air space between the surface and the lamp, was covered with white plastic sheets to prevent gas exchange among tanks and decrease variability in temperature and light within tanks. Plastic sheets also contributed to creating low CO_2 atmospheres (i.e. ambient CO_2 treatments) and CO_2-rich atmospheres (i.e. elevated CO_2 treatments) over the tanks water surface.

Treatments were designed to reflect persistent changes in temperature and CO_2 concentration in mid-intertidal rock pools from the study area, where mean temperatures range between 13–15°C in spring, with daily fluctuations of 2–6°C (Olabarria et al. unpubl.). Thus, the 15°C treatment (low temperature) corresponded to average daily temperatures in the pools and mean (± SE) sea surface temperature values recorded in the Ria de Vigo in April and May between 2006 and 2010 (14.93°C ± 0.38°C, n = 305; <www.meteogalicia.es>). Elevated CO_2 (ca 1000 ppmv) and temperature (5°C increase) were intended to simulate the highest values in future climate change scenarios (IPCC 2007).

Prior to the experiment, assemblages were acclimated at 15°C and ambient CO_2 concentration (ca 380 ppmv) for four days, and then maintained under experimental conditions for 17 days (20 April to 6 May 2010). Each tank was filled with 1-µm-filtered seawater that was renewed once a week. In the high CO_2 treatments, seawater was adjusted to a high CO_2 concentration before it was added to the tanks using bubbling air with 1000 ppmv CO_2 from a gas tank. This method is a very efficient way to manipulate seawater carbonate chemistry in a manner that mimics projected changes due to ocean acidification (Rost et al. 2008). Temperature was controlled using seawater chillers with a UV sterilizer connected to each tank, and CO_2 flow was maintained using individual flowmeters. Ambient air from an open area (ambient CO_2 treatments) and CO_2-rich air from a gas tank (high CO_2 treatments) were bubbled continuously during the experiment using submersible pumps (3000 l h^{-1}) located at the bottom of the tanks, which also ensured water motion. The synthetic assemblages in the tanks were grown under replete-nutrient conditions. They were supplied with a nutrient solution prepared from deionised water and reagent grade salts (42.5 g l^{-1} $NaNO_3$ and 10.75 g l^{-1} Na_2HPO_4) once a week.

Environmental parameters

Temperature was monitored in each tank every 30 min using data loggers. Salinity and pH were measured at least once a day around midday using a glass electrode. Accuracy of the conductivity meter and pH meter was ± 1‰ and ± 0.01 pH units, respectively.

Water samples were taken from each tank at least four times a week to analyse total dissolved inorganic carbon (TDIC). Water samples were filtered through 0.2 µm pore size glass microfiber filters into serum vials that were capped without head space. Samples were analysed the same

day using an infrared gas analyzer, and TDIC was then partitioned into CO_2, bicarbonate and carbonate from salinity, temperature and pH using the program csys.m (Zeebe and Wolf-Gladrow 2001).

Biochemical composition

At the end of the experiment, four samples of each species randomly selected from the different assemblages in each tank were collected, dried at 60°C for 48 h and ground to a fine powder. Total carbon and nitrogen content were determined using a CHN elemental analyser. Total inorganic carbon, $MgCO_3$ and $CaCO_3$ of *L. incrustans* and *Corallina* spp. were also measured using an inductively coupled plasma-optical emission spectrometer. Total organic carbon content was calculated as the difference between total carbon and total inorganic carbon content.

Quantum photosynthetic efficiency

Maximum quantum yield (Fv/Fm) was measured as an indicator of photosynthetic efficiency and physiological stress (Maxwell and Johnson 2000) using a pulse amplitude modulated chlorophyll fluorometer. Measurements were taken three days after the start of the experiment and at the end, i.e. 17 days after starting (hereafter time 1 and time 2, respectively). At each time, three measurements were taken from three different fronds for each species and assemblage. *Chondrus crispus* and *M. stellatus* were considered a group, as it was impossible to distinguish between them while measuring in the dark.

The temporal change in photosynthetic efficiency was calculated as the ratio of values at time 2 to values at time 1. This ratio allowed us to evaluate the physiological condition of macroalgae since values close to 1 indicated a good performance of macroalgae over the experimental period.

Estimation of ecosystem functioning surrogates and incubation procedures

Three ecosystem functioning surrogates were measured per assemblage: 1) productivity estimated as gross primary productivity (GPP); 2) respiration; and 3) biomass (g dry weight). Productivity and respiration were measured twice (i.e times 1 and 2). At time 2, the impact of CO_2 concentration ant temperature on the understory component was also measured independently by removing canopies and incubating only understory assemblages.

Productivity and respiration rates of assemblages were estimated by measuring oxygen fluxes during the dark and light incubation periods (i.e. 30 min) using 15.2-l acrylic incubation chambers submersed in the tanks. Water movement was maintained in the incubation chamber through a submersible pump (300 l h^{-1}) equipped with a diffuser to reduce turbulence. Variations in oxygen concentration were measured every 30 s using a luminescent dissolved oxygen probe connected to a data-logger. Productivity and respiration (measured as mg O_2 h^{-1}) were estimated by regressing oxygen concentration in the chamber over time. Estimates were corrected by volume of seawater inside the chamber to take into account the different volumes of assemblage plates. Control incubations with no macroalgal assemblages were simultaneously performed to correct for bacteria and phytoplankton respiration and productivity rates.

The biomass (g dry weight) of macroalgal assemblages was determined using different methods depending on the species. For encrusting calcareous *L. incrustans*, the dry weight of 40 rock pieces (2×2 cm quadrats covered with the species) was measured before and after a treatment with 0.5 HCl. The average difference was used to obtain the initial biomass of alga per cm^2. Final biomass was estimated by considering the loss of magnesian calcite found in the different treatment combinations (Results). For this species, biomass was estimated without replication within treatment combinations. Initial and final biomass of the rest of the algae was estimated from maximum frond length (L) and circumference (C) (see Supplementary material Appendix 2 for a detailed description). Biomass was measured the day before the start of the experiment and at the end of experiment, i.e. times 0 and 2, respectively.

Data analysis

Response variables were analysed using general linear models (ANOVAs and ANCOVAs). Analyses were performed with the public domain package R (R Development Core Team) and SPSS 16.0 for Windows (SPSS Inc. 1989–2007).

The change in CO_2 concentration among treatments over time was analysed using the model

$$Y_{ij} = \mu + Tr_i + Da_j + [Tr\ Da]_{ij} + \varepsilon_{ij}$$

where Y_{ij} is the value of the response variable from the *i*th level of the factor Tr and the *j*th level of the factor Da, μ is the overall mean and ε_{ij} is the error term. Treatment (Tr; $i = 4$, $CO_2 \times$ temperature combinations) is a fixed factor and Date (Da; $j = 14$) is the random within-subject variable.

Biochemical composition-related variables were analysed at the specific level, photosynthetic efficiency at the morphofunctional group level, and GPP, respiration and biomass at the assemblage level.

The effects of CO_2 and temperature on the biochemical parameters of each species (total carbon, total organic and inorganic carbon, total nitrogen and C/N ratio) were evaluated using the model

$$\begin{aligned} Y_{ijklm} = {} & \mu + Sp_l + CO_i + Te_j + T_{k(ij)} + [CO\ Te]_{ij} \\ & + [Sp\ CO]_{li} + [SpTe]_{lj} + [Sp\ T]_{lk(ij)} \\ & + [Sp\ CO\ Te]_{lij} + \varepsilon_{ijklm} \end{aligned}$$

where Species (Sp; $l = 6$, *L. incrustans*, *Corallina* spp., *Chondrus crispus*, *M. stellatus*, *S. muticum* and *C. tamariscifolia*), CO_2 (CO; $i = 2$, ambient and high) and Temperature (Te; $j = 2$, 15°C and 20°C) are fixed factors and Tank (T; $k = 2$) is a random factor. Total $MgCO_3$ and $CaCO_3$ content were also analysed for the two calcareous species with the same model.

The change in photosynthetic efficiency was analysed using two different models. Model 1 evaluated the effects of CO_2 and temperature on the two canopy species. The model used was

$$\begin{aligned} Y_{ijklm} = {} & \mu + CO_i + Te_j + T_{k(ij)} + Ca_l + [CO\ Te]_{ij} \\ & + [Ca\ T]_{lk(ij)} + [CO\ Ca]_{il} + [Te\ Ca]_{jl} \\ & + [CO\ Te\ Ca]_{ijl} + \varepsilon_{ijklm} \end{aligned}$$

with CO, Te and Canopy (Ca; l = 2, *C. tamariscifolia* and *S. muticum*) as fixed factors and T as a random factor (k = 2).

Model 2 assessed the effects of CO_2, temperature, understory diversity and canopy species identity on the photosynthetic efficiency of each understory component separately (*L. incrustans*, *Corallina* spp. and *C. crispus*/*M. stellatus*). This model was

$$\begin{aligned} Y_{ijklmn} = {} & \mu + CO_i + Te_j + T_{k(ij)} + Undiv_m + Ca_l \\ & + [CO\ Te]_{ij} + [CO\ Undiv]_{im} + [CO\ Ca]_{il} \\ & + [Te\ Undiv]_{jm} + [Te\ Ca]_{jl} + [Undiv\ T]_{mk(ij)} \\ & + [Ca\ T]_{lk(ij)} + [Undiv\ Ca]_{ml} + [CO\ Te\ Undiv]_{ijm} \\ & + [CO\ Te\ Ca]_{ijl} + [CO\ Undiv\ Ca]_{iml} \\ & + [Te\ Undiv\ Ca]_{jml} + [Ca\ Undiv\ T]_{lmk(ij)} \\ & + [CO\ Te\ Undiv\ Ca]_{ijml} + \varepsilon_{ijklmn} \end{aligned}$$

with CO, Te, Understory diversity (Undiv; m = 2, low diversity and high diversity) and Ca as fixed factors and T as a random factor (k = 2). Low and high diversity levels of understory diversity refer to monospecific and mixed understory assemblages (1 and 3 understory morpho-functional groups, respectively).

The effects of CO_2, temperature, understory diversity and canopy species identity on the per capita rates of GPP and respiration of the whole assemblages were analysed for times 1 and 2, separately, using Model 2 of variation in photosynthetic efficiency, with CO, Te, Undiv (m = 4, monospecific *L. incrustans*, monospecific *Corallina* spp., monospecific *C. crispus/M. stellatus* and high diversity) and Ca as fixed factors and T as a random factor (k = 2).

When understory diversity was significant, the correspondent sum of squares from the linear model was partitioned into two orthogonal components to separate the effects of richness (monospecific versus mixed understory assemblages) and identity (differences among monospecific assemblages; see Duffy et al. 2005 for details).

To analyse final biomass, we used this initial model:

$$\begin{aligned} Y_{klm} = {} & \mu + T_k + Ca_l + \beta_1 IB + [T\ Ca]_{kl} + \beta_2 T_k\ IB \\ & + \beta_3 Ca_l\ IB + \beta_4\ [T\ Ca]_{kl}\ IB + \varepsilon_{klm} \end{aligned}$$

where μ is the pooled intercept, T (k = 8 levels) and Ca are fixed factors, Initial biomass (IB) is a covariate and β the regression slopes.

This model differs from that used in analyses of per capita GPP and respiration due to the added complexity of carrying out a model with multiple factors and interactions with a covariate. In this model, the effect of tank from different combinations of CO_2 and temperature and the effect of canopy identity were still tested. The factor Understory diversity could not be included because there were only two replicates per combination of tank and canopy. We fitted this initial model including all factors and interactions with the covariate, and then simplified it by stepwise procedures using the Akaike's (AIC) information criterion (Crawley 2007). The minimal adequate model was finally found by pooling tanks within those combinations of CO_2 and temperature in which no significant differences in slope and intercept were found. This minimal final model was compared to the simplified model (all tank levels included) by using analysis of variance (Supplementary material Appendix 3 Table A1).

Similar procedures were followed to analyse final biomass of the understory component (Supplementary material Appendix 3 Table A2). In this case, we also tested the effect of understory diversity within combinations of CO_2 and temperature as tanks could be pooled. As the initial and final biomass of *L. incrustans* was estimated without replication within tanks, the understory component was excluded from analyses and, therefore, Understory diversity had three levels (monospecific *Corallina* spp., monospecific *C. crispus/M. stellatus* and high diversity).

Prior to all analyses, the homogeneity of variances was examined using Cochran's *C*-test. Data were transformed when necessary to normalise their distribution and remove heteroscedasticity. Type III SS was used for all models. In the analysis of environmental parameters, the assumption of sphericity for repeated measures ANOVA (RM-ANOVA) was tested using Mauchly's criterion. In the analyses of biomass, homogeneity of variances was evaluated by inspection of plots of residuals against x-values (i.e. initial biomass). Student–Newman–Keuls (SNK) tests were performed for a posteriori comparisons of means. All data are reported as means ± SE.

Results

Environmental parameters in tanks

Environmental conditions responded to experimental treatments, although CO_2 concentration values were higher than expected in some treatment combinations, i.e. high CO_2–20°C treatment (Fig. 1, Supplementary material Appendix 4 Table A3). Salinity was similar in all tanks (34.57 ± 0.09‰), and temperature was maintained at 15.30 ± 0.01°C and 20.01 ± 0.05°C in the low and high temperature treatments, respectively. CO_2 values differed significantly between treatments throughout the experiment and were dependent on temperature (RM-ANOVA,

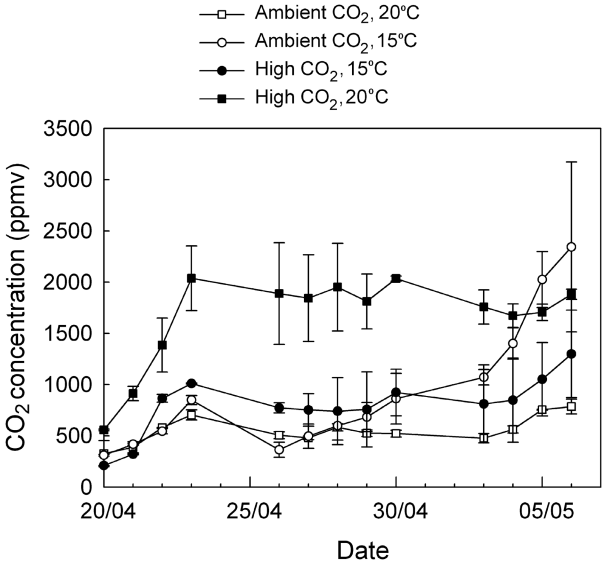

Figure 1. Mean (± SE) values of estimated dissolved CO_2 concentration in each CO_2 and temperature combination (n = 2).

Treatment, $F_{3,4} = 12.5$, $p = 0.017$). The highest CO_2 concentrations were obtained in tanks with high CO_2 and 20°C (Fig. 1). The high CO_2 concentration in this treatment from the first few days of the experiment suggests that assemblages responded to these stressful conditions by increasing respiration rates. In general, CO_2 values increased until day 4 (23 April) and then stabilized in all treatments except in the ambient CO_2–15°C treatment which reached a peak at 2344 ppmv on day 18 (6 May). This drastic increase in CO_2 concentration at the end of the experiment was caused by a failure in the illumination system which reduced daylight hours causing an increase in respiration rates.

pH values remained fairly constant and diel fluctuations were generally less than 0.10 units in all treatments except during the first three days of the experiment when conditions were stabilizing (Supplementary material Appendix 4 Table A3). In the ambient CO_2 treatments, the pH was maintained at 7.99 ± 0.03 and 8.04 ± 0.01 at 15°C and 20°C, respectively, whereas in the high CO_2 treatments the pH was maintained at 7.91 ± 0.05 and 7.68 ± 0.02, respectively.

Biochemical composition of algae

Total carbon content and C/N ratio of fronds varied significantly among species (Species, $F_{5,20} = 106.99$, $p = 0.001$ and $F_{5,20} = 66.13$, $p < 0.0001$, respectively). CO_2 concentration and temperature did not significantly affect total carbon content and C/N ratio ($p > 0.05$), although the C/N ratio tended to decrease in the high CO_2 concentration treatment in *Chondrus crispus* and *Cystoseira tamariscifolia* (data not shown). Total nitrogen content increased significantly with CO_2 in these two species (Species × CO_2, $F_{5,20} = 3.47$, $p = 0.020$, Fig. 2a).

$MgCO_3$ content varied significantly between the two calcareous species and was dependent on CO_2 concentration and temperature. $MgCO_3$ content decreased as CO_2 increased in both species (CO_2, $F_{1,4} = 15.26$, $p = 0.017$, Fig. 2b), and was the greatest in the 15°C treatment in *Lithophyllum incrustans* (Species × Temperature, $F_{1,4} = 12.45$, $p = 0.024$; Fig. 2c). No significant treatment effects were found for total content of organic and inorganic carbon (data not shown).

Quantum photosynthetic efficiency

CO_2 concentration and temperature had a significant effect on the change in photosynthetic efficiency over time in the canopy species (CO_2 × Temperature × Canopy, $F_{1,4} = 7.95$, $p = 0.047$). The change in Fv/Fm was greater in *C. tamariscifolia* than in *Sargassum muticum*, with values remaining fairly constant (close to 1) at ambient CO_2 and 20°C for both species. The greatest reduction was obtained in *C. tamariscifolia* in the ambient CO_2–15°C and high CO_2–20°C treatments (Fig. 3a). The change in Fv/Fm in *Corallina* spp. was also dependent on temperature and CO_2 concentration (significant interaction CO_2 × Temperature; Table 1) with the greatest reduction in photosynthetic efficiency under high CO_2 and 20°C conditions (Fig. 3b).

The change in Fv/Fm in *C. crispus*/ *Mastocarpus stellatus* and *L. incrustans* depended on CO_2 and/or temperature,

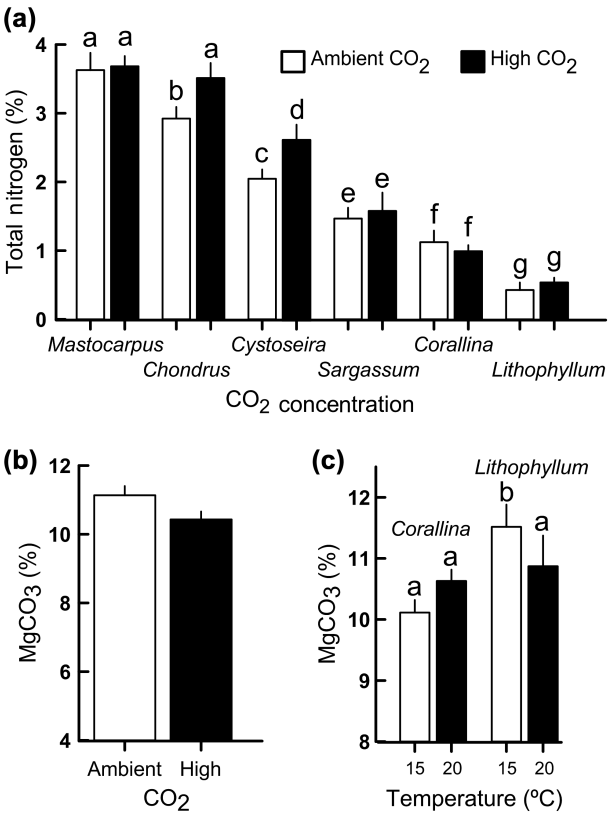

Figure 2. Mean (+ SE) content of: (a) nitrogen in the six species at ambient and high CO_2 conditions (n = 16). SNK tests were calculated from square-root (x + 1) transformed data; (b) $MgCO_3$ in the two calcareous species (*Corallina* spp. and *L. incrustans*) at ambient and high CO_2 conditions (n = 16); and (c) $MgCO_3$ in *Corallina* spp. and *L. incrustans* at 15°C and 20°C (n = 8). Means with a common letter do not differ significantly based on SNK tests at p = 0.05 level. Species labels are *Mastocarpus*: *M. stellatus*, *Chondrus*: *C. crispus*, *Cystoseira*: *C. tamariscifolia*, *Sargassum*: *S. muticum*, *Corallina*: *Corallina* spp. and *Lithophyllum*: *L. incrustans*.

but was modulated by understory diversity and canopy species (Table 1). In the case of *C. crispus*/*M. stellatus*, photosynthetic efficiency remained fairly constant (close to 1) at low understory diversity and ambient CO_2 in the presence of *S. muticum* (i.e. marginally non-significant interaction CO_2 × Understory diversity × Canopy; Table 1, Fig. 3c). The greatest decrease (approximately 30%) was observed in *C. crispus*/*M. stellatus* at high CO_2 and low diversity (Fig. 3c). In the case of *L. incrustans*, photosynthetic efficiency remained fairly constant or even increased over time (i.e. values very close to 1 or above) independent of treatment combinations (e.g. significant interaction CO_2 × Understory diversity; Table 1, Fig. 3d).

Productivity and respiration

Assemblage performance was influenced by CO_2 concentration and temperature, but also by assemblage attributes, i.e. understory composition, and especially canopy species identity (Table 2, Fig. 4).

At time 1, GPP was generally higher in *S. muticum* assemblages than in those with *C. tamariscifolia*, and the

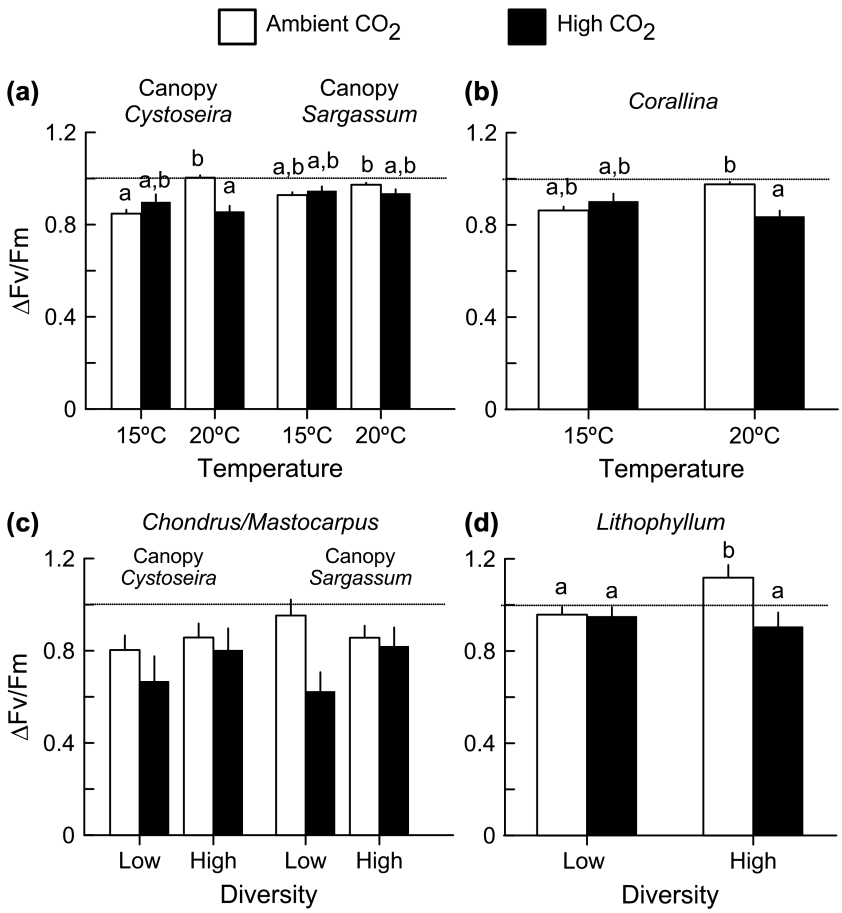

Figure 3. Mean (+ SE) change in photosynthetic efficiency over time. ΔFv/Fm corresponds to the ratio of values at time 2 to values at time 1. (a) Canopy species (n = 16); (b) *Corallina* spp. (n = 8); (c) *C. crispus/M. stellatus* (n = 4); and (d) *L. incrustans* (n = 8). Means with a common letter do not differ significantly from each other based on SNK tests at p = 0.05 level. Interaction was marginally non-significant in *C. crispus/M. stellatus*. Species labels as in Fig. 2.

Table 1. Analyses of variance for change in photosynthetic efficiency (Fv/Fm) over time. Data correspond to the ratio of values at time 2 to values at time 1. Time 1 corresponds to 3 days after the start of the experiment; time 2 is at the end of the experiment (i.e. 17 days after the start of the experiment). *p < 0.05, **p < 0.01, ***p < 0.001, †marginally non-significant. Data were not transformed.

		Corallina spp.		*C. crispus/M. stellatus*		*L. incrustans*	
Source of variation	DF	F	p	F	p	F	p
CO_2 = CO	1,4	4.65	0.097	1.98	0.232	4.20	0.109
Temperature = Te	1,4	1.07	0.359	1.01	0.371	0.11	0.761
Tank (CO_2 × Te) = T (CO × Te)	4,32	0.65	0.629	5.10	0.002**	1.35	0.272
Understory diversity = Undiv	1,4	1.26	0.325	1.23	0.329	4.42	0.103
Canopy = Ca	1,4	0.09	0.784	0.40	0.562	0.00	0.973
CO × Te	1,4	15.13	0.017*	2.31	0.203	0.00	0.996
CO × Undiv	1,4	4.31	0.106	2.06	0.224	14.00	0.020*
CO × Ca	1,4	0.06	0.826	0.83	0.414	2.21	0.211
Te × Undiv	1,4	0.65	0.465	0.77	0.428	0.56	0.496
Te × Ca	1,4	1.82	0.248	0.02	0.902	13.94	0.020*
Undiv × T (CO × Te)	4,32	0.73	0.579	2.22	0.887	0.01	0.360
Ca × T (CO × Te)	4,32	0.84	0.507	1.22	0.321	0.03	0.820
Undiv × Ca	1,4	3.96	0.117	1.17	0.340	9.20	0.038*
CO × Te × Undiv	1,4	0.16	0.708	1.63	0.271	2.71	0.175
CO × Te × Ca	1,4	2.12	0.219	0.94	0.387	0.09	0.778
CO × Undiv × Ca	1,4	2.51	0.188	6.34	0.065†	3.30	0.143
Te × Undiv × Ca	1,4	0.50	0.519	0.25	0.642	2.93	0.162
Ca × Undiv × T (CO × Te)	4,32	0.01	0.420	0.00	0.230	0.02	0.500
CO × Te × Undiv × Ca	1,4	0.85	0.407	0.27	0.631	0.01	0.810
Residual	32						

Table 2. Analyses of variance of gross primary productivity (GPP) and respiration rates at times 1 and 2. Time 1 corresponds to 3 days after the start of the experiment; time 2 is at the end of the experiment (i.e. 17 days after the start of the experiment). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, †marginally non-significant.

		Time 1					Time 2			
		GPP		Respiration			GPP		Respiration	
Source of variation	DF	F	p	F	p	DF	F	p	F	p
CO_2 = CO	1,4	0.22	0.661	2.39	0.197	1,4	8.12	0.046	0.49	0.523
Temperature = Te	1,4	0.06	0.817	2.93	0.162	1,4	3.43	0.138	0.26	0.640
Tank (CO_2 × Temperature) = T (CO × Te)	4,64	4.88	0.002**	4.26	0.004	4,62	3.89	0.007**	13.50	0.000***
Understory diversity = Undiv	3,12	15.30	0.000***	10.41	0.001	3,12	6.91	0.006**	6.91	0.006**
Canopy = Ca	1,4	151.92	0.000***	252.46	0.000***	1,4	37.68	0.004**	1.34	0.312
CO × Te	1,4	10.78	0.030*	0.24	0.652	1,4	0.90	0.019*	0.02	0.897
CO × Undiv	3,12	0.90	0.467	0.11	0.951	3,12	0.78	0.425	0.36	0.782
CO × Ca	1,4	0.08	0.791	0.08	0.785	1,4	0.13	0.234	3.54	0.133
Te × Undiv	3,12	0.73	0.555	0.27	0.846	3,12	0.92	0.731	0.16	0.921
Te × Ca	1,4	0.01	0.926	2.91	0.163	1,4	0.07	0.136	5.76	0.074
Undiv × T (CO × Te)	12,64	1.06	0.409	1.78	0.071	12,62	0.57	0.856	1.14	0.347
Ca × T (CO × Te)	4,64	2.34	0.065	0.48	0.749	4,62	2.18	0.082	0.34	0.852
Undiv × Ca	3,12	9.71	0.002**	2.36	0.123	3,12	0.18	0.066†	1.94	0.177
Ca × Richness		0.05	0.843	–	–		–	–	–	–
Ca × Identity		14.66	0.031*	–	–		–	–	–	–
CO × Te × Undiv	1,12	0.73	0.553	3.69	0.043*	1,12	0.52	0.539	0.79	0.522
CO × Te × Richness	2,12	–	–	5.28	0.040*	2,12	–	–	–	–
CO × Te × Identity	3,12	–	–	2.89	0.095	3,12	–	–	–	–
CO × Te × Ca	1,4	9.26	0.038*	14.10	0.020*	1,4	27.26	0.044*	27.26	0.006**
CO × Undiv × Ca	3,12	3.10	0.067†	0.66	0.592	3,12	0.85	0.768	0.27	0.848
Te × Undiv × Ca	3,12	2.13	0.150	0.38	0.768	3,12	0.75	0.768	0.40	0.753
Ca × Undiv × T (CO × Te)	12,64	0.52	0.895	1.65	0.100	12,62	0.51	0.898	0.85	0.601
CO × Te × Undiv × Ca	3,12	0.64	0.606	1.75	0.209	3,12	0.78	0.714	0.37	0.777
Residual	64					62				
Transformation		Sqrt (x + 1)		None			Ln (x + 1)		Ln (x + 0.1)	

highest rate was obtained in *S. muticum* assemblages at ambient CO_2 and 20°C and high CO_2 and 15°C (significant interaction CO_2 × Temperature × Canopy; Table 2, Fig. 4a). Respiration was also higher in *S. muticum* assemblages than in those with *C. tamariscifolia*, except in the ambient CO_2–15°C treatment (Fig. 4b). Assemblages with *C. tamariscifolia* increased significantly respiration rates in the high CO_2–20°C treatment. The best performance (i.e. the greatest GPP/respiration ratio) was obtained for *S. muticum* assemblages in the ambient CO_2–20°C treatment, whereas the worst performance for both *C. tamariscifolia* and *S. muticum* assemblages was obtained at high CO_2 and 20°C. Furthermore, CO_2 concentration and temperature interacted with understory richness, due to an increase in respiration in high richness assemblages at high CO_2 and 20°C (test a priori, significant interaction CO_2 × Temperature × Understory richness; Table 2).

GPP at time 2 was lower than at time 1 except in *S. muticum* assemblages at ambient CO_2 and 20°C (Fig. 4a, c), and the highest GPP rates were obtained in *S. muticum* assemblages in the ambient CO_2–20°C treatment (significant interaction CO_2 × Temperature × Canopy, Table 2, Fig. 4c). Respiration rates of *C. tamariscifolia* assemblages increased in some treatments (i.e. 15°C treatments) at time 2, whereas *S. muticum* assemblages either decreased or showed almost constant respiration rates compared to time 1. *Cystoseira tamariscifolia* assemblages had the lowest respiration rates at ambient CO_2 and 20°C, whereas *S. muticum* assemblages presented similar rates under all conditions except for the ambient CO_2 and 20°C treatment (Fig. 4d). In *S. muticum* assemblages, the best performance was obtained at ambient CO_2 concentration and 20°C. In *C. tamariscifolia* assemblages, the worst performance was obtained at ambient CO_2 and 15°C, and at high CO_2 and 20°C.

GPP and respiration rates of the understory component were also affected by CO_2 concentration and temperature. GPP varied with CO_2 and temperature, but depended on the identity of the understory species (test a priori, CO_2 × Temperature × Understory identity, $F_{2,12} = 11.08$, $p = 0.001$). Overall, the highest GPP values were obtained in monospecific assemblages of *C. crispus*/*M. stellatus* (Fig. 5). GPP rates were the highest in these assemblages both under ambient CO_2–20°C and high CO_2–15°C. Respiration rates were also the highest in monospecific assemblages of *C. crispus/M. stellatus* (test a priori, Understory identity, $F_{2,12} = 40.4$, $p = 0.0004$; Fig. 5). The best performance for these monospecific assemblages was obtained at ambient CO_2 and 20°C.

Biomass

Biomass remained constant or decreased over time depending on initial biomass and tank (i.e. significant interaction Initial biomass × Tank; Table 3). Biomass remained constant in assemblages at ambient CO_2 and 20°C (i.e. the intercept and slope of the initial-final biomass regression did not differ from 0 and 1, respectively; Table 3, Fig. 6a). However,

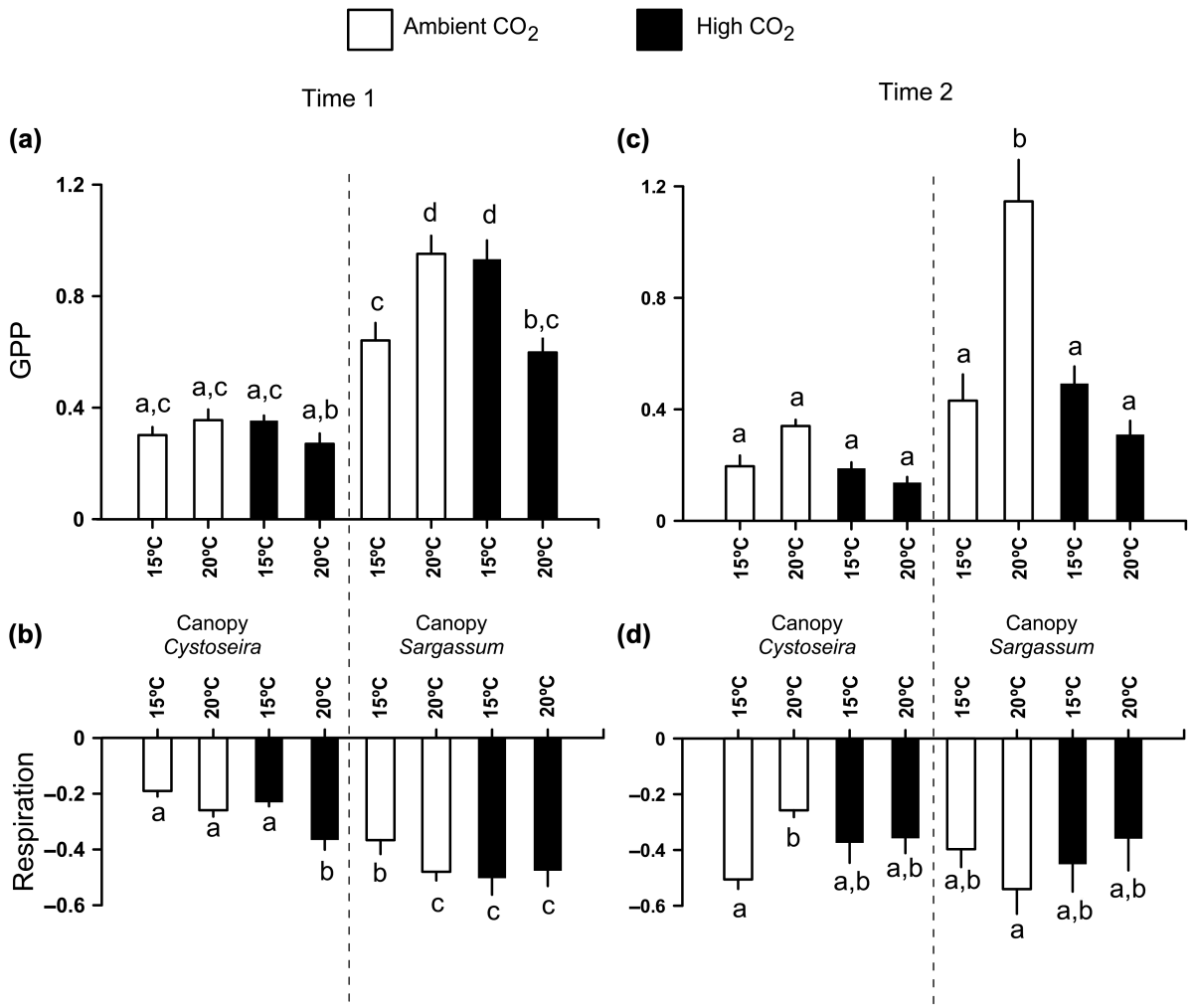

Figure 4. Mean (+ SE) per capita rates of primary productivity (GPP) (a, c) and respiration (b, d) of assemblages with *C. tamariscifolia* and *S. muticum* canopies for each CO_2 and temperature combination (n = 16). Means with a common letter do not differ significantly from each other based on SNK tests at p = 0.05 level. SNK tests were calculated from transformed data (see transformations in Table 2). Time 1 is 3 days after the start of the experiment; time 2 is at the end of the experiment (i.e. 17 days after the start of the experiment). Species labels as in Fig. 2.

assemblages at high CO_2 and 20°C experienced a clear loss of biomass (Table 3, Fig. 6b). Assemblage performance at 15°C and both ambient and high CO_2 corresponded to an intermediate situation (but see tank 1 at high CO_2 and 15°C, Fig. 6c–d). Differences among treatments were generally more evident in assemblages with an initial biomass of over 20–25 g, mainly *C. tamariscifolia* assemblages (Fig. 6a–d).

The biomass of the understory component also experienced greater losses at high CO_2 and 20°C (Table 4, Fig. 6e), mainly in monospecific assemblages of *C. crispus*/*M. stellatus* (Fig. 6e, ANCOVA for the effect of diversity with high CO_2 and 20°C; significant effect of Understory diversity, $F_{2,19} = 17.49$, $p = 0.001$). The identity of the canopy species, however, did not affect the performance of the understory component (Canopy was not included in the final minimal model; Supplementary material Appendix 3 Table A2).

Discussion

To our knowledge, this is the first laboratory study assessing eco-physiological variables to test effects of two climate change factors on macroalgal assemblages of different composition and diversity, including novel assemblages resulting from a successful invasion. Our results indicate that the increase in temperature and CO_2 concentration may interact and affect the functioning of coastal macroalgal assemblages, with effects largely dependent on species composition of assemblages. Although the effects of assemblage richness were mostly negligible, significant differences were found between the response of native and invaded assemblages.

Temperature and/or CO_2 concentration affected the biochemical composition of algae. The N and inorganic carbon (i.e. magnesium calcite) content varied significantly in non-calcareous and calcareous species, respectively. A significantly

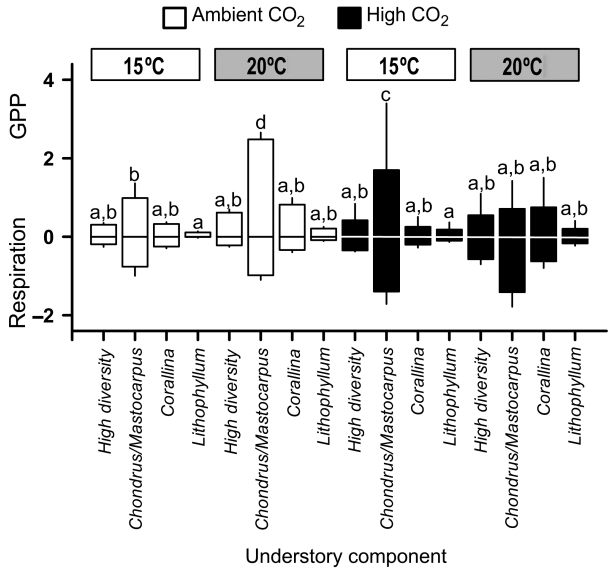

Figure 5. Mean (+ SE) per capita rates of gross primary productivity (GPP) and respiration of the understory component for each CO_2 and temperature combination at the end of the experiment (i.e. 17 days after the start of the experiment) (n = 4). Means with a common letter do not differ significantly from each other based on SNK tests at p = 0.05 level. SNK tests were calculated from ln (x + 1)-transformed data. Species labels as in Fig. 2.

greater nitrogen content was recorded at the high CO_2 concentration in *Chondrus crispus* and *Cystoseira tamariscifolia* which also showed a trend towards lower C:N ratios. Experimental studies have shown that responses of internal N content to CO_2 enrichment vary greatly between different algae (Gordillo et al. 1999, Andría et al. 2001). In algae with CO_2 concentrating mechanisms, photosynthetic performance is related to N concentration and might reflect a demand for carbon to maintain a correct C:N ratio, thereby ensuring optimal operational capacity for cells (Giordano et al. 2005). Here the increase in N by elevated CO_2 under N-sufficient conditions was not correlated with an increase in growth (i.e. both species showed tissue damage). The elevated CO_2 concentration might have stimulated nitrate reductase, the main enzyme in the nitrate assimilatory pathway, inhibiting growth as found in red algae (Mercado et al. 1999).

In agreement with previous studies (but see Kroeker et al. 2010), inorganic carbon content varied according to temperature and/or CO_2 concentration in *Corallina* spp. and *Lithophyllum incrustans*. Specifically, magnesium calcite decreased with increasing CO_2. Biogenic Mg calcite phases are more soluble than calcite and even aragonite, and may initially be more susceptible to rising CO_2 (Martin and Gattuso 2009, Sinutok et al. 2011). Several studies have shown a significant decrease in algal net calcification rate under elevated CO_2 (Gao et al. 1993, Kuffner et al. 2008, Martin and Gattuso 2009). Magnesium calcite also decreased with temperature in *L. incrustans*. Several studies have found that calcification of calcareous algae increases with temperature, although temperatures above the thermal optimum could have a detrimental effect (Martin and Gattuso 2009).

The lowest reduction of photosynthetic efficiency in the canopy species and *Corallina* spp. was generally found under ambient CO_2 concentration and 20°C conditions. *Sargassum muticum* and *L. incrustans* were the species with the smallest changes in photosynthetic efficiency independent of treatment combinations, suggesting that they might be more resilient under stress conditions. Nevertheless, ambient CO_2 concentration positively affected the photosynthetic efficiency of *L. incrustans* under shading conditions experienced in high diversity assemblages (Russell et al. 2011). The interaction of high CO_2 concentration and high temperature (which lowered pH) caused strong changes in photosynthetic efficiency in *C. tamariscifolia* and *Corallina* spp., producing negative effects. In laboratory

Table 3. (a) Final ANCOVA model for the effect of tank and initial biomass on the final biomass of assemblages (log-transformed data). Tanks with ambient CO_2 and 15°C, and tanks with ambient CO_2 and 20°C were respectively pooled; (b) parameter estimates and standard errors (SE) of the final model. The intercept (μ) and the regression slope (β) of ambient CO_2 concentration and 20°C (both tanks pooled) were taken as reference levels. The rest of parameter estimates are the differences between these reference levels and the values from other tanks.

(a) Final ANCOVA model		DF	F	p
Tank (T)		5	5.18	0.000
Initial biomass (IB)		1	168.36	0.000
T × IB		5	6.53	0.000
Residual		111		
(b) Parameter estimates	Estimate	SE	t-value	p
μ reference-ambient CO_2, 20°C (tanks 1–2 pooled)	−0.04	0.11	−0.39	0.728
β reference-ambient CO_2, 20°C (tanks 1–2 pooled)	0.96	0.07	12.97	0.000
μ – ambient CO_2, 15°C (tanks 1–2 pooled)	0.38	0.15	2.48	0.015
μ – tank 1 – high CO_2, 15°C	−0.38	0.18	−2.05	0.043
μ – tank 2 – high CO_2, 15°C	0.33	0.21	1.56	0.122
μ – tank 1 – high CO_2, 20°C	−0.09	0.17	−0.53	0.600
μ – tank 2 – high CO_2, 20°C	−0.43	0.24	−1.78	0.078
β – ambient CO_2, 15°C (tanks 1–2 pooled)	−0.35	0.10	−3.38	0.001
β – tank 1 – high CO_2, 15°C	0.25	0.12	2.05	0.043
β – tank 2 – high CO_2, 15°C	−0.30	0.15	−2.02	0.046
β – tank 1 – high CO_2, 20°C	−0.15	0.12	−1.26	0.210
β – tank 2 – high CO_2, 20°C	0.19	0.17	1.12	0.264

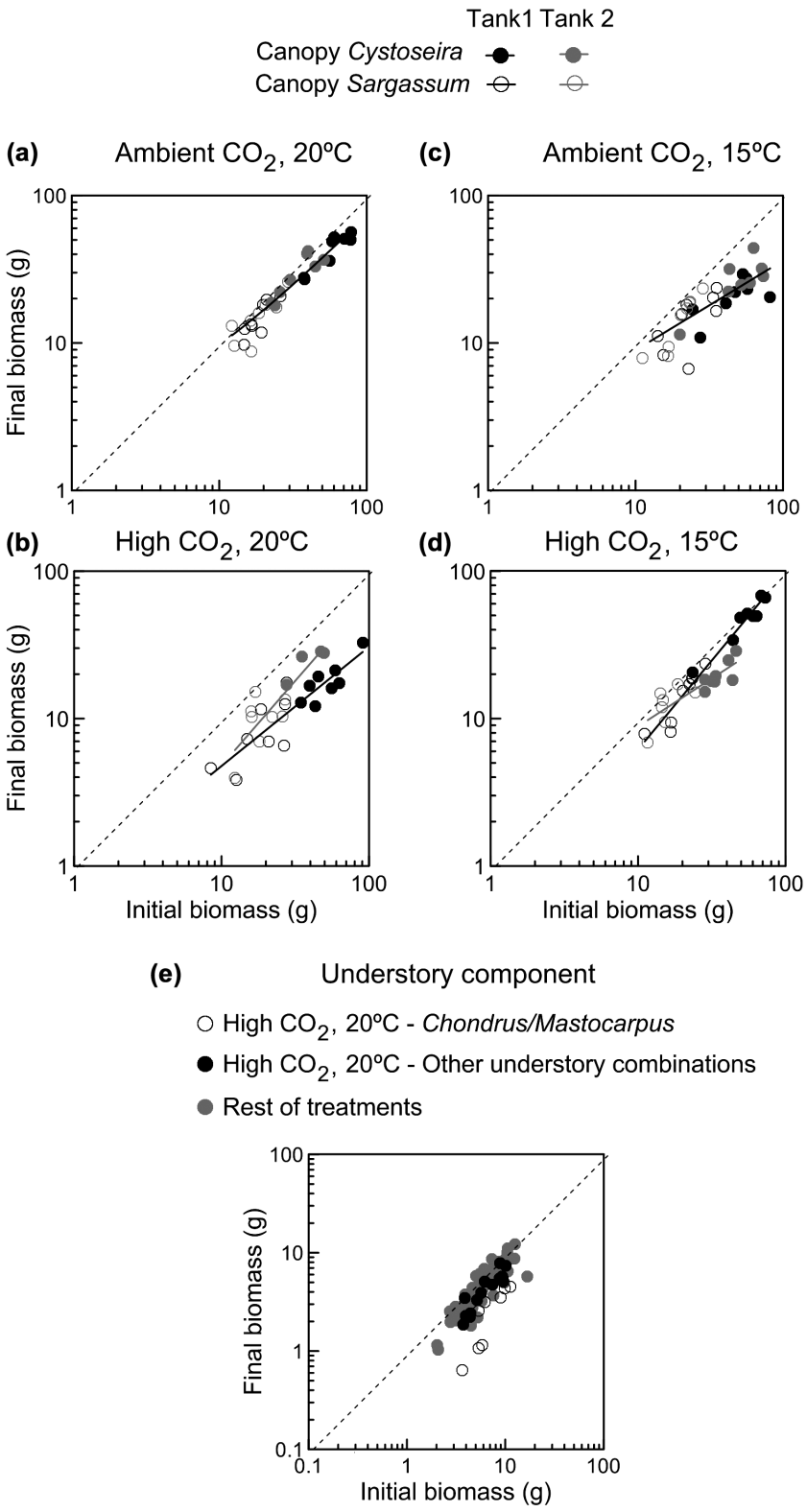

Figure 6. Relationship between final and initial biomass of assemblages with *C. tamariscifolia* and *S. muticum* (n = 8) for each CO_2 and temperature combination (a–d) and relationship between final and initial biomass of the understory component (e): *C. crispus*/*M. stellatus* at high CO_2 and 20°C (n = 8), high diversity and *Corallina* spp. at high CO_2 and 20°C (n = 16) and the rest of treatments (n = 72). Species labels as in Fig. 2.

Table 4. (a) Final ANCOVA model for the effect of tank and initial biomass on the final biomass of the understory component of assemblages (log-transformed data). Tanks with high CO_2 concentration and 20°C, and the rest of the treatments were respectively pooled; (b) parameter estimates and standard errors (SE) of the final model. The intercept (μ) of all pooled tanks except those with high CO_2 and 20°C were taken as the reference level. Intercept estimate for high CO_2 and 20°C (pooled tanks) is the difference from the reference level. Regresssion slope (β) is the overall pooled coefficient.

(a) Final ANCOVA model		DF	F	p
Tank (T)		1	36.45	0.000
Initial biomass (IB)		1	225.05	0.000
Residual		92		
(b) Parameter estimates	Estimate	SE	t-value	p
μ – reference	−0.18	0.06	−3.09	0.003
β	1.09	0.07	15.00	0.000
μ – high CO_2, 20°C	−0.18	0.03	−6.04	0.000

studies, calcifying *Halimeda* species showed lower photosynthetic efficiency under high temperature and high CO_2 (Sinutok et al. 2011). Several studies with terrestrial plants have suggested that elevated CO_2 may increase the capacity of PSII to tolerate high-temperature events. These studies found that the Fv/Fm of plants growing at elevated CO_2 was largely unaffected under high temperature conditions, although the mechanisms underlying this response remain unclear (Huxman et al. 1998, Taub et al. 2000). In contrast, photochemical efficiency of some macroalgae can be downregulated in high CO_2 environments (Mercado et al. 1999). Here, assemblages showed a drastic loss of biomass and some macroalgae, especially *C. tamariscifolia* and *C. crispus*, exhibited tissue damage at high CO_2 and 20°C (Olabarria et al. unpubl.) suggesting that changes in photosynthetic efficiency might be more of a response to stress conditions (i.e. low pH) rather than a downregulation mechanism. In fact, pH stress may negatively affect photosynthetic performance through interference with electron transport (Anthony et al. 2008). Tissue damage under CO_2-enrichment related to decreases in pH has been described for brown and red seaweeds in similar experiments (Israel and Hophy 2002). However, some brown macroalgae may be resistant to acidification under certain conditions. Recently, Roleda et al. (2012) investigated the separate effects of DIC and pH on the germination and growth rates of *Macrocystis pyrifera* meiospores and gametophytes, concluding that these early life-history stages may partially compensate for the acidification of water when medium is enriched with dissolved inorganic carbon.

Temperature and CO_2 also had a significant effect on assemblage performance in all functional proxies examined at the community-level, but the magnitude and intensity of responses differed depending on the functional variable and the type of assemblage considered. At ambient CO_2, the increase in temperature enhanced assemblage productivity by up to 50%, specifically those assemblages with *S. muticum* as canopy species (Fig. 4a, c). Temperature is one of the most important factors modulating growth in macroalgae. A rise of a few degrees in the mean seawater temperature may have significant effects on the life histories and growth of some macroalgae (Beardall et al. 1998). The increase in CO_2 concentration alone did not substantially modify assemblage performance, but in combination with higher temperature, it affected the productivity and respiration of both invaded and non-invaded assemblages. New evidence from transplant experiments in CO_2 vents from the Mediterranean Sea indicated that ocean acidification might aggravate the effects of increasing temperatures on benthic communities (Rodolfo-Metalpa et al. 2011).

Changes in productivity and respiration were not always coupled (Fig. 4). Macroalgae from rock pools show decoupled responses of photosynthesis and respiration to increases in temperature. The Q_{10} value, which is the factor by which performance increases with a 10°C increase in temperature, is generally lower for photosynthesis than for respiration (Morris and Taylor 1983). Terrestrial plants that have a high ratio of maintenance respiration to photosynthetic rates (i.e. slow-growing species) tend to have higher relative growth enhancements with elevated CO_2 (Lloyd and Farquhar 1996). However, in this study, assemblages with *C. tamariscifolia* which is a slow-growing alga compared to *S. muticum*, experienced a greater loss of biomass than assemblages with *S. muticum* in all treatments, especially in the high CO_2–20°C treatment. Ocean acidification might benefit highly invasive macroalgae such as *S. muticum* (Hall-Spencer et al. 2008). Macroalgae often show heterogeneous, species-specific photosynthetic responses to increases in CO_2 and changes in pH (Gao et al. 1991, Beardall et al. 1998, Middelboe and Hansen 2007, Swanson and Fox 2007, Johnson et al. 2012), and very often responses are non-linear. For example, some red and brown seaweeds show increasing growth rates with increasing CO_2, but only under moderate enhancement of CO_2 (Kübler et al. 1999, Diaz-Pulido et al. 2011). Furthermore, CO_2 enrichment can be associated with decreased growth and net photosynthesis vey likely due to a decrease in pH (Martin and Gattuso 2009). In fact, the acidification effect exacerbated by warming has been found in the productivity and growth of crustose coralline and non-calcifying algae (Anthony et al. 2008, Martin and Gattuso 2009, Connell and Russell 2010, Sinutok et al. 2011). Such responses may be partly due to the mechanisms of CO_2 assimilation that vary greatly even among species within same family (Beardall et al. 1998, Moulin et al. 2011).

The identity of the canopy species played an important role in assemblage response, but no relevant effects were found for understory diversity. Previous studies on macroalgal assemblages have also found that species composition has a greater effect on assemblage performance than species richness (Bruno et al. 2005, 2006). Invaded assemblages showed the greatest productivity and respiration rates. Previous studies have reported greater productivity and respiration rates for *S. muticum* than for species of the genus *C. tamariscifolia* (Arenas et al. 1995). The pseudoperennial strategy of *S. muticum*, i.e. the complete loss and regrowth of the branching system every year, could be responsible for these differences. However, the effects of temperature and CO_2 were mainly determined by canopy species, as the performance of invaded assemblages was better than that of non-invaded assemblages under the most adverse

conditions of high temperature and CO_2. Furthermore, the identity of canopy species affected photosynthetic efficiency in *C. crispus*/*M. stellatus* and *L. incrustans* assemblages, although it did not influence productivity and respiration rates in the understory component. The structure of canopy species may affect the availability of resources like light or nutrient uptake for understory components (Tait and Schiel 2011), and consequently, species could modulate/accommodate their photosynthetic activity to these changes (Middelboe and Binzer 2004, Middelboe et al. 2006). Moreover, the performance of the understory component depended on species identity rather than on species richness. Only the photosynthetic efficiency of *C. crispus*/*M. stellatus* and *L. incrustans* seemed to be affected by diversity, although in opposite directions. Thus, most diversity-related effects were generally linked to the identity of species that showed specific responses to CO_2 and temperature, with marked differences within morpho-functional groups (*M. stellatus* vs *C. crispus*, *S. muticum* vs *C. tamariscifolia*). Experimental work has shown that species sharing similar effect traits (i.e. similar effect on one or several ecosystem functions) respond very differently to the same environmental conditions (Dudgeon et al. 1995, Swanson and Fox 2007, Kroeker et al. 2010). For example, *M. stellatus* has higher tolerance to environmental stress (i.e. desiccation, temperature) than *C. crispus* due to differences in the reactive oxygen metabolism (Dudgeon et al. 1995, Collén and Davison 1999). Species of the genus *S. muticum* seem to be more tolerant to increases in pH than species of the genus *C. tamariscifolia* (Porzio et al. 2011). Functional linkages and tradeoffs among specific traits associated with the response of algae to environmental factors may drive such variability (Lavorel and Garnier 2002).

In conclusion, our results highlight the importance of using community-level approaches in mesocosm experiments to integrate biological interactions, although the use of artificial assemblages with a controlled species composition might decrease the variability due to the sampling effect of natural assemblages or communities. Short-term laboratory experiments are unable to detect the effects of chronic exposure to increasing temperature and CO_2 on macroalgal species and their eventual physiological adaptation (Hurd et al. 2009), but they can give us insights into the acclimation process of species. They are also a very convenient way to test the interactions of multiple factors and determine the acute effects and tolerance of species to diverse stressors. Considering the time period in which the experiment was carried out, ocean warming and acidification are actually occurring very slowly. Thus, long-term experiments resembling the slow steady increase of both temperature and CO_2 concentration are needed. While the limitations of extrapolating laboratory data to the natural environment should not be underestimated, some remarks can be made with caution. First, CO_2 alone or in combination with temperature affected the performance of macroalgae at both individual and assemblage level. The combination of high CO_2 concentration and 20°C provoked a drastic loss of biomass in assemblages very likely due to lowered pH, and affected their productivity and respiration rates. Second, assemblage responses to changes in CO_2 concentration and temperature varied according to their composition, and species identity was more important in driving these responses than species richness. Differences in metabolism and functional traits might explain the inter-specific differences in stress tolerance. Third, canopy species played an important role in shaping the overall performance of macroalgal assemblages and in determining the functional responses of assemblages to climate-related changes. Higher functional rates in invaded assemblages suggest modifications in the energy-matter fluxes of these assemblages, differences that might increase, if temperature and CO_2 levels rise as predicted. In this environmental scenario, we predict that macroalgal assemblages invaded by canopy-forming species belonging to the *S. muticum* functional response group (i.e. sharing traits such as life cycle or growth rates) might be favoured. Thus, the responses to increases in temperature and CO_2 discussed here have implications for several potential changes in the structure and functioning of macroalgal assemblages. Finally, this study shows the impossibility of predicting the impact of concurrent stressors from single stressor-study approaches and thus, the importance of testing them simultaneously.

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Supplementary material (available online as Appendix O20825 at <www.oikosoffice.lu.se/appendix>). Appendix 1–4.