Future herbivory: the indirect effects of enriched CO$_2$ may rival its direct effects

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ABSTRACT: Variation in rates of herbivory may be driven by direct effects of the abiotic environment on grazers, as well as indirect effects mediated by their food. Disentangling these direct and indirect effects is of fundamental importance for ecological forecasts of changing climate on species interactions and their influence on biogenic habitat. Whilst elevated atmospheric CO$_2$ may have direct effects on grazers with calcareous structures via 'ocean acidification', it may also have indirect effects via changes caused to their food. In our study we initially tested, and confirmed, that enriched CO$_2$ altered per capita rates of grazing before assessing the relative importance of indirect and direct effects in driving this response. Our results eliminated the model of a direct effect of CO$_2$ enrichment on the grazers themselves and supported the model of an indirect effect driven by a change in the food (i.e. turf algae). We suggest that this indirect effect manifested as grazers responded to the increased nitrogen content (i.e. %N) of algal tissue that resulted under CO$_2$ enrichment. Understanding such indirect effects of modified environmental conditions provide important mechanistic links between climate conditions and the ecological processes they influence.

KEY WORDS: Ocean acidification · Carbon dioxide · Climate change · Turf-forming algae · Habitat loss · Phase-shifts

INTRODUCTION

Natural ecosystems are often maintained by strong consumer control, which is fundamental in promoting the recovery of systems from, and resistance to, natural and human-driven disturbances (Lotze et al. 2001, Bellwood et al. 2004). The capacity of marine herbivores to maintain ecosystems and habitat mosaics, including coral reefs and kelp forests, is a persistent concern for ecologists (Hughes et al. 2007). This focus has recently strengthened because, as human alteration of abiotic conditions intensifies (Harley et al. 2006), so does the potential for human activities to drive variation in herbivory and disrupt ecosystem structure such that phase-shifts occur (O'Connor 2009). While there is a broad consensus that rates of herbivory may change under forecasted conditions of increased CO$_2$ and associated temp-

eratures (Tylianakis et al. 2008), we do not know whether such variation is a consequence of the direct effects of conditions on grazers themselves or the indirect effects on grazers mediated by the response of primary producers (Connell et al. 2011). Indirect effects are often unanticipated because the impact of one component on another requires knowledge of a third that is poorly understood (Wootton 1994).

Herbivory will be sensitive to many of the forecasted changes to abiotic conditions (e.g. Tylianakis et al. 2008). Meta-analyses have suggested that rates of terrestrial herbivory may increase under future climate conditions, including elevated CO$_2$ (Stiling & Cornelissen 2007, Tylianakis et al. 2008, Massad & Dyer 2010). While studies of terrestrial systems have focussed on identifying the effect of enriched CO$_2$ on the interaction of herbivory, in marine systems the focus has centred on identifying direct effects on

individual, calcifying species (e.g. Dupont et al. 2008, Havenhand et al. 2008, Crim et al. 2011). This contrasting focus is largely due to the additional effects of enriched CO_2 in seawater compared to the atmosphere, which are generally known as ocean acidification, and include a reduction in pH and carbonate (CO_3^{2-}) concentration. Previous studies have identified that the effects of ocean acidification on calcifying marine invertebrates may or may not include alterations to activity patterns, particularly feeding activity (with observed responses including suppression: Siikavuopio et al. 2007, sea urchin; negligible change: Poore et al. 2013, amphipod; and expansion: Li & Gao 2012, copepod). Given that calcareous invertebrates, such as urchins and gastropods, are key herbivorous grazers in temperate rocky marine systems (Lubchenco & Menge 1978, Underwood 1980), community structure may be altered where CO_2 enrichment, and consequent ocean acidification, influences the rate of herbivory.

In contrast to the direct effects of ocean acidification, the positive effects of CO_2 enrichment on primary producers may indirectly affect grazers such that rates of herbivory are altered (Stiling & Cornelissen 2007). Various characteristics of primary producers are expected to be modified by CO_2 enrichment, with the proximal factor affecting grazers likely to be their nutrient content, as reflected in the C:N ratio (Bezemer & Jones 1998, Whittaker 2001). In terrestrial systems, experimental manipulation of CO_2 to forecasted levels typically reduces the %N of plant tissues, increasing their C:N ratios (Drake et al. 1997, Reich et al. 2006). Grazers generally respond to this shift in tissue composition by increasing their feeding rates, as a greater biomass needs to be consumed before satiation is achieved (Stiling & Cornelissen 2007). The studies that have quantified the C:N ratios of marine algae exposed to experimentally manipulated CO_2 reveal variable responses, including the counterintuitive, opposite response to terrestrial plants; in certain species, enriched CO_2 reduces the C:N ratio of microalgae and increases the %N (Burkhardt & Riebesell 1997, Burkhardt et al. 1999). While less of this N-rich tissue would need to be consumed for grazers to reach satiation, grazers may actually feed more intensely when the %N of the food source is greater (Tylianakis et al. 2008).

Grazers with calcareous structures contribute disproportionately to the maintenance of community composition along rocky shores and subtidal systems (reviewed in Hawkins & Hartnoll 1983). While human activities (such as those which enrich CO_2) may alter production such that the ability of grazers to maintain

communities is modified, it is also possible that the grazers themselves could be influenced. Understanding the influence of enriched CO_2 on herbivory therefore requires consideration of both the indirect effects that may be mediated by changes to the food consumed, as well as the direct effects on grazers. The purpose of our study was, therefore, 2-fold: (1) to determine whether grazers alter their rates of herbivory under CO_2 enrichment and (2) to determine whether this change represents (a) the direct effect on the grazer or (b) the indirect effect mediated by alterations to the algae.

MATERIALS AND METHODS

Experimental design and set-up

Feeding experiments were conducted in field-based mesocosm experiments in order to assess the effect of a sustained enrichment of CO_2 on herbivory, and whether any change was driven by a direct effect on the grazer or an indirect effect mediated by the algae. The experimental mesocosms utilised were acrylic (A-cast acrylic, Asia Poly) and held 250 l volume (L × W × H: 0.5 × 0.5 × 1 m; algae within these mesocosms were held at 0.6 m depth). The mesocosms were moored at Outer Harbour, Adelaide, SA, Australia (34.473395° S, 138.292184° E) in an open boat harbour protected from the predominant swell by a breakwall. Mesocosms were filled with natural seawater pumped directly from the harbour; therefore, the initial seawater chemistry (before experimental manipulation) was characteristic of these waters (Falkenberg et al. 2012).

Experimental treatment: CO_2 addition

The target CO_2 levels were based on the current ambient (contemporary; 350 to 450 ppm) and the IS92a model scenario for atmospheric CO_2 concentrations in the year 2075 (enriched; 700 to 850 ppm). The pH of mesocosms exposed to the enriched CO_2 treatment was gradually reduced from ambient (8.17 ± 0.01) to the experimental level (mean ± SE: 7.94 ± <0.01, see Table 1 for further detail regarding how our treatments compare with predicted levels). Experimental CO_2 concentrations of seawater in mesocosms were maintained by directly diffusing CO_2 gas into the water column when required and was controlled by temperature-compensated pH probes and automatic solenoid controllers (Sera).

Calibration of probes was checked on a daily basis and, if necessary, recalibrated using NBS calibration buffers to 0.01 pH units. During the experimental period, one-third of the seawater was removed from each mesocosm and replaced with fresh seawater each week to maintain water quality. Total alkalinity (A_T) of seawater in mesocosms was measured weekly using colorimetric titration (Hanna Instruments) and calibrated against a Dickson reference material (Scripps Institution of Oceanography) with a known A_T of 2233.32 ± 0.90 µmol kg^{-1}, this colorimetric method returning 2253.19 ± 42.89 µmol kg^{-1}. Concentrations of pCO_2, CO_3^{2-} and bicarbonate (HCO_3^-) were then calculated from measured A_T, pH, salinity and temperature using the CO2SYS program for Excel (Pierrot et al. 2006) with constants from Mehrbach et al. (1973), as adjusted by Dickson & Millero (1987). The concentration of available nutrients (ammonium, phosphate, nitrite + nitrate) was quantified by regularly collecting water samples using 25 ml sterile syringes, which were filtered (0.45 µm glass fibre) and immediately frozen for subsequent analysis on a Lachat Quickchem 8500 Flow Injection Analyser (Hach).

Experimental grazers and algae

The grazers *Austrocochlea concamerata* used in this study were collected from the shallow subtidal (~0.5 m at low tide) from Fisheries Beach at Cape Jervis, SA (35.38007° S, 138.06502° E). The grazers were placed in holding mesocosms (separate to experimental algae) for 2 wk to enable acclimation to mesocosm conditions. They were then randomly reassigned to experimental mesocosms (n = 20 ind. $mesocosm^{-1}$) and maintained in these treatments (contemporary vs. enriched CO_2) for 3 mo (March– June 2010) prior to feeding experiments. Grazer size was not different among treatments (mass, contemporary CO_2: 3.45 ± 0.10 g, enriched CO_2: 3.44 ± 0.07 g; $F_{1,24}$ = 0.01, p = 0.9247).

The specimens of turf-forming algae used in the feeding experiments were collected from a rocky reef of 2 to 3 m depth near Horseshoe Reef, SA (35.13757° S, 138.46266° E). Here, we use 'turf' as a functional group term to denote mats of low-growing algae <5 cm canopy height in mixed assemblages that were mainly composed of the brown algal genus *Feldmannia*. These assemblages occur throughout much of the littoral zone along the southern Australian coastline, including the low intertidal and shallow subtidal (where the grazers were collected)

to the neritic zone. While we recognise that the environments from which grazers and algae were collected were not exactly the same, we suggest that the fluctuations in physical conditions experienced by these 2 groups in natural environments would be sufficiently similar such that they are acclimated to the same conditions.

Following collection of turfs attached to their natural substratum (approximately the same size, 5 × 5 cm), any attached mesograzers were removed and these samples were placed in holding mesocosms for 8 wk before the experiment commenced to enable acclimation to conditions in the mesocosms. Following this acclimation period, 5 specimens of turf were randomly assigned to each experimental mesocosm, in which conditions were gradually altered over a further 2 wk period until they reached pre-designated experimental levels. The specimens were then exposed to experimental CO_2 (contemporary vs. enriched) conditions for 6 mo (August 2009–March 2010). Following this growth period, settlement panels (5 × 5 cm fibreboard tiles) were provided onto which the algae could recruit. Recruitment occurred over 3.5 mo (March–June 2010). Half of the panels with turf were maintained under experimental conditions to be provided to the grazers in this form; the other half were used to prepare agar suspensions. Quantification of the change in mass and % cover from these substrates (detailed in 'Response variables' below) in the absence of grazers over the 3 d period used for feeding experiments revealed that there was little change and that this change did not vary significantly between treatments (change in mass: contemporary CO_2 = 0.002 ± <0.001 g h^{-1}, enriched CO_2 = 0.002 ± <0.001 g h^{-1}, $F_{1,24}$ = 0.01, p = 0.9290; change in % cover contemporary CO_2 = 0.009 ± 0.032% h^{-1}, enriched CO_2 = 0.005 ± 0.033% h^{-1}, $F_{1,24}$ = 0.01, p = 0.9255).

Agar suspensions of the ground alga were prepared separately for each algal food type (i.e. algae grown under contemporary or enriched CO_2) using the same method. Turf was scraped from the settlement panel on which it had grown, dried and ground, following which 1 g was suspended in 100 ml of filtered (0.45 µm glass fibre) seawater and heated with 1.38 g of BactoAgar (Difco™ agar granulated, Difco Laboratories, Becton, Dickinson and Company) (as in Rietsma et al. 1982, Granado & Caballero 2001). In addition, control agar suspensions were prepared following the method above but did not have any algae added. The media were then poured into 20 ml moulds (round, 5 cm diameter) to solidify and were stored, refrigerated at 5°C until they were presented

to the grazers in feeding experiments. Quantification of feeding marks (defined in 'Response variables' below) produced by grazers presented with the control suspensions (i.e. those containing no algae) over the period of feeding experiments (10 h overnight) revealed that none were produced (n = 3 control suspensions per treatment). This response indicates that grazers were responding to the suspended algae rather than the agar itself.

Experimental design

In the first experiment, we assessed the hypothesis that rates of herbivory would be decreased under enriched CO_2 and associated changes in carbonate chemistry using a mesocosm experiment. To test this hypothesis, grazers *Austrocochlea concamerata* were subjected to alternate CO_2 conditions (contemporary vs. enriched) and provided with turf-forming algae *Feldmannia* spp. grown in the same conditions (see Fig. 1 Row 1). Algae were presented in 1 of 2 forms: either on settlement panels or incorporated into agar suspensions. Each CO_2 treatment was represented by 3 replicate mesocosms (n = 3 mesocosms per treatment), within which 5 replicate panels and 5 replicate agar suspensions were each presented to a single grazer (1 grazer per panel or agar suspension; n = 5 feeding arrays for each form of algae per mesocosm).

In the second experiment, we then tested whether the observed difference in rates of herbivory was due to (1) the direct effect of CO_2 and associated changes in carbonate chemistry on the grazer or (2) the indirect effect mediated by the algae (Fig. 1 Row 2a,b). To test the direct effect of CO_2 enrichment on herbivory, we subjected grazers to the alternate CO_2 conditions (contemporary vs. enriched) and then provided them with algae grown under enriched CO_2. In addition, to test the indirect effect of CO_2 enrichment on herbivory via its effects on algae, we subjected grazers to contemporary CO_2 treatments and presented half with algae from contemporary CO_2 treatments and half with algae from enriched CO_2. Each of these latter experiments involved 3 replicate mesocosms per CO_2 treatment (n = 3 mesocosms per treatment), within which 3 replicate panels and 3 agar suspensions were each presented to a single grazer (1 grazer per panel or agar suspension; n = 3 feeding arrays for each form of algae per mesocosm).

Response variables

In all feeding experiments, each grazer was provided with 1 type of food: either turf on a panel or incorporated into an agar suspension. Grazers that were provided panels were allowed to feed for 3 d (72 h), at which time the response was assessed in terms of change in mass of panels and change in % cover of turf on panels. Change in mass of panels (final–initial measurement) was quantified by gently patting the panels dry and then weighing them at the
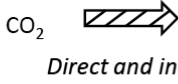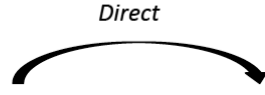

Fig. 1. Conceptual framework used to assess the effects of enriched CO_2 on the rate of herbivory. From left to right, each column represents the model, hypothesis and experimental test, respectively. Row 1 depicts the sequence of logic used to determine whether enriched CO_2 reduces the rate of herbivory. Similarly, Rows 2a and 2b outline the logic used to distinguish whether the observed increase was due to (a) the direct effect of CO_2 on the grazer (i.e. grazers from alternate CO_2 conditions were provided with algae from enriched CO_2) or (b) the indirect effect on the grazer as mediated by altered algal characteristics (i.e. grazers from the contemporary CO_2 conditions were provided algae from alternate CO_2 conditions). CCO_2: contemporary CO_2; ECO_2: enriched CO_2

beginning and end of the feeding period to the nearest 0.01 g. The change in % cover of algae on panels was quantified by visually estimating the % cover of algae at the beginning and end of the feeding period using the point-intercept method (see Drummond & Connell 2005). Grazers provided agar suspensions were allowed to feed overnight (10 h). Agar suspensions were then stored at 5°C until grazing activity was quantified by counting the number of feeding marks in the agar under a microscope. The feeding marks counted were those greater than 5 mm in length (following Valiela et al. 1979, Rietsma et al. 1982, Granado & Caballero 2001).

To quantify the response of algae in terms of chemical composition at the end of the study, turf samples were collected from the specimens following the experimental period. Following collection, the samples were preserved for analysis by being stored frozen at −20°C. They were then rinsed in Milli-Q water to remove contaminants and salts before being placed in an oven at 60°C, where they were dried for 2 d (48 h) and then crushed to a fine power using a mortar and pestle. From each specimen, a sub-sample of the powder weighing 3.5 ± 0.5 mg was measured into a tin capsule (5 × 8 mm) (SerCon). These sub-samples were then analysed using an Isotope-Ratio Mass Spectrometer (IRMS Hydra 2020 ANCA-GSL Version 4.0, SerCon) such that C and N content could be determined.

Analyses

The responses (change in mass, change in % cover and number of bites) were converted to a rate of herbivory by standardising the amount of change per hour and then analysed using ANOVA. Analysis of change in mass, change in % cover, number of bites,

%C, %N and C:N ratio was undertaken using 2-way ANOVA. CO_2 was treated as orthogonal and fixed, with 2 levels (contemporary vs. enriched), and 3 replicate mesocosms nested within these factors (n = 5 replicate samples of panels or agar suspensions per mesocosm). The % cover data from the experiment to compare grazing under contemporary and enriched CO_2 (i.e. Fig. 1 Row 1 & Fig. 2b) was ln(x) transformed prior to analysis to conform to assumptions of homogeneity of variances. Single factor ANOVA with the factor of CO_2 treated as orthogonal and fixed with 2 levels (contemporary vs. enriched) was used to test the water column physicochemical characteristics of mesocosms with measurements averaged across sampling days (n = 32) and mesocosms used as replicates (n = 3). Where significant treatment effects were detected, Student-Newman-Keuls (SNK) post hoc comparison of means were used to determine which factors differed.

RESULTS

Grazer removal of turf

The experiment testing the effects of CO_2 enrichment on per capita rates of herbivory demonstrated an increase under enriched CO_2 conditions. Enriched CO_2 treatments caused grazers to reduce the wet mass and % cover of turf on settlement panels and take bites from agar suspensions at a greater rate than under contemporary CO_2 (Figs. 1 & 2; mass: $F_{1,24} = 9.81$, p = 0.0351; % cover: $F_{1,24} = 9.53$, p = 0.0367; bites from agar: $F_{1,24} = 8.52$, p = 0.0433).

The experiments considering the direct and indirect effects of CO_2 on rates of herbivory did not support the model that enriched CO_2 directly affects grazers but instead supported the model of an indi-
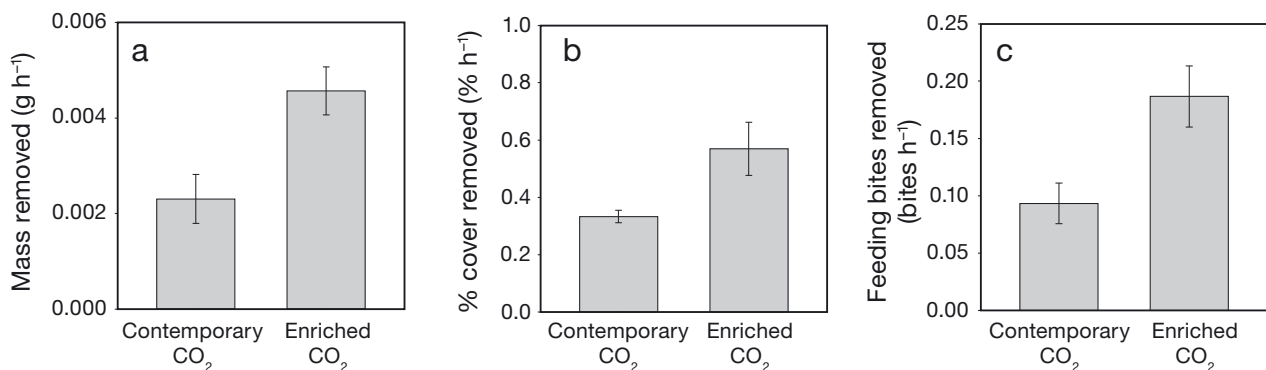

Fig. 2. (a) Mass and (b) % cover removed per hour from panels of turf-forming algae and (c) number of feeding bites taken per hour from agar suspensions incorporating turf-forming algae when both algae and grazer were exposed to the same CO_2 treatment (contemporary vs. enriched) (i.e. Fig. 1 Row 1). Values are means ± SE
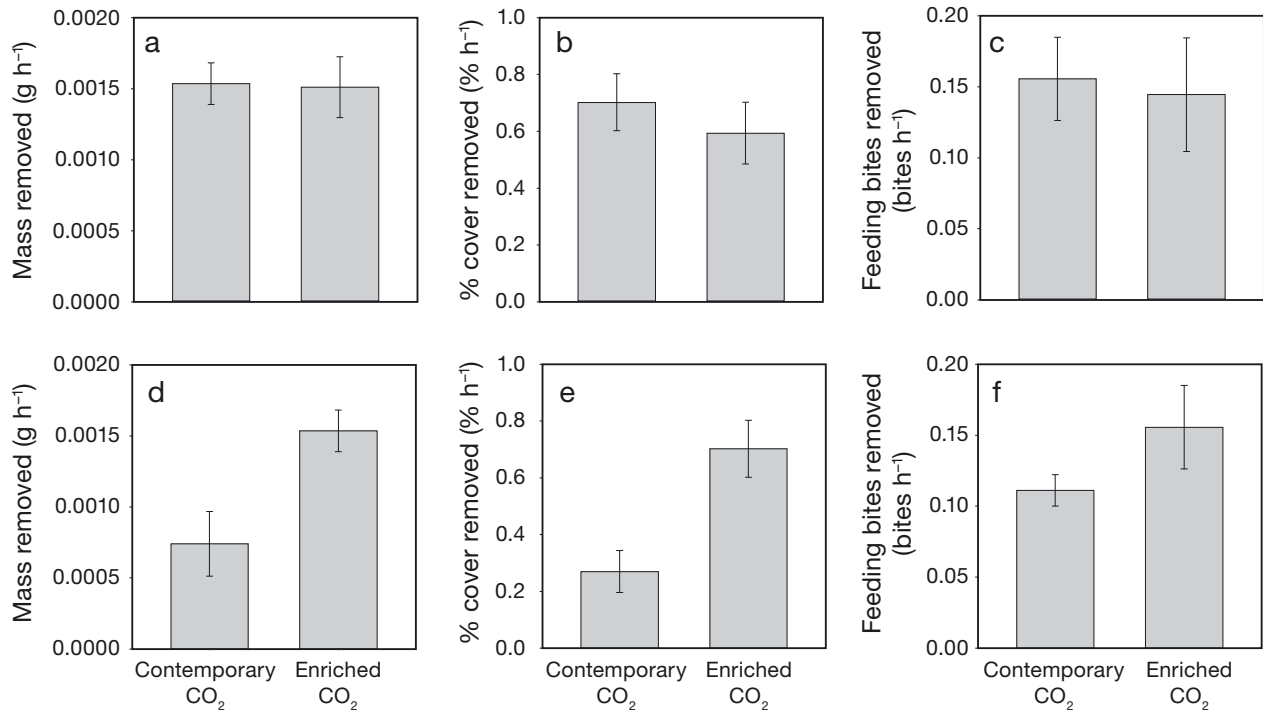
Fig. 3. (a) Mass and (b) % cover removed per hour from panels of turf-forming algae and (c) number of feeding bites taken per hour from agar suspensions incorporating turf-forming algae when (a–c) enriched CO_2 algae was provided to grazers exposed to different concentrations of CO_2 (contemporary vs. enriched) (i.e. Fig. 1 Row 2a), and (d–f) contemporary CO_2 grazers were provided with algae exposed to different concentrations of CO_2 (contemporary vs. enriched) (i.e. Fig. 1 Row 2b). Values are means ± SE

rect grazer response to the effect of CO_2 enrichment on algae (Fig. 1 Row 2a & Fig. 3). Grazers exposed to contemporary and enriched CO_2 did not differ their rates of herbivory when presented with the same type of algae (Fig. 1 Row 2a & Fig. 3a–c; mass: $F_{1,12}$ = 0.01, p = 0.9333; % cover: $F_{1,12}$ = 0.53, p = 0.5060; bites from agar: $F_{1,12}$ = 0.05, p = 0.8340). Grazers did, however, differ in their rates of herbivory when presented with the alternate types of algae. The rate at which grazers removed the mass and % cover of turf algae that recruited to panels was greater under enriched than contemporary CO_2 treatments (Fig. 1 Row 2b & Fig. 3d,e; mass: $F_{1,12}$ = 8.60, p = 0.0427; % cover: $F_{1,12}$ = 12.06, p = 0.0255). Whilst non-significant, there appeared to be a possible trend for bites to be taken at a greater rate from agar suspensions prepared using enriched than contemporary CO_2 algae (Fig. 3f; $F_{1,12}$ = 2.00, p = 0.2302).

Turf C:N ratios

Enriched CO_2 had a positive effect on the %N in the tissues of turf-forming algae (Fig. 4a; $F_{1,24}$ = 9.90, p = 0.0346). The %C was not significantly affected by

enriched CO_2 (Fig. 4b; $F_{1,24}$ = 0.10, p = 0.7625), and the trend for the C:N ratio to be reduced (Fig. 4c) was not significant ($F_{1,24}$ = 4.44, p = 0.1028).

Experimental conditions

The pH and concentration of CO_3^{2-} were reduced under enriched CO_2 compared with contemporary CO_2 treatments (Table 1; $F_{1,4}$ = 240.70, p = 0.0001 and $F_{1,4}$ = 62.47, p = 0.0014, respectively). While there was variation in the quantified pH over time (e.g. at the diurnal scale as illustrated in Fig. A1 in the Appendix), the pH of enriched treatments was consistently lower than that of contemporary treatments. In contrast, the A_T, pCO_2 and HCO_3^- increased under enriched experimental conditions (Table 1; $F_{1,4}$ = 16.42, p = 0.0155; $F_{1,4}$ = 975.38, p < 0.0001; and $F_{1,4}$ = 73.98, p = 0.0010, respectively). Nutrient conditions (ammonium, phosphate and nitrite + nitrate) were also quantified, with results indicating that while there was variability, there was no trend for increasing concentrations over time or in a particular treatment (Fig. A2 in the Appendix).
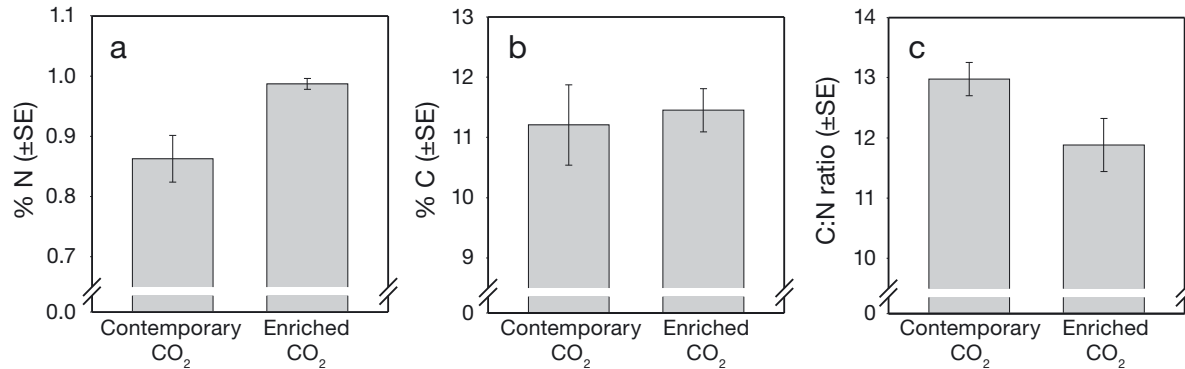
Fig. 4. (a) %N, (b) %C and (c) C:N ratio of turf-forming algae recruited under different CO_2 conditions (contemporary vs. enriched). Values are means ± SE

Table 1. Carbonate parameters, both predicted and measured in experimental mesocosms for each treatment (CCO_2: contemporary CO_2; ECO_2: enriched CO_2). Predicted treatments were calculated from our target pCO_2 (current, 350 to 450; enriched, 700 to 850 ppm) and the associated pH range (current, 8.02 to 8.1; enriched, 7.95 to 7.8). Experimental conditions were quantified by measuring total alkalinity (A_T) and pH simultaneously on 32 occasions, from which concentrations of pCO_2 (ppm), CO_3^{2-} (µmol kg^{-1}) and HCO_3^- (µmol kg^{-1}) were calculated. Values were calculated using constants from Mehrbach et al. (1973), as adjusted by Dickson & Millero (1987). Note: measurements were taken throughout the day, thus include some influence of photosynthesis and respiration, resulting in slight differences between predicted and experimental conditions

Carbonate parameter	Treatment	Predicted condition	Experimental condition Mean (SE)	Max.	Min.
pH	CCO_2	8.02–8.1	8.17 (0.01)	8.53	8.00
	ECO_2	7.8–7.95	7.94 (<0.01)	8.20	7.54
pCO_2	CCO_2	350–450	454 (12)	665	138
	ECO_2	700–850	892 (8)	2337	515
A_T	CCO_2	2458–2523	2516 (35)	3064	1848
	ECO_2	2640–3218	2709 (33)	4571	2042
CO_3^{2-}	CCO_2	205–231	178 (6)	277	97
	ECO_2	141–232	124 (3)	322	55
HCO_3^-	CCO_2	1888–2020	2086 (27)	2585	1502
	ECO_2	2299–2674	2414 (27)	4124	1851

DISCUSSION

Abiotic conditions are a major driver of variation in herbivory which, in turn, has fundamental effects on the organisation and function of ecological systems (O'Connor 2009). Consequently, changes in community structure, including phase-shifts, can often be traced to the direct and indirect effects of human activities on herbivory (Hughes et al. 2005). This study reveals that under enriched CO_2 and associated ocean acidification rates of herbivory may be increased relative to contemporary conditions. Specifically, we show that under enriched CO_2 treatments the rate of turf removal by calcified grazers was approximately double that observed under contemporary CO_2 treatments. While it is possible such change may be due to the direct effects of ocean acidification on grazers resulting from enriched CO_2, our results highlight the indirect effects of plant-mediated herbivory that occur as a function of grazers responding to changes in the algae. This result demonstrates a novel mechanistic link between changing climatic conditions and ecological responses — specifically, indirect effects that are not readily detectable and have been under-represented in ecological studies (but see Poore et al. 2013).

Early research into the ecological consequences of altered environmental conditions primarily focussed on direct effects, reflecting the largely unchallenged idea that it is these direct effects that drive the strongest mechanistic links (see review by Connell et al. 2011). Such studies revealed that enriched CO_2 can have relatively simple and understandable direct effects on numerous physiological processes of invertebrates (e.g. fertilisation, Havenhand et al. 2008; embryonic development, Parker et al. 2009; growth and survival, Dupont et al. 2008, Kurihara et al. 2008; metabolism, Cummings et al. 2011). Although readily detectable, the influence of such direct effects may not exceed that of indirect effects in determining the rate of processes that contribute to shaping communities (including herbivory). A growing number of studies suggest the direct effects of moderate CO_2 enrichment on calcifying organisms may not have profound effects on their feeding rates (e.g. Kurihara

et al. 2008, Gooding et al. 2009, Marchant et al. 2010, Li & Gao 2012), despite early experimentation that manipulated CO_2 to greater concentrations suggesting large negative responses (e.g. Cecchini et al. 2001, Foss et al. 2003, Siikavuopio et al. 2007). We emphasise the counter-intuitive effect we discovered (i.e. a positive response to CO_2), as the identification of such surprising effects is very much the domain of assessments of indirect effects (Menge 1995). Where community organisation is affected by such indirect effects, forecasting the direct effects of changing abiotic conditions will have limited utility (Connell et al. 2011).

What accounts for the indirect effect observed in our study? Analysis of algae from the enriched CO_2 treatments revealed they had a greater %N than algae from contemporary CO_2 conditions. Such elevations in N are well known to increase the rates of grazing in many terrestrial and marine systems (Hillebrand et al. 2000, Silliman & Zieman 2001), including the coastal system considered here (Russell & Connell 2007). Understanding the mechanism by which CO_2 altered the chemical composition of algae is complicated by limited knowledge of pathways of carbon acquisition among different algal species. It is generally true, however, that many species of algae possess carbon concentrating mechanisms (CCMs) that facilitate active influx of CO_2 and/or HCO_3^- (Beardall & Giordano 2002, Giordano et al. 2005, Reinfelder 2011); analysis of stable-isotope ratios has indicated that the *Feldmennia* turf algae considered here does, indeed, have active CCMs (Falkenberg et al. 2013). Under enriched CO_2, this method of carbon acquisition is anticipated to require fewer resources, as the affinity of CCMs for inorganic carbon decreases and algae switch to passive diffusion, enabling the down-regulation of CCMs (Wu et al. 2010, Raven et al. 2011, Reinfelder 2011). Consequently, the requirements of algae for CCMs may be reduced (Drake et al. 1997, Wu et al. 2010, Hepburn et al. 2011), enabling reallocation of resources otherwise used by CCMs (Hamilton et al. 2001). Typically, these resources would be anticipated to be used such that growth rates of the algae increase. However, consideration of the growth and stoichiometric responses of turf algae to enriched CO_2 conditions has indicated that this alga is co-limited by both CO_2 and nutrients, such that enrichment of CO_2 alone may not enable enhanced growth because nutrients remain limiting (particularly in the oligotrophic system considered here) (Falkenberg et al. 2013). Consequently, we suggest the higher carbon availability is channelled into nutrient acquisition rather than

growth, with the resulting increased nutrient resources stored and driving an increased tissue %N (Falkenberg et al. 2013, present study).

While we suggest that this increased %N prompted the altered algal consumption under enriched CO_2, it is also possible that changing CO_2 may have influenced the toughness or some other feature of the palatability, such as secondary metabolites of the algae, to result in the observed indirect effect (for algal example, see Swanson & Fox 2007; seagrass example, Arnold et al. 2012; terrestrial example, Stiling & Cornelissen 2007). Further, while the composition of the turf assemblage appeared to remain stable throughout the experimental period, it is possible that altered conditions drove a shift from one turf species to another which was morphologically similar (e.g. seen in cyanobacteria; Lidbury et al. 2012), with this shift being the change to which grazers were responding. Consequently, we advocate that future studies closely consider the effect of modified conditions on turf individuals and assemblages to determine which feature(s) drive indirect effects.

In the context of the temperate marine habitat considered here (see review by Connell & Gillanders 2007), change to the rate of herbivory under future climatic conditions may have implications for community structure. In the absence of strong herbivory, it is anticipated that enriched CO_2 will facilitate the algae that inhibits recruitment of kelp (e.g. turfs, Connell & Russell 2010) which have caused kelp losses both regionally (within South Australia, Connell et al. 2008) and in Europe (Worm et al. 1999, Eriksson et al. 2002, Airoldi & Beck 2007). Our key result — that herbivores may have greater control of turfs under enriched CO_2 — indicates that herbivory may restrict turf expansion under future conditions and suggest calls to assess the future roles of herbivores have merit (e.g. Post & Pedersen 2008, Rinnan et al. 2009). Where rates of herbivory are strengthened significantly, this process has the potential to change the direct positive effect of CO_2 on the primary producer (i.e. increased net productivity) into an indirect negative response through loss of biomass (Zvereva et al. 2010). The influence of indirect effects resulting via trophic interactions appears to be quite general, with similarly complex effects identified for various environmental conditions in a diversity of systems (e.g. CO_2 in marine systems, present study; temperature in marine systems, O'Connor 2009, O'Connor et al. 2009; rainfall in grasslands, Suttle et al. 2007).

In conclusion, the fundamental role of herbivory in controlling the biomass of primary producers and,

subsequently, maintaining the persistence and resilience of natural systems may be altered under enriched CO_2. The proportion of primary production that is removed by grazers may vary as a consequence of abiotic factors (including climate) that affect not only the grazers themselves (e.g. activity rates, Wood et al. 2008) but also traits of the producers they consume (e.g. net primary production and/or nutritional quality, Cebrian 1999). Our study suggests the effects of future climatic conditions, specifically enriched CO_2, may go well past the direct effects on calcifying grazers. Whilst the indirect effects of climate on species interactions were initially surprising (Sanford 1999), we are coming to recognise they may be more common and rival the more intuitive and easily detectable direct effects of altered climates.

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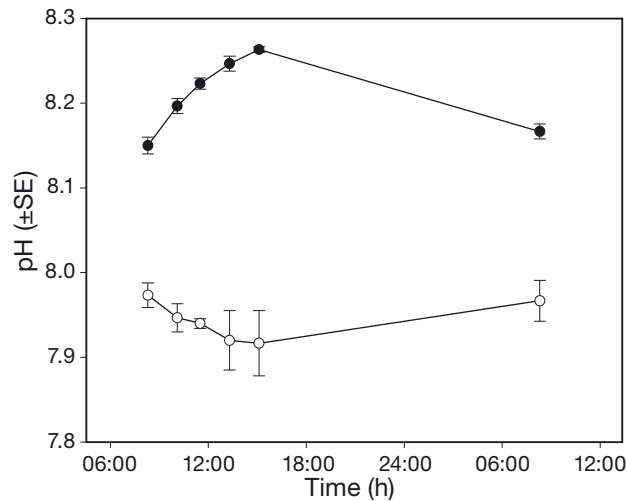
Appendix 1. Experimental conditions

Fig. A1. A representative diurnal pH cycle (6 to 7 Aug 2009; 06:00−06:00 h). ●: current CO$_2$; O: future CO$_2$. Values are means ± SE
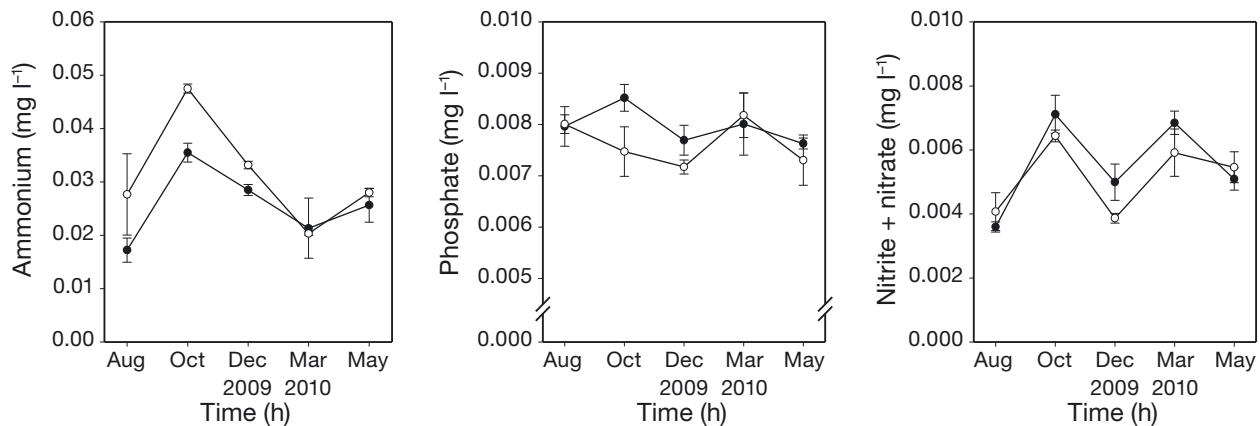

Fig. A2. Nutrient concentrations (ammonium, phosphate, nitrite + nitrate) within mesocosms measured from beginning to end of the experiment. Note the different scales on the *y*-axis. ●: current CO$_2$; O: future CO$_2$. Values are means ± SE