

Herbivory mediates the expansion of an algal habitat under nutrient and CO₂ enrichment

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ABSTRACT: Certain environmental conditions facilitate the control of primary producers by herbivores. Environmental change can, therefore, mediate the strength of consumption relative to production such that the abundance of primary producers is altered, potentially driving phase-shifts from one habitat type to another (e.g. the displacement kelp forests by mats of turfs along temperate coasts). Here, we assessed the extent to which herbivores may counter the increased cover of kelp-competitors (i.e. turfs) anticipated to be caused by enriched nutrients and CO₂. Specifically, we experimentally enriched nutrients and CO₂ and quantified the change in cover of algal turfs in the presence and absence of gastropod grazers. In the absence of grazers, turf algae responded positively to the combination of enriched nutrients and CO₂ such that they occupied >95% of available space. In contrast, where grazers were present, sparse covers of algal turfs were maintained even under CO₂ and nutrient enrichment (i.e. turfs occupied <5% of available space). This result indicates that consumption by grazers increased where enrichment of nutrients and CO₂ facilitated greater algal cover such that cover continued to be largely restricted. By recognising the environmental conditions which will maintain or disrupt the balance between the processes of production and consumption, we may improve forecasts regarding the probability of habitat stasis or shifts.

KEY WORDS: Carbon dioxide · Climate change · Grazers · Nutrients · Ocean acidification · Eutrophication · Turf-forming algae

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INTRODUCTION

The balance between biological processes—particularly the expansion of primary producers to occupy more space and their consumption by herbivores—can determine the occurrence of key species and influence ecosystem composition (O'Connor 2009, Connell et al. 2011). The processes of production and consumption are, however, often differentially influenced by variation in environmental factors (Sanford 2002, O'Connor 2009). Where environmental conditions modified by human activities disrupt the balance between these processes (e.g. if production is facilitated but consumption is not), the occurrence of key primary producers may be altered such that local communities are reorganised and phase-shifts occur (Sanford 1999, Scheffer et al. 2001, Paine & Trimble 2004).

Production is typically increased where human activities enhance the availability of limiting resources, particularly nutrients (Liebig 1842, Harpole et al. 2011). Although greater productivity under conditions of nutrient enrichment may be beneficial in some systems, these effects can act as perturbations where competition for space has a strong role in determining community structure. Enhanced nutrient availabilities enable turfing algae to expand into available space which is created during disturbance events, preventing the replenishment of kelp individuals and driving phase shifts from historically dominant kelp canopies to mats of turf (Connell et al. 2008, Gorman & Connell 2009). As global climatic conditions increase the CO₂ absorbed by coastal waters, carbon may act as a resource among competitors, prompting change in habitat dominance

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(Connell et al. 2013). Specifically, it is anticipated that in the system considered here, increased carbon availability will facilitate not only faster abiotic change but also greater growth of turfs, particularly where nutrients are also enriched (Russell et al. 2009, Falkenberg et al. 2010, 2013b, Cai et al. 2011).

The ecological effects of altered turf growth (e.g. space occupancy) may, however, be modified by the effect of altered conditions on herbivores. Grazers can have strong roles in structuring systems unaffected by human activities (Lotze et al. 2001, Bellwood et al. 2004), as well as those which have experienced moderate nutrient enrichment (Hillebrand et al. 2000). It is unclear, however, how future CO₂ will combine with enriched nutrients to influence rates of consumption. Although early studies indicated the potential for CO₂ enrichment (and the consequent ocean acidification) to disrupt calcification and grazing ability (i.e. Siikavuopio et al. 2007), a growing body of research suggest rates of consumption may be negligibly affected (i.e. Kurihara et al. 2008, Gooding et al. 2009), or even enhanced under future CO₂ conditions (Cummings et al. 2011).

The purpose of this study was, therefore, to investigate whether the increase in spatial cover of algal turfs which is anticipated to manifest under the combined enrichment of nutrients and CO₂ would be mediated by the process of herbivory. We used a model system to test the hypotheses that (1) if nutrients and CO₂ are enriched in combination, then the cover of turfs will increase such that its cover is greater than under ambient conditions, and (2) if grazers are present where nutrients and CO₂ are enriched, then consumption will mediate the expansion of turfs. Identification of this pattern would indicate that the continued presence of grazers may be sufficient to resist ecological change in this coastal system, even under modified abiotic conditions.

MATERIALS AND METHODS

Experimental design

To determine whether the expansion and consumption of turf will remain in balance under forecasted conditions, a laboratory experiment was used to quantify the percentage cover of turf-forming algae in the presence and absence of grazers under manipulated nutrient and CO₂ conditions. Following a crossed design, algae were exposed to combinations of herbivorous grazers (present vs. absent), nutrients (ambient vs. elevated) and CO₂ (current vs. future) over a

period of 133 d. For each combination of treatments there were 2 replicate aquaria, each containing 5 settlement panels which were unoccupied at the start of the experiment (squares of fibreboard, each 5 × 5 cm).

Experimental set-up

During the acclimation period (2 wk), 40 l holding aquaria were filled with sand-filtered seawater which was maintained at conditions similar to the field sites (mean ± SE; temperature = 17°C, salinity = 33.45 ± 0.82‰, pH = 8.15 ± 0.04, irradiance = 33.96 ± 1.61 μmol m⁻² s⁻¹, nutrients: NH₄⁺ = 0.018 ± 0.003 mg l⁻¹, NO_x = 0.022 ± 0.003 mg l⁻¹, PO₄ = 0.011 ± 0.001 mg l⁻¹) (Russell 2007). During both the acclimation and experimental periods, one-third of the seawater in the aquaria was changed weekly, which is sufficient to maintain these conditions and water quality suitable for algal growth (e.g. nutrient levels; Russell et al. 2009). Samples to quantify physicochemical conditions of the water during the acclimation and experimental periods were taken at midday once a week before water changes (values reported in Table A1 in the Appendix).

Experimental algae

Specimens of turf algae (a functional group term used here to denote a mixed assemblage of filamentous algae <1.5 cm in height composed of *Feldmannia* spp.) were collected from shallow subtidal rocky reef near Encounter Bay, Victor Harbor, South Australia (35.34219°S, 138.36599°E). Algal specimens were collected attached to their natural rock substratum and placed in holding aquaria for 4 wk before the experiment commenced. Five replicate natural substrates (with a total mean ± SE = 9.82 ± 0.87 g of turf algae biomass) were then randomly re-assigned to each experimental aquarium to act as a source of propagules for the interspersed settlement panels. Conditions were then gradually altered over a 2 wk period until they reached the pre-designated experimental levels. During the experimental period, turf was allowed to recruit from seawater in the aquaria to the settlement panels.

Experimental treatments: grazer, nutrient and CO₂ manipulation

Grazers were either present, as is observed under contemporary conditions, or absent, which may re-

sult in the future due to continued human exploitation. The grazers used were the herbivorous gastropods *Austrocochlea concamerata* and *A. odontis* collected from the shallow subtidal zone of Fisheries Beach at Cape Jervis, SA (35.38007° S, 138.06502° E). These grazers occur throughout much of the littoral zone along the southern Australian coastline, including the shallow subtidal—which enables their interaction with the turf algae considered here (i.e. assemblages dominated by *Feldmannia* spp.). The grazers were acclimated in holding aquaria (separate to experimental algae) for 2 wk prior to the experiment commencing. As the grazers were observed feeding on turf algae in the field, during the acclimation period they were provided with turf algae growing on natural rock substratum collected from the shallow subtidal. Grazers were then randomly re-assigned to experimental aquaria (3 ind. per 0.15 m², or 133 per m², to reflect densities observed at the collection site and reported from other coastlines of southern Australia [e.g. Underwood & Creese 1976, Walsh et al. 1994], one *A. odontis*, 2 *A. concamerata*; weight of 3.19 ± 0.10 g each; grazer:turf biomass ratio of ~1:1.03 g).

The elevated nutrient treatment was designed to simulate increased concentrations similar to those experienced in waters off the coast of metropolitan Adelaide (Gorman et al. 2009). Nutrients were enhanced using Osmocote Plus® (Scotts) controlled release fertiliser (6 mo release: 15% N, 5% P, 10% K), which was placed in nylon mesh bags (1 mm mesh size) and attached to the bottom of each appropriate aquarium (3 g per aquarium). Concentrations of supplied nutrients were quantified by regularly (before weekly water changes) by collecting water samples using 25 ml sterile syringes, which were then filtered (0.45 µm glass fibre) and frozen before being analysed on a Lachat Quickchem 8200 Flow Injection Analyser (Hach) for ammonia (NH₄⁺), nitrite + nitrate (NO_x) and phosphate (PO₄) (values during acclimation reported in 'Experimental set-up' and those during the experimental period reported in Table A1).

CO₂ concentrations were based on the current ambient (current; ~380 ppm) and IS92a model scenario for atmospheric CO₂ concentrations in the year 2060 (future; ~580 ppm) (Meehl et al. 2007). The CO₂ concentration of seawater was maintained by directly diffusing pure CO₂ into the water column to obtain the pre-determined treatment levels and was controlled by temperature-compensated pH probes and automatic solenoid controllers (Sera). Calibration of probes was checked on a daily basis, and recalibration was performed when necessary. Total

alkalinity (A_T) of seawater was quantified on a weekly basis (before the weekly water change) using colorimetric titrations (Hanna Instruments) with measurements taken from midday (between 12:00 and 13:00 h). The measured A_T , pH (NBS scale), salinity and temperature were used to calculate the partial pressure of CO₂ ($p\text{CO}_2$) and concentration of bicarbonate (HCO₃⁻) using the CO2SYS program for Excel (Pierrot et al. 2006) with constants from Mehrbach et al. (1973), as adjusted by Dickson & Millero (1987) (values reported in Table A1).

Response variables

The response of turf-forming algae to experimental conditions was assessed in terms of percentage cover of turf algae on settlement panels at the completion of the experiment. Following 133 d of treatment, percentage cover of turfs was quantified using the point-intercept method, where the presence or absence of turf algae was recorded under 20 regularly spaced points within a 5 × 5 cm quadrat for each settlement panel, resulting in a measurement resolution of 5%. The physical quadrat was constructed from a transparent piece of plastic, on which the points were marked. The same array was then laid over each settlement panel to enable visual assessment. Consequently, the reported turf cover represents the extent to which the plate was covered by *Feldmannia* spp. and does not distinguish between different turf species which may have been present (for further detail see Drummond & Connell 2005).

Statistical analyses

The response (final % cover turf on settlement panels) was analysed using 4-factor ANOVA. Grazers, CO₂ and nutrients were treated as fixed and orthogonal, with aquaria treated as random and nested within grazers, CO₂ and nutrients. There were 2 levels in each factor (grazers: present vs. absent; CO₂: current vs. future; nutrients: ambient vs. elevated; 2 aquaria) with data for the experimental units (i.e. settlement panels) within each aquaria used as replicates (n = 5). To remove heterogeneity, data was ln(x + 1) transformed prior to analysis. Physicochemical conditions within aquaria were analysed using 1-way ANOVAs in which the factor had 2 levels; the factor of nutrients (ambient vs. elevated) was used to test the nutrient conditions while the factor of CO₂ (current vs. future) was used to test for differences in carbonate condi-

tions. Where significant treatment effects were detected, we then ran Student-Newman-Keuls (SNK) post hoc comparison of means.

RESULTS

Cover of turf algae

The key result was that while enriched nutrients and CO₂ combined to facilitate the greatest increase in turf cover, the presence of grazers limited the expansion (Fig. 1). That is, in the absence of grazers, nutrient and CO₂ enrichment combined to produce a greater increase in turf cover than occurred where either resource was enriched in isolation (Fig. 1). Importantly, there was significantly less cover of turfs where grazers were present than absent under all combinations of nutrients and CO₂ (Table 1; SNK tests of significant CO₂ × Nutrient × Grazer interaction term $F_{1,64} = 8.98$, $p = 0.0172$). This result indicates that under forecasted conditions, the cover of turfs removed by grazers increased (i.e. the percentage turf removed was ~45% under current CO₂, ambient nutrient conditions, ~68% under future CO₂, ~85% under elevated nutrients and ~91% under the combination of future CO₂ and elevated nutrients).

Water column physicochemical variables

The quantified concentrations of NH₄, NO_x and PO₄ were significantly higher in the elevated than ambient

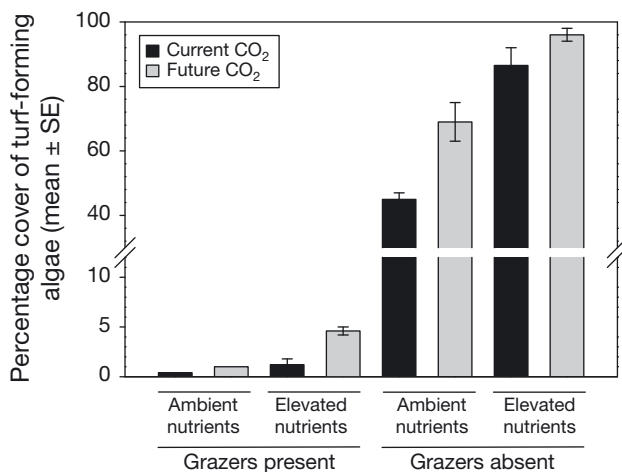


Fig. 1. Percentage cover (\pm SE) of turf-forming algae which recruited to and grew on settlement panels that were exposed to different combinations of grazer (present vs. absent), nutrient (ambient vs. elevated) and CO₂ (current vs. future) conditions

Table 1. ANOVA testing the combined effects of CO₂ (current vs. future), nutrients (ambient vs. elevated) and grazers (present vs. absent) on the percentage cover of turf-forming algae; ns = $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$

Source	df	MS	F	p
CO ₂	1	4.7760	42.61	***
Nutrients (Nutr)	1	7.0499	62.90	***
Grazers (Graz)	1	236.4826	2109.98	***
Tank (CO ₂ × Nutr × Graz)	8	0.1121	1.56	ns
CO ₂ × Nutr	1	0.1171	1.04	ns
CO ₂ × Graz	1	1.0400	9.28	*
Nutr × Graz	1	0.1776	1.58	ns
CO ₂ × Nutr × Graz	1	1.0063	8.98	*
Residual	64	0.0719		

nutrient treatments (NH₄: $F_{1,4} = 8.25$, $p = 0.0454$; NO_x: $F_{1,4} = 12.09$, $p = 0.0254$; PO₄: $F_{1,4} = 8.07$, $p = 0.0468$; Table A1). A_T, pCO₂ and HCO₃⁻ were greater in treatments where CO₂ was experimentally elevated, while pH was lower under future CO₂ compared with current CO₂ conditions (A_T: $F_{1,4} = 157.74$, $p = 0.0002$; pCO₂: $F_{1,4} = 32.92$, $p = 0.0046$; HCO₃⁻: $F_{1,4} = 112.33$, $p = 0.0004$; pH: $F_{1,4} = 41.26$, $p = 0.0030$; Table A1).

DISCUSSION

The balance between the processes of production and consumption contributes to determining the abundance of primary producers. As these processes are anticipated to be differentially affected by alterations of the abiotic environment, human-driven changes may disrupt the current balance, prompting shifts in community organisation and structure (Sanford 2002, O'Connor 2009, Connell et al. 2011). The key result from our model system was that although future nutrient and CO₂ conditions combined to increase the cover of turf algae, its consumption by gastropod grazers was also greater under these modified conditions, with the net result that the spatial extent of turf cover continued to be largely restricted. This result indicates that under forecasted conditions, the process of consumption may be important in mediating the elevated production facilitated by nutrient and CO₂ enrichment.

The interactive effect of nutrient and CO₂ enrichment on the cover of primary producers identified here (i.e. that Nutrients × CO₂ accelerated the expansion of turf cover) aligns closely with the results of previous experimental manipulations (Russell et al. 2009, Falkenberg et al. 2012, 2013a). We suggest the increased cover of turfs where nutrients and CO₂ were both enriched was facilitated by the increased

availability of nutrients and carbon that enabled the co-limitation experienced under current conditions to be overcome and rates of physiological processes increased (Falkenberg et al. 2013b).

The increased cover of algae anticipated to result from future conditions may, however, be mitigated where grazers, and the process of consumption, continue to counter forecasted algal expansion (Falkenberg et al. 2010). That grazers continued to largely restrict the cover of turf algae, even under conditions where turf was promoted, indicates that rates of consumption were not reduced, but actually increased, under nutrient and CO₂ enrichment. We suggest that the increased consumption under enriched nutrient conditions is likely to represent an indirect effect mediated by the quality of the algae consumed by the grazer (Russell & Connell 2007). Under conditions of enriched nutrient availability, turf algae have been found to increase the nutrient content of their tissues (Falkenberg et al. 2013b). While less of this nutrient-rich tissue would need to be consumed to reach satiation, grazers presented with such algae may actually feed more intensely and increase consumption rates (Tylianakis et al. 2008 and references therein). Further, it is also possible that although the composition of the turf assemblage appeared to remain stable throughout the experimental period, enriched nutrients may have prompted a shift from one turf species of the genus *Feldmannia* to another which was morphologically similar (e.g. seen in cyanobacteria; Lidbury et al. 2012), with this shift the change to which grazers were responding. In contrast, enriched CO₂ may have facilitated increased rates of consumption via a direct effect on the grazers as CO₂-driven increases in their metabolic rates (Cummings et al. 2011) can lead to increased resource demands and prompt greater rates of consumption (Li & Gao 2012). Consequently, we suggest that the direct and indirect effects of these altered conditions combined to increase the rate of consumption under future conditions.

In conclusion, while enrichment of nutrients and CO₂ caused the production and cover of turf algae to increase in the absence of grazers, CO₂ also modified consumption such that the key producer response of expansion (i.e. an increase in space occupancy) was largely ameliorated in the presence of grazers. These results clearly suggest that the effects of altered abiotic conditions will go well beyond direct effects on individual taxa to include indirect effects mediated by species interactions. These interactions are, however, likely to be further affected by concomitant changes to the environment (e.g. UV radiation, temperature) and changing species composition and consumer be-

haviour (e.g. species distributions, abundances and per capita feeding rates). By recognising the environmental conditions which modify the processes of production and consumption, and the balance between them, we may be better able to forecast the conditions in which habitat stasis or shifts are more probable.

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Appendix. Table A1. Physicochemical variables measured for each treatment. Reported are means and standard errors (SE). Ammonia (NH₄⁺), nitrite + nitrate (NO_x), phosphate (PO₄), total alkalinity (A_T) and pH were sampled weekly (at midday) on 7 occasions. The measured A_T and pH were then used to calculate the partial pressure of CO₂ (pCO₂) and concentration of bicarbonate (HCO₃⁻) using constants from Mehrbach et al. (1973), as adjusted by Dickson & Millero (1987). Please note that as A_T and temperature measurements were taken at midday they represent values which include some reduction due to photosynthesis, resulting in a slightly lower pCO₂ than the current atmospheric level (e.g. Gao et al. 1991). CCO₂: current CO₂; FCO₂: future CO₂; AN: ambient nutrients; EN: elevated nutrients

Physicochemical variable	Treatment			
	CCO ₂ , AN	CCO ₂ , EN	FCO ₂ , AN	FCO ₂ , EN
NH ₄ (mg l ⁻¹)	0.018 (0.026)	0.025 (0.005)	0.019 (0.005)	0.029 (0.005)
NO _x (mg l ⁻¹)	0.021 (<0.001)	0.031 (0.006)	0.020 (0.001)	0.032 (0.005)
PO ₄ (mg l ⁻¹)	0.011 (<0.001)	0.022 (0.005)	0.011 (<0.001)	0.015 (0.002)
A _T (μmol kg ⁻¹)	2263 (211)	2254 (178)	2585 (82)	2688 (116)
pH	8.14 (0.04)	8.15 (0.05)	7.96 (0.07)	7.97 (0.05)
pCO ₂ (ppm)	300 (29)	290 (23)	557 (23)	585 (25)
HCO ₃ ⁻ (μmol kg ⁻¹)	1795 (173)	1777 (145)	2156 (88)	2300 (101)