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Contrasting resource limitations of marine primary producers: implications for competitive interactions under enriched CO_2 and nutrient regimes

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Abstract

Primary producers rarely exist under their ideal conditions, with key processes often limited by resource availability. As human activities modify environmental conditions, and therefore resource availability, some species may be released from these limitations while others are not, potentially disrupting community structure. In order to examine the limitations experienced by algal functional groups that characterise alternate community structures (i.e. turf-forming algae and canopy-forming kelp), we exposed them to contemporary and enriched levels of CO_2 and nutrients. Turfs responded to the individual enrichment of both CO_2 and nutrients, with the greatest shift in the biomass and C:N ratios observed under their combined enrichment. In contrast, kelp responded to enriched nutrients, but not enriched CO_2. We hypothesise the differing limitations reflect the contrasting physiologies of these functional groups, specifically their methods of carbon acquisition, such as the possession and/or efficiency of a carbon concentrating mechanism (CCM). Importantly, our results reveal that these functional groups, whose interactions structure entire communities, experience distinct resource limitations, with some potentially limited by a single type of resource (i.e. kelp by nutrients), while others may be co-limited (i.e. turf by CO_2 and nutrients). Consequently, the identification of how alternate conditions modify resource availability and limitations may facilitate anticipation of the future sustainability of major ecosystem components and the communities they support.

Key-words: carbon dioxide, co-limitation, kelp, nutrients, turf-forming algae

Introduction

Resource availability has a fundamental role in regulating the productivity of individuals, species and, ultimately, communities (Harpole et al. 2011). As the availability of resources varies both spatially and temporally in most ecosystems, it is rare for organisms to exist under their ideal conditions with key processes, including biomass production, likely to be resource-limited (Andersen and Pedersen 2002). While the concept of resource limitation was initially focussed on identifying the single key limiting resource (Liebig 1842), a shift has recently occurred towards an account of co-limitation as a function of multiple resources (Allgeier et al. 2011; Harpole et al. 2011). The limiting resource(s) can be recognised through the use of manipulative (factorial) experiments in which the relevant factor(s) is added or removed and the response quantified, generally in terms of productivity and/or stoichiometry (Koerselman and Meuleman 1996). Single resource limitation is recognised in individual producers as a change in the rate of processes in response to one resource, while co-limitation is characterised by a greater response to simultaneous modification of multiple factors than enrichment by either factor individually (Davidson and Howarth 2007; Allgeier et al. 2011). As resource limitations are determined, in part, by the ability of organisms to access available resources, they are likely to vary among organisms that have contrasting physiologies. Communities are, therefore, generally comprised of functional groups experiencing diverse limitations, with the potential that some components are limited by a single resource while are others co-limited by multiple resources.

Developing an understanding of the specific limitations experienced by primary producers is of increasing importance as humans continue to alter the availability of resources required for key processes that control productivity, particularly nutrient acquisition and carbon fixation. Of concern is the potential that the influence of enriched CO_2 may be amplified where human activities also increase nutrient loads, a response characteristic of co-limitation. In the marine environment, altered land use and ensuing discharges elevate nutrient concentrations at local scales (Vitousek et al. 1997; Eriksson et al. 2002; Gorman et al. 2009), whilst carbon availability will increase as the ocean absorbs approximately 30 % of CO_2 released to the atmosphere (Gattuso and Buddemeier 2000; Caldeira and Wickett 2003; Feely et al. 2004). Responses to these increasing availabilities are anticipated to reflect the extent to which producers are carbon-limited as a consequence of the physiological mechanisms by which carbon is acquired for use in photosynthesis (Kübler et al. 1999; Hurd et al. 2009; Hepburn et al. 2011), and may be considered using various methods (as outlined in Kraufvelin et al. 2010). While the majority of marine algae have carbon concentrating mechanisms (CCMs) that facilitate active influx of CO_2 and/or HCO_3^- and elevate concentrations at the site of carbon fixation (i.e. Rubisco), a minority use dissolved CO_2 entering by diffusion (Beardall and Giordano 2002; Raven and Beardall 2003; Giordano et al. 2005). Algae with CCMs are predicted to gain little benefit from enriched CO_2 (Hurd et al. 2009), with their response to simultaneous enrichment of CO_2 and nutrients likely to reflect single-resource limitation by nutrients. In contrast, algae that rely on diffusion are anticipated to exhibit increased photosynthetic assimilation and productivity under enriched CO_2 (Kübler et al. 1999). As the relative rates of photosynthetic assimilation and nutrient uptake remain somewhat fixed in accordance with biological stoichiometry (Sterner and Elser 2002), the increased productivity facilitated by elevated photosynthesis under conditions of enriched CO_2 may be constrained by nutrient availability (Pedersen et al. 2010).

The algae that support communities of temperate coastlines, including those of South Australia, are set to be influenced by both nutrient and CO_2 enrichment (Falkenberg et al. 2010). Under conditions of low pollution, these coastlines are dominated by canopies of longlived, morphologically-complex kelp (typically *Ecklonia radiata* (C. Agardh) J. Agardh) (Fowler-Walker and Connell 2002) and seagrass (typically *Posidonia* spp.) (Eriksson et al. 2002; Airoldi and Beck 2007; Connell et al. 2008; Bryars and Rowling 2009). These canopies are a foundation for many marine systems, providing structure that stabilises physical environments, facilitates survival of associated species and provides economic benefit to human societies (Tegner and Dayton 2000; Duarte 2002; Steneck et al. 2002; Orth et al. 2006). While dense mats of low-lying (generally < 5 cm canopy height), finely branched or filamentous algal turfs (typically *Feldmannia* spp. in South Australia) (Gorgula and Connell 2004) are natural components of these communities in many systems, the distributions and abundances of these algae typically vary over seasonal timescales (Coleman 2002; Miller et al. 2009). Under conditions of enriched nutrients the physiology of turfs enables them to rapidly take up the available resources and increase their growth rates (Hein et al. 1995) while, in contrast, canopy-forming kelps and seagrasses tend to store available nutrients in their tissue and maintain relatively consistent growth rates (Lobban and Harrison 1994). Where nutrients are enriched, this difference shifts the competitive balance to favour turfs, enabling them to rapidly occupy available space and persist in fragmented kelp and seagrass canopies (Worm et al. 1999; Kraufvelin et al. 2006, 2010; Kraufvelin 2007; Airoldi et al. 2008; Gorman et al. 2009), inhibit the recruitment of kelp or seagrass (Gorman and Connell 2009; Connell and Russell 2010) and, thereby, facilitate comprehensive loss of canopies (Benedetti-Cecchi et al. 2001; Eriksson et al. 2002; Kraufvelin et al. 2006; Connell et al. 2008). Enrichment of CO_2 may exacerbate this pattern of kelp loss if, as anticipated, turf and kelp experience contrasting CO_2 limitations due to differing mechanisms of carbon acquisition; that is, it has been proposed morphologically-simple algae of low-light habitats, such as turfs, use passive diffusion, while complex canopy-forming species, like kelp, possess CCMs (Hepburn et al. 2011). If enriched nutrients and CO_2 did enable turfs, but not kelp, to overcome their resource limitations, the balance between these algae may be disrupted, promoting phase-shifts from kelp canopies to mats of turf following disturbances that fragment canopies.

In this study, we measured the change in biomass and stoichiometry of turf and kelp following six weeks of exposure to altered CO_2 and nutrients (in crossed combinations) in field-based mesocosms (described in ‘Materials and methods’ below). The aim of this study was to test for the existence of CO_2 and nutrient limitations experienced by turf and kelp. Specifically, we wanted to determine whether these ecological competitors are co-limited by both CO_2 and nutrients, or whether just one of the two resources is limiting. We hypothesised that these competing functional groups, with potentially distinct physiologies, would show contrasting responses to enriched CO_2 and nutrients. Turfs were anticipated to respond with greater increases in biomass and shifts to the C:N ratio under the simultaneous enrichment of CO_2 and nutrients than where either resource was elevated in isolation. Kelps were expected to respond to enrichment of nutrients, but not CO_2. If such contrasting responses do manifest, they will have implications for the relative competitive abilities of these functional groups under conditions of altered resource availability.

Materials and methods

Experimental design and set-up

To determine whether key algae are limited by a single resource or experience resource co-limitation, the responses of turf-forming algae (mainly *Feldmannia* spp.) and kelp (*Ecklonia radiata*) to altered CO_2 and nutrient availability were tested in a field-based mesocosm experiment. Experimental mesocosms were exposed to combinations of CO_2 (current *v.*

future) and nutrients (ambient *v*. elevated) in a crossed design from August to October, 2009. For each functional group, three replicate mesocosms were used per treatment combination, with replicate specimens of turf and kelp in each mesocosm (n = 5 and 3 respectively). The experimental mesocosms (aquaria) utilised were acrylic (A-cast, Asia Poly, Kuala Lumpur, Malaysia) and held 250 L volume (L × W × H: 0.5 × 0.5 × 1 m).

The experiment was conducted in a boat harbour adjacent to the Gulf of St. Vincent at Outer Harbor, Adelaide, South Australia (34.473395° S, 138.292184° E). The boat harbor is protected from the predominant swell by a breakwall, but which has a channel wide enough to allow high flushing rates. Mesocosms were filled with natural seawater pumped directly from the harbour, therefore, the initial seawater chemistry (i.e. before experimental manipulation) was characteristic of these waters (see Table S4 in Electronic Supplemental Material for further detail). During the experimental period, one-third of the seawater was removed from each mesocosm and replaced with fresh seawater weekly to maintain water quality. As the mesocosms had lids, loss of water via evaporation between water changes was minimal. The mesocosms were located in full sunlight and consequently experienced diurnal fluctuations in sunlight and temperature. The light intensity (Photosynthetically Active Radiation, 400 – 700 nm) experienced by the kelp and turf (at a depth of 0.1 and 0.5 m, respectively) was quantified by taking measurements using an underwater radiation sensor (Li-Cor LI-250, Nebraska, USA).

To quantify the effectiveness of Osmocote Plus® (Scotts, Australia) fertiliser at elevating nutrients (see below), a second laboratory-based experiment was conducted in identical mesocosms which did not contain any biota. In this experiment, 10 mesocosms were established in the laboratory and maintained for five weeks between March and April, 2011.

Nutrient enrichment and quantification of water column variables were achieved using the same techniques as in the field-based mesocosms (outlined below).

Experimental algae

Algae used in the experiments were defined as either turf-forming algae or kelp. 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*. Composition of the turf assemblage was monitored throughout the experimental period, and indicated it did not change over time. While the term "kelp" generally corresponds to the group of the larger brown algae of the order Laminariales, here "kelp" refers specifically to the species *Ecklonia radiata*.

Specimens of turf and kelp used in the experiments were collected from rocky reef which had areas of turf adjacent to kelp canopies at Horseshoe Reef, South Australia (35.13757° S, 138.46266° E; collection depth was 2 – 3 m). Turfs were collected from outside the kelp canopy still attached to their natural substratum (approximately the same size, 5 × 5 cm). Individual kelp of approximately the same size (length from bottom of stipe to tip of central lamina, mean ± SE; 32.81 ± 1.92 cm) were collected still attached to their natural substrate.

Following collection, the experimental algae (both turf and kelp) were placed in holding mesocosms for eight weeks before the experiment commenced to enable acclimation to being held in mesocosms. Following this acclimation period, five rocks containing specimens of turf were randomly assigned to the appropriate experimental mesocosms. Also allocated to the appropriate mesocosms were three kelp individuals. Conditions were then gradually altered over a further two week period until they reached the pre-designated experimental levels.

Experimental treatments: CO_2 and nutrient addition

Target CO_2 was based on the current ambient (current; 280 – 380 ppm) and the IS92a model scenario for atmospheric CO_2 concentrations in the year 2050 (future; 550 – 650 ppm). The pH of mesocosms exposed to the future CO_2 treatment were reduced from ambient (mean ± SE; 8.18 ± 0.06) to the experimental level (target: 7.95; measured: mean ± SE; 7.96 ± 0.01, see Table S4). Concentration of CO_2 in the seawater in mesocosms was maintained by directly diffusing CO_2 gas into the water column when required to maintain the experimental level and was controlled using temperature-compensated pH probes and automatic solenoid controllers (Sera, Heinsberg, Germany). Calibration of probes was checked on a daily basis and, if necessary, recalibrated using NBS calibration buffers to 0.01 pH units. Total Alkalinity (TA) of seawater in mesocosms was measured weekly using colorimetric titration (Hanna Instruments, Woonsocket, RI, USA). Concentrations of pCO_2, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) were then calculated from measured TA, 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 and Millero (1987).

The elevated nutrient treatment was designed to result in concentrations similar to moderate enrichments experienced in the otherwise oligotropic waters off the coast of South Australia. The target NO_X (oxidised nitrogen: nitrate + nitrite) was based on the current concentrations in natural catchments under light rainfall (0.013 ± 0.001 mg L^{-1}), while enriched was based on the concentrations adjacent to urban catchments under light rainfall (0.232 ± 0.032 mg L^{-1}) (Gorman, Russell and Connell, unpubl. data). Nutrients were enhanced using Osmocote Plus®

(Scotts, Australia) controlled release fertiliser which releases a combination of nutrients at a set rate over the life of the pellet (6 month release; with a ratio of 15 % N, 5 % P and 10 % K), with the nutrient concentration released proportional to weight of the fertiliser (Worm et al. 2000). Osmocote has successfully been used in previous studies of various systems to manipulate nutrient concentrations (Worm et al. 2000; Nielsen 2001; Pfister and Van Alstyne 2003; Gorgula and Connell 2004; Russell et al. 2009). Osmocote pellets were placed in a nylon mesh bag (1 mm mesh size) and attached to the bottom of each appropriate mesocosm (10 g per mesocosm). The concentration of the supplied nutrients was quantified by regularly collecting water samples using 25 mL sterile syringes, which were filtered (0.45 μm glass fibre) and immediately frozen. Samples were later analysed on a Lachat Quickchem 8500 Flow Injection Analyser (Hach, CO, USA) for ammonia, phosphate and nitrite + nitrate (NO_X).

Experimental responses

At the end of the study, change in biomass (final – initial measurement; October – August measurements) of the algae was quantified by gently patting the samples (i.e. specimens of turf and individual kelp) dry and weighing them using a balance with a measurement resolution of 0.01 g. This response was then standardised per size of the specimen (area of sample in cm^2 and initial length in cm for the turf and kelp, respectively) and converted to a daily growth rate.

To quantify the response in terms of chemical composition at the end of the study, tissue samples were collected from the specimens following the experimental period. Turf was collected by carefully scraping algae from each specimen using a razor blade, while kelp was sampled by removing an area from the youngest lateral of each individual with a razor blade.

Following collection, the samples were preserved for analysis by being stored frozen at -20 °C for four months. They were then rinsed in Milli-Q water to remove contaminants and salts and also hasten the defrosting process. Samples were then placed in an oven at 60 °C where they were dried for two days (48 hours) 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 mm × 8 mm) (SerCon, Cheshire, UK) and then placed into a carousel which, in turn, fed them into an Isotope-Ratio Mass Spectrometer where they were combusted, and the gasses passed through scrubbers prior to entering a Gas Chromatograph where the components of interest were separated (IRMS Hydra 2020 ANCA-GSL Version 4.0, SerCon, Cheshire, UK). The masses of the C and N identified in each sample were used to calculate a C:N ratio. Reported isotope values ($\delta^{13}C$) were calculated for each individual sample as the relative per mille (‰) difference between the sample and recognised international standard (Pee Bee Belemnite limestone carbonate for carbon).

Statistical analyses

Two-way Analysis of Variances (ANOVAs) were used to test the response of algal turfs and kelps to experimental conditions for change in biomass, C:N ratio, % C and % N. The factors of CO_2 and nutrients were both treated as fixed and orthogonal, with two levels in each factor (CO_2: current *v*. future; Nutrients: ambient *v*. elevated). Individual mesocosms were treated as replicates ($n = 3$), with data for individuals within each mesocosm (i.e. multiple specimens of turf or kelp individuals) averaged. Two-way ANOVAs (as described above) were also used to test the water column physicochemical variables of field mesocosms with measurements averaged across days (pH, TA, temperature, pCO_2, HCO_3^-, CO_3^{2-}, $n = 5$ occasions; ammonia, phosphate and NO_X, $n = 4$ occasions; light, $n = 1$ occasion). One-way ANOVAs, using the factor of nutrients as fixed with two levels (ambient *v*. elevated) were used to test for differences in nutrient concentrations in laboratory mesocosms (*n* = 5) with measurements averaged across days (ammonia, phosphate and NO_X in the laboratory, *n* = 20 occasions). Where significant treatment effects were detected, Student–Newman–Keuls (SNK) *post hoc* comparison of means was used to determine which factors differed.

Results

Algal biomass

The biomass of turf was positively influenced by both future CO_2 and elevated nutrients. While kelp biomass was affected by elevated nutrients, which increased biomass, CO_2 did not have a significant influence (Fig. 1; Electronic Supplemental Material Table S1).

Algal C:N ratios, % C and % N

The C:N ratio of turf decreased significantly under both future CO_2 and elevated nutrients (Fig. 2a; Electronic Supplemental Material Table S2a), whereas that of kelp responded only to elevated nutrients (Fig. 2b; Electronic Supplemental Material Table S2b). Underlying these shifts to the C:N ratio were changes to the % C and % N of the algae. The % C of turf algae was increased under elevated nutrients (Fig. 3a; Electronic Supplemental Material Table S3ai), while turf % N was increased by both future CO_2 and elevated nutrients (Fig. 3a; Electronic Supplemental Material Table S3aii). In kelp tissue, the % C was not influenced by enrichment of either CO_2 or nutrients (Fig. 3b; Electronic Supplemental Material Table S3bi), with % N only increased under elevated nutrients (Fig. 3b; Electronic Supplemental Material Table S3bii).

The mean $\delta^{13}C$ of kelp (mean ± SE; -19.8 ± 0.7) was greater than that measured for turf (- 16.5 ± 0.4). There was no significant difference in the $\delta^{13}C$ of either turf or kelp between levels of CO_2 (turf: $F_{1,8} = 0.44$, $P > 0.5$; kelp: $F_{1,8} = 5.31$, $P > 0.05$), nutrients (turf: $F_{1,8} = 3.97$, $P > 0.08$; kelp: $F_{1,8} = 2.32$, $P > 0.15$) or their interaction (i.e. CO_2 × nutrients; turf: $F_{1,8} = 0.21$, $P > 0.6$; kelp: $F_{1,8} = 1.19$, $P > 0.3$).

Water column physicochemical conditions

The concentration of ammonia and phosphate quantified in the field mesocosms was significantly higher in elevated (mean ± SE; ammonia 0.0345 ± 0.0043 mg L^{-1}, phosphate 0.0095 ± 0.0005 mg L^{-1}) than ambient nutrient treatments (ammonia 0.0226 ± 0.0022 mg L^{-1}, phosphate 0.0081 ± 0.0002 mg L^{-1}) (Electronic Supplemental Material Table S4, S5). NO_X (nitrate + nitrite) in elevated nutrient mesocosms was not significantly different compared to ambient nutrient mesocosms (elevated 0.0056 ± 0.0001 mg L^{-1}, ambient 0.0056 ± 0.0004 mg L^{-1}). While the low concentrations in the ambient treatments reflect water quality in the surrounding harbour from which the experimental mesocosms were filled, the low concentrations in the elevated treatments indicate that the available nutrients were being utilised by the algae. This interpretation is supported by the results of the additional laboratory-based mesocosm experiment. While the different source of water used to fill mesocosms prevents direct comparisons with the field study (the ambient concentration is higher in the laboratory than in the field study), the concentrations of all nutrients in laboratory-based mesocosm trials that excluded algae were significantly greater in the elevated (ammonia 0.2652 ± 0.0320 mg L^{-1}, phosphate 0.1285 ± 0.0068 mg L^{-1}, NO_X 0.3796 ± 0.0255 mg L^{-1}) than ambient nutrient treatments (ammonia 0.0346 ± 0.0053 mg L^{-1}, phosphate 0.0272 ± 0.0033 mg L^{-1}, NO_X 0.1222 ± 0.0050 mg L^{-1}) (Electronic Supplemental Material Table S4, S5).

The pH and concentration of carbonate (CO_3^{2-}) were significantly reduced under future CO_2 compared with current CO_2 treatments (Electronic Supplemental Material Table S4, S5). In contrast, the pCO_2 and bicarbonate (HCO_3^-) were significantly increased under future CO_2 conditions (Electronic Supplemental Material Table S4, S5). Temperature was not significantly different under any treatments (Electronic Supplemental Material Table S4, S5). The minimum and maximum temperatures recorded (13.0 °C and 15.9 °C, respectively) highlight the relative stability of this condition during the experimental period. Light was not significantly different under any CO_2 or nutrient enrichment treatment, meaning the algae was under the same light conditions across the different treatments, with kelp exposed to a greater light availability than turf (Electronic Supplemental Material Table S4, S5).

Discussion

Primary producers are limited, to varying extents, by the availability of resources. Historical focus placed emphasis on identifying the single key resource that limits productivity of producers (Liebig 1842), whereas contemporary research has an increasing emphasis on co-limitation by multiple resources (Harpole et al. 2011). Our ecological result, of the contrasting response by distinct functional groups, suggests communities may be comprised of functional groups exhibiting both types of limitations. The kelp response to enrichment of nutrients, but lack of response to CO_2 enrichment, was typical of single-resource limitation as defined by Liebig's Law of the Minimum (Liebig 1842), where production increased when nutrients were enriched, but was not affected by enriched CO_2. This biomass response may have resulted under enriched nutrient conditions as availability of the limiting resource in this marine system, likely nutrients, was increased (Elser et al. 2007; Pedersen et al. 2010). In contrast, the response of turf was characteristic of co-limitation, in that there was a greater biomass response to enrichment of multiple resources, than was identified in response to enrichment of either individually (Allgeier et al. 2011). These limitations, which are specific to each functional group, have implications for the competitive balance of major ecosystem components under conditions of increased resource availability.

The species-specific responses of marine algae to enrichment of particular resources will manifest not simply via changes in productivity, but also through shifts in the nature of resource limitations (Elser et al. 2007). Such alterations may be inferred from the C:N ratios of primary producers which provide an index of the relative amounts of C and N available to algae (Koerselman and Meuleman 1996; Pedersen and Borum 1996, 1997; Craine et al. 2008). The high C:N ratio of kelp under ambient conditions indicates that it is strongly limited by N, while the lower ratio under nutrient enrichment indicates lower N-limitation under these conditions. As increased CO_2 did not produce a shift in the C:N ratio of kelp, this resource may have little influence on the limitation(s) experienced. In comparison to kelp, the C:N ratio of turf was lower under all conditions, indicating that these algae may be less N-limited and more strongly C-limited. Despite this lower turf C:N ratio (*c.f.* kelp), enrichment of CO_2 and nutrients in isolation caused a further reduction, with the greatest reduction occurring when the resources were enriched in combination. Such a response is indicative of strengthened C-limitation under conditions of increased nutrient availability. Importantly, although the low C:N ratio of turf under ambient CO_2 and nutrient conditions is indicative of C-limitation, the fact that both CO_2 and nutrient enrichment influenced these algae suggests it actually falls in the range characteristic of resource co-limitation (*sensu* Koerselman and Meuleman 1996; Craine et al. 2008).

While the combination of biomass and C:N ratio responses reveal the resource-limitation(s) experienced, examination of the absolute content (i.e. % C and % N) provides insight to the availability of resources in the surrounding environment, physiological processes by which resources are acquired and mechanisms by which limitations manifest. For kelp, which the biomass and C:N ratio responses indicate experience single-resource limitation, the only significant effect of resource enrichment on absolute content was increased % N under enriched nutrients, suggesting nutrient enrichment enables these algae to access, and store, more N. Similarly, the non-significant trend for increased % C ($F_{1,8} = 3.76$, $p = 0.0883$), under enriched CO_2 is indicative of the increased environmental availability of C relative to N. This result provides further evidence that kelps do not possess mechanisms by which nutrient availability co-limits the uptake of CO_2 from the environment. Turfs, which appear to be co-limited by CO_2 and nutrients, had a multifaceted pattern of alterations in their absolute resource content. Enriched nutrients resulted in not only a higher % N but also % C in the tissue of algal turfs. Furthermore, CO_2 enrichment had a positive effect on the % N of turfs. Nutrient enrichment may have positively affected the % C of turfs as the increased availability of this resource facilitated synthesis of the pigments required for carbon acquisition/metabolism (Falkowski and Raven 2007). Enriched CO_2 may have resulted in greater % N as, under these conditions, photosynthesis may be more efficient, enabling algae to re-allocate C from photosynthesis to other processes such that tissue % N is increased (Hamilton et al. 2001), as was quantified in these turfs.

The limitation of turf, but not kelp, by CO_2 likely reflects the physiology underlying the acquisition of this resource by the different functional groups. Two key strategies of carbon uptake are utilised by species of marine algae; passive diffusion and active uptake via a carbon concentrating mechanism (CCM). The significant response of turfs to enhanced CO_2

suggests they rely on passive diffusion whereas the absence of a response by kelp indicates they utilise CCMs and so are not reliant on dissolved CO_2 as a source of photosynthetic carbon. This conclusion aligns well with the expectation that morphologically-simple algae would lack CCMs which are proposed to be more common in complex, canopy-forming species (i.e. following Hepburn et al. 2011). Our quantification of the natural abundance of carbon stable isotope values ($\delta^{13}C$) did not, however, support the conclusion that turfs exclusively utilise passive diffusion. The $\delta^{13}C$ indicated that both turf and kelp possess CCMs, as the values were higher than -30‰ which would indicate passive CO_2 diffusion (mean ± SE, -16.5 ± 0.4 for turf, -19.8 ± 0.7 for kelp) (Maberly et al. 1992; Raven et al. 2002; Hepburn et al. 2011). If both types of algae possess CCMs, then the difference in CO_2 limitation may have resulted in one of two ways; 1) the CCMs of turf could be less efficient than those of kelp, meaning that increased CO_2 supplements CCM carbon acquisition, or, 2) kelp are obligate CCM users, whereas turfs can reduce or stop CCM use when increased CO_2 makes it more efficient to use passive diffusion. Such differences may be due to species-specific variation in the CCMs themselves, with diverse efficiencies identified in the CCMs of other producers (Rost et al. 2003; Beardall and Raven 2004). Alternatively, the differing efficiencies may be due to the varied ability of algal species to meet energetic requirements, particularly light, of CCMs, with those algae that can acquire more light able to operate their CCMs at a higher rate (Hepburn et al. 2011). As kelp is a large, canopy-forming algae it may be able to acquire more light energy than the understorey turf (Connell 2003; Russell 2007; Russell et al. 2011). Kelp may, therefore, be able to utilise the greater level of light available to them such that their CCMs operate at a higher rate in both the presence and absence of understorey turf algae. In contrast, if the low-lying turfs do possess CCMs, their activity may be down-regulated due to the low light availability, especially where kelps are present, making these algae more likely to show a stimulation response under CO_2 enrichment

(Beardall and Giordano 2002; Hepburn et al. 2011; Raven et al. 2011). As a consequence of the limited capacity of turfs to acquire light and gain carbon through CCMs, this alga may have a substantial reliance on passive diffusion (Hepburn et al. 2011; Raven et al. 2011). Our results indicate, therefore, that even if turfs do possess CCMs which can facilitate active carbon uptake, it is likely this alga will be more light-limited than kelp, with this potentially being the feature that results in turf showing a greater response to enriched CO_2 than kelp.

Our results indicate that both kelp and turf will increase production under enriched nutrients, with turf further benefitting from CO_2 enrichment. Management to prevent phase-shifts from kelp canopies to mats of turfs would, therefore, ideally restrict enrichment of both CO_2 and nutrients. In practical terms, however, the management strategies developed and implemented will be constrained by issues of cost, time and societal will (Allgeier et al. 2011). Such restrictions are particularly problematic when attempting to manage alterations that occur due to human activities at the global scale. Given that both resources were limiting turf algae, it is possible that controlling the availability of nutrients may be an effective way to prevent the expansion of turfs and consequent phase-shifts under future climate. The potential exists, therefore, for effective local management of nutrients (i.e. water quality guidelines for polluters) to reduce the impact of CO_2 emissions at the global-scale. Where such a management approach is utilised, effective restriction of the local-scale resource represents a powerful tool for managers given the strong ecological responses to nutrients by both kelp and their turf competitors. Indeed, such recognition of the resource limitations experienced by foundation species will be critical not only in managing against, but also forecasting, the phase-shifts anticipated to be favoured under modified conditions.

In conclusion, early research addressing the ecological consequences of resource enrichment primarily focussed on quantifying single resource limitations (Liebig 1842), and has since shifted to identifying the occurrence of co-limitation by multiple resources (Harpole et al. 2011). Our results suggest that communities are comprised of functional groups experiencing diverse limitations, with some components potentially limited by a single resource, while others may be co-limited by multiple resources. In this system, the habitat-forming kelp appear to experience single resource limitation (i.e. nutrients), whereas their turf-forming competitors, which displace kelp canopies under conditions of nutrient pollution, are co-limited by multiple resources (i.e. nutrients and CO_2). Consequently, the human activities that alter resource availability and ensuing limitations may have important implications for the relative competitive abilities of major ecosystem components and the structure of communities they support.

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Figure legends

Fig. 1 The daily change in biomass (g) of (a) turf (per cm^2) and (b) kelp (per lamina length in cm) that were exposed to different combinations of nutrients (ambient or elevated) and CO_2 (current or future levels), mean ± SE. Note the different scales of the y-axes

Fig. 2 The C:N ratio (mean ± SE) of (a) turf and (b) kelp that were exposed to different combinations of nutrients (ambient *v*. elevated) and CO_2 (current *v*. future). Note the different scales of the y-axes

Fig. 3 Nitrogen and carbon composition (mean ± SE) of (a) turf and (b) kelp that were exposed to different combinations of nutrients (ambient *v*. elevated) and CO_2 (current *v*. future). Note the different scales of the y-axes. ● Ambient nutrients, current CO_2; ○ Ambient nutrients, future CO_2; ■ Elevated nutrients, current CO_2; □ Elevated nutrients, future CO_2

Figure 1.

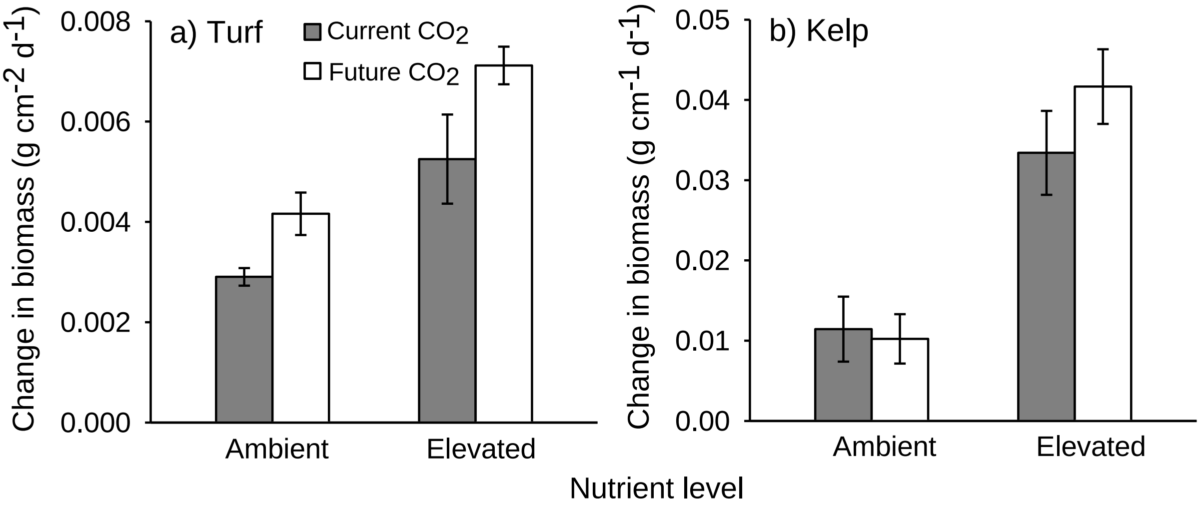

a) Turf
Current CO_2
Future CO_2
Change in biomass (g cm^{-2} d^{-1})
0.008
0.006
0.004
0.002
0.000
Ambient
Elevated
b) Kelp
Change in biomass (g cm^{-1} d^{-1})
0.05
0.04
0.03
0.02
0.01
0.00
Ambient
Elevated
Nutrient level

Figure 2.

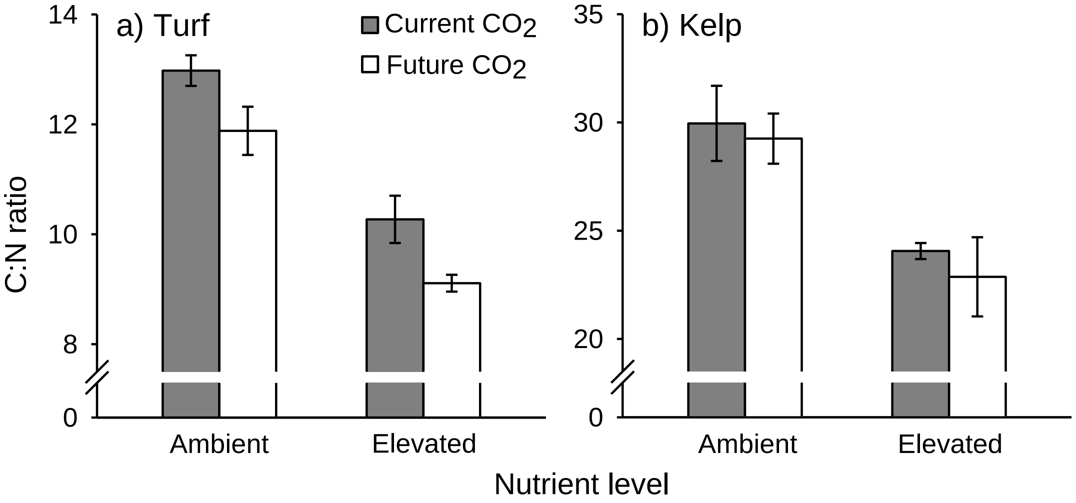
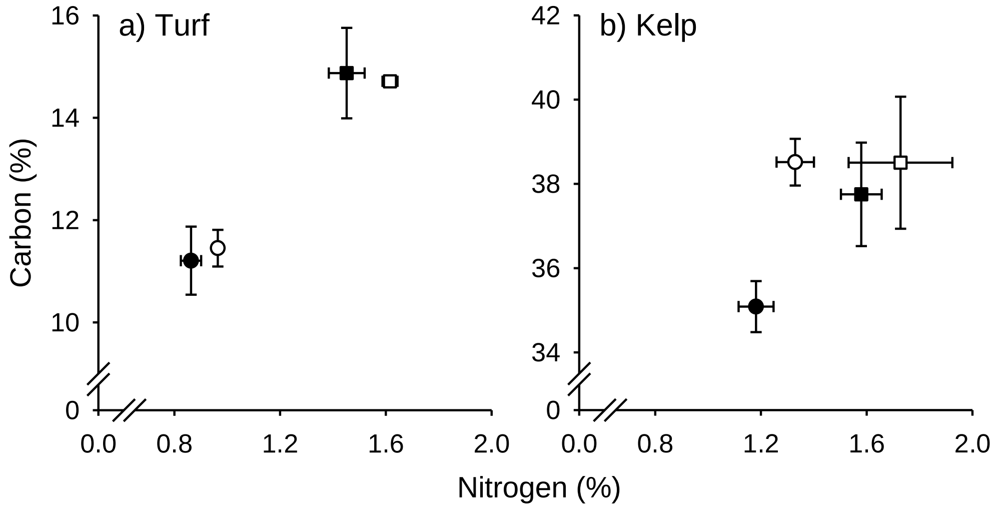

a) Turf
b) Kelp
Current CO2
Future CO2
C:N ratio
14
12
10
8
0
35
30
25
20
0
Ambient
Elevated
Ambient
Elevated
Nutrient level

Figure 3.


a) Turf
b) Kelp
Carbon (%)
Nitrogen (%)
16
14
12
10
0
42
40
38
36
34
0
0.0
0.8
1.2
1.6
2.0
0.0
0.8
1.2
1.6
2.0