MEDIATION OF GLOBAL CHANGE BY LOCAL BIOTIC AND ABIOTIC INTERACTIONS

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DECLARATION

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Laura J. Falkenberg
September, 2012

Cover image: An area of turf-forming algae (dominated by *Feldmannia* spp.) that has colonised much of the free space created by a disturbance within an area of kelp canopy (*Ecklonia radiata*) on the metropolitan Adelaide coastline.
Photo: Sean Connell.

CONTENTS

ABSTRACT

Variation in environmental conditions is a pervasive feature of natural systems that has profound consequences for the structure of ecological communities. As a result of altered local conditions produced by human urbanisation, shifts in marine habitats from kelp forests to mats of turfing algae are increasingly common. Forecasting whether such ecological change will be accelerated or reversed as a function of modified global conditions is a new form of ecological enquiry. Throughout this thesis, I assessed the conceptual model that while cross-scale abiotic stressors can combine to have interactive effects, management of local conditions can counter-balance this change. My experimental manipulations were intended to test the hypotheses that; 1) cross-scale factors (i.e. local and global) will have interactive effects that increase the probability of expansion of turfs but not kelp, and, 2) management of local conditions (e.g. presence of biota, nutrient enrichment) will dampen the effects of global change on turfs (e.g. forecasted CO_2).

Change in ecological communities is anticipated where altered environmental conditions have contrasting effects on interacting taxa that determine their composition and relative abundances. Experimental enrichment of CO_2 and nutrients influenced biomass accumulation of turf and kelp differently, with turf responding positively to enrichment of both resources while kelp responded to enrichment of nutrients but not CO_2. These responses likely reflect resource limitations experienced by the algae, as stoichiometry indicated turf was co-limited by CO_2 and nutrients while kelp appeared to be limited by nutrients but not CO_2. Simultaneous enrichment of these factors would, consequently, be anticipated to facilitate the expansion of turf algae at the expense of established kelp canopies.

Considerable attention has focused on the influence of altered conditions on single taxa in isolation, yet such approaches only elucidate direct response(s). In natural systems, these responses may be mediated by indirect effects resulting from interactions with other taxa. I assessed the model that biotic interactions

(i.e. competition and herbivory) can counter the abiotic drivers of change. Experimental tests revealed the presence of kelp inhibits the synergistic positive effects of stressors (i.e. CO_2 and nutrient enrichment) on their turf competitors, likely due to the modification of physical conditions (i.e. light availability). Similarly, rates of herbivory increased to counter the positive effects of stressors on turfs under enriched CO_2 (i.e. increased grazing of turfs by gastropods). This increase in herbivory was attributable to the changes in stoichiometry of algal turfs under the greater availability of this resource. Together, these results indicate potential for indirect effects, mediated by species interactions, to counter the direct influence of altered environmental conditions.

Where biotic controls are absent, however, such modification of resource availabilities may increase the probability of the expansion of novel habitats. I considered the hypothesis that where human activities combine to synergistically benefit turfs (as occurs where CO_2 and nutrients are enriched), removal of one factor alone may enable further change to be slowed or recovery hastened. Experimental tests that reduced the locally-determined factor (i.e. reducing nutrients under continued CO_2 enrichment) substantially slowed further expansion of turf algae, but the legacy of nutrient enrichment was not entirely eradicated. This result indicates that although management of local environmental conditions may substantially reduce the effects of entrained global change, some effects could be enduring.

In summary, there was broad support for the conceptual model that cross-scale abiotic stressors can combine to interactively affect algal communities, but that such change can be countered by management of local conditions, both biotic (i.e. retaining the processes of competition and herbivory) and abiotic (i.e. removing pollutants). These results represent progress in ecological tests of hypotheses regarding global climate change as they incorporate comprehensive sets of abiotic and biotic community drivers. Further, this thesis contributes new knowledge regarding the anticipated responses of marine communities to local through global scale pollution, and the potential for local conditions to mitigate the effects of global change.

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CHAPTER 2

We thank the many volunteers, including members of the Southern Seas Ecology Laboratories, for assistance with the assembly of the experimental mesocosms, collection of algae and maintenance of experimental conditions. Financial support for this research was provided by an ARC grant to S.D.C. and B.D.R. and an APA grant to L.J.F.

CHAPTER 3

We thank J. T. Taylor for assistance in maintaining experimental conditions and volunteers who assisted with the assembly of experimental mesocosms. We acknowledge the thoughtful insights of the anonymous reviewers and Simon Thrush who added interpretive value to our findings.

CHAPTER 4

We thank volunteers, including members of the Southern Seas Ecology Laboratories, who assisted with the collection of algae and grazers as well as those involved with the assembly and maintenance of experimental mesocosms. Financial support for this research was provided by an ARC grant to Sean Connell and Bayden Russell and an APA to Laura Falkenberg.

CHAPTER 5

We thank volunteers, including members of the Southern Seas Ecology Laboratories, who assisted with the collection of algae and assembly and maintenance of experimental mesocosms. Financial support for this research was provided by an ARC grant to S.D.C. and B.D.R and an APA to L.J.F.

APPENDIX A

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CHAPTER 1

CHAPTER 1

GENERAL INTRODUCTION

Variation in environmental conditions has a central role in determining the dominant community state of numerous ecosystems (Scheffer et al. 2001; Folke et al. 2004). Consequently, anticipating the potential influence of environmental conditions on ecosystem structure has long been a fundamental human concern (Connell et al. 2011). Our need to forecast the ecological consequences of future environmental variation has recently become even more significant as human activities are causing change in numerous conditions to occur at a higher rate, to a greater magnitude and with more directionality than previously occurred due to natural forcing (Solomon et al. 2007; Lindegren et al. 2010). Such alteration of numerous abiotic conditions is anticipated to result in interactive effects whereby the influence of one environmental condition is modified by another to produce 'surprising' outcomes (Wootton 1994; Connell et al. 2011). Developing an improved understanding of the potential consequences of interactive effects will, therefore, be an essential requirement in accurately accounting for observed ecosystem configurations, forecasting ecosystem responses to future change and developing management strategies to limit the effects of human activities.

1.1 CONTEMPORARY ECOLOGICAL CHANGE

The influence of interactive effects is, perhaps, most prominent in structuring ecosystems that undergo phase-shifts. In such systems, the historically-dominant community configurations are typically characterised by an ability to absorb change in factors that can otherwise drive transformations in their structure and function, a trait commonly referred to as 'resistance' (sensu Holling 1973) (Scheffer et al. 2001; Suding et al. 2004; Hughes et al. 2005). Ecosystems do not, however, possess unlimited resistance to change, particularly when they experience the simultaneous modification of multiple structuring factors and the resulting interactive effects. At some point a threshold may be passed and the system will transition to a contrasting state, undergoing a 'phase-shift' (Scheffer et al. 2001; Scheffer & Carpenter 2003; Folke et al. 2004). The alternative, or novel, configuration typically has its own resistance to further changes in structuring forces and their interactive effects, including restoration implemented in an attempt to drive the ecosystem back to its historical state (Suding et al. 2004; Perkol-Finkel & Airoldi 2010; Lotze et al. 2011). This feature leads to the concept of 'resilience', which is concerned with the ease, or difficulty, with which such shifts can be reversed (West & Salm 2003; Suding et al. 2004).

Phase-shifts have been observed in a range of ecological systems (e.g. the shift in arid and semi-arid terrestrial regions from perennial vegetation to bare soil with ephemeral plants, Schlesinger et al. 1990; lakes from clear water with submerged vegetation to turbid phytoplankton-dominated waters, Scheffer et al. 1993; tropical marine reefs from corals to macroalgae, McCook et al. 2007). Although specific details of phase-shifts in each system differ, the contrast between states is usually due to a change in dominance of organisms with different life forms (Scheffer et al. 2001). The newly-established configuration is typically comprised of species perceived to be of less 'value' to human societies than those of the system they replaced, making prevention, or reversal, of such change advantageous (Hobbs et al. 2006; Dudgeon et al. 2010). This pattern of change is exemplified by the degradation of historical kelp habitats and expansion of turf algae observed in many coastal systems (Eriksson et al. 2002; Airoldi & Beck 2007; Connell et al. 2008; Gorman et al. 2009). Where water quality on temperate coastlines remains at its historic baseline canopies of kelp are typically the dominant habitat (Connell et al. 2008). On coastlines adjacent to growing populations, however, resistance has been reduced to an extent that, following natural disturbance events, such as storms, otherwise ephemeral turfs can persist (Reed & Foster 1984; Russell & Connell 2005; Airoldi et al. 2008) to cause intergenerational decline and collapse of the kelp habitat (Connell et al. 2008). Consequently, as humans alter environmental conditions phase-shifts appear to be becoming more frequent and longer-lived (Connell et al. 2008).

Phase-shifts are increasingly observed as a consequence of growing human populations and their impacts, yet uncertainty surrounds the precise mechanisms driving these shifts. It is generally recognised, however, that the structure of ecological communities is determined, at least in part, by abiotic environmental conditions (Scheffer et al. 2001; Sterner & Elser 2002). Where abiotic conditions are altered such that the availability of certain resources are increased some taxa may be released from their limitations while others are not, potentially reducing resistance to further change such that the established balance is disrupted and phase-shifts are favoured (Sterner & Elser 2002). In temperate marine communities, for example, nutrient pollution has increased the availability of this resource, enabling mats of turf algae to expand into available space created during disturbance events and persist past their natural seasonal limits, preventing re-establishment of the historical kelp canopies (Eriksson et al. 2002; Airoldi & Beck 2007; Connell et al. 2008; Gorman et al. 2009). While kelps are likely also nutrient-limited, particularly along oligotrophic coastlines such as those of South Australia (Gorgula & Connell 2004; Russell & Connell 2005), their physiology prevents such rapid utilisation of available resources, placing them at a competitive disadvantage under nutrient enriched conditions (Lobban & Harrison 1994). Recognising the distinct resource limitations experienced, and overcome, by these key habitat-forming taxa is, therefore, important in accounting for observed phase-shifts.

1.2 ANTICIPATED GLOBAL CHANGE AND ITS DIRECT EFFECTS

Variation in global environmental conditions is a natural feature of the Earth and was, over the past 65 million years, largely driven by oscillations in orbital geometry and plate tectonics (Zachos et al. 2001). Recently, however, human activities have emerged as an additional factor forcing variation (Meehl et al. 2007). Unlike that which went before it, the change the Earth is currently undergoing is unique and unprecedented in geological history due to its rate, magnitude and directionality. Specifically, for nearly 400 000 years prior to the Industrial Revolution, atmospheric CO_2 levels varied between 200 – 280 ppm (Feely et al. 2004). In contrast, over the two centuries following the Industrial Revolution, atmospheric CO_2 has increased from ca. 280 to 390 ppm, with levels at the end of century forecasted to fall within the range of 800 – 1000 ppm (Meehl et al. 2007). Much of the CO_2 humans have emitted into the atmosphere has not remained there, however, with 30 – 50 % dissolved into the oceans (Sabine et al.

2004; Doney 2006; Sabine & Feely 2007). When CO_2 dissolves into the oceans, it reduces pH and alters the carbonate chemistry of surface waters (Caldeira & Wickett 2003; Feely et al. 2004). This process, termed ‘ocean acidification’, is associated with an observed contemporary average pH decrease of 0.1 pH units in the surface ocean relative to pre-industrial levels, with a decrease of up to 0.5 pH units probable by the year 2100 (Meehl et al. 2007).

Rapid CO_2 enrichment is anticipated to significantly affect oceanic biota worldwide, with initial concern primarily focused on the potential effects for marine calcifiers due to the associated ocean acidification (see, for example, Orr et al. 2005). While focus has been placed on calcifying organisms, non-calcifiers, including primary producers, may also be influenced (reviewed by Fabry et al. 2008; Connell & Russell 2010; Hepburn et al. 2011; Harley et al. 2012). The response of primary producers to enriched CO_2 will likely vary among taxa due to their contrasting physiologies and inferred carbon limitations (Kübler et al. 1999; Hurd et al. 2009; Hepburn et al. 2011). That is, the majority of marine algae are expected to gain no benefit under enriched CO_2 conditions as they use carbon concentrating mechanisms (CCMs) to facilitate active influx of CO_2, elevating concentrations at the site of carbon fixation (Beardall & Giordano 2002; Raven & Beardall 2003; Giordano et al. 2005; Hurd et al. 2009). A minority of taxa, however, may exhibit increased photosynthetic assimilation and productivity under enrichment as they use CO_2 entering by diffusion (Kübler et al. 1999; Beardall & Giordano 2002; Raven & Beardall 2003; Giordano et al. 2005). In this system, it has been suggested that the fast-growing, understorey turf acquire dissolved CO_2 via diffusion, while the physiologically-complex kelp use CCMs (following Hepburn et al. 2011). Under enriched CO_2, these contrasting physiologies may result in the increased productivity of turf, but not kelp, such that phase-shifts from longer-lived canopy-forming kelp to mats of turf algae will be favoured.

1.3 GLOBAL CHANGE IN A LOCAL CONTEXT

While the potential influence of enrichment of a single factor, determined at either a local or global scale (i.e. nutrients or CO_2, respectively), can be forecasted based on current understanding, substantial uncertainty surrounds predictions of their potential combined influence(s). Currently, conditions modified by relatively local and short-term processes appear to be influencing the resistance of systems such that they are either susceptible to, or protected against, phase-shifts (e.g. storm events and nutrient enrichment, Eriksson et al. 2002; Airoldi & Beck 2007; Connell & Irving 2008; Gorman & Connell 2009). In contrast, it is anticipated that future community composition will be determined, at least in part, by the effects of global change occurring over broad spatial areas and long temporal periods (Harley et al. 2006; Solomon et al. 2007). Potential exists that the effects of such global-scale change will be modified by interactions with small-scale, local heterogeneity, both natural and human-driven (Appendix A; Helmuth et al. 2002; Russell et al. 2009). The resulting interactive effects of structuring forces that manifest over contrasting spatial and temporal scales could, therefore, determine future patterns of ecological change.

1.3.1 POTENTIAL INTERACTIONS BETWEEN ENRICHED RESOURCES

The influence of locally-enriched nutrient pollution is predicted to be amplified as global-scale ocean acidification manifests. Specifically, it is expected that these conditions will combine to benefit algal turfs and result in their synergistic expansion (Russell et al. 2009), strengthening the replacement of kelp canopies and maintaining phase-shifts. Such shifts will be particularly strongly favoured if, as forecasted, enrichment of CO_2 and nutrients does not benefit kelp to a similar extent. Acquiring quantitative data that supports or disproves this model of algal responses to increased resource availability is required in order to confidently forecast the effects of these pollutants on key taxa and communities.

1.3.2 THE MEDIATING ROLE OF BIOTIC INTERACTIONS

The influence of altered environmental conditions in driving phase-shifts will, however, be modified by the presence or absence of key taxa. Key biota involved in strong competitive interactions, such as foundation species or ecological engineers, may be particularly influential as their presence can enhance the stability of the configuration in which they occur (Paine 1980; Power et al. 1996; Stachowicz 2001). In temperate marine systems, for example, the presence of kelp can modify conditions such that the recruitment of its key competitor (i.e. turf algae) is inhibited, enabling the maintenance of the historically-dominant ecosystem composition (Gorman et al. 2009). Given the strength of such biotic interactions, they may also constrain the effect of forecasted global climates (i.e. future CO_2) in polluted systems. Where the interacting taxa have contrasting responses to altered environmental conditions, however, the balance may be disrupted, and phase-shifts facilitated. That is, the competitive dominance of this foundation species may not be maintained if turfs have a synergistic response to the enrichment of nutrients and CO_2 while kelp do not. Potential remains, however, that the competitive effect of foundation species, such as kelp, may be stronger than the synergistic effects of moderate increases in these environmental conditions. If foundation species restrict the effect of altered conditions, the maintenance or restoration of these taxa and their strong interactions may increase resistance to the interactive effects of abitoic environmental change occurring due to human activities at global and local scales.

Although competitive interactions among taxa of primary producers may not be notably modified under altered environmental conditions, it is forecasted that trophic interactions will be more strongly influenced. For example, the predation of mussels by sea stars has been found to occur more rapidly in warmer waters (Sanford 1999; Kordas et al. 2011). The process of consumption is, however, anticipated to be most strongly affected by altered conditions where the interaction involves taxa with highly contrasting physiologies and potentially divergent responses. A pertinent example of such an interaction is that between calcifying gastropod grazers and the primary producers they consume. Under conditions of local nutrient pollution this interaction is commonly strengthened such that rates of herbivory are increased (Silliman & Zieman 2001; Russell & Connell 2007). The increase in herbivory is believed to manifest as, although nutrient enrichment does not directly affect the grazers themselves, it does modify the quality of the algae they consume. That is, under nutrient enriched conditions algae generally have a greater % N and although grazers would need to consume a lower biomass of nutrient-rich tissue to achieve satiation (Stiling & Cornelissen 2007), they often feed more intensely on such high-quality algae (Hillebrand et al. 2000; Silliman & Zieman 2001; Russell & Connell 2007). Consequently, local enrichment of nutrients can influence the algae consumed such that the strength of herbivory is enhanced, and the expansion of turfs otherwise favoured by these conditions is counter-balanced, preventing the occurrence of phase-shifts.

While locally-modified nutrient conditions may enhance the strength of herbivory, the future influence of forecasted globally-modified CO_2 on this process remains ambiguous given the potential for this condition to influence both the grazer and the algae it consumes. It is anticipated that where CO_2 is enriched grazers may be strongly negatively affected. Specifically, CO_2-mediated ocean acidification is expected to decrease the concentration (and therefore availability) of carbonate ions, making it difficult for many calcifying marine organisms to produce their calcium carbonate structures via biomineralisation (reviewed in Doney et al. 2009), negatively affecting their physiology and activity (Pörtner et al. 2004; Dupont et al. 2008; Havenhand et al. 2008; Kurihara et al. 2008; Parker et al. 2009). Importantly, under ocean acidification scenarios, the feeding biology of herbivores may be disrupted (Cecchini et al. 2001; Foss et al. 2003; Siikavuopio et al. 2007), directly reducing their removal of the turf algae. Such a response would enable greater turf covers and phase-shifts to this habitat. Enriched CO_2 may, however, also influence turf algae, potentially facilitating a greater growth rate (as found in Russell et al. 2009) and also modifying chemical composition, with the greater available carbon anticipated to dilute the % N. The influence this shift in nutrient status will have on the process of herbivory is unclear, given the potential for contrasting responses of grazers to altered algal stoichiometry. That is, while grazers would be anticipated to consume more nutrient-poor algae as a greater biomass would need to be consumed to reach satiation (Stiling & Cornelissen 2007), feeding rates may actually be reduced when presented with lower-quality food (Hillebrand et al. 2000; Silliman & Zieman 2001; Russell & Connell 2007). While these potential responses support the broad consensus that rates of herbivory will change under forecasted conditions (Tylianakis et al. 2008), we are currently unable to forecast whether this variation will be driven by direct effects on the herbivores, indirect effects on herbivores mediated by the response of primary producers or both of these effects acting in combination. Identification of the way in which species interactions modify the direct effect of

CO_2 will be necessary to understand how interacting components contribute to determining the ecosystem effect of altered environmental conditions.

1.3.3 DISRUPTING ESTABLISHED INTERACTIONS

Interactions between abiotic factors are recognised for their potential to hasten the expansion of taxa characteristic of the novel configuration, particularly in the absence of biotic controls, yet little is understood regarding their potential influence on the reversal of such change. It is possible that reversing ecological change initially driven by interactive effects will not require all altered conditions to be returned to their initial state, as is the currently dominant paradigm (Suding et al. 2004; Hobbs et al. 2009; Lotze et al. 2011), but rather just a subset of conditions such that the interaction is disrupted (Appendix A; Russell et al. 2009). A key management tool to reduce the impact of interactive effects may, therefore, be the restoration of a single altered factor. Such an approach will be of particular value where future climates contribute to interactions as the spatial and temporal scales over which these stressors are modified means their reversal would be infeasible or even impossible (Matthews & Caldeira 2008; Solomon et al. 2009), making this the context in which any restoration will occur. Given the anticipated strong interactive influence of CO_2 and nutrients on the growth of algal turfs and occurrence of phase-shifts (Russell et al. 2009), effective reduction of the locally-altered factor of enriched nutrients is a potentially powerful tool for managers of this system. Although removing a single factor may disrupt the interaction such that change is reversed, this approach would be ineffective if the self-stabilising feedbacks that maintain community structure of novel assemblages prevent recovery (Suding et al. 2004; Lotze et al. 2011). If disrupting interactions by restoring a locally-modified factor to its historic level does facilitate increased resilience, however, this outcome will highlight the potential for local management to determine the effect of global-scale change, even following the establishment of such conditions.

1.4 STATEMENT OF PURPOSE, SCOPE AND OUTLINE OF THIS THESIS

Interactive effects have the potential to determine the influence of forecasted global and local conditions on ecosystems. In this thesis, I test the model that although cross-scale abiotic pollutants (i.e. global increases in CO_2 and local nutrient enrichment) can have interactive effects that favour ecological change, such alterations can be prevented where local conditions are managed and the interactions disrupted.

Experimental manipulations were used to test the hypotheses that:

1. Enrichment of the global and local pollutants of CO_2 and nutrients will differentially release the key habitat-forming primary producers of marine temperate coastlines (i.e. kelp and turf) from their limitations (Chapter 2)
2. The direct effect(s) of altered environmental conditions (i.e. CO_2 and nutrient enrichment) will be modified by key biotic interactions, specifically competition mediated by foundation species and herbivory of gastropod grazers (Chapters 3 and 4, respectively)
3. Reducing a subset of factors contributing to interactive effects will disrupt their influence such that further expansion of the novel state (i.e. turfing algae) is limited or reduced (Chapter 5)

1.4.1 THESIS SUMMARY

The key models and hypotheses addressed are outlined below.

CHAPTER 2

Individual taxa are anticipated to respond to resource enrichment in contrasting ways contingent on their physiologies (following Hepburn et al. 2011). In Chapter 2 I assess the model that the algal taxa of turf and kelp respond differently to CO_2 and nutrient enrichment. The hypothesis tests for change in biomass and chemical composition (stoichiometry) of the algae, to assess the relative resource limitations experienced by the contrasting taxa, their responses to future conditions and, consequently, potential for shifts in community composition under future abiotic conditions.

CHAPTER 3

The synergistic interactive effect of CO_2 and nutrients on the expansion of turf (as identified in Chapter 2), indicates this taxa will benefit under future conditions, potentially facilitating phase-shifts to the novel configuration it dominates. In Chapter 3 I consider the model that strong biotic interactions can mediate such ecological change (Sanford 1999; O'Connor 2009; O'Connor et al. 2009). Focus is placed on a key interaction structuring communities in temperate marine systems, specifically the competitive dominance of kelp canopies over understorey turf (Reed & Foster 1984; Connell 2005; Russell 2007). I test the hypothesis that if this foundation species is maintained under forecasted CO_2 and nutrient conditions, then it will continue to restrict the expansion of its key competitor (i.e. turfs).

CHAPTER 4

The interactive effect of altered environmental conditions on turf may also be influenced by herbivory (Tylianakis et al. 2008; O'Connor et al. 2009). Where nutrients are locally-enriched algae are typically affected such that grazing rates are increased (Hillebrand et al. 2000; Silliman & Zieman 2001; Russell & Connell 2007). I consider the model that under forecasted conditions of global change, enriched CO_2 may not only influence algae but also the grazers such that the strength of the interaction is modified (reviewed in Pörtner et al. 2004; Doney et al. 2009). Consequently, in Chapter 4 I test the hypotheses that; 1) where CO_2 is enriched the strength of grazing will be modified, and, 2) alteration in the strength of this interaction will be due to a direct effect of CO_2 on the grazer, rather than an indirect effect mediated by a change in the algae.

CHAPTER 5

Interactions between factors altered at global and local scales (considered in various forms in Chapters 2, 3 and 4) are anticipated to strongly influence future community composition and the occurrence of phase-shifts. In Chapter 5 I assess the model that a key management tool to reverse such effects may be the disruption of interactions which led to the initial change (Appendix A; Russell et al. 2009). As returning global conditions to their historical baseline levels will be largely infeasible and impractical once enrichment is established (Matthews &

Caldeira 2008; Solomon et al. 2009), focus will necessarily be placed on managing factors altered at the local-scale. The hypothesis tested was, therefore, that if CO_2 and nutrients combine to facilitate a synergistic increase in turf, then such change will be substantially slowed or recovery hastened where the locally-determined factor of enriched nutrients is removed even under the continuation of CO_2 enrichment.

CHAPTER 6

In Chapter 6 I provide a brief discussion of the preceding data chapters, highlighting how they are associated, and outline directions for future research.

THESIS

Whilst each data chapter (2 – 5) is written in the form of a separate scientific paper that can be read independently, the body of work builds naturally for a comprehensive analysis that is summarised in the discussion chapter (6). Together, these chapters form a thesis which provides insight regarding how conditions altered at contrasting scales (i.e. global v. local) interact to drive ecological change (i.e. the relative abundance of algal species) and provide insight regarding not only the impending impacts of global change, but also the potential for local conditions to determine its influence.

A forest of the perennial algae *Ecklonia radiata* (top) and a mat of turf-forming algae dominated by *Feldmannia* spp. (bottom) growing on subtidal rocky coast in South Australia.

Photos: Sean Connell.

Mesocosms utilised in field-based experiments moored at the Cruising Yacht Club of South Australia.

Photo: Author.

CHAPTER 2

CHAPTER 2

PREAMBLE

Chapter 2 is a co-authored paper which, at the time of printing, was in press with the journal *Oecologia* and available online, with myself as senior author and Bayden D. Russell and Sean D. Connell as co-authors. It is, therefore, written in plural throughout and uses the journal formatting. It is included with kind permission of Springer Science and Business Media (see Appendix B) and can be cited as:

Falkenberg LJ, Russell BD & Connell SD (2013) Contrasting resource limitations of marine primary producers: implications for competitive interactions under enriched CO_2 and nutrient regimes. *Oecologia*, DOI, 10.1007/s00442-012-2507-5.

In this chapter I conducted the study, collected and analysed the data and wrote the manuscript. Sean Connell and Bayden Russell provided funding and assisted with intellectual development, including the initial concept of the study and manuscript evaluation.

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GLOBAL CHANGE ECOLOGY - ORIGINAL RESEARCH

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 these groups to contemporary and enriched levels of carbon dioxide (CO_2) and nutrients. Turfs responded to the individual enrichment of both CO_2 and nutrients, with the greatest shift in the biomass and carbon:nitrogen (C:N) ratios observed under their combined enrichment. In contrast, kelp responded to enriched nutrients, but not enriched CO_2. We hypothesise that the differing limitations reflect the contrasting physiologies of these functional groups, specifically their methods of C 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.

Keywords 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 by 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 the simultaneous modification of multiple factors than to enrichment by either factor individually (Davidson and Howarth 2007; Allgeier et al. 2011). As

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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 composed of functional groups experiencing diverse limitations, with the potential that some components are limited by a single resource while are others are 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 (C) fixation. Of concern is the potential that the influence of enriched carbon dioxide (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), while C 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 C-limited as a consequence of the physiological mechanisms by which C 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 the active influx of CO_2 and/or bicarbonate (HCO_3^-) and elevate concentrations at the site of C 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 long-lived, 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 the 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 the 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 C acquisition; that is, it has been proposed that 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 enabled 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 6 weeks of exposure to altered CO_2 and nutrients (in crossed combinations) in field-based mesocosms (described in the "Materials and methods" section). 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. We anticipated that turfs would respond with greater increases in biomass and shifts in the carbon:nitrogen (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 to that of CO_2. If such contrasting responses were to manifest, they would 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, we tested the responses of turf-forming algae (mainly *Feldmannia* spp.) and kelp (*Ecklonia radiata*) to altered CO_2 and nutrient availability in a field-based mesocosm experiment. Experimental mesocosms were exposed to combinations of CO_2 (current vs. future) and nutrients (ambient vs. 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 a volume of 250 L (L × W × H: 0.5 × 0.5 × 1 m).

The experiment was conducted in a boat harbour adjacent to the Gulf St. Vincent, at Outer Harbor, Adelaide, South Australia (34.473395°S, 138.292184°E). The boat harbour is protected from the predominant swell by a breakwall, but it 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 Electronic Supplemental Material (ESM) Table S4 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 (model LI-250l; Li-Cor, Lincoln, NB).

To quantify the effectiveness of Osmocote Plus® (Scotts Australia, Baulkham Hills, 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, ten mesocosms were established in the laboratory and maintained for 5 weeks between March and April 2011. Nutrient enrichment and quantification of the water column variables were achieved using the same techniques as in the field-based mesocosms (see section "Experimental treatments: CO_2 and nutrient addition").

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 present 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 the results indicated that 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 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 ± standard error (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 8 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 2-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 was reduced from ambient (mean ± SE 8.18 ± 0.06) to the experimental level [target 7.95; measured (mean ± SE) 7.96 ± 0.01; see ESM Table S4]. The 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 the probes was checked on a daily basis and, if necessary, recalibrated using NBS calibration buffers to 0.01 pH units. Total Alkalinity (TA) of the seawater in mesocosms was measured weekly using colorimetric titration (Hanna Instruments, Woonsocket, RI). CO_2 partial pressure (pCO_2) and the concentrations of 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 N: 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, unpublished 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; N:phosphorus:potassium, 15:5:10 %), with the nutrient concentration released proportional to the 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 (mesh size 1 mm) 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 (diameter of glass fibre 0.45 μm) and immediately frozen. Samples were later analysed on a Lachat Quickchem 8500 Flow Injection Analyser (Hach Co, Loveland, CO) for ammonia, phosphate and nitrite + nitrate (NO_X).

Experimental responses

At the end of the study, the change in the 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 square centimetres) and initial length (in centimetres) 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, we collected tissue samples 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 by being stored frozen at −20 °C for 4 months prior to analysis. They were then rinsed in Milli-Q water to remove contaminants and salts and also hasten the defrosting process. The samples were then placed in an oven at 60 °C where they were dried for 2 days (48 h) and then crushed to a fine power using a mortar and pestle. A sub-sample of the powder (3.5 ± 0.5 mg) from each specimen was placed into a tin capsule (5 × 8 mm) (SerCon, Cheshire, UK) which was then placed into a carousel which, in turn, fed each capsule into an isotope-ratio mass spectrometer where it was combusted; the gasses then 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). 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 C).

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, the C:N ratio, and the percentage of C (% C) and N (% N). The factors of CO_2 and nutrients were both treated as fixed and orthogonal, with two levels in each factor (CO_2: current vs. future; nutrients: ambient vs. 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 vs. 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; ESM 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; ESM Table S2a), whereas that of kelp responded only to elevated nutrients (Fig. 2b; ESM Table S2b). Underlying these shifts in 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; ESM Table S3ai), while turf % N was increased by both future CO_2 and elevated nutrients (Fig. 3a; ESM Table S3aii). In kelp tissue, the % C was not influenced by enrichment of either CO_2 or nutrients (Fig. 3b; ESM Table S3bi), with % N only increased under elevated nutrients (Fig. 3b; ESM Table S3bii).

The mean (±SE) $\delta^{13}C$ of kelp (-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. $CO_2 \times$ 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}) (ESM Tables S4, S5). In contrast, the NO_X (nitrate + nitrite) concentration in elevated nutrient mesocosms was not significantly different from that in 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 the quality of the water 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}) (ESM Tables S4, S5).

The pH and concentration of CO_3^{2-} were significantly reduced under future CO_2 compared with current CO_2 treatments (ESM Tables S4, S5). In contrast, the pCO_2 and HCO_3^- concentration were significantly increased under future CO_2 conditions (ESM Tables S4, S5). Temperature was not significantly different under any treatments (ESM Tables S4, S5). The minimum and maximum temperatures recorded (13.0 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 were under the same light conditions across the different

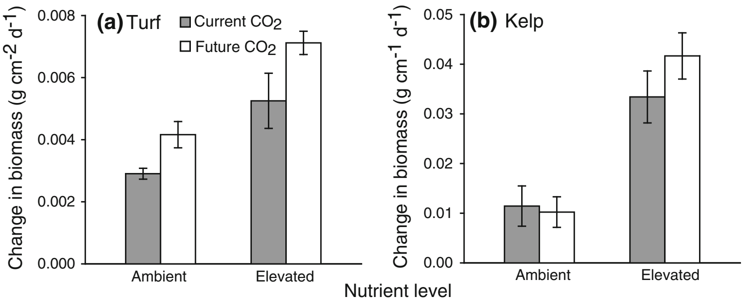


Fig. 1 The daily change in biomass (g) of turf (**a**; per cm^2) and kelp (**b**; per lamina length in cm) that were exposed to different combinations of nutrients (ambient vs. elevated) and carbon dioxide (CO_2; current vs. future levels). Data are presented as the mean ± standard error (SE). Note the different scales of the *y* axes

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

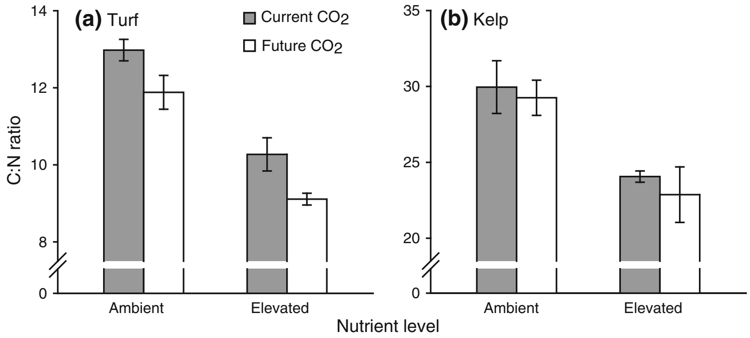
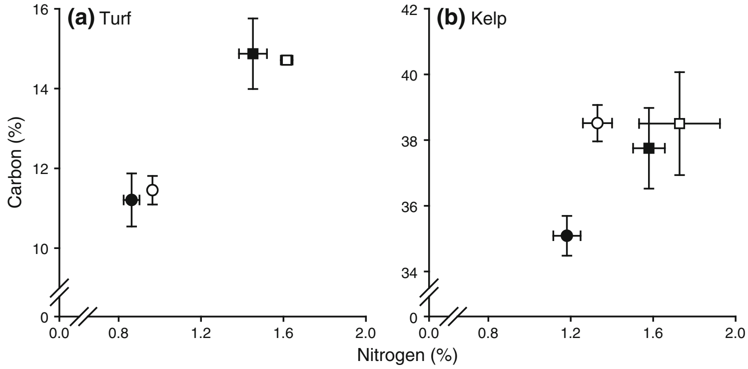


Fig. 3 The N and C composition (mean ± SE) of turf (**a**) and kelp (**b**) that were exposed to different combinations of nutrients (ambient vs. elevated) and CO_2 (current vs. future). Note the different scales of the *y* axes. *Filled circle* Ambient nutrients, current CO_2, *open circle* ambient nutrients, future CO_2, *filled square* elevated nutrients, current CO_2, *open square* elevated nutrients, future CO_2



treatments, with kelp exposed to a greater light availability than turf (ESM Tables 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 the productivity of producers (Liebig 1842), whereas contemporary research places an increasing emphasis on co-limitation by multiple resources (Harpole et al. 2011). Our ecological result, namely, the contrasting response by distinct functional groups, suggests that communities may be comprised of functional groups exhibiting both types of limitations. The kelp response to nutrient enrichment, but lack of response to CO_2 enrichment, is typical of single-resource limitation as defined by Liebig's Law of the Minimum (Liebig 1842), with production increasing when nutrients were enriched, but not being affected by enriched CO_2. This biomass response may have resulted under the enriched nutrient conditions due to increased availability of the limiting resource in this marine system, likely nutrients (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 (cf. 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 into 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 observed 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 both 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 higher % 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 C 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 C uptake are utilised by species of marine algae: passive diffusion and active uptake via a CCM. The significant response of turfs to enhanced CO_2 suggests that 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 C. This conclusion aligns well with the expectation that morphologically simple algae would lack the 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 stable C isotope ($\delta^{13}C$) values did not, however, support the conclusion that turfs exclusively utilise passive diffusion. The $\delta^{13}C$ values 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 C 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 the energetic requirements, particularly light, of CCMs; those algae which can acquire more light are able to operate their CCMs at a higher rate (Hepburn et al. 2011). As kelp is a large, canopy-forming alga, 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 downregulated 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 C 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 C 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 the 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 in our study, 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); this focus has since shifted to identifying the occurrence of co-limitation by multiple resources (Harpole et al. 2011). Our results suggest that communities consist 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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2.8 SUPPLEMENTAL MATERIAL

Table S2.1 ANOVAs testing the combined effects of nutrients (ambient vs. elevated) and CO_2 (current vs. future) on (a) turf and (b) kelp in terms of their daily change in biomass (g) per standard size (sample area in cm^2 and lamina length in cm, respectively).

Source	df	MS	*F*	*P*	df	MS	*F*	*P*
	(a) Turf				(b) Kelp			
CO_2	1	0.0733	8.57	0.0191	1	0.3712	0.66	0.4466
Nutrient	1	0.2109	24.66	0.0011	1	21.3881	38.09	0.0003
CO_2 × nutrient	1	0.0028	0.33	0.5842	1	0.6734	1.20	0.2967
Residual	8	0.0086			8	0.5615		

Table S2.2 ANOVAs testing the combined effects of nutrients (ambient vs. elevated) and CO_2 (current vs. future) on (a) turf and (b) kelp in terms of their C:N ratio.

Source	df	MS	*F*	*P*	df	MS	*F*	*P*
	(a) Turf				(b) Kelp			
CO_2	1	3.8155	10.64	0.0115	1	2.6865	0.46	0.5183
Nutrient	1	22.5067	62.74	< 0.0001	1	113.0616	19.22	0.0023
CO_2 × nutrient	1	0.0032	0.01	0.9269	1	0.1785	0.03	0.8661
Residual	8	0.3587			8	5.8839		

Table S2.3 ANOVAs testing the combined effects of nutrients (ambient vs. elevated) and CO_2 (current vs. future) on (a) turf and (b) kelp in terms of their (i) % C and (ii) % N.

Source	df	MS	*F*	*P*	df	MS	*F*	*P*
	(a) Turf							
	(i) % C				(ii) % N			
CO_2	1	0.0052	0.01	0.9447	1	0.0527	9.94	0.0135
Nutrient	1	35.9875	35.20	0.0003	1	1.1545	218.00	< 0.0001
CO_2 × nutrient	1	0.1233	0.12	0.7374	1	0.0029	0.55	0.4786
Residual	8	1.0224			8	0.0053		
	(b) Kelp							
	(i) % C				(ii) % N			
CO_2	1	13.0955	3.76	0.0883	1	0.0659	1.63	0.2370
Nutrient	1	5.2680	1.51	0.2535	1	0.4755	11.79	0.0089
CO_2 × nutrient	1	5.3782	1.55	0.2490	1	0.0000	0.00	0.9995
Residual	8	3.4796			8	0.0403		

Table S2.4 Physicochemical variables measured in the field (n = 10) and the laboratory (n = 3) for each treatment. Reported are means, standard errors (SE), maximum (Max.) and minimum (Min.) values. Field ammonia, phosphate and NO_X were sampled within treatment tanks weekly on 4 occasions, with the surrounding waters sampled on 1 occasion and laboratory-based mesocosms sampled on alternate days (n = 20 occasions). Total Alkalinity (TA), pH and temperature were simultaneously measured weekly on 5 occasions, from which concentrations of pCO$_2$, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) were calculated. Values were calculated from measured TA and pH using constants from Mehrbach et al. (1973), as adjusted by Dickson and Millero (1987). Light (photosynthetically active radiation, 400 – 700 nm) was measured on 1 occasion.

Physicochemical variable		Treatment	Mean (SE)	Max.	Min.
Ammonia (mg L^{-1})	i) In field	AN, CCO$_2$	0.0186 (0.0006)	0.0450	0.0050
		AN, FCO$_2$	0.0266 (0.0030)	0.0550	0.0068
		EN, CCO$_2$	0.0298 (0.0060)	0.0590	0.0083
		EN, FCO$_2$	0.0391 (0.0059)	0.0915	0.0160
		In surrounding waters	0.0172 (0.0023)	0.0220	0.0044
	ii) In lab	Without nutrients	0.0346 (0.0053)	0.1210	0.0020
		With nutrients	0.2653 (0.0320)	0.6230	0.0110

Phosphate (mg L^{-1})	i) In field	AN, CCO_2	0.0084 (0.0003)	0.0104	0.0051
		AN, FCO_2	0.0078 (0.0003)	0.0094	0.0067
		EN, CCO_2	0.0099 (0.0003)	0.0120	0.0077
		EN, FCO_2	0.0091 (0.0002)	0.0110	0.0067
		In surrounding waters	0.0080 (0.0004)	0.0089	0.0061
	ii) In lab	Without nutrients	0.0272 (0.0033)	0.0600	0.0010
		With nutrients	0.1285 (0.0068)	0.2870	0.0510
NO_X (mg L^{-1})	i) In field	AN, CCO_2	0.0059 (0.0006)	0.0082	0.0028
		AN, FCO_2	0.0053 (0.0006)	0.0078	0.0031
		EN, CCO_2	0.0055 (0.0002)	0.0077	0.0043
		EN, FCO_2	0.0056 (0.0001)	0.0076	0.0039
		In surrounding waters	0.0036 (0.0002)	0.0042	0.0024
	ii) In lab	Without nutrients	0.1222 (0.0050)	0.2080	0.0630
		With nutrients	0.3796 (0.0255)	0.6230	0.1010

pH		AN, CCO$_2$	8.18 (0.06)	8.53	8.02
		AN, FCO$_2$	7.90 (0.00)	8.09	7.75
		EN, CCO$_2$	8.15 (0.03)	8.24	8.05
		EN, FCO$_2$	7.96 (0.02)	8.10	7.73
Temperature (°C)		AN, CCO$_2$	14.4 (0.2)	15.8	13.1
		AN, FCO$_2$	14.6 (0.1)	15.9	13.2
		EN, CCO$_2$	14.6 (0.2)	15.8	13.2
		EN, FCO$_2$	14.6 (0.1)	15.9	13.0
TA (µmol kg^{-1})		AN, CCO$_2$	2276 (63)	2723	1848
		AN, FCO$_2$	2357 (57)	2723	2091
		EN, CCO$_2$	2211 (55)	2529	1897
		EN, FCO$_2$	2341 (43)	2723	2091
pCO$_2$ (ppm)		AN, CCO$_2$	408 (59)	574	138
		AN, FCO$_2$	893 (17)	1301	515
		EN, CCO$_2$	400 (19)	450	334
		EN, FCO$_2$	738 (68)	1460	490
HCO_3^- (µmol kg^{-1})		AN, CCO$_2$	1890 (92)	2261	1502
		AN, FCO$_2$	2141 (48)	2454	1906
		EN, CCO$_2$	1859 (28)	2076	1652
		EN, FCO$_2$	2093 (48)	2550	1901
CO_3^{2-} (µmol kg^{-1})		AN, CCO$_2$	157 (21)	277	97
		AN, FCO$_2$	88 (4)	135	55
		EN, CCO$_2$	142 (12)	195	95
		EN, FCO$_2$	101 (3)	134	73
Light (µmol m^{-1} s^{-2})	Experienced by turf	AN, CCO$_2$	547.23 (147.83)	886.50	242.60
		AN, FCO$_2$	377.08 (82.66)	737.00	171.93
		EN, CCO$_2$	463.67 (124.67)	786.70	242.10
		EN, FCO$_2$	581.91 (117.77)	898.46	300.90

Experienced by kelp	AN, CCO_2	1321.35 (83.17)	1552.40	1037.10
	AN, FCO_2	1204.88 (259.40)	1625.00	665.60
	EN, CCO_2	1479.46 (159.39)	1897.70	965.10
	EN, FCO_2	1432.42 (237.00)	1841.80	635.50

AN, ambient nutrients; EN, elevated nutrients; CCO_2, current CO_2; FCO_2, future CO_2.

Table S2.5 Results from different ANOVAs, testing the combined effect of nutrients (ambient vs. elevated) and CO_2 (current vs. future) on the 9 physicochemical variables measured in the field and effect of nutrients (ambient vs. elevated) on the 3 measured in the laboratory. Field ammonia, phosphate and NO_X were sampled weekly on 4 occasions, with laboratory-based mesocosms sampled on alternate days (n = 20 occasions). Total Alkalinity (TA), pH and temperature were measured weekly on 5 occasions, from which concentrations of $p$$CO_2$, bicarbonate ($HCO_3^-$) and carbonate ($CO_3^{2-}$) were calculated. Values were calculated from measured TA and pH using constants from Mehrbach et al. (1973), as adjusted by Dickson and Millero (1987). Light (photosynthetically active radiation, 400 – 700 nm) was measured on 1 occasion.

Physicochemical variable (response variable)	CO_2		Nutrients		CO_2 × Nutrients	
	F	*P*	*F*	*P*	*F*	*P*
Ammonia	3.80	0.0870	**7.12**	**0.0284**	0.02	0.8917
Phosphate	2.10	0.1854	**8.00**	**0.0222**	0.03	0.8607
NO_X	0.27	0.6167	0.01	0.9294	0.58	0.4681
Ammonia (lab)			**1066.79**	**< 0.0001**		
Phosphate (lab)			**124.69**	**< 0.0001**		
NO_X (lab)			**382.33**	**< 0.0001**		
pH	**44.40**	**0.0002**	0.24	0.6363	1.37	0.2753
TA	3.71	0.0904	0.55	0.4802	0.20	0.6686
Temperature	0.15	0.7123	0.32	0.5895	0.22	0.6495
pCO_2	**77.08**	**< 0.0001**	3.04	0.1194	2.45	0.1564
HCO_3^-	**16.96**	**0.0034**	0.45	0.5230	0.02	0.8860
CO_3^{2-}	**20.36**	**0.0020**	0.01	0.9421	1.27	0.2928
Light (turf)	0.05	0.9966	0.25	0.0821	1.44	0.6872
Light (kelp)	0.95	0.3571	0.17	0.6896	0.03	0.8647

For field-based measurements two-factor ANOVAs with tanks (n = 3) as replicates; for laboratory-based measurements one-factor ANVOAs with tanks (n = 5) as replicates. Bold values indicate a significant difference (at $p < 0.05$ level).

A forest of the perennial algae *Ecklonia radiata* growing on subtidal rocky coast in South Australia.

Photo: Sean Connell.

CHAPTER 3

CHAPTER 3

PREAMBLE

Chapter 3 is a co-authored paper published in the journal *PLoS One*, with myself as senior author and Bayden D. Russell and Sean D. Connell as co-authors. It is, therefore, written in plural throughout and uses the journal formatting. It is included with permission from PLoS (see Appendix B) and can be cited as:

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In this chapter I conducted the study, collected and analysed the data and wrote the manuscript. Sean Connell and Bayden Russell provided funding and assisted with intellectual development, including the initial concept of the study and manuscript evaluation.

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Stability of Strong Species Interactions Resist the Synergistic Effects of Local and Global Pollution in Kelp Forests

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Abstract

Foundation species, such as kelp, exert disproportionately strong community effects and persist, in part, by dominating taxa that inhibit their regeneration. Human activities which benefit their competitors, however, may reduce stability of communities, increasing the probability of phase-shifts. We tested whether a foundation species (kelp) would continue to inhibit a key competitor (turf-forming algae) under moderately increased local (nutrient) and near-future forecasted global pollution (CO_2). Our results reveal that in the absence of kelp, local and global pollutants combined to cause the greatest cover and mass of turfs, a synergistic response whereby turfs increased more than would be predicted by adding the independent effects of treatments (kelp absence, elevated nutrients, forecasted CO_2). The positive effects of nutrient and CO_2 enrichment on turfs were, however, inhibited by the presence of kelp, indicating the competitive effect of kelp was stronger than synergistic effects of moderate enrichment of local and global pollutants. Quantification of physicochemical parameters within experimental mesocosms suggests turf inhibition was likely due to an effect of kelp on physical (i.e. shading) rather than chemical conditions. Such results indicate that while forecasted climates may increase the probability of phase-shifts, maintenance of intact populations of foundation species could enable the continued strength of interactions and persistence of communities.

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Introduction

A few strong interactions often contribute disproportionately to maintaining the composition and function of an ecosystem by modifying both the physical conditions and species interactions within [1,2,3]. Key species can maintain ecosystem composition not only by forming biological habitats whose physical environment facilitates their own recruitment, but also by dominating competitors that would otherwise inhibit this process. Such organisms, variously called 'foundation species' or 'ecosystem engineers', create stable conditions for other dependent species [3,4]. The inhibition of competitors associated with contrasting physical conditions and species interactions, therefore, enhances the stability of systems centered on these foundation species [5].

As human activities continue to modify abiotic conditions, there is increasing concern that such strong interactions will be altered (e.g. the sea *Pisaster ochraceus* may be less effective at consuming mussels [6]). Reduction in the strength of interactions could disrupt the persistence of entire biological communities, ranging from kelp forests to seagrasses and coral reefs in the marine realm, and grasslands to forested ecosystems in the terrestrial realm. In the marine realm, the coastal zone is an area in which high productivity and species diversity coincide with human activity and this area is set to be further influenced by the effects of a changing climate [7]. Altered land use and ensuing discharges to the marine environment elevate nutrient concentrations at local scales, with the extent of change ranging from strong enrichment in urban areas to little or no change in agricultural and natural systems [8,9,10]. These waters will also absorb approximately 30 percent of the atmospheric CO_2 produced by human populations globally, leading to gradual ocean acidification [11,12]. While there is recent recognition that these alterations of the physical environment will affect species interactions [13,14,15,16] experiments to date have not progressed sufficiently to identify how they will affect biological communities dominated by foundation species such as kelp.

Australian kelp are habitat-forming species whose persistence has been enabled by their self-facilitation of recruitment through the competitive exclusion of opportunistic turf-forming algae [17]. When kelp canopies are lost, turfs rapidly colonise space and their sediment-trapping morphology inhibits the recruitment of juvenile kelp and re-formation of kelp forests [18,19]. Under conditions of severely elevated nutrients, these naturally-ephemeral turfs persist in fragmented canopies [10,20] to cause intergenerational decline and collapse of the kelp community [8]. Turfs, therefore, mediate the effect of nutrient-driven loss of kelp forests and often constitute a vital component in the indirect effects of pollution on habitat loss.

Under moderate scenarios of nutrient pollution, it is possible that kelp forests can persist by continuing to exclude turfs [10]. Similarly, the elevation of CO_2 over the near-future may not alter the strength by which kelp suppress turfs. While susceptible to many other human-altered conditions, kelp meiospores are anticipated to germinate successfully under near-term enrichment of CO_2 conditions [21]. Furthermore, productivity of ensuing recruits and subsequent individuals may be increased under elevated CO_2 [22]. Evidence to date, however, suggests moderate increases of CO_2 facilitate greater covers and biomass of turf, potentially turning them from ephemeral to persistent habitats [16,23]. It remains unknown whether the competitive dominance of kelp over turf, (i.e. an interaction of particular concern to the regeneration of kelp) is likely to be reduced or increased under the combined influence of moderate nutrient and CO_2 pollution. We consider the model that elevated CO_2 may assist kelp sustainability despite the greater potential for turfs to persist.

We tested the hypothesis that a foundation species would continue to suppress its key competitor under conditions of moderate forecasted levels of pollution which have the potential to favour its competitor's expansion. That is, we assessed if the competitive dominance of kelp over turfs [17] would continue under moderate forecasted levels of local (i.e. nutrient) and near-term global pollution (i.e. CO_2) and their known synergy [16].

If the strength of interactions involving foundation species are maintained despite the increasingly novel conditions brought about by human activities, then phase-shifts may be avoided. Such phase-shifts are not uncommon, but anticipating them has been problematic because many involve indirect effects [24] for which the impact of one species (e.g. kelp) on another (e.g. turf) requires knowledge of a third element that is inadequately understood (e.g. synergies among pollutants). Our study addresses a reasonably widespread challenge of forecasting the ecology of phase-shifts under future climates.

Materials and Methods

Experimental design

The effects of kelp removal (*Ecklonia radiata*), increased CO_2 and elevated nutrients were tested on the turf-forming algae in a mesocosm experiment conducted in an open boat harbour located within Gulf of St. Vincent at Outer Harbour, Adelaide, South Australia (34.473395°S, 138.292184°E) (detail in "Experimental mesocosms" below). The effects of treatments on mesocosm water column physicochemical parameters were also quantified. Experimental mesocosms had combinations of kelp (present *v.* absent), CO_2 (current *v.* future) and nutrients (ambient *v.* elevated) in a crossed design. Three replicate mesocosms were used per treatment combination, with replicate specimens of algal turfs in each mesocosm ($n = 5$). Treatments were maintained for 90 days between August and November 2009. Kelp were either present at densities similar to those observed at the collection site (9–11 m^{-2}, or 3 kelp per mesocosm) or absent, as is observed on many developed coastlines, including Adelaide [10,20]. Target [CO_2] were 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), which is derived from model predictions by Meehl et al. [25] (Table S4). The elevated nutrient treatment was designed to result in concentrations similar to those moderate enrichments experienced in waters off the coast of metropolitan Adelaide [10].

Turf-forming algae

The specimens of turf-forming algae used in the experiments were collected from rocky reef with areas of turfs adjacent to kelp canopies at Horseshoe Reef, Gulf of St. Vincent, South Australia (35.13757°S, 138.46266°E). Turfs (mainly *Feldmannia* spp.) were collected from outside the kelp canopy still attached to their natural substratum (approximately the same size, 5×5 cm) and placed in holding mesocosms for eight weeks before the experiment commenced to enable acclimation to conditions in the mesocosms. Following this acclimation period five specimens of turf-forming algae were randomly assigned to each experimental mesocosm in which conditions were gradually altered over a further two week period until they reached the pre-designated experimental levels. Turf response to treatments was quantified in terms of change in percentage cover, final percentage cover and dry mass per standard area. To quantify the percentage cover of turf on each experimental specimen, a 2.5×2.5 cm quadrat was placed over the specimen within which the percentage cover was visually estimated to the nearest 5 percent. This measurement was made at the beginning (day 0; mean ± s.e. across all samples, $28.83 \pm 1.97\%$; three-way ANOVA detected no significant difference among samples placed in the different treatments, all $p > 0.05$) and end (day 64) of the experimental period (see [26]). Change in percentage cover was then calculated by subtracting the initial percent cover from the final percent cover, while final percentage cover was that measured on day 64. Dry mass of algae was measured at the completion of the experiment (day 90) from a standard area of each specimen (2.5×2.5 cm). All algae was carefully scaped from the specimen using a razor into a pre-weighed aluminium tray, rinsed with fresh water to remove excess salt and dried to a constant weight at 60°C for 48 h before weighing (see [16,23]).

Experimental treatments: kelp, CO_2 and nutrient addition

Kelp used in the experiments were collected from rocky reef adjacent to the location from which turfs were collected. 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 and acclimated in holding mesocosms for eight weeks before the experiment commenced. Three individual kelp were then placed in each of the appropriate treatment mesocosms. The effect of kelp on light in the tanks was quantified by taking measurements using an underwater radiation sensor (Li-Cor LI-250, Nebraska, USA).

Experimental [CO_2] of seawater in mesocosms was maintained by directly diffusing CO_2 gas into mesocosms when required 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. The pH of mesocosms exposed to the elevated CO_2 treatment was gradually reduced from ambient (8.15) to the experimental level (target: 7.95; measured: 7.91–7.95, see Table S4 for detail) over a two-week period (approximately 0.01 pH units per day). Total Alkalinity (TA) of seawater in mesocosms was measured weekly using colorimetric titration (Hanna Instruments, Woonsocket, RI, USA). Concentrations of pCO_2 and bicarbonate (HCO_3^-) were then calculated from measured TA, pH, salinity and temperature using the CO2SYS program for Excel [27] with constants from Mehrbach et al. [28], as adjusted by Dickson and Millero [29].

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: 15, 5, 10 N-P-K), with the nutrient concentration released proportional to weight of the fertiliser [30]. Osmocote has successfully been used in previous studies of this system to manipulate nutrient concentrations (e.g. [16,31]). 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 NO_X (nitrite+nitrate). Additionally, to quantify the effect of elevated nutrients in the absence of biota, a trial was conducted whereby 10 mesocosms identical to the field mesocosms were established in the laboratory and maintained for five weeks between March and April 2011. Using the same methods as in the field, 10 g of Osmocote was added to half of these tanks, with water samples being regularly analysed from all mesocosms.

Experimental mesocosms

The closed, experimental mesocosms were moored in a boat harbour adjacent to the Gulf of St. Vincent at Outer Harbour, Adelaide, South Australia. The boat harbour is protected from the predominant swell by a breakwall, but which has a channel wide enough to allow high flushing rates. The mesocosms were moored alongside a system of floating pontoons that move up and down with the tides, and held in place by an array of vertical pilings. Mesocosms (L×W×H: 0.5×0.5×1 m) 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. While this water is not different from that adjacent to the harbour and is representative of the oligotrophic coastlines of South Australia, the quality of water used in the mesocosm experiments may not have been ambient relative to the collection site. During the experimental period one-third of the seawater was removed from each mesocosm and replaced with fresh seawater weekly to maintain water quality. The mesocosms were located in full sunlight and consequently experienced diurnal and seasonal fluctuations in sunlight and temperature.

Analyses

Three-factor Analysis of Variance (ANOVA) was used to test the response of algal turfs to experimental conditions (change in percentage cover, final percentage cover and dry mass per area of turfs). The three factors of kelp, CO_2 and nutrients were treated as fixed and orthogonal, with two levels in each factor (Kelp: present *v.* absent; CO_2: current *v.* future; Nutrient: ambient *v.* elevated). Data for the five algal specimens within each mesocosm were averaged and analysed with mesocosms as replicates ($n = 3$). Three-factor ANOVA (as described above) was used to test the water column physicochemical parameters of mesocosms with measurements averaged across days (pH, TA, pCO_2, HCO_3^- and temperature $n = 8$ days; light $n = 1$ day; ammonia, phosphate and NO_X in field $n = 6$ days; ammonia phosphate and NO_X in laboratory $n = 20$ days) and mesocosms used as replicates ($n = 3$ for field; $n = 5$ for laboratory). Where significant treatment effects were detected, Student–Newman–Keuls (SNK) *post hoc* comparison of means was used to determine which factors differed. The magnitude of effects (ω^2) was calculated [32,33] to assess which factor, or combination of factors, primarily contributed to the response of turfs (in terms of change in percentage cover, final percentage cover and dry mass per area) under experimental treatments.

Results

Turf-forming algae

A key result was that the negative response of turfs to canopies was of similar magnitude across all treatments of pollution and their combinations. There was no change in the percentage cover of turfs under 'ambient conditions' (i.e. the experimental treatments of ambient nutrients and current CO_2 and no kelp canopy) from the beginning to end of the experimental period (Figure 1). The treatment of largest influence was the presence or absence of kelp canopies ($\omega^2 = 0.53$; Table S1). In the absence of kelp, elevated nutrients and CO_2 positively affected percentage cover in a multiplicative rather than additive manner (Figure 1; Table S1; SNK test of Kelp×Nutrient×CO_2 interaction). In the presence of kelp, the percentage cover of turfs was reduced below that of 'ambient conditions', with neither elevated CO_2 or nutrients having a significant effect, either in isolation or combination (Figure 1; Table S1; SNK test of Kelp×Nutrient×CO_2 interaction).

A synergistic interaction occurred between the simultaneous effects of kelp loss and multiple pollutants (i.e. CO_2 and nutrients), with these treatments resulting in percentage covers (Figure 2; mean ± SE; 69.25±5.88%) which cannot be predicted from the independent effects of kelp in the absence of elevated CO_2 and nutrients (i.e. kelp absent – present = 23.50%), future CO_2 in the absence of kelp and elevated nutrients (i.e. future CO_2 – ambient $CO_2 = -5.00\%$) and elevated nutrients in the absence of kelp and elevated CO_2 (i.e. elevated nutrients – ambient nutrients = 14.67%). Elevated CO_2 alone had no detectable effect in the absence of kelp, but caused greater covers of turfs when combined with elevated nutrients (Figure 2; Table S2; SNK test of Kelp×Nutrient×CO_2 interaction). The treatment of largest influence was the presence or absence of kelp canopies ($\omega^2 = 0.78$; Table S2). Canopies of kelp restricted the cover of turf to an average of 19.84% less than 'ambient conditions', and 54.76% less than the combination of elevated CO_2 and nutrients (Figure 2), demonstrating the strong competitive effects of kelp over turfs under both ambient and forecasted conditions.

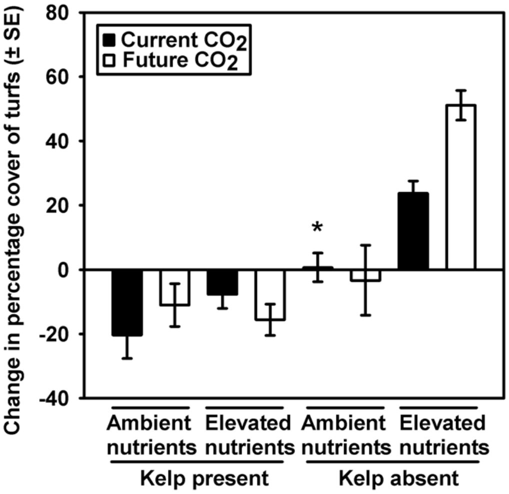

Figure 1. The change in percentage cover of turf-forming algae (final – initial measurement) that were transplanted from ambient conditions* to different combinations of Kelp (present *v.* absent), Nutrients (ambient *v.* elevated) and CO_2 (current *v.* future). * Ambient conditions = turfs growing in canopy gaps under ambient concentrations of nutrients and CO_2.
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Importantly, this competitive effect was consistent across the treatments of elevated CO_2 and nutrients both when they were manipulated in isolation and combination (Figure 2; Table S2; SNK tests).

The removal of kelp and elevation of CO_2 and nutrients had positive effects on the dry mass of turf, with the greatest mass (0.07 ± 0.02 g) when they were manipulated in combination (Figure 3; Table S3). While the presence or absence of kelp was the treatment of largest influence ($\omega^2 = 0.54$; Table S3), nutrients and the kelp×nutrient term also contributed strongly ($\omega^2 = 0.15$ for both; Table S3). Kelp and nutrients interacted such that the mass of turf was greater under elevated than ambient nutrient conditions, with this effect restricted in the presence of kelp (Table S3; SNK of significant Kelp×Nutrient interaction).

Water column physicochemical parameters

The concentration of ammonia, phosphate and NO_X (nitrate+nitrite) quantified in the field mesocosms was significantly higher in elevated (mean ± SE; ammonia 0.0406 ± 0.0025 mg L^{-1}, phosphate 0.0091 ± 0.0002 mg L^{-1}, NO_X 0.0060 ± 0.0002 mg L^{-1}) than ambient nutrient treatments (ammonia 0.0296 ± 0.0021 mg L^{-1}, phosphate 0.0079 ± 0.0001 mg L^{-1}, NO_X 0.0054 ± 0.0002 mg L^{-1}) (Table S4, S5; Figure S1a, c, e). These relatively small differences (e.g. $NO_X < 0.0001$ mg L^{-1}) indicate the elevated nutrients were being used by the algae. This interpretation is supported by the additional laboratory-based mesocosm trials, testing the effects of nutrient enrichment in the absence of algae. That is, the measurable concentrations of nutrients in the elevated nutrient treatments were substantially greater (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}) (Table S4, S5; Figure S1b, d, f).

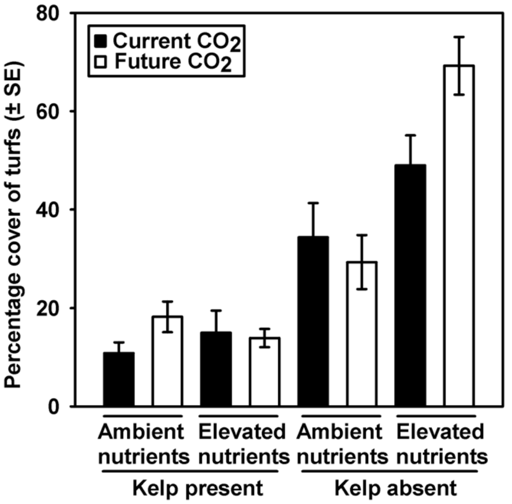

Figure 2. The final percentage cover of turf-forming algae that were transplanted from ambient conditions (as defined in Figure 1) to different combinations of Kelp (present *v.* absent), Nutrients (ambient *v.* elevated) and CO_2 (current *v.* future).
doi:10.1371/journal.pone.0033841.g002

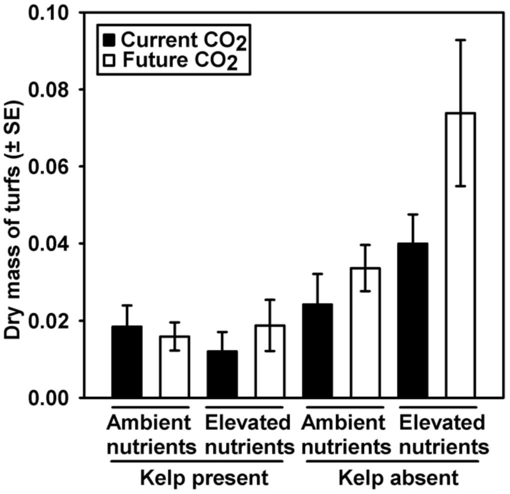

Figure 3. The dry mass of turf-forming algae on natural rock substrate that were transplanted from ambient conditions (as defined in Figure 1) to different combinations of Kelp (present *v.* absent), Nutrients (ambient *v.* elevated) and CO_2 (current *v.* future).
doi:10.1371/journal.pone.0033841.g003

TA, pCO_2, and HCO_3^- were increased in treatments where CO_2 was experimentally elevated (Table S4, S5; Figure S2b, c, d), while pH was reduced under future CO_2 compared with current CO_2 conditions (Table S4, S5; Figure S2a). Light was reduced where kelp were present (70.34 ± 11.15 µmol m^{-2} s^{-1}) compared to where they were absent (1316.44 ± 59.57 µmol m^{-2} s^{-1}) (Table S4, S5). Temperature was not significantly different among treatments (Table S4, S5).

Discussion

Over 30 years ago, Harrison [34] suggested that there was a need to understand not only the behavior of a community under 'normal or good conditions', but also its response to unusual or stressful conditions. Since then, research considering the effects of stressful conditions created by human activities has often focused on identifying the community response to highly-modified conditions (e.g. [35,36]). A more pressing contemporary concern, however, is whether moderate near-term alterations will be of a sufficient magnitude to drive changes in community interactions. Potential exists that near-term future conditions may reduce the capacity of foundation species to suppress competitors whose expansion would otherwise cause communities to shift to, and be maintained in, a contrasting state (e.g. [19]). Whilst severe pollution, such as nutrient conditions associated with urban coasts [10], is known to reduce the capacity of kelp forests to recover from disturbance (i.e. resilience) [19], intact kelp forests may be quite stable in the face of similar sets of stressors, of a lesser magnitude, such as coasts associated with agriculture [10]. Although near-term forecasted environmental conditions are anticipated to facilitate competitors and increase the probability of loss of foundation species (e.g. the strong positive synergistic effect of increasing nutrient and CO_2 concentrations on turf [16]), the current study suggests that where kelp canopies are retained their mere presence may be sufficient to continue to suppress a key competitor (e.g. turfs), despite the synergistic effects of moderate elevation of local (i.e. elevated nutrients) and global pollutants (i.e. forecasted CO_2). As the conditions that promote community resistance may be different from those that favour resilience, recognizing the factors that affect persistence rather than recovery could assist in forecasting their effects on these normally robust and diverse natural systems [37].

The synergistic responses of kelp competitors to multiple pollutants (i.e. turf response to $CO_2 \times$nutrients ([16], this study), supports the model that multiple stressors can combine to produce conditions which increase the likelihood of phase-shifts [38]. Consequently, researchers have been increasing their focus to identify those sets of stressors which combine to produce effects that cannot be anticipated by adding their isolated effects [39]. The frequency and magnitude of non-additive responses are surprisingly common, to the extent that our concept of resource limitation has shifted from an earlier paradigm of single-resource limitation [40] towards that of co-limitation by multiple resources [41,42]. While 'limitation' can be experimentally recognised by changing the rate of processes through addition or reduction of the single relevant factor, 'co-limitation' is recognised as the greater response to simultaneous enrichment of multiple factors than would be expected from the sum of their individual responses [42]. The repeated observation of an interaction between CO_2 and nutrients ([16], this study) indicates nutrients are not available in great excess relative to CO_2, as a modest addition of CO_2 quickly produces a limitation on nutrients. It also appears CO_2 is not in great excess relative to nutrients, as an addition of nutrients quickly provokes a limitation on CO_2. When CO_2 and nutrients are added together, CO_2 and nutrient limitation may alternate in numerous small incremental steps, ultimately producing a synergistic effect. This model may account for the observed synergy between CO_2 and nutrients in a similar way Davidson and Howarth [43] account for the prevalence of nitrogen and phosphorous interactions [44]. Whilst this synergy would appear relevant for canopy-gaps or locations experiencing canopy loss, it is less likely to be relevant in disrupting the persistence of intact kelp forests

The mechanisms that allow kelp to suppress their competitors under conditions that would otherwise facilitate their spread may be useful to understand. Quantification of physiochemical conditions within the experimental mesocosms indicates that the mechanism driving kelp inhibition is alteration of the physical (i.e. shading) rather than chemical (i.e. nutrient or carbonate) conditions experienced by understorey species. The presence of kelp did not appear to modify either the nutrient status (i.e. ammonia, phosphate, NO_X) or carbonate chemistry of water within the mesocosms (i.e. pH, TA, pCO_2, HCO_3^-; see also Figure S3 for diurnal pH variation). We suspect, however, that the accelerated growth of turf in the absence of kelp is likely to obscure this potential effect by utilising the relatively moderately elevated nutrients. On biomass basis, turfs are naturally more productive (i.e. 44–77%) than surrounding canopy-forming algae in this system [45]. We consider that shading by kelp canopies provides a more powerful explanation of the suppression of turfs. This explanation is derived from classical experiments showing the effects of canopy-shade on understorey communities [46] and covers of turfs [17,47]. Where perennial canopy species are removed, algae adapted to high light conditions, such as turfs, are then able to utilise the increased light to expand their covers [46,48]. In contemporary algal assemblages the presence of intact kelp canopies reduces light reaching the substratum to a similar extent as that which was observed in our experimental mesocosms (i.e. a ~95% reduction) [17,47].

The retention of populations of foundation species seems critical in ensuring maintenance of the primary mechanism that enables the continued dominance of kelp over its competitors, in this case shading. We do, however, recognise that this conclusion is based on the assumption that communities will remain intact, maintaining the strength of interactions, a particularly important assumption for assemblages whose structure is determined by a small number of interactions centred on a single foundation species [49]. The biotic factors that influence shading tend to vary, especially when the impacts of human activities, such as canopy removal, are considered [50]. While the delivery of light flecks to the understorey during canopy movement appears important in maintaining understorey productivity, when large amounts of light become available, such as when entire plants are removed from the substratum and a gap in the canopy is produced, the influence of the canopy may be reduced and persistence of ecosystems disrupted [51]. For example, as kelp canopies are thinned, reduced in size or fragmented, the associated environmental conditions (including light) become more similar to those experienced outside the canopy [52]. Under these conditions, turfs can expand to dominate space in assemblages and inhibit the recruitment of kelp [19,23], leading to phase-shifts over multiple generations [53].

Key species can maintain ecosystem composition through strong interactions that are often self-stabilising because they create conditions that facilitate the persistence of entire ecosystems [54]. Given that species interactions are often mediated by environmental conditions [55,56], human activities which modify the abiotic environment have the potential to disrupt these interactions and alter the species composition of ecosystems [7,15]. Where strong interactions maintain community structure by retarding the effects of environmental forcing, management of key species may assist in the retention of communities, even under forecasted global conditions (i.e. large-scale pollution and climate change).

In conclusion, our results show the interaction between kelp and turf may be maintained under near-term future conditions, indicating the retention of intact forests may reduce the effect of moderate pollutant enrichment in these communities. Many communities are governed by a few strong interactions (e.g. presence of kelp forests) which exert disproportionately strong community-wide effects [3]. The maintenance of intact populations of foundation species may enable these habitats to persist despite forecasted climates that would otherwise appear to increase the probability of their loss.

Supporting Information

Table S1 ANOVA testing the combined effect of Kelp (present *v.* absent), Nutrients (ambient *v.* elevated) and CO_2 (current *v.* future) on the change in percentage covers of turf-forming algae.
(TIF)

Table S2 ANOVA testing the combined effect of Kelp (present *v.* absent), Nutrients (ambient *v.* elevated) and CO_2 (current *v.* future) on the final percentage covers of turf-forming algae.
(TIF)

Table S3 ANOVA testing the combined effect of Kelp (present *v.* absent), Nutrients (ambient *v.* elevated) and CO_2 (current *v.* future) on the final weight per area of turf-forming algae.
(TIF)

Table S4 Physicochemical parameters of mesocosms measured in the field ($n = 9$) and the laboratory ($n = 3$) for each treatment. Reported are means, standard errors (S.E.),

maximum and minimum values. Field ammonia, phosphate and NO_X were sampled weekly on six occasions, with laboratory-based mesocosms sampled on alternate days ($n = 20$ occasions). Total Alkalinity (TA), pH and temperature were simultaneously measured weekly on eight occasions, from which concentrations of pCO_2 (ppm) and bicarbonate (HCO_3^-) (μmol kg^{-1}) were calculated using constants from Mehrbach et al. [28], as adjusted by Dickson and Millero [29]. Light was measured on one occasion.
(TIF)

Table S5 Results from ANOVA, testing the combined effect of Kelp (present *v.* absent), Nutrients (ambient *v.* elevated) and CO_2 (current *v.* future) on the 9 physico-chemical parameters measured in the field and effect of Nutrients (ambient *v.* elevated) on the 3 measured in the laboratory. Field ammonia, phosphate and NO_X were sampled weekly on six occasions, with laboratory-based mesocosms sampled on alternate days ($n = 20$ occasions). Total Alkalinity (TA), pH and temperature were simultaneously measured weekly on eight occasions, from which concentrations of pCO_2 (ppm) and bicarbonate (HCO_3^-) (μmol kg^{-1}) were calculated using constants from Mehrbach et al. [28], as adjusted by Dickson and Millero [29]. Light was measured on one occasion.
(TIF)

Figure S1 Nutrient concentrations within field (a, c, e) and laboratory (b, d, f) based mesocosms measured from beginning to end of the experiment. Ammonia (a, b), phosphate (c, d) and NO_X (e, f) under ambient nutrients (filled circles) and elevated nutrients (empty circles). Data presented are means across CO_2 and kelp treatments. Note the different scales on the y-axes.
(TIF)

Figure S2 Carbonate chemistry parameters in field-based experimental mesocosms measured weekly from beginning to end of the experiment. pH (a), TA (b), pCO_2 (c), HCO_3^- (d) in mesocosms under current CO_2 (filled circles) and future CO_2 (empty circles). Total Alkalinity (TA) and pH were measured weekly on eight occasions, from which concentrations (μmol kg^{-1}) of pCO_2, and bicarbonate (HCO_3^-) were calculated. Values were calculated from measured TA and pH using constants from Mehrbach et al. [28], as adjusted by Dickson and Millero [29]. Data presented are means across different nutrient and kelp treatments.
(TIF)

Figure S3 A representative diurnal cycle (Oct 9–10, 2009; 0630-0630) of pH for all treatment combinations. CCO_2, current CO_2; FCO_2, future CO_2; KP, kelp present; KA, kelp absent; AN, ambient nutrients; EN, elevated nutrients.
(TIF)

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Author Contributions

Conceived and designed the experiments: LJF BDR SDC. Performed the experiments: LJF. Analyzed the data: LJF. Contributed reagents/materials/analysis tools: BDR SDC. Wrote the paper: LJF BDR SDC.

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3.10 SUPPORTING INFORMATION

Table S3.1 The combined effect of Kelp (present v. absent), Nutrients (ambient v. elevated) and CO_2 (current v. future) on the change in percentage covers of turf-forming algae

Source	df	MS	F	P	ω^2
Kelp	1	6016.67	51.17	***	0.53
Nutrients	1	2741.34	23.31	***	0.24
CO_2	1	231.26	1.97	ns	0.01
Kelp × Nutrients	1	1802.67	15.33	***	0.15
Kelp × CO_2	1	181.50	1.54	ns	0.01
Nutrients × CO_2	1	75.26	0.64	ns	0.00
Kelp × Nutrients × CO_2	1	888.17	7.55	*	0.07
Residual	16	117.59			

ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$.

The magnitude of effects (ω^2) were calculated for each of the three factors tested (Vaughan & Corballis 1969; Graham & Edwards 2001).

Table S3.2 The combined effect of Kelp (present v. absent), Nutrients (ambient v. elevated) and CO_2 (current v. future) on the final percentage covers of turf-forming algae

Source	df	MS	F	P	ω^2
Kelp	1	5760.83	81.64	***	0.78
Nutrients	1	1111.57	15.75	***	0.14
CO_2	1	174.24	2.47	ns	0.01
Kelp × Nutrients	1	1122.95	15.91	***	0.01
Kelp × CO_2	1	30.00	0.43	ns	0.00
Nutrients × CO_2	1	105.56	1.50	ns	0.01
Kelp × Nutrients × CO_2	1	426.45	6.04	*	0.05
Residual	16	70.56			

ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$.

The magnitude of effects (ω^2) were calculated for each of the three factors tested (Vaughan & Corballis 1969; Graham & Edwards 2001).

Table S3.3 ANOVA testing the combined effect of Kelp (present v. absent), Nutrients (ambient v. elevated) and CO_2 (current v. future) on the final weight per area of turf-forming algae.

Source	df	MS	F	P	ω^2
Kelp	1	0.0043	19.89	***	0.54
Nutrients	1	0.0013	5.98	*	0.15
CO_2	1	0.0011	5.04	*	0.12
Kelp × Nutrients	1	0.0013	5.84	*	0.15
Kelp × CO_2	1	0.0005	2.47	ns	0.04
Nutrients × CO_2	1	0.0003	1.27	ns	0.01
Kelp × Nutrients × CO_2	1	0.0001	0.46	ns	0.00
Residual	16	0.0002			

ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$.

The magnitude of effects (ω^2) were calculated for each of the three factors tested (Vaughan & Corballis 1969; Graham & Edwards 2001).

Table S3.4 Physicochemical parameters of mesocosms measured in the field ($n = 9$) and the laboratory ($n = 3$) for each treatment.

Physicochemical parameter		Treatment	Mean (S.E.)	Max	Min
Ammonia (mg L^{-1})	i) In field	CCO_2, KP, AN	0.0260 (0.0051)	0.0810	0.0050
		CCO_2, KP, EN	0.0355 (0.0046)	0.0735	0.0083
		CCO_2, KA, AN	0.0262 (0.0041)	0.0650	0.0010
		CCO_2, KA, EN	0.0371 (0.0054)	0.0810	0.0111
		FCO_2, KP, AN	0.0298 (0.0034)	0.0550	0.0068
		FCO_2, KP, EN	0.0468 (0.0059)	0.1010	0.0160
		FCO_2, KA, AN	0.0366 (0.0039)	0.0815	0.0135
		FCO_2, KA, EN	0.0408 (0.0031)	0.0695	0.0209
	ii) In lab	Without nutrients	0.0346 (0.0053)	0.1210	0.0020
		With nutrients	0.2652 (0.0320)	0.6230	0.0110
Phosphate (mg L^{-1})	i) In field	CCO_2, KP, AN	0.0082 (0.0003)	0.0104	0.0051
		CCO_2, KP, EN	0.0094 (0.0003)	0.0120	0.0074
		CCO_2, KA, AN	0.0077 (0.0002)	0.0088	0.0065
		CCO_2, KA, EN	0.0088 (0.0003)	0.0110	0.0063

		FCO_2, KP, AN	0.0080 (0.0002)	0.0102	0.0067
		FCO_2, KP, EN	0.0093 (0.0003)	0.0120	0.0067
		FCO_2, KA, AN	0.0077 (0.0003)	0.0110	0.0051
		FCO_2, KA, EN	0.0088 (0.0004)	0.0130	0.0069
	ii) In lab	Without nutrients	0.0272 (0.0033)	0.0600	0.0010
		With nutrients	0.1285 (0.0068)	0.2870	0.0510
NO_X (mg L^{-1})	i) In field	CCO_2, KP, AN	0.0058 (0.0004)	0.0082	0.0028
		CCO_2, KP, EN	0.0056 (0.0003)	0.0079	0.0043
		CCO_2, KA, AN	0.0053 (0.0004)	0.0085	0.0031
		CCO_2, KA, EN	0.0062 (0.0004)	0.0095	0.0036
		FCO_2, KP, AN	0.0060 (0.0004)	0.0086	0.0031
		FCO_2, KP, EN	0.0064 (0.0004)	0.0103	0.0039
		FCO_2, KA, AN	0.0049 (0.0003)	0.0067	0.0033
		FCO_2, KA, EN	0.0057 (0.0003)	0.0088	0.0035
	ii) In lab	Without nutrients	0.1222 (0.0050)	0.2080	0.0630
		With nutrients	0.3796 (0.0255)	0.6230	0.1010

pH	CCO$_2$, KP, AN	8.15 (0.03)	8.53	8.02
	CCO$_2$, KP, EN	8.15 (0.01)	8.24	8.05
	CCO$_2$, KA, AN	8.10 (0.01)	8.21	8.04
	CCO$_2$, KA, EN	8.17 (0.02)	8.33	8.04
	FCO$_2$, KP, AN	7.91 (0.01)	8.2	7.75
	FCO$_2$, KP, EN	7.95 (0.01)	8.1	7.73
	FCO$_2$, KA, AN	7.95 (0.02)	8.13	7.84
	FCO$_2$, KA, EN	7.92 (0.02)	8.02	7.70
TA (µmol kg^{-1})	CCO$_2$, KP, AN	2298 (53)	2723	1848
	CCO$_2$, KP, EN	2196 (46)	2529	1897
	CCO$_2$, KA, AN	2209 (58)	2626	1848
	CCO$_2$, KA, EN	2194 (52)	2626	1848
	FCO$_2$, KP, AN	2575 (123)	4571	2091
	FCO$_2$, KP, EN	2488 (96)	4134	2091
	FCO$_2$, KA, AN	2307 (50)	2723	1945
	FCO$_2$, KA, EN	2372 (88)	3647	1945
pCO$_2$ (ppm)	CCO$_2$, KP, AN	307 (15)	412	133
	CCO$_2$, KP, EN	276 (5)	382	222
	CCO$_2$, KA, AN	317 (15)	393	224
	CCO$_2$, KA, EN	267 (18)	412	157
	FCO$_2$, KP, AN	647 (25)	975	352
	FCO$_2$, KP, EN	545 (26)	1040	335
	FCO$_2$, KA, AN	504 (26)	767	336
	FCO$_2$, KA, EN	574 (37)	1090	399
HCO$_3^-$ (µmol kg^{-1})	CCO$_2$, KP, AN	1773 (37)	2073	1282
	CCO$_2$, KP, EN	1700 (31)	1916	1545
	CCO$_2$, KA, AN	1754 (53)	2120	1470
	CCO$_2$, KA, EN	1682 (55)	2093	1366
	FCO$_2$, KP, AN	2208 (95)	3924	1801
	FCO$_2$, KP, EN	2104 (80)	3575	1770
	FCO$_2$, KA, AN	1950 (45)	2387	1641
	FCO$_2$, KA, EN	2030 (77)	3095	1655

Light (µmol m^{-2} s^{-1})	CCO_2, KP, AN	93.26 (21.92)	150.21	49.91
	CCO_2, KP, EN	47.51 (13.03)	82.26	11.18
	CCO_2, KA, AN	1285.02 (79.35)	1503.40	999.50
	CCO_2, KA, EN	1162.21 (265.40)	1569.00	597.60
	FCO_2, KP, AN	85.24 (13.62)	124.40	20.25
	FCO_2, KP, EN	55.37 (24.72)	151.75	16.55
	FCO_2, KA, AN	1426.12 (165.41)	1862.70	911.10
	FCO_2, KA, EN	1392.42 (235.85)	1815.60	587.50
Temperature (°C)	CCO_2, KP, AN	16.1 (0.1)	17.4	14.5
	CCO_2, KP, EN	15.9 (0.1)	17.3	14.6
	CCO_2, KA, AN	15.9 (0.2)	17.3	14.4
	CCO_2, KA, EN	16.1 (0.1)	17.5	14.6
	FCO_2, KP, AN	16.0 (0.1)	17.6	14.5
	FCO_2, KP, EN	16.1 (0.1)	17.4	14.6
	FCO_2, KA, AN	15.8 (0.1)	17.1	14.5
	FCO_2, KA, EN	16.0 (0.1)	17.3	14.4

CCO_2, current CO_2; FCO_2, future CO_2; KP, kelp present; KA, kelp absent; AN, ambient nutrients; EN, elevated nutrients.

Table S3.5 Results of ANOVAs testing the combined effect of Kelp (present v. absent), Nutrients (ambient v. elevated) and CO_2 (current v. future) on the 9 physicochemical parameters measured in the field and effect of Nutrients (ambient v. elevated) on the 3 measured in the laboratory

Physicochemical parameter (response variable)	Kelp		Nutrient		CO_2		Kelp × Nutrient		Kelp × CO_2		Nutrient × CO_2		Kelp × Nutrient × CO_2	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Ammonia	0.65	0.4324	**54.50**	**0.0000**	**28.48**	**0.0001**	2.74	0.1175	0.03	0.8646	0.20	0.6581	3.98	0.0634
Phosphate	2.97	0.1043	**18.72**	**0.0005**	0.03	0.8725	0.08	0.7828	0.03	0.8694	0.04	0.8480	0.00	0.9594
NO_X	3.79	0.0692	**5.08**	**0.0386**	0.01	0.9426	2.66	0.1224	4.12	0.0592	0.36	0.5557	0.57	0.4594
Ammonia (lab)			**1066.79**	**0.0000**										
Phosphate (lab)			**124.69**	**0.0000**										
NO_X (lab)			**382.33**	**0.0000**										
pH	0.05	0.8290	0.98	0.3375	**142.02**	**0.0000**	0.03	0.8604	0.47	0.5027	0.49	0.4923	**4.65**	**0.0466**
TA	2.30	0.1489	0.23	0.6416	**6.64**	**0.0203**	0.48	0.4981	0.91	0.3555	0.06	0.8124	0.02	0.8800
pCO_2	1.38	0.2577	1.14	0.3016	**102.70**	**0.0000**	1.89	0.1887	1.47	0.2436	0.17	0.6873	3.06	0.0994

HCO_3^-	1.89	0.1886	0.42	0.5275	**23.93**	**0.0002**	0.38	0.5453	1.23	0.2835	0.15	0.7033	0.37	0.5491
Light	**154.15**	**0.0000**	0.33	0.5712	0.85	0.3692	0.04	0.8429	0.86	0.3685	0.07	0.7970	0.03	0.8576
Temperature	0.76	0.3974	0.55	0.4691	0.07	0.8014	1.84	0.1941	0.85	0.3709	0.24	0.6336	0.55	0.4691

For field-based measurements three-factor ANOVAs with tanks (n = 3) as replicates; for laboratory-based measurements two-factor ANVOAs with tanks (n = 5) as replicates. Bold values indicate significance (at $p < 0.05$ level).

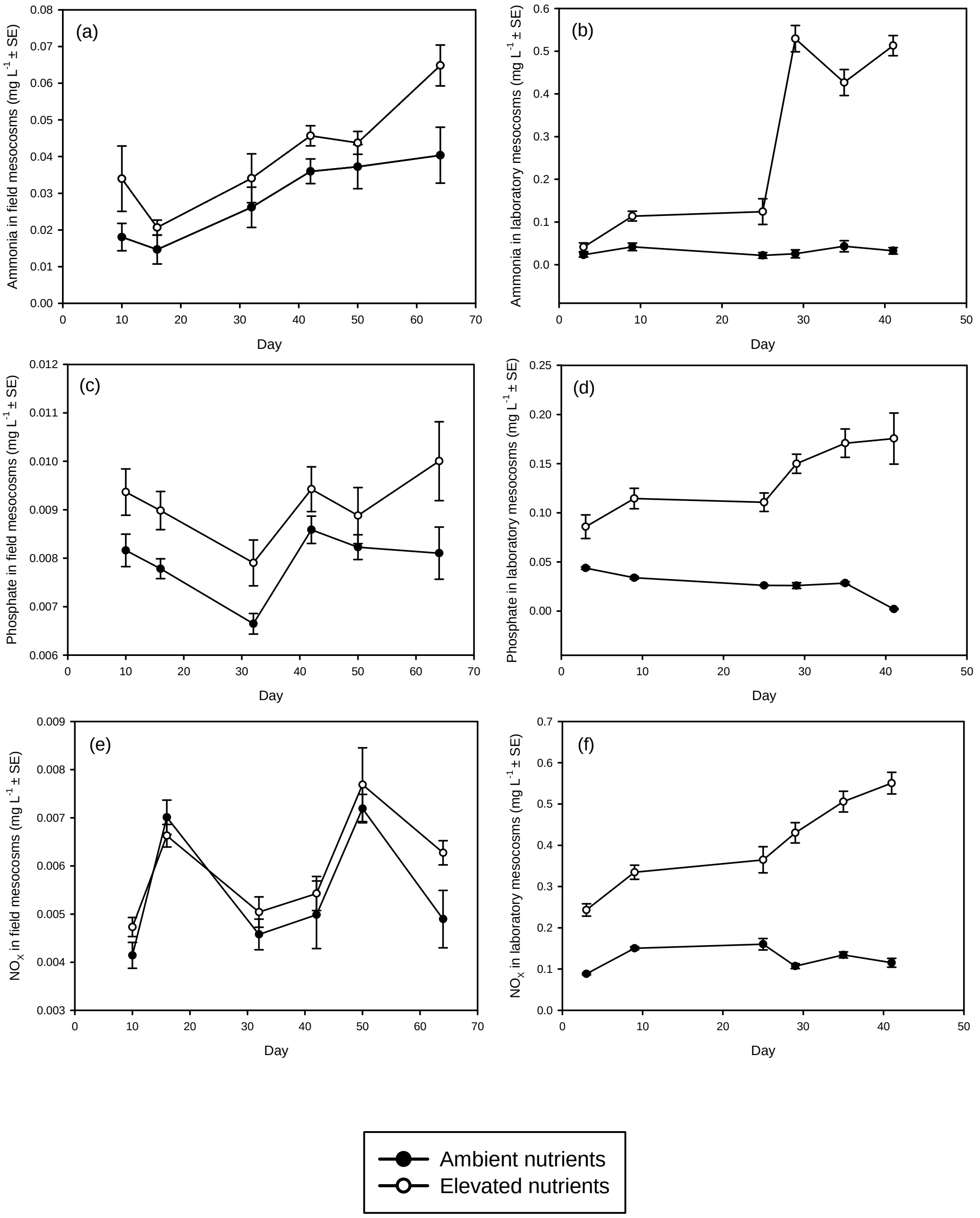

Figure S3.1 Nutrient concentrations within field (a, c, e) and laboratory (b, d, f) based mesocosms measured from beginning to end of the experiment

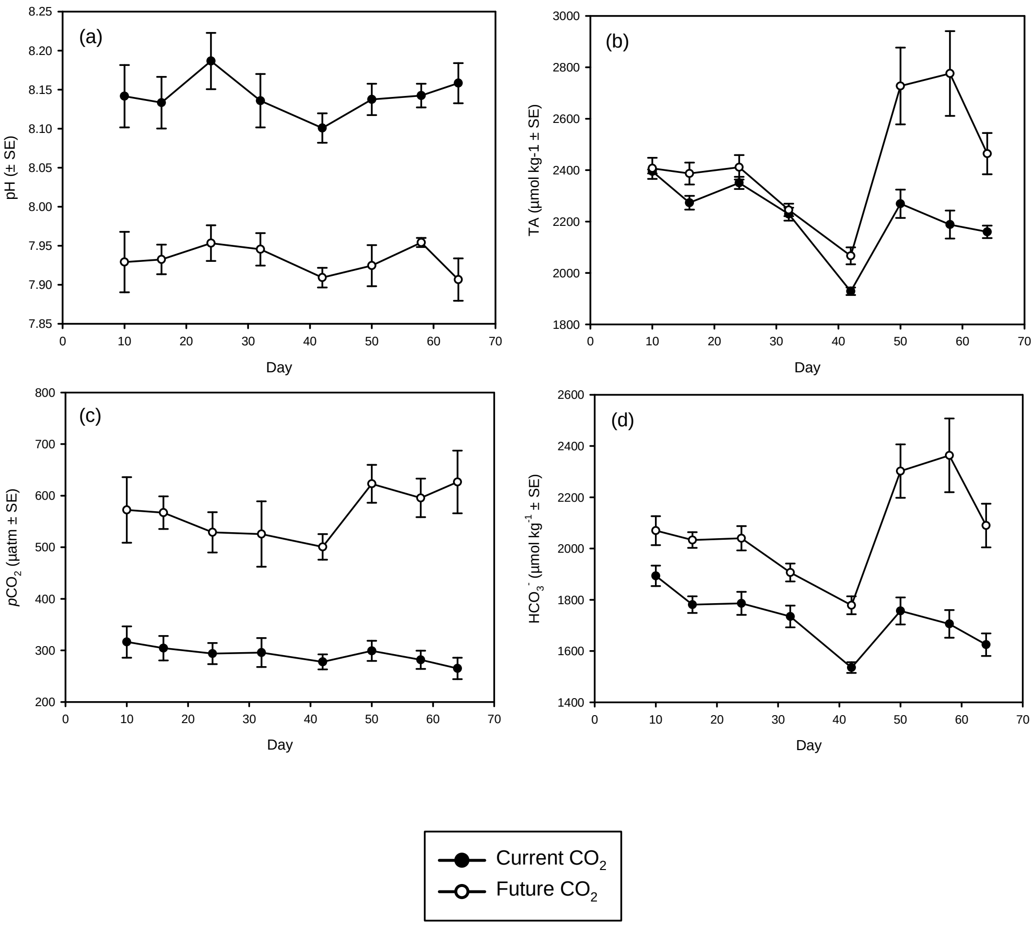

Figure S3.2 Carbonate chemistry parameters in field-based experimental mesocosms measured weekly from beginning to end of the experiment.

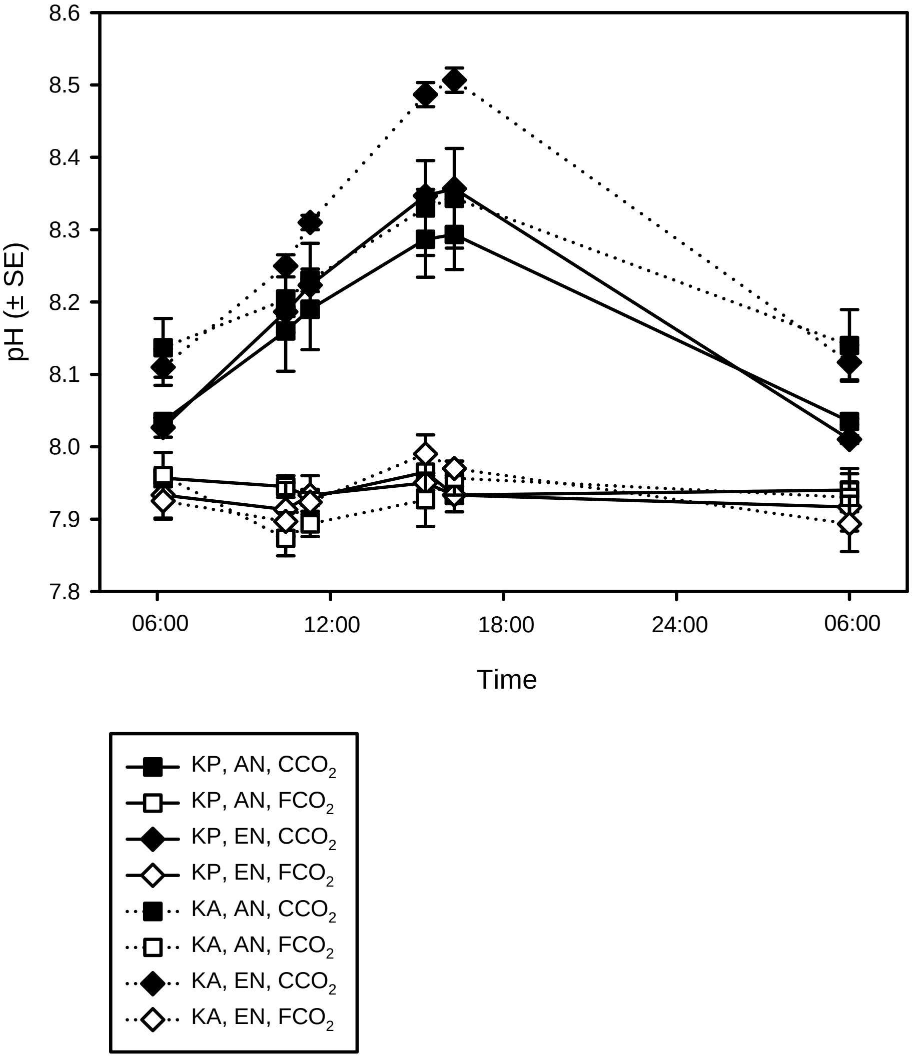

Figure S3.3 A representative diurnal cycle (Oct 9-10, 2009; 0630-0630) of pH for all treatment combinations

A representation of the varied conditions experienced at the Cruising Yacht Club of South Australia. These photos were taken on the 17th and 20th of October 2010.

Photos: Author.

CHAPTER 4

CHAPTER 4

PREAMBLE

Chapter 4 is a co-authored paper which, at the time of printing, was under peer-review with the journal *Marine Ecology Progress Series*, with myself as senior author and Bayden D. Russell and Sean D. Connell as co-authors. It is, therefore, written in plural throughout and uses the journal formatting.

In this chapter I conducted the study, collected and analysed the data and wrote the manuscript. Sean Connell and Bayden Russell provided funding and assisted with intellectual development, including the initial concept of the study and manuscript evaluation.

Signatures of co-authors:

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CHAPTER 4

FUTURE HERBIVORY: THE INDIRECT EFFECTS OF ENRICHED CO_2 MAY RIVAL ITS DIRECT EFFECTS

4.1 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 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.

4.2 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 temperatures (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 molluscs may include alterations to activity patterns, particularly feeding activity (with observed responses including both suppression: Siikavuopio et al. 2007, and expansion: Li & Gao 2012). 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, twofold; (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.

4.3 MATERIALS AND METHODS

4.3.1 EXPERIMENTAL DESIGN AND SET-UP

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, feeding experiments were conducted in field-based mesocosm experiments. The experimental mesocosms utilised were acrylic (A-cast acrylic, Asia Poly, Kuala Lumpur, Malaysia) 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 (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 (i.e. before experimental manipulation) was characteristic of these waters (Falkenberg et al. 2012).

4.3.2 EXPERIMENTAL TREATMENT: CO_2 ADDITION

The target CO_2 levels were based on the current ambient (contemporary; 350 – 450 ppm) and the IS92a model scenario for atmospheric CO_2 concentrations in the year 2075 (enriched; 700 – 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 (measured; mean ± SE: 7.94 ± < 0.01, see Table 4.1 for detail). 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, Heinsberg, Germany). 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, Woonsocket, RI, USA) and calibrated against a Dickson reference material (Scripps Institution of Oceanography, University of California, San Diego, CA, USA) with a known A_T of mean ± S.E. 2233.32 ± 0.90 µmol kg^{-1} this colorimetric method returning

2253.19 ± 42.89 µmol kg^{-1}. Concentrations of pCO_2, carbonate (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, CO, USA).

4.3.3 EXPERIMENTAL GRAZERS AND ALGAE

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, South Australia (35.38007 ° S, 138.06502 ° E). The grazers were placed in holding mesocosms (separate to experimental algae) for two weeks to enable acclimation to mesocosm conditions. They were then randomly reassigned to experimental mesocosms ($n = 20$ individuals per mesocosm) and maintained in these treatments (contemporary vs. enriched CO_2) for three months (March – June 2010) prior to feeding experiments. Grazer size was not different among treatments (mass, mean ± S.E., 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 – 3 m depth near Horseshoe Reef, South Australia (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 (i.e. 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 two 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 eight weeks before the experiment commenced to enable acclimation to conditions in the mesocosms. Following this acclimation period, five specimens of turf were randomly assigned to each experimental mesocosm in which conditions were gradually altered over a further two week period until they reached pre-designated experimental levels. The specimens were then exposed to experimental CO_2 (contemporary vs. enriched) conditions for six months (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 three and a half months (March – June 2010). Half of the panels with turf were maintained under experimental conditions to be provided to the grazers in this form, with the other half 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 period used for feeding experiments (i.e. 3 days) revealed that there was little change, and that this change did not vary significantly between treatments (change in mass, mean ± S.E., 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, Maryland, USA) (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 (i.e. 10 hours 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.

4.3.4 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. 4.1, row 1). Algae were presented in one of two forms; either on settlement panels or incorporated into agar suspensions. Each CO_2 treatment was represented by three replicate mesocosms (*n* = 3 mesocosms per treatment), within which five replicate panels and five replicate agar suspensions were each presented to a single grazer; i.e. one 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 (a) the direct effect of CO_2 and associated changes in carbonate chemistry on the grazer or (b) the indirect effect mediated by the algae (Fig. 4.1, row 2a and 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 three replicate mesocosms per CO_2 treatment (*n* = 3 mesocosms per treatment), within which three replicate panels and three agar suspensions were each presented to a single grazer; i.e. one grazer per panel or agar suspension (*n* = 3 feeding arrays for each form of algae per mesocosm).

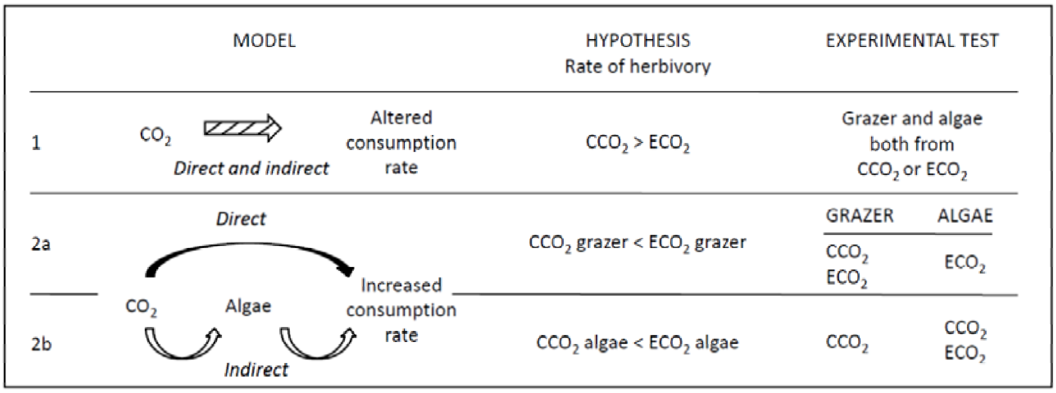


Figure 4.1 The conceptual framework used to assess the effects of enriched CO_2 on the rate of herbivory. From left to right, each column represents the model, hypothesis and experimental test respectively. Row 1 depicts the sequence of logic used to determine whether enriched CO_2 reduces the rate of herbivory. Similarly, Rows 2a and b outline the logic used to distinguish whether the observed change (i.e. 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). Abbreviations: CCO_2 – contemporary CO_2; ECO_2 – enriched CO_2

4.3.5 RESPONSE VARIABLES

In all feeding experiments each grazer was provided with one type of food, either turf on a panel or incorporated into an agar suspension. Grazers provided panels were allowed to feed for 3 days (72 hours), following which time the response was assessed in terms of change in mass of panels and change in percentage 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 beginning and end of the feeding period to the nearest 0.01 g. The change in percentage cover of algae on panels was quantified by visually estimating the percentage 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 hours). Following this time the agar suspensions were 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 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). The tin capsules containing sample material were 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 components of interest were separated (IRMS Hydra 2020 ANCA-GSL Version 4.0, SerCon, Cheshire, UK) such that C and N content could be determined.

4.3.6 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 Analysis of Variance (ANOVA). Analysis of change in mass, change in % cover, number of bites, % C, % N and C:N ratio was undertaken using two-way ANOVA. CO_2 was treated as orthogonal and fixed, with two levels (contemporary vs. enriched), and three 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. 4.1, row 1; Fig. 4.2b) 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 two 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.

4.4 RESULTS

4.4.1 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 percent cover of turf on settlement panels and take bites from agar suspensions at a greater rate than under contemporary CO_2 (Fig. 4.1 and Fig. 4.2; mass: $F_{1,24} = 9.81$, $p = 0.0351$; percent 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 indirect grazer response to the effect of CO_2 enrichment on algae (Fig. 4.1, row 2a and Fig. 4.3). Grazers exposed to contemporary and enriched CO_2 did not differ their rates of herbivory when presented with the same type of algae (Fig. 4.1, row 2a and Fig. 4.3a; mass: $F_{1,12} = 0.01$, $p = 0.9333$; percent 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 percent cover of turf algae that recruited to panels was greater under enriched than contemporary CO_2 treatments (Fig. 4.1 row 2b and Fig. 4.3bi and ii; mass: $F_{1,12} = 8.60$, $p = 0.0427$; percent 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. 4.3biii; $F_{1,12} = 2.00$, $p = 0.2302$)

4.4.2 TURF C:N RATIOS

Enriched CO_2 had a positive effect on the % N in the tissues of turf-forming algae (Fig. 4.4a; $F_{1,24} = 9.90$, $p = 0.0346$). The % C was not observed to be significantly affected by enriched CO_2 (Fig. 4.4b; $F_{1,24} = 0.10$, $p = 0.7625$), and the trend for the C:N ratio to be reduced (Fig. 4.4c) was not significant ($F_{1,24} = 4.44$, $p = 0.1028$).

4.4.3 EXPERIMENTAL CONDITIONS

The pH and concentration of carbonate (CO_3^{2-}) were reduced under enriched CO_2 compared with contemporary CO_2 treatments (Table 4.1; $F_{1,4} = 240.70$, $p = 0.0001$ 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. S4.1 in the supplement), the pH of enriched treatments was consistently lower than that of contemporary treatments. In contrast, the A_T, pCO_2 and bicarbonate (HCO_3^-) increased under enriched experimental conditions (Table 4.1; $F_{1,4} = 16.42$, $p = 0.0155$; $F_{1,4} = 975.38$, $p = < 0.0001$; 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. S4.2 in the supplement).

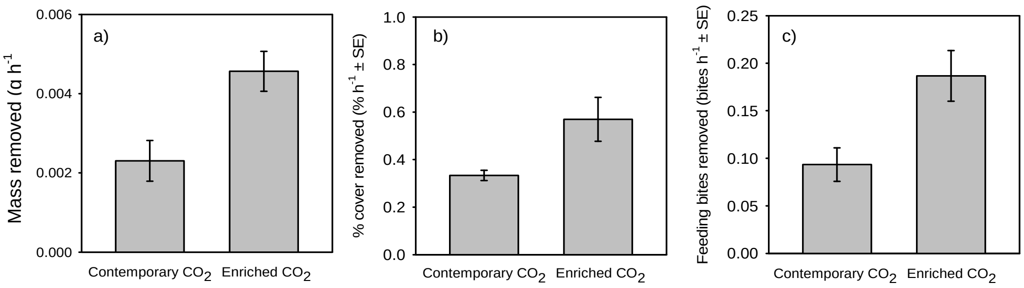


Figure 4.2 The a) mass and b) % cover removed per hour from panels of turf-forming algae and c) number of feeding bites taken per hour from agar suspensions incorporating turf-forming algae when both algae and grazer exposed to the same CO_2 treatment (contemporary vs. enriched) (i.e. Fig.4.1, Row 1).

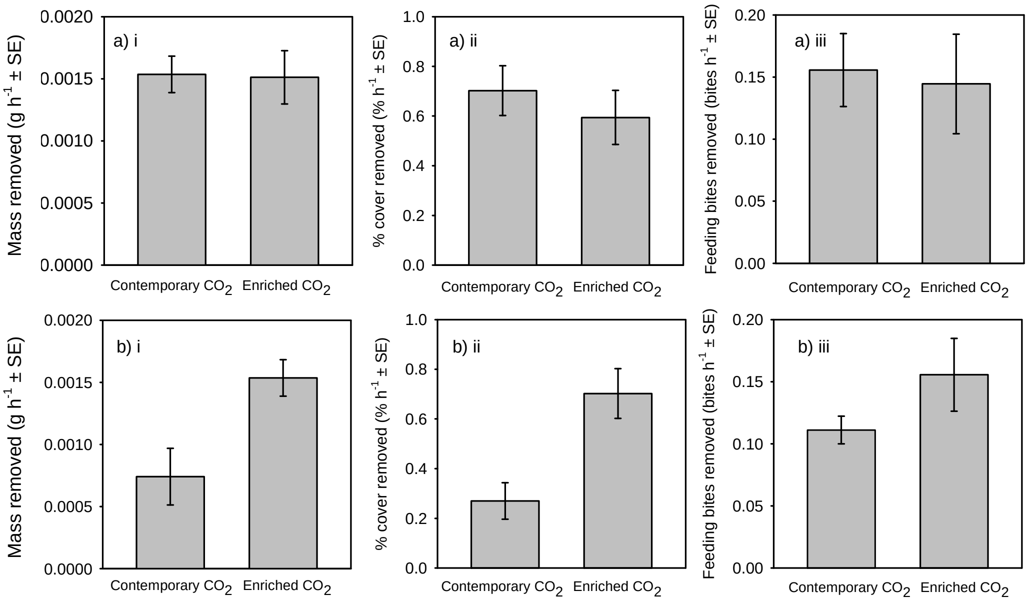


Figure 4.3 The i) mass and ii) % cover removed per hour from panels of turf-forming algae and iii) number of feeding bites taken per hour from agar suspensions incorporating turf-forming algae when a) enriched CO_2 algae was provided to grazers exposed to different concentrations of CO_2 (contemporary vs. enriched) (i.e. Fig. 4.1, Row 2a) and b) contemporary CO_2 grazers were provided with algae exposed to different concentrations of CO_2 (contemporary vs. enriched) (i.e. Fig. 4.1, Row 2b).

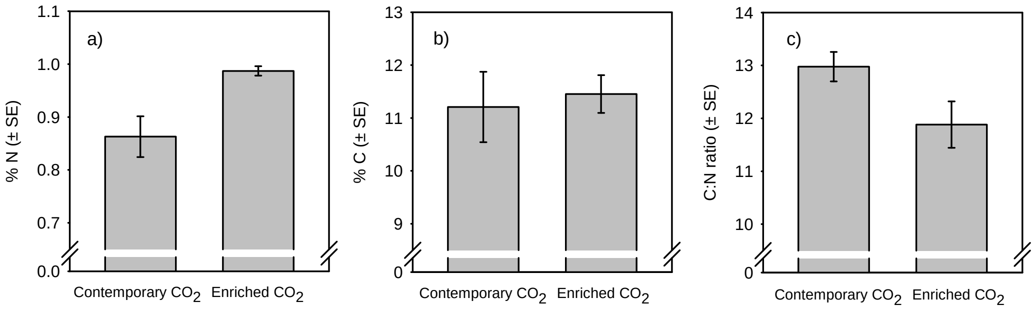


Figure 4.4 The a) % N, b) % C and c) C:N ratio of turf-forming algae recruited under different CO_2 conditions (contemporary vs. enriched).

Table 4.1 Carbonate parameters measured in experimental mesocosms for each treatment. Reported here are means, (and standard errors (S.E.)), maximum and minimum values. Total Alkalinity (A_T) and pH were measured simultaneously on 32 occasions, from which concentrations of pCO_2 (ppm), carbonate (CO_3^{2-}) (μmol kg^{-1}) and bicarbonate (HCO_3^-) (μmol kg^{-1}) were calculated. Values were calculated from measured A_T and pH using constants from Mehrbach *et al.* (1973), as adjusted by Dickson and Millero (1987).

Carbonate parameter	Treatment	Mean (S. E.)	Maximum	Minimum
pH	CCO_2	8.17 (0.01)	8.53	8.00
	ECO_2	7.94 (< 0.01)	8.20	7.54
A_T	CCO_2	2516 (35)	3064	1848
	ECO_2	2709 (33)	4571	2042
pCO_2	CCO_2	454 (12)	665	138
	ECO_2	892 (8)	2337	515
CO_3^{2-}	CCO_2	178 (6)	277	97
	ECO_2	124 (3)	322	55
HCO_3^-	CCO_2	2086 (27)	2585	1502
	ECO_2	2414 (27)	4124	1851

CCO_2, contemporary CO_2; ECO_2, enriched CO_2

4.5 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, change 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 resulting due to enriched CO_2 on grazers, 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.

Early research into the ecological consequences of altered environmental conditions primarily focused 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 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 counterintuitive 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). It is widely believed that brown algae, such as the *Feldmennia* turf considered here, will possess CCMs (Hepburn et al. 2011), with analysis of stable-isotope ratios indicating that this algae 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 (this study; Falkenberg et al. 2013). 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 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 enriched CO_2 will facilitate the algae that inhibits recruitment of kelp (i.e. turfs, Connell & Russell 2010) which have caused kelp losses both regionally (i.e. 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 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 (i.e. CO_2 in marine systems, this 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.

4.6 SUPPLEMENTARY MATERIAL

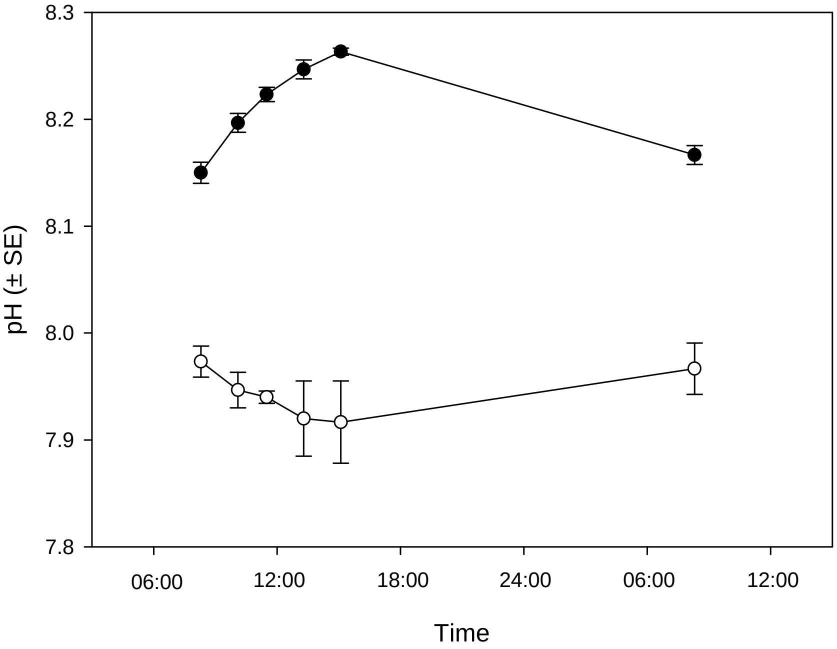


Figure S4.1 A representative diurnal pH cycle (6 – 7 Aug 2009; 0600 – 0600). Filled circles, current CO_2; empty circles, future CO_2

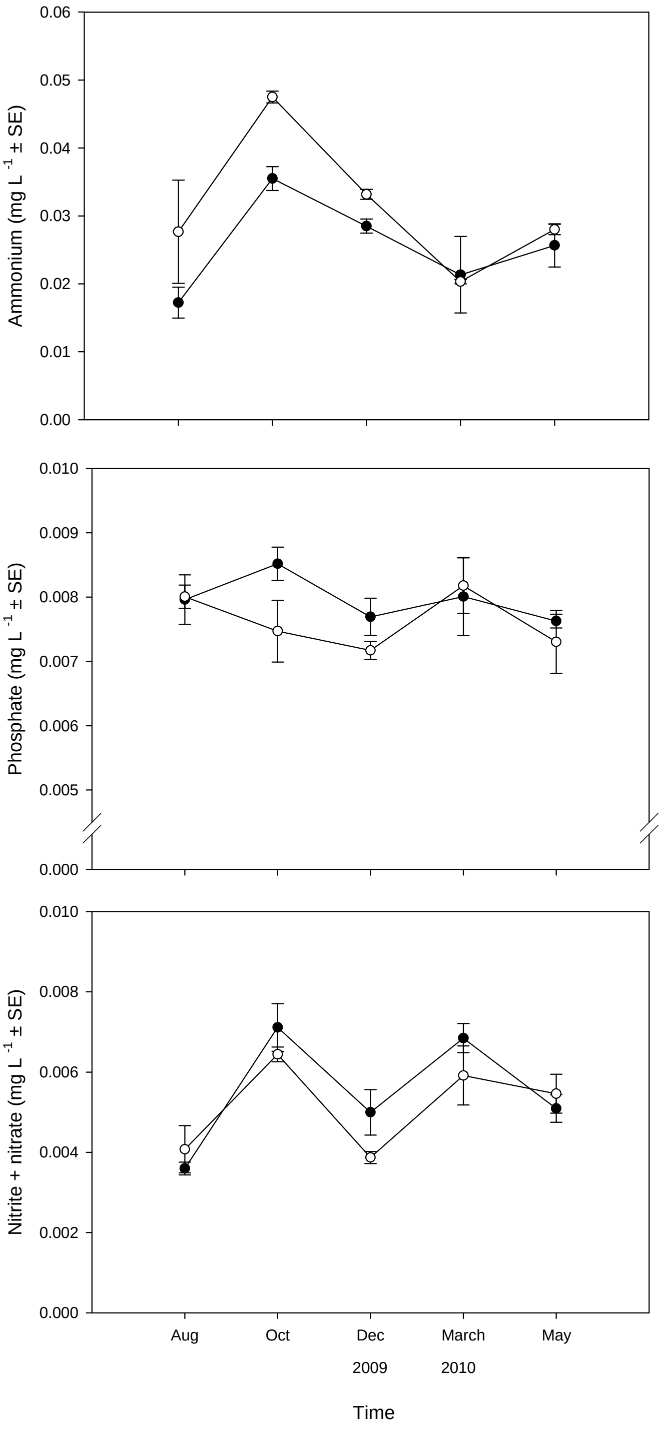
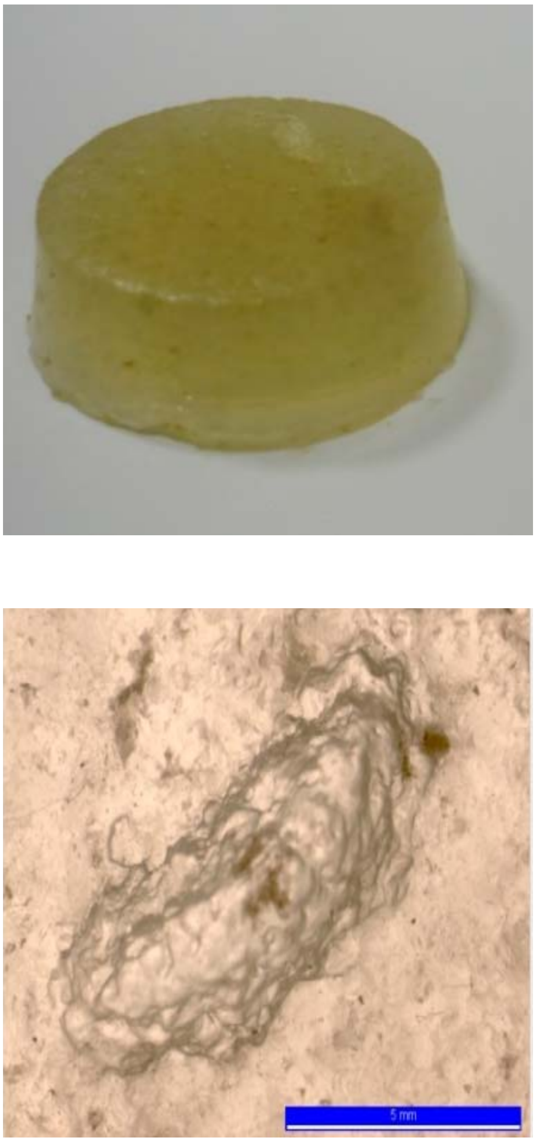


Figure S4.2 Nutrient concentrations (ammonium, phosphate, nitrite + nitrate) within mesocosms measured from beginning to end of the experiment. Note the different scales on the y-axis. Filled circles, current CO_2; empty circles, future CO_2



An agar suspension used (in part) to quantify the feeding rates of *Austrocochlea concamerata* with a feeding mark as it appears to the naked eye (top) and as viewed under a light microscope (bottom).

Photos: Author.

CHAPTER 5

CHAPTER 5

PREAMBLE

Chapter 5 is a co-authored paper published in the journal *Journal of Applied Ecology*, with myself as senior author and Sean D. Connell and Bayden D. Russell as co-authors. It is, therefore, written in plural throughout and uses the journal formatting. It is included with permission from John Wiley and Sons (see Appendix B) and can be cited as:

Falkenberg LJ, Connell SD & Russell BD (2013) Disrupting the effects of synergies between stressors: improved water quality dampens the effects of future CO_2 on a marine habitat. *Journal of Applied Ecology*, **50**, 51-58.

In this chapter I conducted the study, collected and analysed the data and wrote the manuscript. Sean Connell and Bayden Russell provided funding and assisted with intellectual development, including the initial concept of the study and manuscript evaluation.

Signatures of co-authors:

Sean D. Connell

Bayden D. Russell

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Disrupting the effects of synergies between stressors: improved water quality dampens the effects of future CO_2 on a marine habitat

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Summary

1. Synergies among stressors drive unanticipated changes to alternative states, yet little has been done to assess whether alleviating one or more contributing stressors may disrupt these interactions. It would be particularly useful to understand whether the synergistic effects of global and local stressors could be alleviated, leading to slower change or faster recovery, if conditions under the control of local management alone were managed (i.e. nutrient pollution).

2. We utilized field-based mesocosms to manipulate CO_2 (i.e. forecasted global concentrations) and nutrients (i.e. local pollution) to test the hypothesis that, where synergies exist, reducing one contributing stressor would limit the effect of the other. In testing this hypothesis, we considered the response of turfing algae, which can displace kelp forests on urbanized coastlines.

3. Initial manipulations of CO_2 and nutrient enrichment produced an anticipated synergistic effect on the biomass of turfing algae.

4. Following exposure of algal turfs to a combination of enriched nutrients and CO_2, a subsequent reduction in nutrients was able to substantially slow further increases in turf growth. Despite this substantial effect, the historical legacy of previous nutrient enrichment was evident as greater turf was maintained relative to ambient conditions (i.e. ambient CO_2 and nutrients). Such legacies of past stressors may be stubborn (e.g. persist as intergenerational change) where the alternative state (i.e. turf algae) has substantial resilience to restorative actions.

5. *Synthesis and applications*. As stressors accumulate across global to local scales, some combine to produce synergistic effects which cause changes of disproportionate ecological magnitude. While strong synergies attract heavy scrutiny, there remains substantial merit in assessing whether their influence can be ameliorated by managing a contributing stressor. Of note, we show that by reducing a locally determined stressor (nutrients), its synergistic effects with a globally determined stressor (CO_2 enrichment) on a key taxon (turf algae) may be substantially reduced. These results suggest that in the face of changing climate (e.g. ocean acidification), the management of local stressors (e.g. water pollution) may have a greater contribution in determining the dominant state than current thinking allows.

Key-words: carbon dioxide, climate change, habitat loss, nutrients, ocean acidification, pollutants, turf-forming algae

Introduction

Novel environmental conditions created by human activities are, with increasing frequency, transforming ecosystems into new, non-historical habitats (Scheffer *et al.* 2001; Hobbs, Higgs & Harris 2009 and examples within). Many of these seemingly abrupt shifts are prompted as altered environmental conditions push systems over a threshold (or unstable equilibrium) that marks the border between the 'basins of attraction' of alternative states (Holling 1973; Scheffer *et al.* 2001). The newly established habitats, or alternative states, typically comprise species, interactions and functions perceived to be of less 'value' to human societies than those of the system they replaced

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(Dudgeon *et al.* 2010). Subsequent ecological restoration commonly aims to mitigate the numerous abiotic changes which led to transformations (i.e. stressors) such that resilience (i.e. the basin of attraction of the novel state) is reduced and systems are pushed back towards their historical arrangement (Suding, Gross & Houseman 2004; Hobbs, Higgs & Harris 2009; Lotze *et al.* 2011; Morecroft *et al.* 2012). Defining the success of such restoration is, however, complicated as definitions of 'historical' and 'non-historical' habitats are rarely clear due to natural variability in ecosystems and the pervasiveness of human influence (Connell *et al.* 2008; Hobbs, Higgs & Harris 2009). Where human experiences and values lead to the selection of a non-pristine baseline, reaching this management target does not necessarily mean that the system has been restored, but merely that it is improved relative to its novel configuration (Connell *et al.* 2008). Recent experimental work indicates that while restorative actions can enable recovery to the defined historical habitats in some degraded systems, others are resilient to restoration efforts as they have either undergone a shift to an alternative persistent state with a strong basin of attraction or the dynamic equilibrium between alternative states has shifted (Beisner, Haydon & Cuddington 2003; Suding, Gross & Houseman 2004; Lotze *et al.* 2011 and examples within). It is the existence of these alternative states and their resilience to restorative actions which underlie many of the difficulties in anticipating the potential for re-establishment of historical habitats (Beisner, Haydon & Cuddington 2003). The permanence of novel habitats and effectiveness of post-disturbance management in restoring historical habitats are therefore poorly understood areas of applied ecology, representing major challenges for research and management.

The approaches that have most successfully returned systems to their defined historical habitats are typically those which simultaneously ameliorate the multiple stressors that caused the initial transition such that the alternative state's basin of attraction is lessened (Scheffer *et al.* 2001; Beisner, Haydon & Cuddington 2003; Lotze *et al.* 2011). The management of stressors modified over particularly large spatial and temporal scales, however, may be impossible or infeasible (Solomon *et al.* 2009), meaning that only a subset of altered conditions can be effectively managed (Falkenberg *et al.* 2010; Morecroft *et al.* 2012). Due to the irreversibility and persistence of global climate change, these conditions are anticipated to be the backdrop against which any local-scale change, either degradation or restoration, occurs. Interactions between, and among, global and local stressors created by human activities influence transitions to novel habitats (Hobbs, Higgs & Harris 2009; Pettorelli 2012). Further complicating this process is the potential for interactive effects which range from additive (where the response can be predicted based on the effects of individual stressors) to synergistic or antagonistic (where the response is greater or lesser than would be predicted from adding the independent effects of stressors, respectively) (Crain, Kroeker & Halpern 2008; Darling & Côté 2008). While the role of interactive effects in determining transitions to novel habitats has been considered for a number of combinations of stressors and systems (reviewed in Crain, Kroeker & Halpern 2008), less attention has been given to the influence of such interactions on recovery to the historical habitat. As these interactions are anticipated to drive phase-shifts to alternative states that themselves have substantial resistance to change (i.e. a strong basin of attraction), removing one stressor alone may be insufficient to force a transition back to the original state (Scheffer *et al.* 2001). It is coming to be recognized, however, that restoring a system to its historical state may not require reversal of change which drove the initial transition (Suding, Gross & Houseman 2004). In terms of synergies, it has been proposed that the effects of these interactions may be disrupted by restoring a subset of the altered conditions (Russell *et al.* 2009). If such disruption of synergistic interactions is possible, it would indicate the potential for effective local management to facilitate a return to the historical ecosystems despite the irreversibility and persistence of altered global stressors.

Degradation and replacement of historical habitats has occurred in many coastal ecosystems influenced by local-scale human activities, including wetlands, seagrass beds, coral reefs and kelp forests (Bellwood *et al.* 2004; Lotze *et al.* 2006; Airoldi & Beck 2007). Temperate coastlines of southern Australia are typically dominated by canopies of long-lived, topographically complex algae (Connell & Irving 2008). In comparison with this baseline condition, which was widely observed until the 1970s when coastal development was accelerated and is still observed in regional areas where human impacts are negligible, sites in southern Australia impacted by nutrient enrichment are characterized by comprehensive loss of kelp canopies and their replacement by mats of turfing algae (Connell *et al.* 2008; Gorman, Russell & Connell 2009). Such change manifests owing to elevated nutrients which enable the normally ephemeral turfs to persist among fragmented canopies and compete against kelp for space such that recruitment is inhibited (Gorman & Connell 2009) and kelp forests are displaced (Connell & Irving 2008). This persistence and expansion of turfs may be further facilitated by future atmospheric enrichment of CO_2 (Connell & Russell 2010; Falkenberg *et al.* 2010), with the simultaneous enrichment of nutrient and CO_2 pollution anticipated to enable a synergistic increase in the spatial cover and biomass of turf (as identified in Russell *et al.* 2009; Falkenberg, Russell & Connell 2012). Conditions that enhance the potential for turfs to become spatially dominant and reduce the area available for kelp recruitment following its removal by storm events promote transitions from the historical kelp-dominated habitat to the novel one associated with mats of turfing algae. Therefore, where the combination of enriched local (nutrients) and global (CO_2) conditions facilitates a synergistic increase in turfs, restoration of the historical habitat will require feedbacks between turf and these stressors to be broken such that its dominance is reduced and space is again available for kelp recruitment.

The aim of this study was therefore to assess whether the alleviation of a local stressor under local governance could effectively slow or reverse the increase in abundance of taxa that are forecasted to dominate under future climate conditions. Specifically, we wanted to determine whether, following exposure to enriched nutrient and CO_2 conditions, the change in biomass of a species which characterizes the novel habitat on temperate coastlines would be dampened when nutrients were reduced, but CO_2 enrichment was maintained. We hypothesized that where enriched nutrients and CO_2 combined to drive a synergistic increase in turf algae, this effect would be limited by reducing elevated nutrients alone.

Materials and methods

EXPERIMENTAL SITE AND SET-UP

Experimental mesocosms were moored in an open boat harbour protected from the predominant swell by a breakwall adjacent to the Gulf St. Vincent at Outer Harbour, South Australia (34·473395° S, 138·292184° E). The 250-L experimental mesocosms (L × W × H: 0·5 × 0·5 × 1 m; A-cast brand transparent acrylic, Asia Poly, Kuala Lumpur, Malaysia, see Russell, Passarelli & Connell 2011; for spectral properties) were filled with natural seawater pumped directly from the harbour; therefore, initial seawater chemistry (i.e. before experimental manipulation) was characteristic of these waters. During the experimental period, one-third of the seawater was removed from each mesocosm and replaced with fresh seawater weekly to maintain water quality.

EXPERIMENTAL ALGAE

This experiment was conducted using turf-forming algae (see Appendix S1 in Supporting Information for the definition of turf used here) initially collected attached to their natural substratum from rocky reef at Horseshoe Reef, Gulf St. Vincent, South Australia (35·13757° S, 138·46266° E). Samples of turf approximately the same size (5 × 5 cm) were placed in field-based holding mesocosms for 8 weeks before the experiment commenced to enable acclimation to mesocosm conditions.

EXPERIMENTAL DESIGN

The experiment had two key components which together ran for 12 months from August 2009–August 2010. First, we assessed whether enriched CO_2 and nutrients would have a synergistic effect on the biomass of turf algae. To do this, turf algae were subjected to nutrients (ambient: current concentrations adjacent to natural catchments of 0·013 ± 0·001 mg L^{-1} NO_X vs. elevated: concentrations adjacent to urban catchments of 0·232 ± 0·032 mg L^{-1} NO_X; Gorman, Russell & Connell, unpubl. data) and CO_2 (current: current ambient of 280–380 ppm vs. future: IS92a model scenario for 2050 of 550–650 ppm) in a crossed design. Three replicate mesocosms were used per treatment combination, with ten turf samples randomly assigned to each experimental mesocosm following the initial acclimation period. Conditions were then gradually altered (as described in the 'Experimental treatments: nutrient and CO_2 addition' subsection below) over a further 2-week period until they reached pre-designated experimental levels. These initial experimental conditions were maintained for 6 months between August 2009 and February 2010.

In the second phase of the experiment, we assessed whether the change in biomass of algae exposed to the combination of elevated nutrients and future CO_2 would be limited if the local-scale factor of nutrients was reduced, while CO_2 enrichment was maintained. To do this, the specimens initially exposed to elevated nutrients with future CO_2 were reallocated, either to elevated nutrients with future CO_2 (i.e. nutrients maintained) or to ambient nutrients with future CO_2 (i.e. nutrients reduced). In addition, the control treatment of ambient nutrient with current CO_2 was continued to provide a contemporary baseline for biomass under 'ambient' conditions. Three replicate mesocosms were used per treatment combination, with replicate specimens of algal turfs in each mesocosm ($n = 5$). These experimental conditions were then maintained for a further 6 months between February and August 2010.

In addition, to determine how closely change in turf cover in the control mesocosms matched that occurring in the field, we compared turf cover in the contemporary control mesocosms with that in the field throughout the experimental period (details in Appendix S2).

EXPERIMENTAL TREATMENTS: NUTRIENT AND CO_2 ADDITION

Nutrients were enriched to concentrations similar to those experienced in waters off the coast of metropolitan Adelaide (target NO_X: mean ± SE, 0·232 ± 0·032 mg L^{-1}; measured in laboratory; 0·3796 ± 0·0255, see Table S3 for further detail) using Osmocote Plus® (Scotts, Australia) controlled release fertilizer (6-month release: 15, 5, 10 N-P-K). Osmocote pellets (10 g per mesocosm) were placed in a nylon mesh bag (1-mm mesh size) and attached to the bottom of each appropriate mesocosm (i.e. those of elevated nutrients; in the ambient and reduced nutrient treatments, nutrients were simply not added). The concentration of 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) (for results, see Appendix S4 and Table S3, S4). Additionally, to quantify the effect of elevated nutrients in the absence of biota, a trial was conducted whereby 10 mesocosms identical to the field mesocosms were established in the laboratory and maintained for 5 weeks between March and April 2011. Using the same method as in the field, 10 g of Osmocote was added to half of these mesocosms, with water samples regularly collected and analysed from all mesocosms (for results, see Appendix S4 and Table S3, S4).

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 was reduced from ambient (mean ± SE: 8·17 ± 0·02) to the experimental level (target: 7·95; measured: mean ± SE; 7·94 ± 0·01, see Table S3). The CO_2 concentration of seawater within mesocosms was maintained by directly diffusing CO_2 gas into the water column as required to maintain the experimental level and was controlled using temperature-compensated pH probes and automatic solenoid controllers (Sera, Heinsberg, Germany). Total alkalinity (A_T) of seawater in mesocosms was measured weekly using colorimetric titration (Hanna Instruments, Woonsocket, RI, USA). Concentrations of pCO_2 and bicarbonate (HCO_3^-) were then calculated from measured A_T, pH, salinity and temperature using CO2SYS for Excel (Pierrot, Lewis & Wallace 2006) with constants from Mehrbach *et al.* (1973), as adjusted by Dickson & Millero (1987) (results summarized in Appendix S3 and Table S3, S4).

EXPERIMENTAL RESPONSES

Change in percentage cover of turf was calculated for the first experimental phase (i.e. final – initial percentage cover; August 2009–February 2010) by quantifying the percentage cover of turf at both time points by overlaying a 2·5 × 2·5 cm quadrat over each algal specimen, within which the percentage cover was visually estimated to the nearest 5 per cent (Drummond & Connell 2005). In addition, we also quantified the final mass following the initial experimental period (i.e. February 2010) by carefully scraping all turf biomass from a standard area of the individual specimens (1 × 1 cm) using a razor into a pre-weighed aluminium tray, rinsing with fresh water to remove excess salt and dried to a constant weight at 60 °C for 48 h before weighing.

In order to quantify the change in turf biomass over time following nutrient reduction, we measured the change in fresh weight from the time treatments were altered at the start of phase two (February 2010) until the end of the experimental period (August 2010). Change in fresh weight was quantified by gently patting the specimens dry and then weighing them to the nearest 0·01 g using an electronic balance.

STATISTICAL ANALYSES

The response of algal turfs to experimental conditions was analysed using analysis of variance (ANOVA). A two-way ANOVA was used to test the effect on the change in percentage cover and dry mass of turf algae following the initial enrichment, while a repeated-measures (mixed split-plot design) ANOVA was used to test the change in fresh weight of turfs over time following reallocation of samples and implementation of the nutrient reduced treatment. The water column physicochemical parameters were also analysed using ANOVAS. Where significant treatment effects were detected, *post hoc* comparison of means was used to determine which factors differed (details of the specific ANOVAS and *post hoc* comparisons are provided in Appendix S3).

Results

ALGAL RESPONSE

Following enrichment, the greatest change in turf cover and dry mass was observed when elevated nutrients and future CO_2 were experienced in combination (Fig. 1a and 1b; Table S1a and S1b). This treatment caused turf cover and biomass to increase synergistically, that is, by a greater magnitude than would be anticipated by adding their isolated effects (Fig. 1a and 1b). Specifically, the detectable effects of nutrients in the absence of future CO_2 (i.e. elevated nutrients – ambient nutrients under current CO_2 = 14%, 0·004 g) and the effects of CO_2 in the absence of elevated nutrients (i.e. future CO_2 – current CO_2 under ambient nutrients =2%, 0·001 g) do not add to their combined effect (i.e. future CO_2 and elevated nutrients – current CO_2 and ambient nutrients =37%, 0·009 g), because their combined effect is multiplicative (i.e. 131 and 80% greater than their additive effects, for change in percentage cover and dry mass, respectively).

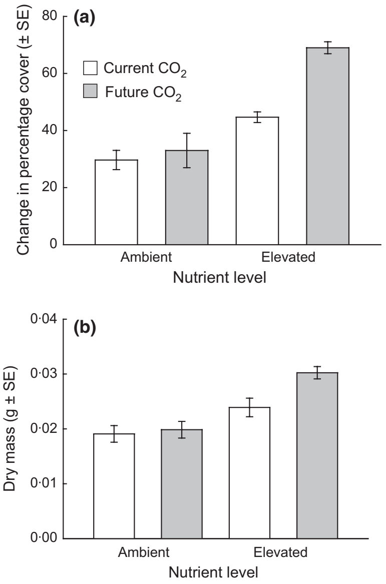

Fig. 1. The (a) change in percentage cover and (b) dry mass of turf-forming algae per standard area (1 × 1 cm) on natural rock substrates that were exposed to different combinations of nutrients (ambient *vs.* elevated) and CO_2 (current *vs.* future).

When the nutrient reduction treatment was implemented, the change in turf biomass (fresh weight) was significantly affected by a treatment × time interaction (Fig. 2; Table S2). Initially, the change in fresh weight was not significantly different between the treatments (day 22: ambient nutrients, current CO_2 = reduced nutrients, future CO_2 = elevated nutrients, future CO_2; $P > 0·05$, Tukey HSD), but within 2 months all treatments had diverged (day 51: ambient nutrients, current CO_2 < reduced nutrients, future CO_2 < elevated nutrients, future CO_2; $P < 0·05$, Tukey HSD). The fresh weight in the elevated nutrients, future CO_2 treatment continued to increase rapidly throughout the experimental period, with the change in this treatment significantly greater than the other two at all but one subsequent measurement times (the exception was day 108, elevated nutrients, future CO_2 = reduced nutrients, future CO_2, $P = 0·067$, Tukey HSD). While the change in fresh weight was not as substantial in the other treatments (i.e. nutrient reduced, future CO_2

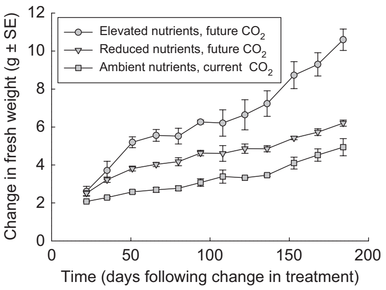

Fig. 2. The change in fresh weight of specimens of turf-forming algae exposed to either elevated nutrients and future CO_2, reduced nutrients and future CO_2 or ambient nutrients and current CO_2.

and ambient nutrients, current CO_2), they also increased consistently, with the fresh weight of turf in the nutrient reduced, future CO_2 treatment tracking higher than that of the ambient control (Fig. 2).

COMPARISON TO FIELD CONDITIONS

The percentage cover of turf algae in the mesocosms was similar to that quantified in the field at each time point (Fig. S1). In both mesocosms and the field, percentage cover of turf gradually increased throughout the experimental period (Fig. S1).

Discussion

Interactions among stressors created by human activities have the potential to drive transitions to novel habitats. Observed examples of shifts between alternative states include the switch in terrestrial deserts from perennial vegetation to bare soil with ephemeral plants, lakes from clear water with submerged vegetation to turbid phytoplankton-dominated waters and tropical marine reefs from corals to fleshy macroalgae (Scheffer *et al.* 2001 and references within). Stressors often interact synergistically to influence key taxa, and also ecosystems, more strongly than would be anticipated based on the addition of their isolated effects (Crain, Kroeker & Halpern 2008). For example, temperature, salinity and ultraviolet radiation combine to increase the embryonic mortality of gastropods (Przeslawski, Davis & Benkendorff 2005), potentially limiting their ability to continue providing their current ecosystem function which is the removal of key primary producers (Lotze, Worm & Sommer 2001; Russell & Connell 2007). Similarly, where these stressors alter the occurrence of key habitat-forming taxa that are involved in strong interactions, their combined effects can hasten transitions to novel habitats (Hobbs, Higgs & Harris 2009). The results of our initial manipulation provide evidence that such interactions may also be prevalent in rocky temperate coastlines when nutrients and CO_2 are simultaneously enriched, with the abundance of turf algae increasing synergistically (as identified in Russell *et al.* 2009; Falkenberg, Russell & Connell 2012). As algal turfs characterize the novel habitat of this system, such an increase would be anticipated to promote transitions away from the historical habitat dominated by kelp canopies.

The influence of multiple stressors on transitions from historical to novel habitats has been heavily discussed (e.g. the influence of overfishing, declining water quality and climate change on the shift from coral to macroalgae; Bellwood *et al.* 2004). The same is not true, however, for the influence of interactions, especially synergies, on the converse transitions from novel to historical habitats (e.g. the reverse shift from macroalgae to coral, but see Dudgeon *et al.* 2010). It has been suggested that reversing shifts between habitat states may be particularly difficult where only one of the conditions that drove the initial shift can be restored, as the effect of such restorative actions may be insufficient to weaken the 'basin of attraction' of the alternative state. Consequently, we assessed the results of our initial enrichment to gain an indication of the extent to which locally modified nutrient conditions determined the synergistic response of turf algae, and whether nutrient reduction had the potential to limit the expansion of turf even where CO_2 enrichment was maintained (c.f. restorative actions after the shift has occurred). The results indicate that while enriched CO_2 and nutrients combine to produce a synergistic increase in abundance of turfs, future CO_2 would have little effect in the absence of elevated nutrients (as identified in Russell *et al.* 2009; Falkenberg, Russell & Connell 2012). Specifically, we found that elevated nutrients prompted an increase in turf under both current and future CO_2 conditions. In contrast, although future CO_2 facilitated an increase in turfs under elevated nutrients, it had little effect where nutrients were maintained at their ambient level. Such a response is characteristic of incremental co-limitation of nutrients and CO_2, whereby processes determining growth are firstly restricted by the primarily limiting resource until it is in adequate supply, in this case nutrients, at which time the limiting resource switches to be a second factor, in this case CO_2 (Davidson & Howarth 2007; Allgeier, Rosemond & Layman 2011). Based on the results of our initial enrichment, we anticipated therefore that nutrient reduction could disrupt the synergistic effects of preceding nutrient and CO_2 enrichment.

The response of turfs to restorative management of nutrient concentrations under maintained enrichment of CO_2 supports our suggestion that where these stressors have synergistic effects on turf algae, it will be possible to substantially reduce their effects by restoring a subset of those conditions that were initially altered. Following reduction in nutrients and continuation of CO_2 enrichment, the increase in turf biomass was limited relative to that which occurred where both nutrient and CO_2 enrichment were maintained. Such a response was likely observed as the reduction in nutrients caused limitation to an extent that continued enrichment of CO_2 could not maintain an elevated growth response. This key result indicates that where the factors of nutrients and CO_2 interact to influence turfs in a synergistic manner, appropriate local management of nutrients can disrupt the feedbacks that maintain this novel composition, even following the establishment of globally altered CO_2 conditions. This result demonstrates the key role of local environmental conditions in determining the response of systems to forecasted global stressors, and highlights the potential for local management, including that which prevents nutrient inputs, to reduce the effect of irreversible global climate change.

While reduction in local stressors following the establishment of altered global conditions could disrupt synergies and limit further change to the system, delayed action may not be as effective as a proactive approach that precludes these interactions. We show that a reduction in nutrients following establishment of enriched CO_2 conditions substantially reduced the rate of increase in turf biomass. Of concern, however, is that there appeared to be a legacy from the historical conditions of enriched nutrients because biomass did not reduce to levels quantified under ambient conditions (i.e. ambient nutrients, current CO_2). This legacy may represent a positive feedback whereby the greater biomass established under combined nutrient and CO_2 enrichment was self-sustaining. Such an effect may be long-lasting and produce intergenerational change where the novel state (e.g. mats of turf algae) has substantial resilience to restorative actions (Scheffer *et al.* 2001; Beisner, Haydon & Cuddington 2003). Our results suggest, however, that although further expansion of turf algae may be prevented where local management reduces nutrient pollution under future CO_2 conditions, the initial expansion of turfs may be avoided where future climates manifest under good local water quality. Consequently, the establishment of effective management of local conditions, such as nutrients, may be most beneficial before forecasted climate conditions become established.

Forecasting the potential effects of anticipated change occurring at both global and local scales currently requires the use of mesocosms that enable manipulation of environmental factors impossible to modify in the field. Conditions within such mesocosms are, however, an imperfect approximation of those in natural ecosystems (Carpenter 1996). As such, limitations are typically placed on the interpretation of results from such experimental approaches (Wernberg, Smale & Thomsen 2012). We had concerns that the sheltered conditions within our mesocosms could minimize the removal of turf associated with water movement and that turf expansion would be greater in mesocosms than in the field. Our data reveals, however, that turf cover in control mesocosms (i.e. ambient nutrients, current CO_2) increased at a similar rate and magnitude to turfs in the adjacent Gulf St. Vincent. Therefore, the conditions that strongly influenced turf growth in our mesocosms were likely to be representative of conditions throughout the Gulf St. Vincent during the experimental period. Our mesocosm experiment therefore not only enables comparisons to be made between the responses of turfs under ambient conditions with that of their future counterparts, but also allows confidence in predictions regarding the magnitude of change in turf cover under future management scenarios.

The striking ecosystem shifts that occur where human-driven stressors combine to produce synergistic effects often provide the impression that prevention of further change, or its reversal, will be difficult to achieve (Lotze *et al.* 2011). To date, effective management of such change has typically involved amelioration of a broad suite of stressors (Lotze *et al.* 2011). Our results indicate, however, that disrupting the effects of synergies (e.g. between nutrients and CO_2 on turf algae) may not actually require all stressors to be alleviated, but rather the local-scale stressor(s) that strongly drives the initial interaction (e.g. nutrients). These findings empower local policy makers (e.g. Department of Environment, Water and Natural Resources) and managers of water quality (e.g. SA Water) who are implementing policy initiatives to decrease nutrient pollution. South Australia is a global leader in the use of ecological sciences to inform policy initiatives that aim to reduce nitrogen loads (i.e. by 75% in coastal waters) in line with improving coastal ecology. Measures implemented to achieve these targets include the increased effectiveness of wastewater treatment plants (i.e. Environment Improvement Plan, SA Water) and development of infrastructure for water recycling (i.e. 27 000 ML per year, or 30% of total wastewater flows). We caution, however, that disrupting the effects of synergies will be far more difficult to achieve where the key stressors result due to human activities over large spatial and temporal scales that are not easily reversed, such as anticipated levels of enriched CO_2 (Solomon *et al.* 2009; Pettorelli 2012).

In conclusion, as environmental conditions are altered across global to local scales, some will combine in synergistic ways to cause change of disproportionate ecological magnitude. We show that by reducing a locally determined stressor (i.e. nutrient pollution), its synergistic effects with a globally determined stressor (i.e. CO_2 enrichment) on turf-forming algae may be substantially reduced. Consequently, management actions to reduce the total load of nutrients released into marine systems (e.g. improved wastewater treatment and recycling) may be of particular importance in determining the occurrence of this taxon and the ecological structure of coastal marine systems in southern Australia. The detectable legacy effect where nutrients were removed following the establishment of a synergy with enriched CO_2 suggests that proactive management strategies which prevent such interactions may be more effective than approaches to disrupt them. Importantly, these results suggest that in the face of changing climate (e.g. ocean acidification), effective management of local stressors (e.g. water pollution) may have a greater contribution in determining natural habitats than currently anticipated.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Definition of 'turf-forming algae'.

Appendix S2. Comparison of turf growth in mesocosms to that in the field.

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Figure S1. The percentage cover of turf-forming algae in each Austral season over the experimental period.

Table S1. ANOVA testing the combined effects of nutrients (ambient vs. elevated) and CO_2 (current vs. future) on the (a) change in percentage cover and (b) dry mass of turf-forming algae.

Table S2. Summary of ANOVA testing the effects of treatment (ambient nutrients and current CO_2, reduced nutrients and future CO_2 or enriched nutrients and future CO_2) over time on the change in fresh weight of turf-forming algae.

Table S3. Physicochemical parameters measured in the field before the switch (i.e. reduction in nutrients), in the field after the switch and in the laboratory.

Table S4. Results from ANOVA testing the effect of nutrients (ambient vs. elevated) and CO_2 (current vs. future) on the physicochemical parameters measured in the field before the switch (i.e. reduction in nutrients), the effect of treatment on those measured after the switch and the effect of nutrients (ambient vs. elevated) on the parameters measured in the laboratory.

5.9 SUPPORTING INFORMATION

Appendix S5.1 Definition of 'turf-forming algae'

Here, we use 'turf' as a functional group term to denote mats of low-growing algae < 5 cm in canopy height. These mats, which are ecologically important within Australia due to their occupation of space, were primarily composed of the brown algal genus *Feldmannia*. Within the genus *Feldmannia* there are 24 species currently accepted taxonomically. Of these species, 8 are reported to occur in Australia, with 4 reported from South Australia (Guiry 2012). While there is anecdotal evidence that turf growth is slow in winter (i.e. at the start and end of this experimental period), and increases over summer (i.e. in the middle of this experimental period, when the 'switch' was implemented), this pattern is yet to be quantified. Indeed, results from the quantification of turf cover (Fig. S5.1), indicate that turf cover increased steadily throughout the experimental period. As the cover of turf and physicochemical conditions in this period cannot be compared with those of another year, the underlying mechanism cannot be stated with confidence. We suggest, however, that this pattern may be attributable to relatively consistent water temperatures that can dampen seasonal patterns. In addition to monitoring the cover of turf assemblage, its composition was also monitored throughout the experimental period, and observed to remain constant over time.

Appendix S5.2 Comparison of turf growth in mesocosms to that in the field

To determine how closely the effect of change in turf cover in the control mesocosms matched that occurring in the field, percentage cover of turf was quantified within the contemporary control mesocosms and in the field in each Austral season over the experimental period. In the mesocosms, percentage cover was quantified for each algal specimen by overlaying a 2.5 × 2.5 cm quadrat within which the percentage cover was visually estimated to the nearest 5 percent (Drummond & Connell 2005). Abundance (percentage cover) of the same algal turf habitats was measured at three field sites within the Gulf St. Vincent; the collection site of Horseshoe reef as well as reefs both north and south (Hallet Cove and Noarlunga, respectively). At each site, the percentage cover of algae was quantified using the line intercept method for a pair of 20 m transects.

Appendix S5.3 Detailed statistical analyses

The response of algal turfs to experimental conditions was analysed using Analysis of Variance (ANOVA). Analysis of the change in percentage cover of turf and dry mass of turf proceeded in two steps. First, a three-way ANOVA was used to identify whether there was any difference in experimental effects between replicate mesocosms. Both nutrients and CO_2 were treated as fixed and orthogonal, with two levels in each factor (nutrient: ambient vs. elevated; CO_2: current vs. future), and three replicate mesocosms were nested within these factors ($n = 5$ replicate samples of algae per mesocosm). No differences were detected between replicate mesocosms within treatments (i.e. no 'tank' effects). Therefore, to avoid pseudoreplication within mesocosms, data for the five algal specimens within each mesocosm were averaged, and data reanalysed using two-way ANOVAs; the two factors of nutrients and CO_2 were treated as fixed and orthogonal, with two levels in each factor (nutrient: ambient vs. elevated; CO_2: current vs. future; $n = 3$ mesocosms). A repeated measures (mixed split-plot design) ANOVA was used to test the change in fresh weight of turfs over time following reallocation of samples and implementation of the nutrient reduced treatment. Again, the analysis proceeded in two steps. In the first step, no 'tank' effects were detected for any of the time points. In the subsequent repeated measures ANOVA, the independent variables included treatment, with three levels (elevated nutrients + future CO_2, reduced nutrients + future CO_2, ambient nutrients + current CO_2) and time, with 12 levels (the 12 different sampling days). In all ANOVAs where significant treatment effects were detected *post hoc* comparison of means were used to determine which factors differed; Student-Newman-Keuls (SNK) tests were used for the change in percentage cover of turf and dry mass of turf while Tukey's HSD was used for the change in fresh weight over time.

The water column physicochemical parameters were also analysed using ANOVAs. The two-way ANOVA design outlined above (i.e. nutrients and CO_2 were fixed and orthogonal, with two levels in each; nutrient: ambient vs. elevated; CO_2: current vs. future) was used to analyse parameters measured during the initial enrichment. One-way ANOVAs were used to analyse parameters measured following the 'switch' in treatments (i.e. the reduction of nutrients and continuation of other treatments), with the factor of treatment treated as fixed with three levels (ambient nutrients + current CO_2, reduced nutrients + future CO_2, elevated nutrients + future CO_2). One-way ANOVAs, with the factor of nutrients fixed and having two levels (ambient vs. elevated) were used to test nutrient conditions of mesocosms in the laboratory experiment. In all analyses measurements were averaged across days (the nutrient parameters of ammonia, phosphate and NO_X were measured $n = 5$ times before the switch, $n = 4$ times after the switch and $n = 20$ times in the laboratory study while the carbonate parameters of pH, A_T, temperature, pCO_2 and HCO_3^- were measured $n = 5$ times before the switch and $n = 5$ times after the switch). Mesocosms were used as replicates ($n = 3$ for both field components; $n = 5$ for laboratory component). Where significant treatment effects were detected, SNK *post hoc* comparison of means were used to determine which factors differed.

Appendix S5.4 Results of statistical tests on water column physicochemical conditions

In the initial enrichment experiment, pH was significantly reduced under future CO_2 compared with current CO_2 treatments (Table S5.4, S5.5, Supporting information; SNK test). In contrast, the *p*CO_2 and bicarbonate (HCO_3^-) were significantly increased under future experimental CO_2 conditions (S5.4, S5.5, Supporting information; SNK test). Following the switch in treatment conditions (i.e. reduction of nutrients) pH was still reduced under all future CO_2 treatments compared to the current CO_2 treatment (Table S5.4, S5.5, Supporting information; SNK test), with the *p*CO_2 and bicarbonate (HCO_3^-) again increased under the future CO_2 treatments (Table S5.4, S5.5, Supporting information; SNK test). Temperature was not significantly different among any treatments for either experimental period (Table S5.4, S5.5, Supporting information; SNK test).

The concentration of ammonia, phosphate and NO_X (nitrate + nitrite) quantified in the field mesocosms during the enrichment experiment were significantly higher in the elevated than ambient nutrient treatments (Table S5.4, S5.5, Supporting information; SNK test). Following the switch in treatments, phosphate and NO_X in the nutrient reduced treatment were reduced to levels not significantly different to ambient conditions, both of which were lower than that measured in the elevated nutrient treatment (Table S5.4, S5.5, Supporting information; SNK test of significant ‘treatment’ term). The low concentrations of these nutrients, even in elevated treatments, indicate available nutrients were being utilised by the algae. This interpretation is supported by results of the additional laboratory-based mesocosm trials that excluded algae, within which the concentrations of all nutrients in were significantly greater in the elevated than ambient nutrient treatments (Table S5.4, S5.5, Supporting information; SNK test).

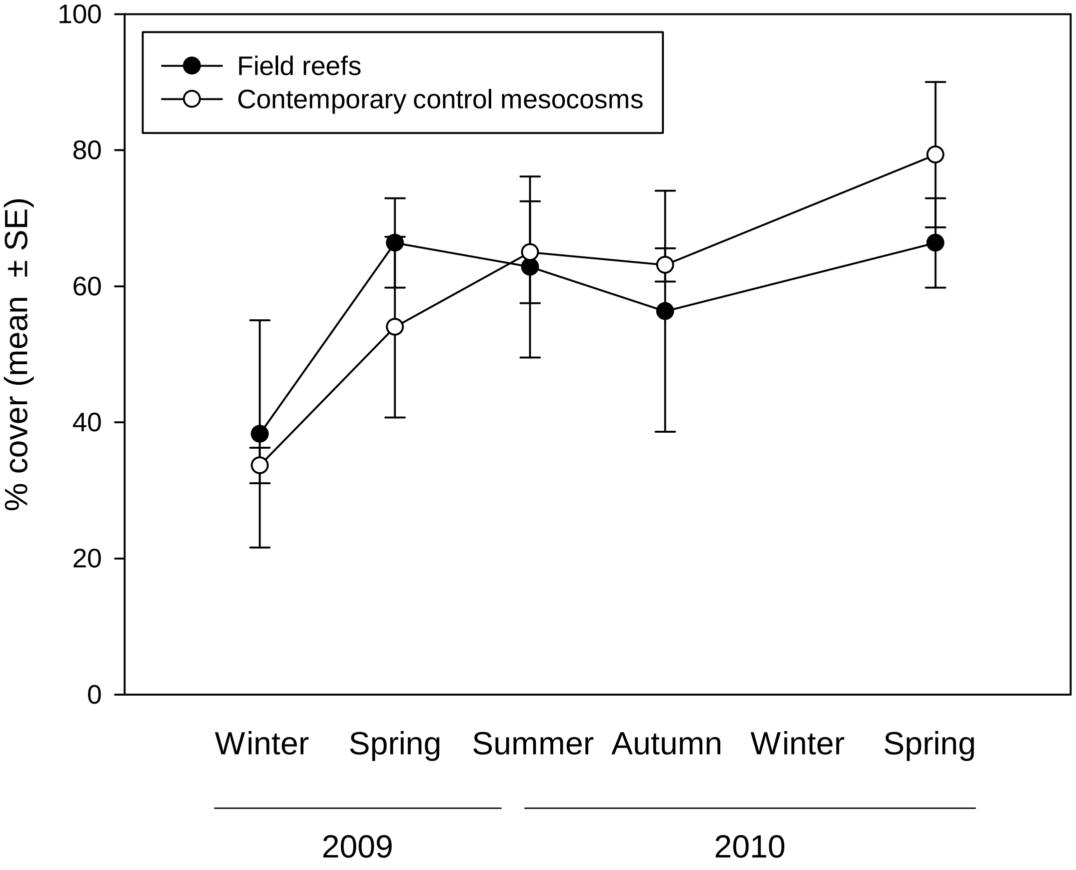

Figure S5.1 The percentage cover of turf-forming algae in each Austral season (except Winter 2010) over the experimental period on specimens in the contemporary control mesocosms (i.e. ambient nutrients and current CO_2) and in three reefs in the field near the collection site within the Gulf St. Vincent.

Table S5.1 ANOVA testing the combined effects of nutrients (ambient vs. elevated) and CO_2 (current vs. future) on the (a) change in percentage cover and (b) dry mass of turf-forming algae per standard area (1 × 1 cm) of natural rock substrate.

Source of variation	Sum of squares	df	Mean squares	F	*P*
(a) change in percentage cover					
Nutrients	1875.00	1	1875.000	42.45	0.0002
CO_2	533.33	1	533.333	12.08	0.0084
Nutrients × CO_2	363.00	1	363.000	8.22	0.0209
Residual	353.33	8	44.167		
(b) dry mass					
Nutrients	173.381	1	173.381	26.50	0.0009
CO_2	37.689	1	37.689	5.76	0.0432
Nutrients × CO_2	23.157	1	23.157	3.54	0.0967
Residual	52.338	8	6.542		

Table S5.2 Summary of ANOVA testing the effects of treatment (ambient nutrients and current CO_2, reduced nutrients and future CO_2 or enriched nutrients and future CO_2) over time on the change in fresh weight of turf-forming algae on natural rock substrate.

Source of variation	Sum of squares	df	Mean squares	F	*P*
Treatment	187.037	2	93.519	56.41	< 0.001
Time	191.449	11	17.404	67.62	< 0.001
Treatment × time	40.219	22	1.828	7.10	< 0.001
Between error (treatment)	9.946	6	1.658		
Within error (time)	16.988	66	0.257		

Table S5.3 Physicochemical parameters measured in the field before the switch (i.e. reduction of nutrients), in the field after the switch and in the laboratory for each treatment (*n* = 3 mesocosms for all). Reported are means, standard errors (S.E.), maximum and minimum values. Field ammonia, phosphate and NO_X were sampled on 5 occasions before the switch (i.e. reduction of nutrients) and on another 4 occasions after the switch. Laboratory-based mesocosms sampled on alternate days (*n* = 20 occasions). Total Alkalinity (A_T), pH and temperature were simultaneously measured on 5 occasions before the switch and on another 5 occasions after the switch, from which concentrations of pCO_2 (ppm) and bicarbonate (HCO_3^-) (µmol kg^{-1}) were calculated using constants from Mehrbach et al. (1973), as adjusted by Dickson and Millero (1987).

Physicochemical parameter		Treatment	Mean (S.E.)	Max.	Min.
Ammonia (mg L^{-1})	i) In field, before switch	AN, CCO_2	0.0309 (0.0011)	0.0400	0.0010
		AN, FCO_2	0.0362 (0.0016)	0.0500	0.0135
		EN, CCO_2	0.0387 (0.0017)	0.0435	0.0111
		EN, FCO_2	0.0389 (0.0010)	0.0480	0.0209
	ii) In field, after switch	AN, CCO_2	0.0277 (0.0013)	0.0550	0.0102
		RN, FCO_2	0.0282 (0.0022)	0.0655	0.0005
		EN, FCO_2	0.0344 (0.0036)	0.0615	0.0180
	iii) In lab	Without nutrients	0.0346 (0.0053)	0.1210	0.0020
		With nutrients	0.2652 (0.0320)	0.6230	0.0110

Phosphate (mg L^{-1})	i) In field, before switch	AN, CCO_2	0.0081 (0.0004)	0.0091	0.0066
		AN, FCO_2	0.0079 (0.0001)	0.0086	0.0051
		EN, CCO_2	0.0098 (0.0002)	0.0125	0.0063
		EN, FCO_2	0.0090 (0.0001)	0.0105	0.0069
	ii) In field, after switch	AN, CCO_2	0.0078 (0.0003)	0.0097	0.0067
		RN, FCO_2	0.0082 (0.0003)	0.0110	0.0064
		EN, FCO_2	0.0094 (0.0002)	0.0125	0.0075
	iii) In lab	Without nutrients	0.0272 (0.0033)	0.0600	0.0010
		With nutrients	0.1285 (0.0068)	0.2870	0.0510
NO_X (mg L^{-1})	i) In field, before switch	AN, CCO_2	0.0067 (0.0001)	0.0082	0.0001
		AN, FCO_2	0.0062 (0.0001)	0.0067	0.0001
		EN, CCO_2	0.0075 (0.0002)	0.0095	0.0001
		EN, FCO_2	0.0074 (0.0003)	0.0088	0.0001
	ii) In field, after switch	AN, CCO_2	0.0062 (0.0002)	0.0080	0.0053
		RN, FCO_2	0.0061 (0.0001)	0.0076	0.0052
		EN, FCO_2	0.0077 (0.0002)	0.0092	0.0063

	ii) In lab	Without nutrients	0.1222 (0.0050)	0.2080	0.0630
		With nutrients	0.3796 (0.0255)	0.6230	0.1010
pH	i) In field, before switch	AN, CCO_2	8.18 (0.06)	8.53	8.02
		AN, FCO_2	7.90 (0.00)	8.09	7.75
		EN, CCO_2	8.15 (0.03)	8.24	8.05
		EN, FCO_2	7.96 (0.02)	8.10	7.73
	ii) In field, after switch	AN, CCO_2	8.16 (0.01)	8.24	8.09
		RN, FCO_2	7.95 (0.01)	7.99	7.91
		EN, FCO_2	7.95 (0.00)	7.99	7.91
A_T (µmol kg^{-1})	i) In field, before switch	AN, CCO_2	2276 (63)	2723	1848
		AN, FCO_2	2357 (57)	2723	2091
		EN, CCO_2	2211 (55)	2529	1897
		EN, FCO_2	2341 (43)	2723	2091
	ii) In field, after switch	AN, CCO_2	2623 (44)	2942	2334
		RN, FCO_2	2744 (72)	3380	2480
		EN, FCO_2	2748 (82)	3234	2504
pCO$_2$ (ppm)	i) In field, before switch	AN, CCO_2	408 (59)	574	138
		AN, FCO_2	893 (17)	1301	515
		EN, CCO_2	400 (19)	450	334
		EN, FCO_2	738 (68)	1460	490
	ii) In field, after switch	AN, CCO_2	475 (7)	567	346
		RN, FCO_2	853 (10)	1031	726
		EN, FCO_2	855 (30)	1140	718
HCO_3^- (µmol kg^{-1})	i) In field, before switch	AN, CCO_2	1890 (92)	2261	1502
		AN, FCO_2	2141 (48)	2454	1906
		EN, CCO_2	1859 (28)	2076	1652
		EN, FCO_2	2093 (48)	2550	1901
	ii) In field, after switch	AN, CCO_2	2194 (36)	2533	1840
		RN, FCO_2	2454 (62)	3068	2165
		EN, FCO_2	2456 (76)	2941	2211

Temperature (°C)	i) In field, before switch	AN, CCO_2	14.4 (0.2)	15.8	13.1
		AN, FCO_2	14.6 (0.1)	15.9	13.2
		EN, CCO_2	14.6 (0.2)	15.8	13.2
		EN, FCO_2	14.6 (0.1)	15.9	13.0
	ii) In field, after switch	AN, CCO_2	16.0 (0.0)	21.6	12.0
		RN, FCO_2	16.0 (0.0)	21.7	11.9
		EN, FCO_2	16.0 (0.1)	22.2	11.8

CCO_2, current CO_2; FCO_2, future CO_2; AN, ambient nutrients; EN, elevated nutrients; RN, reduced nutrients.

Table S5.4 Results from ANOVA (detailed above) testing the combined effect of nutrients (ambient vs. elevated) and CO_2 (current vs. future) on the 9 physicochemical parameters measured in the field before the switch (i.e. reduction of nutrients), the effect of treatment on those measured after the switch and the effect of nutrients (ambient vs. elevated) on the 3 measured in the laboratory. Field ammonia, phosphate and NO_X were sampled weekly on 5 occasions before the switch and on a further 4 occasions after the switch, with laboratory-based mesocosms sampled on alternate days (n = 20 occasions). Total Alkalinity (A_T), pH and temperature were simultaneously measured on 5 occasions before the switch and on another 5 occasions after the switch, from which concentrations of pCO$_2$ (ppm) and bicarbonate (HCO_3^-) (µmol kg^{-1}) were calculated using constants from Mehrbach et al. (1973), as adjusted by Dickson and Millero (1987).

For field-based measurements three-factor ANOVAs with tanks (n = 3) as replicates; for laboratory-based measurements two-factor ANVOAs with tanks (n = 5) as replicates. Bold values indicate significance (at $p < 0.05$ level).

	Physicochemical parameter (response variable)	CO_2		Nutrients		CO_2 × Nutrients		Treatment	
		F	*P*	F	*P*	F	*P*	F	*P*
In field, before switch	Ammonia	4.13	0.0765	**14.78**	**0.0049**	3.66	0.0920		
	Phosphate	4.94	0.0570	**35.67**	**0.0003**	1.67	0.2325		
	NO_X	1.36	0.2765	**22.23**	**0.0015**	0.70	0.4274		

	pH	**44.40**	**0.0002**	0.24	0.6363	1.37	0.2753		
	A_T	3.71	0.0904	0.55	0.4802	0.20	0.6686		
	Temperature	0.15	0.7123	0.32	0.5895	0.22	0.6495		
	pCO_2	**77.08**	**0.0000**	3.04	0.1194	2.45	0.1564		
	HCO_3^-	**16.96**	**0.0034**	0.45	0.5230	0.02	0.8860		
In field, after switch	Ammonia							2.13	0.2001
	Phosphate							**9.16**	**0.0150**
	NO_X							**27.59**	**0.0009**
	pH							**349.76**	**0.0000**
	A_T							1.10	0.3931
	Temperature							0.05	0.9495
	pCO_2							**134.69**	**0.0000**
	HCO_3^-							**6.24**	**0.0342**
In lab	Ammonia (lab)			**1066.79**	**0.0000**				
	Phosphate (lab)			**124.69**	**0.0000**				
	NO_X (lab)			**382.33**	**0.0000**				

Retrieving algae from inside the mesocosms to enable quantification of its response(s) to experimental treatments.

Photo: Anja Richards.

A forest of the perennial alga *Ecklonia radiata* growing on subtidal rocky coast in South Australia.

Photo: Owen Burnell.

CHAPTER 6

CHAPTER 6

GENERAL DISCUSSION

Identifying the stressors that combine to drive observed change in ecosystems has long been a central human concern, yet our ability to anticipate the effects of forecasted conditions remains limited. Throughout this thesis, key features of ecosystems altered along rocky temperate coastlines, both resource enrichment and direct removal of biota, were experimentally manipulated to identify their likely effects on the currently dominant kelp canopy and opportunistic turf-forming algae. Deliberately recurring themes are; 1) identification of interactive effects between forecasted global-scale stressors and locally-degraded conditions, and, 2) consideration of local management approaches which may alter interactions such that the effects of forecasted global change are limited or reduced. Initial CO_2 and nutrient enrichments revealed algal taxa may experience, and be released from, contrasting resource limitations such that turf benefit more so than kelp under highly modified conditions (i.e. enriched CO_2 and nutrients, primarily Chapter 2 but also see Chapters 3 and 5), potentially enabling turf to replace kelp canopies as the dominant habitat. While the synergistic increase in turf cover that typically occurs under enrichment of CO_2 and nutrients suggests managing against these conditions will be difficult (Chapters 2, 3 and 5; Russell et al. 2009), maintenance of biotic controls such as a key competitor (i.e. kelp, Chapter 3) or herbivore (i.e. gastropods, Chapter 4) appear sufficient to resist this environmental forcing. Similarly, the effects of this synergy could be disrupted even following establishment (i.e. expansion of turf slowed) if local management effectively reduces just one of the altered conditions contributing to the interaction (e.g. limiting nutrient pollution under future CO_2 climates, Chapter 5). Together, these results indicate that while stressors may interact to drive changes in the dominant habitat, recognising their potential influence can enable management that effectively prevents ecological change. This Chapter (6) provides a discussion of these key points, explores links among them and suggests directions for future research.

6.1 INTERACTIVE EFFECTS OF ENRICHED RESOURCE AVAILABILITIES

The ecological implications of altered resource availabilities are potentially substantial, as modified conditions may effectively release certain primary producers, but not others, from their limitations such that phase-shifts and loss of ecosystem services are promoted (Harpole et al. 2011). It is prudent, therefore, to begin considering the potentially contrasting responses of key taxa to altered resource conditions. Experimental manipulation of the CO_2 and nutrient conditions which kelp and turf experienced indicated this pair of physiologically-contrasting taxa probably experience distinct resource limitations. Specifically, kelp biomass increased under elevated nutrient conditions, a response which was not further influenced by modified CO_2 (Chapter 2). This kelp response to nutrients but not CO_2 is characteristic of single-resource limitation as derived from the classical concept outlined in Liebig's Law of the Minimum (Liebig 1842; Harpole et al. 2011). In contrast, the turf response to nutrient and CO_2 enrichment appeared to represent the more recently developed concept of co-limitation, whereby individual enrichment of each resource had an effect, with the greatest change identified where they were manipulated in combination (Chapter 2; Davidson & Howarth 2007; Allgeier et al. 2011). The results from my initial manipulations of these resources suggest, therefore, that ecological communities are comprised of taxa with contrasting limitations, with some experiencing single resource limitation while others are co-limited by multiple resources.

The contrasting responses of taxa to altered abiotic conditions typically manifest as a consequence of physiological characteristics that influence their ability to access and/or utilise the resources required for key processes controlling production, specifically nutrient acquisition and carbon fixation (Sterner & Elser 2002). The physiological processes by which limitations manifest are indicated by the stoichiometry of primary producers (Koerselman & Meuleman 1996), a characteristic that was quantified here to develop an understanding of the mechanisms driving kelp and turf responses to altered conditions (Chapter 2). The effects of nutrient modification on kelp and turf were comparable as under enriched conditions both taxa increased biomass production and had a shifted % N. This result indicates that, while these taxa appear disparate, both were able to access and utilise the greater amount of available nutrients. Although their responses to nutrients were similar, kelp and turf were differentially affected by the enrichment of CO_2, revealing that acquisition of this resource is a key way in which their physiologies contrast. That is, the biomass and stoichiometry of turf, but not kelp, was influenced by enriched CO_2, indicating that while turf is currently carbon-limited, kelp is not. CO_2 saturation has been proposed to manifest in kelps and other morphologically-complex algae as they are believed to possess carbon concentrating mechanisms (CCMs) and, therefore, are not reliant on dissolved CO_2 as their source of photosynthetic carbon (Beardall et al. 1998; Hurd et al. 2009; Hepburn et al. 2011). In contrast, simpler algae such as turfs may acquire CO_2 via passive diffusion rather than the use of CCMs, a photosynthetic process whose efficiency is typically limited by the availability of CO_2 (Kübler et al. 1999; Hepburn et al. 2011). It is also possible that turfs do possess CCMs (as indicated by their $\delta^{13}C$ values, Chapter 2) (Maberly et al. 1992; Raven et al. 2002; Hepburn et al. 2011), but that they are more strongly CO_2 limited than those of kelps due to taxa-specific features such as; 1) inherently low CCM efficiency, or, 2) an inability to acquire the resources (i.e. light) that regulate this process (Beardall & Giordano 2002; Rost et al. 2003; Beardall & Raven 2004; Raven et al. 2011; Russell et al. 2011). A promising area for future research is, therefore, identification of the photosynthetic mechanisms used by these algal taxa, as such work will not only contribute to an improved understanding of their physiologies but also enhance forecasts of community responses to anticipated resource availabilities.

6.2 BIOTIC CONTROLS OVER THE MANIFESTATION OF INTERACTIVE EFFECTS

While considering the influence of altered environmental conditions on individual taxa enables elucidation of their direct response(s) to such change, in natural systems these responses will be modified by indirect effects resulting from interactions with other taxa. Although indirect effects were initially surprising (Sanford 1999), we are now coming to recognise they exist for various environmental conditions in a diversity of systems (Suttle et al. 2007; O'Connor 2009; O'Connor et al. 2009) such that their influence may rival that of the more intuitive and easily detected direct effects (Connell et al. 2011).

6.2.1 COMPETITION MEDIATED BY FOUNDATION SPECIES

Competition has long been recognised as an important process contributing to the determination of community structure (Clements et al. 1926; Tilman 1982; Schoener 1983). Where space is fully occupied, as routinely occurs in intertidal marine systems, it is often the most important limiting resource (Dayton 1971). While competitive interactions for space can result in the establishment of clear dominance hierarchies, human activities that modify abiotic conditions may result in their disruption. For example, where mussels and barnacles co-exist, mussels have been suggested to be the competitive dominant (Dayton 1971). Although studies that consider the response of mussels and barnacles to enriched CO_2 in isolation indicate direct effects may not modify resulting community structure (as both have been found to be negatively affected: Beesley et al. 2008; McDonald et al. 2009, respectively), indirect effects mediated by their interaction may drive change. Specifically, consideration of mussels and barnacles in combination revealed that exposure to reduced pH associated with a rise in atmospheric CO_2 negatively affected mussels and reduced their abundance while barnacles benefitted from the weakened competition to such an extent that their abundance actually increased (Wootton et al. 2008; Armstrong 2009). This effect would not have been predicted from experiments that consider the isolated response of mussels and barnacles to enriched CO_2. Consequently, such results indicate that although the potential for competitive hierarchies to be disrupted under future conditions is coming to be more widely recognised, the nature of change remains largely unpredictable. For the turf-kelp interaction considered here, potential exists that their contrasting resource limitations (as identified in Chapter 2) may result in future conditions having stronger positive effects on turf than kelp, reducing the capacity of this foundation species to continue to suppress its competitor. The response of turf to enriched CO_2 and nutrients in the presence and absence of kelp revealed that where a canopy was retained, its presence was sufficient to suppress the expansion of turf algae, even under the combined enrichment of CO_2 and nutrients which would otherwise lead to a synergistic expansion of turf (Chapter 3). Therefore, while the direct effects of increased resource availabilities may be relevant in determining the turf response to altered conditions in the absence of canopies, they are less likely to be relevant where intact kelp forests are maintained and indirectly determine the influence of resource enrichment.

Quantification of physicochemical conditions in the presence of kelp canopies suggests their continued inhibition of turfs is likely to be due to an effect on physical (shading) rather than chemical conditions (Chapter 3). While the potential effect of kelp on water quality was investigated as the mechanism driving turf suppression under forecasted conditions, a more powerful explanation was derived from classical experiments showing the effects of canopy-shade on understorey communities (Reed & Foster 1984) and covers of turfs (Connell 2005; Russell 2007). It is recognised, however, that this conclusion is dependent on the shading conditions utilised in experimental manipulations. For example, another study considering the role of shading under forecasted conditions found that where CO_2 is enriched the structuring role of light availability may be weakened (Russell et al. 2011). I suggest, however, that inhibition of turf was weaker in Russell et al. (2011) as the ‘shading’ treatments may have reduced light conditions to a lesser extent than occurs in the presence of kelp (B. D. Russell, pers. comm.). Such potential for differences in light regimes to modify ecological responses is relatively well-recognised from field studies considering the removal of canopies. Where canopies are disturbed, either by natural storms or human activities such that they are thinned, reduced in size or fragmented, environmental conditions become more similar to those outside the canopy and light availability increases (Bruno & Bertness 2001). Turfs typically respond to the altered conditions created by the loss of canopies by expanding and dominating space to drive phase-shifts over multiple generations (Connell & Irving 2008; Gorman & Connell 2009). The expansion of turfs is, however, determined not only by the proximate triggers (e.g. kelp loss and increased light availability), but also gradual changes in conditions (Paine et al. 1998; Scheffer et al. 2001; Perkol-Finkel & Airoldi 2010). In this system, the expansion of turf algae following kelp loss is particularly strongly favoured where human activities increase the availability of other resources that limit turf production (e.g. nutrients), such that these algae are able to take advantage the higher light availabilities (Chapter 3). These results suggest, therefore, that maintenance of intact populations of foundation species may enable environmental conditions to be moderated such that the historical ecosystem state persists, even under forecasted conditions otherwise anticipated to increase the likelihood of their loss.

6.2.2 CONSUMER CONTROL MEDIATED BY GRAZERS

Natural communities are often maintained by strong consumer control which is itself affected by alterations to the abiotic environment (Paine 1966; Tylianakis et al. 2008; O'Connor et al. 2009). Key species interactions may be particularly sensitive to certain conditions such that relatively small changes in the environment generate large changes in communities (Sanford 1999). An example of this phenomenon is the strengthened effects of the predatory sea star on its principle prey of mussels under increased temperatures (Sanford 1999). This interaction will, however, not only be influenced by increased temperatures, but also enriched CO_2.While extreme CO_2 enrichment has been found to reduce consumption rates of mussels by both sea stars and shore crabs (Appelhans et al. 2012), more moderate enrichments have revealed a positive but non-significant effect on feeding rates of sea stars (Gooding et al. 2009). Although such results were initially surprising, given that strongly enriched CO_2 conditions suppressed the feeding activity of invertebrates (as in Cecchini et al. 2001; Foss et al. 2003; Siikavuopio et al. 2007), a growing number of studies indicate moderate CO_2 enrichment may have negligible effects on feeding rates (e.g. Kurihara et al. 2008; Marchant et al. 2010; Landes & Zimmer 2012), or could even enhance this process (Cummings et al. 2011). My results contribute to this body of work as they indicate rates of herbivory by marine gastropods may increase under CO_2 enrichment (i.e. > 95 % more turf mass was removed under enriched compared to ambient CO_2 conditions, Chapter 3). Such a response has the potential to change the mild direct effect of CO_2 on the consumed taxa (i.e. slightly impaired functioning of the invertebrate prey; reviewed in Fabry et al. 2008, Doney et al. 2012, or increased production of turf; Chapters 2 and 3, Russell et al. 2009, Connell & Russell 2010) into an indirect negative response (i.e. greater biomass removed). Predictions of community responses to modified conditions will, consequently, need to be developed in recognition of indirect effects driven by interactions that exist between taxa of different trophic levels.

While the result that enriched CO_2 increases rates of consumption contributes to a relatively large body of work assessing the response of this process to future conditions, there has been little consideration as to whether the mechanism of this effect is direct (i.e. an effect on the consumer) or indirect (i.e. an effect on the consumer mediated by the food). Both direct and indirect effects have been invoked to account for the change in rates of predation, with the reduced consumption under strong CO_2 enrichment attributed to a direct negative effect on the consumer itself (whether it be a sea star or crab; Appelhans et al. 2012), while it has been suggested increased consumption may result under moderate CO_2 enrichment scenarios due to an indirect effect mediated by the food (specifically the structure of its calcified parts; Gooding et al. 2009; Dupont et al. 2010). The relative influence of these effects has not, however, been experimentally assessed. In order to tease apart the competing models of direct and indirect effects for the process of herbivory, feeding experiments were conducted in which various combinations of grazers and algae exposed to either current or moderate future CO_2 conditions were considered (for detail see Fig. 4.1). These experimental tests did not support the model that increased herbivory occurred due to a direct effect on the grazer, but rather provided evidence for an indirect effect mediated by the algae (Chapter 4). Various characteristics of primary producers are anticipated to be modified by CO_2 enrichment, with the proximal factor affecting grazers, and therefore rates of herbivory, likely their nutrient content. Under enriched CO_2 conditions the % N of turf tissue was increased, a factor known to increase rates of herbivory in many terrestrial and marine systems (Hillebrand et al. 2000; Silliman & Zieman 2001), including the system considered here (Russell & Connell 2007). If the % N of algal tissue is, indeed, the factor driving modified rates of herbivory, it is suggested this process will be even more strongly affected where CO_2 is enriched in combination with nutrients. Although the rate of herbivory was only directly considered when CO_2 was altered (Chapter 4), quantification of turf stoichiometry when both CO_2 and nutrients were manipulated revealed it is under the concurrent enrichment of these conditions that the % N of turf will be greatest (Chapter 2). Consequently, it is likely that grazers will remove turf at the greatest rate under these enriched conditions. Indirect effects on herbivores mediated by the algae may, therefore, have a strong role in determining rates of consumption under forecasted conditions.

6.3 DISRUPTING ESTABLISHED SYNERGISTIC INTERACTIONS

Once ecological change is entrained, management to slow this process would ideally restore all altered conditions with the view to push the system back towards its historical configuration (Suding et al. 2004; Hobbs et al. 2009; Lotze et al. 2011). An emerging concern is that once certain stressors are established, such as globally-enriched CO_2, they will be irreversible (Matthews & Caldeira 2008; Solomon et al. 2009), meaning only locally-altered conditions can be restored to historical levels (Russell et al. 2009). While the role of interactions in hastening the expansion of novel habitats is recognised, it is unclear if their effects can be dampened where only a subset of the contributing conditions is effectively managed (e.g. nutrient pollution). Given that the turf which characterise novel habitats is co-limited by both CO_2 and nutrients (Chapters 2, 3 and 5), it was hypothesised the interactive effect of these conditions may be disrupted if the subset which could be managed in natural systems (i.e. nutrients) was effectively reduced while irreversible, global-scale change was maintained at a forecasted level (i.e. CO_2). Following establishment of the synergistic turf response to enriched CO_2 and nutrients, reduction of nutrients (despite continued CO_2 enrichment) did substantially slow the increase of turf biomass relative to the treatment in which nutrients and CO_2 both continued to be enriched (Chapter 5). This response was likely observed as, given the CO_2 and nutrient co-limitation of turf (Chapters 2, 3 and 5), removal of nutrients caused this resource to be limiting such that continued CO_2 enrichment was insufficient to maintain the elevated growth response. Effective local management may, therefore, be sufficient to limit the influence of CO_2 emissions at the global scale, but the legacy of past pollution may endure for some time.

The potential remains, however, that although disruption of synergies may slow the rate of further ecological change, the influence of preceding interactions between altered conditions could continue to be felt where the novel configuration is not easily removed. It was identified here that while the rate of increase in turf biomass was reduced when enrichment of nutrients was prevented under future CO_2 conditions, the biomass of turf in this treatment did not reach the lower level maintained under ambient CO_2 and nutrient conditions (Chapter 5). Consequently, an approach developed to disrupt synergies may not favour the historical habitat as strongly as a proactive approach that prevents the initial expansion of novel habitats by ensuring future climates manifest in combination with relatively unpolluted local conditions. Together, these results indicate interactions experienced, both currently and in the past, contribute to determining the dominant habitat configuration.

6.4 FUTURE RESEARCH

As with most ecological research, while testing the original hypotheses a number of issues were encountered which warrant more sophisticated investigation. With this focus, the discussion now addresses potential areas for future research that are perceived to be most critical.

Initial studies considering the influence of forecasted stressors typically aimed to identify the general features of change (e.g. direction and magnitude of change in specific response variables) anticipated to occur when they are modified (as was assessed throughout this thesis for enrichment of resources and removal of key biota). While the results of such studies indicate whether or not particular stressors contribute to the determination of community structure, they cannot suggest how ecological systems will change as stressors are gradually modified, nor can they be used to infer whether thresholds exist for the stressor of interest. In order to identify these trends and thresholds, future research should manipulate the stressors recognised as important in structuring communities to various levels. Similarly, given that the conditions which lead to initial degradation are not necessarily the same as those that facilitate recovery (Scheffer et al. 2001; Beisner et al. 2003), research considering the reversal of such change should incorporate a variety of levels to which conditions could be restored. Identifying the specific conditions at which disproportionate change is both initiated and reversed will be important for understanding ecosystem responses and selecting management targets.

While the effects of altered environmental conditions were identified for adult organisms, it is likely that the susceptibility of other life history stages will be variable (Kroeker et al. 2010). Consequently, the generality of the conclusion that future global CO_2 conditions will have little effect on either kelp (Chapter 2) or grazers (Chapter 4) may be limited. Future research should, therefore, directly consider the potential effects of enriched CO_2 and consequent ocean acidification on additional life history stages (e.g. eggs, sperm, larvae and juveniles) of these species, as has already been considered for a range of others (see, for example, meiospore germination in the giant kelp, Roleda et al. 2011; sperm limitation in the red sea urchin, Reuter et al. 2011; larval development and survival in the northern abalone, Crim et al. 2011). Not only will such research facilitate a better physiological understanding of each individual species, but it may also provide insight regarding the generality of responses in these early life history stages. Furthermore, such research may indicate the potential for these taxa to structure future ecosystems. That is, if altered conditions do significantly influence early life-history stages, population sizes may be reduced such that the ecological role of key taxa is modified, restricting the effectiveness of biotic interactions in limiting the effects of environmental forcing such that ecological change is facilitated.

Consideration of these susceptible life-history stages would lead to identification of cross-generational responses to altered environmental conditions and indicate the adaptation potentials of taxa to altered environmental conditions. While adaptation potentials have been assessed for a few taxa to date (see, for example, Sunday et al. 2011), there is a need for this characteristic to be assessed across a wider variety of marine organisms. Consequently, future studies should quantify this feature for an increasing diversity of taxa, with a particular focus on the key biota that contribute to determining ecosystem structure.

Throughout this thesis, an emphasis has been placed on the importance of interactions between biota in mediating direct responses to altered conditions. Although interactions between key taxa were considered, the size of mesocosms utilised inherently dictated, and limited, the complexity of the ecological system considered. In order to assess the ways in which direct effects identified from single-taxa experiments may be modified by biotic interactions, future studies should be conducted using methods that facilitate consideration of more complex assemblages approaching those of natural systems. One method would be the use of larger mesocosms such that space becomes a weaker limitation. The artificial assemblages contained within such aquaria will, however, always be approximations of those in natural systems (Wernberg et al. 2012). An alternative approach that incorporates the entire community of interest is the quantification of biota along naturally-occurring CO_2 gradients created by volcanic vents/seeps (e.g. Hall-Spencer et al. 2008; Fabricius et al. 2011; Porzio et al. 2011; Johnson et al. 2012). While this method enables consideration of whole-community responses to longer-term CO_2 enrichment, it also has limitations. In contrast to the carefully controlled and maintained conditions achieved in closed mesocosm experiments, those of field studies are inherently more variable. Not only are the communities exposed to fluctuating CO_2 concentrations from the vents, but they are also open to surrounding waters which can act as a source of biota unaffected by the acidified conditions (Cigliano et al. 2010; Hall-Spencer 2011). Consequently, the conditions experienced by measured biota may not be as severe as indicated by the mean pH condition, potentially leading to an under-estimation of the severity of the effects of future CO_2 enrichment (Hall-Spencer 2011). Despite these limitations, field-based methods should be used to compliment carefully-controlled mesocosm-based approaches to facilitate a better understanding of community response to forecasted change.

Interactions among the taxa that comprise ecological systems considered in future research will be influenced by the many stressors created as a consequence of human activities. Although it would be ideal to consider all stressors both in isolation and combination via a factorial design, the number and diversity of conditions being modified by human activities mean such an approach is impossible. Consequently, future research should draw on understanding acquired through natural history (including observations of high-CO_2 volcanic vent environments) and single-factor experiments, such that combinations of stressors forecasted to have the greatest influence are initially manipulated and studied. As the main drivers of ecosystems vary spatially, it is suggested that the stressors considered in experiments should reflect the local ecosystem.

6.5 CONCLUSIONS

As human activities modify an ever-expanding number and diversity of drivers of ecological communities, forecasting their potential interactive effects is of increasing value to science and management. The data presented throughout this thesis demonstrate that understanding how conditions altered at contrasting scales (i.e. global v. local) interact to drive ecological change (i.e. the relative abundance of algal species) provides insight regarding not only the impending impacts of global change, but also the potential for effective local management to determine its influence. While the influence of global conditions (i.e. future CO_2) was restricted where local conditions (i.e. nutrients) were maintained at their ambient level, of concern is the potential that concurrent enrichment of these resources (i.e. future CO_2 and nutrient pollution) may release certain taxa (i.e. turfs), but not others (i.e. kelps), from their limitations such that phase-shifts are favoured. It is possible, however, that local management which retains key biotic interactions (e.g. competition mediated by foundation species or herbivory mediated by grazers) could mitigate the effect of enriched resources. Furthermore, while local management is anticipated to be most beneficial if implemented prior to the manifestation of future global conditions such that interactive effects are prevented, its post-hoc establishment may also be sufficient to disrupt interactions between factors such that further change is slowed. Consequently, while it is often suggested little can be done in the face of entrained global change, the results obtained here suggest it is not only possible, but probable, that effective local management could mitigate many effects of global change.

My office and laboratory at the Cruising Yacht Club of South Australia
(it was also used by other members as a BBQ area).

Photo: Author.

APPENDIX A

APPENDIX A

PREAMBLE

Appendix A is a co-authored paper published in the journal *Sustainability*, with myself as senior author and Owen W. Burnell, Sean D. Connell and Bayden D. Russell as co-authors. It is, therefore, written in plural throughout and uses the journal formatting. It is included with permission from MDPI – Open Access Publishing (See Appendix B), and can be cited as:

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In this Appendix I primarily conducted the initial literature review, wrote much of the initial manuscript and contributed to its revision. Owen Burnell also reviewed the literature and evaluated the manuscript, while Sean Connell and Bayden Russell provided critical evaluations of the manuscript.

Signatures of co-authors:

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Communication

Sustainability in Near-shore Marine Systems: Promoting Natural Resilience

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Abstract: Accumulation of atmospheric CO_2 is increasing the temperature and concentration of CO_2 in near-shore marine systems. These changes are occurring concurrently with increasing alterations to local conditions, including nutrient pollution and exploitation of selected biota. While the body of evidence for the negative effects of climate change is rapidly increasing, there is still only limited recognition that it may combine with local stressors to accelerate degradation. By recognizing such synergies, however, it may be possible to actively manage and improve local conditions to ameliorate the effects of climate change in the medium-term (e.g., by reducing nutrient pollution or restoring populations of herbivores). Ultimately, however, the most effective way to increase the sustainability of near-shore marine systems into the future will be to decrease our reliance on carbon-based sources of energy to reduce the negative effects of climate change.

Keywords: ecosystem shift; climate change; carbon dioxide; algae; amelioration

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1. Introduction

As the human population has grown, so too has the extent and rate at which the environment is modified [1]. While it has long been recognized that human activities directly alter local-scale processes, such as changes to primary productivity through nutrient-pollution or its consumption through fishing of foragers [2-4], it is only recently that global-scale influences have started to receive recognition [5,6]. We now recognize that human activity is driving unprecedented change to climate beyond that attributable to natural variation [7-9]. While the body of evidence for the negative effects of climate change on natural systems is rapidly increasing, there is still only limited understanding of how multiple stressors, such as increasing CO_2 and temperature, may combine to accelerate degradation [10-13]. More worrying, however, is that the impact of climate change will manifest at local scales, and as such, will also interact with local stressors that have been degrading the environment for decades, potentially accelerating change to natural systems.

2. Synergistic Effects and Accelerated Degradation

The oceans currently absorb ~30% of the CO_2 emitted into the atmosphere. It is now well established that the resulting reduction in pH (or ocean acidification) has negative effects on calcifying organisms [14-18]. An important recognition, however, is that in marine systems multiple climate parameters (e.g., temperature and pH) are inherently linked to one another via atmospheric CO_2 concentration [19,20], and may create complex outcomes which cannot be predicted through studying variables independently [13,21,22].

Most research into the effects of climate change on marine systems has considered acidification or temperature in isolation and there is a lack of knowledge about the extent of these interactions [23,24]. The few studies that have focussed on the interactions between these two parameters have demonstrated conflicting responses, showing possibilities of both synergistic negative effects on organisms [11,12] and others where the effects are not of a multiplicative nature [25]. Importantly, while organisms may show some resistance to independent stressors, their sensitivity is often altered under the concurrent application of multiple changes, resulting in effects of a larger magnitude than anticipated from the study of independent stressors [11-13,22]. Further, there is recent evidence that combinations of climate factors, such as elevated CO_2 and temperature, may have synergistic positive effects on some non-calcareous algae which facilitate ecosystem shifts, thus producing a negative ecological outcome [10].

Of even greater concern is how changing climates will combine with local environmental impacts. Numerous stressors, particularly nutrient pollution and exploitation of selected biota, have resulted from human activities and produced environmental conditions distinct from those experienced at any other time in history [26]. As the effects of climate change will manifest at similar scales to these local stressors, it is likely that they will combine to alter conditions that maintain system function through amplifying feedbacks, compounding effects and synergies [27,28]. For example, in temperate marine waters canopies of algae form forests analogous to tropical rainforests. These canopies are a foundation for marine systems, providing structure that enables stabilization of physical environments, survival of associated species and economic benefit for human societies [29,30]. On many coasts of the world, however, these canopies are being replaced by small filamentous algal turfs [31-33], causing massive loss of biodiversity and ecological function. This current decline is being driven by elevated nutrients from land-derived sources [34-36]. Furthermore, it has only recently been recognized that increasing [CO_2] may increase the productivity of non-calcareous algae, particularly the opportunistic species which facilitate system shifts, suggesting that the decline of algal canopies will be accelerated into the future as local nutrient pollution interacts with increasing [CO_2] to increase the abundance of turf-forming algal species which inhibit the recovery of algal forests [10,37].

Nutrient-driven loss of the dominant habitat forming taxa is also well documented in tropical ecosystems, where macroalgae overgrow and smother reef-building corals [38]. As with kelp dominated systems, this habitat loss is accelerated under a combination of perturbations such as increased nutrients and harvesting of herbivores [39,40]. With the increasing body of literature demonstrating the negative effects of increasing [CO_2] on corals, it is likely that the interaction between increasing [CO_2] and nutrients will cause a synergistic negative effect on coral reefs from two directions, the negative effect on corals and the positive effect on non-calcareous macroalgae. Therefore, while the study of climate stressors on individual organisms provides insights into species level responses and adaptation, an understanding of local-to-global scale interactions between multiple stressors on communities is required to identify mechanisms of increasing resilience of systems into the future.

3. Promoting Natural System Resilience

Current projections of climate driven change to oceanic pH are based primarily upon ocean physics, with the biological components of these predictions currently lacking in sophistication [20]. Subsequently, the current understanding of the potential for natural biological interactions to provide both regional and global resilience to ocean acidification remains limited. Increasing CO_2 and the associated reduction in pH is influenced by the photosynthesis and respiration of marine organisms [18,41-43]. For example, diurnal pH fluctuations in coastal sub-tidal zones caused by photosynthesis and respiration can be in the range of 0.2–0.3 units [44]. However, it is the very cause of this variation that may ameliorate some of the negative effects of climate change; it has been predicted that future CO_2 may enhance the productivity of marine plants and potentially some non-calcifying algae [24,45-47]. Implementing conservation measures to protect large habitat-forming algae and seagrasses (c.f. algal turfs which reduce habitat complexity) could allow this adaptive capacity to moderate ocean acidification and buffer against some of the negative effects associated with increased [CO_2]. While this buffering effect is likely to provide global benefits via increased carbon sequestration, additional local actions to maintain biomass of photosynthetic organisms in systems (e.g., kelp forests or seagrass meadows) may help decrease the effects of elevated CO_2 within these localities.

Another management tool to enhance the resilience of systems to climate change would be to remove the potential for synergies between climate change and local conditions that have been altered by human activities. The presence of herbivores can moderate the effect of local pollution (*i.e.*, elevated nutrients) through consumption of bloom-forming macroalgae that drive ecosystem shifts at the expense of complex habitat-formers [40,48-51]. There is also evidence that the presence of herbivores may increase the resilience of systems to climate-related stressors, as seen with the recovery of coral reefs from bleaching events [52]. Therefore, protection of herbivores from harvesting, such as through the establishment of Marine Protected Areas, could increase the resilience of natural systems to both local and global stressors.

This moderating effect of herbivory can, however, be overwhelmed by longer-term eutrophication events [48,50,53]. Further, it can be reasonably predicted that ocean acidification may disrupt the feeding biology of some herbivores [54,55], restricting their ability to effectively control the nutrient or climate-enhanced growth of algae. Therefore, reducing the potential for synergies between local conditions and climate change may only be possible by reducing the nutrient load entering marine waters from terrestrial sources [37]. Recognition of the need to increase resilience in natural systems has lead to more proactive management of local stressors in some regions. In South Australia, local government now recognizes the global-local connections of future change, and has implemented long-term policy to upgrade wastewater treatment plants and to recapture storm-water to produce recycled water for residential and industrial use. While concerned mainly with the provision of freshwater for human use, this policy also reduces the nutrient rich discharge that has primarily contributed to phase shifts on metropolitan reefs from kelp to turf-dominated [32,34].

While recent experimental work indicates that the reduction of pollution or supporting populations of herbivores may increase resilience of near-shore marine systems [37,52], it is likely that the regional biological context will be an important consideration. In regions which have naturally eutrophic waters (e.g., upwelling zones) and strong herbivory it may be more appropriate to use Marine Protected Areas to support natural populations of herbivores. Conversely, elevated nutrients can have disproportionately large effects in regions which have oligotrophic waters and weak herbivory [56] so restricting terrestrial based sources of nutrient pollution may be more affective in maintaining system resilience under future climates. Further, regional assessments of the potential impact of climate change show that it’s effects will vary geographically and that actions to ameliorate climate change will need to differ among regions [57,58]. Therefore, to maximize the effectiveness of actions to increase the resilience of natural systems to climate change, it will be necessary to identify the regional drivers of systems (e.g., nutrients *vs.* herbivory) and the likely drivers of future change (e.g., temperature *vs.* increasing [CO_2])

What we are yet to identify is whether local mitigation measures are likely to be more effective when implemented before forecasted climates arrive [59]. Recruitment of habitat-forming species is key to ecosystem resilience [60], and both local and global perturbations tend to promote species which inhibit recruitment of habitat-forming species [31,34,40,52]. Therefore, it is likely that locations which have already undergone loss of habitats driven by anthropogenic perturbations will be less likely to recover to their “natural” state if local amelioration actions are implemented after global stressors have passed a threshold where recruitment of habitat-forming species is inhibited.

4. Conclusions

The anticipated synergies between local conditions and increasing temperature and CO_2 have clear implications for the function of marine systems globally. In the medium-term, it may be possible to promote the resilience of natural systems by either reducing local stressors or supporting populations of organisms which will alter the effects of these stressors. Management which limits nutrient addition (*i.e.*, stops waste water outfall) or protects herbivores from fishing (e.g., Marine Protected Areas) will restrict the growth of bloom-forming algae and possibly prevent system shifts to less desirable states, especially if such strategies are implemented in unison. Such management actions cannot, however, ameliorate the effect of negative synergies between climate factors (e.g., CO_2 and temperature) and ultimately the only way to mitigate the effects of climate change is to reduce reliance on carbon-based sources of fuel [61]. This understanding is particularly important as managing global-scale changes is inherently difficult because their sheer magnitude requires an international effort to implement policy change and because their effects are so long-lasting, if not permanent.

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Sunrise at the Cruising Yacht Club of South Australia.

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