Title
Responses of marine macroalgae to short and long-term changes in nutrient availability under varying environmental conditions

Author
Clausing, Rachel

Publication Date
2014

Peer reviewed|Thesis/dissertation

University of California
Los Angeles

RESPONSES OF MARINE MACROALGAE TO SHORT AND LONG-TERM CHANGES IN NUTRIENT AVAILABILITY UNDER VARYING ENVIRONMENTAL CONDITIONS

A dissertation submitted in partial satisfaction
of the requirements for the degree
Doctor of Philosophy in Biology

by

Rachel Joy Clausing

2014

Abstract of the Dissertation

RESPONSES OF MARINE MACROALGAE TO SHORT AND LONG-TERM CHANGES IN NUTRIENT AVAILABILITY UNDER VARYING ENVIRONMENTAL CONDITIONS

by

Rachel Joy Clausing

Doctor of Philosophy in Biology

University of California, Los Angeles, 2014

Professor Peggy M. Fong, Chair

While macroalgae provide habitat and trophic support in many marine ecosystems, excessive proliferation is often considered an indicator of an impacted system, particularly in tropical reef ecosystems. How the processes structuring these macroalgal communities are affected by anthropogenic impacts, particularly within the context of spatial and temporal environmental heterogeneity, remains unclear. I conducted a series of short-term and long-term experiments in both tropical and intertidal temperate ecosystems examining the role of nutrient enrichment and its interactions with other anthropogenic stressors (reduction of herbivores, sediments) in regulating macroalgal populations and structuring macroalgal communities.

On an impacted tropical reef, I manipulated nutrient availability on the dominant reef flat macroalgal species at various times after rainfall. Nutrient limitation rapidly switched from nitrogen to phosphorous to no limitation over very short time scales, highlighting the dynamic relationship with environmental context. Additionally, field and lab experiments examined how terrestrial sediment loads on these algal thalli disturbed nutrient and herbivory control. I found that environmental conditions created by sediment loads had variable effects on algal biomass accumulation of different species, suggesting effects by different mechanisms. On an intertidal temperate reef, I manipulated nutrient availability and herbivory on macroalgal communities for two years, encompassing the heterogeneous nature of

rocky reefs. Grazers had more dramatic and immediate effects, increasing cover by >10x in the first year. However, nutrients influenced the community in nearly all metrics in the second year.

Overall, my results indicated that nutrient control of tropical reef macroalgae is more complex than previously recognized and depends on both the species and context under consideration. Moreover, sediment loads may strongly modulate controls on macroalgal dynamics by altering, among other things, nutrient availability and herbivory. Finally, on intertidal temperate reefs, where nutrient control remains a matter of debate, my results showed that nutrient addition and herbivore reduction have complex effects on algal diversity and structure that changed over time and depended on habitat complexity. Together, these results indicate the importance of considering timescales and environmental context when determining the consequences of anthropogenic alteration to controls of macroalgal dynamics on both tropical and temperate reefs.

The dissertation of Rachel Joy Clausing is approved.

Paul H. Barber

Richard F. Ambrose

Peggy M. Fong, Committee Chair

University of California, Los Angeles

2014

To my parents

for their enduring love, patience, and support

Table of Contents

1 INTRODUCTION **1**

References **7**

2 DYNAMIC TEMPORAL CHANGES IN NUTRIENT LIMITATION OF TROPICAL MACROALGAE DEPEND ON LIFE HISTORY STRATEGIES AND ENVIRONMENTAL CONTEXT **17**

2.1 Abstract 17

2.2 Introduction 18

2.3 Methods 22

2.3.1 *Study site* 22

2.3.2 *Environmental data* 22

2.3.3 *Experimental design* 23

2.3.4 *Tissue nutrient collections* 25

2.3.5 *Data analysis* 26

2.4 Results 26

2.4.1 *Environmental context of experimental bioassays* 26

2.4.2 *Experimental results* 28

2.4.3 *Tissue nutrient collections* 29

2.5 Discussion 30

2.6 Acknowledgments 35

References **40**

3 SEDIMENTS DIFFERENTIALLY ALTER DRIVERS OF TWO DOMINANT

MACROALGAL SPECIES BIOMASS ACCUMULATION ON A FRINGING CORAL REEF **47**

3.1 Abstract 47

3.2 Introduction 48

3.3 Methods 51

3.3.1 *Study site and species* 51

3.3.2 *Experimental approaches* 54

3.3.3 *Tissue nutrients* 58

3.3.4 *Calcification versus nutrient content of P. boryana* 58

3.4 Results 59

3.4.1 *Characteristics of the study system and species* 59

3.4.2 *Experimental results* 60

3.4.3 *Calcification and nutrient content of P. boryana* 62

3.5 Discussion 63

Appendices **76**

3.A Calculations of percent change in tissue nutrient mass 76

3.B Additional experimental results 77

References **82**

4 DELAYED BUT EXTENSIVE EFFECTS OF NUTRIENT ADDITION ON PRODUCER COMMUNITIES IN A TOP-DOWN DOMINATED ECOSYSTEM **88**

4.1 Abstract 88

4.2 Introduction 89

4.3 Methods 94

4.3.1 *Study site* . . . 94
4.3.2 *Experimental design and set up* . . . 94
4.3.3 *Measuring response variables and treatment maintenance* . . . 97
4.3.4 *Data analysis* . . . 98
4.4 Results . . . 100
4.4.1 *Environmental parameters* . . . 100
4.4.2 *Algal diversity indices* . . . 100
4.4.3 *Algal functional form cover* . . . 101
4.4.4 *Algal successional trajectory* . . . 103
4.5 Discussion . . . 104

Appendices . . . **119**
4.A Site details and justification . . . 119
4.B Experimental methods . . . 121
4.B.1 Algal methods . . . 121
4.B.2 Herbivore methods . . . 121
4.B.3 Herbivores biomass . . . 123
4.C Treatment efficacy and environmental parameters . . . 125
4.C.1 Treatment efficacy . . . 125
4.C.2 Environmental parameters . . . 131
4.D Additional experimental results . . . 133

References . . . **140**

5 CONCLUSION . . . **151**

List of Figures

2.1 Daily a) rainfall and b) daytime solar radiance during experimentation . . . 37

2.2 Growth response of 3 algal species to addition of nitrogen (N) and phosphorus (P) . . . 38

2.3 Tissue nutrient data collected for each species at three times after varying periods post rainfall. . . . 39

3.1 *Padina boryana* and *Galaxaura fasciculata* under ambient sediment loads on Gump Reef in Cook's Bay, Mo'orea, French Polynesia . . . 68

3.2 Tissue nutrient data of *Padina boryana* and *Galaxaura fasciculata* and the environmental history of rainfall prior to each experiment . . . 69

3.3 The net effect of sediments on biomass accumulation of A) *Padina boryana* and B) *Galaxaura fasciculata* and tissue nutrient content of *Padina boryana* 70

3.4 The interacting effects of natural sediments and added nutrients on biomass accumulation of A) *Padina boryana* and B) *Galaxaura fasciculata* . . . 73

3.5 The individual and interacting effects of ambient sediments and herbivory on biomass accumulation and tissue nutrients of *P. boryana* and *G. fasciculata* . 74

3.6 Percent (A) calcification and (B) nitrogen of thallus sections of *P. boryana* . 75

3.B.1 Nutrient content of sediments collected from thalli of *P. boryana* and *G. fasciculata* on the experimental reef in April 2013 . . . 77

3.B.2 Tissue nutrient content of *G. fasciculata* with ambient and removed sediment loads . . . 80

3.B.3 Change in tissue nutrient content of *P. boryana* and *G. fasciculata* with manipulation of sediments and water nutrient content . . . 81

4.1 Site location in Cook Strait on the South Coast of Wellington, New Zealand. 109

4.2 (A) Pielous Evenness and (B) Shannon-Wiener Diversity of algal functional form percent cover over time 110

4.3 Species Richness of (A) common species (>1% cover) and (B) rare species (<1% cover) over time 112

4.4 Functional group composition of the algal community at the start and end of experimentation 114

4.5 Percent cover over time of the four dominant functional forms: (A) foliose (B) corticated (C) crustose and (D) CCA 115

4.6 Algal community succession among treatments visualized as (A) ordination of multivariate centroids and (B) PCA of the dominant taxonomic groups . . 118

4.C.1 Water column nutrient levels taken throughout the year at varying times after experimental nutrient addition. (A) nitrate (NO_3) and (B) ammonium (NH_4). 126

4.C.2 Biomass of herbivores in plots with ambient herbivore communities in both enriched and ambient nutrient treatments 130

4.D.1 Percent cover of (A) total algal cover and (B) open space over the experimental duration 136

4.D.2 Percent cover of algal film over the experimental duration 138

List of Tables

2.1 Two-factor PERMANOVA results on each N vs. P limitation bioassay per species and time combination 36

3.1 Characteristics of sediment collected from the thalli of *P. boryana* and *G. fasciculata* 68

3.2 Two-way ANOVAs on the effects of sediments and nutrient addition on growth of *P. boryana* and *G. fasciculata* 70

3.3 Two-way ANOVAs on the interacting effects of sediments and herbivory on growth and tissue nutrient stores of *P. boryana* and *G. fasciculata* 71

3.4 Mixed effects model on A) % calcification and B) % nitrogen of *P. boryana* thallus sections 72

3.B.1 Net Effect Field Experiment: mean tissue %N and %P with ambient and removed sediments 77

3.B.2 Sediment x Nutrient Mesocosm Experiment: interacting effects of sediments and nutrient addition on tissue nutrient stores 78

3.B.3 Sediment x Nutrient Mesocosm Experiment: mean tissue %N and %P with and without sediments and nutrient addition 79

3.B.4 Sediment x Herbivore Field Experiment: mean tissue %N and %P with ambient and removed sediment loads and herbivory 79

4.1 Linear mixed models of functional form-based measures of algal community diversity: (A) Pielou's Evenness and (B) Shannon-Wiener diversity. Complexity refers to topographic complexity. 111

4.2 Linear mixed models of (A) common and (B) rare species richness 113

4.3 Linear mixed models of % cover of algal functional forms: (A) foliose, (B) corticated, (C) non-calcareous crustose, and (D) CCA 116

4.B.1 Functional form groupings for observed taxonomic units of algae 122

4.B.2 Percent composition of total herbivore biomass in initial surveys 123

4.B.3 Contribution of the dominant herbivores to the average % biomass composition in ambient grazers plots across all sampling dates 124

4.B.4 Regression coefficients for the relationship between herbivore length and biomass 124

4.C.1 Percent loss by weight of osmocote after two weeks diffusing nutrients in the field 127

4.C.2 Timeline of nutrient addition and water sampling events 128

4.C.3 Results of a linear mixed model on nitrate (NO_3) concentrations above ambient vs. enriched plots 129

4.C.4 Results of a linear mixed model on ammonium (NH_4) concentrations above ambient vs enriched plots 129

4.C.5 Results of a linear mixed model on herbivore biomass in the ambient herbivore treatment 130

4.C.6 Environmental (habitat) data for each plot 132

4.D.1 Shannon Wiener Diversity (H) (utilizing all species percent cover including rare species at 0.5%) 133

4.D.2 Pielou's Evenness (J) (utilizing all species percent cover including rare species at 0.5%). 134

4.D.3 Results of a linear mixed effect model on total algal percent cover. 137

4.D.4 Results of a linear mixed effect model on percent cover of open space. . . . 137

4.D.5 Results of a permutational manova on algal functional form percent cover data. 138

4.D.6 Results of a linear mixed effect model on percent cover of algal film. 139

4.D.7 PCA loadings for the ten species most aligned with PCA1 and PCA2 . . . 139

Acknowledgments

So many people have been an integral part of this learning and growing process in so many ways. I owe both its completion and my own professional and personal development to each and every one of them, and more unnamed here.

First and foremost, thank you to my advisor, Peggy Fong, who has been not only an academic and intellectual guide, but also a source of support and encouragement. Her energy is boundless, and she is a fount of ideas and creative solutions. Moreover, she gave me the option and support to pursue an outside path in my research, and stuck with me when those decisions resulted in challenges. I'm indebted to her in so many ways.

I'm also thankful for a supportive committee in Rich Ambrose, Paul Barber and Rick Vance, all of whom have provided valuable alternative perspectives to my tendency toward tunnel-vision. I'm especially grateful to Rich Ambrose who spent extra time introducing me to the rocky intertidal - in the field, with insights in on-campus meetings, and grandfathering me into the MARINe community and the many wonderful people there.

Jeff Shima and Nicole Phillips require an extra special thanks. Their sponsorship got me in the door with Fulbright New Zealand, and their continued support and advice for two + years at the Victoria University Coastal Ecology Lab (VUCEL) truly made my research possible. Their generosity is unparalleled. The School of Biological Sciences at Victoria University of Wellington housed me during my Fulbright tenure, provided financial support and access to the facilities at VUCEL, and allowed me to continue with my research long after my Fulbright had ended; I am grateful to all the Vic staff. Daniel McNaughtan and John VanderSman provided logistical aid, and Ken Ryan allowed me to use his fluorometer.

Heather and Nigel Roberts unsuspectingly shared their lovely home with me for a few days and never complained when I stayed for a month. And kept coming back. And regaled them with stories of limpets. You are truly my "New Zealand parents".

A number of individuals have given me their time and expertise. Jay Smith, Carol Blanchette, and Steve Lee thank you all for helpful discussion, guidance, and fieldwork fun in

various rocky intertidal locations. Sean Vogt and I had many adventures in Orange County identifying algae by headlamp.

The University of California Reserve system provided access to and facilities at protected field sites. In particular, the managers of the UC Rancho Marino Reserve, Don Canestro, and the UC Santa Cruz Island Reserve, Lyndal Laughrin, provided logistical support, patience, great stories and fantastic waffles. The team at Te Papa in Wellington welcomed me warmly to their Phycology group. Jenn Dalen and Wendy Nelson in particular helped with algal ids. I am thankful for you all.

A shout out to my cohort: Keith, Pam, Gilene, Princess, David, Christene, Chris, Ranjan, Raul and Hilton. We've made this journey together, and though we are now all over the globe, we remain intact. Thanks for all the shared fun, laughs, tears, and support.

Without my friends and labmates, this work could not have been done, and it sure would have been a lot less fun. To the Fong lab - Rachel, Lauri, Sarah, Tonya, Sara, Ranjan, Sarah Joy and my fellow algal enthusiast, Stacie - my time here has been enriched by our conversations about science and life, the outdoor adventures, and your help in navigating graduate school. Jamie and Sandra - within a short time in New Zealand, you became my co-conspirators and collaborators - both in research, and in dedicated coffee drinking. The Chocolate Fish will never be the same without us. I am incredibly appreciative of Tim Jones and Brant Faircloth who both provided statistical advice as well as ideas, assistance, and humor in the field, and Shane Geange who was never too busy to lend a helping hand. Caitlin Fong and Sarah Bittick provided much insight, field help, and laughter in the execution of chapter two.

In rain, 3+ meter waves, and wind gusts of over 100 kmph, my New Zealand field team never said 'die'. Katie Clemens-Seely and Nicky Fitzgibbon, I can confidently say there are no two tougher and more dedicated women out there. May the Coolah live on! Jeff, Bird, Rebecca and countless others - I would still be out at the quarry with the hammer drill if it weren't for you. Likewise at UCLA - without the boundless enthusiasm of Tim White and the patient diligence of Courtney Van Gorden, Jordan Fong and Kendal Fong, as

well as the generous donation of Sarah Joy's algae task force, I would still be grinding algae and scoring photos. My heartfelt gratitude goes to Jocelyn Yamadera, without whom I am certain no one in EEB would ever get paid or graduate.

There are a few special people scattered around the globe that I am honored to call both mentors and friends: Mark Roberts, Chun Ti, Athena McKown, and Rachel Kennison - your perspectives and insights have kept me sane and motivated, each in very different ways! Gia Marson, our conversations have included many lightbulb moments and always give me the reality check I need; I have grown so much through them.

Finally, my gratitude goes to those family and friends who have been there for me each in their own way: my sisters Abby, who has on countless occasions spent her vacation helping me in the field or helping me move, and Katie, who always has an encouraging word or a photo of her adorable kids ready when I need cheering up; Rachel Venema - for all the times in Chicago and good conversation - they are always a breath of fresh air; Rachel Lagestee - for great roadtrips and your enduring friendship; my roomies Emily Barkley-Levenson and Erin Streff - for shared laughs, meals and spontaneous fun; and last, to Vlad, for his uncanny ability to make me laugh even when I'm crying, and for showing me that I can work hard, play hard and still take time to relax and eat a good brunch now and again.

I have been very fortunate to be supported by the UCLA Ecology and Evolutionary Biology Chair's Fellowship, GAANN, the UCLA Graduate Division Chancellor's Prize and the Dissertation Year Fellowship, the National Science Foundation Graduate Research Fellowship, and the US Fulbright IIE and Fulbright New Zealand programs. Additional research funds were provided by the Mildred E. Mathias Graduate Student Research Grant, Pacific Rim Fellowship Program, Sea Space, Western Aquatic Plant Management Society, and the Department of Ecology and Evolutionary Biology at UCLA. Phil Ender, Andy Lin, and others at the UCLA Statistical Consulting Center provided hours of invaluable advice.

Last, I am forever grateful to my parents, who have been the picture of diligence, hard work, faith, and compassion in all they do. I still have a lot to learn from you.

Vita

2002–2006	B.S. in Biology and in Marine Science University of Miami, Miami, Florida
2003	Research assistant, Operation Wallacea Hoga Research Station, Indonesia
2004	Research Assistant James Cook University, Australia
2005	Research Intern, Honors Summer Research Program Rosenstiel School of Marine & Atmospheric Science, Miami, FL
2006	Honors Researcher, Department of Biological Sciences University of Miami, FL
2007-2008	UCLA Chancellor's Prize Recipient University of California Los Angeles
2008	Workshop Overseer Center for Ocean Sciences Education Excellence West, NSF
2008, 2012	Teaching Assistant, Dept of Ecology & Evolutionary Biology University of California, Los Angeles
2010	Fulbright IIE Recipient Victoria University of Wellington, New Zealand
2010-2013	National Science Foundation Graduate Research Fellow University of California, Los Angeles
2013-2014	Dissertation Year Fellow University of California, Los Angeles

Publications and Presentations

Obaza, A.K., R. Hoffman, B. Chesney and **R.J. Clausing** (2014). Long-term fish community stability in urban estuarine eelgrass habitat, *in review for Estuaries and Coasts.*

Clausing, R.J. and P. Fong (2014). Dynamic changes in nutrient limitation of tropical macroalgae depend on algal identity and environmental drivers, *in review at Oecologia.*

Clausing, R.J., C. Annunziato, G. Baker, C. Lee and P. Fong (2014). Herbivorous fish ameliorate sediment-linked hydrogen sulfide production in algal turf communities, *in revision for MEPS.*

Lirman, D., G. Deangelo, J. Serafy, A. Hazra, D. Smith Hazra, J. Herlan, J. Luo, S. Bellmund, J. Wang and **R.J. Clausing** (2008). Seasonal changes in the abundance and distribution of submerged aquatic vegetation in a highly managed coastal lagoon. *Hydrobiologia* 596: 105-120.

Clausing, R.J. and P. Fong. 6 Aug 2013. *Context-dependent effects of sedimentation on top-down and bottom-up control of tropical algae.* Oral presentation at Ecological Society of America Annual Meeting, Minneapolis, MN.

Clausing, R.J. 24 Nov 2010. *Effects of anthropogenic nutrient inputs on biodiversity of New Zealand rocky shores.* Oral presentation at Fulbright New Zealand, NZ.

Clausing, R.J. and P. Fong. 6 Jul 2010. *Life history strategies drive macroalgal responses to terrestrial nutrient inputs: an example from a fringing reef in Mo'orea, French Polynesia.* Oral presentation at New Zealand Marine Science Society, NZ.

Clausing, R.J. and P. Fong. 6 Nov 2009. *Nutrient limitation in tropical macroalgae depends on life history strategies and watershed influences.* Poster presentation at Coastal Estuarine Research Federation, Portland, OR.

Clausing, R.J., et. al. 25 Aug 2009. *A model of coral/algal competition in reef environments.* Oral presentation at National Center for Coral Reef Research, Miami, FL.

CHAPTER 1

INTRODUCTION

Two of the primary controls of macroalgal abundance across all ecosystems are the availability of nutrients primarily nitrogen (N) and phosphorus (P) and the strength of herbivory (e.g. Fong and Paul, 2011). However, humans have altered nutrient content (Valiela et al., 1992; Vitousek et al., 1997; Tilman et al., 2001; Suding et al., 2005) and reduced abundance or diversity of herbivores in nearly every ecosystem worldwide (Steneck, 1998; Jackson et al., 2001). The effects of these changes to nutrient and herbivore control on macroalgal dynamics can be hard to predict, particularly as they are modulated by spatial and temporal variability in both external environmental drivers (e.g. rainfall) and inherent physical characteristics (e.g. sediments, complexity). Moreover, different strategies among macroalgal forms or species with regard to morphology and physiology (e.g. nutrient use: Pedersen and Borum, 1997; Pedersen et al., 2010) suggest that changes in nutrient availability or herbivory may have differential affects among them. Thus, studies are needed examining how the roles of nutrients and herbivory in macroalgal species and community dynamics change with spatial and temporal variability in environmental heterogeneity.

Importance of nutrients for macroalgae

Nutrients (N and P) have been shown to limit primary productivity in nearly every ecosystem worldwide (reviewed by Downing et al., 1999; Elser et al., 2007; Harpole et al., 2011); yet how changes in nutrient availability over time affect growth response of macroalgae, particularly in connection to environmental drivers, remains understudied. In tropical reef systems, macroalgal proliferation is often attributed to release from nutrient limitation with enrichment (McManus and Polsenberg, 2004; Fabricius, 2005). However, evidence of

nutrient-stimulated growth is mixed (Smith et al., 2010, for reviews, see Fong and Paul, 2011; Mejia et al., 2012), with some species responding to enrichment (e.g. Lapointe et al., 1992; Larned, 1998; Smith et al., 2001; Vermeij et al., 2010), others showing mixed species responses (Thacker et al., 2001; Fong et al., 2003) and some finding no response (Kuffner and Paul, 2001; Koop et al., 2001). In contrast, temperate reefs are traditionally considered nutrient replete. Currents and wave action were thought to prevent depletion of relatively high nutrient levels by increasing advective supply (Mann, 1973; Probyn and Chapman, 1983; Leigh et al., 1987, but see Hanisak, 1983), suggesting rocky reef communities should be resistant to enrichment. In addition, early studies nearly exclusively demonstrated the dominance of top-down control (e.g. Connell, 1961; Paine, 1966; Dayton, 1971; Menge, 1976, 1978; Lubchenco and Menge, 1978, reviewed by Menge, 2000). In the past decade, however, continued increases in coastal nutrient loading worldwide (Vitousek et al., 1997) have prompted studies examining the effects of nutrient enrichment on shallow subtidal and intertidal temperate reefs; but these also led to mixed effects (e.g. Lotze et al., 2001; Nielsen, 2001, 2003; Bracken, 2004; Kraufvelin et al., 2006; Korpinen et al., 2007; Masterson et al., 2008; Guerry et al., 2009; Bulleri et al., 2012; Williams et al., 2013). Thus, consequences of nutrient enrichment on temperate reefs are relatively unknown.

Environmental drivers of nutrient availability

Predicting community consequences of altered nutrient availability in coastal marine systems may be complicated by unpredictable spatial and temporal variability in nutrient supplies due to the often localized and typically pulsed nature of nutrient loading events (McCook, 1999; Fong et al., 2001; Fry et al., 2003). Despite knowledge of the temporal variability of water column nutrients levels, few studies of nutrient enrichment on reefs have been explicitly replicated over time (but see Lapointe, 1987; Delgado and Lapointe, 1994, for replication in winter and in summer). Moreover, none have examined changes in algal limitation within the context of rapid environmental changes, particularly on the short-term scale of variability known to occur in water column nutrient levels on tropical reefs (McCook, 1999). Widespread changes in nutrient regimes necessitate focus on the patterns of

limitation and the role of environmental context in how species respond to temporally varying nutrient loads associated with anthropogenic inputs. In contrast, the dynamic nature of temperate reefs suggests that periodic nutrient events may be rapidly diluted, and only long-term nutrient disturbances (e.g. Kraufvelin et al., 2006) or those occurring on recently opened substrate (e.g. Guerry, 2008) may result in community-wide changes.

Differential effects of nutrients across varying species strategies

Perhaps most problematic for predicting macroalgal responses to changes in nutrient and herbivore control are species-specific variations in life history strategies, particularly with regard to morphology and nutrient use (Pedersen and Borum, 1996; Fong and Paul, 2011; Gordillo, 2012). These different strategies may result in strongly heterogeneous responses to enrichment. For example, opportunist algal species have the ability to take up nutrients rapidly when they become available and store them in their tissues for future growth during low nutrient periods (Hanisak, 1983; Fujita and Goldman, 1985; Fong et al., 2003), highlighting the importance of nutrient history in understanding limitation. Thus, enrichment response may depend on internal stores of nutrients, where these opportunistic species only exhibit limitation when internal stores are depleted (e.g. Schaffelke and Klumpp, 1998; Fong et al., 2003; Beach et al., 2006). This adaptation may be particularly advantageous in naturally low-nutrient tropical systems when nutrient inputs are pulsed (Fong and Paul, 2011). In contrast, slower-growing perennial species may have very limited storage capacities, but lower nutrient requirements enable them to persist under low nutrient conditions (Gordillo, 2012) typical of tropical reefs. Thus growth is more directly dependent on availability of low but more consistent supplies of nutrients in the environment (Delgado and Lapointe, 1994; Fong et al., 2003). Theory (Cloern, 2001) and some studies (e.g. Worm and Lotze, 2006; Masterson et al., 2008) also suggest that nutrient enrichment on temperate reefs should increase the abundance and dominance of early-colonizing, opportunist species at the expense of slower-growing, later-successional perennial forms (see also Kraufvelin et al., 2006, 2010). Thus the nutrient-use strategies of the species in question must be considered in attempts to understand the consequences of nutrient enrichment, particularly in systems where pulses

of anthropogenic enrichment may result in fluctuations in or widespread changes to nutrient regimes.

Interactions of nutrients with herbivores – theory and evidence

Extension from theoretical models (Huston, 1979, 1994; Tilman, 1994; Kondoh, 2001) predict that the effects of changes in nutrient supply on algal diversity should vary depending on levels of herbivory and vice versa (Proulx and Mazumder, 1998; Worm et al., 2002). In spite of the strong nutrient-herbivore interactions predicted by theory and general consensus that these effects need to be considered together, empirical evidence is limited (e.g. Hillebrand, 2003; Nielsen, 2003; Korpinen et al., 2007; Guerry et al., 2009; Atalah and Crowe, 2010; Williams et al., 2013 but see Worm et al., 2002; Worm and Lotze, 2006). On intertidal temperate reefs, some studies suggest that interactions may be found depending on the timescale (e.g. Guerry, 2008) or component of the algal community (e.g. opportunist species, biomass, cover: Nielsen, 2001; Guerry et al., 2009; Bulleri et al., 2012). Guerry 2008 found that at low nutrient levels (ambient), herbivores reduced species richness, but only in the first year of the experiment; this effect was not seen after two years. On tropical reefs, herbivory, particularly by intact fish communities, is likely to compensate for increased macroalgal growth with enrichment in the short term (e.g. Burkepile and Hay, 2006; Heck and Valentine, 2007; Rasher et al., 2012 but see Vermeij et al., 2010), but it is unclear how broadly and to what extent these interactions occur, particularly with continued enrichment (Littler et al., 2006; Smith et al., 2010; Rasher et al., 2012). Overall, these studies suggest that interactive effects, if occurring, may be most prominent on total algal cover or biomass, particular components of the community (e.g. opportunist species) and certain stages (e.g. early successional communities). Moreover, they indicate the importance of scale – both timescale and level of organization (individual, population, community) – in detecting effects, and in the generalizations that can be made from them.

Role of environmental context: sediments and stress

Variation in the strength of nutrient and herbivore control may result from spatial

and/or temporal variation in environmental factors that cause stress. On tropical reefs, a main stressor of concern is sediments (Fabricius, 2005; Mora, 2008). Despite increasing concern of the impacts of anthropogenic sediment loads on coral reefs (Richmond et al., 2007), it is relatively unknown how sediments interplay with the dominant drivers of algal communities, nutrients and herbivory, to control their dynamics. One of the primary effects of increasing sediment loads may be changes in the availability of resources for photosynthesis that ultimately affect algal growth, abundance and community composition. Effects may be positive by providing a nutrient source (e.g. Schaffelke, 1999; Stimson and Larned, 2000; Eyre and Ferguson, 2002) or negative, by blocking light and gas or nutrient exchange (Airoldi, 2003). Sediments may also alter processes that affect algal loss by blanketing thalli and thereby interfering with herbivory (e.g. Bellwood and Fulton, 2008; Goatley and Bellwood, 2012, 2013). However, sediment loads on algal thalli may vary spatially due to localized sources or different thallus structure, and temporally with inputs (e.g. rainfall). Heterogeneity in environmental factors is characteristic of intertidal temperate reefs (Menge and Olson, 1990). In particular, physical stress from high temperatures and desiccation are well-documented, important drivers of community dynamics (Morelissen and Harley, 2007; Bertocci et al., 2010; Gedan et al., 2011; Williams et al., 2013). At the local scale, topographical heterogeneity and its effects on water motion and retention may alleviate thermal stress (Jackson et al., 2013), and in doing so, differentially alter the effects of nutrient and herbivory on algal communities (Werner and Matthiessen, 2013). Thus, in order to understand how nutrients and herbivores act to control macroalgal diversity in realistic systems, studies are needed that incorporate heterogeneity in the environment (e.g. sediments, stress) while accounting for the changes that they cause.

I investigated the role of nutrient enrichment and its interactions with other anthropogenic stressors (reduction of herbivores, sediments) in regulating macroalgal populations and structuring macroalgal communities on tropical and intertidal temperate reefs within the context of environmental heterogeneity. To do so, I conducted a series of field and laboratory experiments on a tropical reef in Moorea, French Polynesia, and on a temperate intertidal reef in

Cook Strait, New Zealand.

In Chapter 1, I manipulated nutrient availability on the dominant inshore tropical reef macroalgal species at various times after rainfall to examine the relationship between environmental context and nutrient response across species. In Chapter 2, I conducted field and laboratory experiments examining how ambient sediment loads on thalli of two dominant tropical algal species from the reef flat disturbed nutrient and herbivory control. In Chapter 3, I conducted two-year manipulations of nutrient availability and herbivory strength on a structurally heterogenous intertidal temperate reef to determine their effects on macroalgal communities across variability in both spatial and temporal environmental parameters.

References

Airoldi, L. 2003. The effects of sedimentation on rocky coast assemblages. Pages 161–236 *in* R. N. Gibson and R. J. A. Atkinson, editors. Oceanography and Marine Biology: an Annual Review, volume 41. Taylor and Francis, 41 edition.

Atalah, J., and T. P. Crowe. 2010. Combined effects of nutrient enrichment, sedimentation and grazer loss on rock pool assemblages. Journal of Experimental Marine Biology and Ecology **388**:51–57.

Beach, K. S., L. J. Walters, and H. B. Borgeas. 2006. Irradiance and nutrient limitation of Dicytota spp. populations on Conch Reef, Florida Keys, USA. Journal of Experimental Marine Biology and Ecology **329**:101–112.

Bellwood, D. R., and C. J. Fulton. 2008. Sediment-mediated suppression of herbivory on coral reefs: Decreasing resilience to rising sea levels and climate change? Limnology and Oceanography **53**:2695–2701.

Bertocci, I., E. Maggi, S. Vaselli, and L. Benedetti-Cecchi. 2010. Resistance of rocky shore assemblages of algae and invertebrates to changes in intensity and temporal variability of aerial exposure. Marine Ecology Progress Series **400**:75–86.

Bracken, M. E. S. 2004. Invertebrate-mediated nutrient loading increases growth of an intertidal macroalga. Journal of Phycology **40**:1032–1041.

Bulleri, F., B. D. Russell, and S. D. Connell. 2012. Context-dependency in the effects of nutrient loading and consumers on the availability of space in marine rocky environments. PloS one **7**:e33825.

Burkepile, D. E., and M. E. Hay. 2006. Herbivore vs. nutrient control of marine primary producers: Context-dependent effects. Ecology **87**:3128–3139.

Cloern, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series **210**:223–253.

Connell, J. H. 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology **42**:710–723.

Dayton, P. K. 1971. Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. Ecological Monographs **41**:351–389.

Delgado, O., and B. E. Lapointe. 1994. Nutrient-limited productivity of calcareous versus fleshy macroalgae in a eutrophic, carbonate-rich tropical marine environment. Coral Reefs **13**:151–159.

Downing, J. A., C. W. Osenberg, and O. Sarnelle. 1999. Meta-analysis of marine nutrient-enrichment experiments: variation in teh magnitude of nutrient limitation. Ecology **80**:1157–1167.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters **10**:1135–1142.

Eyre, B., and A. Ferguson. 2002. Comparison of carbon production and decomposition, benthic nutrient fluxes and denitrification in seagrass, phytoplankton, benthic microalgae- and macroalgae-dominated warm-temperate Australian lagoons. Marine Ecology Progress Series **229**:43–59.

Fabricius, K. E. 2005. Eects of terrestrial runo on the ecology of corals and coral reefs: review and synthesis. Marine Pollution Bulletin **50**:125–146.

Fong, P., K. E. Boyer, K. Kamer, and K. A. Boyle. 2003. Influence of initial tissue nutrient status of tropical marine algae on response to nitrogen and phosphorus additions. Marine Ecology Progress Series **262**:111–123.

Fong, P., K. Kamer, K. E. Boyer, and K. A. Boyle. 2001. Nutrient content of macroalgae

with differing morphologies may indicate sources of nutrients for tropical marine systems. Marine Ecology Progress Series **220**:137–152.

Fong, P., and V. J. Paul, 2011. Coral reef algae. Pages 241–272 *in* Z. Dubinsky and N. Stambler, editors. Coral reefs: an ecosystem in transition. Springer.

Fry, B., A. Gace, and J. W. McClelland. 2003. Chemical indicators of anthropogenic nitrogen loading in four Pacific estuaries. Pacific Science **57**:77–101.

Fujita, R. M., and J. C. Goldman. 1985. Nutrient flux and growth of the red alga *Gracilaria-tikvahiae* mclachlan (Rhodophyta). Botanica Marina **28**:265–268.

Gedan, K. B., J. Bernhardt, M. D. Bertness, and H. M. Leslie. 2011. Substrate size mediates thermal stress in the rocky intertidal. Ecology **92**:576–82.

Goatley, C. H. R., and D. R. Bellwood. 2012. Sediment suppresses herbivory across a coral reef depth gradient. Biology letters **8**:1016–8.

Goatley, C. H. R., and D. R. Bellwood. 2013. Ecological consequences of sediment on high-energy coral reefs. PloS one **8**:e77737.

Gordillo, F. J. L., 2012. Environment and algal nutrition. Chapter 4, pages 67–86 *in* C. Wiencke and K. Bischof, editors. Seaweed biology. Springer-Verlag, Berlin, Heidelberg, ecological edition.

Guerry, A. D. 2008. Interactive effects of grazing and enrichment on diversity; conceptual implications of a rocky intertidal experiment. Oikos **117**:1185–1196.

Guerry, A. D., B. A. Menge, and R. A. Dunmore. 2009. Effects of consumers and enrichment on abundance and diversity of benthic algae in a rocky intertidal community. Journal of Experimental Marine Biology and Ecology **369**:155–164.

Hanisak, D. M., 1983. The nitrogen relationships of marine macroalgae. Pages 699–730 *in* E. J. Carpenter and D. G. Capone, editors. Nitrogen in the marine environment. Academic Press, New York.

Harpole, W. S., J. T. Ngai, E. E. Cleland, E. W. Seabloom, E. T. Borer, M. E. S. Bracken, J. J. Elser, D. S. Gruner, H. Hillebrand, J. B. Shurin, and J. E. Smith. 2011. Nutrient co-limitation of primary producer communities. Ecology Letters **14**:852–62.

Heck, K. L., and J. F. Valentine. 2007. The primacy of top-down effects in shallow benthic ecosystems. Estuaries and Coasts **30**:371–381.

Hillebrand, H. 2003. Opposing effects of grazing and nutrients on diversity. Oikos **100**:592–600.

Huston, M. 1979. A general hypothesis of species diversity. The American Naturalist **113**:81–101.

Huston, M. 1994. Biological diversity: The coexistence of species on changing landscapes. Cambridge University Press.

Jackson, A. C., R. J. Murphy, and A. J. Underwood. 2013. Biofilms on rocky shores: Influences of rockpools, local moisture and temperature. Journal of Experimental Marine Biology and Ecology **443**:46–55.

Jackson, J. B. C., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury, R. Cooke, J. Erlandson, and J. A. Estes. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science **293**:629–637.

Jara, V. C., J. H. S. Miyamoto, B. A. P. Gama, M. Molis, M. Wahl, and R. C. Pereira. 2006. Limited evidence of interactive disturbance and nutrient effects on the diversity of macrobenthic assemblages. Marine Ecology Progress Series **308**:37–48.

Kondoh, M. 2001. Unifying the relationships of species richness to productivity and disturbance. Proceedings of the Royal Society of London Series B-Biological Sciences **268**:269–271.

Koop, K., D. Booth, A. Broadbent, J. Brodie, D. Bucher, D. Capone, J. Coll, W. Dennison, M. Erdmann, P. Harrison, O. Hoegh-Guldberg, P. Hutchings, G. B. Jones, a. W.

Larkum, J. O'Neil, A. Steven, E. Tentori, S. Ward, J. Williamson, and D. Yellowlees. 2001. ENCORE: the effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. Marine Pollution Bulletin **42**:91–120.

Korpinen, S., V. Jormalainen, and T. Honkanen. 2007. Effects of nutrients, herbivory, and depth on the macroalgal community in the rocky sublittoral. Ecology **88**:839–852.

Kraufvelin, P., A. Lindholm, M. F. Pedersen, L. A. Kirkerud, and E. Bonsdorff. 2010. Biomass, diversity and production of rocky shore macroalgae at two nutrient enrichment and wave action levels. Marine Biology **157**:29–47.

Kraufvelin, P., F. E. Moy, H. Christie, and T. L. Bokn. 2006. Nutrient addition to experimental rocky shore communities revisited: Delayed responses, rapid recovery. Ecosystems **9**:1076–1093.

Kuffner, I. B., and V. J. Paul. 2001. Effects of nitrate, phosphate and iron on the growth of macroalgae and benthic cyanobacteria from Cocos Lagoon, Guam. Marine Ecology Progress Series **222**:63–72.

Lapointe, B. E. 1987. Phosphorus-and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. Marine Biology **93**:561–568.

Lapointe, B. E., M. M. Littler, and D. S. Littler. 1992. Nutrient availability to marine macroalgae in siliciclastic versus carbonate-rich coastal waters. Estuaries and Coasts **15**:75–82.

Larned, S. T. 1998. Nitrogen-versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae. Marine Biology **132**:409–421.

Leigh, E. G., R. T. Paine, J. F. Quinn, and T. H. Suchanek. 1987. Wave energy and intertidal productivity. Proceedings of the National Academy of Sciences of the United States of America **84**:1314–1318.

Littler, M. M., D. S. Littler, and B. L. Brooks. 2006. Harmful algae on tropical coral reefs: bottom-up eutrophication and top-down herbivory. Harmful Algae **5**:565–585.

Lotze, H. K., B. Worm, and U. Sommer. 2001. Strong bottom-up and top-down control of early life stages of macroalgae. Limnology and Oceanography **46**:749–757.

Lubchenco, J., and B. A. Menge. 1978. Community development and persistence in a low rocky Intertidal Zone. Ecological Monographs **48**:67–94.

Mann, K. H. 1973. Seaweeds: their productivity and strategy for growth. Science **182**:975–981.

Masterson, P., F. A. Arenas, R. C. Thompson, and S. R. Jenkins. 2008. Interaction of top down and bottom up factors in intertidal rockpools: Effects on early successional macroalgal community composition, abundance and productivity. Journal of Experimental Marine Biology and Ecology **363**:12–20.

McCook, L. J. 1999. Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. Coral Reefs **18**:357–367.

McManus, J. W., and J. F. Polsenberg. 2004. Coral-algal phase shifts on coral reefs: Ecological and environmental aspects. Progress in Oceanography **60**:263–279.

Mejia, A. Y., G. N. Puncher, and A. H. Engelen, 2012. Macroalgae in tropical marine coastal systems. Chapter 16, pages 329–357 *in* C. Wiencke and K. Bischof, editors. Seaweed biology, volume 219 of *Ecological Studies*. Springer-Verlag, Berlin, Heidelberg.

Menge, B. A. 1976. Organization of the New England rocky intertidal community: Role of predation, competition, and environmental heterogeneity. Ecological Monographs **46**:355–393.

Menge, B. A. 1978. Predation intensity in a rocky intertidal community - effect of an algal canopy, wave action and desiccation on predator feeding rates. Oecologia **34**:17–35.

Menge, B. A. 2000. Top-down and bottom-up community regulation in marine rocky intertidal habitats. Journal of Experimental Marine Biology and Ecology **250**:257–289.

Menge, B. A., and A. M. Olson. 1990. Role of scale and environmental factors in regulation of community structure. Trends in Ecology & Evolution **5**:52–7.

Mora, C. 2008. A clear human footprint in the coral reefs of the Caribbean. Proceedings. Biological sciences / The Royal Society **275**:767–73.

Morelissen, B., and C. D. Harley. 2007. The effects of temperature on producers, consumers, and plantherbivore interactions in an intertidal community. Journal of Experimental Marine Biology and Ecology **348**:162–173.

Nielsen, K. J. 2001. Bottom-up and top-down forces in tide pools: test of a food chain model in an intertidal community. Ecological Monographs **71**:187–217.

Nielsen, K. J. 2003. Nutrient loading and consumers: Agents of change in open-coast macrophyte assemblages. Proceedings of the National Academy of Sciences of the United States of America **100**:7660.

Paine, R. T. 1966. Food web complexity and species diversity. The American Naturalist **100**:65–75.

Pedersen, M. F., and J. Borum. 1996. Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Marine Ecology Progress Series **142**:261–272.

Pedersen, M. F., and J. Borum. 1997. Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. Marine Ecology Progress Series **161**:155–163.

Pedersen, M. F., J. Borum, and F. L. Fotel. 2010. Phosphorus dynamics and limitation of fast- and slow-growing temperate seaweeds in Oslofjord, Norway. Marine Ecology Progress Series **399**:103–115.

Probyn, T. A., and A. R. O. Chapman. 1983. Summer growth of *Chordaria-flagelliformis* (Muell,O.F.) C-ag - physiological strategies in a nutrient stressed environment. Journal of Experimental Marine Biology and Ecology **73**:243–271.

Proulx, M., and A. Mazumder. 1998. Reversal of grazing impact on plant species richness in nutrient-poor vs. nutrient-rich ecosystems. Ecology **79**:2581–2592.

Rasher, D. B., S. Engel, V. Bonito, G. J. Fraser, J. P. Montoya, and M. E. Hay. 2012. Effects of herbivory, nutrients, and reef protection on algal proliferation and coral growth on a tropical reef. Oecologia **169**:187–98.

Richmond, R. H., T. Rongo, Y. Golbuu, S. Victor, N. Idechong, G. Davis, W. Kostka, L. Neth, M. Hamnett, and E. Wolanski. 2007. Watersheds and coral reefs: conservation science, policy, and implementation. BioScience **57**:598.

Schaffelke, B. 1999. Short-term nutrient pulses as tools to assess responses of coral reef macroalgae to enhanced nutrient availability. Marine Ecology Progress Series **182**:305–310.

Schaffelke, B., and D. Klumpp. 1998. Short-term nutrient pulses enhance growth and photosynthesis of the coral reef macroalga *Sargassum baccularia*. Marine Ecology Progress Series **170**:95–105.

Smith, J. E., C. L. Hunter, and C. M. Smith. 2010. The effects of top-down versus bottom-up control on benthic coral reef community structure. Oecologia **163**:497–507.

Smith, J. E., C. M. Smith, and C. L. Hunter. 2001. An experimental analysis of the effects of herbivory and nutrient enrichment on benthic community dynamics on a Hawaiian reef. Coral Reefs **19**:332–342.

Steneck, R. S. 1998. Human influences on coastal ecosystems: does overfishing create trophic cascades? Trends in Ecology & Evolution **13**:429–430.

Stimson, J., and S. Larned. 2000. Nitrogen efflux from the sediments of a subtropical bay and the potential contribution to macroalgal nutrient requirements. Journal of experimental marine biology and ecology **252**:159–180.

Suding, K. N., S. L. Collins, L. Gough, C. Clark, E. E. Cleland, K. L. Gross, D. G. Milchunas, and S. Pennings. 2005. Functional-and abundance-based mechanisms explain diversity loss due to N fertilization. Proceedings of the National Academy of Sciences **102**:4387–4392.

Thacker, R. W., D. W. Ginsburg, and V. J. Paul. 2001. Effects of herbivore exclusion and nutrient enrichment on coral reef macroalgae and cyanobacteria. Coral Reefs **19**:318–329.

Tilman, D. 1994. Competition and biodiversity in spatially structured habitats. Ecology **75**:2–16.

Tilman, D., J. Fargione, B. Wolff, C. D'Antonio, A. Dobson, R. Howarth, D. Schindler, W. H. Schlesinger, D. Simberloff, and D. Swackhamer. 2001. Forecasting agriculturally driven global environmental change. Science **292**:281–284.

Valiela, I., K. Foreman, M. LaMontagne, D. Hersh, J. Costa, P. Peckol, B. DeMeo-Andreson, C. DïAvanzo, M. Babione, and C. H. Sham. 1992. Couplings of watersheds and coastal waters: Sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. Estuaries and Coasts **15**:443–457.

Vermeij, M. J. a., I. van Moorselaar, S. Engelhard, C. Hörnlein, S. M. Vonk, and P. M. Visser. 2010. The effects of nutrient enrichment and herbivore abundance on the ability of turf algae to overgrow coral in the Caribbean. PloS one **5**:e14312.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications **7**:737–750.

Werner, F. J., and B. Matthiessen. 2013. Temperature indirectly affects benthic microalgal diversity by altering effects of top-down but not bottom-up control. Oikos **122**:52–63.

Williams, S. L., M. E. S. Bracken, and E. Jones. 2013. Additive effects of physical stress and herbivores on intertidal seaweed biodiversity. Ecology **94**:1089–101.

Worm, B., and H. K. Lotze. 2006. Effects of eutrophication, grazing, and algal blooms on rocky shores. Limnology and Oceanography **51**:569–579.

Worm, B., H. K. Lotze, H. Hillebrand, and U. Sommer. 2002. Consumer versus resource control of species diversity and ecosystem functioning. Nature **417**:848–851.

CHAPTER 2

DYNAMIC TEMPORAL CHANGES IN NUTRIENT LIMITATION OF TROPICAL MACROALGAE DEPEND ON LIFE HISTORY STRATEGIES AND ENVIRONMENTAL CONTEXT

2.1 Abstract

Nitrogen (N) or phosphorus (P) has been shown to limit primary productivity in nearly every ecosystem worldwide; yet how this limitation changes over time, particularly in connection to variation in environmental drivers, remains understudied. We evaluated temporal variability in the relative importance of nitrogen and phosphorus limitation among coral reef macroalgae in two-factor bioassay experiments conducted twice after rains of differing magnitude and twice after dry conditions to explore connections to environmental drivers. We studied three common macroalgal species: a fast-growing opportunist, *Dictyota bartayresiana*, and two slower-growing calcifying species, *Galaxaura fasciculata*, and *Padina boryana*. Nutrient limitation was extremely variable over time and among species. After light rain or dry conditions, *D. bartayresiana* grew most rapidly (up to ∼60% in three days) with little indication of nutrient-limitation, while *P. boryana* and *G. fasciculata* rapidly shifted between N, P, or no limitation. All species grew slowly or lost mass after a large storm, presumably due to unfavorable conditions on the reef caused by the storm prior to the experiment. *P. boryana* and *G. fasciculata* both became nutrient-limited three days post-storm, while *D. bartayresiana* did not. Altogether, these results suggest *D. bartayresiana* has a greater capacity for rapid nutrient uptake and storage, while *P. boryana* and

G. fasciculata show little potential for nutrient storage and thus reduced influence of nutrient history, yet higher tolerance to unfavorable conditions. These findings highlight the dynamic relationship between environmental context and nutrient limitation. Moreover, the great variability in species responses indicates that patterns of nutrient limitation are more complex than previously recognized, and broad generalizations about N vs. P limitation of a given system inaccurately portray the inherent complexity in governing conditions and processes.

2.2 Introduction

The importance of nutrient limitation in controlling primary productivity and structuring producer communities is well-documented in terrestrial, freshwater, and marine systems (reviewed by Downing et al., 1999; Elser et al., 2007; Harpole et al., 2011); yet, the relative importance and spatio-temporal variance of nitrogen (N) vs. phosphorus (P) limitation or co-limitation remains a debate in most ecosystems worldwide (reviewed by Downing et al., 1999; Harpole et al., 2011). Historically, studies have aimed at making generalizations about the nutrient limitation status of the system in question. Even within the past decade, studies commonly examine N and P in isolation (e.g., LeBauer and Treseder, 2008) although recent meta-analyses have demonstrated the importance of synergistic and interactive effects of N and P across terrestrial and aquatic habitats (Elser et al., 2007; Vitousek et al., 2010; Harpole et al., 2011). In aquatic systems, early paradigms of nutrient limitation were developed, in part, by creating system budgets from water column N:P ratios (e.g. Redfield et al., 1963; Ryther and Dunstan, 1971) and later by producer tissue C:N:P (e.g. Atkinson and Smith, 1983), a more integrated measure of water column nutrient supply (Cohen and Fong, 2006). These paradigms suggested that P is limiting in freshwater systems, while marine primary production is limited by N (for reviews, see Hecky and Kilham, 1988; Howarth, 1988; Howarth and Marino, 2006). In the 1980s and 90s, studies utilizing experimental enrichment to assess limitation began to cast doubt on these paradigms, suggesting that nutrient limitation is more complex, both spatially and temporally (e.g. Lapointe, 1987; Lapointe

and Connell, 1989; Littler et al., 1991). Some generalities about marine systems developed: N tends to limit coastal temperate regions (Taylor et al., 1995; Valiela et al., 1997; Lyngby et al., 1999) while P may limit shallow tropical systems (Smith, 1984; Lapointe, 1987; Lapointe et al., 1992). Now there is growing recognition that generalizations within and across aquatic ecosystems about N vs. P limitation are hampered by the dynamic nature of nutrient supply and cycling compared to snap-shot measures of limitation (reviewed in Fong, 2008; Fong and Paul, 2011).

Understanding variability in patterns of nutrient limitation and the causes underlying them has become critical as human alteration of both local and large-scale nutrient regimes has extended to nearly every ecosystem worldwide (Vitousek et al., 1997; Cloern, 2001; García-Reyes and Largier, 2010). Anthropogenic nutrient inputs from terrestrial sources to coastal marine systems have been implicated in major ecosystem changes and degradation in estuaries, lagoons and coral reefs since the early 1970s (see Vitousek et al., 1997; Smith et al., 1999; Fong, 2008). In the past decade, however, the scale of degradation has increased, with vast tracts of varied coastlines deemed eutrophic (Rabalais et al., 2009), from Southern Californian lagoons and estuaries (McGlathery et al., 2012), to various Caribbean reefs (e.g. Lapointe et al., 2005), and even part of the Baltic Sea (Paerl et al., 2008). Coastal marine systems that are affected by nutrient enrichment may also have unpredictable spatial and temporal variability in nutrient supplies due to the often localized and typically pulsed nature of nutrient loading events (McCook, 1999; Fong et al., 2001; Fry et al., 2003). These widespread changes in nutrient regimes have intensified focus on the patterns of limitation and the role of environmental context in how species respond to temporally varying nutrient loads associated with anthropogenic inputs.

In tropical reef systems, the relative importance of N vs. P limitation remains an open question due to the widely mixed results of empirical tests on macroalgae (Smith et al., 2010, for reviews, see Fong and Paul, 2011; Mejia et al., 2012) that may be due, in part, because temporal patterns are rarely explored. Proponents of a P-limited paradigm point to benthic-pelagic coupling that reduces P availability due to adsorption of P by carbonate reef sediments (Littler et al., 1991; Delgado and Lapointe, 1994; McGlathery et al., 1994).

In concordance, many studies have found P more limiting for tropical macroalgae than N (e.g. Lapointe and Connell, 1989; Lapointe et al., 1992). Conversely, algae in the historically eutrophic Kaneohe Bay were found to be primarily N limited (e.g. Larned and Stimson, 1996; Larned, 1998), supporting the view that N-limitation becomes increasingly important along a gradient of increasing nutrient supply (Downing et al., 1999). Several studies, however, showed responses vary among species (e.g. Schaffelke, 1999; Fong et al., 2003). Still other short-term (Delgado et al., 1996; Kuffner and Paul, 2001) and long-term cited Koop2001 experiments found tropical systems may show a complete lack of algal response to nutrient enrichment, which is suggested to be caused by high advective supply rates compensating for low concentrations (Hatcher, 1988; McCook, 1999). In spite of the large number of studies examining tropical algal nutrient limitation with factorial N and P enrichment in the lab or field (Fong et al., 2003; Teichberg et al., 2008 numerous others - for a review, see Fong and Paul, 2011), few of these studies were explicitly replicated over time (but see Lapointe, 1987; Delgado and Lapointe, 1994, for replication in winter and in summer). Moreover, none have examined changes in algal limitation within the context of rapid environmental changes, particularly on the short-term scale of variability known to occur in water column nutrient levels on tropical reefs (McCook, 1999).

Perhaps most problematic for system-wide generalizations derived from studies of macroalgae are species-specific variations in life histories with regard to nutrient strategies, including different nutrient requirements, uptake rates, storage capacities, and growth rates (Pedersen and Borum, 1996; Fong and Paul, 2011; Gordillo, 2012). These different strategies may result in strongly heterogeneous nutrient limitation, precluding a unified, community-wide response to enrichment. Thus, the assessment of limitation may depend on the species chosen. For example, algal species having an opportunistic strategy have the ability to take up nutrients rapidly when they become available (termed surge uptake) and store them in their tissues for future growth during low nutrient periods (Hanisak, 1983; Fujita, 1985; Fong et al., 2003), highlighting the importance of nutrient history in understanding limitation. Research in estuarine systems has shown that opportunistic species such as *Ulva* spp. have rapid nitrate uptake rates and large short-term storage capabilities (Kennison et al.,

2011). Thus, enrichment response may depend on internal stores of nutrients, where these opportunistic species only exhibit limitation when internal stores are depleted (e.g. Schaffelke and Klumpp, 1998; Fong et al., 2003; Beach et al., 2006). Likewise, this adaptation may be particularly advantageous in naturally low-nutrient tropical systems when nutrient inputs are pulsed (Fong and Paul, 2011). In contrast, slower-growing perennial species may have very limited storage capacities, but lower nutrient requirements enable them to persist under low nutrient conditions (Gordillo, 2012). Growth in species having this persister strategy is more directly dependent on availability of low but more consistent supplies of nutrients in the environment. For example, slow-growing calcified species on tropical reefs may be less able than opportunistic forms to take advantage of pulsed nutrient events, making them less dependent on nutrient history (Delgado and Lapointe, 1994; Fong et al., 2003). Thus the nutrient-use strategies of the species in question must be considered in attempts to understand the role of nutrient-limitation, particularly in systems where pulses of anthropogenic enrichment may result in fluctuations in or widespread changes to nutrient regimes.

Our objective was to fill gaps in understanding of temporal dynamics of nutrient limitation, both how these dynamics vary between algal species with different nutrient strategies and how they vary with environmental context. We predicted nutrient-limitation status may be constantly in flux due to temporal variability in nutrient supplies in impacted habitats and associated environmental variability that may affect a species ability to take up nutrients. Our approach was to repeat N vs. P limitation laboratory bioassay experiments four times after changing environmental conditions associated with rainfall using three algal species with differing nutrient-use strategies. We tested whether (1) the importance of N and P limitation varies between macroalgal species with distinct nutrient-use strategies, (2) these patterns change over time, and (3) if temporal variation in patterns of nutrient limitation is connected to changes in environmental context associated with rainfall that may drive species ability to respond to nutrients.

2.3 Methods

2.3.1 *Study site*

We conducted bioassay experiments using algae from a fringing reef on the island of Mo'orea, French Polynesia, in the South Pacific. On this volcanic island, the rugged terrain limits urban development to a narrow fringe along the coast, concentrated in the northeast and within the two northern bays, Cook's Bay and Opunohu Bay. In developed tropical bays such as these, rainfall is often a major driver of nutrient dynamics by increasing run-off of terrestrial sediments as well as seepage from septic systems (Holthuss et al., 1989). In Mo'orea, run-off from the 250 hectares of pineapple farms as well as additional agriculture in the island interior is funneled from the watershed to the head of each bay. There is also direct run-off of fertile sediments from the surrounding steep mountainsides (Adjeroud and Salvat, 1996). The fringing reef from which algae were collected for this study was on the north shore of Mo'orea at the mouth of Cooks Bay (17 °32'S 149 °50'W). At this site, sediment plumes develop over near-shore fringing reefs after major rain events, with plumes visible after heavy rainfall due to coastal run-off (R. J. Clausing, P. Fong pers. obs.). The research was performed at University of Californias Gump Biological Research Station.

2.3.2 *Environmental data*

Rainfall and solar radiation data were obtained with permission from the Mo'orea Coral Reef Ecosystem Long Term Ecological Research (LTER) (Washburn and Brooks of Moorea Coral Reef LTER, 2012). Sample data were collected every five minutes by sensors deployed at Gump Research Station in Cook's Bay. The dataset was subsetted for each time period during which nutrient limitation experiments were conducted and algae were collected for tissue nutrient analysis. Each time period included two weeks prior to the collection through the experimental duration, where applicable, to show the context that the algae were subject to prior to and during each event (19 Apr – 26 May 2008 for experiments; 1 – 31 May 2010 and 21 Apr – 20 May 2012 for tissue nutrient collections). We summed

the accumulated rainfall (mm) over 24 hours to give total daily rainfall. Solar radiation data were collected as photons in the range of 300–1200 nm wavelengths (kilowatts m^{-2} s^{-1}) and were averaged over daylight hours (06:00–18:00 daily) to give daytime estimates of irradiance. Although these data include more than just photosynthetically active radiation (PAR: 400–700 nm), their day to day variation parallels that of PAR (Washburn and Brooks of Moorea Coral Reef LTER, 2012). Because light data were collected above water and turbidity resulting from rainfall may further reduce irradiance at the surface of the reef, light data are reported to give context rather than suggest causation. Averages of both rainfall and daytime radiation were calculated for both the week prior to and during each experiment. Light and rain conditions prior to each experiment may have indirectly affected growth during the experiment by influencing algal tissue nutrient content at the onset of the experiment (see below; Fong et al., 1994, 2003). We expected rain to increase nutrient availability and therefore tissue stores for opportunistic algae, although low light conditions may limit uptake and storage in the field and thereby affect growth during the bioassay regardless of increased nutrient availability.

2.3.3 *Experimental design*

We conducted two-factor dose-response nutrient addition experiments as a bioassay to assess temporal variability in N and P limitation of three species: *Dictyota bartayresiana*, *Galaxaura fasciculata*, and *Padina boryana* collected from a common site on the fringing reef. These bioassay experiments assessed each algal species ability to respond to ambient and nutrient-enriched seawater from this site based on the environmental context on the reef prior to each experiment and their respective nutrient-use strategies. We performed these experiments four times for each of the three species (12 experiments) after varying amounts of rainfall over the course of a month from Apr to May 2008. The design was factorial, with two levels of N enrichment (+/- addition) crossed with two levels of P (+/- addition). This gave a total of four water treatments: Ambient (no addition, A), N addition (+N), P addition (+P), and addition of both nutrients (+N+P). The ambient treatment water was taken from the reef just prior to each experiment and thus represented the snapshot

conditions of nutrients at that time as they varied in response to environmental drivers of oceanic and terrestrial sources.

The three species of algae were chosen because they are all locally abundant and yet represent differing life-history strategies, particularly with regard to nutrient-use. *Dictyota* is a genus of fast-growing, sheet-like brown algae (Steneck and Watling 1982; Littler and Littler 1984; Steneck and Dethier 1994) having an opportunistic strategy (Fong et al. 2003). This means that it is capable of rapid growth in response to nutrient pulses and also can store nutrients in its tissues (Fong et al. 2003) during these pulses to use for later growth when nutrient concentrations in the water are low (Aisha et al. 1995; see also Beach et al. 2006). *Galaxaura* is a genus of calcified, branching red algae (Taylor 1945) with a slower-growing, persister strategy (Fong and Paul 2011) and a reduced capacity for tissue nutrient storage (Aisha et al. 1995). *Padina* is a genus of lightly calcified, foliose brown algae (Taylor 1966; NYeurt and Payri 2006), some species of which have been shown to grow rapidly in direct response to externally supplied rather than internally stored nutrients (e.g. Kuffner and Paul 2001). Thus it displays characteristics of both strategies, and we hypothesize it has an intermediate strategy to *Dictyota* and *Galaxaura*. All three species are common and persistent in Mo'orea throughout both the wet and dry season. These three genera are also globally abundant (e.g. McClanahan et al. 2004; Fox and Bellwood 2007) and increasing on reefs worldwide (Lirman and Biber 2000; reviewed by Fong and Paul 2011).

Immediately prior to the onset of each experiment, we collected all algae from one location on the reef, adjacent to the research station (hereafter called Gump Reef) to ensure all specimens had been exposed to the same nutrient history and environmental conditions. At the time of collection, we also collected 80 L of water from the site. Water was divided into 4 subsets, one of which served as the ambient treatment. Nutrient addition treatments were created by adding inorganic N (as sodium nitrate, $NaNO_3$) and/or inorganic P (as sodium dihydrogen phosphate, NaH_2PO_4) to ambient seawater to enrich it by 20μM and 2μM of N and P, respectively. This enabled us to examine how exposure to environmental conditions during and after rainfall in the field prior to collection affected the way each algal species responded to experimental nutrient enrichment.

Collected algae were immediately cleaned of epiphytes, epifauna, and sediment, spun for one minute in a salad spinner to standardize removal of excess water, and wet weighed. Each experimental unit (500 mL plastic beaker) received 2 g of either *P. boryana* or *D. bartayresiana*, or 3 g of *G. fasciculata*. Calcification in *G. fasciculata* required more biomass to approximate equal volumes across species. Care was taken to ensure that algae placed in experimental units included apical tips. Each experimental unit received two nutrient dosings: the initial 400 mL plus an exchange of treatment water (400 mL) after the first 24 h. Experimental units were randomly placed in a flow-through outdoor water table to maintain constant temperature. A shade cloth reduced sunlight by 30% to simulate light levels in the upper meters on the reef (Fong et al. 2003). Replication was fivefold, giving a total of 20 experimental units for each species. After three days, algae were again spun and weighed. Growth was estimated by percent change in wet biomass. It is important to note that these experiments are bioassays, meant only to assess N and/or P limitation based on differential response to enrichment. They do not simulate natural supply and flow rates, nor competition among species. Thus they are not predictors of how algae might grow in the field.

2.3.4 *Tissue nutrient collections*

To examine the relationship between environmental conditions, specifically rain, and algal tissue nutrient stores, we collected replicate specimens of the three algal species from the study reef in a time series (three dates) after rainfall events (*Dictyota bartayresiana* in May 2010; *Padina boryana* and *Galaxaura fasciculata* in May 2012). As for the experiments, algae were collected from a common location. Five specimens of each species were cleaned of epiphytes and epifauna, rinsed in freshwater, and dried at 60 °C. Samples were then analyzed for tissue %N (all species) and %P (*P. boryana* and *G. fasciculata* only) at UC Davis (*D. bartayresiana* at the Stable Isotope Facility, and *P. boryana* and *G. fasciculata* at the Davis Analytical Laboratory). We were unable to analyze tissue P for *D. bartayresiana* due to limited sample mass. Total N was analyzed by converting all organic and inorganic N in the sample to N_2 or NO_x via oxidation by flash combustion and measuring the gases using

thermal conductivity and IR detection. Total P was analyzed with nitric acid digestion and determination by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Sah and Miller, 1992).

2.3.5 *Data analysis*

All data were analyzed using the R programming language (version 2.15.1, R Development Core Team 2012). Bioassay experiments were analyzed individually using PERMANOVA (*adonis* function, vegan package; Oksanen et al., 2012) to test for the main effects and interaction of addition of the two nutrients (N and P) on percent growth for each species at each time ($n = 5$ except where noted in figure captions). PERMANOVA was used because several experiments could not be transformed to meet assumptions of normality, an assumption which PERMANOVA does not make (Anderson, 2001). Tissue nutrient data met assumptions of parametric statistics and were analyzed with ANOVA followed by Tukeys HSD test when significant. Tukeys comparisons with p values < 0.05 were considered significant and those $0.10 >$ p values > 0.05 were considered marginally significant.

2.4 Results

2.4.1 *Environmental context of experimental bioassays*

Overall, differences in the environmental context prior to each bioassay experiment defined the four experimental conditions as: 1) short, intense rain but high light; 2) nearly dry but low light; 3) prolonged intense rain with low light; 4) light rain with low light. Based on previous research, we reasoned that the environmental context during the week prior to each experiment may influence the nutrient limitation of the algae, which we assessed through the bioassay experiments. The first experiment was conducted after intense rainfall over a short duration, beginning 29 Apr 2008. Prior to this experiment, there were two days of rain (21.8 mm total fell prior to algal collections) (Figure 2.1a) with the majority (19.1 mm total) during the 24 h period before the experiment. A further 8.5 mm fell after collections

were made on 29 Apr. In spite of the short, heavy rains directly prior to the experiment, most of the week previous was relatively sunny with average daytime solar radiation of 0.44 kw·m^{-2}s^{-1} (Figure 2.1b). During the first day of the experiment, continued rain reduced light levels, while remaining days were clear with an average daytime solar radiation of 0.41 kw·m^{-2}s^{-1}.

We performed the second experiment after six nearly dry days (5.3 mm in total) beginning 5 May (Figure 2.1a). Thus the algae collected were exposed to very little terrestrial run-off in the preceding week, but periodic cloud cover reduced averaged solar radiation to 0.39 kw·m^{-2}s^{-1} (Figure 2.1b). Together, these conditions may have reduced the ability of the algae to take up nutrients prior to the experiment. During the experiment, however, there was little rain or cloud cover, with 0.45 kw·m^{-2}s^{-1} average daytime solar radiation.

The third experiment began on 19 May, after a week of prolonged intense rain adding up to 41.1mm (Figure 2.1a). During this week a large plume of fine terrigenous sediment was observed over the fringing reef in Cook's Bay. Levels of irradiance penetrating the water column would certainly have been lower than surface values, although average solar radiation was also low at 0.38 kw·m^{-2}s^{-1} (Figure 2.1b). Thus, despite large amounts of rain that presumably resulted in increased nutrient supplies, algae may have had limited energy for uptake. In addition, there was cloud cover periodically throughout the experiment and rain for most of the last day, resulting in lower average daytime solar radiation of 0.36 kw·m^{-2}s^{-1}.

We conducted the fourth experiment beginning the same day the third experiment ended, 22 May, three rainless days after a week of storms (Figure 2.1a). Although there was light rain prior to experimental collection on 22 May (4.1 mm), the turbidity of the overlying water had begun to settle (R. Clausing, P. Fong pers. obs.). Average solar radiation prior to the experiment remained low, however, at 0.38 kw·m^{-2}s^{-1} (Figure 2.1b). There was no rain during the course of the experiment, but sporadic cloud cover resulted in an average daytime solar radiation of 0.40 kw·m^{-2}s^{-1}.

2.4.2 *Experimental results*

2.4.2.1 Short, intense rainfall but high light

Each algal species responded uniquely to N and P addition (Figure 2.2a). After two days of heavy rainfall in the field, *D. bartayresiana* did not show a significant response to either nutrient (Table 2.1a). Rather, it grew rapidly in all experimental units regardless of treatment, increasing its biomass by 55 ± 3.2% over the course of three days. This suggests nutrient content stored in tissues and/or contained in ambient seawater after rainfall were sufficient to sustain rapid growth for three days, and that light levels were also sufficient both during (for growth) and prior to (for uptake) the experiment. In contrast, both N and P stimulated increased growth in *G. fasciculata*, but an interaction indicated the effects of one nutrient depended on the presence of the other. The addition of P led to particularly rapid growth at 38 ± 6.5% in three days, a nearly 40-fold increase compared to growth in ambient nutrient supplies. Algae in experimental units with only N addition grew at half the rate of those with P alone. *P. boryana* responded significantly to N but not to P, and with overall slower growth rates than the other species. The addition of N increased biomass five-fold over treatments lacking it (A, +P).

2.4.2.2 Nearly dry but low light

After six days with very little rain but reduced solar radiation prior to the experiment, growth rates in *D. bartayresiana* were only about one-third of those after the short, intense rain (Figure 2.2b). However, there was marginally significantly more growth in experimental units with P addition (Table 2.1b). Reduced growth compared to the first bioassay and a trend of responding to P enrichment suggest that nutrient stores were being depleted, and P limitation was imminent. *G. fasciculata*, on the other hand, was severely N-limited after five days without rain, as positive growth was only observed in treatments with added N. Without N addition, biomass was lost, suggesting respiration exceeded photosynthesis in these treatments. This contrasted with the previous experiment where P stimulated growth more than N and growth rates were up to nearly 40%. *P. boryana* showed no significant

response to nutrient addition, suggesting a lack of nutrient limitation, though variability was high in this experiment. Overall, *D. bartayresiana* remained nutrient sufficient, *G. fasciculata* was N-limited, and *P. boryana* had variable responses. Prolonged rainfall with low light

2.4.2.3 Prolonged rainfall with low light

After nearly a week of storms and reduced light levels in the field, experimental results suggest limitation switched from nutrients to some other energetic constraint related to conditions on the reef, possibly light (Table 2.1c, Figure 2.2c). Biomass changes were small throughout, with some species showing growth and others losing biomass (-9 to 7%). The only significant, though small, effect of nutrient addition was that of N on *P. boryana*, and this was negative (Table 2.1c).

2.4.2.4 Little rain with low light

After three days in the field with little rain following the weeklong storm, negative effects of N addition on *D. bartayresiana* were significant, resulting in very small losses of biomass. In contrast, *G. fasciculata* was again strongly nutrient limited and *P. boryana* was nearing limitation (Table 2.1d, Figure 2.2d). *G. fasciculata* grew significantly more with N addition, at nearly 20% – three times the rate in the ambient treatment. In contrast to three days prior, *P. boryana* grew in all treatments, with marginally higher growth with P addition indicating near P limitation. These results suggest that *D. bartayresiana* may be less tolerant to environmental conditions associated with the storm than the other species.

2.4.3 *Tissue nutrient collections*

Specimens of *D. bartayresiana* were collected on 16 May 2010, two days after 71 mm of rainfall, and again on 26 and 27 May, with little intervening rain except two incidences of light rains of 12-15 mm (Figure 2.3a). Tissue analysis showed that, at 1.50 ± 0.08%, tissue N was significantly higher 16 May after the short but intense storm (ANOVA $F_{(2,6)} = 14.47$,

$p = 0.005$) and had declined 20% by 26 May (1.20 ± 0.03%), with no further significant declines by 27 May (Figure 2.3b).

G. fasciculata was collected on 2 May 2012, three days after a very large four-day storm producing 175mm of rain (Figure 2.3c). *P. boryana* was collected on 4 May, five days after the storm. Both species were collected again on 7 May and 16 May, eight and seventeen dry days post-storm. Both tissue N and P appeared to be substantially higher for *P. boryana* five days after the storm compared to later collections (Figure 2.3d,f; ANOVA for N: $F_{(2,11)} = 22.6$, $p = 0.0001$; P: $F_{(2,10)} = 3.88$, $p = 0.053$). Tissue N was 1.14 ± 0.007% on 4 May and decreased 25% by 7 May (0.86 ± 0.026%) at which level it remained nine days later. Similarly, tissue P was 33% lower on both subsequent dates compared to 4 May, but high variability resulted in non-significant comparisons among dates. In *G. fasciculata*, tissue N also decreased significantly over time from the storm (ANOVA $F_{(2,10)} = 10.8$, $p = 0.003$; Figure 2.3e), losing 14% of tissue N stores by 16 May (from 1.20 ± 0.03% on 2 May). In contrast, %P showed no change over time (Figure 2.3g).

2.5 Discussion

We found that nutrient limitation shifted radically between nutrients and among species on the scale of days to weeks. This finding implies that single snapshot assessments of producer limitation cannot fully characterize complex systems, particularly those subjected to pulsed or rapidly changing nutrient supplies. This supports recent conclusions from large-scale meta-analyses across terrestrial and aquatic systems that changes in nutrient supply may not only increase production but also alter the nature of limitation (Elser et al. 2007). Thus, generalizations about nutrient limitation are particularly problematic in areas where human alteration of nutrient supply may alter community and ecosystem dynamics (Downing et al. 1999; Hooper and Johnson 1999; Vitousek et al. 2010). However, the drastic changes in nutrient limitation we observed over short timescales are indicative not of broad shifts in dynamics, but of rapidly evolving interactions among nutrients and among species. Recent research in terrestrial systems has broadened from proximate nu-

trient limitation – a direct response to addition with increased productivity – to ultimate limitation, wherein limitation is assessed by a nutrients ability to transform the ecosystem (Vitousek et al. 2010). For example, if both N and P addition in a lake cause a growth response, but only P enrichment causes that body of water to become eutrophied, then only P is an ultimate limiting nutrient. However, in marine systems, increased salinity has been shown to inhibit N fixation (e.g. Souza and Yoch 1997; Magalhes et al. 2005), which may complicate the mechanisms by which ultimate limitation is proposed to occur. Moreover, in tropical reef systems, where nutrient supply may be highly pulsed and responses hugely variable over time, ultimate limitation across the community is unlikely to occur. Instead, maintenance of diverse communities depends upon varying strategies to adapt to short term pulsed supply. Thus, while appropriate for terrestrial or freshwater systems, the concept of ultimate limitation may not be useful for coastal marine systems, especially those with anthropogenic inputs. If true, this will have profound implications for mitigation of nutrient impacts and management strategies in coastal marine ecosystems.

The high variability in nutrient limitation shown by each species in the current study may also shed some light on the wide-ranging and seemingly contradictory results of previous studies on tropical algae. For example, experimental enrichments of *Dictyota* spp. on various tropical reefs have produced the gamut of conclusions, from no effects (Delgado and Lapointe 1994; Kuffner et al. 2006), to weak (Aisha et al. 1995) or conditional (Beach et al. 2006) nutrient limitation, to strong growth response of *Dictyota* spp. to both N and P addition (Lapointe et al. 1987; Fong et al. 2003; Littler et al. 2006). Fong et al. (2003) suggested that limitation in *Dictyota* spp. may depend on the frequency of nutrient pulses in its habitat. Few studies have explicitly examined temporal variability in nutrient limitation, but in seasonal comparisons, Lapointe (1987) found greater effects of P-enrichment on *Gracilaria* in the summer than the winter, and Fujita et al. (1989) found that response to N addition in *Pelvetiopsis limitata* depended on upwelling season, though this study was in a temperate system (see also Fisher et al. 1999 for a study on seasonal limitation in phytoplankton in temperate Chesapeake Bay). Our results demonstrate that the processes affecting algal nutrient limitation are operating on much finer temporal scales than previously considered,

and research under relevant spatial and temporal scales is imperative to understanding or characterizing nutrient limitation in any system.

The observed dynamic temporal shifts in nutrient limitation in our experiments may be driven, at least in part, by changes in environmental conditions that affect nutrient supply, uptake ability, and growth. All species were more nutrient limited after longer periods of dry weather and less so after short rains, implicating an external nutrient source consistent with pulses of terrestrial run-off after rainfall events. Changes in initial internal nutrient stores associated with storm-linked nutrient inputs on the reef prior to experimentation are a likely reason for the rapid shifts in patterns of nutrient limitation. Increased tissue nutrient stores in our field-collected algae after rainfall corroborate this conclusion as well as the findings of Schaffelke and Klumpp (1998) and Fong et al. (2003) that growth response of various macroalgal forms to nutrient addition depended on pre-assay tissue nutrient levels. They also support the suggestion of Littler et al. (1991) that differences in algal nutrient limitation may be linked to run-off and decomposition of organics after rainfall. Overall lower growth rates after the long storm, however, suggest watershed-related causes, possibly toxins from run-off or light limitation from low light levels both prior to and during the experiment. Reduced salinity in the water column could also have negatively affected growth if it was carried over into the experimental treatment water. Negative effects of nitrogen observed in each species, though small in effect, may have resulted from toxic levels of nutrients (e.g. Haines and Wheeler 1978; Lotze and Schramm 2000; Fong et al. 2004) and/or energetic costs either directly of the algae or from the associated microbial community. After the long storm, the associated extensive period of low light may have constrained energy for uptake and storage of nutrients on the reef, creating light limitation. Thus, during the experiment, when specimens were cleaned and kept in shallow units, energy was likely to be directed to nutrient uptake across all treatments to support future growth or fill depleted nutrient stores (see Gordillo 2012). This is particularly true of N, as tissue data showed N became more depleted than P in all species. These results are substantiated by recent evidence on benthic algae in lakes suggesting that, even in oligotrophic conditions, organic matter in the water column may be a better indicator of productivity than nutrient levels

because light availability may have primacy over nutrient availability and constrain growth regardless of nutrient supply (Karlsson et al. 2009). Thus our findings suggest that adverse environmental conditions affecting algal ability to take up and process nutrients may trump nutrient availability, particularly on turbid reefs.

Algae with different life-history strategies showed different strengths and types (N vs. P) of nutrient limitation even though they were taken from the same habitat and therefore subjected to the same nutrient and environmental history. This finding contrasts with a study of five morphologically variable tundra plant species where soil P content determined rates of uptake regardless of form (Kielland and Chapin 1994). Furthermore, it may provide another explanation for mixed results of previous experiments on tropical algae. For example, the higher growth rates in *G. fasciculata* after the storm and more rapid return to N limitation three days after the rains ended suggest an overall greater tolerance to conditions associated with rainfall in *G. fasciculata* than in either brown algal species. *D. bartayresiana*, in contrast, was able to buffer the effects of variable nutrient supplies, presumably by storage, but was most affected by the storm. These findings corroborate a study in Taiwan (Su et al. 2009) that found blooms of *G. oblongata* were common in areas with high turbidity and another study suggesting that *Dictyota* spp. cannot acclimate to low irradiance levels (Beach et al. 2006). These mixed results contrast, however, with other studies such as Larned (1998) who found nearly uniform N-limitation in eight of nine macroalgal species in Kaneohe Bay (see also Littler et al. 1991). In Kaneohe Bay, however, this is likely related to the long history of sewage pollution with a low N:P ratio and the high residence time of phosphorus in the sediment (Larned and Stimson 1996). Moreover, our findings do correspond to work by Aisha et al. (1995) who found that *Galaxaura* was more strongly nutrient-limited than *Dictyota* in the Red Sea. Thus, our results seem to corroborate assumptions that under moderate enrichment in the tropics, opportunists may benefit more than species with other nutrient-use strategies with release from limitation and capability for rapid growth rates (Littler and Littler 1984), yet they suggest that under chronic or prolonged disturbances affecting nutrient loads or supply rates, species more tolerant of variable environmental conditions may be best adapted. Consequently, algal community response to

increasing or changing environmental stressors may depend on the magnitude and duration of these disturbances.

The mechanisms underlying the observed differences in nutrient limitation among species may relate directly to differences in physiology, particularly with regard to nutrient uptake and storage strategies (see Pedersen and Borum 1997; Fong and Paul 2011). Despite exposure to the same nutrient history, our variable results among species were likely caused by differing stores of nutrients in their tissues at experimental onset. In fact, over the short timescales in which we observed extreme changes in nutrient limitation, we also observed drastic changes in tissue nutrient stores in the field collections, providing a probable explanation for those patterns. Some opportunist species have been shown to maintain enriched tissue nutrient stores for up to ten days following a nutrient pulse (e.g. Fong et al. 1998; Fong et al. 2001). As in Fong et al. (2003), we found that *D. bartayresiana* was responsive to and adapted to pulsed nutrient supply. In fact, the marginal P-limitation after six days without rain suggests a capacity for P storage of at least one week. In contrast, species with persister strategies (such as *G. fasciculata*) are restricted by external availability and growth is based more on uptake from what is directly available in the environment at that time (e.g. Larned and Stimson 1996), rather than internal stores (Gordillo 2012), which reflect earlier environmental conditions. Our patterns corroborate the findings of Aisha et al. (1995) that *Galaxaura* spp. both lacks the capability for surge uptake in response to nutrient pulses and has a lower storage capacity relative to *Dictyota* spp. Likewise, severe nutrient limitation in *G. fasciculata* while *D. bartayresiana* remained unlimited indicates that nutrient history has little effect on growth of persister species like *G. fasciculata*. This is supported by evidence from Fong et al. (2003) that algal species with differing growth and storage capacities respond variably to nutrient addition only when initial tissue nutrient stores are enriched, but respond uniformly with increased growth when tissue nutrient stores are initially depleted. Our study highlights the importance of considering storage capacities as well as nutrient history when predicting nutrient limitation across species assemblages.

In conclusion, we showed that limitation by N and P was extremely variable over short timescales and among species, even though these species were exposed to the same

nutrient history. This indicates that broad generalizations about N vs. P limitation of a given system inaccurately portray the inherent complexity in the governing conditions and processes. Furthermore, this is the first study to examine tropical algal limitation in connection with distinct changes in environmental conditions, and it highlights the need to consider the appropriate temporal and spatial framework for the question and system under investigation.

2.6 Acknowledgments

We are thankful to Gump Biological Research Station for providing the facilities to conduct this research and the UCLA undergraduate students of the Marine Biology Quarter field course for field and laboratory assistance. We are indebted to the Center for Oceanic Science Education Excellence West for funding and support. We also thank Richard Ambrose and Rick Vance for comments that greatly improved an earlier version of the manuscript. Research was funded in part by the UCLA Office of Instructional Development and the UCLA Department of Ecology and Evolutionary Biology. Writing was completed with support by the National Science Foundation Graduate Research Fellowship Program. Data on rainfall and solar radiation were provided by the Mo'orea Coral Reef Ecosystem LTER, funded by the US National Science Foundation (OCE-0417412).

Table 2.1: Main effects (N, P) and interaction term (N·P) from 2-factor PERMANOVA (euclidean distance with 9,999 permutations) run for each species and time combination.

	Species	**Source**	***df***	**SS**	***F* ratio**	***P***
a. Exp 1: short rain, high light	*Dictyota*	N	1	343	0.058	0.82
		P	1	104	0.921	0.38
		N·P	1	64.2	0.230	0.65
	Galaxaura	N	1	108.9	1.41	0.26
		P	1	2880.0	37.16	0.0001
		N·P	1	802.2	10.35	0.0059
	Padina	N	1	701.2	19.52	0.0002
		P	1	3.7	0.1	0.79
		N·P	1	80.1	2.23	0.15
b. Exp 2: 6d dry, low light	*Dictyota*	N	1	0.22	0.009	0.85
		P	1	92.2	3.858	0.077
		N·P	1	6.88	0.288	0.6
	Galaxaura	N	1	320	14.4	0.002
		P	1	2.2	0.1	0.71
		N·P	1	20	0.9	0.36
	Padina	N	1	211.2	0.399	0.77
		P	1	361.2	0.682	0.59
		N·P	1	11.2	0.021	0.98
c. Exp 3: long rain, low light	*Dictyota*	N	1	20	2.67	0.22
		P	1	5	0.67	0.41
		N·P	1	0	0	1
	Galaxaura	N	1	0.56	0.018	0.82
		P	1	93.9	3.045	0.097
		N·P	1	45	1.459	0.23
	Padina	N	1	125	8	0.0062
		P	1	5	0.32	0.54
		N·P	1	20	1.28	0.25
d. Exp 4: little rain, low light	*Dictyota*	N	1	80	8.53	0.015
		P	1	20	2.13	0.14
		N·P	1	5	0.53	0.38
	Galaxaura	N	1	320	8.113	0.012
		P	1	142.2	3.606	0.074
		N·P	1	20	0.507	0.51
	Padina	N	1	11.3	1.2	0.26
		P	1	31.3	3.3	0.12
		N·P	1	1.3	0.13	0.57

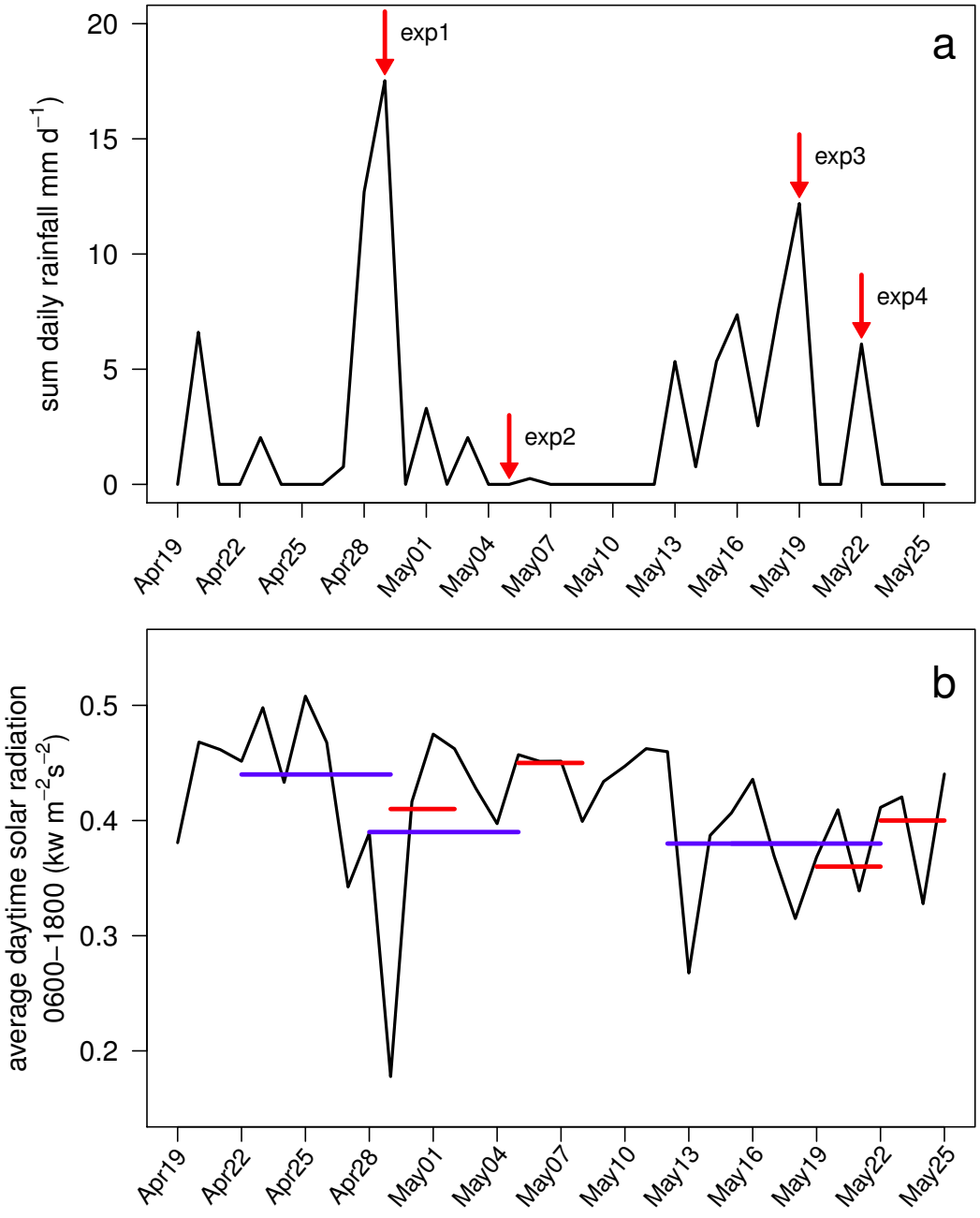

Figure 2.1: a) Summed daily rainfall in mm during the course of experimentation (19 Apr – 27 May 2008). Red arrows indicate the start date of each experiment, where algal nutrient limitation status is influenced by the environmental conditions on the reef prior to the experiment as they may determine nutrient uptake and storage. b) Average daytime solar irradiance over the course of each experiment. Values are calculated from measurements taken between 06:00–18:00 and measured in kilowatts·m^{-2} s^{-1}. Red lines indicate duration of experiment and average value of solar radiation. Blue lines indicate average value of solar radiation in the week prior to each experiment.

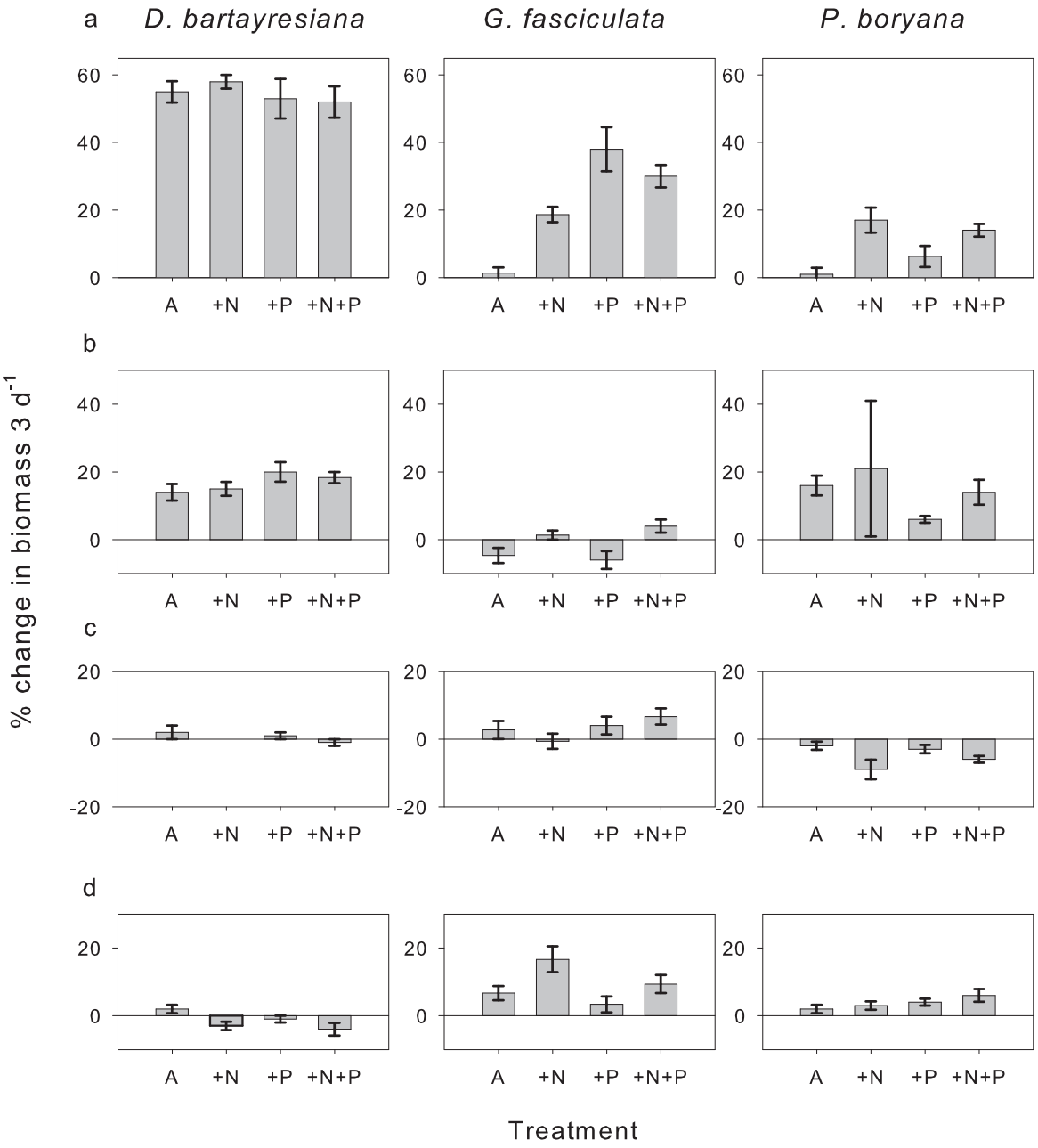

Figure 2.2: Results from two factor bioassay experiments conducted on three algal species, *Dictyota bartayresiana*, *Galaxaura fasciculata*, and *Padina boryana* across four times with varying environmental conditions (rows a-d). Growth response to nutrient addition is measured as mean % change in biomass ± SE, where A is the ambient treatment, +N is the nitrogen addition, +P is phosphorus addition, and +N+P is the treatment with both nutrients added. a) 29 Apr 2008 after short, intense rainfall but high light (19.1mm). *P. boryana* treatment +P had $n = 4$. b) 5 May 2008 after six days of nearly dry but low light conditions. Treatments +N and +P in *D. bartayresiana* had $n = 4$; +N+P had $n = 3$. c) 19 May 2008 after prolonged rainfall with low light (48.1mm). The +N treatment of *D. bartayresiana* showed no change across treatments, giving a mean and standard error of zero. d) 22 May 2008 after little rain with low light.

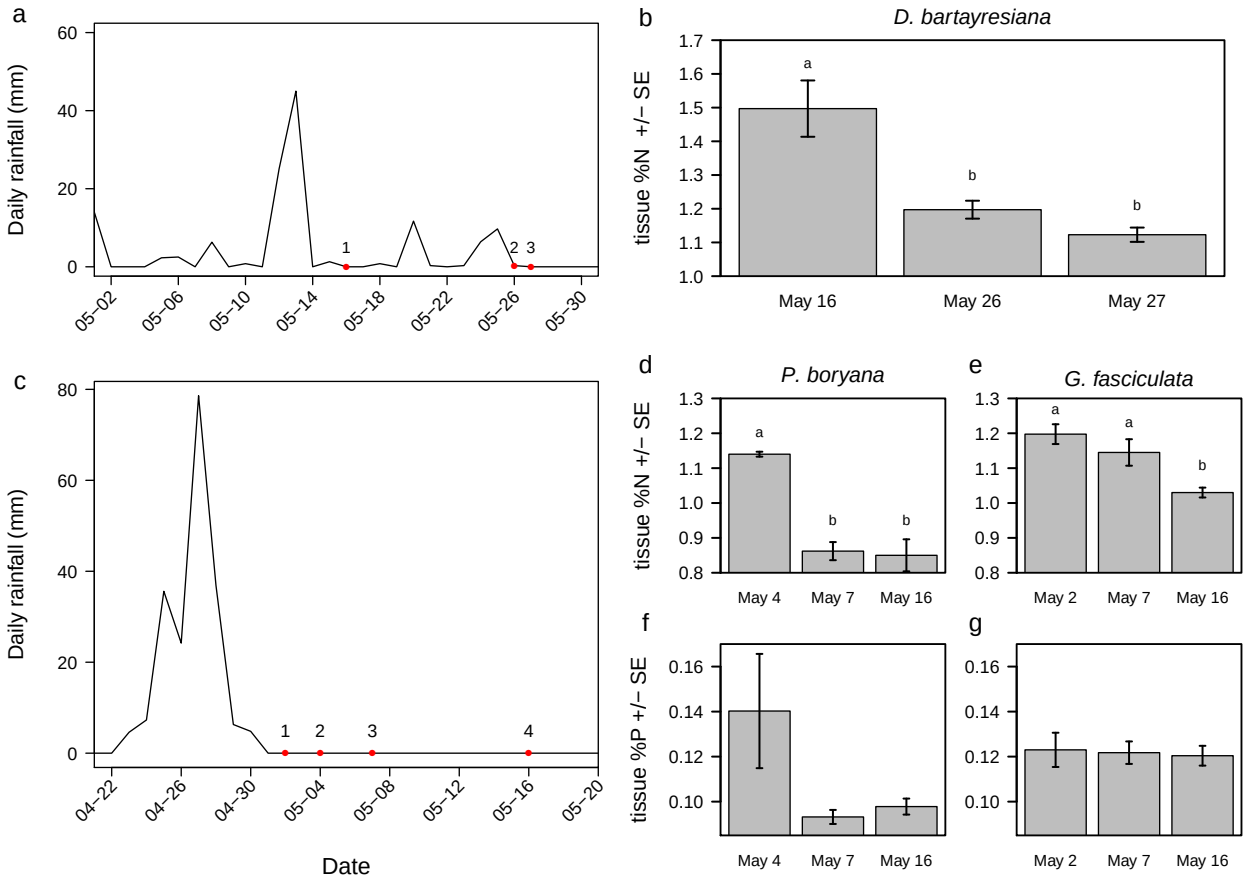

Figure 2.3: 1-3. Tissue nutrient data collected for each species at three times (May 2010 and 2012) after varying periods post rainfall ($n = 5$ for all species and date combinations). a) Summed daily rainfall in mm over the course of May 2010, where red dots indicate days on which *D. bartayresiana* specimens were collected. b) Tissue nitrogen (N) content of *D. bartayresiana* from samples collected on the dates indicated in (a) ($n = 3$). c) Summed daily rainfall in mm from 20 Apr – 31 May 2012, with red dots indicating dates on which *G. fasciculata* (1,3,4) and *P. boryana* (2, 3, 4) were collected. d) Tissue N content of *P. boryana* and e) *G. fasciculata* at three times post-storm indicated in (c) ($n = 5$). f) Tissue phosphorus (P) content of *P. boryana* and g) *G. fasciculata* at three times post-storm indicated in (c) ($n = 5$). In panels b, d, e, f, and g letters indicate significant differences as determined by Tukeys HSD test, and bars are means ± SE.

References

Adjeroud, M., and B. Salvat. 1996. Spatial patterns in biodiversity of a fringing reef community along Opunohu Bay, Mo'orea, French Polynesia. Bulletin of Marine Science **59**:175–187.

Anderson, M. J. 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences **58**:626–639.

Atkinson, M. J., and S. V. Smith. 1983. C:N:P ratios fo benthic marine plants. Limnology and Oceanography **28**:568–574.

Beach, K. S., L. J. Walters, and H. B. Borgeas. 2006. Irradiance and nutrient limitation of Dicytota spp. populations on Conch Reef, Florida Keys, USA. Journal of Experimental Marine Biology and Ecology **329**:101–112.

Cloern, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series **210**:223–253.

Cohen, R. A., and P. Fong. 2006. Using opportunistic green macroalgae as indicators of nitrogen supply and sources to estuaries. Ecological Applications **16**:1405–1420.

Delgado, O., and B. E. Lapointe. 1994. Nutrient-limited productivity of calcareous versus fleshy macroalgae in a eutrophic, carbonate-rich tropical marine environment. Coral Reefs **13**:151–159.

Delgado, O., C. Rodrigues-Prieto, E. Gacia, and E. Ballesteros. 1996. Lack of severe nutrient limitation in *Caulerpa taxifolia* (Vahl) C. Agardh, an introduced seaweed spreading over the oligotrophic northwestern Mediterranean. Botanica Marina **39**:61–67.

Downing, J. A., C. W. Osenberg, and O. Sarnelle. 1999. Meta-analysis of marine nutrient-enrichment experiments: variation in teh magnitude of nutrient limitation. Ecology **80**:1157–1167.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters **10**:1135–1142.

Fong, P., 2008. Macroalgal-dominated ecosystems. Chapter 20, pages 917–947 *in* D. G. Capone, D. A. Bronk, M. R. Mulholland, and E. J. Carpenter, editors. Nitrogen in the marine environment. Academic Press, San Diego, 2nd edition.

Fong, P., K. E. Boyer, K. Kamer, and K. A. Boyle. 2003. Influence of initial tissue nutrient status of tropical marine algae on response to nitrogen and phosphorus additions. Marine Ecology Progress Series **262**:111–123.

Fong, P., R. M. Donohoe, and J. B. Zedler. 1994. Nutrient concentration in tissue of the macroalga Enteromorpha as a function of nutrient history: an experimental evaluation using field microcosms. Marine Ecology Progress Series **106**:273–281.

Fong, P., K. Kamer, K. E. Boyer, and K. A. Boyle. 2001. Nutrient content of macroalgae with differing morphologies may indicate sources of nutrients for tropical marine systems. Marine Ecology Progress Series **220**:137–152.

Fong, P., and V. J. Paul, 2011. Coral reef algae. *in* Z. Dubinsky and N. Stambler, editors. Coral reefs: an ecosystem in transition. Springer.

Fry, B., A. Gace, and J. W. McClelland. 2003. Chemical indicators of anthropogenic nitrogen loading in four Pacific estuaries. Pacific Science **57**:77–101.

Fujita, R. M. 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. Journal of Experimental Marine Biology and Ecology **92**:283–301.

García-Reyes, M., and J. Largier. 2010. Observations of increased wind-driven coastal upwelling off central California. Journal of Geophysical Research **115**:C04011.

Gordillo, F. J. L., 2012. Environment and algal nutrition. Chapter 4, pages 67–86 *in* C. Wiencke and K. Bischof, editors. Seaweed biology. Springer-Verlag, Berlin, Heidelberg, ecological edition.

Hanisak, D. M., 1983. The nitrogen relationships of marine macroalgae. Pages 699–730 *in* E. J. Carpenter and D. G. Capone, editors. Nitrogen in the marine environment. Academic Press, New York.

Harpole, W. S., J. T. Ngai, E. E. Cleland, E. W. Seabloom, E. T. Borer, M. E. S. Bracken, J. J. Elser, D. S. Gruner, H. Hillebrand, J. B. Shurin, and J. E. Smith. 2011. Nutrient co-limitation of primary producer communities. Ecology Letters **14**:852–62.

Hatcher, B. G. 1988. Coral reef primary productivity: A beggar's banquet. Trends in Ecology & Evolution **3**:106–11.

Hecky, R. E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. Limnology and Oceanography **33**:796–822.

Holthuss, P. E., J. E. Maragos, and C. W. Evans. 1989. Coral reef recovery subsequent to the freshwater kill of 1965 in Kaneohe Bay, Oahu, Hawaii. Pacific Science **43**:122–134.

Howarth, R. W. 1988. Nutrient limitation of net primary production in marine ecosystems. Annual Review of Ecology and Systematics **19**:89–110.

Howarth, R. W., and R. Marino. 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. Limnology and Oceanography **51**:364–376.

Kennison, R. L., K. Kamer, and P. Fong. 2011. Rapid nitrate uptake rates and large short-term storage capacities may explain why opportunistic green macroalgae dominate shallow eutrophic estuaries. Journal of Phycology **47**:483–494.

Kuffner, I. B., and V. J. Paul. 2001. Effects of nitrate, phosphate and iron on the growth of macroalgae and benthic cyanobacteria from Cocos Lagoon, Guam. Marine Ecology Progress Series **222**:63–72.

Lapointe, B. E. 1987. Phosphorus-and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. Marine Biology **93**:561–568.

Lapointe, B. E., P. J. Barile, M. M. Littler, D. S. Littler, B. J. Bedford, and C. Gasque. 2005. Macroalgal blooms on southeast Florida coral reefs I. Nutrient stoichiometry of the invasive green alga *Codium isthmocladum* in the wider Caribbean indicates nutrient enrichment. Harmful Algae **4**:1092–1105.

Lapointe, B. E., and J. O. Connell. 1989. Nutrient-enhanced growth of *Cladophora prolifera* in Harrington Sound , Bermuda: eutrophication of a confined, phosphorus-limited marine ecosystem. Estuarine Coastal and Shelf Science **28**:347–360.

Lapointe, B. E., M. M. Littler, and D. S. Littler. 1992. Nutrient availability to marine macroalgae in siliciclastic versus carbonate-rich coastal waters. Estuaries and Coasts **15**:75–82.

Larned, S. T. 1998. Nitrogen-versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae. Marine Biology **132**:409–421.

Larned, S. T., and J. Stimson. 1996. Nitrogen-limited growth in the coral reef chlorophyte *Dictyosphaeria cavernosa*, and the effect of exposure to sediment-derived nitrogen on growth. Marine Ecology Progress Series **145**:95–108.

LeBauer, D. S., and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology **89**:371–379.

Littler, M. M., D. S. Littler, and E. a. Titlyanov. 1991. Comparisons of N- and P-limited productivity between high granitic islands versus low carbonate atolls in the Seychelles Archipelago: a test of the relative-dominance paradigm. Coral Reefs **10**:199–209.

Lyngby, J. E., S. Mortensen, and N. Ahrensberg. 1999. Bioassessment techniques for monitoring of eutrophication and nutrient limitation in coastal ecosystems. Marine Pollution Bulletin **39**:212–223.

McCook, L. J. 1999. Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. Coral Reefs **18**:357–367.

McGlathery, K., R. Marino, and R. Howarth. 1994. Variable rates of phosphate uptake by shallow marine carbonate sediments: mechanisms and ecological significance. Biogeochemistry **25**:127–146.

McGlathery, K. J., K. Sundback, and P. Fong, 2012. Estuarine benthic algae. Chapter 7, pages 203–234 *in* J. W. Day, B. C. Crump, W. M. Kemp, and A. Yanez-Arancibia, editors. Estuarine ecology. John Wiley & Sons, Inc., Hoboken, N.J., second edi edition.

Mejia, A. Y., G. N. Puncher, and A. H. Engelen, 2012. Macroalgae in tropical marine coastal systems. Chapter 16, pages 329–357 *in* C. Wiencke and K. Bischof, editors. Seaweed biology, volume 219 of *Ecological Studies*. Springer-Verlag, Berlin, Heidelberg.

Oksanen, J., R. Blanchet, Guillaume F. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, and H. Wagner, 2012. vegan: Community Ecology Package. R package version 2.0-5. http://CRAN.R-project.org/package=vegan.

Paerl, H., J. Joyner, A. Joyner, K. Arthur, V. Paul, J. ONeil, and C. Heil. 2008. Co-occurrence of dinoflagellate and cyanobacterial harmful algal blooms in southwest Florida coastal waters: dual nutrient (N and P) input controls. Marine Ecology Progress Series **371**:143–153.

Pedersen, M. F., and J. Borum. 1996. Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Marine Ecology Progress Series **142**:261–272.

R Core Team, 2012. R: A language and environment for statistical computing.

Rabalais, N. N., R. E. Turner, R. J. Diaz, and D. Justic. 2009. Global change and eutrophication of coastal waters. ICES Journal of Marine Science **66**:1528–1537.

Redfield, A. C., B. H. Ketchum, and F. A. Richards, 1963. The influence of organisms on the composition of seawater. Pages 26–77 *in* M. Hill, editor. The sea. Wiley, New York, volume 2 edition.

Ryther, J. H., and W. M. Dunstan. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science **171**:1008–1013.

Sah, R. N., and R. O. Miller. 1992. Spontaneous reaction for acid dissolution of biological tissues in closed vessels. Analytical Chemistry **64**:230–233.

Schaffelke, B. 1999. Short-term nutrient pulses as tools to assess responses of coral reef macroalgae to enhanced nutrient availability. Marine Ecology Progress Series **182**:305–310.

Schaffelke, B., and D. Klumpp. 1998. Short-term nutrient pulses enhance growth and photosynthesis of the coral reef macroalga *Sargassum baccularia*. Marine Ecology Progress Series **170**:95–105.

Smith, J. E., C. L. Hunter, and C. M. Smith. 2010. The effects of top-down versus bottom-up control on benthic coral reef community structure. Oecologia **163**:497–507.

Smith, S. V. 1984. Phosphorus versus nitrogen limitation in the marine environment. Limnology and Oceanography **29**:1149–1160.

Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution **100**:179–196.

Taylor, D., S. Nixon, S. Granger, and B. Buckley. 1995. Nutrient limitation and the eutrophication of coastal lagoons. Marine Ecology Progress Series **127**:235–244.

Teichberg, M., S. E. Fox, C. Aguila, Y. S. Olsen, and I. Valiela. 2008. Macroalgal responses to experimental nutrient enrichment in shallow coastal waters: growth, internal nutrient pools, and isotopic signatures. Marine Ecology Progress Series **368**:117–126.

Valiela, I., J. McClelland, J. Hauxwell, P. J. Behr, D. Hersh, and K. Foreman. 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnology and Oceanography **42**:1105–1118.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications **7**:737–750.

Vitousek, P. M., S. Porder, B. Z. Houlton, and O. a. Chadwick. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecological applications : a publication of the Ecological Society of America **20**:5–15.

Washburn, L., and A. Brooks of Moorea Coral Reef LTER, 2012. MCR LTER:.

CHAPTER 3

SEDIMENTS DIFFERENTIALLY ALTER DRIVERS OF TWO DOMINANT MACROALGAL SPECIES BIOMASS ACCUMULATION ON A FRINGING CORAL REEF

3.1 Abstract

Despite increasing concern that anthropogenic sediment loads will facilitate algal dominance on tropical reefs, it is relatively unknown how sediments interplay with the dominant drivers of algal communities, nutrients and herbivory, to control their dynamics. We examined the effects of ambient sediment loads on two increasingly abundant genera of fleshy macroalgae, *Galaxaura fasciculata* and *Padina boryana*, in a bay subject to high flux of terrestrial sediment inputs in Mo'orea, French Polynesia. Field experiments examining net effects of ambient sediments and interacting effects of sediments (ambient/removal) and herbivores (caged/uncaged) on growth and tissue nutrients demonstrated strong but opposite effects of sediments on growth of the two species, and no evidence of inhibition of herbivory. Across experiments, *G. fasciculata* only achieved positive growth with ambient sediment loads, and lost biomass in removal treatments. In contrast, removal of sediments increased growth of *P. boryana* by 50% in the net effect experiment, but did not affect growth in the factorial experiment; rather, herbivores appeared to over-compensate for increased tissue nutrient stores in the presence of sediments by preferential consumption of nutrient-rich tissues. In laboratory experiments testing interactions of added nutrients and thallus sediment loads on growth and tissue nutrient stores, *G. fasciculata* grew at equivalent rates with organically

rich sediment (∼8%) as with additions of 20 μM nitrate and 2 μM phosphate; however, effects of nutrients and sediment were not additive. Sediments again decreased growth of *P. boryana* by 50%, while nutrients had no effect. Results demonstrated that increasing sediment loads on reefs have the capacity to strongly alter processes controlling macroalgal community dynamics including, but not limited to, herbivory and nutrient availability, and as such, will have substantial but varying effects on changes in abundance and composition of tropical reef macroalgal communities.

3.2 Introduction

The global occurrence of phase shifts on coral reefs toward macroalgal dominance (Pandolfi et al., 2003; Hughes et al., 2007) has heightened the need to elucidate how anthropogenic impacts affect the drivers of macroalgal biomass accumulation. Two of the primary macroalgal structuring processes that humans have altered are nutrient availability and herbivory, particularly by fish populations (for a review see Fong and Paul, 2011). Anthropogenic increases in nutrient loads may release macroalgae from nutrient-limitation, increasing growth and abundance, particularly of opportunistic species (e.g. Littler et al., 2006). A concurrent reduction in herbivores lessens their ability to compensate for increased productivity and maintain low levels of biomass (e.g. Thacker et al., 2001). However, sedimentation on reefs is also increasing (Fabricius, 2005), particularly on fringing reefs exposed to the stresses of urban development and agricultural land use (Mora, 2008). On some reefs, these changes have become so widespread that many believe the baseline has shifted toward a new ambient condition of heightened sedimentation (and associated particulate organic matter) (McCulloch et al., 2003; Richmond et al., 2007; Prouty et al., 2010) and reduced herbivory (Hughes et al., 2010). There is substantial correlative evidence that macroalgae (>1 cm) are more abundant under higher sediment loads (McCook, 1996, 1999, 1997; De'ath and Fabricius, 2010), yet it is not known how increased sediment loads interact with nutrients and herbivory, the dominant drivers of macroalgal communities, to influence their dynamics.

One of the primary effects of increasing sediment loads may be changes in the avail-

ability of resources for photosynthesis that ultimately affect algal growth, abundance and community composition. Reef sediments, particularly those with a high terrigenous component, are often high in organic and inorganic nutrients (Weber et al., 2006), and release nutrients when water column levels are low (Stimson and Larned, 2000). There is some evidence that certain species may utilize nutrients in sediment for growth (e.g. Schaffelke, 1999; Stimson and Larned, 2000; Eyre and Ferguson, 2002). Studies of direct effects of sediment on macroalgal growth on tropical reefs, however, are rare and primarily limited to *Sargassum* spp. (e.g. Schaffelke, 1999). One study on *Sargassum* spp. on the Great Barrier Reef found that sediments benefitted the alga by providing nutrients (Schaffelke, 1999), while another demonstrated that experimentally increased sediment loads reduced recruitment, growth, survival, and vegetative regeneration (Umar et al., 1998). On temperate reefs, where the effects of sediment loads on macroalgae are better established, most studies suggest the effects on growth are primarily negative (Airoldi, 2003; Schiel et al., 2006, Kawamata et al., 2011, but see), as sediments may block light and gas or nutrient exchange (Airoldi, 2003). *Sargassum duplicatum*, a temperate species in Japan, exhibited reduced settlement in addition to lower growth rates with sediment, although mortality was only significantly affected under sediment depths greater than 2 mm (Kawamata et al., 2012). Additionally, in limiting diffusion of metabolic waste products, sediment burial may result in production of hydrogen sulfide (H_2S). Production of H_2S under sediment has been shown to limit growth and survival of the temperate macroalga *Fucus serratus* (Chapman and Fletcher, 2002) and algal turf in the South Pacific (Clausing et al., in revision for MEPS). Yet, on a temperate subtidal reef in Southwest Australia, Gorgula and Connell (2004) found that nutrient enrichment in both the water column and the sediments independently increased algal turf % cover. Thus, while sediments may significantly alter resource control of reef macroalgae, including altering nutrient limitation, it is unclear whether the net effect is positive or negative.

In addition to altering algal growth potential, sediments may also affect herbivory of algal tissue by blanketing thalli and decreasing tissue access or palatability. For example, on a reef flat in the Great Barrier Reef, experimental reduction of sediment loads on algal turf led to a 225% increase in herbivory rates of the five most abundant fish species (Bellwood

and Fulton, 2008). Subsequent research demonstrated that increased fish bite rates with experimental sediment removal extended across 3 reef zones including the reef crest and flat (Goatley and Bellwood, 2012, but see Bonaldo and Bellwood, 2011). Moreover, a pulse of sediment on the reef crest increased turf growth to equivalent rates as individuals caged from herbivores (Goatley and Bellwood, 2013). However, a recent study on the back reef flat in Mo'orea, French Polynesia showed that moderate levels of sediment only inhibited fish herbivory when it resulted in anoxia, indicating that these effects may depend on sediment depth as well as environmental context (Clausing et al., in press; see also Bonaldo and Bellwood, 2011). On a temperate reef in Japan, *Sargassum duplicatum* has been shown to settle and thrive only where a thin layer of sediment (<2 mm) protects new recruits from herbivory by urchins (Kawamata et al., 2011), suggesting constant but thin sedimentation may support macroalgal dominance by inhibiting herbivory of early life stages of macroalgae in particular. Thus, most studies of the effects of sediments on fish herbivory have been done in temperate systems (e.g. Kawamata et al., 2011) or on turf algae (<1 cm) in tropical systems with mixed results (e.g. Bellwood and Fulton, 2008; Bonaldo and Bellwood, 2011; Goatley and Bellwood, 2012); we know little about the impacts of sediments on macroalgal grazing by fish, particularly in tropical reef ecosystems.

Although sediments are likely to interact with the macroalgal controlling forces of herbivory and nutrients, how and to what extent sediments alter these processes may depend on different morphologies and nutrient use strategies among species (Airoldi, 2003). Connell (2005) showed extreme variability in impacts of sediments on differing morphological forms of a temperate algal community, where crustose coralline algae was strongly inhibited by sediments, turfs were tolerant of heavy sediment loads, and articulated corallines actually required sediment to grow and persist. In contrast, Begin et al. (2013) showed that, along with well-documented harm to corals, increased sediment loads had negative effects on abundance of two morphological algal forms: macroalgae and turf. Some species are adapted to use nutrients from sediment porewater (Larned and Stimson, 1996), and other species have been shown to increase growth rates with deposition of particulate matter due to nutrient provision Schaffelke (1999). Species such as these may gain competitive advan-

tage with increasing sediment loads. McClanahan et al. (2005) also found that two species of brown frondose algae benefitted from addition of particulate organic matter, although whether this was due in part to provision of nutrients or only attributable to the inhibition of small herbivorous fish was unclear. These results, combined with evidence of sediment providing nutrients and inhibiting herbivory, suggest that benefits of sediments may exist, but they are likely to strongly vary between algal forms. Yet few studies have experimentally examined these effects, and almost none examine underlying mechanisms (e.g. Umar et al., 1998; Kawamata et al., 2011, but see Schaffelke, 1999).

The present study examines the impact of sediment loads on growth and biomass accumulation of tropical reef macroalgae and whether these effects are due to changes in the controlling forces of herbivory or nutrients. Specifically, we addressed the following questions in two dominant fringing reef macroalgal species that differ in morphology and nutrient use: 1) Do sediment loads affect biomass accumulation and if so, is the effect consistently positive or negative? 2) Do sediments provide a source of nutrients? 3) Does the presence of sediment inhibit herbivory? We hypothesized that sediments would benefit both species of fleshy macroalgae, but that the positive effect may vary quantitatively among species, where those with more complex morphologies on which more sediments are trapped may have stronger positive effects.

3.3 Methods

3.3.1 *Study site and species*

Experiments were conducted from 2 May – 23 May 2012 on the island of Mo'orea, French Polynesia, on a fringing reef near the University of California Gump Biological Research Station (hereafter termed Gump Reef) in Cook's Bay (17 °32′S 149 °50′W). In Cook's Bay, sediment comes both from agricultural run-off from the island interior via a riverine watershed at the head of the bay, and from direct run-off of sediments from adjacent developed land and steep mountainsides (up to 900 m) (Adjeroud and Salvat, 1996). Sediment

plumes over the reef are frequent after large rain events (R. J. Clausing, P. Fong, pers. obs.), and the proportion of terrestrial sediments increases with proximity to the head of the bay (King et al., unpubl. data).

All experiments were conducted on *Padina boryana* and *Galaxaura fasciculata* because both genera have become dominant on inshore reefs globally (e.g. Beach et al., 2006; Fox and Bellwood, 2007, reviewed by Fong and Paul, 2011; Rasher et al., 2012, Fong and Fong in press.), and are found in areas with higher sediment loads, yet differ in aspects of their morphology and ecology. Both species are fleshy macroalgae, but *G. fasciculata* is a highly branched red alga with a robust calcified skeleton and is generally unpalatable. In contrast, *P. boryana* is a foliose brown alga with fan shaped blades with rings of light calcification that is readily consumed by fish herbivores (Mantyka and Bellwood, 2007, Fong and Fong in press). In a previous study on Gump Reef, *G. fasciculata* was found to respond directly to water column nutrient supplies and quickly become nutrient limited, while *P. boryana* was less limited and showed more variable response to nutrient addition (Clausing et al. in review; see also Kuffner and Paul, 2001). In addition, studies have demonstrated that species of *Galaxaura* have a low capacity for internal nutrient storage (Aisha et al., 1995) and are adapted to reduced light levels in the lab (Su et al., 2009), with corresponding positive correlation between field abundances and turbidity (Su et al., 2009), while *Padina* spp. often dominate sandy reef flats (Fox and Bellwood, 2007; Rasher et al., 2012) and increase in abundance with addition of particulate organic matter (McClanahan et al., 2005). In Mo'orea, fine sediment has been observed to adhere to fine hairs on the thallus surface in these species on the reef flat (Figure 3.1). Sediments retained on *G. fasciculata* at this site have lower % calcification compared to adjacent benthic sediments (78.1% vs 93.4%, respectively; Fong, unpubl. data), indicating that, to a large extent, the terrestrial sediments are being retained on the algal thalli rather than settling on the benthos. These species characteristics suggest that sediments may interact with herbivory and nutrient control of each species differently.

To characterize the environmental conditions prior to and during experiments, we obtained rainfall and solar radiation data at Gump Research Station with permission from

the Mo'orea Coral Reef Ecosystem LTER (Washburn and Brooks of Moorea Coral Reef LTER, 2012). Light and rain conditions prior to the experiment may have indirectly affected growth during the experiment by altering algal tissue nutrient content at the experimental onset. Rain may increase nutrient availability, and therefore we expect tissue nutrients to increase after rain except when extreme low light conditions limit uptake regardless of availability. We summed accumulated rainfall (mm) to give daily estimates and averaged solar radiation data (kilowatts $m^{-2}s^{-1}$ in the range of 300–1200 nm) over daylight hours (06:00–18:00) to give relative estimates of daytime irradiance. We calculated averages of both parameters during each experiment and during the week prior. Because light data is collected above water, and turbidity resulting from rainfall may further reduce irradiance at the surface of the reef, light and rainfall data must be considered together to evaluate the environmental context on the reef. In order to characterize initial tissue N and P content prior to each experiment, we saved subsamples of algal thalli collected for each experiment to process for nutrient content (methods below).

In order to measure the sediment load on the thalli of each species as well as the nature of the sediment (grain size, organic matter and nutrient content), we collected sediment samples from individual thalli of *G. fasciculata* and *P. boryana* using Ziploc bags to capture all sediment on the surface. We cleaned sediment off each thallus individually using seawater and decanted and dried the resulting slurry at 60 °C. We rinsed cleaned algae in fresh water and dried it at 60 °C to calculate gram dry weight (DW) sediment $\cdot$ gram^{-1} algal DW (*G. fasciculata* $n = 2$; *P. boryana* $n = 1$). We collected additional samples of each species ($n = 3$) the following year (2013) to compare patterns (see Appendix Figure 3.B.1).

To characterize the sediments on each species, we analyzed subsamples of dried sediment for organic content, grain size, and nutrients (N, P) (Table 3.1; see caption for sample sizes). Organic content was determined by the change in weight of each sample after burning off organic material in a muffle furnace at 400 °C for 12 h. Grain size (where sample mass was sufficient – see Table 3.1 caption) was measured using the hydrometer method (Bouyoucos, 1962). Sediment nutrient analysis was performed at the UC Davis Analytical Laboratory (DANR). Total Nitrogen (TN) was determined by flash combustion in which

all organic and inorganic compounds are instantaneously oxidized to combustion gases (N_2, NO_x) and subsequently measured using a thermal conductivity detector. The quantity of bioavailable inorganic ortho-phosphate (PO_4-P) was determined using the Olsen method in which phosphate is extracted with sodium bicarbonate, reacting with other ions to produce a blue complex whose absorbance at 880 nm is proportional to the sample PO_4-P concentration (Olsen and Sommers, 1982).

3.3.2 *Experimental approaches*

To examine the consequences of thallus sediment loads for biomass accumulation of two dominant species of macroalgae on the reef flat and identify possible underlying mechanisms, we conducted a series of field and lab experiments. We assessed 1) the net effect of sediments on biomass accumulation; 2) how effects of sediments compare to those of nutrient addition on algal growth, and if sediments modify nutrient-stimulated algal growth; 3) how sediments alter the effects of herbivory on algal biomass accumulation. Because we focused on the effects of naturally occurring (ambient) sediment loads on algal thalli at the experimental reef, we used the ambient reef condition (defined here as +sediment, -nutrients, +herbivores) as the reference in all experiments.

3.3.2.1 Sediment net effect field experiment

To examine the net effect of natural accumulation of sediment on algal thalli (hereafter referred to as sediment load) on change in biomass of *P. boryana* and *G. fasciculata*, we conducted an in situ sediment removal experiment. Treatments were ambient sediment loads (Amb.) and sediment removal (-Sed). We collected algae from one location on Gump reef to ensure replicate thalli had been exposed to the same nutrient history in the field, thereby minimizing differences in tissue nutrient stores. We cleaned algae of sediment and epibiota, spun it for one minute in a salad spinner to constant wet weight (to standardize removal of excess water after Fong et al., 2003), and recorded weights of individual thalli. Initial weights varied (20-30 g *G. fasciculata*, 11-17 g *P. boryana*) between replicates to maintain

intact thalli. In all collections for all experiments, we used entire thalli with little fouling and retained apical growing tips. We attached thalli to 10 cm x 10 cm metal mesh using cable ties and randomly assigned a treatment ($n = 15$). Five additional cleaned thalli of both species were rinsed in freshwater and dried at 60 °C in a forced air oven to serve as initials for tissue N and P content (all subsequent tissue samples treated equivalently).

We secured experimental replicates haphazardly to an 8 m x 8 m area of substrate (primarily hard bottom with a layer of fine sediment) with 10 cm masonry nails. We allowed natural sediment to accumulate on algal thalli in ambient treatments and manually removed accumulated sediment in removal treatments by disturbing the water above thalli or gently brushing thalli each day. *G. fasciculata* was deployed from 2 May until 9 May; *P. boryana* from 4 May until 11 May. After 7 days, we collected replicates, removed the sediment, and re-spun and weighed thalli. Five random replicates of each treatment were retained for subsequent tissue nutrient analysis (analytical methods below).

We analyzed differences in percent change in biomass between treatments with a bootstrapped t-test because data marginally met assumptions of normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) (Manly, 1997).

3.3.2.2 Sediment x nutrient laboratory experiment

To examine the effects and interactions of sediment and nutrients on growth of *G. fasciculata* and *P. boryana*, we performed two-factor laboratory dose-response experiments. The design was factorial: with and without nutrient addition and sediments. Thus treatments included ambient (Amb.: addition of sediment alone), no sediment (-Sed: no sediments added), nutrient addition (+Nut, sediments and nutrients added), and manipulation of both relative to ambient field conditions (-S+N). We applied each treatment combination to eight replicate algal samples, giving 64 total experimental units. The experiment ran for six days from 7 May – 14May.

On 6 and 7 May we collected and prepared *G. fasciculata* and *P. boryana*, respectively, while retaining, sieving and decanting thallus sediment loads (other preparation as described

previously). We trimmed *G. fasciculata* thalli to 8 g subsamples to standardize weight as a way of minimizing differences in surface area, which is the basis for nutrient uptake. To avoid shredding the more delicate *P. boryana*, we retained entire thalli, allowing initial weights to vary between 8.0 g–9.3 g. We retained five additional samples of each species for initial tissue nutrient content.

Immediately prior to the experiment, we collected water from the reef to serve as ambient treatment water. For the enriched treatment, we added inorganic N (as sodium nitrate, $NaNO_3$) and inorganic P (as sodium dihydrogen phosphate, NaH_2PO_4) to increase N and P by 17μM and 1.7μM, respectively. The 10:1 ratio of N:P prevents secondary limitation in one nutrient due to the abundance of the other. We mixed a sediment slurry from ambient treatment water and sediment collected from *G. fasciculata* the previous day. Each experimental unit received 700 mL of treatment water and ambient treatments also received 15 mL of sediment slurry, a volume that trials showed to approximate sediment loads observed in the field. Upon sediment addition, we agitated each unit to allow sediment to settle naturally on the algal thalli as occurs during turbulent water conditions or settling of terrestrial run-off. We placed experimental units haphazardly in a flow-through water bath 10 cm deep to maintain constant temperature. To simulate water movement and shifting sediment loads as may naturally occur in the field, we re-suspended the sediment after 2 days by agitating each unit for 5 s.

To determine the impacts of sediment load on algae grown, after six days, we cleaned, spun, and reweighed algae. We estimated growth by percent change in wet biomass. While these experiments do not simulate natural nutrient supply and flow rates, nor competition among species, and thus are not predictors of how algae may grow in the field, results can be compared among treatments as all units were treated identically. Five of the eight algal replicates for each treatment group were saved for analysis of nutrient content, as above. We analyzed the change in biomass among treatments using a two-way factorial ANOVA. Data were not transformed as assumptions of normality and homogeneity of variance were met when checked with Shapiro-Wilk and Levene's tests.

3.3.2.3 Interacting sediment and herbivory field experiment

To isolate the consequences of sediments for herbivore and nutrient control of *P. boryana* and *G. fasciculata*, we conducted a factorial field experiment examining change in biomass and tissue N and P content under manipulated herbivory and sediment conditions (two levels each: ambient vs. reduced). Thus, treatments included ambient (Amb.), reduced sediment (-Sed), reduced herbivory (-Herb), and reduced sediment and herbivory (-S-H). This experiment ran for 6d from 16 – 23 May on both *G. fasciculata* and *P. boryana* on Gump reef at the collection site.

We collected algae from Gump reef, cleaned and spun it as before, and trimmed it into 5 g (*P. boryana*) and 8 g (*G. fasciculata*) subsamples. Equal weights within species roughly standardized the surface area for uptake among replicates, while weight differences between algal species standardized volume to avoid differences in the potential attractiveness to herbivores. We retained and processed five additional cleaned thalli of each species as before for initial tissue nutrient content.

We cable-tied random algal subsamples to the bottom of 1 cm metal mesh cylindrical cages (D x H of 10 cm x 15 cm) for reduced herbivory treatments and to 10 cm x 10 cm squares of 1 cm metal mesh for replicates open to herbivory. We attached replicates to ropes in a random array. The ropes secured experimental units loosely to the reef benthos while allowing cages or squares to be individually agitated as needed for sediment removal. Sediment was allowed to accumulate naturally on Amb and -Herb replicates and removed on -Sed and -S-H replicates daily as described above. On the sixth day, we collected experimental units and cleaned, re-spun, and wet weighed thalli. We kept all forty thalli of each species for tissue nutrient analysis. We analyzed growth as percent change in wet biomass using a two-way ANOVA for each species as data met assumptions (Shapiro-Wilk and Levene's Tests).

3.3.3 *Tissue nutrients*

To determine the effects of sediments on tissue nutrient content, we analyzed all tissue samples from the field and laboratory experiments for N and P content at the UC Davis Analytical Laboratory (DANR). Total N was analyzed using the combustion method in which organic and inorganic N is converted to N_2 or NO_x gases via oxidation by flash combustion and subsequently measured using thermal conductivity and IR detection. Total P was analyzed using nitric acid digestion in a closed vessel and determination by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Sah and Miller, 1992).

In each experiment, we calculated change in tissue nutrients of algal thalli as percent change in N and P mass between the onset and end of each experiment. Tissue concentrations alone are confounded by growth, which can dilute nutrient concentration even when uptake is occurring (see Appendix 3.A for calculations). We analyzed percent change in nutrient mass using the same model used for each experiment (bootstrapped two-sample comparison for the net effect experiment, and two-factor ANOVAs for the laboratory and factorial field experiment). All data met assumptions of normality and homogeneity of variance where necessary (ANOVA models) and thus were not transformed.

3.3.4 *Calcification versus nutrient content of P. boryana*

To assess partitioning of calcification and nutrient storage with age in the thalli of *P. boryana*, we collected algal thalli ($n = 9$) in April 2013 from Gump reef, and cleaned, rinsed, and dried thalli at 60 °C. Prior to drying, we partitioned thalli into three parts along concentric rings of calcification at 2 cm intervals to ensure division by age within and between thalli. The base of the thallus is the oldest tissue and the apical part, where the meristem is located, is the newest. The apical area is typically thinner, less tough and degraded, and we hypothesized increasing calcification and decreasing relative nutrient content with age (toward the base). After removing roughly 0.01 g of each sample for nutrient analysis, we weighed and treated the remaining sample with dilute hydrochloric acid (0.1 M) to dissolve calcium carbonate, rinsed it in deionized water, and dried it at 60 °C overnight. We calculated

percent calcification by the change in weight compared to the initial weight. Tissue N content was analyzed at the UC Davis Stable Isotope Facility (SIF) using combustion. We analyzed both calcification and nutrient data with mixed effects models using the function lme in the add-on package nlme in *R* (Pinheiro et al., 2012). The model included section of the thallus (outer, middle or inner) as a fixed factor and replicate thalli as a random effect. We performed all data analyses in *R* (R Core Team, 2012).

3.4 Results

3.4.1 *Characteristics of the study system and species*

Conditions on the reef prior to and during experimentation varied among the three experiments, but the most prominent event was a large, weeklong storm (23–30 April; 198 mm rain) just prior to the onset of any experimentation (Figure 3.2). Net effect experiments began one (*G. fasciculata*) or three (*P. boryana*) days after the storms conclusion. Thus algae on Gump reef had been subjected to reduced light levels during the storm (0.23 average daytime $kw{\cdot}m^{-2}s^{-1}$), although solar radiation was back at high levels (0.44 and 0.42 average daytime $kw{\cdot}m^{-2}s^{-1}$, respectively) through the net effect experiments and remained relatively high with minor fluctuations for the remainder of the experimental duration (ranging 0.42–0.45 $kw{\cdot}m^{-2}s^{-1}$ average daytime solar radiance through 23 May; Figure 3.2). The laboratory experiment examining nutrient and sediment effects was conducted after one week of completely dry conditions post-storm and the final field experiment begun after 16 days with no rain (Figure 3.2). Thus there would have been little potential for fresh terrestrial sedimentation on Gump reef as well as for nutrient inputs associated with runoff. Tissue nutrients in thalli of both *P. boryana* and *G. fasciculata* collected directly prior to each experiment confirmed decreasing nutrient stores in algal tissue with time from the storm (Figure 3.2C–H), which implies nutrient supplies were reduced. For both *P. boryana* and *G. fasciculata*, tissue N concentration was significantly higher prior to the first field experiment conducted after the storm than before the second field experiment two weeks later (Figure 3.21C–D). There was a trend of decreasing %P in *P. boryana*, but no trend was evident

for *G. fasciculata* (Figure 3.2E–F) or either species N:P ratios (Figure 3.2G–H).

Sediment loads on thalli of *G. fasciculata* and *P. boryana* were similar (0.24 and 0.25 g DW sediment g^{-1} DW algae, respectively; $n = 1$) and patterns remained in samples collected the following year (0.27 $\pm$ 0.03 and 0.29 $\pm$ 0.03 g sediment g^{-1} algae $\pm$ SE, respectively; $n = 3$). Organic content appeared to be higher in sediments collected from thalli of *G. fasciculata* compared to those from *P. boryana*, but small sample size precluded statistical analysis (Table 3.1). Sediments on the thalli of *G. fasciculata* were roughly half sand, with the remainder split between silt and clay (Table 3.1). Samples collected from sediment loads on *P. boryana* thalli were not sufficient for grain size analysis. Total N and P appeared higher in sediments collected from *G. fasciculata* than those from *P. boryana*, although only one sample was taken (Table 3.1). Sediment samples from the following year ($n = 3$) suggest marginal differences, although more striking is the lower nutrient content in all samples compared to 2012 (Appendix Figure 3.B.1).

3.4.2 *Experimental results*

3.4.2.1 Net effect field experiment

The ambient accumulation of sediment significantly decreased accumulation of biomass in *P. boryana* (bootstrapped $p = 0.009$). Thalli where sediment was removed daily grew $\sim$50% more than thalli accumulating natural sediment loads (Figure 3.3). In contrast, ambient sediment loads on thalli of *G. fasciculata* had a significant positive effect on biomass accumulation (bootstrapped $p = 0.014$), as *G. fasciculata* only gained biomass in the presence of sediment (Figure 3.3).

Sediments also had a negative effect on tissue nutrient content of *P. boryana* (Appendix Table 3.B.1). The total mass of N contained in the thallus showed a significant gain when ambient sediments were removed compared to a loss with ambient sediment accumulation (Figure 3.3; bootstrapped $p < 0.001$). Tissue P mass also increased with removal of sediments compared to sedimented thalli (Figure 3.3, bootstrapped $p = 0.02$). Tissue nutrient content (mass of both N and P) of *G. fasciculata*, however, was variable across

treatments with no significant trend (Appendix Figure 3.B.2). Nutrient percentages of algal tissue are reported in Appendix Table 3.B.1.

3.4.2.2 Sediment x nutrient laboratory experiment

P. boryana grew significantly more when sediments were removed (Table 3.2, Figure 3.4). Growth increased by ∼50% when sediments were removed and nutrients were ambient (Amb vs. -Sed) but only 10% when nutrient levels were enriched (+Nut compared to -S+N). Although there appeared to be a positive effect of nutrients on growth of *P. boryana*, this effect was not significant. Further, tissue N and P content showed little pattern (Appendix Table 3.B.2 and Appendix Figure 3.B.3A,C).

In contrast, *G. fasciculata* showed a significant positive interaction of sediment and nutrients on growth (Table 3.2), where the absence of sediments decreased growth by more than half, but only in the ambient nutrient condition (Figure 3.4). In contrast, when nutrients were added in the absence of sediments (-S+N), growth occurred at a rate similar to that of the ambient condition. Thus, this interaction suggests that sediments provide a similar benefit as added nutrients in low nutrient conditions, but may have little effect on growth when nutrients are replete (ambient compared to -S+N). Growth was lowest in treatments with no sediment or added nutrients (-Sed), and intermediate when nutrients were added alone (+Nut). Despite effects on biomass, no trends were observed in either tissue N or P changes (Appendix Table 3.B.2 and Figure 3.B.3B,D). Nutrient percentages of algal tissue are reported in Appendix Table 3.B.3.

3.4.2.3 Interacting top-down and bottom-up field experiment

Protection from herbivory had a significant positive effect on biomass accumulation of *P. boryana* (Table 3.3, Figure 3.5). Caged thalli increased roughly an order of magnitude compared to uncaged units where herbivory resulted in negligible biomass accumulation. In contrast to the previous field and laboratory experiment, sediment had no effect on *P. boryana*, regardless of access to herbivores. The difference in effect of sediments from the net

effect experiment may relate to the different environmental conditions on the reef prior to experimentation (as evidenced by substantially reduced tissue nutrient stores in Figure 3.2). Examination of changes in tissue N mass, however, revealed a significant interaction between sediment and herbivory where sediment loads increased tissue N content in the absence of herbivores but decreased N content when herbivores were present (Table 3.3, Figure 3.5). When herbivores were present, sedimented thalli (ambient) contained less than 30% of the tissue N than those without, suggesting the herbivores were mediating the accumulation of N in the tissue. For tissue P, sediment removal in the presence of herbivores appeared to have no effect, while sediment removal without herbivores appeared to decrease tissue P stores, resulting in a marginally significant trend for an interaction (Figure 3.5). Herbivores alone had no effect on P mass.

G. fasciculata either lost or maintained initial biomass (Figure 3.5), likely related to initially lower tissue N concentrations for this experiment resulting in strong nutrient limitation and respiration exceeding photosynthesis (Figure 3.2). While *G. fasciculata* appeared to have less loss of biomass with sediments, this difference was only marginally significant (Table 3.3). Herbivory had no main or interactive effects, as there was no measurable herbivory on *G. fasciculata*. Changes in tissue N and P mass did not follow the patterns in biomass changes (Figure 3.5E,F). Herbivores appeared to have a negative effect on tissue N mass, increasing loss of N by >50% compared to either caged treatment (Figure 3.5E), though this effect was only marginally significant (Table 3.3B). Herbivores caused negligible gain or loss of P mass (Figure 3.5F), while treatments without herbivory were able to maintain or gain P. In contrast to N, sediments had a marginal positive effect on P (Table 3.3B). Actual tissue nutrient percentages are reported in Appendix Table 3.B.4.

3.4.3 *Calcification and nutrient content of P. boryana*

Calcification in *P. boryana* was significantly higher in the older, inner portion of the thallus with roughly 10% greater calcification by weight than in either the mid (linear mixed effects model, $p = 0.001$) and outer sections ($p = 0.002$) of the thallus (Figure 3.6,

Table 3.4A). Nutrient content showed the opposite pattern, with significantly greater %N by weight in the outer portion of the thallus where the outer section had 30% more N than the mid and 25% more than the inner thallus sections (linear mixed effects model, $p < 0.001$) (Figure 3.6B; Table 3.4B).

3.5 Discussion

The net effect of sediments on biomass accumulation of two dominant coral reef macroalgal species were neither universal in strength nor direction; rather they varied among species and with environmental conditions. Despite gross morphological similarities and co-dominance in back reef flat habitats, sediments consistently benefitted *G. fasciculata*, while effects on *P. boryana* were generally negative. Contrasting effects of sediment may result from smaller-scale morphological and physiological differences between species that result in unique properties of each species sediment load and their response to it. Although particulate matter covers the thallus of both species, and the fan-shape of *P. boryana* facilitates sediment settlement (Schaffelke, 1999), *G. fasciculata* particularly retains small particles in its dense filamentous hairs (up to 4mm in length) to the extent of appearing as an amorphous brown mass (R. Clausing, pers. obs.). Retention of smaller particles is the likely explanation for higher nutrient and organic content of sediment loads on *G. fasciculata* than on *P. boryana*, although more samples are needed to confirm this hypothesis. Schaffelke (1999) also found that, in five species, a species of *Padina* had the heaviest sediment load ($\sim$0.284 g DW g^{-1} DW algae compared to $\sim$0.29 g on Gump Reef) but lowest sediment nutrient content (0.34% N and 0.05% P). Moreover, sediments on a species of *Sargassum* that doubled growth rates with intact thallus sediments contained $\sim$100% more nutrients (0.72%N and 0.1%P; Schaffelke, 1999). Sustained rapid growth of sedimented *P. boryana* across all our experiments, contrasted with the dependence of *G. fasciculata* on sediment for growth in the field, suggest different mechanisms of persistence under increasing sediment deposition. Abundance of *P. boryana* is likely due to either overall tolerance to sediments or indirect benefits in spite of the costs to growth and tissue nutrients, whereas *G. fasciculata* appears to

have adaptations to benefit from sediments. Combined with evidence of strong herbivory on *P. boryana*, which may be restricted to refuge areas with low grazing pressure (Mantyka and Bellwood, 2007), and low/no herbivory on *G. fasciculata* (see also Mantyka and Bellwood, 2007), our results suggest that similarly tolerant, yet palatable species such as *P. boryana* may increase in abundance on sedimented reefs if herbivory is reduced. In contrast, less palatable species adapted to benefit from sediments such as *G. fasciculata* may become more dominant if herbivory is strong. Overall, these results imply that sediment may shape algal community structure on impacted reefs, and future work should focus on uncovering the underlying causes.

Environmental conditions such as the history of rainfall and light may drive the nature of the effects of sediments, particularly if these conditions affect sediment load or organic content or algal species nutrient supply. In both species, the effects of sediments on growth in the field were qualitatively or quantitatively different directly after a weeklong storm compared to 16 completely dry days later. Negative effects of sediments on *P. boryana* were no longer detectable after two dry weeks. *G. fasciculata* consistently benefitted from sediment loads, but only maintained positive growth after the storm when its tissue N stores were substantially higher, indicating it required sediments under particular conditions to thrive. However, it is unknown whether the source of nutrients for both species came from the water column or from fresh terrestrial sediment deposition (see also Begin et al., 2013 on Caribbean reefs) via the sediment plume over the reef after the storm (R. Clausing, pers. obs.). Particularly on developed coasts, storm plumes commonly deposit organic-rich sediments on the reef (Nemeth and Nowlis, 2001; Fabricius et al., 2006). A recent study on Gump Reef found that temporal patterns of nutrient limitation in *G. fasciculata* and *P. boryana* were strongly linked to rainfall patterns, with nutrient limitation developing during dry conditions, particularly rapidly in *G. fasciculata* (Clausing and Fong, in review). One explanation may be that *P. boryana* is only inhibited by sediment at a certain critical load or past a threshold of organic content. Organic content of sediment is linked to the sediment source (Weber et al., 2006; Begin et al., 2013) and strongly influences the effect sediments have on reef assemblages (Weber et al., 2006). High organic content (>5% weight

after combustion or ∼1.5% organic carbon) has been shown to increase mortality of tissues beneath it by promoting low oxygen conditions, the production of H_2S, and microbial activity (Weber et al., 2006, 2012). Yet, in our study, species thrived under sediments containing nearly 8% organic content. Moreover, identical and simultaneous field experiments conducted on a fringing reef in a neighboring Mo'orean bay with comparatively reduced terrestrial influences (organic content in sediment: 6.0% ± 0.05) found no effect (positive or negative) of sediments on growth of *G. fasciculata* (R. Clausing, unpubl. data). This suggests that *G. fasciculata* may be dependent on external nutrient inputs onto the reef to maintain positive growth, and, while sediments positively affect growth, the extent of its benefits may depend on other environmental conditions. Thus, the effects of sediments may not only be species-specific, but also temporally dependent upon the environmental conditions, particularly those associated with sediment inputs. More research is needed to understand how these effects may change temporally, particularly with regard to changes in sediment load or depth and sediment composition.

Our results did not support earlier work demonstrating that sediments inhibited herbivory. One explanation may be that previous studies were conducted primarily on algal turf, which can form a dense matrix with trapped sediments (e.g. Bellwood and Fulton, 2008; Goatley and Bellwood, 2012). This maxrix may render algal tissue more difficult for herbivores to access than macroalgae alone. Another possible cause could be that inhibitory effects of sediment on herbivory require threshold depths or load of sediment or organic content. For example, 7-18 mm of sediment substantially reduced fish herbivory on algal turfs in one study on the Great Barrier Reef (Bellwood and Fulton, 2008, see also Goatley and Bellwood, 2012, 2013), but a recent study in the South Pacific showed that 4 mm sediment only inhibited fish herbivory on algal turfs if low oxygen conditions developed (Clausing et al., in review). McClanahan et al. 2005 found that small fish herbivores were inhibited by organic particulate matter but not by inorganic, and thus the presence of organic matter indirectly allowed proliferation of fleshy macroalgae which were otherwise removed by grazers. Altogether, our results combined with the findings of those previous suggest that the effects of sediments may vary depending on environmental context, depth, and species.

Rather than inhibiting herbivory, sediments affected nutrient retention capability and this resulted in targeted herbivory on enriched thallus portions. When herbivores were present, sediments appeared to reduce tissue nutrient content in *P. boryana*. However, manipulation of sediment and herbivore presence in a factorial design revealed that in the absence of herbivores, sediments resulted in increased tissue nutrients (only marginally so for *G. fasciculata*), indicating increased nutrient availability. Thus, intact herbivore populations over-compensated for increased tissue nutrients. No difference in the amount of herbivory was observed, however, suggesting differences may have been caused by preferential consumption of nutrient-rich tissues. Although not causative, patterns of decreased calcification and increased nutrient content in younger tissues of the thallus of *P. boryana* provide supporting evidence for this hypothesis. Thus, this partitioning of nutrients and $CaCO_3$, and its effects on fish consumption rates, may account for the net negative relationship between sediment and tissue nutrients when herbivores were present. Targeted consumption of young meristematic tissue is likely to have extensive long-term negative effects on abundance of *P. boryana*, particularly if herbivory rates increase.

We were not able to determine the mechanism/nature of the positive sediment effect on *G. fasciculata*, but one possible explanation is that sediments provided nutrient subsidies. Increases in tissue nutrient content with sediments in both species when herbivores were reduced provide provisional support for this hypothesis. Furthermore, sediments caused an equivalent boost in growth of *G. fasciculata* as the addition of nutrients in the lab experiment, suggesting sediment nutrient provision. Differences in suggested nutrient benefits between species are not surprising, as nutrient benefits have been shown to depend on each species ability to utilize sediment nutrients (e.g. Larned, 1998), and are expected to be more pronounced in species with lower uptake and storage capacities of water column nutrients. Previous findings that *Galaxaura* lacks the capacity for surge uptake in response to pulsed nutrient supplies and has low capacity for tissue nutrient storage (Aisha et al., 1995), combined with evidence that *G. fasciculata* was more frequently and severely nutrient limited than *P. boryana* in the absence of sediments (Clausing and Fong in review) suggests that sediment nutrients could be an important mechanism increasing abundance of *G. fasciculata*.

However, unlike the field experiments, tissue nutrient content in the mesocosm experiment was variable and did not indicate a sediment nutrient benefit. It is possible that though capable of utilizing sediment nutrients, algae prefer to use water column nutrients when available, or are more effective at obtaining sediment nutrients when low water column nutrients levels cause sediments to release nutrients, acting as a slow-release fertilizer (Stimson and Larned, 2000; Kamer et al., 2004). Gorgula and Connell (2004) found that although both water and sediment nutrient sources increased percent cover of a temperate turf, the effect was six times greater from water column nutrients than those of sediment. Overall, further tests are needed to determine if sediments confer a nutrient benefit to either species.

This research suggests that sediments will have substantial but varying effects on changes in abundance and composition of tropical reef macroalgal communities. No single mechanism was discovered for either species much less both. Instead, our results demonstrated that increasing sediment loads on reefs have the capacity to strongly alter processes controlling macroalgal community dynamics including, but not limited to, herbivory and nutrient availability. Moreover differential effects among species have the potential to lead to unexpected effects in impacted communities, particularly if the focus remains on nutrient and herbivore control of algae without considering sediment modulation of these processes. In light of recent studies demonstrating reduced coral reef resilience to phase shifts under multiple human stressors (e.g. Fung et al., 2011), it may be particularly important to manage sediment inputs onto reefs, as they may disrupt nutrient cycles and patterns of herbivory.

Figure 3.1: Ambient sediment loads on thalli of *Padina boryana* and *Galaxaura fasciculata* at Gump Reef in Cook's Bay, Mo'orea, French Polynesia.

Table 3.1: Characteristics of sediment collected from the thalli of each species, where $n = 1$ for all samples except *G. fasciculata* organic content and grain size ($n = 2$).

		P. boryana	***G. fasciculata***
Nutrient content	%N	0.25	0.32
	%P	0.033	0.048
	N:P	16.2	14.8
Organic content	mean	6.97	8.06
	SE	–	0.093
Grain size	Sand	–	0.532
	Silt	–	0.275
	Clay	–	0.193

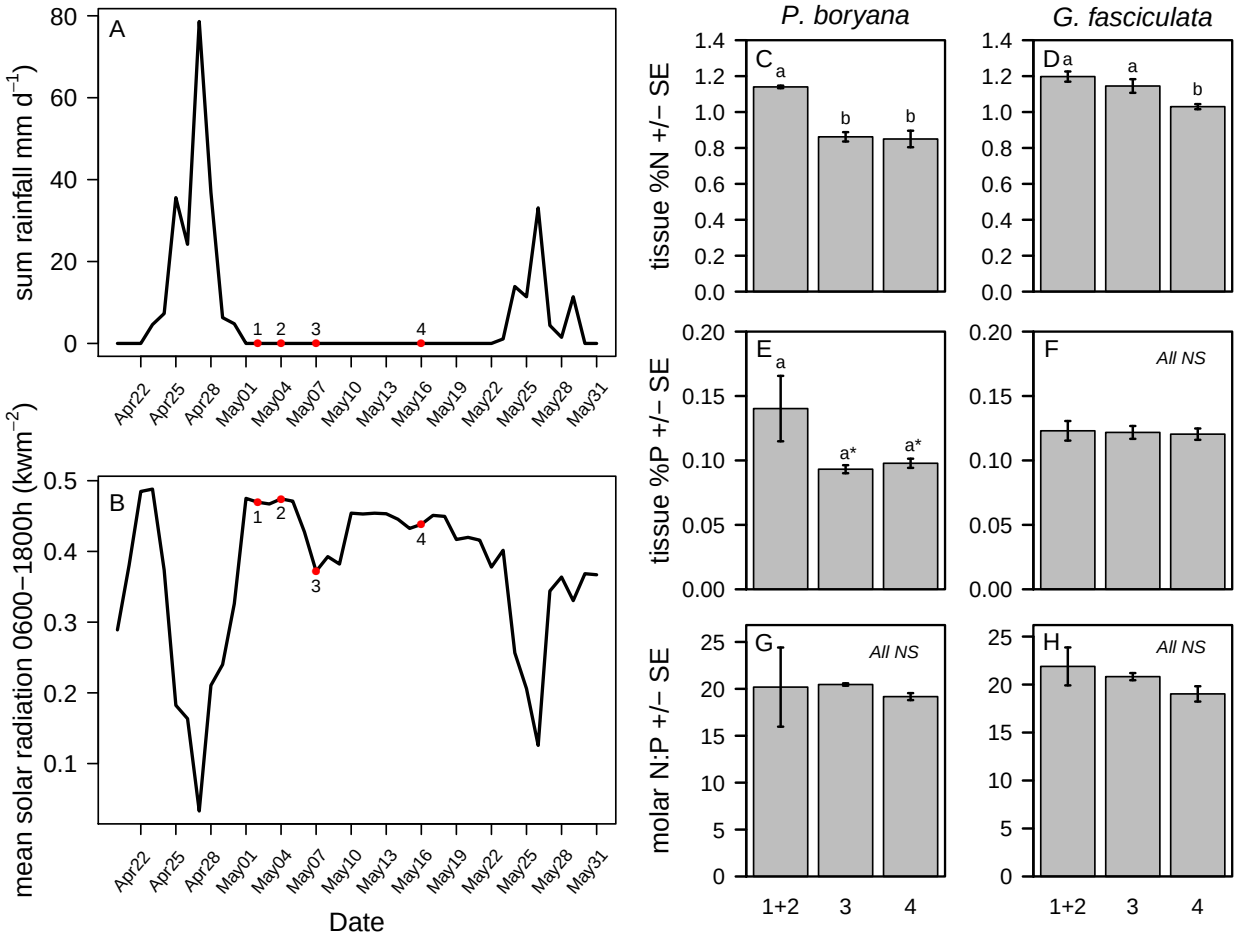

Figure 3.2: Environmental data describing the conditions in the field during the experimental period (A,B) and the resulting algal tissue nutrient status (C-G) prior to each experiment. (A) Daily rainfall and (B) average daytime solar irradiance and over the course of each experiment. Rainfall is summed over each 24 h period; Solar radiation is recorded at Gump Station as kilowatts·m^{-2}s^{-1}) and values are averaged from 06:00–18:00 h daily. Red dots indicate onset of each experiment (numbered as follows) with the average daily radiation in the week prior to experimentation: 1) Net effect field experiment on *G. fasciculata*, 0.23 kw·m^{-2}s^{-1}; 2) Net effect field experiment on *P. boryana*, 0.32 kw·m^{-2}s^{-1}; 3) Bottom-up laboratory experiment, 0.45 kw·m^{-2}s^{-1}; 4) Top-down bottom-up field experiment, 0.44 kw·m^{-2}s^{-1}. Each experiment ran for 6 days. (C-H) Tissue nutrient concentrations: %N (C,D); %P (E,F); N:P (G,H) in *P. boryana* and *G. fasciculata* at the onset of each experiment. Letters indicate differences using Tukeys HSD test, where different letters indicate significance at $p < 0.05$ and asterisks indicate differences at $p < 0.1$. Bars are means ± SE.

Table 3.2: **Sediment x Nutrient Mesocosm Experiment:** Two-way analyses of variance on % change in biomass in *P. boryana* and *G. fasciculata*, comparing the impact of sediment and nutrient addition on growth.

Source of variation	df	MS	F	p	
P. boryana					
Nutrient	1	68.22	2.719	0.11	
Sediment	1	192.56	7.676	0.010	**
Sediment:Nutrient	1	22.56	0.899	0.351	
Error	28	25.09			
Total	32				
G. fasciculata					
Sediment	1	107.9	1.966	0.172	
Nutrient	1	320.4	5.838	0.022	
Sediment:Nutrient	1	1026.6	18.708	<0.001	****
Error	28	54.9			
Total	32				

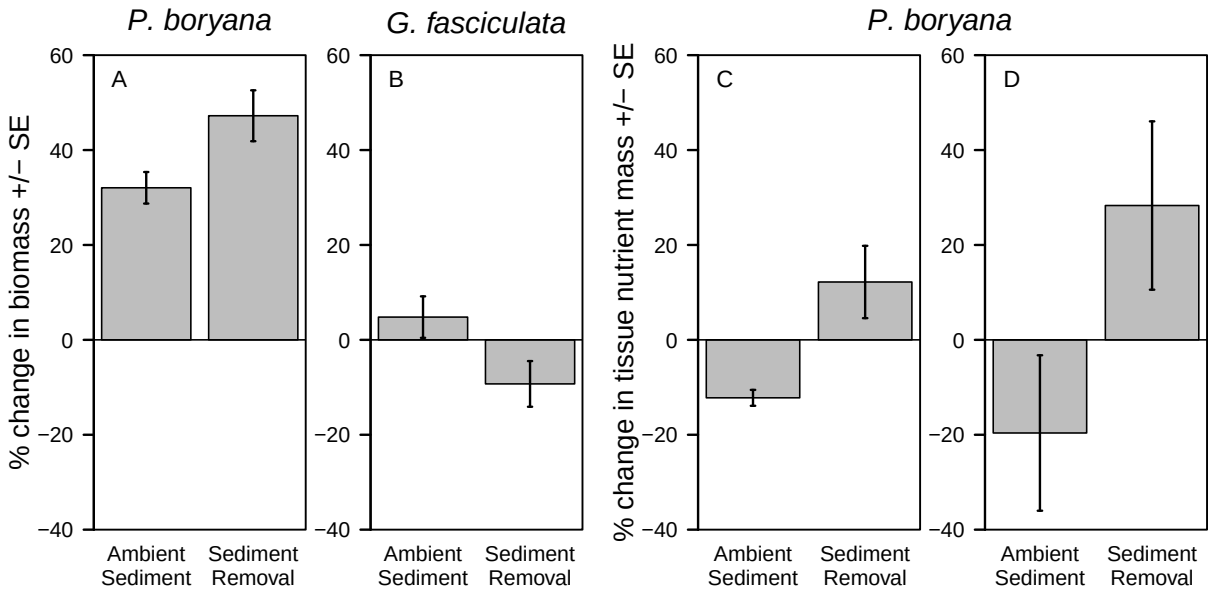

Figure 3.3: The net effect of ambient sediments vs. sediment removal on mean biomass accumulation over 7 days of A) *Padina boryana* and B) *Galaxaura fasciculata* and the tissue nutrient content of *P. boryana* only, where nutrients are C) nitrogen (N) mass and D) phosphorus (P) mass. All data were analyzed with bootstrapped two-sample comparisons and were significant at less than $p = 0.05$. Bars are means ± SE.

Table 3.3: Factorial Field Experiment: Two-way analyses of variance on % changes in biomass and % changes in tissue N and P mass in A) *P. boryana* and B) *G. fasciculata*, comparing the impact of sediment removal and herbivore removal on growth and tissue nutrient stores.

	Source of variation	df	MS	*F*	*p*	
A) Padina boryana	**biomass**					
	Sediment	1	31.2	0.115	0.737	
	Herbivores	1	2550	9.379	0.004	***
	Sediment:Herbivores	1	50.3	0.185	0.67	
	Error	33	271.9			
	N mass					
	Sediment	1	7.74	0.043	0.837	
	Herbivores	1	135.67	0.758	0.39	
	Sediment:Herbivores	1	1445.41	8.074	0.008	***
	Error	33	179.02			
	P mass					
	Sediment	1	919.08	3.67	0.064	*
	Herbivores	1	95.01	0.379	0.542	
	Sediment:Herbivores	1	874.3	3.491	0.071	*
	Error	33	250.45			
A) Galaxaura fasciculata	**biomass**					
	Sediment	1	600.6	4.014	0.053	*
	Herbivores	1	56.4	0.377	0.543	
	Sediment:Herbivores	1	30.6	0.205	0.654	
	Error	36	149.6			
	N mass					
	Sediment	1	81.08	0.419	0.522	
	Herbivores	1	772.74	3.993	0.053	*
	Sediment:Herbivores	1	229.57	1.186	0.283	
	Error	36	193.55			
	P mass					
	Sediment	1	621.12	3.9011	0.056	*
	Herbivores	1	998.7	6.2726	0.017	**
	Sediment:Herbivores	1	0.02	0.0001	0.991	
	Error	36	159.22			

Table 3.4: Mixed effects model on A) % calcification and B) % nitrogen (N) of *P. boryana* thallus sections of differing age.

A) % Calcification

Random effects	Model: ∼1|plot	
	(Intercept)	Residual
StdDev:	0.0002	2.658

Fixed effects	Model: % calcification ∼ thallus section					
	estimate	SE	df	t-value	p-value	
(Intercept)	69.856	1.002	14	69.728	< 0.0001	****
mid section	-5.603	1.377	14	-4.069	0.0011	***
outer section	-5.428	1.417	14	-3.831	0.0018	***

B) % Nitrogen

Random effects	Model: ∼1|plot	
	(Intercept)	Residual
StdDev:	0.037	0.075

Fixed effects	Model: % nitrogen ∼ thallus section					
	estimate	SE	df	t-value	p-value	
(Intercept)	0.827	0.030	14	27.839	< 0.0001	****
mid section	0.058	0.038	14	1.546	0.1445	
outer section	0.365	0.041	14	8.986	< 0.0001	****

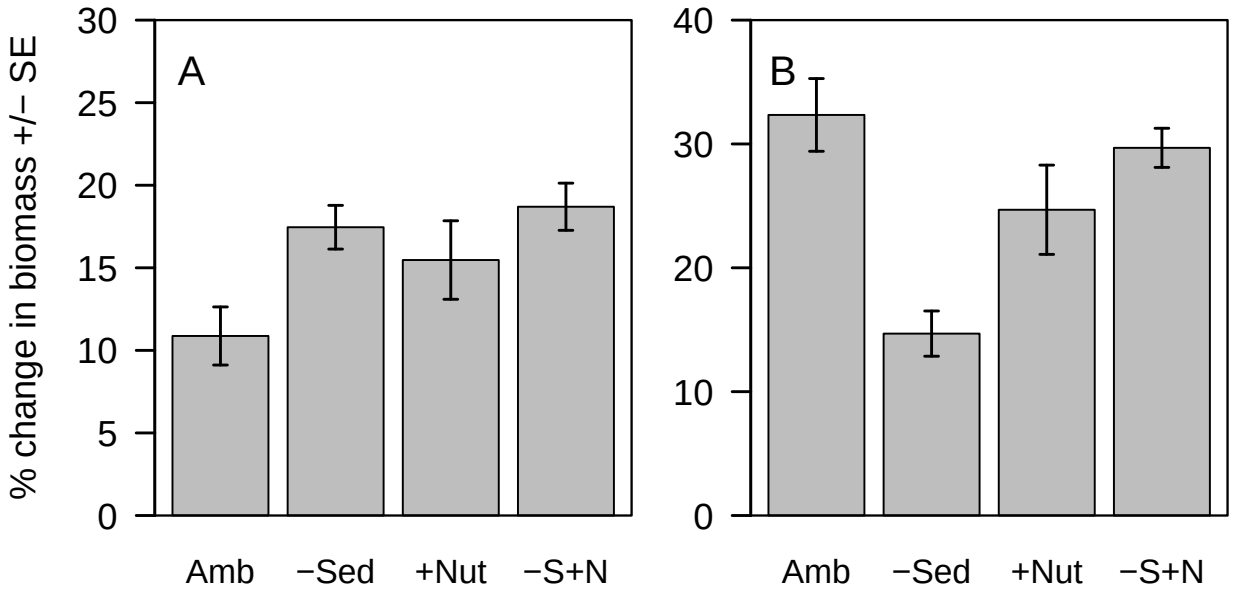

Figure 3.4: The individual and interacting effects of sedimentation and nutrient addition on growth of A) *Padina boryana* and B) *Galaxaura fasciculata* over 6 d in laboratory experimental units, where the ambient treatment (Amb) simulates conditions on the reef (natural sediment loads, ambient water), -Sed indicates the absence of sediment, +N represents nutrient addition (with ambient sediments), and -S+N indicates the absence of sediment with added nutrients, respectively. Bars are means ± SE.

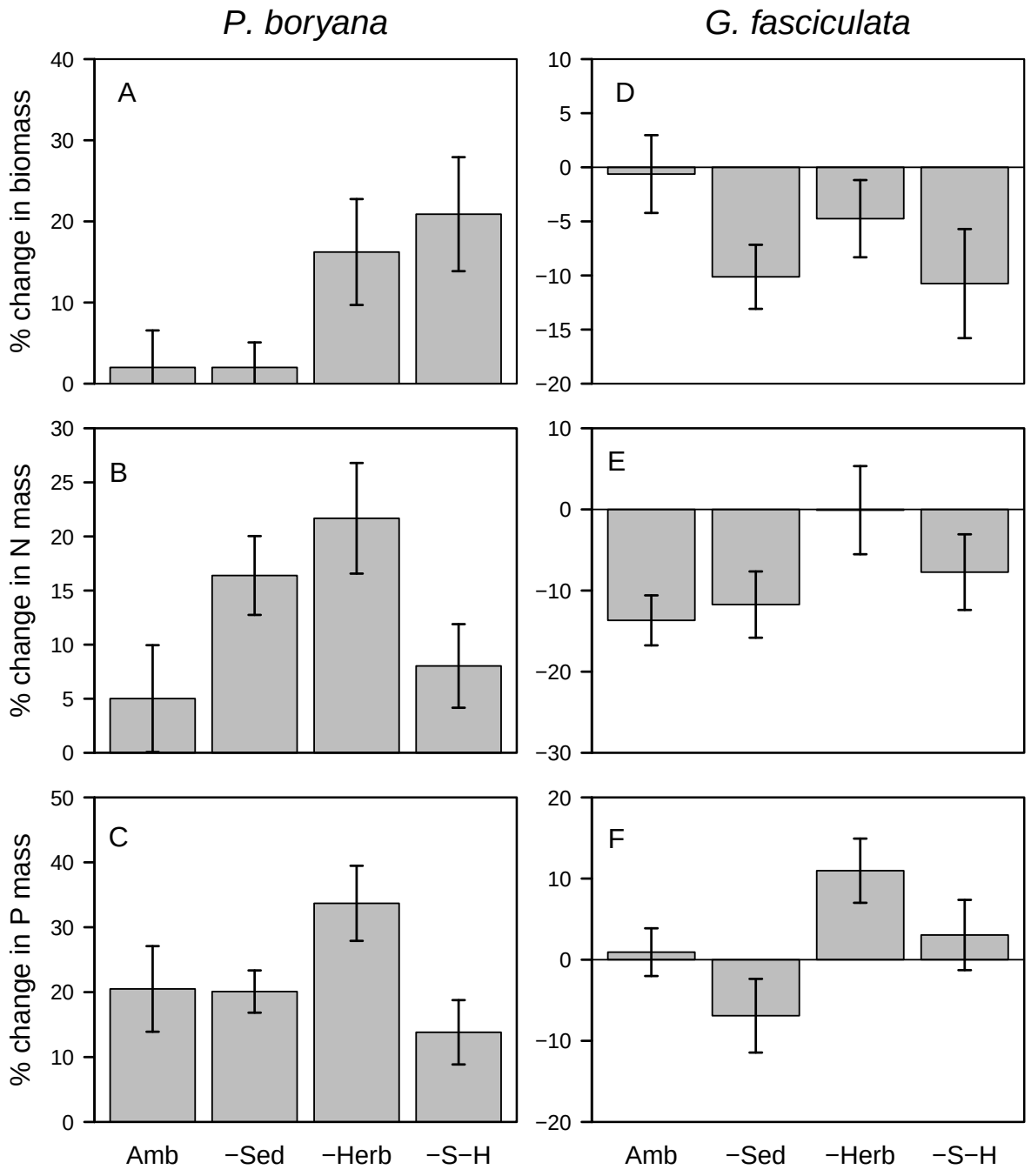

Figure 3.5: The individual and interacting effects of ambient sediments and herbivory on biomass accumulation and tissue nutrient stores of A,B,C) *P. boryana* and D,E,F) *G. fasciculata*. Tissue nutrients are % change in tissue nutrient mass of B) N and C) P on *P. boryana*, and E) N and F) P on *G. fasciculata*. All responses are % changes over a 6 d experimental duration where the ambient treatment (Amb) is unmanipulated to experience ambient reef conditions (natural sediment loads and herbivory), -Sed indicates the removal of accumulating sediment, -Herb represents thalli caged from herbivorous fish, and -S-H both removal of sediments and cages to prevent herbivory. Bars are means ± SE.

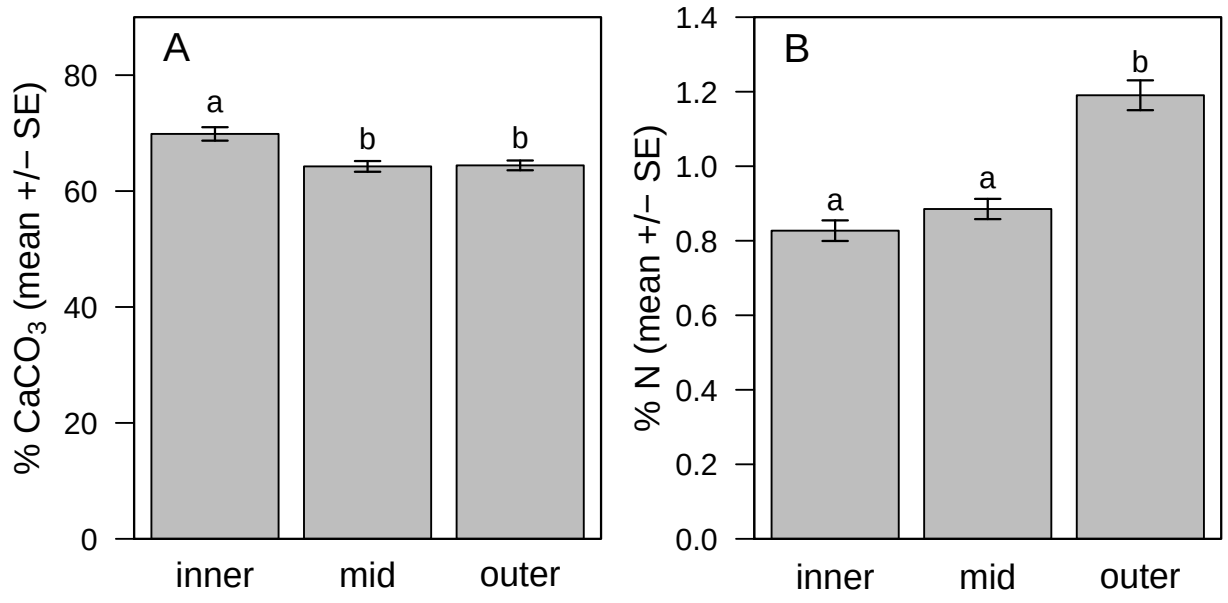

Figure 3.6: Percent calcification (A) and percent nitrogen (B) of thallus sections of *P. boryana*, where thalli were partitioned along concentric rings of calcification and the innermost section is the oldest tissue and the outer section is the newest growth.

Appendix

3.A Calculations of percent change in tissue nutrient mass

Initial mass of tissue nutrients for each experimental sample was calculated using the average % N and %P in the initial samples (replicate subsamples of the algae collected for each experiment) and an estimate of the initial dry weight of the experimental samples using the following series of equations:

$$change\ in\ N\ mass_i = 100 \times \frac{Final\ N\ mass_i - Initial\ N\ mass_i}{Initial\ N\ mass_i} \tag{3.1}$$

where

$$Final\ N\ mass_i = \%N_i \times Final\ dry\ weight_i \tag{3.2}$$

and

$$Initial\ N\ mass_i = [\frac{1}{k}\sum_{j=1}^{k}\ initial\ \%N_j\ ] \times Initial\ dry\ weight_i \tag{3.3}$$

where

$$Initial\ dry\ weight_i = Initial\ wet\ weight_i \times R_{Final} \tag{3.4}$$

where

$$R_{Final} = \frac{1}{n}\sum_{i=1}^{n}\frac{Final\ dry\ weight_i}{Final\ wet\ weight_i} \tag{3.5}$$

Here i is one of n final samples saved for tissue nutrient analysis, and j is one of k (typically 5) initial samples saved for tissue nutrient analysis prior to experimentation. Estimation of initial dry weights of samples was necessary to determine percent change in nutrient mass, as algae could not be dried and weighed before experimentation. Thus we used the average final dry to wet weight ratio to calculate the initial dry weight from the initial wet weight.

3.B Additional experimental results

Table 3.B.1: **Net Effect Field Experiment:** Tissue %N and %P in a random subset ($n = 5$) of the experimental specimens after the end of the experiment, where % change in % N/P is calculated as [100 × (Final %N/P − Initial %N/P) ÷ Initial %N/P]

Spp	Trt	Mean %N	SE	%chg %N	SE	Mean %P	SE	%chg %P	SE
P. boryana	Amb	0.93	0.06	-28.9	1.14	0.126	0.006	-33.1	10.4
	-Sed	0.81	0.01	-26.8	1.85	0.094	0.015	-18	4.84
G. fasciculata	Amb	1.05	0.04	-22.3	5.2	0.114	0.018	2.24	5.22
	-Sed	0.83	0.02	-12.5	3.04	0.115	0.007	-7.64	14.4

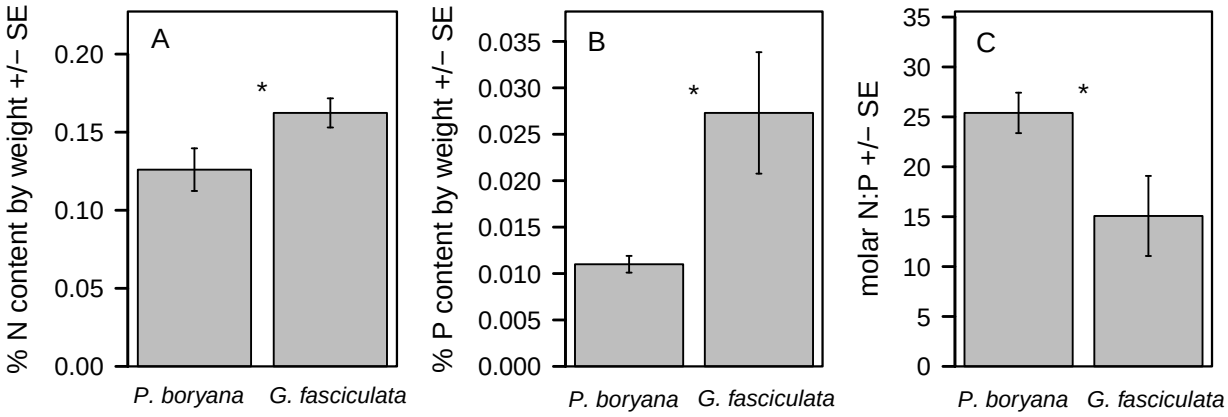

Figure 3.B.1: Nutrient content of sediments collected from thalli of *P. boryana* and *G. fasciculata* on the experimental reef in April 2013 ($n = 3$): A) nitrogen (N); B) phosphorus (P); C) ratio of molar N:P. Relative patterns of nutrient content (higher % in G. fasciculata than P. boryana) are similar to the previous year, but absolute values are substantially lower. Organic content of G. fasciculata (7.72 ± 1.36) was similar to 2012. Bars are means ± SE.

Table 3.B.2: **Sediment x Nutrient Mesocosm Experiment:** interacting effects of sediments and nutrient addition on tissue nutrient stores of *P. boryana* and *G. fasciculata* in a laboratory experiment.

		Source of variation	df	MS	*F*	*p*
Tissue N mass	***P. boryana***	Sediment	1	118.766	1.139	0.302
		Nutrient	1	0.45	0.004	0.949
		Sediment:Nutrient	1	1.287	0.012	0.913
		Error	15	104.244		
		Total	19			
	G. fasciculata	Sediment	1	35.31	0.068	0.798
		Nutrient	1	596.05	1.14	0.302
		Sediment:Nutrient	1	82.9	0.159	0.696
		Error	16	523.07		
		Total	20			
Tissue P mass	***P. boryana***	Sediment	1	67.78	1.385	0.258
		Nutrient	1	34.699	0.709	0.413
		Sediment:Nutrient	1	9.218	0.188	0.671
		Error	15	48.956		
		Total	19			
	G. fasciculata	Sediment	1	3.92	0.0067	0.936
		Nutrient	1	861.42	1.484	0.241
		Sediment:Nutrient	1	156.43	0.269	0.611
		Error	16	580.62		
		Total	20			

Table 3.B.3: **Sediment x Nutrient Mesocosm Experiment:** Tissue %N and %P in a random subset ($n = 5$) of the experimental specimens after the end of the experiment, where % change in % N/P is calculated as [100 × (Final %N/P − Initial %N/P) ÷ Initial %N/P]

Spp	Trt	Mean %N	SE	%chg %N	SE	Mean %P	SE	%chg %P	SE
P. boryana	Amb	0.9	0.05	4.4	6.2	0.085	0.003	-9.2	2.8
	-Sed	0.85	0.01	-1.6	1	0.081	0.002	-13.3	2.3
	+Nut	0.88	0.03	1.6	3.2	0.085	0.002	-8.8	2.2
	-S+N	0.87	0.02	0.93	2.5	0.084	0.002	-9.7	2.7
G. fasciculata	Amb	1.05	0.03	-8.5	2.7	0.12	0.004	-2.8	3.6
	-Sed	1.11	0.02	-2.7	1.4	0.12	0.005	1.8	3.9
	+Nut	1.1	0.04	-3.6	3.6	0.12	0.006	1.2	5.1
	-S+N	1.07	0.03	-6.2	2.4	0.12	0.003	-4.7	2.1

Table 3.B.4: **Sediment x Herbivore Field Experiment:** Mean tissue %N and %P of all specimens after the end of the experiment, where % change in % N/P is calculated as [100 × (Final %N/P − Initial %N/P) ÷ Initial %N/P]

Spp	Trt	Mean %N	SE	%chg %N	SE	Mean %P	SE	%chg %P	SE
P. boryana	Amb	0.78	0.03	-7.7	3.3	0.1	0.004	5.5	3.7
	-Sed	0.85	0.02	-0.47	1.7	0.1	0.001	2.7	1.4
	-Herb	0.81	0.03	-4.6	3.2	0.1	0.003	4.6	3.3
	-S-H	0.86	0.02	1.3	1.9	0.1	0.002	5.1	2.1
G. fasciculata	Amb	0.88	0.01	-14.5	1.4	0.12	0.003	0.33	2.5
	-Sed	1.02	0.03	-0.58	3.1	0.13	0.003	4.5	2.7
	-Herb	0.88	0.02	-14.2	2.1	0.12	0.002	-4.1	2
	-S-H	0.98	0.03	-4.7	3	0.13	0.003	6.6	2.3

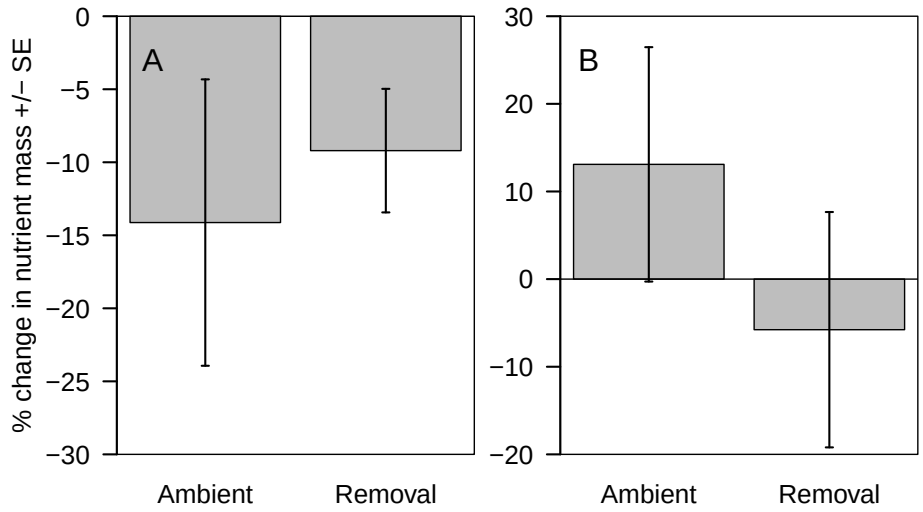

Figure 3.B.2: Change in tissue nutrient content of *G. fasciculata* after 6 d with ambient vs. removed thallus sediment loads (net effect experiment). A) % change in total mass of N; B) % change in total mass of P. Bars are means ± SE. Data were non-significant (bootstrapped $p = 0.29$ and 0.14 for N and P, respectively).

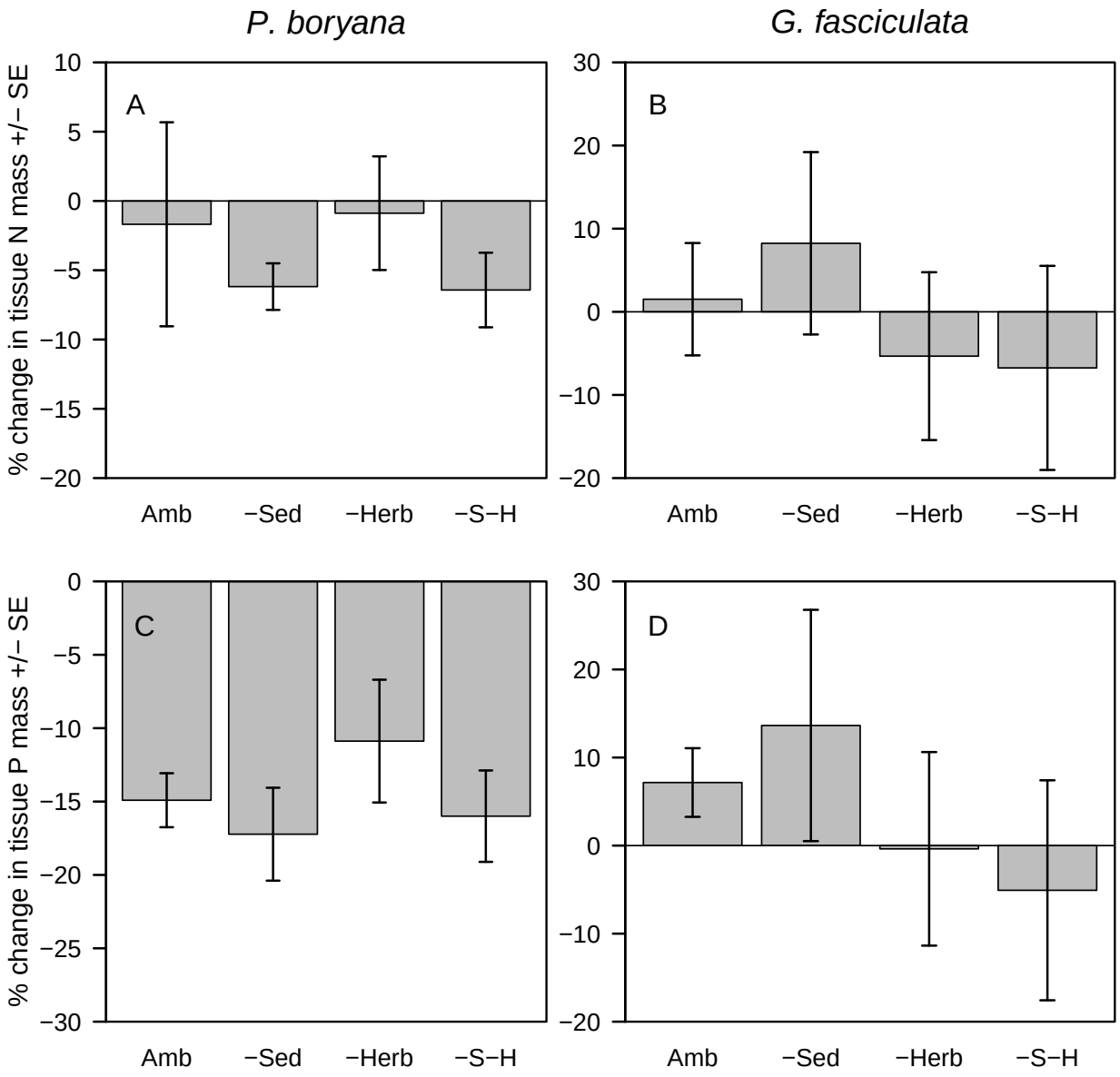

Figure 3.B.3: Change in tissue nutrient content of *P. boryana* and *G. fasciculata* with manipulation of sediments and water nutrient content, where A) and B) are N and C) and D) are P. The ambient treatment (Amb) simulates conditions on the reef (natural sediment loads, ambient water), -Sed indicates the absence of sediment, +N represents nutrient addition with ambient sediments, and -S+N indicates the absence of sediment with added nutrients, respectively. Bars are mean % change in nutrient mass ± SE.

References

Adjeroud, M., and B. Salvat. 1996. Spatial patterns in biodiversity of a fringing reef community along Opunohu Bay, Mo'orea, French Polynesia. Bulletin of Marine Science **59**:175–187.

Airoldi, L., 2003. The effects of sedimentation on rocky coast assemblages. Pages 161–236 *in* R. N. Gibson and R. J. A. Atkinson, editors. Oceanography and Marine Biology: an Annual Review, volume 41. Taylor and Francis, 41 edition.

Aisha, K. A., E. F. Shabana, M. S. El-abyad, I. A. Kobbia, and F. Schanz. 1995. Pulse feeding with nitrate and phosphate in relation to tissue composition and nutrient uptake by some macroalgae from the Red Sea at Ghardaqa (Egypt). Journal of Basic Microbiology **35**:135–145.

Beach, K. S., L. J. Walters, and H. B. Borgeas. 2006. Irradiance and nutrient limitation of *Dicytota* spp. populations on Conch Reef, Florida Keys, USA. Journal of Experimental Marine Biology and Ecology **329**:101–112.

Begin, C., J. Wurzbacher, and I. M. Cote. 2013. Variation in benthic communities of eastern Caribbean coral reefs in relation to surface sediment composition. Marine Biology **160**:343–353.

Bellwood, D. R., and C. J. Fulton. 2008. Sediment-mediated suppression of herbivory on coral reefs: Decreasing resilience to rising sea levels and climate change? Limnology and Oceanography **53**:2695–2701.

Bonaldo, R. M., and D. R. Bellwood. 2011. Spatial variation in the effects of grazing on epilithic algal turfs on the Great Barrier Reef, Australia. Coral Reefs **30**:381–390.

Bouyoucos, G. J. 1962. Hydrometer method improved for making particle size analyses of soils. Journal of Agronomy **54**:464–465.

Chapman, A. S., and R. L. Fletcher. 2002. Differential effects of sediments on survival and growth of *Fucus serratus* embryos (Fucales, Phaeophyceae). Journal of Phycology **38**:894–903.

Connell, S. 2005. Assembly and maintenance of subtidal habitat heterogeneity: synergistic effects of light penetration and sedimentation. Marine Ecology Progress Series **289**:53–61.

De'ath, G., and K. Fabricius. 2010. Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. Ecological applications : a publication of the Ecological Society of America **20**:840–50.

Eyre, B., and A. Ferguson. 2002. Comparison of carbon production and decomposition, benthic nutrient fluxes and denitrification in seagrass, phytoplankton, benthic microalgae- and macroalgae-dominated warm-temperate Australian lagoons. Marine Ecology Progress Series **229**:43–59.

Fabricius, K. E. 2005. Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. Marine Pollution Bulletin **50**:125–146.

Fabricius, K. E., Y. Golbuu, and S. Victor. 2006. Selective mortality in coastal reef organisms from an acute sedimentation event. Coral Reefs **26**:69–69.

Fong, P., K. E. Boyer, K. Kamer, and K. A. Boyle. 2003. Influence of initial tissue nutrient status of tropical marine algae on response to nitrogen and phosphorus additions. Marine Ecology Progress Series **262**:111–123.

Fong, P., and V. J. Paul, 2011. Coral reef algae. Pages 241–272 *in* Z. Dubinsky and N. Stambler, editors. Coral reefs: an ecosystem in transition. Springer.

Fox, R. J., and D. R. Bellwood. 2007. Quantifying herbivory across a coral reef depth gradient. Marine Ecology Progress Series **339**:49–59.

Fung, T., R. M. Seymour, and C. R. Johnson. 2011. Alternative stable states and phase shifts in coral reefs under anthropogenic stress. Ecology **92**:967–82.

Goatley, C. H. R., and D. R. Bellwood. 2012. Sediment suppresses herbivory across a coral reef depth gradient. Biology letters **8**:1016–8.

Goatley, C. H. R., and D. R. Bellwood. 2013. Ecological consequences of sediment on high-energy coral reefs. PloS one **8**:e77737.

Gorgula, S. K., and S. D. Connell. 2004. Expansive covers of turf-forming algae on human-dominated coast: the relative effects of increasing nutrient and sediment loads. Marine Biology **145**:613–619.

Hughes, T. P., N. a. J. Graham, J. B. C. Jackson, P. J. Mumby, and R. S. Steneck. 2010. Rising to the challenge of sustaining coral reef resilience. Trends in ecology & evolution **25**:633–42.

Hughes, T. P., M. J. Rodrigues, D. R. Bellwood, D. Ceccarelli, O. Hoegh-Guldberg, L. McCook, N. Moltschaniwskyj, M. S. Pratchett, R. S. Steneck, and B. Willis. 2007. Climate change, human impacts, and the resilience of coral reefs. Current Biology **17**:360–365.

Kamer, K., P. Fong, R. Kennison, and K. Schiff. 2004. Nutrient limitation of the macroalga *Enteromorpha intestinalis* collected along a resource gradient in a highly eutrophic estuary. Estuaries and Coasts **27**:201–208.

Kawamata, S., S. Yoshimitsu, T. Tanaka, T. Igari, and S. Tokunaga. 2011. Importance of sedimentation for survival of canopy-forming fucoid algae in urchin barrens. Journal of Sea Research **66**:76–86.

Kawamata, S., S. Yoshimitsu, S. Tokunaga, S. Kubo, and T. Tanaka. 2012. Sediment tolerance of *Sargassum* algae inhabiting sediment-covered rocky reefs. Marine Biology **159**:723–733.

Kuffner, I. B., and V. J. Paul. 2001. Effects of nitrate, phosphate and iron on the growth of macroalgae and benthic cyanobacteria from Cocos Lagoon, Guam. Marine Ecology Progress Series **222**:63–72.

Larned, S. T., and J. Stimson. 1996. Nitrogen-limited growth in the coral reef chlorophyte *Dictyosphaeria cavernosa*, and the effect of exposure to sediment-derived nitrogen on growth. Marine Ecology Progress Series **145**:95–108.

Larned, S. T. 1998. Nitrogen-versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae. Marine Biology **132**:409–421.

Littler, M. M., D. S. Littler, and B. L. Brooks. 2006. Harmful algae on tropical coral reefs: bottom-up eutrophication and top-down herbivory. Harmful Algae **5**:565–585.

Manly, B. F. J. 1997. Randomization, bootstrap and Monte Carlo methods in biology. 2nd edition. Chapman and Hall, Boca Raton.

Mantyka, C., and D. R. Bellwood. 2007. Macroalgal grazing selectivity among herbivorous coral reef fishes. Marine Ecology Progress Series **352**:177–185.

McClanahan, T. R., R. S. Steneck, D. Pietri, B. Cokos, and S. Jones. 2005. Interaction between inorganic nutrients and organic matter in controlling coral reef communities in Glovers Reef Belize. Marine Pollution Bulletin **50**:566–75.

McCook, L. 1996. Effects of herbivores and water quality on *Sargassum* distribution on the central Great Barrier Reef:cross-shelf transplants. Marine Ecology Progress Series **139**:179–192.

McCook, L. J. 1997. Effects of herbivory on zonation of Sargassum spp. within fringing reefs of the central Great Barrier Reef. Marine Biology **129**:713–722.

McCook, L. J. 1999. Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. Coral Reefs **18**:357–367.

McCulloch, M., S. Fallon, T. Wyndham, and E. Hendy. 2003. Coral record of increased sediment flux to the inner Great Barrier Reef since European settlement. Nature **42**:727–730.

Mora, C. 2008. A clear human footprint in the coral reefs of the Caribbean. Proceedings. Biological sciences / The Royal Society **275**:767–73.

Nemeth, R. S., and J. S. Nowlis. 2001. Monitoring the effects of land development on the near-shore reef environment of St. Thomas, USVI. Bulletin of Marine Science **69**:759–775.

Olsen, S. R., and L. E. Sommers, 1982. Phosphorus. Pages 403–430 *in* A. L. Page and E. Al., editors. Methods of soil analysis: Part 2. Chemical and microbiological properties. American Society of Agronomy, Madison, WI, 2nd edition.

Pandolfi, J. M., R. H. Bradbury, E. Sala, T. P. Hughes, K. A. Bjorndal, R. G. Cooke, D. McArdle, L. McClenachan, M. J. H. Newman, and G. Paredes. 2003. Global trajectories of the long-term decline of coral reef ecosystems. Science **301**:955.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R. Development Core Team. 2012. nlme: Linear and Nonlinear Mixed Effects Models. R package version **3.1-105**.

Prouty, N. G., M. E. Field, J. D. Stock, S. D. Jupiter, and M. McCulloch. 2010. Coral Ba/Ca records of sediment input to the fringing reef of the southshore of Moloka'i, Hawai'i over the last several decades. Marine pollution bulletin **60**:1822–35.

R Core Team, 2012. R: A language and environment for statistical computing.

Rasher, D. B., S. Engel, V. Bonito, G. J. Fraser, J. P. Montoya, and M. E. Hay. 2012. Effects of herbivory, nutrients, and reef protection on algal proliferation and coral growth on a tropical reef. Oecologia **169**:187–98.

Richmond, R. H., T. Rongo, Y. Golbuu, S. Victor, N. Idechong, G. Davis, W. Kostka, L. Neth, M. Hamnett, and E. Wolanski. 2007. Watersheds and coral reefs: conservation science, policy, and implementation. BioScience **57**:598.

Sah, R. N., and R. O. Miller. 1992. Spontaneous reaction for acid dissolution of biological tissues in closed vessels. Analytical Chemistry **64**:230–233.

Schaffelke, B. 1999. Short-term nutrient pulses as tools to assess responses of coral reef macroalgae to enhanced nutrient availability. Marine Ecology Progress Series **182**:305–310.

Schiel, D. R., S. A. Wood, R. A. Dunmore, and D. I. Taylor. 2006. Sediment on rocky intertidal reefs: Effects on early post-settlement stages of habitat-forming seaweeds. Journal of Experimental Marine Biology and Ecology **331**:158–172.

Stimson, J., and S. Larned. 2000. Nitrogen efflux from the sediments of a subtropical bay and the potential contribution to macroalgal nutrient requirements. Journal of experimental marine biology and ecology **252**:159–180.

Su, S.-w., I.-c. Chung, and T.-m. Lee. 2009. Temporal dynamics of rocky-shore macroalgal assemblage structures in relation to coastal construction threats in Orchard Island (Taiwan): impacts of turbidity and nutrients on the blooms of *Galaxaura oblongata* and a red alga-sponge symbiose. Kuroshio Science **3**:63–80.

Thacker, R. W., D. W. Ginsburg, and V. J. Paul. 2001. Effects of herbivore exclusion and nutrient enrichment on coral reef macroalgae and cyanobacteria. Coral Reefs **19**:318–329.

Umar, M. J., L. J. McCook, and I. R. Price. 1998. Effects of sediment deposition on the seaweed *Sargassum* on a fringing coral reef. Coral Reefs **17**:169–177.

Washburn, L., and A. Brooks of Moorea Coral Reef LTER, 2012. MCR LTER:.

Weber, M., D. de Beer, C. Lott, L. Polerecky, K. Kohls, R. M. M. Abed, T. G. Ferdelman, and K. E. Fabricius. 2012. Mechanisms of damage to corals exposed to sedimentation. Proceedings of the National Academy of Sciences of the United States of America **109**:E1558–67.

Weber, M., C. Lott, and K. E. Fabricius. 2006. Sedimentation stress in a scleractinian coral exposed to terrestrial and marine sediments with contrasting physical, organic and geochemical properties. Journal of Experimental Marine Biology and Ecology **336**:18–32.

CHAPTER 4

DELAYED BUT EXTENSIVE EFFECTS OF NUTRIENT ADDITION ON PRODUCER COMMUNITIES IN A TOP-DOWN DOMINATED ECOSYSTEM

4.1 Abstract

Two of the predominant anthropogenic impacts on coastal ecosystems, nutrient enrichment and the reduction or removal of key consumers, are predicted to interact in their effects on producer diversity, with outcomes depending on baseline resource availability. Evidence in rocky intertidal systems, however, is mixed, and comparison is limited by temporal and spatial differences in experimental design. We conducted a long-term experiment examining the interactive effects of nutrients and herbivores in a markedly low-productivity environment encompassing and explicitly accounting for the small-scale heterogeneity characteristic of rocky shores. Rapid and dramatic shifts in diversity metrics and cover of algal communities in the first year were driven by experimental removal of benthic herbivores. Within two months after exclusion of herbivores, algal cover increased from less than 10% to over 100%, regardless of nutrient addition, and both diversity and evenness plummeted while species richness rose. In contrast to theoretical predictions, however, strong interactions between top-down and bottom-up forces on measures of diversity were rare, and the addition of nutrients negatively impacted diversity. Instead nutrient levels drove distinct patterns of succession in communities in which herbivores were removed. Enriched communities followed an inhibition model with foliose species maintaining dominance, whereas

later-successional corticated species eventually became prominent under ambient nutrients, suggesting a tolerance model. Topographic complexity, a proxy for environmental heterogeneity that modulates stress, also strongly influenced composition and increased richness of the community. Positive effects of complexity were likely due to provision of a refuge from the indiscriminate herbivory of limpets. Overall, our results suggest that nutrient addition and herbivore reduction exert strong control on community biodiversity regardless of environmental heterogeneity; however, context dependency in seasonality, stress, and successional stage also played an important role, indicating the importance of longer-term field studies and environmental context to distinguish the delayed yet extensive effects of nutrients.

4.2 Introduction

Understanding the relationship between diversity and the forces that control it is becoming increasingly important as anthropogenic modification of the environment has become ubiquitous in ecosystems worldwide. Two of the predominant anthropogenic impacts on ecosystems include 1) nutrient addition and resultant increases in productivity (Valiela et al., 1992; Vitousek et al., 1997; Tilman et al., 2001; Suding et al., 2005) and 2) the reduction or removal of key species, particularly those in upper trophic levels (Steneck, 1998; Jackson et al., 2001). These human alterations of resource availability (Smith et al., 1999; Suding et al., 2005) and key components of trophic webs (Duffy, 2003) can disrupt and cause dramatic shifts in the relative roles of community structuring processes (Vitousek et al., 1997; Smith et al., 1999; Hughes et al., 2003; Folke et al., 2004; Suding et al., 2005; Worm and Lotze, 2006), with major influence on community diversity (Hillebrand, 2003). Thus, a current focus in ecology is how the relative strength of consumer vs. resource effects varies across a range of both natural and anthropogenically-altered baseline conditions (Denno et al., 2005).

Current theoretical models indicate that effects of productivity (and the resources driving productivity) and disturbances (including the biotic disturbance of consumption) on communities strongly interact, and thus must be considered together in order to predict

their consequences on diversity (Kondoh, 2001; Worm et al., 2002). Huston (1979; 1994) first proposed an interactive model (Dynamic Equilibrium Model) that suggested it is the interaction of disturbance (whether abiotic or biotic) and resource supply that results in varying individual effects on diversity. Applying these predictions to the effects of herbivores and nutrient supply on plants, Proulx and Mazumder (1998) performed a meta-analysis demonstrating that the effects of grazers on plant species richness do indeed depend on levels of nutrient availability. Mathematical elaboration of these concepts, incorporating competitive ability and colonization rates, resulted in the current interactive models (Kondoh, 2001; Tilman, 1994; Worm et al., 2002). These models predict the nature of interactions between consumption and resource supply, suggesting that peak diversity shifts toward higher levels of nutrient supply with increasing consumer pressure, thus making outcomes on diversity dependent on baseline levels of productivity.

In the past decade, temperate rocky shores, with their long history of ecological research, easily manipulated communities, and large-scale gradients in nutrient supply, have been recognized as a prime system in which this theory may be tested. The urgency of understanding the interplay between these top-down and bottom-up forces has been amplified by recent insights into the importance of bottom-up forcing in this system, which has historically been considered top-down controlled (Connell, 1961; Paine, 1966; Dayton, 1971; Menge, 1976; Lubchenco and Menge, 1978, reviewed by Menge, 2000). Together, a growing body of theory and evidence for the importance of benthic-pelagic links (Menge, 1997; Menge et al., 2003, 2004; Schoch et al., 2006) and supply-side ecology on open coasts (Underwood and Fairweather, 1989; Underwood and Keough, 2001; Menge et al., 2010) in governing community structure and diversity led to a proliferation of studies examining nutrient-herbivore interactions on various coasts worldwide.

In spite of the strong interactions predicted by theory and the general consensus that these top-down and bottom-up effects need to be considered together, empirical tests in benthic marine systems leave limited evidence of strong interactions. An early study by Worm et al. (2002) tested these predictions in rocky shore communities in the NW Atlantic and eutrophic Baltic Sea (see also Worm and Lotze, 2006) and found interactive effects

of nutrients and consumers on both % cover and diversity that differed between high and relatively lower productivity sites. However, in the past decade since Worm et al. (2002), few studies have found the prominent herbivore-nutrient interactions on measures of diversity predicted by the models (e.g. Nielsen, 2003; Hillebrand, 2003; Korpinen et al., 2007; Guerry et al., 2009; Atalah and Crowe, 2010; Williams et al., 2013). Guerry (2008) found that at low nutrient levels (ambient), herbivores reduced species richness, but only in the first year of the experiment; this effect was not seen after two years. Several studies have found nutrient benefits for measures of ecosystem function when herbivores were removed (e.g. productivity: Nielsen, 2001; Masterson et al., 2008 and biomass, particularly of ephemeral species: Nielsen, 2001; Guerry et al., 2009; Bulleri et al., 2012). Overall, these studies suggest that interactive effects, if occurring, may be most prominent on total algal cover, particular components of the community (e.g. ephemeral species) and associated ecosystem functions (e.g. biomass and productivity) rather than measure of diversity. One possible explanation for the paucity of observed interactions on algal diversity, and lack of strong effects of nutrients in general, may be that, despite the evidence that baseline productivity alters the outcome of nutrient and herbivore effects, studies on relatively unproductive coasts are rare (but see Guerry et al., 2009; Bulleri et al., 2012). Nutrients may simply not be limiting in the more productive systems typically studied. Together these results suggest that interactive effects may be occurring at a level of productivity rarely studied and on aspects of community structure that may not be evident in traditional measures of diversity.

In addition to affects on diversity, changes in forces of nutrient supply or herbivory may also alter successional processes, which have been shown to be strong drivers of rocky intertidal community composition (Dayton, 1971; Connell and Slatyer, 1977; Lubchenco and Menge, 1978; Murray and Littler, 1978; Sousa, 1979; Farrell, 1991). For example, in a system with inhibition succession, a decrease in herbivory may allow the persistence of early colonists, preventing later successional species from establishing (Connell and Slatyer, 1977; Lubchenco and Menge, 1978). In developed late-successional communities, physical disturbances remove established species and open space for rapid colonizers, while the biotic disturbance of herbivory has stronger effects on early successional communities composed

of more palatable algae (Hay and Fenical, 1988), providing space for slower-growing, later-successional species and thus accelerating succession (Sousa, 1979). Nutrient enrichment concurrent with recent disturbance is likely to greatly increase biomass of opportunistic algal taxa with rapid growth potential (Worm and Lotze, 2006), whereas in later successional communities nutrient effects without disturbance may be somewhat buffered due to the relatively small area of open space for colonization by opportunistic forms. Most studies of nutrient-herbivore interactions in the rocky intertidal have been conducted on cleared or artificial substrate (e.g. tiles) (e.g. Worm et al., 2002; Worm and Lotze, 2006; Freidenburg et al., 2007; Masterson et al., 2008; Bulleri et al., 2012) and thus reflect effects on successional changes. However, successional processes and well-demonstrated seasonality in rocky intertidal communities (e.g. Underwood and Jernakoff, 1984) may also cause changes in nutrient and herbivore effects over time due to substantial time lags in recruitment (e.g. Oliveira et al., 2011). Moreover, successional processes can be strongly influenced by the timing and scale of disturbance (Dayton, 1971; Paine, 1988; Blanchette, 1996) as well as other environmental factors (Gray and Christie, 1983; Currie and Parry, 1999; Breves-Ramos et al., 2005). Thus, studies should be conducted over long-enough timescales to account for this variability.

While the influences of nutrients and herbivores on community structure may vary temporally with seasonal and successional processes, they may also vary spatially with habitat heterogeneity (Gripenberg and Roslin, 2007; Jackson et al., 2013). Heterogeneity is characteristic of rocky intertidal systems, from microscale topographical heterogeneity to large-scale environmental gradients (see Menge and Olson, 1990), and much effort has been devoted to examining their roles in structuring communities (e.g. Menge et al., 1985). At the local scale, one of the most important ways in which topographical heterogeneity may influence communities is by alleviating thermal stress, which may differentially alter the effects of nutrient and herbivory on algal diversity (Werner and Matthiessen, 2013) and drive intertidal community dynamics (Morelissen and Harley, 2007; Bertocci et al., 2010; Gedan et al., 2011; Williams et al., 2013). Many studies aim to reduce heterogeneity within the experimental design by limiting site selection to a relatively uniform location (e.g. to smooth rocky bench) or to certain pre-defined characteristics. However, minimizing habitat hetero-

geneity in order to better determine effects of altered nutrient or herbivory regimes may not give an accurate picture of the overall effects these changes will have on heterogeneous landscapes (see Jackson et al., 2013). Conversely, not accounting for these factors may impair the ability to detect real effects (e.g. Bracken et al., 2011). Thus, studies are needed that both allow/encompass realistic levels of habitat heterogeneity while accounting for the changes in environmental conditions that they cause.

Our goal was to conduct a multi-year experiment examining the interactive effects of nutrients and grazers in a low-productivity environment encompassing and explicitly accounting for the small-scale heterogeneity characteristic of rocky shores. The coastal waters around New Zealand, where this study was conducted, are relatively nutrient poor in comparison with the upwelling regimes of the eastern boundary currents such as the Northeast Pacific, Chile and South Africa (Viner and Wilkinson, 1988; Menge et al., 2003; Schiel, 2004), where many of the studies examining top-down and bottom-up interactions have been conducted (see also Appendix 4.A). Based on observations of low algal cover, naturally low nutrient conditions, and the abundance of limpets we expected strong herbivore control. Thus, we hypothesized that reduction of herbivory would increase algal diversity, although biomass may also be limited by other factors such as environmental stress. However, as predicted by interactive models, we expected strong interactions between nutrients and herbivory such that nutrients negate the positive effect of herbivory on diversity. We also predicted that nutrient effects would be strongest on forms associated with early stages of succession, although interactions due to concurrent increased consumption might mask these effects in the presence of herbivores. Finally, we hypothesized that environmental heterogeneity would also exert strong effects on community structure, and accounting for heterogeneity would better enable detection of both individual and interacting effects of nutrients.

4.3 Methods

4.3.1 *Study site*

We performed experiments on a moderately exposed intertidal rocky reef in the Cook Strait Region in Wellington, New Zealand (Lat: -41.349205, Long: 174.74079; Figure 4.1; see Appendix 4.A for site details). The study site was 1 km from the nearest road, adjacent to the Taputeranga Marine Reserve, and 3 km away from the nearest watershed outflow or source of terrestrial runoff. The experiment was conducted in the upper-mid intertidal community, which is likely to be most vulnerable to anthropogenic nutrient changes (Bracken, 2004, see also Appendix 4.A).

Algal communities in this tidal range are relatively sparse, with an average cover of $6.38\% \pm 0.975$ composed, on average, of 5.22 ± 0.33 species (mean $\pm$ SE, $n = 46$; R. Clausing, unpubl. data). The dominant space occupiers are crusts of various forms: crustose calcareous algae (CCA), *Hildenbrandia* spp., *Apophlaea sinclairii*, *Ralfsia* spp. and *Hapalospongidion gelatinosum*. Small amounts of the red corticated *Gelidium* spp. and cyanobacterial films are also present. In contrast, biomass of herbivores, though limited in species numbers, is high at 67.57 ± 7.83 (mean $\pm$ SE g tissue m^{-2}, $n = 46$; R. Clausing, unpubl. data). The dominant herbivores are three species of limpets in the genus *Cellana*, particularly *Cellana denticulata*, which comprised 82% of all herbivore biomass (Appendix Table 4.B.2) and was observed up to 52 mm in length. Other common herbivores include the spotted top snail (trochid) *Diloma aethiops*, the littorinid *Austrolittorina cincta*, and the pulmonate limpet *Siphonaria australis* (see also Appendix 4.B).

4.3.2 *Experimental design and set up*

In order to evaluate the potential consequences of nutrient enrichment as well as the effects of changing consumer pressure on algal diversity and community composition, we conducted a long-term (2 year) field experiment within existing natural mid-high zone rocky intertidal communities from March 2010 – February 2012. The 2-factor, fully crossed

design manipulated nutrient supply and herbivore abundance to include four treatments: 1) ambient nutrients and ambient herbivores (control; -N+G), 2) elevated nutrients and ambient herbivores (+N+G), 3) ambient nutrients and herbivore removal (-N-G), 4) elevated nutrients and herbivore removal (+N-G). We initiated experimental treatments on plots with already established but naturally low algal biomass communities, whereas the majority of previous studies have utilized either cleared or already bare substrate (e.g. Worm et al., 2000*a*, 2002; Worm and Lotze, 2006; Freidenburg et al., 2007; Masterson et al., 2008; Bulleri et al., 2012, but see Guerry et al., 2009) or mesocosm communities (Bokn et al., 2003; Kraufvelin et al., 2006). Logistical constraints (elevational zone, size >0.6 m) limited available substrate for choice of plots, but within suitable area, we haphazardly selected plot locations to meet *a priori* criteria for variation in relative slope (∼0–40 degrees from horizontal) and distance to prevent cross contamination of enriched vs. ambient plots (minimum 1m, Guerry et al., 2009). We allowed topographic complexity to vary between plots as it did naturally. The use of circular plots (0.45m^2 in diameter) reduced edge effects and minimized unequal nutrient diffusion from a central dispenser. We assigned treatments to plots randomly.

We used slow-release fertilizer (Osmocote Exact, in N:P:K ratios of 15:4:9) to diffuse nutrients to plots (Worm et al., 2000*b*). Incoming tides cause nutrient release from the resin balls via diffusion and simulate pulsed supplies typical of rocky intertidal ecosystems (Nielsen, 2001). Nutrients were dispensed from PVC cylinders with screw-on caps that were attached to the center of each plot with 10 cm lag screws and epoxy (after Williams et al., 2013). In enriched treatment plots, we placed nylon stockings with 75 g Osmocote in dispensers, while ambient plots had empty stockings. Fertilizer masses throughout the experiment were comparable to other studies (reviewed by Worm et al., 2000*b*). After one month we added 15 g of urea mixed into the Osmocote, with the intention of creating an initial pulse of nutrients that may mimic a pulse input in coastal waters after a storm. Initially, we replaced nutrients every three weeks. Due to small loss in weight of osmocote over 3 weeks (Appendix Table 4.C.1) and small measurable increases in water column nutrient levels (Appendix Figure 4.C.1), we increased the volume of the dispenser with PVC extenders in October 2010 to hold 230 g Osmocote which we changed every 6 weeks. We continued to

add urea every two weeks in ∼20 g amounts (Appendix Table 4.C.2).

We measured nutrient levels at various times after fertilizer addition by taking 15 mL water samples 5–10 cm above the center of each plot on an incoming tide when water was roughly 20 cm deep (generally one sample per plot per event). Samples were kept on ice in the field and immediately frozen upon return to the laboratory. In this location, there is very little particulate matter in the water column. Because early tests (R. Clausing, unpubl. data) showed no difference in nutrient readings between filtered samples (Whatman GF/C) and unfiltered ones, we did not filter samples. We analyzed sample nitrate (NO_3), nitrite (NO_2), phosphate (PO_4) and ammonia (NH_3) with an autoanalyser (Skalar ++) with replicate runs to check for technical error. Though highly variable among sampling events, water column NO_3 and NH_3 were significantly higher above enriched plots (Appendix Table 4.C.3, 4.C.4). Furthermore, increases in nutrient levels were similar from 1 day after nutrient addition up to 16 days after addition (Appendix Figure 4.C.1).

A 4 cm wide boundary of Z-spar marine epoxy painted with copper anti-fouling paint delineated plots and prevented immigration and emigration of limpets and chitons (Cubit, 1984; Freidenburg et al., 2007). At the onset of the experiment, we removed all benthic macroherbivores (chitons, limpets, snails) from the exclusion plots. Although copper paint does not effectively prevent movement of trochids (top-snails) or littorinids (periwinkles), we removed these herbivores manually at least every other week, and they were a small percentage of the total grazer biomass observed in each plot (3.33% ± 0.45 trochids and 1.90% ± 0.25g littorinids; means ± SE, $n = 24$; Appendix 4.B.3). We did not remove isopods and amphipods as they were initially uncommon and Lotze and Worm (2002) showed that these grazers do not consume detectable biomass, and may actually have positive effects on algal recruit densities at low nutrient levels (but see Duffy, 1990). Herbivorous fish (e.g. kyphosids and *Odax pullus*) were not commonly seen or observed grazing in plots during high tide surveys. In ambient grazer treatments, we left herbivore communities intact and stocked limpets as needed to maintain natural abundances as determined by initial surveys. Due to their mobility, variability in numbers, and relatively small proportion of the total biomass, we did not stock other herbivores (*D. aethiops, S. australis, A. cincta, R. varia* and

chitons; see Appendix 4.B.2, 4.B.3). Limpet transplants included only *C. denticulata* and *C. radians* due to high mortality in transplanting *C. ornata*, which exhibits strong homing (Morton and Miller, 1973).

We recorded various habitat measures characterizing habitat heterogeneity to include in statistical models. These included slope and relative measures of habitat complexity, water motion, light and temperature. Methods are described in full and plots measurements given in Appendix Table 4.C.6.

4.3.3 *Measuring response variables and treatment maintenance*

We measured percent algal cover within each plot using point-contact surveys with 100 random points at the experimental onset (initial), monthly for the first three months, and quarterly thereafter. We also visually scanned plots for 1 minute for the occurrence of rare species that were not found using the point-contact method; we assigned these species 0.5% (e.g. Masterson et al., 2008; Atalah and Crowe, 2010, Appendix 4.B.1). To account for layering, usually of smaller or crustose algae beneath canopy forming species, we recorded all species lying below each point, allowing total percent cover to sum to greater than 100%. We identified species to lowest possible resolution but grouped them into functional forms based on a modification of Steneck and Dethiers (1994) classification system (Appendix Table 4.B.1).

We counted total herbivore abundances (limpets, snails, chitons) in each plot in the field. We identified all individuals to species except limpets less than 10 mm, which we classified simply as a limpet. Because herbivore pressure is a function of size as well as abundance, we measured the length of all species to the nearest half mm except *A. cincta* and *Risselopsis varia*, which were simply counted. All individuals were 5 mm or less, however, and commonly found but highly variable in occurrence and numbers (Appendix 4.B.2). We counted herbivores twice monthly, stocking as needed to maintain initial densities, and conducted full surveys counting and measuring all individuals (except *A. cincta* and *R. varia* as above) on the same dates as algal sampling.

4.3.4 *Data analysis*

To examine the effects of changes in nutrient supply and grazing pressure on algal diversity and community structure, we ran linear mixed-effects models on all univariate responses and PERMANOVA (Anderson, 2001) on cover of algal functional groups, followed by individual mixed models on each form. We included nutrient and herbivore treatments and time and their interactions as fixed factors (nutrients and herbivore as categorical, time as continuous), and environmental parameters as fixed continuous predictors. To account for residual variation among plots, plot was treated as a random effect. As no correlations (Spearman Rank) between any of the environmental parameters were greater than 0.7, we included all variables in each model. Season, specified as a sinusoidal function, was also included as a fixed factor, ($a \cdot \sin \omega t$; where $\omega = \frac{2\pi}{12}$, t = time of sampling as months since experimental onset, and a is the parameter to be estimated). After running the initial model, we simplified each model to the lowest possible value (ω) of Akaike Information Criterion. We used ANOVAs to ensure model simplification did not lose significant terms (Crawley, 2007). We checked assumptions by examining plots of standardized residuals vs. fitted values (homogeneity of variance) and QQ plots of residuals (normality) (Quinn and Keough, 2002). Due to large numbers of zeroes, analyses of individual functional forms required an arcsin square root transformation to meet assumptions.

Univariate measures of algal community dynamics included diversity, evenness, richness, total % cover and, after performing PERMANOVA, changes in cover of each of the dominant functional groups. We calculated diversity from % cover of functional groupings using the Shannon-Wiener Index (hereafter referred to as diversity)

$$H' = -\sum p_i \cdot \ln p_i \tag{4.1}$$

where p_i = (% cover for form i) $\div$ (total cover of p forms). Evenness was examined using Pielous Evenness Index (hereafter termed evenness)

$$J' = \frac{H'}{H'_{max}} \tag{4.2}$$

where H'_{max} is the theoretical maximum value of H' assuming all species are equally abundant. Because rare species (Bracken and Low, 2012) as well as species identity (Best et al.,

2014) may have disproportionately important effects on upper trophic levels, we assigned 0.5% to all rare species - those which were observed in plots but did not lie beneath one of the survey point contacts (Masterson et al., 2008; Atalah and Crowe, 2010; see also Appendix 4.C). In addition, we examined species richness data as species with >1% cover (common) and those <1% (rare species) separately, as observation suggested patterns of change with manipulation differed between common and rare species, which overall species counts did not differentiate. Because filamentous and leathery forms and articulated calcareous algae were low in abundance across all times (<9%, <2% and <0.5% cover on average in any treatment at any sampling event, respectively) analyses of functional forms focused on foliose, corticated, and crustose forms (non-calcareous and CCA analyzed separately).

Community trajectories among treatments over the course of the experiment were depicted using principal components analysis (see also Smith et al., 2010). We constructed Bray-Curtis dissimilarity matrices using square root transformed cover (proportion) data, which we analyzed using principal components analysis (PCA). We extracted the multivariate centroids in two dimensions for each plot by time combination, averaged these by treatment group, and plotted them according to the first two PCA axes. These data provide a visualization of how the successional trajectories of algal communities in each treatment differed from one another.

Because abundance of herbivores is a highly imprecise measure of the force of herbivory, we calculated biomass per plot by transforming size (in most cases, shell length) to weight (tissue wet weight without shell) for each individual in each plot and then summing them across all species (Methods in Appendix 4.B).

All data were analyzed with the programming language R (R Core Team, 2012). Mixed models were run using the package nlme (Pinheiro et al., 2012), and multivariate analyses were run using the vegan package (Oksanen et al., 2012).

4.4 Results

4.4.1 *Environmental parameters*

Average ambient water column nutrient levels encompassing both summer and winter sampling dates were generally low (<1 μM each for NO_3 + NO_2 and PO_4) except for NH_4 (9 μM), although measurements ranged nearly an order of magnitude among sampling times (Appendix 4.A, 4.C). All environmental/habitat measures were relative excepting slope (ranged from 10.5 ° to 38.5 ° incline from horizontal). These data are found in Appendix 4.C: environmental parameters, Table 4.C.6.

4.4.2 *Algal diversity indices*

Nutrients strongly decreased evenness, but only in the absence of herbivory and only in the first year of experimentation, resulting in a three-way interaction. While the pattern was similar for diversity, it was more variable and thus only 2-way interactions with time resulted (Figure 4.2a; Table 4.1a). Strong effects of nutrient addition over time are reflected in the relatively stable evenness in ambient treatments (-N+G), while plots with ambient herbivores but added nutrients had pronounced variability. Herbivore removal caused rapid, drastic reduction of evenness, associated with the development of extensive algal films in areas that were previously bare. All herbivore removal (-G) plots subsequently increased in evenness, although to a lower level than the initial (G-:time interaction); however, recovery in ambient nutrient (-N) plots was immediate, while enriched plots slowly increased over the following year. Seasonality was also prominent in patterns of evenness, with higher evenness in late summer and fall months. Topographic complexity increased evenness (Table 4.1a).

Diversity of functional groupings of algae showed similar overall trends as evenness (Figure 4.2b; Table 4.1b), with the ambient treatment (-N+G) remaining the most constant over time, and a large initial drop in diversity after herbivore removal (G-:time interaction). However, variability in diversity remained throughout the entire experiment, whereas evenness became more stable in the second year in all treatments except ambient grazers with

added nutrients (+N+G). In particular, the addition of nutrients to ambient grazer plots (+N+G) appeared to increase variability over time and decrease diversity (relative to ambient plots) (N+: time interaction). Diversity and evenness based on individual species data rather than functional groupings were significantly affected only by topographic complexity and nutrient effects over time, respectively. These results are discussed in Appendix 4.D (see also Table 4.D.1, 4.D.2).

Both common and rare species numbers were strongly controlled by herbivores (Figure 4.3; Table 4.2), but with opposing effects. While the removal of grazers rapidly quadrupled the abundance of common species (effect of G-), regardless of nutrient treatment, reduction of herbivory caused decreases in numbers of rare species that became more prominent over time (G-:time interaction). With ambient herbivores, the addition of nutrients (+N+G) slowed the increase of common species observed in ambient treatments, but numbers of both treatments merged and leveled in the second year. After the initial increase in common species with herbivore removal, numbers remained roughly constant for the remaining 20 months with the exception of seasonal trends of lower numbers in winter. Common species were also substantially greater numerically in more topographically complex plots (Table 4.2A). In contrast, numbers of rare species appeared to increase with nutrient enrichment although this trend was not significant. Total numbers of species ranged from 0 to 20 per plot over the duration of the experiment (median = 9).

4.4.3 *Algal functional form cover*

Changes in total algal cover were driven by the reduction of herbivores (Figure 4.4; Appendix Figure 4.D.1A, Table 4.D.3), which increased cover from <10% to over 100% in two months time, effectively covering all open space (Appendix Figure 4.D.1B, Table 4.D.4). Algal cover also increased slowly but consistently in ambient herbivore (+G) plots, ending at 40-55% cover compared to 135% with removal of herbivores (-G) (Figure 4.4). Moreover, in herbivore removal plots, algal cover changed from primarily encrusting (∼70%) to upright forms of mixed successional stages (∼80%), whereas the increase in cover in ambient her-

bivore plots consisted primarily of crusts (CCA and other crusts). PERMANOVA showed significant interactions of the main effects, nutrients and herbivores, as well as significant interactions of each main effect with time (Appendix Table 4.D.5). Thus functional forms were analyzed individually with linear mixed models.

Films increased in cover immediately in herbivore removal plots (Appendix Figure 4.D.2, Table 4.D.6), nearing 100% cover. However, they had decreased to <20% within six months as they were replaced by foliose algae. Foliose algae increased from roughly 0% to between 45-70% cover in 6 months in herbivore removal plots (-G) and remained high for the remainder of the experimental period excepting June 2011, resulting in highly significant grazer by time interaction (Figure 4.5A; Table 4.3A). In plots with intact herbivore populations, cover of foliose algae never reached 5% (median = 1.00%). Without herbivores, nutrients had a significant positive effect that increased dramatically in the first 6 months (Table 4.3A, N+:time interaction), with enriched plots (+N-G) maintaining higher foliose cover than those without (-N-G). There were also seasonal effects, with peak cover in spring and summer. Topographic complexity reduced the occurrence of foliose algae.

In contrast, cover of corticated species showed no discernible increases in cover during the first seven months of experimentation. Beginning in January 2011, cover increased steadily in herbivore removal, ambient nutrient plots (-N-G) (Figure 4.5B; Table 4.3B; G-:time interaction) and to a lesser degree in N+G and +N-G plots. Corticated cover in both ambient nutrient treatments was double that of enriched plots with the same herbivore treatment, resulting in an interaction of nutrients and time. Cover of corticated species also increased in ambient plots (-N+G) roughly in parallel with enriched, reduced herbivore plots (+N-G), suggesting nutrients may have a similar negative impact as the positive effect of herbivore removal. Finally, topographic complexity increased cover, the opposite effect it had on foliose species.

Encrusting algal cover (not calcareous) initially increased in all plots from 0-2% to a plateau between 6-19% (Figure 4.5C; Table 4.3C). After 10 months, cover increased in herbivore removal plots (-G) at rates triple those in plots with intact herbivores (+G), resulting in strong grazer by time interaction. Increases in cover were nearly parallel among

plots within grazer treatments, regardless of nutrient addition, and peaked at the end of the experiment, with 50% cover in -G plots and 27% cover in +G plots. Seasonality was the only other significant effect, with peak cover occurring in late fall and winter.

CCA was the only major algal functional group that did not benefit from reduced herbivory (Figure 4.5D; Table 4.3D). Rather, in the presence of herbivores, nutrient addition negatively impacted cover. Cover also remained the lowest in enriched herbivore removal plots (+N-G), although it was not significantly lower than ambient ones (-N-G) (N+:G- interaction). Topographic complexity had strong positive impacts on cover, and, excepting the -N+G treatment, most plots showed little variation over time, staying between 1.1 to 6.8% cover despite that seasonality was a significant predictor in the model.

4.4.4 *Algal successional trajectory*

Successional trajectories diverged distinctly between plots with ambient grazers compared to those without (Figure 4.6A). Initial change in composition in herbivore removal (-G) plots showed a change along the PCA2 axis, driven primarily by algal and bacterial films (Figure 4.6B; Appendix Table 4.D.7). Subsequent change toward negative values on the PCA1 axis are consistent with increases in *Ulva* spp., primarily *Ulva intestinalis*, and more so in the added nutrient treatment (+N-G) than the ambient (-N-G). Finally, the last year the trajectories suggest the majority of the change is in the corticated canopy-forming species, *Scytothamnus australis*, and the encrusting species *Hapalospongidion gelatinosum*, *Diplura australis*, and *Hildenbrandia* spp. In contrast, plots with intact herbivore populations changed little initially, decreasing along both PCA1 and PCA2, indicating change lies primarily in increases in the encrusting species. However, in both herbivore treatments, divergence between enriched and ambient nutrient plots occurred primarily during the second year, suggesting effects on successional processes.

4.5 Discussion

In the first year, our results demonstrated an extreme level of herbivore control beyond previous findings on intact communities (e.g. Bulleri et al., 2012; Kraufvelin et al., 2006). It has been suggested that the effect of herbivory is strongest where high herbivore abundances are concurrent with low barnacle and/or slow algal colonization and growth (Freidenburg et al., 2007). The conspicuous lack of algal biomass in this system suggested strong herbivory; however, we expected high thermal stress to limit algal biomass even in the absence of herbivores, which did not occur. Thus, although a large number of studies examining nutrient and herbivore control in rocky intertidal algal communities systems have also found top-down control (e.g. Masterson et al., 2008; Atalah and Crowe, 2010; Williams et al., 2013) the magnitude of the control we found (from <10% to >100% in 2 months) was unprecedented (but see Guerry et al., 2009). Extreme top down control here is likely a result of high density of generalist limpet herbivores, which indiscriminately rasp the substrate clean of microalgae and germlings of larger algal species (Hawkins and Hartnoll, 1983), which are functionally equivalent at that stage (Steneck and Watling, 1982). Although limpets are frequently more numerous than the densities in our studies (e.g. more than 10x higher limpet density in Guerry et al., 2009, individual size was much greater in our study (e.g. 3x higher mean size and maximum size of 52 mm compared to 25 mm in Guerry et al., 2009). Moreover, limpets exceeding 35 mm were present in nearly every plot in our study. Thus, our site was high in terms of herbivore biomass. Rather than low rates of algal colonization contributing to the strength of top-down forcing, this suggests that high rates of algal settlement may be occurring to support high rates of consumption. Overall, these results suggest that the force of herbivory at this location is high on the global spectrum; drastic changes in cover when grazers are removed indicate that grazers alone drive algal biomass standing stock.

In contrast to theoretical predictions of interacting consumption-diversity and productivity-diversity models (Kondoh, 2001; Huston, 1994), strong interactions between top-down and bottom-up forces on measures of diversity were rare in this system, and the addition of nutrients negatively impacted diversity. Despite expectations of theory, interactions of nutrients

and grazers have rarely been found on algal diversity (e.g. Worm et al., 2000*a*; Nielsen, 2003; Hillebrand, 2003; Guerry et al., 2009; Atalah and Crowe, 2010; Williams et al., 2013), with instead a prevalence of main or dual effects. Interactions are most frequently observed on specific species e.g. opportunists (e.g. Korpinen et al., 2007) or in measures of total algal cover or biomass (e.g. Bulleri et al., 2012). We observed interactions of bottom-up and top-down control on open space and total algal cover, where nutrients enhanced cover and decreased open space without herbivory, but grazers were able to compensate for this increase in algal productivity (see also Nielsen, 2001). Moreover, in systems with naturally low nutrient levels and high rates of consumption, theory predicts that increasing nutrient loads would boost diversity (Worm et al., 2002) by increasing resources to support more complex assemblages including rapid colonizers or reverse the negative effects of grazers (Proulx and Mazumder, 1998). Guerry (2008) found partial support of this grazer-reversal hypothesis on terracotta pots placed in a highly productive intertidal system in Oregon, but only in the first year of experimentation. In our study, however, the effects of enrichment on algal diversity were negative (or minimal) on all metrics except numbers of rare species. One possibility may be that, at this level of enrichment, herbivory increases proportionally with nutrient-stimulated growth of palatable foliose species or even increases consumption beyond growth. This may be particularly true if herbivores are food-limited and/or if nutrient enrichment increases palatability of all species. Evidence of higher algal cover in ambient plots with intact herbivores (-N+G) compared to enriched plots (+N-G) supports this hypothesis.

Communities with herbivores removed underwent distinct patterns of succession (Connell and Slatyer, 1977), which followed different models depending on nutrient levels. Open space was initially colonized by biofilms, followed by sporelings of foliose forms (*Ulva* spp. and *Porphyra* spp.). Here, nutrient enrichment appeared to slow down or halt the succession process by maintaining the dominance of these ephemeral species. Thus nutrients facilitated an inhibition model of succession by maintaining communities of ephemeral foliose species, which thus inhibited later successional species colonization by pre-emption of space (Connell and Slatyer, 1977; Lubchenco, 1978). In contrast, communities without nutrient addition followed a tolerance model where later successional corticated species were able to dominate

the substrate over time (beginning in earnest after a year) as the ephemeral species turned over. Subsequently, as the canopy-forming brown alga *Scytothamnus australis* dominated cover in unenriched plots, it facilitated growth of a full understory of fleshy crusts. Nutrient enrichment of high biomass intact communities may also favor opportunist species over later successional perennials (Kraufvelin et al., 2010). Kraufvelin et al. (2006) found that high biomass communities of the canopy-forming perennial alga *Fucus sp.* crashed after 5 years of nutrient addition, giving way to opportunist green algae. Evidence that late-successional species are unable to colonize unless herbivory on ephemeral forms opens up substrate (Lubchenco, 1983; Worm et al., 2001; Korpinen et al., 2007) may only be applicable in our study system when nutrient levels are enriched (see also Masterson et al., 2008). Overall, these results suggest that, given sufficient time, nutrient enrichment may drive community development in fundamentally different ways that are likely to result in strongly heterogenous ecosystem properties.

Our results indicate that, in dynamic rocky shores, effects of nutrients may be dominant only in later stages of succession. Thus, nutrient effects may only be important under chronic nutrient disturbances, particularly as short-term disturbances may also be confounded by seasonality. This may at least partially explain why nutrient effects often appear highly variable in experiments conducted over short timescales. Substantial time lags in effects, particularly of nutrients, suggest that results from experiments performed under shorter durations may only be indicative of changes under pulse or short-term disturbances. The lag times we observed were consistent with both seasonal and successional processes, where herbivore effects were immediate on biofilms and opportunists, but effects on later successional species and crusts were not evident until the second year. Strong seasonality in almost all measures of diversity and cover of functional forms suggests that main effects in experiments conducted less than 2 years may be dependent upon seasonal processes. For example, in all reduced herbivory plots, initial drops in diversity due to development of films after grazer removal were not indicative of the longer term, overall patterns of herbivore reduction. The consistent increases in non-calcareous encrusting species showed periodic jumps in cover likely associated with seasonal recruitment. For example, several brown crust species

including *Colpomenia* spp. recruit heavily in winter months (McNaughtan, 2007). Timing of herbivore and algal recruitment may also play a role in seasonal patterns (Dethier, 1982; Underwood and Jernakoff, 1984). Overall, the evidence for strong seasonal patterns as well as successional processes highlight the importance of experimental design and consideration of the types of disturbances (both duration, as well as community condition) being simulated and what generalizations can be made from it.

Topographic complexity, which modulates physical stress, played an important role in the composition and richness of the community. Positive effects of topographic complexity on species richness are likely due to provision of a refuge from limpet herbivores, which rasp all exposed microalgae and settled propagules from the substrate. Thus, increases in the second year of mid/late-successional crusts and corticated species, which initially were rare, suggest the use of refugia to grow to a size that is impervious to grazing (see Lubchenco, 1983). Strong benefits of complexity to the poor colonizers - corticated species and to CCA - support the refugia theory. Studies have shown that CCA is strongly inhibited by aerial exposure (Benedetti-Cecchi, 2006), which crevices may relieve by reducing irradiance, temperature and evaporation (see also Jackson et al., 2013 for effects on microphytobenthos). Furthermore, rapid increases in fleshy encrusting algae that were nearly synchronous with establishment of the dominant space-occupying, canopy-forming alga *S. australis* (between Jan-Mar 2011) also point to the importance of stress in this system, as the algal canopy likely reduced temperature and increased water retention. Although physical stress was not directly manipulated as in some studies (e.g. Williams et al., 2013; Dethier et al., 2005; Bulleri et al., 2012 and Korpinen et al., 2007 via depth), this study encompassed some of the heterogeneity that is so characteristic of rocky intertidal systems. Including this variation implicitly in the design creates conservative estimates of the factors of interest (nutrients and herbivory), and indicates their larger effects across some of the larger within-site variability seen in rocky shores, and also allows for estimation of their effects as covariates.

Herbivory was unequivocally the driving force of algal cover in the upper mid intertidal in this system. However, both nutrient enrichment and reduction of herbivory had demonstrable yet complex effects on algal diversity and structure that were mediated by

time and by habitat complexity. While grazers had the more dramatic and immediate effects, nutrients influenced the community in nearly all metrics in the second year. Furthermore, the effect of nutrients to slow successional processes has important implications for the recovery trajectory once natural herbivory and nutrient levels are restored. Less diverse, early-successional communities occurring with herbivore reduction and concurrent enrichment may recover more quickly than later-successional communities developed under reduced herbivory with ambient nutrient loads. Overall, our results suggest that improved mechanistic understanding of the effects of nutrients and herbivores on community biodiversity will necessitate refinement of resource- and consumption-diversity models, likely incorporating further context dependency in additional factors such as seasonality, stress, and successional stage (Pfaff et al., 2010).

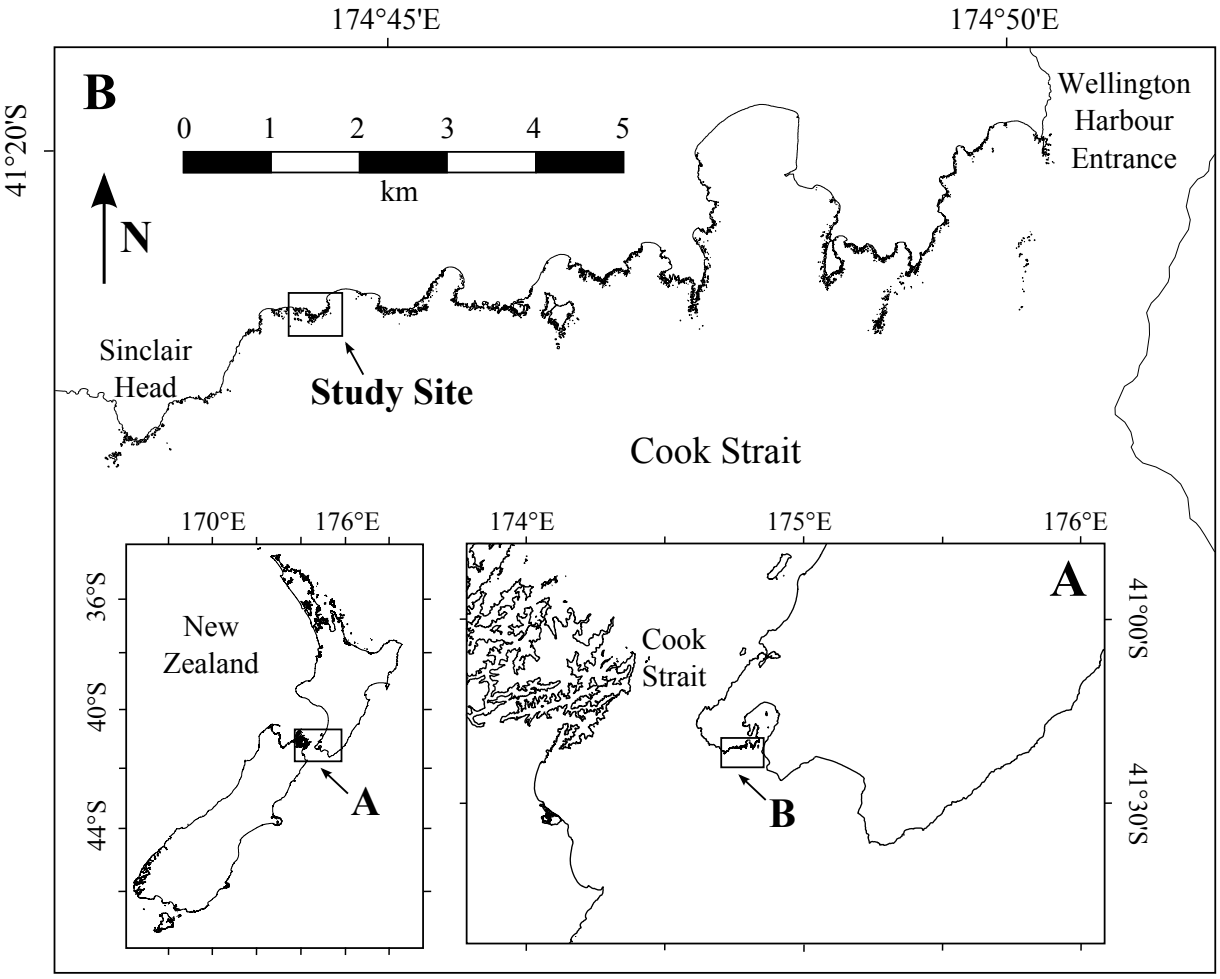

Figure 4.1: Site location in Cook Strait on the South Coast of Wellington, New Zealand.

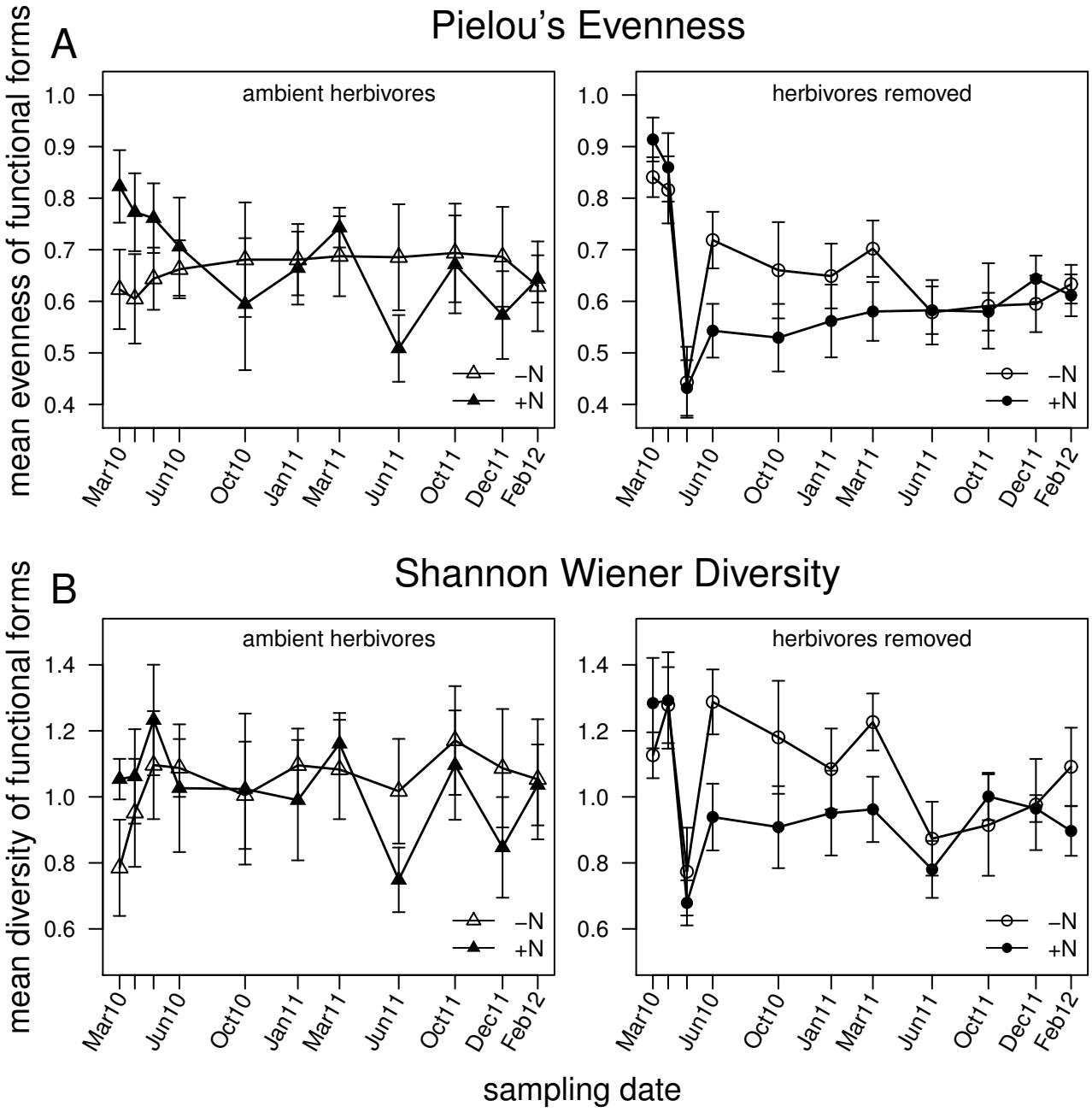

Figure 4.2: (A) Pielous Evenness (J) and (B) Shannon-Wiener Diversity (H) of algal functional form percent cover in each treatment from initial community composition (March 2010) through the experimental endpoint (February 2012). Austral winter is roughly May to October; austral summer is roughly November to April.

Table 4.1: Linear mixed models of functional form-based measures of algal community diversity: (A) Pielou's Evenness and (B) Shannon-Wiener diversity. Complexity refers to topographic complexity.

A) Pielou's Evenness (J)

Random effects	Model: ∼1|plot	
	(Intercept)	Residual
StdDev:	0.075	0.152

Fixed effects	Model: J ∼ N * G * time + season + complexity					
	estimate	SE	df	t-value	p-value	
(Intercept)	0.614	0.049	225	12.5	0	
N+	0.132	0.066	18	2	0.06	
G-	0.083	0.066	18	1.26	0.223	
time	0	0	225	0.67	0.505	
season	-0.049	0.015	225	-3.31	0.001	**
complexity	0.822	0.344	18	2.39	0.028	*
N+:G-	-0.14	0.091	18	-1.55	0.139	
N+:time	0	0	225	-2.67	0.008	**
G-:time	0	0	225	-2.07	0.040	*
N+:G-:time	0	0	225	2.1	0.037	*

B) Shannon-Wiener Diversity (H)

Random effects	Model: ∼1|plot	
	(Intercept)	Residual
StdDev:	0.16	0.259

Fixed effects	Model: H ∼ N * G * time + season + complexity					
	estimate	SE	df	t-value	p-value	
(Intercept)	-1.0927	0.8127	225	-1.345	0.18	
N+	0.1593	0.1272	18	1.253	0.226	
G-	0.194	0.1267	18	1.531	0.143	
time	0.0002	0.0001	225	1.14	0.256	
season	-0.0482	0.0261	225	-1.851	0.066	
complexity	1.7878	0.6956	18	2.57	0.019	*
N+:G-	-0.1808	0.1751	18	-1.033	0.316	
N+:time	-0.0004	0.0002	225	-2.167	0.031	*
G-:time	-0.0004	0.0002	225	-2.151	0.033	*
N+:G-:time	0.0004	0.0003	225	1.494	0.137	

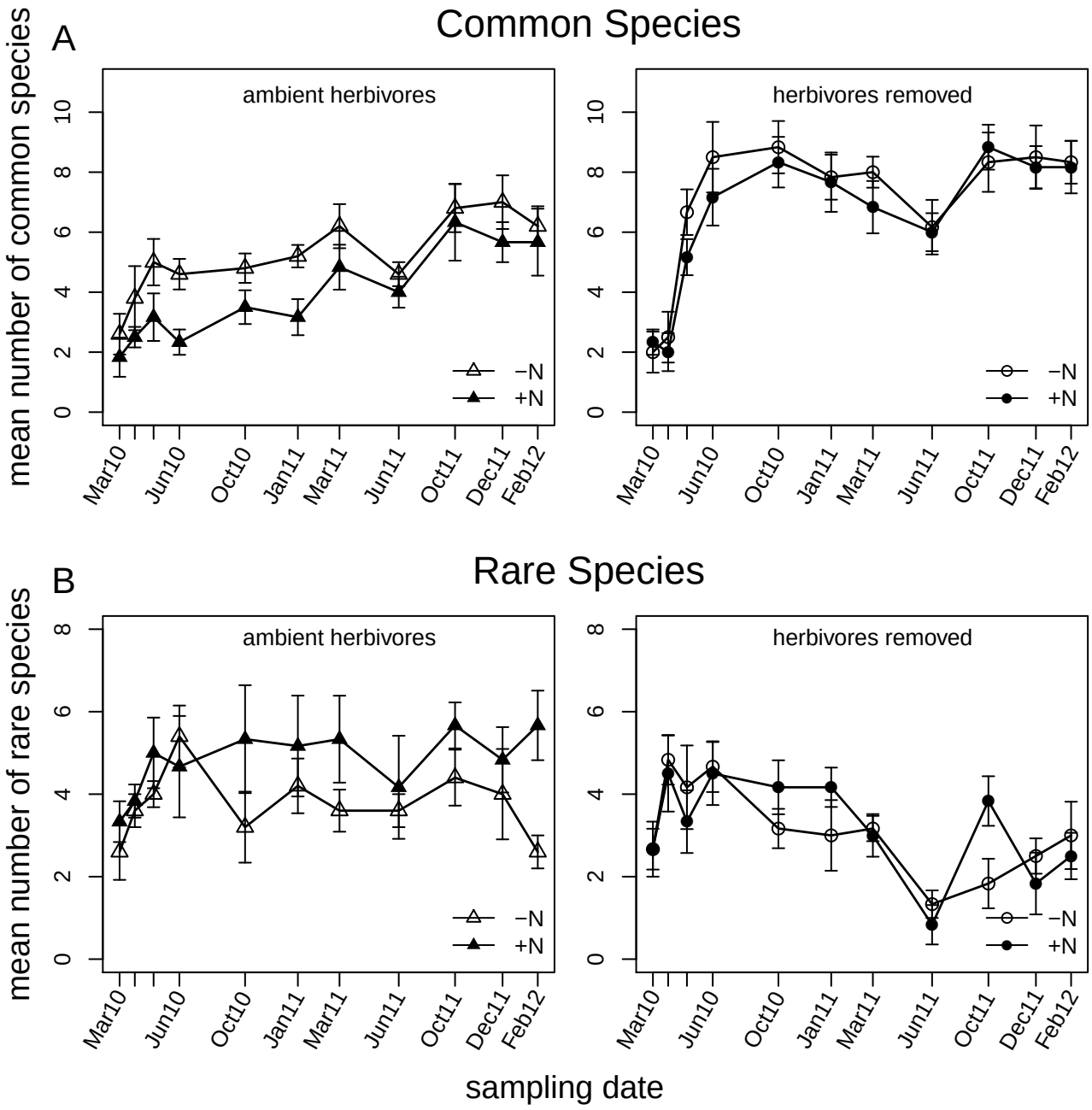

Figure 4.3: Species richness of (A) only species with >1% cover (common species) and (B) those species with <1% cover (rare species) across treatments from initial community composition (March 2010) through the experimental endpoint (February 2012).

Table 4.2: Linear mixed models of measures of algal species richness: (A) common species richness (>1% cover) and (B) rare species richness (<1% cover). Motion refers to water movement and complexity refers to topographic complexity.

A) Common Species Richness

Random effects	Model: ∼1|plot	
	(Intercept)	Residual
StdDev:	0.651	1.884

Fixed effects	Model: Common ∼ N * G + time + season + complexity					
	estimate	SE	df	t-value	p-value	
(Intercept)	3.736	0.447	228	8.354	0	
N+	-1.004	0.536	18	-1.875	0.077	
G-	1.882	0.533	18	3.533	0.002	**
time	0.004	0.001	228	7.926	0.000	***
season	0.909	0.194	228	4.691	0.000	***
complexity	11.484	3.355	18	3.423	0.003	**
N+:G-	1.045	0.736	18	1.418	0.173	

B) Rare Species Richness

Random effects	Model: ∼1|plot	
	(Intercept)	Residual
StdDev:	0.488	1.735

Fixed effects	Model: Rare ∼ N + G + time + motion + N:time + G:time					
	estimate	SE	df	t-value	p-value	
(Intercept)	4.26	0.399	226	10.671	0	
N+	0.016	0.426	19	0.038	0.97	
G-	-0.139	0.417	19	-0.333	0.743	
time	0	0.001	226	-0.18	0.857	
season	0.267	0.179	226	1.495	0.136	
motion	0.028	0.014	19	1.957	0.065	
N+:time	0.001	0.001	226	1.276	0.203	
G-:time	-0.003	0.001	226	-3.682	0.000	***

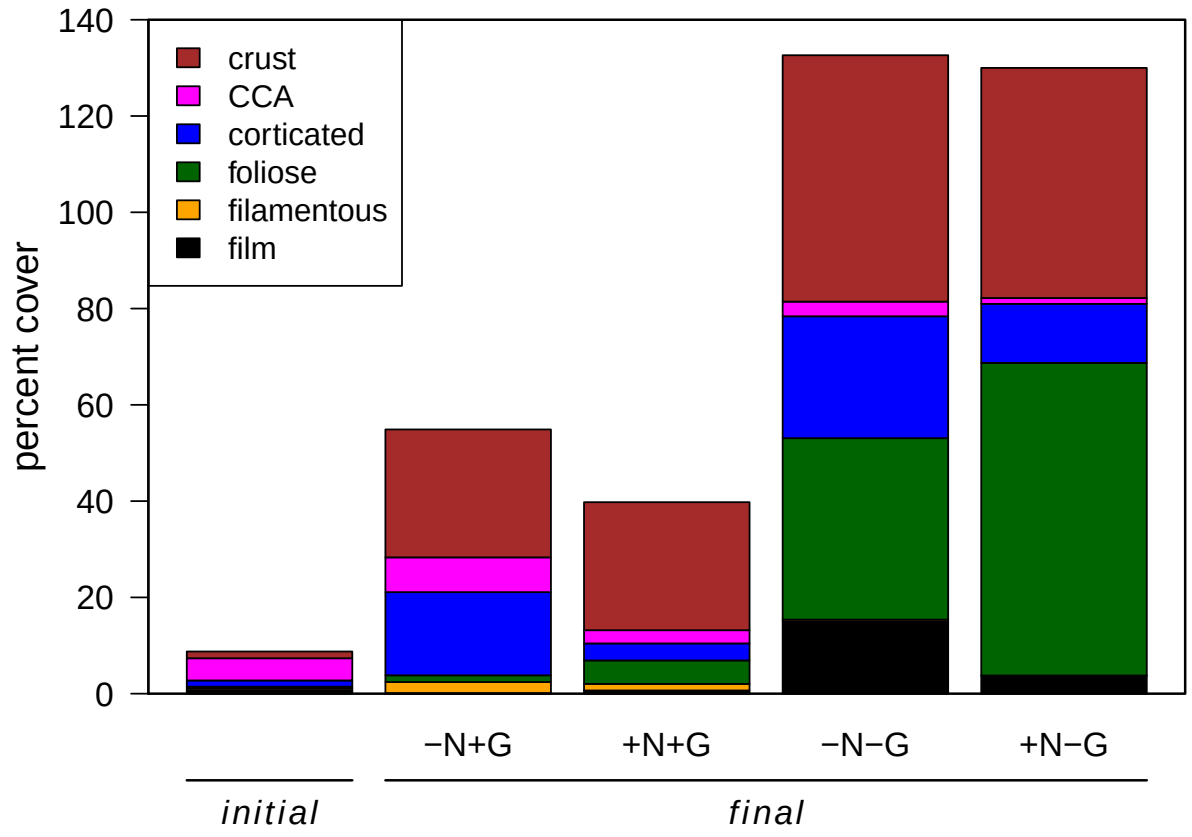

Figure 4.4: Composition of the algal community by functional groupings within each treatment combination at the beginning of the experiment (initial: March 2010) and at the end (final: February 2012). The leathery group is omitted as average % cover within a treatment was always less than 1% (0–0.69% range; 0.02% average cover at initial time).

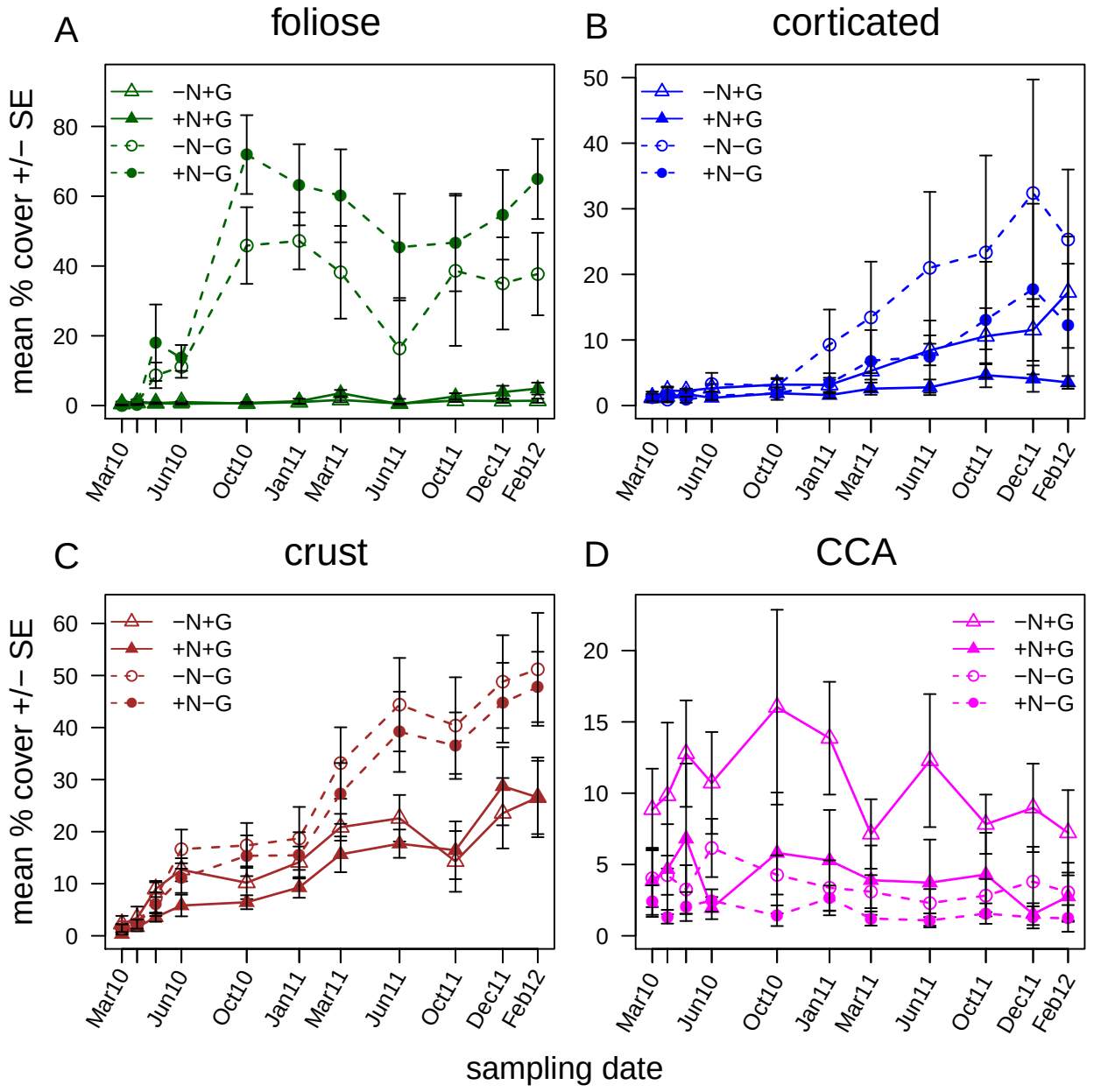

Figure 4.5: Percent cover of the four dominant functional forms: (A) foliose (B) corticated (C) crustose and (D) CCA across each treatment combination from the beginning of the experiment (March 2010) to the end (February 2012). Note scale changes on Y-axes.

Table 4.3: Linear mixed models of abundance (percent cover) of algal functional forms: (A) foliose, (B) corticated, (C) non-calcareous crustose, and (D) CCA. Data is square root transformed prior to analyses so estimates do not represent changes in % cover between treatments. Motion refers to water movement and complexity refers to topographic complexity.

(a) Cover of foliose forms

Random effects	Model: ∼1|plot	
	(Intercept)	Residual
StdDev:	0.089	0.176

Fixed effects:	foliose ∼ N + G + time + season + complexity + N:time + G:time					
	estimate	SE	df	t-value	p-value	
(Intercept)	1.8351	0.4695	226	3.91	0	
N+	-0.0333	0.0542	19	-0.61	0.546	
G-	0.1337	0.0535	19	2.5	0.022	*
time	-0.0001	0.0001	226	-1.57	0.119	
season	0.0867	0.0188	226	4.61	0	***
complexity	-1.4852	0.4002	19	-3.71	0.002	**
N+:time	0.0002	0.0001	226	2	0.046	*
G-:time	0.0006	0.0001	226	6.29	0	***

(b) Cover of corticated forms

Random effects	Model: ∼1|plot	
	(Intercept)	Residual
StdDev:	0.102	0.116

Fixed effects:	corticated ∼ N + G + time + slope + complexity + N:time + G:time					
	estimate	SE	df	t-value	p-value	
(Intercept)	-1.0646	0.4935	227	-2.16	0.032	
N+	0.025	0.0516	18	0.48	0.635	
G-	0.0072	0.0523	18	0.14	0.891	
time	0.0003	0.0001	227	6.29	0	***
slope	-0.0051	0.0032	18	-1.62	0.122	
complexity	1.1134	0.4356	18	2.56	0.02	*
N+:time	-0.0003	0.0001	227	-4.3	0	***
G-:time	0.0002	0.0001	227	4.09	0	***

(c) Cover of non-calcareous encrusting forms

Random effects	Model: ∼1|plot	
	(Intercept)	Residual
StdDev:	0.088	0.117

Fixed effects:	crust ∼ N + G + time + season + motion + complexity + G:time					
	estimate	standard error	df	t-value	p-value	
(Intercept)	-0.4276	0.4298	227	-0.99	0.321	
N+	-0.0016	0.0445	18	-0.04	0.972	
G-	0.0321	0.0459	18	0.7	0.493	
time	0.0006	0	227	12.49	0	***
season	0.043	0.0124	227	3.47	0.001	**
motion	-0.0032	0.0019	18	-1.63	0.12	
complexity	0.6329	0.3745	18	1.69	0.108	
G-:time	0.0004	0.0001	227	6.07	0	***

(d) Cover of CCA

Random effects	Model: ∼1|plot	
	(Intercept)	Residual
StdDev:	0.07	0.063

Fixed effects:	CCA ∼ N + G + time + season + slope + complexity + N:G					
	estimate	standard error	df	t-value	p-value	
(Intercept)	-1.38	0.333	228	-4.14	0	
N+	-0.121	0.045	17	-2.67	0.016	*
G-	-0.086	0.046	17	-1.89	0.076	
time	0	0	228	-4.96	0	***
season	0.015	0.006	228	2.29	0.023	*
slope	-0.005	0.002	17	-2.44	0.026	*
complexity	1.581	0.295	17	5.36	0	***
N+:G-	0.118	0.062	17	1.9	0.074	

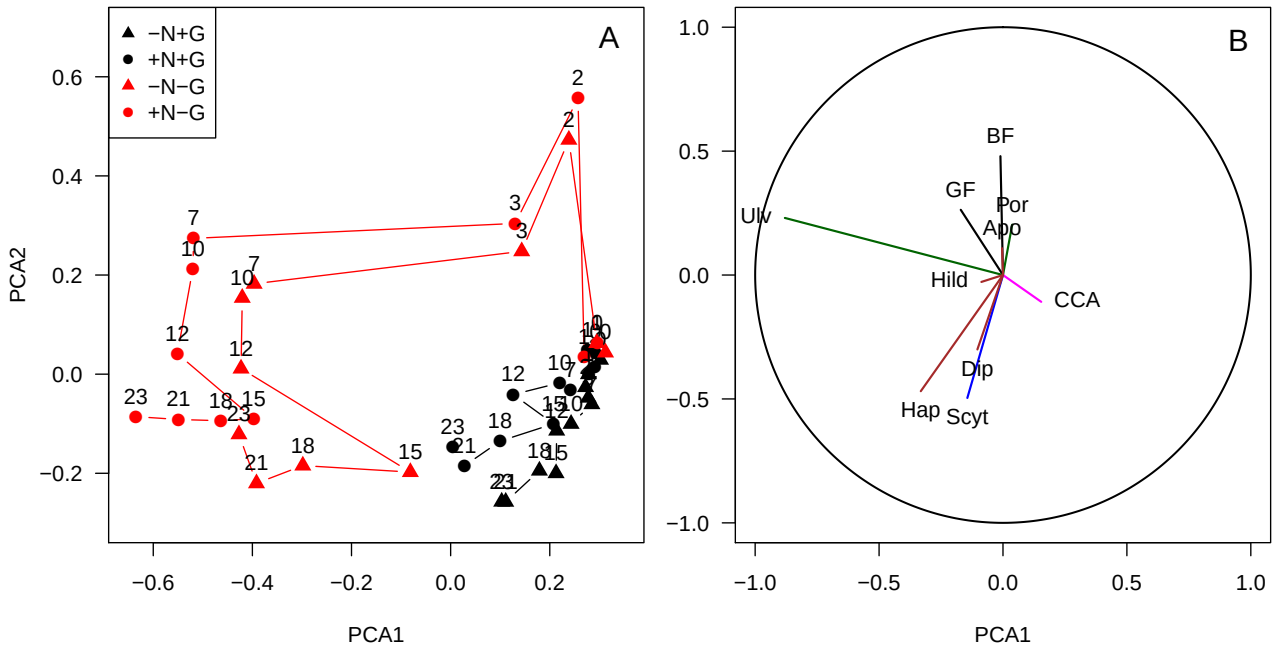

Figure 4.6: Visualization of algal community succession among treatments plotted as multivariate ordination of multivariate centroids calculated using principal components analysis (PCA). (A) Succession trajectory following average centroids plotted by each time x treatment combination, where numbers represent months since experimental onset. (B) PCA showing the 10 dominant taxonomic groups responsible for the 2 principal axes (PCA1 and PCA2), where the length of the vector indicates the strength – its contribution to that component. Vectors are colored by the functional forms they represent. Black indicates films (BF = black film and GF = green film); green indicates foliose species (Ulv = *Ulva intestinalis* and Por = *Porphyra* spp.); blue indicates corticated species (Scyt = *Scytothamnus australis*); pink indicates CCA; and brown indicates other encrusting species (Hap = *Hapalospongidion gelatinosum*; Dip = *Diplura australis*; Apo = *Apophlaea sinclairii*; Hild = *Hildenbrandia* spp.)

Appendix

4.A Site details and justification

The vast majority of past studies examining interactions of resource supply and herbivory on rocky shore algal community dynamics and diversity have been conducted in areas with relatively high natural levels of resource supply and productivity, such as eastern boundary currents or the eutrophic Baltic Sea (e.g. Nielsen, 2001; Worm et al., 2002; Guerry, 2008; Williams et al., 2013). In addition, those studies conducted in the relatively lower productivity North Atlantic have been on shallow subtidal rocky shore locations (e.g. Bokn et al., 2003; Kraufvelin et al., 2006). However, there is strong evidence that thermal stress may differentially alter the effects of nutrient and herbivory on algal diversity (Werner and Matthiessen, 2013) and drive intertidal community dynamics (Morelissen and Harley, 2007; Bertocci et al., 2010; Gedan et al., 2011). These studies as well as those explicitly examining variation in herbivore and nutrient effects on algal communities with height on shore (e.g. Bulleri et al., 2012; Williams et al., 2013) suggest that conclusions of studies conducted in shallow subtidal rocky shore communities (e.g. Worm et al., 2002; Kraufvelin et al., 2006; Korpinen et al., 2007; Kraufvelin et al., 2010) may not be relevant for communities higher on the shore, where both environmental stress (see Williams et al., 2013) and nutrient limitation (due to limited time for uptake; Bulleri et al., 2012) are more pronounced.

New Zealand was selected as the larger experimental region for its relatively low productivity (Viner and Wilkinson, 1988; Bradford-Grieve et al., 1999; Schiel, 2004), and specifically rocky intertidal shores in Cook Strait (Figure 4.1). New Zealand is not on an Eastern Boundary Current and, although some generalizations have been drawn about oceanography of the South Island (upwelling West coast, downwelling East coast; see Menge et al., 2003), in the Cook Strait Region coastal oceanography is complex (Schiel, 2004). Regional differences in nutrient supply are more likely to be driven by tidal dynamics and wind forcing through Cook Strait (Bradford et al., 1986; Walters et al., 2010) or localized terrestrial runoff (Barr, 2007). Overall, with low water column nutrient content and low

number of species, this site represents a relatively low-productivity, low-diversity location in comparison to many temperate rocky reefs worldwide.

The experiment was conducted in the upper-mid intertidal, where communities are likely to be most vulnerable to anthropogenic change (Bracken, 2004). The rationale is that, at higher tidal heights, increasing nutrient levels are unlikely to sufficiently ameliorate harsh physical extremes to cause large shifts in these low-diversity communities. This may be particularly true in New Zealand where levels of UV are high and biotic cover in the upper intertidal is sparse compared to other temperate rocky reefs. In the low intertidal, communities may be less affected by nutrients due to high rates of flushing. In the mid-intertidal zone, conversely, moderate daily exposure times ameliorate environmental extremes, but limit uptake to submerged periods. Thus this zone may be considered a nutrient-poor habitat in comparison to the larger system (Bracken, 2004). The lower range of the zone of study was established using the low intertidal species *Hormosira banksii* to determine the functional elevation (after Harley and Helmuth, 2003).

Average ambient water column nutrient levels (taken across summer and winter sampling dates) were 0.48 $\pm$ 0.043 μM NO_3, 0.12 $\pm$ 0.016 μM NO_2 and 9.46 $\pm$ 0.34 μM NH_4 (mean $\pm$ SE, $n = 62$). Measurements ranged nearly an order of magnitude, however, among sampling times (0.034 to 4.35 μM NO_3; 0.0081 to 0.68 μM NO_2; 1.00 to 34.18 μM NH_4). Levels of PO_4 were 0.39 $\pm$ 0.037 μM on average but ranged from 0.011 to 3.26 μM.

4.B Experimental methods

4.B.1 Algal methods

All algae beneath a point-intercept in the surveys were identified to the lowest possible taxonomic unit and classed within a functional form grouping. Groupings were based on a modification of Steneck and Dethier's (1994) classification system where the commonly occurring crustose calcareous algae (CCA) was grouped with the very rare articulated coralline species rather than the crustose algae. This was done because fleshy crustose algae became prevalent in plots without herbivores, while CCA (and articulated corallines) were always confined to cracks and crevices, and thus seemed to occupy a very different niche that warranted distinguishing. Algal species present in plots but not recorded with the point-intercept surveys were recorded as above but assigned 0.5%. (Each point-intercept represented 1%). Although this is commonly practiced (e.g. Masterson et al., 2008; Guerry et al., 2009), we ran a sensitivity analysis to check that the assigned value did not arbitrarily affect the model outcome. We found that that the assumed abundance assigned to rare species (checked for values between 0.2-0.8) neither affected the terms retained in the model nor the parameter estimates (qualitatively). P-values remained relatively constant. Thus all results were reported with 0.5% assigned to rare species.

4.B.2 Herbivore methods

Diloma aethiops, *Austrolittorina cincta*, and *Risselopsis varia* were not manipulated due to their high variability in occurrence and small size (latter two). In initial surveys ($n = 46$), these three species represented only 7.6%, 1.15%, and 0.15% of herbivore biomass respectively (Table 4.B.2), although *D. aethiops* was only 3.3% of biomass in experimental surveys (Table 4.B.3). In reality, the biomass percentage for littorinids is much lower because we were unable to remove the snail tissue from the shell and thus this species is the only herbivore in which the biomass estimates per individual include shell mass (0.013-0.016 g per individual, calculated as a 95% confidence interval bootstrapped from 50 sampled

Table 4.B.1: Functional form groupings for the observed distinct taxonomic units of algae, based on a modification of Steneck and Dethiers (1994) classification system.

Species or taxonomic unit		**Functional group**
brown film (diatom film)	1	Film/Microalgae
green film	1	Film/Microalgae
Cladophora spp.	2	Filamentous algae
Polysiphonia spp.	2	Filamentous algae
green, red, or brown filamentous	2	Filamentous algae
Ulva spp. (foliose)	3	Foliose algae
Ulva spp. (tubular)	3	Foliose algae
Porphyra spp.	3	Foliose algae
Blidingia sp.	3	Foliose algae
unidentified foliose	3	Foliose algae
Endarachne binghamiae	3.5	Corticated foliose
Gelidium caulacantheum	4	Corticated macrophytes
Gelidium pusillum	4	Corticated macrophytes
Caulacanthus ustulatus	4	Corticated macrophytes
Nothogenia fastigiata	4	Corticated macrophytes
Nothogenia pulvinata	4	Corticated macrophytes
Scytothamnus australis	4	Corticated macrophytes
Colpomenia peregrina	4	Corticated macrophytes
Colpomenia sinuosa	4	Corticated macrophytes
Leathesia spp.	4	Corticated macrophytes
Splachnidium rugosum	4	Corticated macrophytes
unidentified red turf	4	Corticated macrophytes
unidentified branched brown	4	Corticated macrophytes
Hormosira banksii	5	Leathery macrophytes
coralline (erect)	6	Erect coralline algae
crustose coralline algae	6.5	Encrusting coralline algae
Apophlaea sinclairii	7	Crustose algae
Diplura australis	7	Crustose algae
Hapalospongidion gelatinosum	7	Crustose algae
Ralfsia spp.	7	Crustose algae
Hildenbrandia spp.	7	Crustose algae
Pseudolithoderma spp.	7	Crustose algae
unidentified brown or red crust	7	Crustose algae

individuals). Moreover, these species movement is uninhibited by copper paint. Due to their mobility, we did not attempt to maintain any specified density in ambient grazer plots, but we did remove them from herbivore removal plots. For *A. cincta* and *R. varia*, we recorded numbers only because the size range was not drastic enough to cause biologically significant variation in per plot biomass. Each individual of *A. cincta* was multiplied by the average biomass for collected individuals, 0.0147 g. Only one individual of *R. varia* was collected due to the uncommonness with which they were found. Maximum shell diameter was 5.3 mm with a total weight of 0.0364 g of which 0.008 g was tissue biomass. Across sampling dates, numbers of *A. tincta* per ambient herbivore plot were, on average (mean individuals per plot ± SE and equivalent biomass (g) ± SE), 11.70 ± 1.52 (0.179g ± 0.027), *R. varia* were 1.81 ± 0.32 (0.015g ± 0.0026), and *D. aethiops* were 1.35 ± 0.30 (0.33g ± 0.050). Chitons were rare and highly variable in occurrence, with 0.122 ± 0.032 individuals per plot at an average length (± SE) of 14.5 ± 1.2 mm.

Table 4.B.2: Percent composition of total herbivore biomass in initial surveys

Species	**% of total biomass**
C. denticulata	81.03
C. radians	6.31
C. ornata	2.45
D. aethiops	7.6
S. australis	1.31
A. cincta	1.15
R. varia	0.15

4.B.3 Herbivores biomass

Because abundance of herbivores is a highly imprecise measure of the force of herbivory, the size and number of individuals was used to calculate a total measure of biomass in each plot. To do so, we developed relationships between shell length and wet tissue biomass for the main herbivore species. Tissue mass of individuals within a plot could then

Table 4.B.3: Average % biomass composition in ambient grazers plots across all sampling dates (Mar10-Feb12): where % biomass contribution of each species in each ambient grazer plot was averaged for a sampling event ($n = 11$) and then all sampling events ($n = 11$) were averaged together.

Species	**Mean % of biomass**	**SE**
C. denticulata	82.07	1.33
C. radians	4.79	0.9
C. ornata	2.76	1.03
D. aethiops	3.33	0.45
S. australis	4.98	0.81
A. cincta	1.9	0.25
R. varia	0.17	0.032

be summed to give per plot estimates of herbivore biomass, and thus the force of herbivory. We collected and dissected individuals (*C. denticulata* $n = 36$; *C. radians* $n = 27$; *C. ornata* $n = 18$; *D. aethiops* $n = 19$; *S. australis* $n = 15$) and removed tissue from the shell. Exponential relationships were used to relate the measured shell length (or height for *D. aethiops*) to the weight of tissue (g) (equations and R^2 values in Table 4.B.4).

Table 4.B.4: Regression coefficients representing the relationship between herbivore length (or height: *D. aethiops*) and biomass, where biomass $= a \cdot e^{length \cdot b}$.

Species	**a**	**b**	**R^2**
C. denticulata	0.0154	0.1456	0.95662
C. radians	0.0111	0.1505	0.95285
C. ornata	0.0104	0.1923	0.91397
D. aethiops	0.0154	0.1713	0.87887
S. australis	0.0074	0.2119	0.87326

4.C Treatment efficacy and environmental parameters

4.C.1 Treatment efficacy

Small weight percentages of osmocote lost while deployed in the field (Table 4.C.1) prompted increased dosage of nutrients. Initially, only osmocote was added in 75 g amounts. After 1 month, we mixed 15 g of urea with the osmocote to simulate a nutrient pulse. In November 2010, after 8 months, we increased the volume of the dispensers with PVC extenders and began adding 230 g osmocote, which was changed every 6 weeks. We continued to add urea every other week, but in 20 g amounts, through the duration of the experiment (for a timeline, see Table 4.C.2).

Despite variation in ambient NO_3 and NH_4 concentrations among dates (date accounted for 50% and 24% of the residual variation of NO_3 and NH_4, respectively), linear mixed models showed that both nutrients were significantly higher in enriched plots than in ambient plots ($p = 0.0027$ and $p = 0.0019$, respectively; Figure 4.C.1, Table 4.C.3, 4.C.4). Nutrient enrichment resulted in a 0.3 μM increase in NO_3, on average, and a 4.8 μM increase in NH_4. Models accounted for nutrient treatment and its interaction with the number of days since the last fresh nutrient addition (see Table 4.C.2) as well as relative water motion in each plot (as measured by clod cards described below; all fixed factors) and random variability in baseline/ambient nutrient levels introduced by different dates of sampling (random factor). Concentrations of PO_4 were variable across times and treatments with no significant effect of enrichment. Levels of NO_2 were extremely low and are not shown here.

At the experimental onset and prior to herbivore removals, the mean value of herbivore biomass was 9.28 $\pm$ 1.66 g tissue per plot (58.3 $\pm$ 10.42 g m^{-2}). Herbivore density and biomass stayed relatively constant throughout the experimental duration (Figure 4.C.2), and limpets were transplanted in as needed. Linear mixed models showed that there was no difference in herbivore biomass between nutrient treatments (+N+G and N+G) at any time ($p = 0.76$). Even with incomplete removal (-G plots always had a couple *A. littorina* or *D. aethiops* at the time of sampling, which were subsequently removed), herbivore biomass was orders of magnitude higher in +G plots ($p < 0.0001$) (Table 4.C.5).

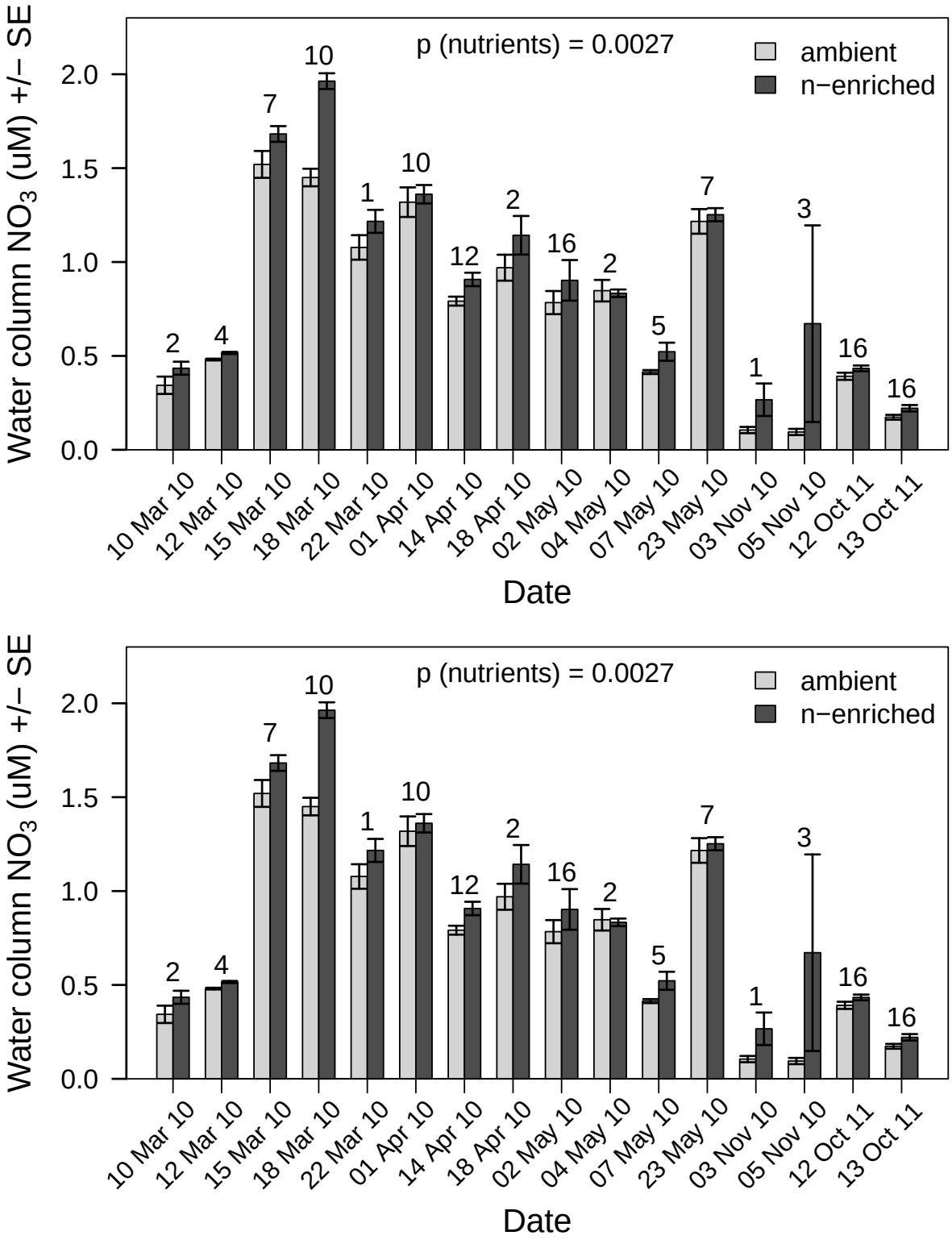

Figure 4.C.1: Water column nutrient levels taken throughout the year at varying times after experimental nutrient addition. (A) nitrate (NO_3) and (B) ammonium (NH_4).

Table 4.C.1: Percent loss by weight of osmocote after two weeks diffusing nutrients in the field ($n = 18$). Small losses prompted increases in dispenser volume and osmocote weight as well as doubling the number of holes in the diffuser cylinder.

Start Date	**End Date**	**Mean % weight loss**	**SE**	**Days in oven at 60 °C**
2-Apr-10	16-Apr-10	3.90	0.42	9
16-Apr-10	2-May-10	6.22	0.32	7
2-May-10	16-May-10	5.98	0.50	7

Table 4.C.2: Timeline of nutrient addition in terms of (A) date on which fertilizer was exchanged for fresh, (B) weight (g) of fertilizer added, (C) dates on which water samples were taken, with number of plots sampled in parentheses and (D) number of days since last nutrient exchange when water samples were taken. $(\cdots)$ indicate nutrients continued to be added in the intervals and amounts specified.

A. Osmocote Change Dates	**B. Amount**	**C. Water Sample Dates**	**D. Days after Change**
		8-Mar-2010 (36)	0 (initial)
		10-Mar-2010 (36)	2
8-Mar-10	50 osmo	12-Mar-2010 (12)	4
		15-Mar-2010 (35)	7
		18-Mar-2010 (12)	10
		22-Mar-2010 (36)	1
20-Mar-10	75 osmo	1-April-2010 (12)	10
		8-Apr-2010 (12)	6
2-Apr-10	75 osmo	14-April-2010 (36)	12
16-Apr-10	75 osmo	18-April-2010 (36)	2
		2-May-2010 (36)	16
2-May-10	75 osmo, 15 urea	4-May-2010 (12)	2
		7-May-2010 (12)	5
16-May-10	75 osmo, 15 urea		
31-May-10	75 osmo, 15 urea	23-May-2010 (12)	7
14-Jun-10	75 osmo, 15 urea		
$\cdots$	75 osmo, 15 urea		
26-Sep-10	75 osmo, 15 urea		
7-Oct-10	87.5 osmo, 17.5urea		
21-Oct-10	87.5 osmo, 17.5urea		
		3-Nov-2010 (35)	1
2-Nov-10	230 osmo, 20 urea	5-Nov-2010 (20)	3
6-Dec-10	230 osmo, 20 urea		
$\cdots$	230 osmo, 20 urea		
10-Aug-11	230 osmo, 20 urea		
		12-Oct-2011 (17)	16
26-Sep-11	230 osmo, 20 urea	13-Oct-2011 (25)	16
8-Nov-11	230 osmo, 20 urea		
$\cdots$	230 osmo, 20 urea		

Table 4.C.3: Results of the linear mixed model on nitrate (NO_3) concentrations above ambient vs. enriched plots at varying times during the experimental duration. Samples were taken during various conditions (surgy and calm to represent conservative estimates).

Random effects	Model: ∼1 | Date	
	Intercept	**Residual**
Standard deviation	0.331	0.325

Fixed effects	Model: NO3 ∼ N addition · Days since addition + water motion				
	Value	**SE**	**df**	**t-value**	**p-value**
Intercept	0.744	0.209	350	3.555	0.0004
N1	0.202	0.052	350	3.862	0.0001
days	0.007	0.022	14	0.31	0.7612
water motion	-0.001	0.002	350	-0.52	0.6031
N : days	-0.009	0.006	350	-1.655	0.0989

Table 4.C.4: Results of the linear mixed model on ammonium (NH_4) concentrations above ambient vs enriched plots at varying times during the experimental duration.

Random effects	Model: ∼1 | Date	
	Intercept	**Residual**
Standard deviation	1.685	5.346

Fixed effects	Model: NH_4 ∼ N addition · Days since addition + water motion				
	Value	**SE**	**df**	**t-value**	**p-value**
Intercept	13.889	1.867	209	7.438	0
N1	3.219	1.023	209	3.147	0.0019
days	0.067	0.116	15	0.578	0.572
water motion	-0.098	0.035	209	-2.828	0.0051
N : days	-0.212	0.121	209	-1.761	0.0797

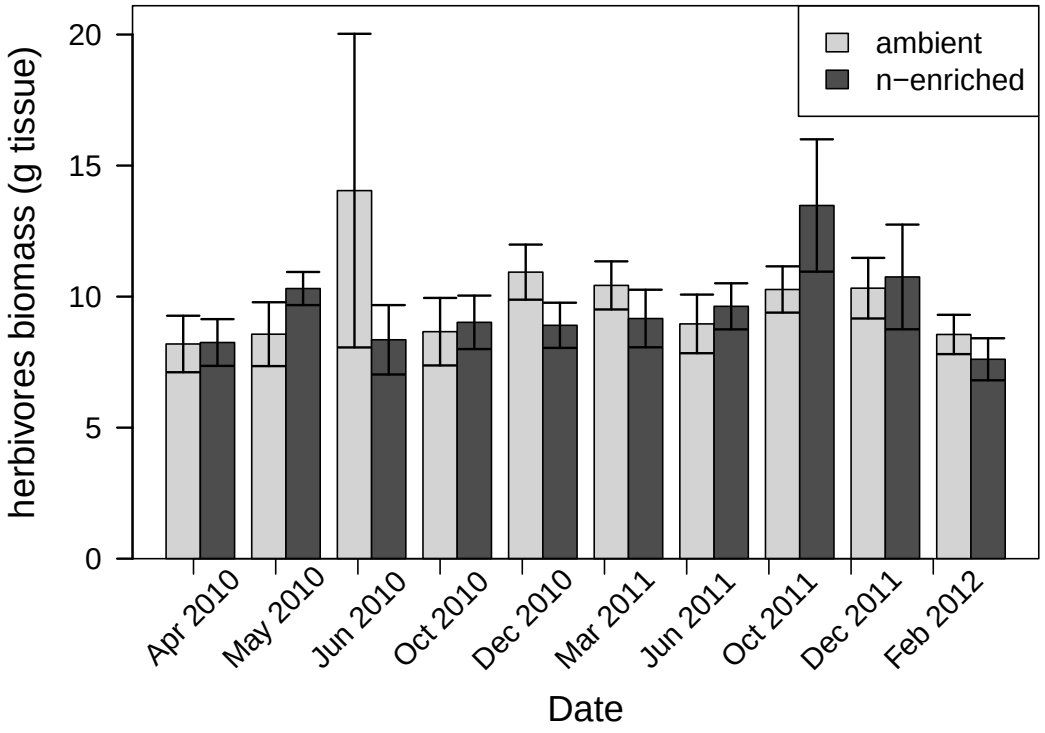

Figure 4.C.2: Biomass of herbivores in plots with ambient herbivore communities in both enriched and ambient nutrient treatments. Bars are means averaged across all +G plots of each nutrient treatment (ambient and +N) ± SE.

Table 4.C.5: Results of the linear mixed model on herbivore biomass examining if there were inadvertent differences in ambient grazer numbers at sampling times during the experimental duration.

Random effects	Model: ∼1 | Date	
	Intercept	**Residual**
Standard deviation	0.381	2.790

Fixed effects	Model: biomass ∼ N · G + time				
	Value	**SE**	**df**	**t-value**	**p-value**
Intercept	-0.008	0.538	206	-0.014	0.989
N+	-0.228	0.571	19	-0.399	0.694
G-	9.992	0.599	19	16.676	0
Time	0.099	0.066	206	1.5	0.135
N1:G1	-0.324	0.828	19	-0.392	0.700

4.C.2 Environmental parameters

Plots were established on heterogeneous habitat with varying environmental parameters. Various measures characterizing habitat heterogeneity were taken to include in statistical models (Table 4.C.6). Parameters included relative habitat complexity, measured as the length of a fine-link chain draped across the diameter of each plot relative to the absolute diameter (0.45m). We took three regular measurements horizontally across the plot and three from top to bottom, averaging all six measurements to give an estimate of complexity. Slope was determined by averaging three measurements taken using a protractor attached to a level. Relative water motion was determined using clod cards (Jokiel and Morrissey, 1982; Thompson and Glenn, 1994). Clod cards were made of hydrocal gypsum cement, which has slower dissolution rates than plaster, and were constructed in mini muffin tins to weigh approximately 40 g. Clod cards were placed adjacent to each plot and left in the field until one reached roughly $\frac{1}{3}$ its original size ($\sim$3 days). Dissolution has been shown to occur disproportionately rapidly once a critical mass is lost ($\sim$70% loss; Jokiel and Morrissey, 1982). At this time, all clod cards were collected, dried, and reweighed to determine % loss. Relative light and temperature were measured using data loggers (HOBO) placed in each plot for 24 h over the course of a week and standardized by a control, which remained in one location throughout the week to account for between day differences in light intensity.

Table 4.C.6: Environmental (habitat) data for each plot, where the treatment assignment is represented by N (nutrients, two levels: with (+) and without (-)) and G (grazers, two levels: stocked with ambient (+) and removal (-)). Slope is degrees from horizontal. Relative water motion is clod card percent mass lost in 3 d. Topographic complexity is represented by actual diameter as measured by a fine-link chain divided by absolute diameter (0.45 m). Light and temperature are both relative measures representing proportion (light) or difference in summation (temperature) over 24 h relative to and standardized by a control plot.

Plot	**N**	**G**	**slope**	**water motion**	**topographic complexity**	**light**	**temperature**
1	+	-	30.5	48.82	1.12	1.15	74.9
2	-	-	33	47.19	1.13	1.26	41.8
3	-	+	25.5	47.6	1.15	1.15	138.8
4	+	+	32.5	68.5	1.24	0.92	-154.8
5	-	-	38.5	59.18	1.15	1.1	-271.7
6	+	+	16.5	68.7	1.09	1.03	9.4
7	+	-	19	78.04	1.05	1.15	-136.5
8	-	-	31.5	52.64	1.15	1.05	-93.1
9	+	+	10.5	44.09	1.21	1.25	222.5
10	-	+	17	47.93	1.19	0.74	-578.9
11	+	-	26	52.98	1.17	0.63	-529
12	+	+	21	41.33	1.09	1	0
13	-	-	23	40.15	1.07	1.23	173.3
14	+	-	31	49.17	1.06	1.2	55.3
15	-	+	22	33.44	1.12	1.05	249.8
16	-	-	20	55.85	1.2	1.41	-93.7
17	-	+	37	47.38	1.25	0.81	-203.2
18	+	-	36	43.22	1.17	1.16	-60.6
19	+	+	37.5	61.51	1.13	0.74	-416
20	-	-	26.5	40.35	1.18	1.06	-102.6
21	+	+	22.5	38.43	1.07	0.99	-2.8
22	-	+	26	38.64	1.09	0.98	108.6
23	+	-	13.5	37.19	1.04	1.15	-2

4.D Additional experimental results

In addition to functional diversity and evenness, calculated on the functional groupings of algae as outlined in Appendix 4.B, calculations of diversity and evenness were also made on the full resolution dataset, i.e. all species data (or lowest taxonomic grouping). Shannon-Wiener diversity was not affected by either nutrients or herbivores over the duration of the experiment, but was driven by positive effects of increased topographic complexity (Table 4.D.1). Seasonal patterns had marginally significant effects with increased diversity during late spring and summer months. Total numbers of species ranged from 0 to 20 per plot over the duration of the experiment (median = 9), with a maximum of 14 species having >1% cover (common species; median = 5) and a maximum of 11 rare species (<1% cover; median = 4).

In contrast, patterns of evenness were strongly driven by nutrient addition, where nutrients decreased diversity over time, but were unaffected by herbivore reduction (Table 4.D.2). Season had similar effects with increased evenness in late spring and summer months.

Table 4.D.1: Shannon Wiener Diversity (H) (utilizing all species percent cover including rare species at 0.5%)

Random effects	Model: ∼1 | Date	
	Intercept	**Residual**
Standard deviation	0.252	0.34

Fixed effects	Model: H ∼ N + G + time + season + topographic complexity				
	Value	**SE**	**df**	**t-value**	**p-value**
(Intercept)	-1.6965	1.2274	228	-1.382	0.168
N+	0.0772	0.12	19	0.643	0.528
G-	0.007	0.118	19	0.06	0.953
time	0.0002	0.0001	228	1.738	0.084
season	0.0659	0.0363	228	1.817	0.071
complexity	2.6276	1.0478	19	2.508	0.021*

Table 4.D.2: Pielou's Evenness (J) (utilizing all species percent cover including rare species at 0.5%).

Random effects	Model: ∼1 | Date	
	Intercept	**Residual**
Standard deviation	0.075	0.155

Fixed effects	Model: J ∼ N · G · time + season + water motion + complexity					
	Value	**SE**	**df**	**t-value**	**p-value**	
(Intercept)	-0.1794	0.4046	225	-0.443	0.658	
N+	0.1609	0.07	17	2.3	0.034	*
G-	0.0586	0.0676	17	0.868	0.398	
time	0.0001	0.0001	225	0.976	0.33	
season	-0.0375	0.0151	225	-2.476	0.014	*
water motion	0.0027	0.0019	17	1.451	0.165	
complexity	0.5565	0.3522	17	1.58	0.133	
N+:G-	-0.1541	0.0929	17	-1.66	0.115	
N+:time	-0.0003	0.0001	225	-2.675	0.008	**
G-:time	-0.0001	0.0001	225	-1.226	0.221	
N+:G-:time	0.0002	0.0002	225	1.446	0.15	

Total algal cover was driven by the absence of grazers (Figure 4.D.1A), with a lag time in response creating a significant grazer by time interaction (Table 4.D.3). In the absence of grazers, cover rapidly increased to over 100%. Prior to experimentation, substrate had 8.8 ± 1.4% algal cover, nearly 70% of which was encrusting (CCA 51%, other crusts 19%) and confined to micro complexities in the rock (R. Clausing, pers. obs), with upright macroalgae covering only 2.8% of the substrate. In contrast, after 2 years of experimental removal of herbivores, over 80% of the substrate was covered by upright macroalgae.

Nutrients were also important, interacting with herbivory such that when herbivores were removed, the addition of nutrients initially caused further increases in cover, whereas in the presence of herbivores, nutrient addition resulted in reduced algal cover throughout the experiment. Significant seasonality suggests that total cover peaks in late winter/early spring.

The percentage of open space roughly mirrored that of total cover, with some exceptions due to layering (Figure 4.D.1B). The removal of grazers decreased open space from >90% to 10% within two months (Table 4.D.4). Nutrients again interacted with herbivory, causing further reductions to nearly 0% in herbivore removal (-G) plots where nutrients were added, but increasing open space relative to ambient nutrient levels (-N+G) in the presence of grazers (+N+G). Even plots with herbivores showed a decline from 76 ± 9.6% to 60 ± 5.6% over the two years. No environmental parameters had an effect on the percentage of either open space or total algal cover, indicating the heterogeneity in the environmental affects the composition of the community and the diversity, but not the total cover.

In each functional group, 1 or 2 species made up the majority of cover and showed similar patterns to their group (foliose: Ulva spp., ∼60% after the first 3 months; corticated: the NZ endemic *Scytothamnus australis*, ∼60% after and ∼50% during the first 3 months; crustose: *Hapalospongidion gelatinosum* and *Diplura australis*, ∼45% after the first 3 months. The effects of nutrients at the experimental end were most visible in their effects on corticated and foliose cover when herbivores were removed, driving communities of differing composition in spite of similar cover. With ambient grazers, enriched plots differed from ambient ones primarily in reduced cover of CCA and corticated species.

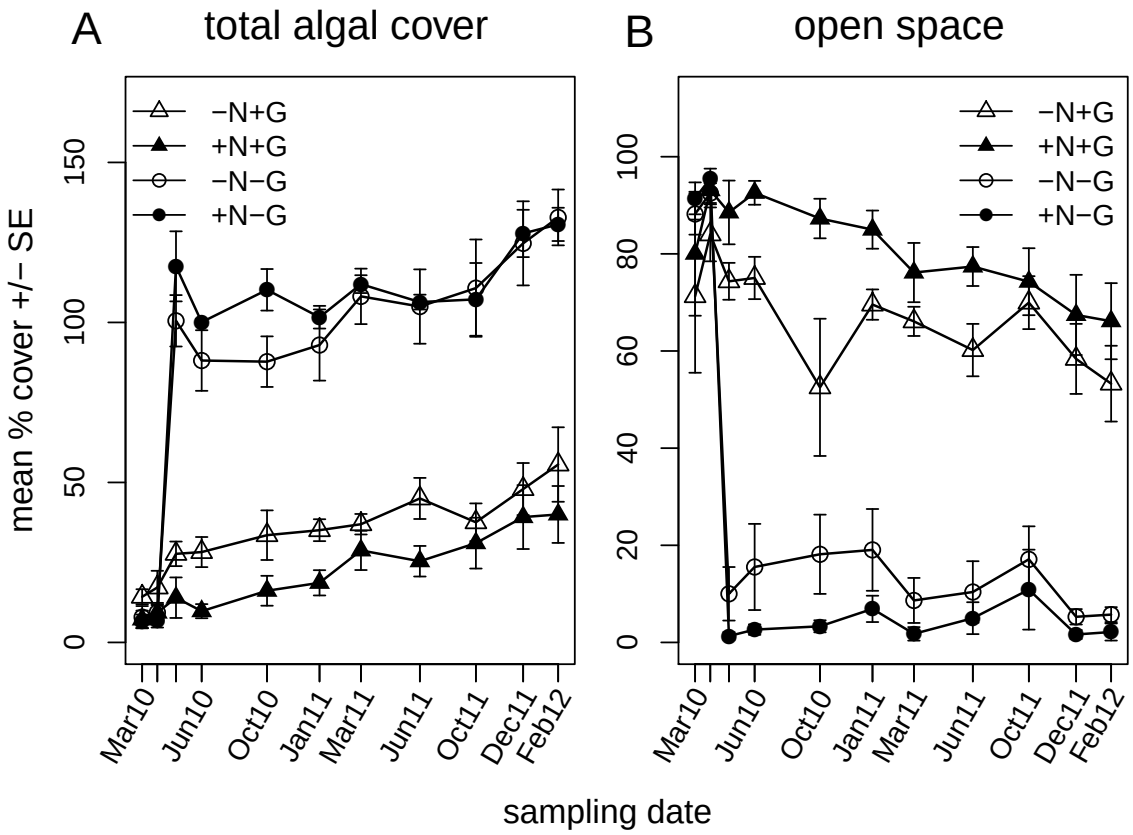

Figure 4.D.1: Percent cover of (A) total algal cover and (B) open space across each treatment combination from the beginning of the experiment (March 2010) to the end (February 2012). Note scale changes on Y-axes.

Table 4.D.3: Results of a linear mixed effect model on total algal percent cover.

Random effects	Model: ∼1 | Date	
	Intercept	**Residual**
Standard deviation	0.045	0.167

Fixed effects	Model: cover ∼ N + G + time + season + slope + N:G + G:time					
	Value	**SE**	**df**	**t-value**	**p-value**	
N+	-0.1143	0.0417	18	-2.74	0.013	*
G-	0.182	0.0501	18	3.64	0.002	**
time	0.0004	0.0001	227	5.75	0.000	***
season	0.0524	0.0161	227	3.25	0.001	**
slope	-0.0031	0.0019	18	-1.62	0.124	
N+:G-	0.1289	0.0573	18	2.25	0.037	*
G-:time	0.0004	0.0001	227	5.07	0.000	***

Table 4.D.4: Results of a linear mixed effect model on percent cover of open space.

Random effects	Model: ∼1 | Date	
	Intercept	**Residual**
Standard deviation	0.004	23.20

Fixed effects	Model: open space ∼ N · G + time + slope + G:time					
	Value	**SE**	**df**	**t-value**	**p-value**	
N+	14.659	4.315	18	3.4	0.003	**
G-	-25.744	5.772	18	-4.46	0	***
time	-0.029	0.009	228	-3.35	0.001	**
slope	0.35	0.196	18	1.78	0.092	
N+:G-	-19.885	5.936	18	-3.35	0.004	**
G-:time	-0.05	0.012	228	-4.14	0	***

Table 4.D.5: Results of a permutational manova (10 000 permutations) on algal percent cover data, grouped by functional forms.

Model: functional form cover ∼ N · G · time					
	df	**MS**	F **value**	P **value**	
N	1	1.49	9.32	0.0001	***
G	1	12.63	78.89	0.0001	***
time	1	9.83	61.41	0.0001	***
N:G	1	0.73	4.59	0.0012	**
N:time	1	0.36	2.24	0.0474	*
G:time	1	1.44	9	0.0001	***
N:G:time	1	0.11	0.69	0.6414	
Residuals	245	0.16	0.6		

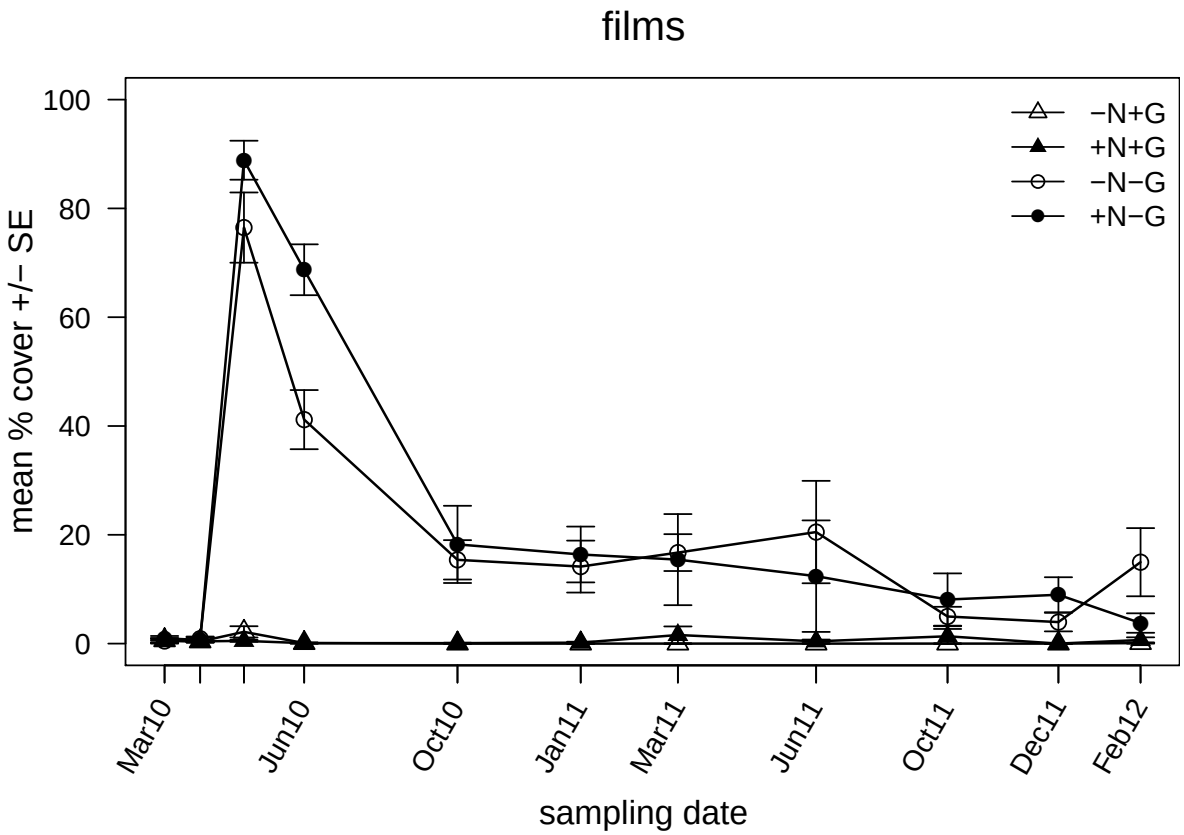

Figure 4.D.2: Cover of algal films throughout the experimental duration from March 2010 – February 2012.

Table 4.D.6: Results of a linear mixed effect model on percent cover of algal film.

Random effects	Model: ∼1 | Date	
	Intercept	**Residual**
Standard deviation	0.000	0.251

Fixed effects	Model: Films ∼ N · G · time + season + G:time					
	Value	**SE**	**df**	**t-value**	**p-value**	
N+	0.019	0.032	20	0.58	0.568	
G-	0.477	0.052	20	9.178	0	***
time	0	0	227	0.434	0.665	
season	0.103	0.025	227	4.106	0	***
G-:time	0	0	227	-2.868	0.005	**

Table 4.D.7: PCA loadings for the ten species most aligned with PCA1 and PCA2 (longest vector length).

Code	**Species**	**Functional group**	**pca1**	**pca2**	**length**
BrF	brown film	Film	-0.010	0.480	0.480
GF	green film	Film	-0.170	0.263	0.314
Rfo	*Porphyra spp.*	Foliose	0.037	0.203	0.207
Ent	*Ulva intestinalis*	Foliose	-0.880	0.230	0.910
Scyt	*Scytothamnus australis*	Corticated	-0.144	-0.496	0.517
CCA	CCA	CCA	0.154	-0.108	0.188
Apo	*Apophlaea sinclairii*	Crustose	-0.004	0.108	0.108
Dipl	*Diplura australis*	Crustose	-0.103	-0.300	0.318
Hap	*Hapalospongidion gelatinosum*	Crustose	-0.332	-0.469	0.574
Hild	*Hildenbrandia spp.*	Crustose	-0.087	-0.028	0.092

References

Anderson, M. J. 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences **58**:626–639.

Atalah, J., and T. P. Crowe. 2010. Combined effects of nutrient enrichment, sedimentation and grazer loss on rock pool assemblages. Journal of Experimental Marine Biology and Ecology **388**:51–57.

Barr, N. G., 2007. Aspects of nitrogen metabolism in the green alga *Ulva*: developing an indicator of seawater nitrogen loading.

Benedetti-Cecchi, L. 2006. Understanding the consequences of changing biodiversity on rocky shores: How much have we learned from past experiments? Journal of Experimental Marine Biology and Ecology **338**:193–204.

Bertocci, I., E. Maggi, S. Vaselli, and L. Benedetti-Cecchi. 2010. Resistance of rocky shore assemblages of algae and invertebrates to changes in intensity and temporal variability of aerial exposure. Marine Ecology Progress Series **400**:75–86.

Best, R., A. Chaudoin, and M. Bracken. 2014. Plant-animal diversity relationships in a rocky intertidal system depend on invertebrate body size and algal cover. Ecology **95**:1308–1322.

Blanchette, C. A. 1996. Seasonal patterns of disturbance influence recruitment of the sea palm, *Postelsia palmaeformis*. Journal of Experimental Marine Biology and Ecology **197**:1–14.

Bokn, T. L., C. M. Duarte, M. F. Pedersen, N. Marba, F. E. Moy, C. Barron, B. Bjerkeng, J. Borum, H. Christie, S. Engelbert, F. L. Fotel, E. E. Hoell, R. Karez, K. Kersting, P. Kraufvelin, C. Lindblad, M. Olsen, K. A. Sanderud, U. Sommer, and K. Sorensen. 2003. The response of experimental rocky shore communities to nutrient additions. Ecosystems **6**:577–594.

Bracken, M. E. S. 2004. Invertebrate-mediated nutrient loading increases growth of an intertidal macroalga. Journal of Phycology **40**:1032–1041.

Bracken, M. E. S., and N. H. N. Low. 2012. Realistic losses of rare species disproportionately impact higher trophic levels. Ecology letters **15**:461–7.

Bracken, M. E. S., E. Jones, and S. L. Williams. 2011. Herbivores, tidal elevation, and species richness simultaneously mediate nitrate uptake by seaweed assemblages. Ecology **92**:1083–1093.

Bradford, J. M., P. P. Lapennas, R. A. Murtagh, F. H. Chang, and V. Wilkinson. 1986. Factors controlling summer phytoplankton production in greater Cook Strait, New Zealand. New Zealand Journal of Marine and Freshwater Research **20**:253–279.

Bradford-Grieve, J., P. Boyd, F. Chang, S. Chiswell, M. Hadfield, J. Hall, M. James, S. Nodder, and E. Shushkina. 1999. Pelagic ecosystem structure and functioning in the Subtropical Front region east of New Zealand in austral winter and spring 1993. Journal of Planktonic Research **21**:405–428.

Breves-Ramos, A., H. P. Lavrado, A. D. R. Junqueira, and S. H. G. da Silva. 2005. Succession in rocky intertidal benthic communities in areas with different pollution levels at Guanabara Bay (RJ-Brazil). Brazilian Archives of Biology and Technology **48**:951–965.

Bulleri, F., B. D. Russell, and S. D. Connell. 2012. Context-dependency in the effects of nutrient loading and consumers on the availability of space in marine rocky environments. PloS one **7**:e33825.

Connell, J., and R. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. American Naturalist **111**:1119–1144.

Connell, J. H. 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology **42**:710–723.

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. Science **199**:1302–1310.

Crawley, M. 2007. The R book. John Wiley & Sons, Ltd., West Sussex, England.

Cubit, J. D. 1984. Herbivory and the seasonal abundance of algae on a high intertidal rocky shore. Ecology **65**:1904–1917.

Currie, D. R., and G. D. Parry. 1999. Changes to benthic communities over 20 years in Port Phillip Bay, Victoria, Australia. Marine Pollution Bulletin **38**:36–43.

Dayton, P. K. 1971. Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. Ecological Monographs **41**:351–389.

Denno, R. F., D. Lewis, and C. Gratton. 2005. Spatial variation in the relative strength of top-down and bottom-up forces: causes and consequences for phytophagous insect populations. Annales Zoologici Fennici **42**:295–311.

Dethier, M. 1982. Pattern and process in tidepool algae: factors influencing seasonality and distribution. Botanica Marina **XXV**:55–66.

Dethier, M., S. Williams, and A. Freeman. 2005. Seaweeds under stress: manipulated stress and herbivory affect critical life-history functions. Ecological Monographs **75**:403–418.

Duffy, J. E. 1990. Amphipods on seaweeds: partners or pests? Oecologia **83**:267–276.

Duffy, J. E. 2003. Biodiversity loss, trophic skew and ecosystem functioning. Ecology Letters **6**:680–687.

Farrell, T. M. 1991. Models and mechanisms of succession: An example from a rocky intertidal community. Ecological Monographs **61**:95–113.

Folke, C., S. Carpenter, B. Walker, M. Scheffer, T. Elmqvist, L. Gunderson, and C. S. Holling. 2004. Regime shifts, resilience, and biodiversity in ecosystem management. Annual Review of Ecology and Systematics **35**:557–581.

Freidenburg, T. L., B. A. Menge, P. M. Halpin, M. Webster, and A. Sutton-Grier. 2007. Cross-scale variation in top-down and bottom-up control of algal abundance. Journal of Experimental Marine Biology and Ecology **347**:8–29.

Gedan, K. B., J. Bernhardt, M. D. Bertness, and H. M. Leslie. 2011. Substrate size mediates thermal stress in the rocky intertidal. Ecology **92**:576–82.

Gray, J. S., and H. Christie. 1983. Predicting long-term changes in marine benthic communities. Marine Ecology-Progress Series **13**:87–94.

Gripenberg, S., and T. Roslin. 2007. Up or down in space? Uniting the bottom-up versus top-down paradigm and spatial ecology. Oikos **116**:181–188.

Guerry, A. D. 2008. Interactive effects of grazing and enrichment on diversity; conceptual implications of a rocky intertidal experiment. Oikos **117**:1185–1196.

Guerry, A. D., B. A. Menge, and R. A. Dunmore. 2009. Effects of consumers and enrichment on abundance and diversity of benthic algae in a rocky intertidal community. Journal of Experimental Marine Biology and Ecology **369**:155–164.

Harley, C., and B. Helmuth. 2003. Local-and regional-scale effects of wave exposure, thermal stress, and absolute versus effective shore level on patterns of intertidal zonation. Limnology and Oceanography **48**:1498–1508.

Hawkins, S. J., and R. G. Hartnoll. 1983. Grazing of intertidal algae by marine invertebrates. Oceanography and Marine Biology **21**:195–282.

Hay, M., and W. Fenical. 1988. Marine plant-herbivore interactions: the ecology of chemical defense. Annual review of ecology and systematics **19**:111–145.

Hillebrand, H. 2003. Opposing effects of grazing and nutrients on diversity. Oikos **100**:592–600.

Hughes, T. P., A. H. Baird, D. R. Bellwood, M. Card, S. R. Connolly, C. Folke, R. Grosberg, O. Hoegh-Guldberg, J. B. C. Jackson, and J. Kleypas. 2003. Climate change, human impacts, and the resilience of coral reefs. Science **301**:929–933.

Huston, M. 1979. A general hypothesis of species diversity. The American Naturalist **113**:81–101.

Huston, M. 1994. Biological diversity: The coexistence of species on changing landscapes. Cambridge University Press.

Jackson, A. C., R. J. Murphy, and A. J. Underwood. 2013. Biofilms on rocky shores: Influences of rockpools, local moisture and temperature. Journal of Experimental Marine Biology and Ecology **443**:46–55.

Jackson, J. B. C., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury, R. Cooke, J. Erlandson, and J. A. Estes. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science **293**:629–637.

Jara, V. C., J. H. S. Miyamoto, B. A. P. Gama, M. Molis, M. Wahl, and R. C. Pereira. 2006. Limited evidence of interactive disturbance and nutrient effects on the diversity of macrobenthic assemblages. Marine Ecology Progress Series **308**:37–48.

Jokiel, P. L., and J. Morissey. 1982. Water motion on coral reefs: evaluation of the 'clod card' technique. Marine Ecology Progress Series **93**:175–181.

Kondoh, M. 2001. Unifying the relationships of species richness to productivity and disturbance. Proceedings of the Royal Society of London Series B-Biological Sciences **268**:269–271.

Korpinen, S., V. Jormalainen, and T. Honkanen. 2007. Bottom-up and cascading top-down control of macroalgae along a depth gradient. Journal of Experimental Marine Biology and Ecology **343**:52–63.

Korpinen, S., V. Jormalainen, and T. Honkanen. 2007. Effects of nutrients, herbivory, and depth on the macroalgal community in the rocky sublittoral. Ecology **88**:839–852.

Kraufvelin, P., A. Lindholm, M. F. Pedersen, L. A. Kirkerud, and E. Bonsdorff. 2010. Biomass, diversity and production of rocky shore macroalgae at two nutrient enrichment and wave action levels. Marine Biology **157**:29–47.

Kraufvelin, P., F. E. Moy, H. Christie, and T. L. Bokn. 2006. Nutrient addition to experimental rocky shore communities revisited: delayed responses, rapid recovery. Ecosystems **9**:1076–1093.

Lotze, H. K., and B. Worm. 2002. Complex interactions of climatic and ecological controls on macroalgal recruitment. Limnology and Oceanography **47**:1734–1741.

Lubchenco, J. 1978. Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. The American Naturalist **112**:23–39.

Lubchenco, J. 1983. *Littornia* and *Fucus*: effects of herbivores, substratum heterogeneity, and plant escapes during succession. Ecology pages 1116–1123.

Lubchenco, J., and B. A. Menge. 1978. Community development and persistence in a low rocky Intertidal Zone. Ecological Monographs **48**:67–94.

Masterson, P., F. A. Arenas, R. C. Thompson, and S. R. Jenkins. 2008. Interaction of top down and bottom up factors in intertidal rockpools: Effects on early successional macroalgal community composition, abundance and productivity. Journal of Experimental Marine Biology and Ecology **363**:12–20.

McNaughtan, D., 2007. The distribution and influence of the introduced alga *Colpomenia bullosa* in the rocky intertidal. Master of science, Victoria Univeristy of Wellington.

Menge, B. A. 1976. Organization of the New England rocky intertidal community: role of predation, competition, and environmental heterogeneity. Ecological Monographs **46**:355–393.

Menge, B. A. 1997. Detection of direct versus indirect effects: were experiments long enough? The American Naturalist **149**:801–823.

Menge, B. A. 2000. Top-down and bottom-up community regulation in marine rocky intertidal habitats. Journal of Experimental Marine Biology and Ecology **250**:257–289.

Menge, B. A., C. Blanchette, P. Raimondi, T. Freidenburg, S. Gaines, J. Lubchenco, D. Lohse, G. Hudson, M. Foley, and J. Pamplin. 2004. Species interaction strength: testing model predictions along an upwelling gradient. Ecological Monographs **74**:663–684.

Menge, B. A., M. M. Foley, J. Pamplin, G. Murphy, and C. Pennington. 2010. Supply-side ecology, barnacle recruitment, and rocky intertidal community dynamics: Do settlement surface and limpet disturbance matter? Journal of Experimental Marine Biology and Ecology **392**:160–175.

Menge, B. A., J. Lubchenco, and L. R. Ashkenas. 1985. Diversity, heterogeneity and consumer pressure in a tropical rocky intertidal community. Oecologia **65**:394–405.

Menge, B. A., J. Lubchenco, M. E. S. Bracken, F. Chan, M. M. Foley, T. L. Freidenburg, S. D. Gaines, G. Hudson, C. Krenz, H. Leslie, D. N. L. Menge, R. Russell, and M. S. Webster. 2003. Coastal oceanography sets the pace of rocky intertidal community dynamics. Proceedings of the National Academy of Sciences of the United States of America **100**:12229–12234.

Menge, B. A., and A. M. Olson. 1990. Role of scale and environmental factors in regulation of community structure. Trends in Ecology & Evolution **5**:52–7.

Morelissen, B., and C. D. Harley. 2007. The effects of temperature on producers, consumers, and plantherbivore interactions in an intertidal community. Journal of Experimental Marine Biology and Ecology **348**:162–173.

Morton, J. E., and M. Miller. 1973. The New Zealand seashore. 2nd edition. Collins, London.

Murray, S. N., and M. M. Littler. 1978. Patterns of algal succession in a perturbated marine inter-tidal community. Journal of Phycology **14**:506–512.

Nielsen, K. J. 2001. Bottom-up and top-down forces in tide pools: test of a food chain model in an intertidal community. Ecological Monographs **71**:187–217.

Nielsen, K. J. 2003. Nutrient loading and consumers: Agents of change in open-coast macrophyte assemblages. Proceedings of the National Academy of Sciences of the United States of America **100**:7660.

Oksanen, J., R. Blanchet, Guillaume F. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, and H. Wagner, 2012. vegan: Community Ecology Package. R package version 2.0-5. http://CRAN.R-project.org/package=vegan.

Oliveira, J., I. Bertocci, G. Weber, and I. Sousa-Pinto. 2011. Type and timing of disturbance modify trajectories of recovery of rockpool assemblages at Aguda (NW Portugal). Journal of Experimental Marine Biology and Ecology **399**:135–141.

Paine, R. T. 1966. Food web complexity and species diversity. The American Naturalist **100**:65–75.

Paine, R. T. 1988. Habitat suitability and local-population persistence of the sea palm textitPostelsia palmaeformis. Ecology **69**:1787–1794.

Pfaff, M. C., C. Hiebenthal, M. Molis, G. M. Branch, and M. Wahl. 2010. Patterns of diversity along experimental gradients of disturbance and nutrient supply: the confounding assumptions of the Intermediate Disturbance Hypothesis. African Journal of Marine Science **32**:127–135.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R. Development Core Team. 2012. nlme: Linear and Nonlinear Mixed Effects Models. R package version **3.1-105**.

Proulx, M., and A. Mazumder. 1998. Reversal of grazing impact on plant species richness in nutrient-poor vs. nutrient-rich ecosystems. Ecology **79**:2581–2592.

Quinn, G. G. P., and M. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge.

R Core Team, 2012. R: A language and environment for statistical computing.

Schiel, D. R. 2004. The structure and replenishment of rocky shore intertidal communities and biogeographic comparisons. Journal of Experimental Marine Biology & Ecology **300**:309–342.

Schoch, G. C., B. A. Menge, G. Allison, M. Kavanaugh, S. A. Thompson, and S. A. Wood. 2006. Fifteen degrees of separation: Latitudinal gradients of rocky intertidal biota along the California Current. Limnology and Oceanography **51**:2564–2585.

Smith, J. E., C. L. Hunter, and C. M. Smith. 2010. The effects of top-down versus bottom-up control on benthic coral reef community structure. Oecologia **163**:497–507.

Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution **100**:179–196.

Sousa, W. P. 1979. Disturbance in marine intertidal boulder fields: the nonequiibrium manteinance of species diversity. Ecology **60**:1225–1239.

Steneck, R. S. 1998. Human influences on coastal ecosystems: does overfishing create trophic cascades? Trends in Ecology & Evolution **13**:429–430.

Steneck, R. S., and M. N. Dethier. 1994. A functional group approach to the structure of algal-dominated communities. Oikos **69**:476–498.

Steneck, R. S., and L. Watling. 1982. Feeding capabilities and limitation of herbivorous molluscs: A functional group approach. Marine Biology **68**:299–319.

Suding, K. N., S. L. Collins, L. Gough, C. Clark, E. E. Cleland, K. L. Gross, D. G. Milchunas, and S. Pennings. 2005. Functional-and abundance-based mechanisms explain diversity loss due to N fertilization. Proceedings of the National Academy of Sciences **102**:4387–4392.

Thompson, T., and E. Glenn. 1994. Plaster standards to measure water motion. Limnology and Oceanography **39**:1768–1779.

Tilman, D. 1994. Competition and biodiversity in spatially structured habitats. Ecology **75**:2–16.

Tilman, D., J. Fargione, B. Wolff, C. D'Antonio, A. Dobson, R. Howarth, D. Schindler, W. H. Schlesinger, D. Simberloff, and D. Swackhamer. 2001. Forecasting agriculturally driven global environmental change. science **292**:281–284.

Underwood, A., and P. Jernakoff. 1984. The effects of tidal height, wave-exposure, seasonality and rock-pools on grazing and the distribution of intertidal macroalgae in New South Wales. Journal of Experimental Marine Biology and Ecology **75**:71–96.

Underwood, A. J., and P. G. Fairweather. 1989. Supply-side ecology and benthic marine assemblages. Trends in ecology & evolution **4**:16–20.

Underwood, A. J., and M. J. Keough, 2001. Supply-side ecology: the nature and consequences of variations in recruitment of intertidal organisms. Pages 183–200 *in* Marine Community Ecology. Sinauer Associates Inc, Sunderland.

Valiela, I., K. Foreman, M. LaMontagne, D. Hersh, J. Costa, P. Peckol, B. DeMeo-Andreson, C. DïAvanzo, M. Babione, and C. H. Sham. 1992. Couplings of watersheds and coastal waters: Sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. Estuaries and Coasts **15**:443–457.

Viner, A. B., and V. H. Wilkinson. 1988. Uptake of nitrate and ammonium, and distribution of related variables, in the upwelled plume of western Cook Strait/Taranaki Bight, New Zealand. New Zealand Journal of Marine and Freshwater Research **22**:565–576.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications **7**:737–750.

Walters, R. a., P. a. Gillibrand, R. G. Bell, and E. M. Lane. 2010. A study of tides and currents in Cook Strait, New Zealand. Ocean Dynamics **60**:1559–1580.

Werner, F. J., and B. Matthiessen. 2013. Temperature indirectly affects benthic microalgal diversity by altering effects of top-down but not bottom-up control. Oikos **122**:52–63.

Williams, S. L., M. E. S. Bracken, and E. Jones. 2013. Additive effects of physical stress and herbivores on intertidal seaweed biodiversity. Ecology **94**:1089–101.

Worm, B., and H. K. Lotze. 2006. Effects of eutrophication, grazing, and algal blooms on rocky shores. Limnology and Oceanography **51**:569–579.

Worm, B., H. K. Lotze, H. Hillebrand, and U. Sommer. 2002. Consumer versus resource control of species diversity and ecosystem functioning. Nature **417**:848–851.

Worm, B., H. K. Lotze, and U. Sommer. 2000*a*. Coastal food web structure, carbon storage, and nitrogen retention regulated by consumer pressure and nutrient loading. Limnology and Oceanography **45**:339–349.

Worm, B., H. K. Lotze, and U. Sommer. 2001. Algal propagule banks modify competition, consumer and resource control on Baltic rocky shores. Oecologia **128**:281–293.

Worm, B., T. B. H. Reusch, and H. K. Lotze. 2000*b*. In situ nutrient enrichment: methods for marine benthic ecology. International Review of Hydrobiology **85**:359–375.

CHAPTER 5

CONCLUSION

The role of nutrients in regulating macroalgal populations and communities, as well as its interactions with other important controlling factors such as herbivory, remains strongly debated on both tropical and intertidal temperate reefs. This research demonstrated that, at various scales across both temperate and tropical reefs, change in nutrient availability was a strong driver of macroalgal dynamics. However, its effects were, at times, overshadowed by overwhelming strength of herbivory. Moreover, responses to nutrient addition and rates of consumption varied among species or functional forms of algae and with environmental context, which often resulted in changes over both short and long timescales. On a tropical reef, environmental variability in sediment loads played a key role in altering control of macroalgal growth, whereas spatial variation in topographic complexity strongly altered the effects of nutrients and herbivory across algal community metrics on an intertidal temperate reef. Thus, across both tropical and intertidal temperate reefs, spatial and temporal environmental variability modulate a species-specific framework of nutrient and herbivore control. These results have important implications for understanding the complex and seemingly contradictory results of many previous studies in these systems. They suggest that sweeping generalizations of the consequences of nutrient enrichment and its interplay with herbivore control in any system are likely inappropriate. Instead, their effects may only be determined within its environmental context. This highlights the need to consider the appropriate temporal and spatial framework for the question and system under investigation.

In chapter 1, I demonstrated that nutrient limitation plays an important although highly dynamic role in determining algal species growth rates on an impacted tropical reef. The extreme variability in response to nutrients between species and rapid shifts over short

timescales demonstrated that the processes affecting algal nutrient limitation are operating on much finer temporal scales than previously considered. This is surprising in part because not only were all species exposed to the same nutrient and environmental history on the reef, but also all of these species are abundant and thrive on the reef flat. The variation in nutrient response suggests that they do so by differing mechanisms. The dynamic temporal shifts within species, on the other hand, appeared to be caused by changes in environmental conditions that affect nutrient supply, uptake ability, and growth. This is the first study to examine tropical algal limitation in connection with distinct changes in environmental conditions, and overall, our findings highlight the dynamic relationship between environmental context and macroalgal response to increasing nutrient loads.

In chapter 2, I showed that the environmental context of sediment loads altered control of reef flat macroalgae by modifying nutrient storage, patterns of herbivory, and growth. However, these effects varied among species and with environmental conditions that are likely to affect the supply of sediments and its characteristics (e.g. grain size, organics). In two dominant species on the reef flat, one appeared dependent on sediment loads to sustain positive growth, while the other exhibited temporally variable negative sediment effects, either by reducing growth potential or stimulating targeted herbivory on enriched apical meristems that are critical for growth. This research demonstrates that understanding the role of nutrients and herbivory on control of tropical reef macroalgae may be insufficient to predict changes in impacted communities unless temporal variability in environmental modulation of these processes, particularly the effects of sediments, is also taken into account.

In chapter 3, results indicated that nutrients and herbivory exert strong control on algal community diversity regardless of environmental heterogeneity. However, environmental context with regard to seasonality, stress (as modulated by topographic complexity), and successional stage also played an important role in driving compositional changes in the community, at least in part by shaping the effects of nutrients. In the initial six months, an unprecedented level of herbivore control eclipsed detection of nutrient effects, driving changes in biomass over an order of magnitude. Over the course of two years, in contrast,

nutrients influenced the community in nearly every metric and slowed successional processes where herbivores were reduced, maintaining communities dominated by early-successional species. Overall, these results indicate the importance of longer-term field studies implicitly measuring environmental context to distinguish the delayed yet extensive effects of nutrients, while elucidating its critical importance in differential community development that is likely to drive ecosystem properties.

This body of work demonstrates first and foremost, that anthropogenic changes in nutrient availability are likely to have strong effects across tropical and intertidal temperate reefs, particularly due to complex interactions or modifications by species-specific responses, herbivory, and environmental context. In doing so, it indicates the importance of a question- and context-specific framework for designing studies to assess the consequences of anthropogenic alteration to controls of macroalgal dynamics, regardless of the system under examination.