Title

Nutrient Enrichment Promotes Eutrophication in the Form of Macroalgal Blooms Causing Cascading Effects in Two Anthropogenically Disturbed Coastal Ecosystems

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Nutrient Enrichment Promotes Eutrophication in the Form of Macroalgal Blooms Causing Cascading Effects in Two Anthropogenically Disturbed Coastal Ecosystems

A dissertation submitted in partial satisfaction

of the requirements for the degree

Doctor of Philosophy in Biology

by

Tiara Nydia Moore

2019

ABSTRACT OF THE DISSERTATION

Nutrient enrichment promotes eutrophication in the form of macroalgal blooms causing cascading effects in two anthropogenically disturbed coastal ecosystems

by

Tiara Nydia Moore

Doctor of Philosophy in Biology

University of California, Los Angeles, 2019

Professor Peggy Marie Fong, Chair

Humans are impacting almost every major ecological process that structures communities and ecosystems. Examples of how human activity can directly control key processes in ecosystems include destruction of habitat changing trophic structure, nutrient pollution altering competitive outcomes, overharvesting of consumers reducing top down control, and now climate change impacting virtually every global biogeochemical cycle. These human impacts may have an independent effect on the ecosystem, but they also have the potential to cause cascading effects and promote subsequent stressors. Also, these impacts are not limited to a particular system or geographic location making research on their overall effects vital for management practices. For example, tropical reefs have been transitioning from coral to mixed communities dominated by macroalgae, motivating research on how macroalgae respond to anthropogenic stressors and interact with each other during these stressful events. Further, while eutrophication

of coastal estuaries due to increased anthropogenic supplies of nutrients has been of critical global concern for decades, the potential for eutrophication to drive new stressors is a growing concern. To address these knowledge gaps, I investigated how human stressors impact two different and major coastal ecosystems known to be vulnerable to anthropogenic disturbances.

In chapter 1, I demonstrate that anthropogenic stressors in the form of increased nutrients in the water and sediments have strong impacts on interspecific interactions of coral reef macroalgae. Abiotic stressors such as nutrients have been linked to phase-shifts from coral to algal domination on tropical reefs. However, few studies have considered how these stressors impact changes in the biotic and abiotic constituents of dominant species of calcifying macroalgae, and how this may be mediated by species-species interactions. I conducted 4 mesocosm experiments to examine whether different nutrient sources (water column vs. terrestrial sediment) as well as species interactions (alone vs. mixed species) affected total mass (biomass + calcium carbonate ($CaCO_3$)) of two common calcifying macroalgae (*Padina boryana* and *Galaxaura fasciculata*). *P. boryana* gained total mass with increased water column nutrients but declined with increased nutrients supplied by the sediment. Conversely, *G. fasciculata* gained total mass with increased nutrients in the sediment but declined with increased water column nutrients. In both interactions, the "winner" (i.e., *G. fasciculata* in the sediment experiment) also had a greater % of thallus mass comprised of $CaCO_3$, potentially due to the subsequent decomposition of the "loser" as this result was not found in the alone treatments. These findings ultimately suggest that nutrient stressors can cause cascading effects, such as promoting calcification and biomass growth or loss in these macroalgal communities, and the potential for domination or decline is based on the nutrient source and community composition.

In chapter 2, I demonstrate that decomposition of macroalgal blooms cause a sequence of biogeochemical processes that can drive acidification in shallow coastal estuaries, and that these processes are mediated by a dynamic microbial community. Eutrophication and ocean acidification are both widely acknowledged as major human-induced stressors in marine environments. While the link between eutrophication and acidification has been established for phytoplankton, it is unclear whether eutrophication in the form of macroalgal blooms can cause cascading effects like acidification in shallow eutrophic estuaries. I conducted seasonal field surveys and assessed microbial communities and functional genes to evaluate changes in biotic and abiotic characteristics between seasons that may be associated with acidification in Upper Newport Bay, CA, USA. Acidification, measured as a drop in pH of 0.7, occurred in summer at the site with the most macroalgal cover. Microbial community composition and functional gene expression provide evidence that decomposition processes contributed to acidification, and also suggest that other biogeochemical processes like nitrification and degradation of polyphosphate also contributed to acidification. To my knowledge, my findings represent the first field evidence that eutrophication of shallow coastal estuaries dominated by green macroalgal blooms can cascade to acidification.

In chapter 3, I demonstrate that macroalgal blooms in shallow estuaries are strong drivers of key microbially-mediated biogeochemical processes that can cause cascading effects, such as acidification and nutrient fluxing, regardless of simulated tidal flushing. Estuaries are productive and diverse ecosystems and are vulnerable to eutrophication from increased anthropogenic nutrients. While it is known that enhanced tidal flushing can reduce adverse effects of anthropogenic disturbances in larger, deeper estuarine ecosystems, this is unexplored for eutrophication in shallow coastal estuaries where macroalgae usually dominate. I simulated

eutrophication as a macroalgal bloom in a mesocosm experiment, varied tidal flushing (flushed daily vs unflushed), and assessed the effects on water column and sediment biogeochemical processes and the sediment microbial community. While flushing did not ameliorate the negative effects of the macroalgal bloom, it caused transient differences in the rate of change in biogeochemical processes and promoted increased fluxes of nutrients from the sediment. In the beginning, the macroalgal bloom induced basification and increased total alkalinity, but during decomposition, acidification and the accumulation of nutrients in the sediment and water column occurred. The findings from this chapter ultimately suggest that macroalgal blooms have the potential to be the cause of, yet may also offer a partial solution to, global ecological changes to biogeochemical processes.

Overall, my results indicate that anthropogenic disturbances, particularly in the form of increased nutrients, can cause cascading effects like macroalgal blooms that in turn cause acidification, basification, increased interspecific interactions, nutrient depletion, and nutrient fluxing in multiple ecosystems. These data advance our current understanding of the ecological consequences of eutrophication in the form of macroalgal blooms in different ecosystems. It also provides mechanistic links to microbial communities and biogeochemical processes not previously identified for shallow coastal estuaries. As human population and subsequent nutrient pollution increases in watersheds globally, ecological phenomenon such as eutrophication will only be intensified, and macroalgal communities will continue to dominate. Consequently, this dominance, especially during decomposition as shown here, can drive a multitude of subsequent stressors that can impact the entire ecosystem.

The dissertation of Tiara Nydia Moore is approved.

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2019

DEDICATION

To all the little black girls, just keep swimming

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The following chapters were prepared as manuscripts and have been or will be submitted to peer reviewed journals. Below is a list of co-authors and their contributions, funding sources, and specific acknowledgments for each chapter.

Chapter 1: Moore, T and Fong, P. Anthropogenic stressors and interspecific interactions alter biotic and abiotic constituents of calcifying coral reef macroalgae. TM and PF were affiliated with University of California Los Angeles (UCLA), Department of Ecology and Evolutionary Biology (EEB) during the time research was conducted. TM and PF contributed equally to experimental design. TM conducted research and analysis with assistance from PF. TM wrote the first manuscript draft and PF edited subsequent drafts. We wish to thank the Diversity Project funded by NSF PIRE Grant (#OISE1243541) and the University of California's HBCU initiative for travel and research support and Paul Barber, Benjamin Cuker, Camille Gaynus, Amanda

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2016: Leasi F, Gaynus C, Mahardini A, **Moore T**, Norenburg J, & Barber P. Spatial and ecological distribution of neglected microinvertebrate communities across endangered ecosystems: Meiofauna in Bali (Indonesia). *Marine Ecology.*

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2014: "*Abundance and Diversity of Meiofauna in Bali, Indonesia*."
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**promotes diversity in science

CHAPTER 1:

Anthropogenic stressors and interspecific interactions alter biotic and abiotic constituents of calcifying coral reef macroalgae

ABSTRACT

Abiotic stressors such as nutrients, which may be supplied to the water column or via sedimentation, have been linked to phase-shifts from coral to algal domination on tropical reefs. However, few studies have considered how these stressors impact changes in the biotic and abiotic constituents of dominant species of calcifying macroalgae, and how this may be mediated by species-species interactions. We conducted 4 mesocosm experiments to identify if different nutrient sources (water column vs. terrestrial sediment) as well as species interactions (alone vs. mixed species) affected total mass (biomass + calcium carbonate ($CaCO_3$)) of two common reef calcifying macroalgae (*Padina boryana* and *Galaxaura fasciculata*). *P. boryana* gained total mass with increased water column nutrients but declined with increased nutrients supplied by the sediment. Conversely, *G. fasciculata* gained total mass with increased nutrients in the sediment but declined with increased water column nutrients. In both of these interactions, the "winner" (i.e., *G. fasciculata* in the sediment experiment) also had a greater % of thallus mass comprised of $CaCO_3$. This increase may be due to the subsequent dissolution of *P. boryana*, as it was not found in the alone treatments, suggesting nutrient stressors can cause cascading effects in these macroalgal communities. Our findings indicate that anthropogenic stressors in the form of increased nutrients in the water and sediments have strong impacts on macroalgal interspecific interactions, with potential for domination or decline based on the nutrient source and community composition.

INTRODUCTION

Tropical reefs have been transitioning from coral to mixed communities dominated by macroalgae (Sangil & Guzman 2016, Cheal et al 2010, Fong & Paul 2011), motivating research on how macroalgae respond to anthropogenic stressors and interact with each other during these stressful events. Macroalgae provide essential ecosystem services such as primary productivity, nutrient cycling, sediment accretion, and carbon (C) storage (Duarte & Cebrián 1996, Smale et al. 2013). In addition, some macroalgae, due to their calcium carbonate ($CaCO_3$) structure, also contribute to the production of $CaCO_3$ sediment and the accretion of coral reefs (Chisholm 2003, Delgado & Lapointe 1994). Carbonate (CO_3^{2-}) precipitation is a major C sink (Renforth et al. 2009) and calcifying macroalgae are important players in global C sequestration (Krause-Jensen & Duarte 2016). However, as their growth combines both biotic and abiotic processes, predicting how increasing anthropogenic impacts will affect these vital organisms is quite challenging.

Increased nutrient loading due to watershed development is a major anthropogenic stressor to marine systems (Valiela et al. 1997, Conley et al. 2000, Wallace et al. 2014). These excess nutrients, particularly nitrogen (N) and phosphorus (P), increase primary productivity and can lead to a buildup of organic matter (i.e. eutrophication) in the form of algal blooms (Nixon 1995, Diaz 2001). It is well-documented that macroalgal blooms have increased in temperate marine systems subjected to anthropogenic nutrient inputs (e.g. Kennison et al. 2011, Peckol & Rivers 1995, Raven & Taylor 2003). Conversely, in tropical systems, studies linking nutrient enrichment to macroalgae have mixed results, with some studies showing macroalgal proliferation while others have no or adverse effects of nutrients (Hughes et al. 2003, Clausing et al. 2016, Lapointe et al. 2004, Smith et al. 2010, Rasher et al. 2012). As tropical reefs tend to be nutrient limited (Odum & Odum 1955), nutrient enrichment may affect how these algae allocate

energy between uptake and growth (see Fong & Paul 2011) potentially driving these mixed results. In addition, calcifying macroalgae must also allocate energy for calcification, further complicating interpretation of the relationship between anthropogenic nutrient enrichment and growth.

Increasing terrestrial sedimentation due to watershed development contributes another anthropogenic stressor to marine systems generally, and tropical reefs, in particular (De'ath & Fabricius 2010, reviewed in Bainbridge et al. 2018). For example, terrigenous material comprised ~65% of material from sediment traps in a study measuring sedimentation to nearshore waters in the Virgin Islands (Pait et al. 2018). This increased sedimentation can have both positive and negative effects on macroalgae as sediments can carry nutrients (Weber et al 2006) and reduce grazing (Kawamata et al. 2011), promoting algal growth. However, sediments can also block light (Airoldi 2003), reducing algal growth.

In temperate systems, increased sediment loads generally cause negative effects on macroalgae by limiting nutrient and gas exchange (reviewed in Airoldi 2003, Kawamata et al. 2012). Again, the few studies that address sedimentation effects on macroalgal growth have mixed results in the tropics, ranging from positive benefits (Schaffelke 1999), to no effects (Johnson et al 2018), to negative impacts (Umar et al. 1998). Moreover, responses can be species specific, with sediments being required for growth of one macroalgal species, but not affecting growth for the other species (Clausing et al. 2016). Additionally, this study showed that sediments provided the same benefit to growth as nutrient additions to the water column, suggesting the alga can utilize sediment nutrients for growth. Building on Clausing et al. (2016), we measured the effects of nutrients and sediments on algal growth, but also examined these

effects in mixed communities and assessed these effects on both the biotic and abiotic ($CaCO_3$) constituents of the algal thallus.

Interspecific interactions may also affect the response of macroalgae to changing environmental conditions. Theory predicts that interspecific interactions such as competition should be strong within algal communities as species share multiple resources such as light, nutrients, and space (Connell 1983). Under the stress gradient hypothesis, however, competition can transition to facilitation in communities impacted by multiple stressors (Bertness and Callaway 1994). As stressors increase due to human impacts, it is possible that algal interactions will be altered as well, e.g., moving from competition to facilitation.

Although some studies have observed outcomes of competitive interactions between macroalgae (Young & Gobler 2017, Bennet et al. 2015), few have considered how outcomes may change with fluctuating environmental factors such as nutrients or sedimentation (Hofmann et al. 2015, Fong & Fong 2018). However, when measuring increased nutrients and CO_2 effects on a calcifying macroalgal species and its noncalcifying epiphyte, Hofmann et al. (2015) found that the epiphyte growth increased, while the calcifying macroalga's growth declined. Another study with two competing macroalgae exposed to elevated CO_2 found increased growth when alone, but when together growth of one species declined (Young & Gobler 2017). In these interactions, we can clearly identify the species with increased growth as the "winner" and the declining species as the "loser", but we don't know the effects the "losers" may have on the "winner." As multiple stressors continue to impact macroalgal-dominated tropical reef communities, species interactions and the potential for cascading effects warrants further investigation.

In this study we examine whether different nutrient sources (water column vs. terrestrial sediment) as well as species interactions (alone vs. mixed communities) have unique effects on calcifying macroalgal species total mass (abiotic + biotic). We hypothesized that nutrient enrichment would facilitate increased growth while increased sedimentation would hinder growth, and these effects may be influenced by interspecific interactions.

MATERIALS & METHODS

To accomplish these objectives, we conducted 4 mesocosm experiments assessing the main and interactive effects of different sources of nutrients (water column vs. terrestrial sediment) as well as interspecific interactions on change in mass of two species of calcifying macroalgae commonly found on coral reefs. In the first two mesocosms (one for each of the 2 species), we evaluated the impacts of increased nutrient loading to the water column (+/- enriched water column) and the presence of a potential interacting species (+/- interspecific interactions) on growth of the two macroalgae. The enriched water treatment simulated increased nutrient inputs to the water column due to runoff. To partition out the responses of calcifying algae between biomass and calcified portions of the algal thalli, our response variables were both total mass and the $CaCO_3$ component. In the second two experiments (one for each species) we evaluated the role of nutrient-enriched sedimentation (+/- enriched sediments on algal thali) and interspecific interactions (+/-). The enriched sediment treatment simulated terrestrial sediments entering the bay due to watershed development and settling on the algal thalli.

Study species and rationale

We conducted experiments during the dry season, in June and July of 2015, in Mo'orea, French Polynesia at the Gump South Pacific Research Station at the mouth of Cook's Bay

(17.4905° S, 149.8264° W). For all experiments, two common species of calcifying coral reef macroalgae were used: *Galaxaura fasciculata* (hereafter, *Galaxaura*) and *Padina boryana* (hereafter, *Padina*). These species can dominate shallow portions of fringing reefs of Mo'orea (Fong & Fong 2014) and can dominate some reef zones of the Great Barrier Reef (Fox & Bellwood 2007). *Galaxaura* is a branching red calcareous macroalga that can switch rapidly from being nutrient limited to replete depending on environmental conditions (Clausing & Fong 2016) and is generally unpalatable to herbivores (Mantyka & Bellwood 2007), possibly due to its $CaCO_3$ structure. While *Padina* is the only genus of brown macroalgae that calcifies, it is relatively palatable, and switches between N & P limitation depending on nutrient supplies (Clausing & Fong 2016, Fong & Fong 2014). In the rainy season (Dec-March), terrestrial sediment and nutrients are introduced to the bays of the north shore of Mo'orea via run off of storm water from developed watersheds (Adjeroud & Salvat 1996, Clausing & Fong 2016). During the dry season, there is wind-driven resuspension and deposition of sediments onto algal thalli on fringing reefs within Cook's Bay (personal observation).

Enriched water column mesocosm

For the two 2-factor fully-crossed experiments varying nutrient concentration of the water column (ambient/enriched) and interaction with a second species (alone/together), we collected algae and seawater from a fringing reef in Cook's Bay, Mo'orea in July 2015. Algae were cleaned of debris, placed in nylon mesh bags, and spun in a salad spinner for 30 seconds to remove excess water and attain a standardized initial wet weight. Experimental units were 300 mL jars filled with 275 mL of either enriched or ambient sea water and either 2 g of algae for the alone treatment or 1 g of each algal species for the together treatment (Fong & Fong 2014). To enrich the seawater, we added inorganic nutrients to half of the collected seawater to enrich it by

20 µM NO_3 and 2 µM PO_4; the other half was left at ambient seawater concentrations (as in Fong & Fong 2014). Each of the 4 treatment combinations was replicated 10 times and randomly placed in a flow-through outdoor water table for the duration of the experiment. Units were re-randomized daily to equalize any light variation within the water table, and water was changed in all units on the fourth day of the experiment to relieve potential nutrient limitation within the mesocosms.

After 7 days, algae were reweighed (as above) and % change in total algal mass from initial calculated. Subsamples from experimental units were taken to quantify the $CaCO_3$ content of each algal thalli using an acid washing method. We dried subsamples at 60 ^oC until constant weight and then placed them in a 10% hydrochloric acid bath. We replaced acid until samples stopped bubbling, indicating $CaCO_3$ was fully dissolved, then redried and reweighed each sample. To determine % $CaCO_3$, we subtracted the final decalcified dry weight from the initial dry weight, divided by the initial subsample dry weight and multiplied by 100. As data met the assumptions of parametric statistics, 2-factor ANOVA's were conducted in RStudio v. 3.5.1 to test for main and interactive effects for both % change in total mass and % $CaCO_3$ for each species separately.

Enriched sediment mesocosm

In the second set of 2-factor fully-crossed experiments, we varied nutrient content of sediments (ambient/enriched) and the presence of a second species (alone/together). To model terrestrial inputs of sediments that can settle on algae in the bay, we collected sediment from the head of the bay where a major river enters. The algae and seawater were collected from the same fringing reef as described above. To enrich the sediment, we added 20 g of slow release fertilizer (Osmocote) to 1 liter of collected sediment 48 hours prior to the start of the experiment while the

other half was left as collected for the ambient treatment. The 300 mL experimental units were then filled with 275 mL of seawater, 2 grams of algae (*Padina*, *Galaxaura*, or together), and the algal thalli in each unit was sprinkled with either ~3.75 mL (by volume) (Clausing et al. 2016) of enriched or ambient sediment. Units were randomly placed in an outside water bath for the duration of the experiment, location re-randomized daily, and after 7 days the experiment was completed as above. As data met the assumptions of parametric statistics, 2-factor ANOVA's were conducted in RStudio v. 3.5.1 to test for main and interactive effects for both % change in total mass and % $CaCO_3$ for each species separately.

RESULTS

Enriched water column mesocosm

Overall *Padina* gained total mass (biomass + $CaCO_3$) in all treatments (Figure 1.1a). There was a positive effect of enhanced water column nutrients on growth of *Padina* that only occurred when *Padina* was grown alone, resulting in a significant interaction (2-factor ANOVA, $p = 0.047$ for the interaction). When grown alone and enriched, growth of *Padina* was ~ 2.5-times higher than in the other 3 treatments. There was a significant positive effect of interspecific interactions on % $CaCO_3$ that occurred when *Padina* was grown with *Galaxaura* regardless of nutrient treatment (Figure 1.1b; 2-factor ANOVA, $p = 0.048$ for species presence). Overall % $CaCO_3$ was ~1.2-times higher in *Padina* in the treatments with interspecific interactions compared to when grown alone.

In contrast, there was an overall loss in total mass of *Galaxaura* in all treatments (Figure 1.2a). Loss was the least when *Galaxaura* was grown with *Padina* in ambient nutrients, but not in enriched, resulting in a significant interaction (2-factor ANOVA, $p = 0.034$ for the

interaction). Overall % $CaCO_3$ was similar in each treatment and not affected by either factor (Figure 1.2b, 2-factor ANOVA, $p > 0.05$ for all factors and the interaction).

Enriched sediment mesocosm

When terrestrial sediments were added to mesocosms *Padina* lost total mass in all treatments (Figure 1.3a). Effects of sediments on total mass of *Padina* were more negative when sediments were enriched compared to ambient and occurred both when *Padina* was grown alone and with *Galaxaura* (2-factor ANOVA, $p = 0.001$ for sediment treatment). In the enriched sediment treatments, *Padina* lost ~2-times more total mass than in the ambient treatments. Overall there was about half the $CaCO_3$ in the together treatments compared to when grown alone (Figure 1.3b). Thus, there was a negative effect of interspecific interactions on % $CaCO_3$ regardless of sediment nutrient treatment (2-factor ANOVA, $p = 0.002$ for species presence). Comparing $CaCO_3$ content of *Padina* in this and the previous experiment (Figure 1.1b) suggests that $CaCO_3$ dissolution occurred.

In contrast, *Galaxaura* gained total mass in all treatments (Figure 1.4a). There was a positive effect of interspecific interactions on *Galaxaura* that only occurred with ambient sediments (2-factor ANOVA, $p = 0.001$ for the interaction). With ambient sediment nutrients, *Galaxaura* gained ~2-times more total mass when grown with *Padina* than when grown alone. Overall % $CaCO_3$ in *Galaxaura* was twice as high in the together compared to the alone treatments (Figure 1.4b). Thus, there was a positive effect of interspecific interactions on % $CaCO_3$, where *Galaxaura* had more $CaCO_3$ when grown with *Padina* regardless of nutrients (2-factor ANOVA, $p < 0.0001$ for species presence). Overall *Galaxaura* had lower $CaCO_3$ in this experiment compared to the water enrichment experiment (compare Figure 1.2b and Figure 1.4b), especially in the alone treatments.

DISCUSSION

We uncovered a novel ecological process whereby interspecific interactions control $CaCO_3$ production in calcifying algae; this finding has global implications because $CaCO_3$ production is one means of sequestering excess C associated with climate change (Renforth et al. 2009). For both species tested, interspecific interactions greatly increased the % of the thallus' mass comprised of $CaCO_3$, up to doubling the $CaCO_3$ content in "winners" when conditions were favorable for growth. While it is well documented that photosynthesis and calcification are linked (e.g. Jensen et al. 1985, Borowitzka & Larkum 1987, Gattuso et al. 1999), few studies have assessed whether interspecific interactions affect this relationship. In the few studies that have assessed species interactions, the historical focus has been on how the photosynthesis rates of coral symbionts affect rates of coral calcification (e.g. Stambler et al. 1991). More recently, studies of calcification are focused on ocean acidification (OA), and identify shifts in water chemistry, specifically things that alter the concentration of $CaCO_3$, as drivers of calcification rate (Langdon & Atkinson 2005, Peach et al. 2017). Here we discovered that interspecific interactions can also drive this complex mechanism. As $CaCO_3$ precipitation is an important sink for C (Renforth et al. 2009), our work has important and novel implications for understanding processes that may ameliorate the effects of climate change.

One possible mechanism that may drive the relationship between interspecific interactions and $CaCO_3$ production is biotically-mediated changes in water chemistry that are similar to the abiotic changes that occur during OA. One line of evidence supporting this mechanism is that increased calcification in one species was always linked with loss in mass of the other species in our study. Loss in mass for primary producers can occur when respiration exceeds photosynthesis or during microbial decomposition; both processes lead to increased

concentrations of CO_2 in the water (Lloyd et al. 1977). This increased CO_2 can be utilized for photosynthesis by the "winner", and subsequently support calcification (Koch et al. 2013). For example, in a study on *Halimeda spp.* (calcifying green macroalgae), increased photosynthesis due to CO_2 uptake led to an increased pH environment conducive for calcification (de Beer & Larkum 2001). Thus, respiring or decomposing algae may have changed the carbonate water chemistry, which could have benefited the "winner" species in our study. This result is supported by studies of OA that suggest some photosynthetic organisms may benefit from OA in the short-term due to the spike in CO_2 (Peach et al. 2017, Zou & Gao 2009), although in the long-term these effects have shown negative impacts to $CaCO_3$ producing organisms (Russell 2009, Hofmann & Bischof 2014). As CO_2 continues to increase globally, research on understanding mechanisms of consumption/production by calcifying organisms must also increase in order to combat continued degradation of reefs.

Another possible explanation for positive effects of interspecific interactions on $CaCO_3$ production is dissolution of $CaCO_3$ in the "loser", freeing CO_3^{2-} ions for use by the "winner". For example, if *Padina* was losing $CaCO_3$ by dissolution, this would produce free CO_3^{2-} ions, increase pH (Millero 2007), and support higher calcification in *Galaxaura*. This example may not explain the $CaCO_3$ increase for *Padina*, as dissolution did not appear to occur in *Galaxaura.* However, as the $CaCO_3$ structure and calcification sites are different in the two species (reviewed in Koch et al. 2013), it is likely their responses may be unique as well. Overall, we demonstrate that the calcification process is complex and controlled by varying factors, many which can be affected by species interactions.

Our results imply that *Padina* may dominate overfished coral reefs with increased supplies of nutrients to the water column, although this dominance may be mediated by

interspecific interactions. Our findings support other mesocosm and field studies that documented *Padina* responding favorably to enhanced water column nutrients (e.g. Clausing & Fong 2016, Fong & Fong 2014, Johnson et al. 2018, Thacker et al. 2001). A field study in Hawaii also found *Padina* and other tropical macroalgal (i.e. *Sargassum*, *Dictyosphaeria*, and *Gracilaria*) growth increased with added nutrients (Larned 1998). This study also concluded that due to *Padina*'s flat form, advection of water column nutrients was the primary nutrient source rather than sediments. Less understood is the response of *Padina* when exposed to interspecific interactions.

In one of the few studies that address interspecific interactions, Fong & Fong (2018) documented a negative impact on *Padina* when grown with multiple species compared to when grown alone in both ambient and enriched nutrient treatments (Fong & Fong 2018). Even though the growth in our experiment was lower with species interactions, *Padina* still grew in all treatments suggesting under increasing nutrient supplies to the water column we will see continued proliferation. These results may explain the worldwide trend of increased abundance of brown algae on topical reefs (reviewed in Fong & Paul 2011).

Our results also suggest that *Galaxaura* may become the dominant macroalga on overfished tropical reefs if reefs continue to be subjected to increased terrestrial sources of sediments. This finding also supports the few studies that have documented *Galaxaura*'s increased growth in response to sedimentation (Clausing et al. 2016, Su et al. 2009). Due to *Galaxaura*'s coarsely branched structure covered in filaments, fine sediments adhere to the thallus and provide nutrients, which may create a positive feedback with subsequent growth (Clausing et al. 2016). *Galaxaura* must allocate energy between nutrient uptake and growth and this may also be affected by interspecific interactions. In our mixed community treatments,

where *Galaxaura* was the "winner", energy appears to be allocated to biomass accumulation in ambient nutrients. Conversely when nutrients were added, *Galaxaura*'s energy allocation may shift to uptake and $CaCO_3$ mass accumulation. This suggests that sediments, enriched or ambient, contribute to both biotic and abiotic growth, which is mediated by interspecific interactions. With sediment loads increasing to the water column due to development and deforestation globally (Maina et al. 2013, reviewed in Bainbridge et al. 2018), *Galaxaura* thriving in this environment will only add to macroalgal-dominated reef systems. However, on a *Galaxaura*-dominated reef, food chain support may be disrupted as this is a less palatable species than many others (Fong & Fong 2014), suggesting this shift may compromise the community, and break down the trophic structure.

Our findings indicate that anthropogenic disturbances in the form of increased sediments and nutrients have strong effects on macroalgal species-species interactions, with potential for domination or decline based on the nutrient source and community composition. This may contribute to our difficulty in predicting how nutrients will affect growth of calcifying algae. For these species, growth is comprised of both the biotic and abiotic portions of the thalli, which are differently affected by sediments and nutrients. These complex interactions are likely mediated by links between CO_2 and CO_3^{2-} availability. Consequently, reefs affected by enhanced anthropogenic stressors in this climate change era will be vulnerable to macroalgal dominance, but how this will affect net calcification warrants further investigation. Ultimately, however, as species either "win" or "lose", these interactions may cause local environmental changes that could alter the overall reef community structure.

FIGURES

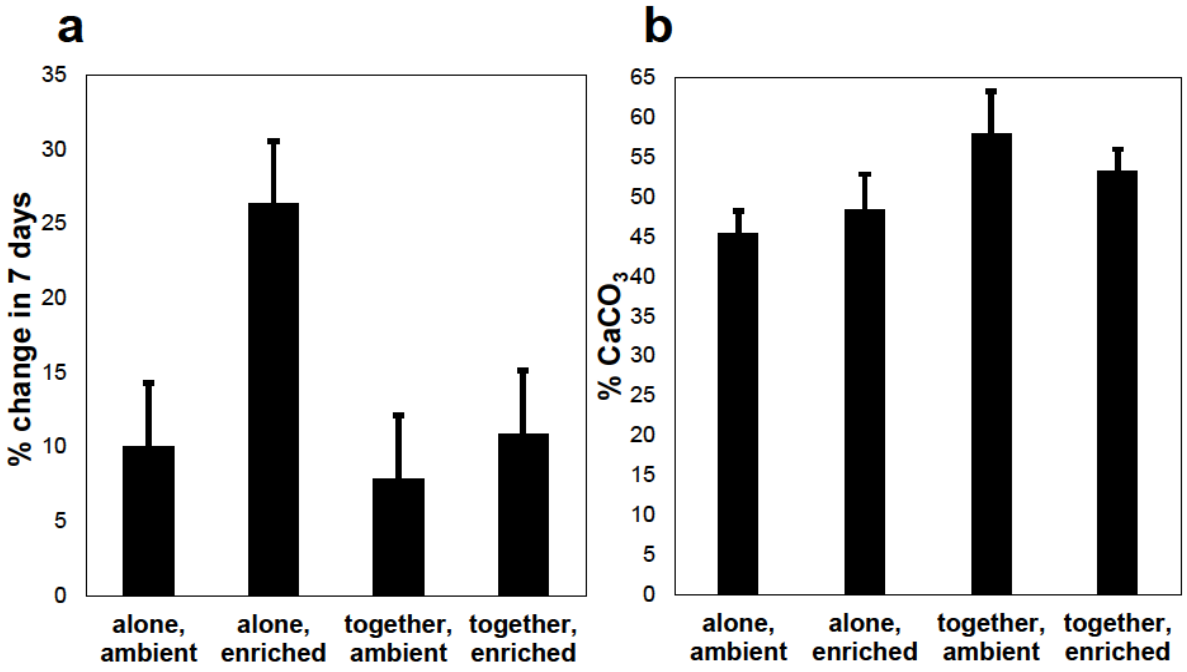

Figure 1.1. Results of a 2 factor experiment varying water column nutrient supplies and interspecific interactions for a) *Padina* and b) % calcium carbonate ($CaCO_3$) in algal thalli after 7 days of experimental treatments. Bars are means ± SE.

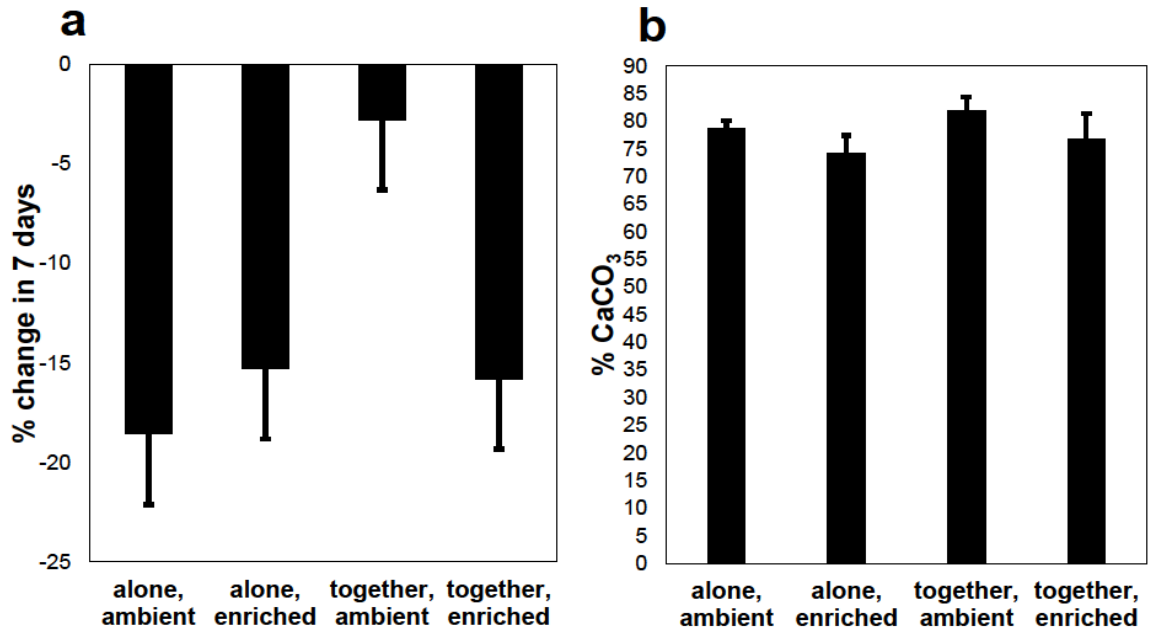

Figure 1.2. Results of a 2 factor experiment varying water column nutrient supplies and interspecific interactions for a) *Galaxaura* b) % calcium carbonate ($CaCO_3$) in algal thalli after 7 days of experimental treatments. Bars are means ± SE.

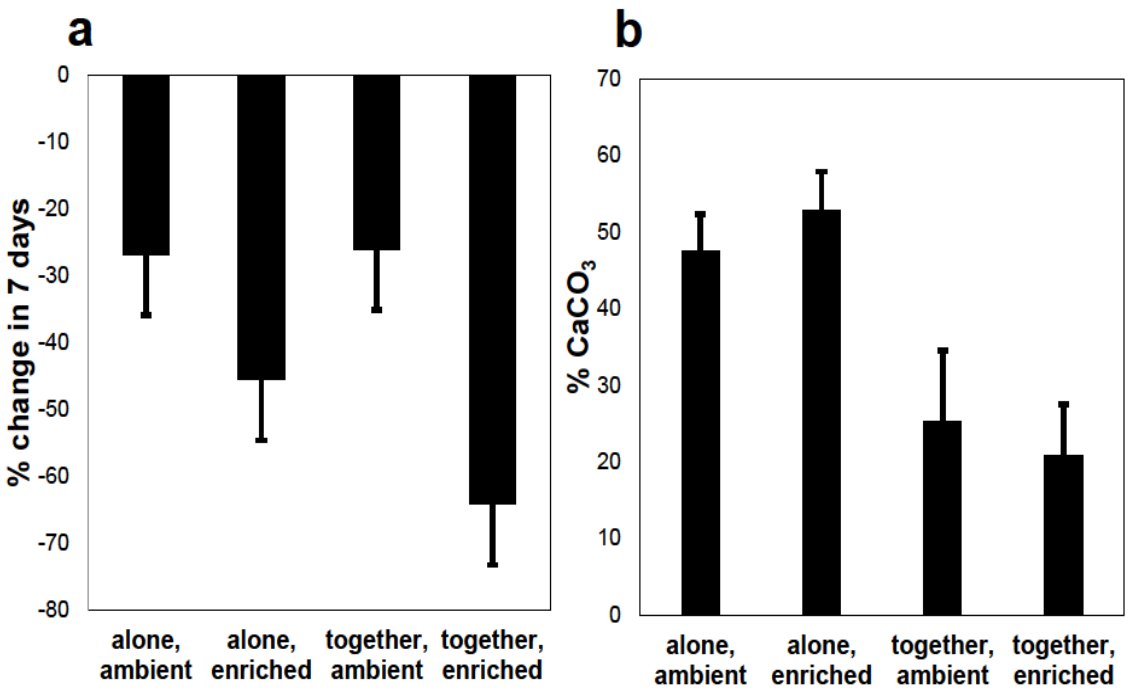

Figure 1.3. Results of a 2 factor experiment varying sediment nutrients and interspecific interactions for a) *Padina* b) % calcium carbonate ($CaCO_3$) in algal thalli after 7 days of experimental treatments. Bars are means ± SE.

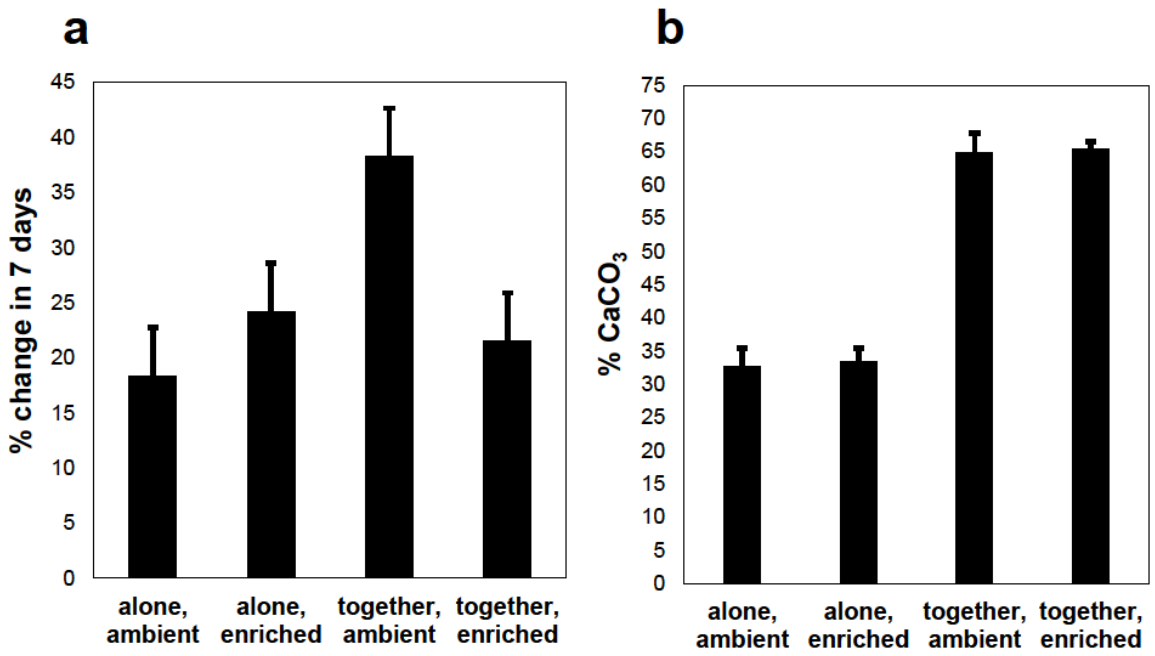

Figure 1.4. Results of a 2 factor experiment varying sediment nutrients and interspecific interactions for a) *Galaxaura* b) % calcium carbonate ($CaCO_3$) in algal thalli after 7 days of experimental treatments. Bars are means ± SE.

REFERENCES

Adjeroud M, Salvat B (1996) Spatial patterns in biodiversity of a fringing reef community along Opunohu Bay, Moorea, French Polynesia. Bull Mar Sci 59:175–187

Airoldi L (2003) The effects of sedimentation on rocky coast assemblages. Oceanogr Mar Biol Annu Rev 41:161-236

Bainbridge Z, Lewis S, Bartley R, Fabricius K and others (2018) Fine sediment and particulate organic matter: A review and case study on ridge-to-reef transport, transformations, fates, and impacts on marine ecosystems. Mar Pollut Bull 135:1205-1220

Bergman JL, Dang BN, Tabatabaee MM, Mcgowan MM and others (2016) Nutrients induce and herbivores maintain thallus toughness, a structural anti-herbivory defense in Turbinaria ornata. Mar Ecol Prog Ser 559:35-43

Bertness MD, Callaway R (1994) Positive interactions in communities. Trends Ecol Evol 9:187–191

Bittick SJ, Clausing RJ, Fong CR, Fong P (2016) Bolstered physical defences under nutrient-enriched conditions may facilitate a secondary foundational algal species in the South Pacific. J Ecol 104:646–653

Borowitzka MA, Larkum AWD (1987) Calcification in algae: Mechanisms and the role of metabolism. CRC Crit Rev Plant Sci 6:1–45

Cheal AJ, MacNeil MA, Cripps E, Emslie MJ and others (2010) Coral–macroalgal phase shifts or reef resilience: links with diversity and functional roles of herbivorous fishes on the Great Barrier Reef. Coral Reefs 29:1005-1015

Chisholm JRM (2003) Primary productivity of reef-building crustose coralline algae. Limnol Oceanogr 48:1376–1387

Clausing RJ, Bittick SJ, Fong CR, Fong P (2016) Sediments influence accumulation of two macroalgal species through novel but differing interactions with nutrients and herbivory. Coral Reefs 35:1297–1309

Clausing RJ, Fong P (2016) Environmental variability drives rapid and dramatic changes in nutrient limitation of tropical macroalgae with different ecological strategies. Coral Reefs, 35:669–680

Conley DJ, Kaas H, Mohlenberg F, Rasmussen B, Windolf J (2000) Characteristics of Danish estuaries. Estuaries 23:820–837

Connell JH (1983) On the prevalence and relative importance of interspecific competition: evidence from field experiments. Am Nat 122:661-696

De'ath G, Fabricius K (2010) Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. Ecol Appl 20:840–850

De Beer D, Larkum AWD (2001) Photosynthesis and calcification in the calcifying algae Halimeda discoidea studied with microsensors. Plant Cell Environ 24:1209-1217

Delgado O, Lapointe BE (1994) Nutrient-limited productivity of calcareous versus fleshy macroalgae in a eutrophic, carbonate-rich tropical marine environment. Coral Reefs 13:151-159

Diaz R (2001) Overview of hypoxia around the world. J Environ Qual 30:275-281

Duarte CM, Cebrián J (1996) The fate of marine autotrophic production. Limnol Oceanogr, 41:1758–1766

Fong CR, Fong P (2014) Why species matter: An experimental assessment of assumptions and predictive ability of two functional-group models. Ecology 95:2055–2061

Fong CR, Fong P (2018) Nutrient fluctuations in marine systems: Press versus pulse

nutrient subsidies affect producer competition and diversity in estuaries and coral reefs. Estuaries and Coasts 41:421-429

Fong P, Paul V (2011) Coral reef algae. In: Dubinsky Z, Stambler N (eds) Coral reefs: An ecosystem in transition. Springer, Amsterdam, p 241–272

Fox RJ, Bellwood DR (2007) Quantifying herbivory across a coral reef depth gradient. Mar Ecol Prog Ser 339:49–59

Gattuso JP, Allemand D, Frankignoulle M (1999) Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: A review on interactions and control by carbonate chemistry. Amer Zool 39:160-183

Hofmann LC, Bischof K (2014) Ocean acidification effects on calcifying macroalgae. Aquat Biol 22:261–279

Hofmann LC, Bischof K, Baggini C, Johnson A and others (2015) CO2 and inorganic nutrient enrichment affect the performance of a calcifying green alga and its noncalcifying epiphyte. Oecologia 177:1157-1169

Hughes TP, Baird AH, Bellwood DR, Card M and others (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301:929-933

Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D and others (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. Curr Biol 17:360–365

Jensen PR, Gibson RA, Littler MM, Littler DS (1985) Photosynthesis and calcification in four deep-water Halimeda species (Chlorophyceae, Caulerpales). Deep-Sea Res 32:451-464

Johnson GC, Pezner AK, Sura SA, Fong P (2018) Nutrients and herbivory, but not sediments, have opposite and independent effects on the tropical macroalga, *Padina* boryana. J Exp Mar Bio Ecol 507:17–22

Kawamata S, Yoshimitsu S, Tanaka T, Igari T, Tokunaga S (2011) Importance of sedimentation for survival of canopy-forming fucoid algae in urchin barrens. J Sea Res 66:76–86

Kawamata S, Yoshimitsu S, Tokunaga S, Kubo S, Tanaka T (2012) Sediment tolerance of Sargassum algae inhabiting sediment covered rocky reefs. Mar Biol 159:723–733

Kennison RL, Kamer K, Fong P (2011) Rapid nitrate uptake rates and large short-term storage capacities may explain why opportunistic green macroalgae dominate shallow eutrophic estuaries. J Phycol 47:483–494

Koch M, Bowes G, Ross C, Zhang XH (2013) Climate change and ocean acidification effects on seagrasses and marine macroalgae. Glob Chang Biol 19:103–132

Krause-Jensen D, Duarte CM (2016) Substantial role of macroalgae in marine carbon sequestration. Nature Geoscience 9:737-742

Langdon C, Atkinson MJ (2005) Effect of elevated pCO2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/ irradiance and nutrient enrichment. J Geophys Res Oceans 110:1–16

Lapointe B, (2004) Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. J Exp Mar Biol Ecol 308:23-58

Larned ST (1998) Nitrogen-versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae. Mar Biol 132:409–421

Lloyd NDH, Canvin DT, Culver DA (1977) Photosynthesis and photorespiration in algae. Plant Physiol 59:936-940

Maina J, de Moel H, Zinke J, Madin J and others (2013) Human deforestation outweighs future climate change impacts of sedimentation on coral reefs. Nat Commun 4:1986

Mantyka C, Bellwood DR (2007) Macroalgal grazing selectivity among herbivorous coral reef fishes. Mar Ecol Prog Ser 352:177–185

Millero FJ (2007) The marine inorganic carbon cycle. Chem Rev 107:308–341

Nixon SW (1995) Coastal marine eutrophication: A definition, social causes, and future concerns. Ophelia 41:199-219

Odum HT, Odum EP (1955) Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. Ecol Monogr 25:291–320

Pait AS, Galdo FR, Hartwell SI, Apeti DA, Mason AL (2018) An assessment of nutrients and sedimentation in the St. Thomas East End Reserves, US Virgin Islands. Environ Monit Assess 190:270

Peach KE, Koch MS, Blackwelder PL, Manfrino C (2017) Calcification and photophysiology responses to elevated pCO2 in six Halimeda species from contrasting irradiance environments on Little Cayman Island reefs. J Exp Mar Bio Ecol 486:114–126

Peckol P, Rivers JS (1995) Physiological responses of the opportunistic macroalgae Cladophora vagabunda (L.) van den Hoek and Gracilaria tikvahiae (McLachlan) to environmental disturbances associated with eutrophication. J Exp Mar Bio Ecol 190:1–16

Rasher DB, Engel S, Bonito V, Fraser GJ, Montoya JP, Hay ME (2012) Effects of herbivory, nutrients, and reef protection on algal proliferation and coral growth on a tropical reef. Oecologia 169:187–198

Raven JA, Taylor R (2003) Macroalgal growth in nutrient-enriched estuaries: A biogeochemical and evolutionary perspective. Water Air Soil Pollut 3:7–26

Renforth P, Manning DAC, Lopez-Capel E (2009) Carbonate precipitation in artificial soils as a sink for atmospheric carbon dioxide. Appl Geochemistry 24:1757–1764

Russell BD, Thompson JI, Falkenberg LJ, Connell SD (2009) Synergistic effects of climate change and local stressors: CO2 and nutrient-driven change in subtidal rocky habitats. Glob Chang Biol 15:2153–2162

Sangil C, Guzman HM (2016) Macroalgal communities on multi-stressed coral reefs in the Caribbean: Long-term changes, spatial variations, and relationships with environmental variables. J Sea Res 117:7-19

Schaffelke B (1999) Particulate organic matter as an alternative nutrient source for tropical Sargassum species (Fucales, Phaeophyceae). J Phycol 35:1150-1157

Smale DA, Burrows MT, Moore P, O'Connor N, Hawkins SJ (2013) Threats and knowledge gaps for ecosystem services provided by kelp forests: A northeast Atlantic perspective. Ecol Evol 3:4016-4038

Smith JE, Hunter CL, Smith CM (2010) The effects of top–down versus bottom–up control on benthic coral reef community structure. Oecologia, 163:497-507

Stambler N, Popper N, Dubinsky Z, Stimson J (1991) Effect of nutrient enrichment and water motion on the coral Pocillopora damicornis. Pac Sci 45:299–307

Su SW, Chung IC, Lee TM (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-spongesymbiose Ceratodictyon/Haliclona. Kuroshio Science 3:63-80

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

Umar MJ, McCook LJ, Price IR (1998) Effects of sediment deposition on the seaweed Sargassum on a fringing coral reef. Coral Reefs 17:169-177

Valiela I, McClelland J, Hauxwell J, Behr PJ and others (1997) Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. Limnol Oceanogr 42:1105–1118

Wallace RB, Baumann H, Grear JS, Aller RC, Gobler CJ (2014) Coastal ocean acidification: The other eutrophication problem. Estuar Coast Shelf Sci 148:1–13

Weber M, Lott C, Fabricius KE (2006) Sedimentation stress in a scleractinian coral exposed to terrestrial and marine sediments with contrasting physical, organic and geochemical properties. J Exp Mar Bio Ecol 336:18–32

Young CS, Gobler CJ (2017) The organizing effects of elevated CO2 on competition among estuarine primary producers. Sci Rep 7:7667

Zou D, Gao K (2009) Effects of elevated CO2 on the red seaweed Gracilaria lemaneiformis (Gigartinales, Rhodophyta) grown at different irradiance levels. Phycologia 48:510–517

CHAPTER 2:

Macroalgal-driven acidification of a shallow coastal estuary is mediated by multiple alterations in microbial processes

ABSTRACT

Eutrophication and ocean acidification are both widely acknowledged as major human-induced stressors in marine environments. While the link between eutrophication and acidification has been established for phytoplankton, it is unclear whether eutrophication in the form of macroalgal blooms can cause cascading changes in biogeochemical processes that lead to acidification in shallow eutrophic estuaries dominated by macroalgal blooms. We conducted seasonal field surveys and assessed microbial communities and functional genes to evaluate changes in biotic and abiotic characteristics between seasons that may be associated with acidification in Upper Newport Bay, CA, USA. Acidification, measured as a drop in pH of 0.7, occurred in summer at the site with the most macroalgal cover, which reached ~90%. All evidence indicated that acidification was likely driven by the same excessive productivity/decomposition cycle found for phytoplankton bloom-induced acidification. Microbial community composition and functional gene expression provide evidence that decomposition processes contributed to acidification, and also suggest that other biogeochemical processes like nitrification and degradation of polyphosphate contributed to acidification. Overall, our results show macroalgal blooms cause a sequence of biogeochemical processes that ultimately can drive acidification in shallow coastal estuaries, and that these processes are mediated by a dynamic microbial community.

INTRODUCTION

Humans are impacting almost every major ecological process that structures communities and ecosystems. Examples of how human activity can directly control key processes in ecosystems include destruction of habitat changing trophic structure (Gieswein, Hering, & Feld, 2017), nutrient pollution altering competitive outcomes (Liu, Deng, Du, Zhang, & Hou, 2019), overharvesting of consumers reducing top down control (Zhang & Vincent, 2019), and now climate change impacting virtually every global biogeochemical cycle (Duarte et al., 2013; Hargrave, Foggo, Pessarrodona, & Smale, 2017). Importantly, several meta-analyses have demonstrated that multiple stressors acting simultaneously often interact in complex ways (e.g., Crain, Kroeker, & Halpern, 2008; Jackson, Loewen, Vinebrooke, & Chimimba, 2016), making net effects difficult to predict.

One meta-analysis, focused on multiple stressors in marine ecosystems, found that most stressor pairs had synergistic effects, suggesting that complex interactions between co-occurring stressors will have larger effect sizes than simply the addition of the effects of single stressors (Crain et al., 2008). However, a second meta-analysis in freshwater ecosystems found the opposite results, with combined stressors most often having smaller effect sizes than the sum of single stressors, an interaction termed antagonistic (Jackson et al., 2016). Yet a third review determined that antagonistic or additive (if stressors A and B affect a species independently, then the total effect on the species is A plus B) relationships are more prevalent than synergistic relationships when dealing with multiple stressors (Côté, Darling, & Brown., 2016). These conflicting results suggest that stressors may interact differently in different ecosystems, or even within an ecosystem. What remains unclear is the mechanism that may drive these differences. Even less explored is if one stressor can result in cumulative or cascading effects that trigger

other stressors, and lead to unforeseen effects or "ecological surprises" (uncertainty about the impact of multiple new and extreme stressors, *sensu* Jackson et al., 2016).

One of the best-studied human stressors is eutrophication (Cloern, 2001; Hu et al., 2017; Moore & Cuker, 2018; Wasson et al., 2017a). Defined as the build-up of organic matter in aquatic systems (Nixon, 1995), eutrophication usually results from excess nutrient (nitrogen (N) and phosphorus (P)) loading. Numerous studies link increased human populations and watershed development with increased nutrient supplies to aquatic systems (Kemp, Testa, Conley, Gilbert, & Hagy, 2009; Valiela et al., 1997; Wallace, Baumann, Grear, Aller, & Gobler, 2014; Wasson et al., 2017b). In turn, these nutrients increase primary productivity and the accumulation of organic matter in the form of phytoplankton and macroalgal blooms (Cloern, 2001; Kennison & Fong, 2014; Lapointe, Burkholder, & Van Alstyne, 2018; Strain, Thomson, Micheli, Mancuso, & Airoldi, 2014; Testa et al., 2017). Thus, many marine ecosystems associated with human-dominated landscapes are eutrophic, including major systems such as Chesapeake Bay (US, Kemp et al., 2005; Testa et al., 2017), Chinhae Bay (South Korea, Lim, Diaz, Hong, & Schaffner, 2006), and the Baltic Sea (Northern Europe, Murray et al., 2019).

Consequences of excessive algal blooms include habitat loss, decreased water quality, changes in sediment chemistry, and depletion of oxygen (O_2) (i.e., hypoxia) in marine systems (Green, Sutula, & Fong, 2014; Hu et al., 2017; Kemp et al., 2009; Rabalais, Turner, Díaz, & Justić, 2009; Tango & Batiuk, 2016). Although similar adverse effects may occur in the open ocean, the magnitude of the impacts are greater in coastal waters and estuaries as these systems are typically shallower and semi-enclosed (Cloern, 2001; McCrackin, Jones, Jones, & Moreno-Mateos, 2017).

Another stressor in marine systems is ocean acidification (OA), which occurs when excess carbon dioxide (CO_2), usually from anthropogenic sources to the atmosphere, increases the formation of carbonic acid in seawater. Dissociation of carbonic acid to bicarbonate and carbonate decreases pH through proton release (Millero, 2007), thereby affecting the solubility of essential elements and their subsequent geochemical cycling (Russell, Thompson, Falkenberg, & Connell, 2009). Research on the biotic effects of OA has generally focused on organisms that produce carbonate shells or skeletons such as oysters, clams, and corals (Evensen & Edmunds, 2018; Gobler, DePasquale, Griffith, & Baumann, 2014). More recently, studies focus on the effect OA may have on primary producers (reviewed in Koch, Bowes, Ross, & Zhang, 2013), some even suggesting OA may stimulate primary productivity (Harley et al., 2012; Koch et al., 2013; Peach, Koch, Blackwelder, & Manfrino, 2017; Wahl et al., 2018).

The preponderance of OA research has focused on the paradigm that acidification occurs from atmospheric input of CO_2 (Evensen & Edmunds, 2018; Peach et al., 2017; Poore et al., 2013; Russell et al., 2009). However, an alternative mechanism is an increase in CO_2 and subsequent acidification due to microbial respiration driven by eutrophication in the form of primary producers blooms (Cai et al., 2011; Laurent et al., 2017; Sunda & Cai, 2012; Wallace et al., 2014). This sequence implies that one stressor (in this case eutrophication) can drive/cause another stressor (e.g., acidification) as suggested by Jackson et al. (2016) for other stressor pairs. Here, large algal blooms can dominate the water column and, during decomposition, fuel microbial degradation of released organic matter, which produces CO_2 and leads to acidification.

Cai et al. (2011) modeled an end of the century pH drop of 0.74 units in coastal estuaries globally with nearly half attributed to respiration processes following phytoplankton blooms resulting from eutrophication. This eutrophication-driven acidification has been increasingly

observed for phytoplankton blooms (Laurent et al., 2017; Sunda & Cai, 2012; Wallace et al., 2014), but has yet to be linked to macroalgal blooms in the field. However, diel fluctuations with decreases in pH at night have been observed during macroalgal blooms (Krause-Jensen et al., 2015), suggesting that the same processes leading to day time acidification may occur for large macroalgal blooms.

In shallow estuaries, eutrophication often takes the form of macroalgal blooms (reviewed in Fong, 2008). Such blooms occur worldwide including such geographically diverse places as Australia (Valesini et al., 2019), Denmark (Rasmussen, Dromph, Göke, & Krause-Jensen, 2015), China (Li et al., 2016), and the US (Lewis & DeWitt, 2017). Macroalgal blooms are usually comprised of opportunistic red or green species of algae that respond with rapid growth to increased nutrient supplies (Fong, 2008), tolerate a wide range of environmental conditions (Hargrave et al., 2017; Kamer & Fong, 2001; Sangil & Guzman, 2016), and undergo episodic or seasonal blooms (Cui et al., 2018; Kennison & Fong, 2014). These blooms often detach from the benthos and form floating rafts that can cover up to 70% of the surface area of shallow estuaries (Green et al., 2014).

Negative effects during the growth phase of blooms include loss of trophic support (Green, Blumstein, & Fong, 2015; Wasson et al., 2017a) and adverse effects on foundational seagrass communities (Bittick, Sutula, & Fong, 2018). During decomposition, blooms can cause fish and invertebrate kills due to reduced O_2 content in the water and sediment, and the accumulation of toxic level of sulfides in the sediment (Green & Fong, 2016). Also, during the decomposition phase, algal mats become negatively buoyant and sink to the seafloor where they lead to the accumulation of organic matter in the sediment (García-Robledo & Corzo, 2011; Kamer, Fong, Kennison, & Schiff, 2004). This large input of labile organic matter can fuel

microbial respiration as the macroalgal bloom degrades (García-Robledo, Corzo, García de Lomas, & van Bergeijk, 2008; Herbert, 1999) and may contribute to acidification by producing CO_2 in excess (e.g., Figure 2.1), as documented in phytoplankton blooms (Sunda & Cai, 2012; Wallace et al., 2014).

In shallow estuarine systems, water and benthic processes are strongly linked, therefore microbial community composition and activity in the sediment may play key roles in eutrophication-driven acidification fueled by macroalgal blooms (Figure 2.1). Many characteristics of bloom-forming macroalgae have the potential to drive changes in microbial community structure and function (Hardison, Canuel, Anderson, & Veuger, 2010), such as their capacity to store N and polyphosphate (poly P) in excess of growth requirements (Fong, 2008; Valiela et al., 1997) and the organic carbon (C) sources such as cellulose, starch, pectin, and lignin found in their cells walls (Holzinger, Herburger, Kaplan, & Lewis, 2015). Certain microbes can use these C sources via respiration or fermentation, resulting in production of CO_2 during decomposition (Hu et al., 2017), which can directly contribute to acidification.

Decomposition processes also promote rapid N and P cycling, which could indirectly contribute to acidification. For example, microbial decomposition of algal organic matter promotes release of orthophosphate (PO_4^{3-}) via poly P degradation (Joshi et al., 2015; Zhang et al., 2015) as well as rapid ammonification, where the organic N is microbially transformed to ammonium (NH_4^+) (Herbert, 1999). The production of NH_4^+ fuels the next step of the N cycle, nitrification, where NH_4^+ is oxidized to nitrate (NO_3^-), and protons are released (Wolf-Gladrow, Zeebe, Klaas, Kortzinger, & Dickson, 2007). The protons and NO_3^- are then reduced via denitrification, and N gas (N_2) is produced (Herbert, 1999).

Normally the coupling of nitrification-denitrification not only prevents the build-up of excess NH_4^+, but also keeps protons balanced (Herbert, 1999; Marchant et al., 2016; Wolf-Gladrow et al., 2007). However, in eutrophic systems these processes often become uncoupled (Duarte et al., 2013; Howarth et al., 2011), which can contribute to acidification. For example, one study found nitrification alone contributed up to 34% of proton production in a coastal lake (Hagens, 2015), although this was not during an algal bloom. The potential for strong links between degradation of macroalgal blooms driven by microbial communities and acidification motivates further exploration into these processes.

One approach that may reveal the microbial link between macroalgal blooms and acidification is to evaluate the functional capacity of the microbial community associated with an acidification event. It is becoming clearer in ecological research that in order to truly link processes we must also consider functional gene expression (e.g., Nostrand, Yin, Wu, Yuan, & Zhou, 2016; Yang et al., 2016) in the field in real time. Thus, our objectives were to 1) determine if macroalgal blooms, like phytoplankton blooms, are associated with acidification in a shallow eutrophic estuary, 2) assess microbial community structure and diversity and relate these to standard abiotic and biotic measures of estuarine health, and 3) evaluate microbial functional gene expression before and during an acidification event.

MATERIALS & METHODS

We conducted seasonal field surveys to characterize changes in biotic and abiotic characteristics between seasons that may be associated with acidification in Upper Newport Bay, CA, USA. During the two sampling events, one each in the wet and dry seasons (December 2017 and June 2018, respectively) we conducted benthic surveys of algal cover, and collected water

and sediment samples to measure water quality and assess the microbial community composition. Finally, in the site that acidified in one season, we collected samples for functional genes in both seasons to compare functional activity.

Upper Newport Bay (UNB, 33.6454° N, 117.8861° W) is a 304-ha estuary located 56 km south of Los Angeles with San Diego Creek draining into the bay. The surrounding 271 km^2 watershed is 71% urbanized with only 5% developed by agriculture (Kennison & Fong, 2014), which leads to increased nutrient input and subsequent macroalgal blooms (Kennison, Kamer, & Fong, 2011). While lower Newport Bay has been dredged to form an embayment for marinas, the upper portion is an ecological reserve with only the main channel dredged. We conducted our surveys along tidal creeks, which have not been dredged, at the same sites used in Kennison and Fong (2013). These sites are at the head of the estuary where there is freshwater inflow, the mouth of the estuary (nearest the ocean inlet), and mid-way between (Figure 2.2).

Environmental analysis

We conducted surveys at low tide in both the wet (December) and dry (June) seasons to maximize measurement of nutrient-rich fresh water entering the estuary and to enable comparison to previous studies in this estuary. During each survey, water quality measurements, including temperature (Oakton portable meter), pH (Oakton portable meter), and salinity (handheld refractometer) were recorded. We collected five water samples in 10 mL syringes and placed them on ice in a dark cooler. These samples were transported to the laboratory within 4 h of collection, filtered (Whatman 0.45 µm), and frozen until analysis (within 1 week of collection). NH_4^+ and PO_4^{3-}, both inorganic nutrient species from recycled sources, were measured photometrically (Grasshoff, Ehrhardt, & Kremling, 1999). Total alkalinity (TA), used

here to assess biogeochemical cycling (Wolf-Gladrow et al., 2007), was determined by acid titration (Dale et al., 2011).

Benthic surveys of algal cover were conducted at each site along a 30 m transect parallel to the waterline and 1 m downslope from the vascular vegetation (Kamer & Fong, 2001; Kennison & Fong, 2014). At 10 randomly selected points along each transect macroalgal cover was assessed using the point intercept technique within a 0.25 m^2 quadrat with 49 intercepts. Macroalgal percent cover was calculated by dividing the number of intersections that covered algae by 49 and multiply by 100.

To measure sediment organic content, we collected surface sediment (top 3 cm) from five of the ten quadrats, dried it for 24 hours at 100 °C, and measured loss after ignition in a 400 °C muffle furnace. We also collected sediment cores in 50 mL centrifuge tubes that were placed in dark coolers and transported back to the lab. Upon return, samples were centrifuged for 20 mins at 10,000 rpms to separate the porewater from the sediment. After centrifugation, the overlying water was removed from each core and analyzed as above for NH_4^+, PO_4^{3-}, and TA.

Survey data were analyzed with 2-factor ANOVA's, with factors being site and season. All data that did not meet parametric assumptions were log transformed and then analyzed with 2-factor ANOVA. Tukey's post hoc tests were conducted after ANOVA detected significant differences. These analyses were conducted in RStudio v. 3.5.1 using package: *car*.

Microbial community analysis

To assess the microbial community diversity, metabarcoding was employed, which uses universal primers that target specific DNA loci that will amplify certain groups of taxa. Using sterile gloves and spatulas, 2 mL sediment samples were collected in triplicate from the top 1 cm of sediment within 5 quadrats at each site in winter and 3 quadrats during summer for

environmental DNA (eDNA) analysis. One field blank was also collected each season by opening an empty tube, swiping the air, and closing the tube within 5 seconds. These samples were also kept cold, transported to the lab and kept frozen at -80°C until analysis (within 2 weeks of collection).

The triplicate samples were pooled, and DNA was extracted using the QIAGEN DNeasy PowerSoil Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions). Blank tubes were also extracted with each set of samples as a negative extraction control for a total of 5 controls. Extracted DNA was amplified in triplicate by polymerase chain reaction (PCR) using the *16S* (515f and 806r; Caporaso et al., 2012) primers with a Nextera adaptor modification.

PCR amplification for each replicate and a PCR negative was performed in 15 µL reactions: 7.5 µL of QIAGEN Multiplex Taq PCR 2x Master Mix, was combined with 0.25 µL of each primer, 2 µL of template DNA and 5 µL of purified water. The reaction was performed under the following thermocycler settings: 15 mins of initial denaturation at 95°C; 13 touchdown cycles of 30 s at 94°C, 30 s at 69.5°C and 60 s at 72°C; 35 amplification cycles of 30 s at 94°C, 30 s at 50°C and 60 s at 72°C; 10 min of final extension at 72°C; hold at 10°C. PCR amplification was conducted 3 times for each sample and successful amplification was visualized using gel electrophoresis in 2% agarose gels with 200 mL of 1x Tris-Acetate-EDTA buffer and stained with 6 µL of SyberSafe (Thermo Fisher, Waltham, MA, USA).

Samples were cleaned using AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA), quantified on a Victor 3V Plate Reader, and indexed using dual i7 and i5 Illumina Nextera indices (Illumina, San Diego, CA, USA). The clean library was sent to the University of California, Santa Cruz Paleogenomics lab and sequenced on an Illumina MiSeq v3 2x300

platform. The raw sequence reads are available in the National Center for Biotechnology Information (NCBI) database under bioproject (ID pending).

We used the newly developed *Anacapa Toolkit* for taxonomy assignment using its default parameters (see version 1.1 supplemental in Curd et al., 2018). In brief, quality control was performed, and chimeras were removed. Then amplicon sequence variants (ASVs) were assigned using *DADA2* (Callahan et al., 2016), next taxonomy was assigned using *Bowtie 2* with the Bayesian Least Common Ancestor (BCLA) method (Langmead & Salzberg, 2012).

Final results tables were generated by modifying the output tables of reads per site sample for each taxon. First, the NCBI-based classification was converted to follow recent revision (Ruggiero et al., 2015) which produced more complete taxonomic strings for estimating phylogenetic diversity at different taxonomic hierarchies. Samples were decontaminated by removing any taxa found in 3 or more reads in the controls (i.e., PCR negatives, extraction blanks, and field blanks). Lastly, taxa only present in one or two reads total across all samples, and any unknowns were also removed.

The final ASV tables and metadata were converted to *Phyloseq* objects (McMurdie & Holmes, 2013) which merges the taxonomy data with the environmental data collected in the field surveys for community analysis. Data were normalized for analysis by rarefying samples to retain 628 reads each, and a new object summarized to taxonomic family was created. The low number of reads was due to the conservative decontamination step explained above. OTU (operational taxonomic unit) tables were extracted to identify the most abundant (defined here as >10 % of relative abundance) taxa between sites and seasons and visualize differences. A dominance test, using function “dominant”, was used to determine the most dominant taxa in each sample. Alpha (species) diversity of each community was assessed at each site and season

using the Shannon Index, and analyzed with a 2-factor ANOVA. Beta diversity, which accounts for spatial differences of the community, was assessed with a principal coordinate analysis (PCoA) using the Bray-Curtis distance index and analyzed with a PERMANOVA with factors being site and season. A constrained analysis of principal coordinates (CAP) was also conducted to observe how the environmental data contributed to the community structure. Site means of all measured variables were used for this analysis; the model and variable significance were analyzed using function "anova." These analyses were conducted in RStudio v. 3.5.1 using packages: dplyr, *ranacapa*, *phyloseq*, *vegan*, *microbiome*, and, *ggplot2*.

We chose to quantify functional activity of the benthic microbe community where acidification occurred; thus, only the middle site was chosen for functional gene exploration. Two subsamples (winter, non-acidified and summer, acidified) from the middle site's extracted DNA (1µg) were sent to the University of Oklahoma Institute for Environmental Genomics and analyzed using GeoChip 5.0 (Glomics Inc., Norman, OK, USA). We used this microarray-based metagenomic tool to assess functional gene diversity as it is comprised of 167,000 oligonucleotide probes covering 1500 functional gene families involved in processes such as C, N, and P cycling. The two subsamples were processed as described in Nostrand et al., (2016), which included amplification, labeling, hybridization, and data preprocessing. Removal of poor-quality spots and normalization of spot signal intensity were conducted as described in (Liang et al., 2010) and provided by Glomics Inc. as a customer service. In brief, spots were scored as positive and retained if the signal-to-noise ratio [SNR = (signal mean – background mean)/background standard deviation] was ≥ 2.0, the coefficient of variation (CV) of the background was < 0.8, and signal intensity 1.3 times the background. In addition, spots with signal intensity less than 200 were discarded (Nostrand et al., 2016).

Functional gene data were uploaded to the microarray data manager provided by Glomics Inc. (ieg.ou.edu/microarray). Data were normalized by dividing the total intensities of the probes per gene by the number of probes expressed in each gene. Intensity represents how many probes expressed a particular gene, and therefore is an estimate of prevalence/frequency of occurrence. As there were only 2 samples total, with one for each season, no statistical analysis was conducted. Visual analysis of gene diversity between the seasons was conducted graphically.

RESULTS

Environmental analysis

Percent cover of *Ulva spp.* did not differ between seasons but was different among sites in the estuary (Figure 2.3A; 2-factor ANOVA, $p = 0.029$ for site). *Ulva spp.* cover appeared the highest in the middle site with an average of 82% cover across both seasons, though mouth and middle sites did not differ statistically. TA was highest in the middle site in winter and at the head site during summer (Figure 2.3B), resulting in a statistical interaction (2-factor ANOVA, $p < 0.0001$ for the site by season interaction). Following the same pattern as TA, PO_4^{3-} changed from being the highest in the middle during the winter to the head in the summer (Figure 2.3C), also resulting in an interaction (2-factor ANOVA, $p < 0.0001$ for the interaction). NH_4^+ was also highest in the middle site during the winter and varied among sites, (1-factor ANOVA, $p < 0.0001$ for site), but was not measurable at any site in the summer (Figure 2.3D).

pH in the middle site dropped from 8.3 in the winter to 7.6 in summer, suggesting acidification. In contrast, pH in the head site increased from 8.17 to 8.26 and in the mouth site from 8.31 to 8.55, which are above the oceanic baseline of 8.1.

Overall sediment organic content was higher in the summer (Figure 2.4A; 2-factor ANOVA, $p = 0.02$ for season) and consistently highest at the middle site (2-factor ANOVA, $p < 0.0001$ for site), with no interaction. There were no differences due to site or season in sediment porewater TA (Figure 2.4B; 2-factor ANOVA, $p > 0.05$ for both factors and the interaction). PO_4^{3-} was higher in the summer (2-factor ANOVA, $p = 0.005$ for season) but similar across sites (Figure 2.4C). In contrast, NH_4^+ was lower in the summer (2-factor ANOVA, $p = 0.004$ for season), but also with no differences between sites (Figure 2.4D).

Microbial community analysis

We identified 246 families of bacteria and archaea across our sites. Families Flavobacteriaceae, Chromatiaceae, Cyclobacteriaceae, Desulfobulbaceae, Cytophagaceae, Pseudomonadaceae, Planctomycetaceae, and Desulfobacteraceae each comprised at least 10% of the relative abundance among samples (Figure 2.5). Of those families, Flavobacteriaceae dominated the head and mouth during the winter and summer, while Chromatiaceae and Cyclobacteriaceae dominated the middle.

There was no significant difference in the microbial community alpha diversity between sites or seasons (ANOVA, p-value>0.05). However, there was an interaction between site and season for beta diversity (PERMANOVA, p-value=0.003 for the interaction) with the middle and head sites grouped together regardless of season, while diversity at the mouth separated seasonally (Figure 2.6).

Microbial community structure at the middle site in summer was associated with porewater PO_4^{3-}; while numerous variables (porewater NH_4^+, water column NH_4^+, PO_4^{3-}, and TA, organic content, and % *Ulva spp.*) were related with the middle community in the winter

(Figure 2.7). pH was associated with the community at the mouth in winter, but not in summer, while the head appeared not to be associated with any of the variables.

Functional genes that mediate several major biogeochemical and metabolic processes were detected in our samples, including those involved in C, N, and P cycling (Figure 2.8). Expression of these genes, measured as signal intensity, was higher in summer, when acidification occurred, than in winter.

Functional genes for C degradation were detected for 5 key C sources, with 5 for starch, 4 for chitin, 11 for pectin, 5 for cellulose, and 5 for lignin (Figure 2.9). One gene for pectin degradation, *pel_Cdeg,* was nearly an order of magnitude higher than the other genes and reached the highest values in the summer when *Ulva spp.* was also the most abundant.

Functional genes for the entire N cycle (2 for ammonification, 2 for annamox, 4 for assimilatory N reduction, 5 for denitrification, 2 for dissimilatory N reduction, 2 for nitrification, and 1 for N fixation) were detected (Figure 2.10). One nitrification gene, *amoA*, had the highest signal intensity overall and expression was highest in summer followed by the ammonification gene *gdh*. Genes representing other N cycling processes, such as denitrification, though present, expressed lower signal intensities than those for nitrification and ammonification.

P cycle genes including one each for phytic acid hydrolysis, polyphosphate synthesis, polyphosphate degradation, and P oxidation were detected (Figure 2.11); degradation genes (i.e., *ppx*) expressed the highest intensity and were highest in the summer.

DISCUSSION

To our knowledge, our findings represent the first field evidence that eutrophication of shallow coastal estuaries dominated by green macroalgal blooms can cascade to acidification.

Previously, acidification in estuaries has only been documented as a result of phytoplankton blooms (e.g., Wallace et al., 2014) or for macroalgae at night (Krause-Jensen et al., 2015). In our study, acidification occurred during the summer at the site where there was a persistent *Ulva spp.* bloom. Observed water pH was 0.71 units lower than measured in the winter, which is on the higher end of acidification observed during phytoplankton blooms. For example, during a red tide in the Bohai Sea, pH was 0.29 units lower between June and August (Zhai, Zhao, Zheng, & Xu, 2012). Further, differences of < 0.5 pH units were observed between surface and bottom waters during a phytoplankton bloom in the Gulf of Mexico (Cai et al., 2011). Larger decreases of > 1 pH unit were measured in eutrophic estuaries across the northeast US (Wallace et al., 2014).

One key requisite for the large drops in pH noted in these latter studies was that phytoplankton bloom-induced microbial respiration was coupled with summer stratification and hypoxia, which isolates and intensified microbial activity in the water column (Laurent et al., 2017). As stratification doesn't occur in most shallow coastal estuaries (Day & Smith, 1988, but see Nezlin et al. 2009 for temporary stratification that can occur at the mouth of upper Newport Bay), the acidification in our study must have occurred when water and sediment processes were coupled. Thus, the macroalgal bloom-induced acidification measured in our study was likely influenced by microbial processes in both the sediment and the water column. That acidification can occur in response to macroalgal blooms and without stratification in shallow estuaries has global implications, as more estuarine systems are becoming impacted by mat-forming macroalgal blooms (Strain et al., 2014).

Acidification in our study was likely driven by the same excessive productivity/decomposition cycle found for phytoplankton-induced acidification. However, in

our case excessive accumulation of organic matter was in the form of macroalgae. Degradation of *Ulva spp.* was indicated by a high sediment organic content, and confirmed by the expression of C degradation genes in the summer, particularly those that degrade pectin, a large component of *Ulva spp*'s cell wall (Holzinger et al., 2015). This finding adds to other laboratory and field studies globally that have observed macroalgal blooms contributing to organic matter availability in the sediments following degradation (Hardison et al., 2010; García-Robledo & Corzo, 2011; Sfriso, Pavoni, Marcomini, & Orio, 1992). Further, a 2-fold increase in C degradation gene expression compared to the control site was found in the Xiamen Sea during a phytoplankton bloom (Yang et al., 2016), although they did not measure subsequent acidification. Taken together, these studies suggest that macroalgal blooms, like phytoplankton blooms, will result in degradation processes that can produce CO_2 in excess and contribute to acidification.

Another explanation for the acidification observed in our study could be that degradation of the macroalgal bloom released excess nutrients that intensified and uncoupled N cycle processes. For example, our results suggest that ammonification onset during decomposition, as functional genes responsible for ammonification were higher during acidification. In addition, NH_4 produced during ammonification likely drove increased nitrification as these genes had the highest signal intensity.

In contrast denitrification genes did not express as highly during acidification, suggesting the uncoupling of the nitrification/denitrification processes. This uncoupling may have led to acidification as protons that were released during nitrification were not utilized for denitrification (Wolf-Gladrow et al., 2007). Presence of Gammaproteobacteria in the Chromatiaceae family provide additional support that nitrification was occurring, as some genera are chemolithoautotrophs that oxidize inorganic N (i.e., nitrification), and are termed ammonia-

oxidizing bacteria (Klotz et al., 2006). Similarly, another field study measured increases of ammonia-oxidizing archaea during the post-phytoplankton bloom stage (Zhou et al., 2018), supporting that algal organic matter will stimulate nitrification. Other studies have observed uncoupling of nitrification-denitrification in eutrophic estuaries, but it is usually the reverse effect with nitrification, an oxygen consuming process, reduced due to phytoplankton bloom-induced hypoxia (Duarte et al., 2013; Howarth et al., 2011). As both the water column and sediment in shallow estuaries interact with the atmosphere, O_2 depletion may not be as severe, and therefore O_2 would be available to fuel nitrification and subsequent acidification.

It is possible that degradation of poly P during the macroalgal bloom also contributed to the acidification measured in our study. Degradation of poly P during acidification was indicated in our study by an increase in porewater PO_4^{3-} and confirmed by the high expression of poly P degradation genes. Numerous field and laboratory studies have observed increased PO_4^{3-} fluxes during blooms (Boynton & Kemp, 1985; García-Robledo & Corzo, 2011; Joshi et al., 2015; Nędzarek & Rakusa-Suszczewski, 2004), and specifically during poly P degradation (Zhang et al., 2015). Further, the presence of Bacteroidetes from the family Cyclobacteriaceae support this explanation, as some genera that are chemoorganotrophs with fermentative metabolism that degrade polysaccharides (Pinnaka & Tanuku, 2014) are commonly associated with phytoplankton blooms (Eckert, Salcher, Posch, Eugster, & Pernthaler, 2012; Salcher, Posch, & Pernthaler, 2013).

There is some evidence that live *Ulva spp.* could have contributed to acidification through internal poly P degradation associated with severe nutrient limitation (Kumari, Kumar, Reddy, & Jha, 2014), and we found extremely low concentrations of PO_4^{3-} in the water column. One study found *Ulva lactuca* exhibited internal poly P degradation with increased acid

phosphatase activity when exposed to PO_4^{3-} concentrations (Lee, 2000) comparable to those in our field survey (1 vs 2 µM, respectively). Thus, it is likely that multiple processes contributed to the acidification found in our field study.

Overall, our results show macroalgal blooms cause a sequence of biogeochemical processes that ultimately can drive acidification in shallow coastal estuaries, and that these processes are mediated by a dynamic microbial community. It must be noted that macroalgal blooms occurred in all of the sites and in both of the seasons at Upper Newport Bay, albeit not in as great a magnitude as the site with acidification. Further, all sites had similar water column and porewater characteristics and shared many microbial community members and functions. Thus, it is likely that all of the sites had the capacity to acidify, if the macroalgal bloom abundance was greater. As human population and subsequent nutrient pollution increases in watersheds globally, ecological phenomenon such as eutrophication will only be intensified. These adverse effects leading to acidification will be exacerbated in shallow coastal estuaries dominated by macroalgal blooms and tightly linked sediment-water interfaces. We assert that more research must be conducted on the long-term effects of eutrophication and the cascading stressors that follow.

FIGURES

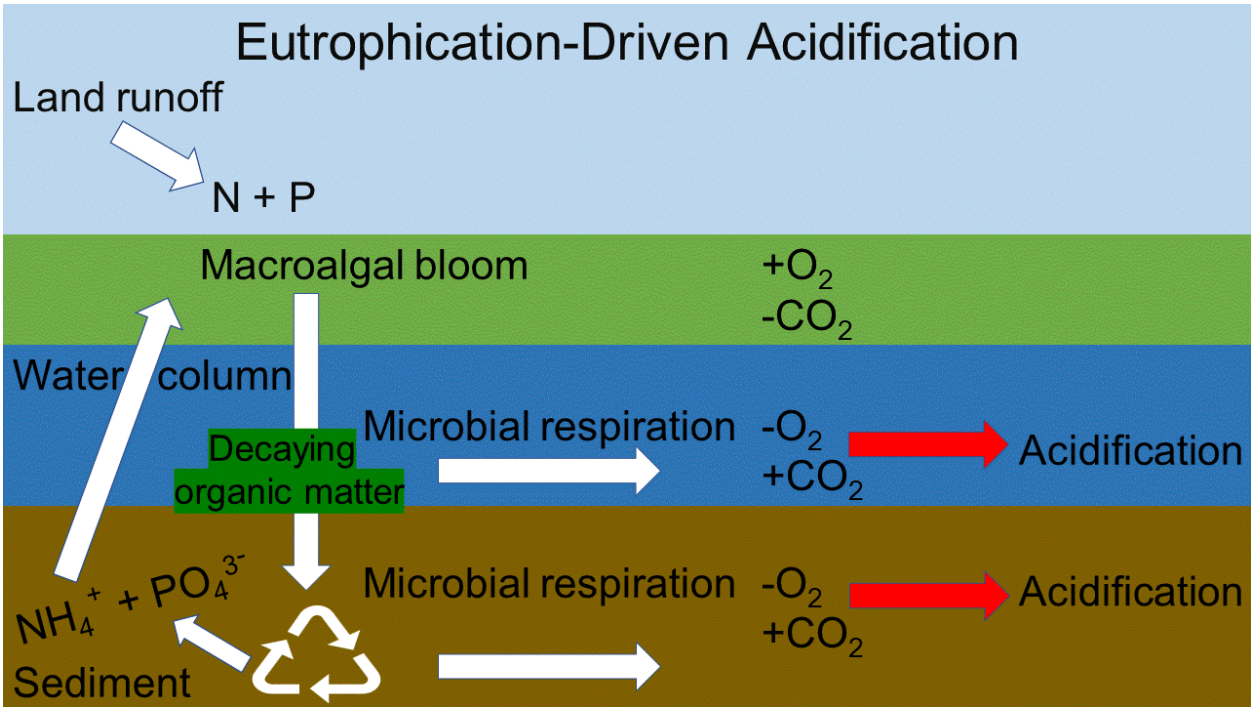

Figure 2.1. Conceptual diagram of the processes that can lead to eutrophication-driven acidification in shallow coastal estuaries. Nitrogen (N) and phosphorus (P) enter the water column via land run off and fuel the macroalgal bloom, which results in oxygen (O_2) production and carbon dioxide (CO_2) consumption. Decaying organic matter sinks down the water column fueling microbial respiration and the consumption of O_2 and production of CO_2. Subsequently the decaying organic matter lies on the sediment and nutrients like ammonium (NH_4^+) and orthophosphate (PO_4^{3-}) are recycled in the sediment and flux out to the water column. Additional microbial respiration in the sediment produces acidification.

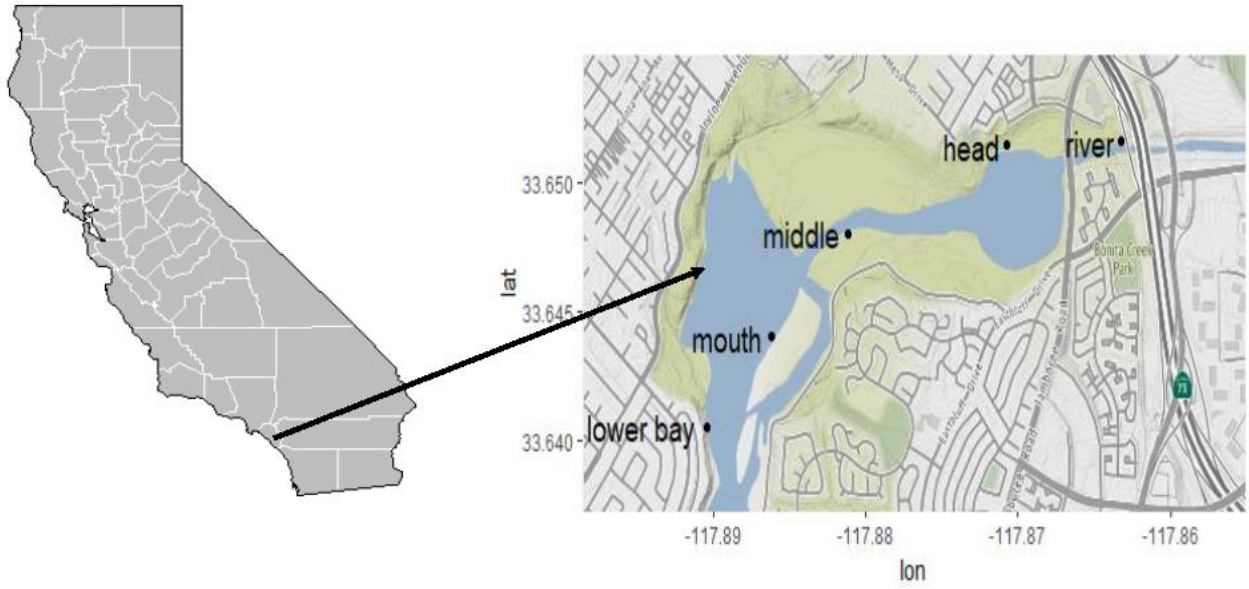

Figure 2.2. Sampling sites in Upper Newport Bay, CA (RStudio v. 3.5.1; package *ggmap*).

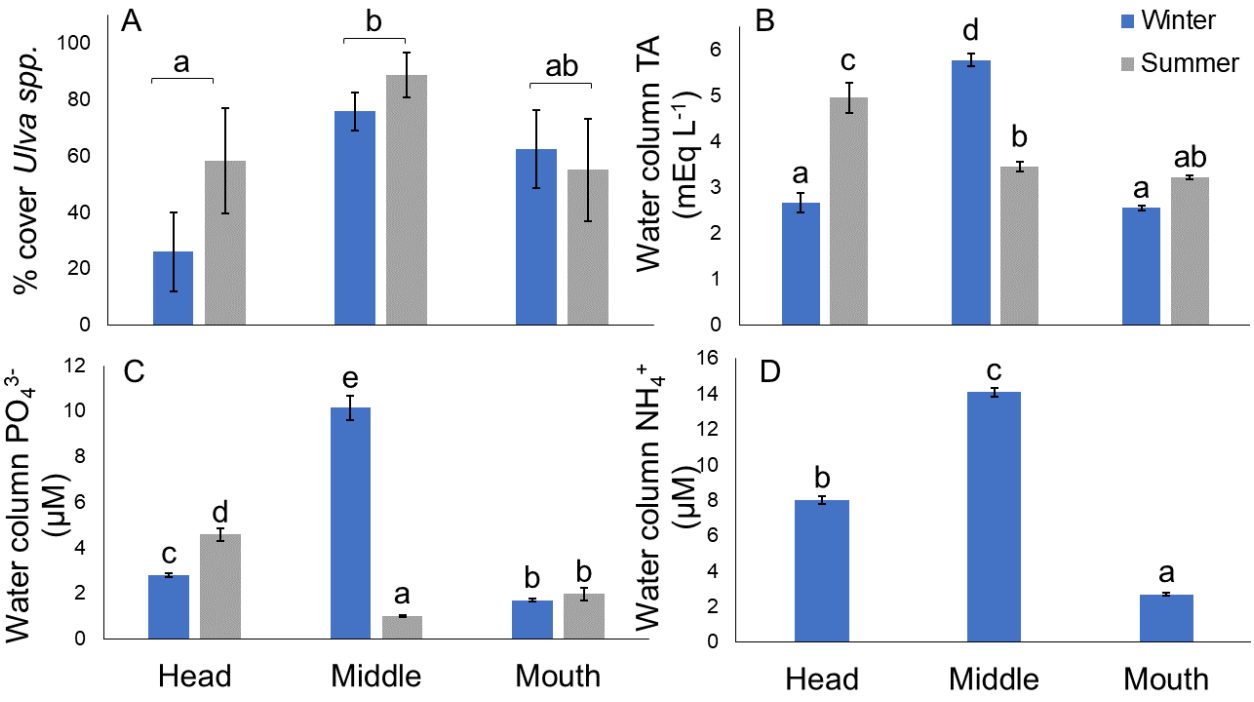

Figure 2.3. Results of benthic surveys and water column water quality measurements. A) % cover *of Ulva spp.* B) total alkalinity (TA) C) orthophosphate (PO_4^{3-}) D) ammonium (NH_4^+) with Tukey posthoc results. Bars are means ± SE, n = 5. *note NH_4^+ was not detected in summer.

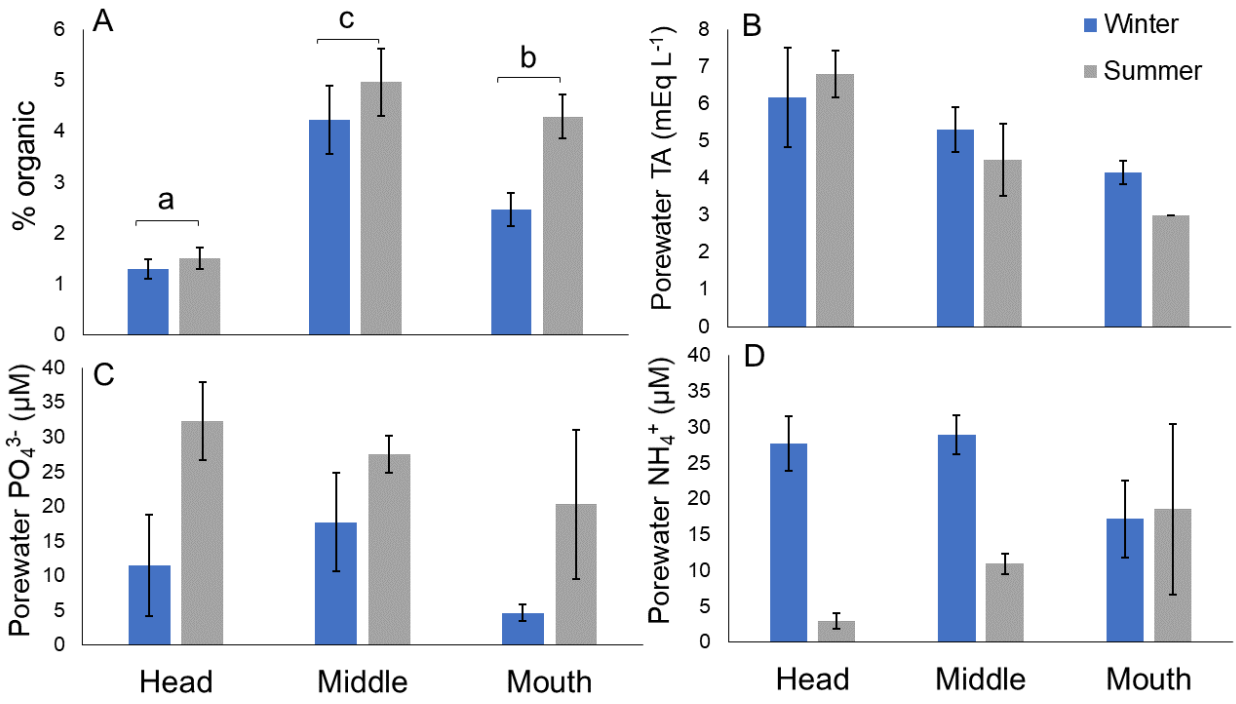

Figure 2.4. Results of sediment analysis. A) organic content B) total alkalinity (TA) C) orthophosphate (PO_4^{3-}) D) ammonium (NH_4^+); winter (n = 5), summer (n = 3; TA: head n = 2, mouth n = 1; PO_4^{3-}: head n = 2; NH_4^+: head n = 2, mouth n = 2). Bars are means ± SE.

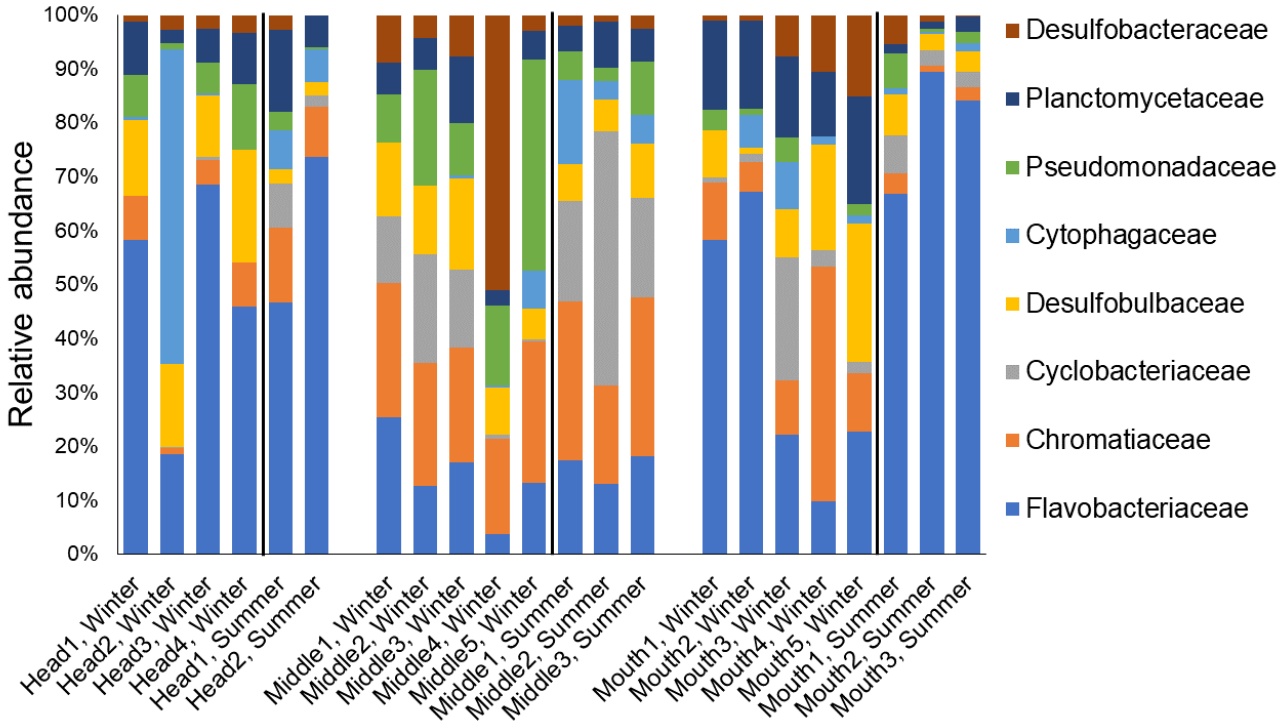

Figure 2.5. Family composition of the microbial community comprising >10% of the relative abundance. Head winter n=4; head summer n=2.

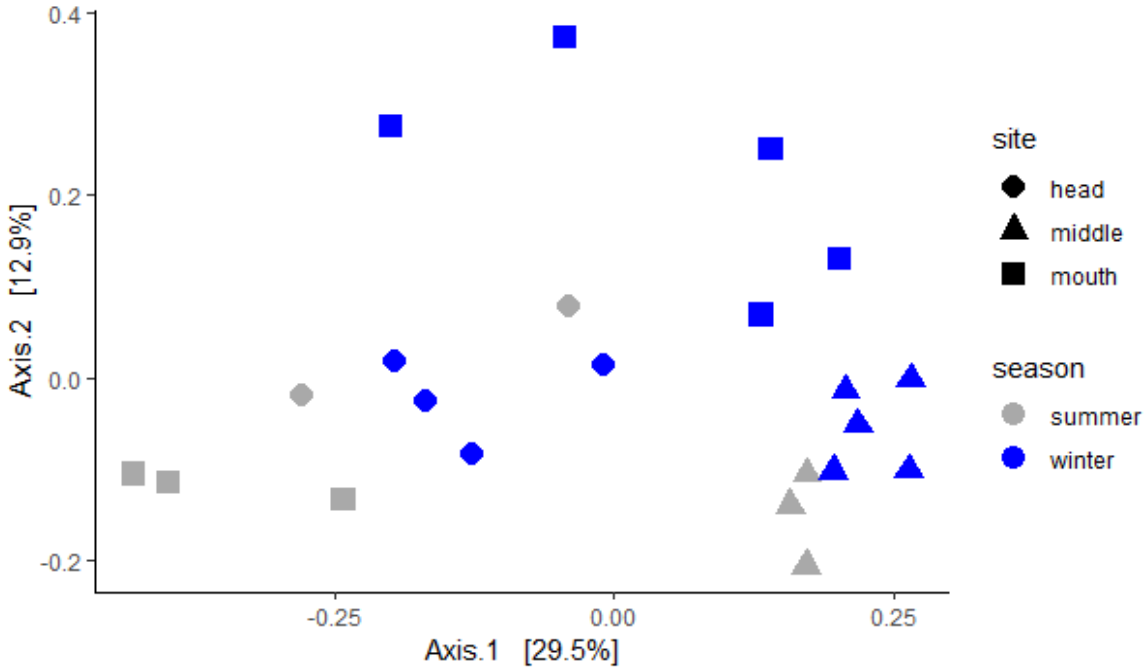

Figure 2.6. Principal Coordinate Analysis (PCoA) plot for the beta diversity of microbial families.

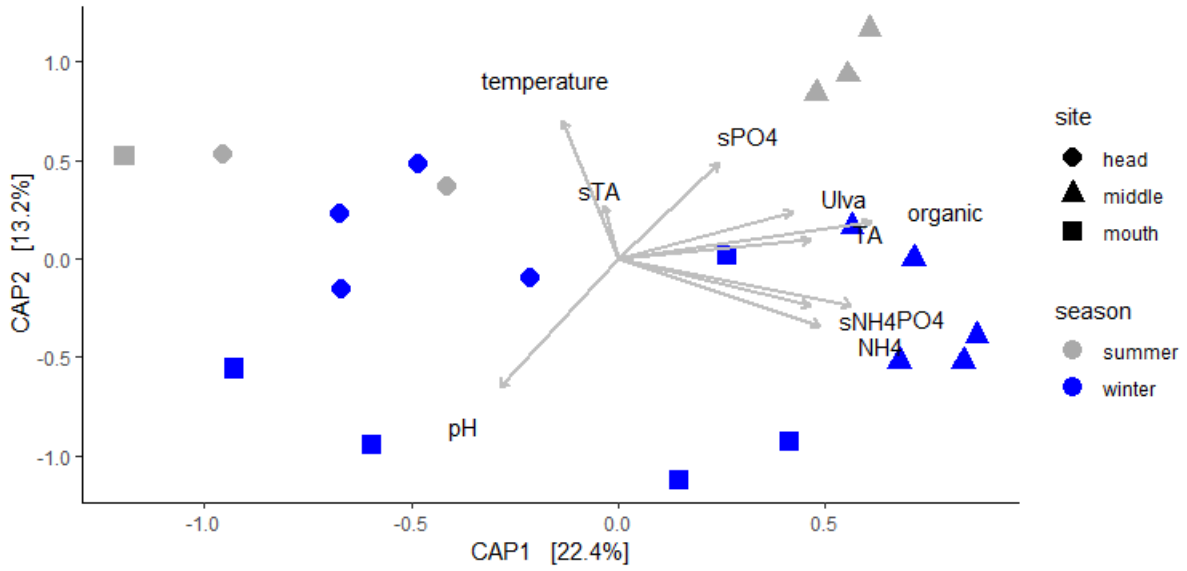

Figure 2.7. Constrained analysis of principal coordinates (CAP) plot. Arrows indicate factors influencing community composition.

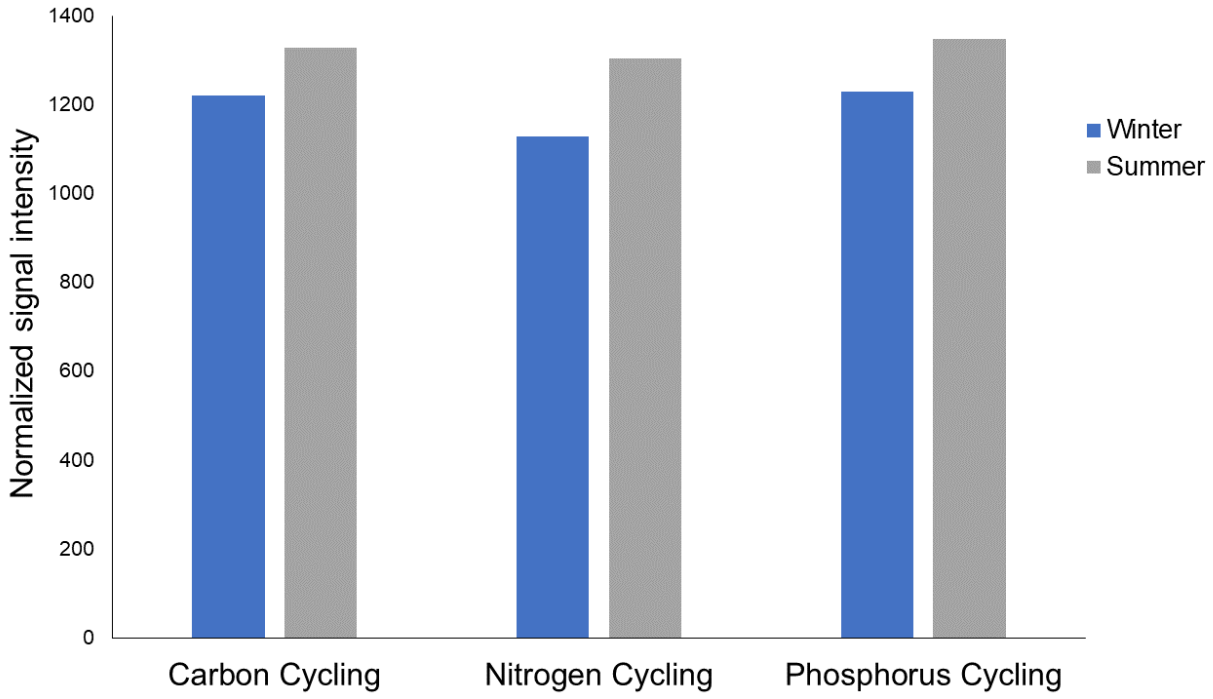

Figure 2.8. The normalized signal intensity of observed functional gene categories for the middle site. *not all data displayed

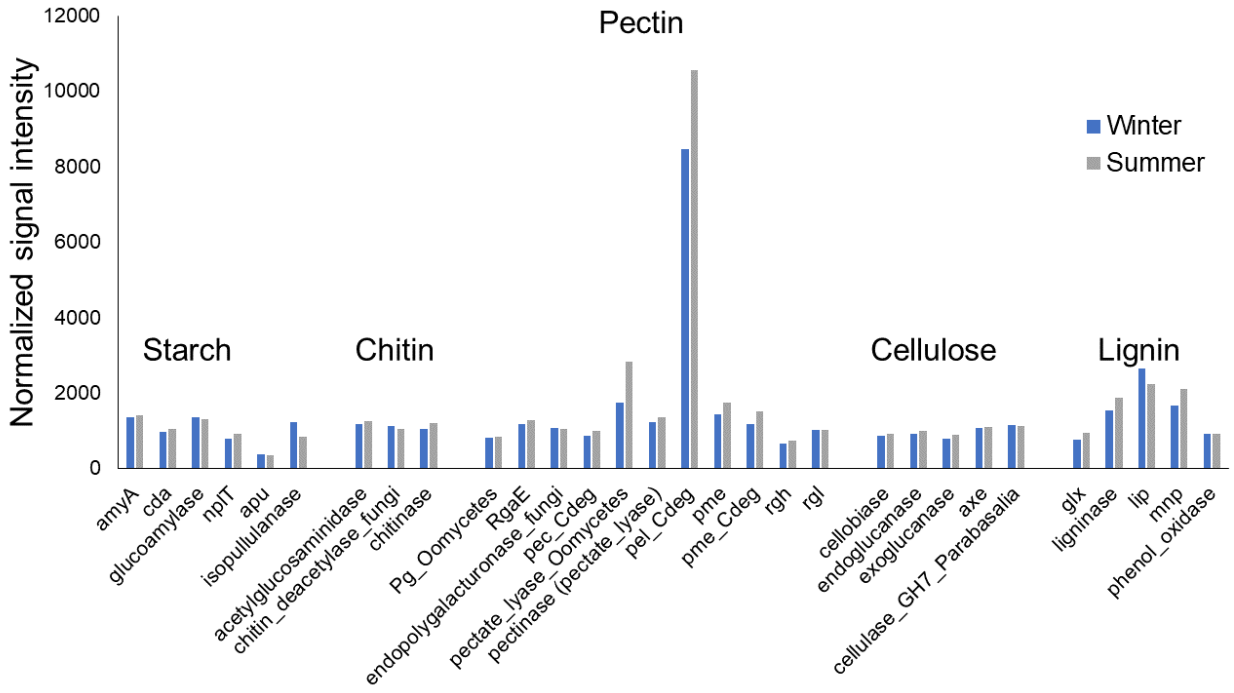

Figure 2.9 The normalized signal intensity of carbon (C) degradation functional genes with C source indicated.

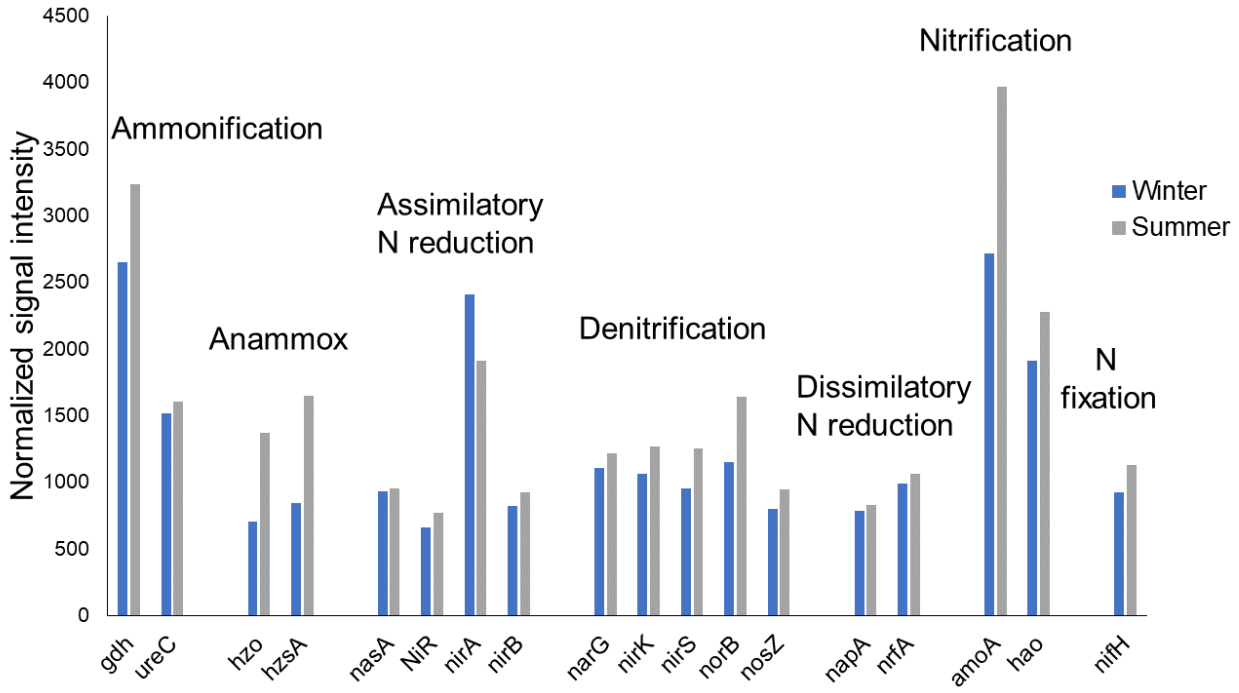

Figure 2.10 Normalized signal intensity of functional genes involved in the nitrogen cycle with processes indicated.

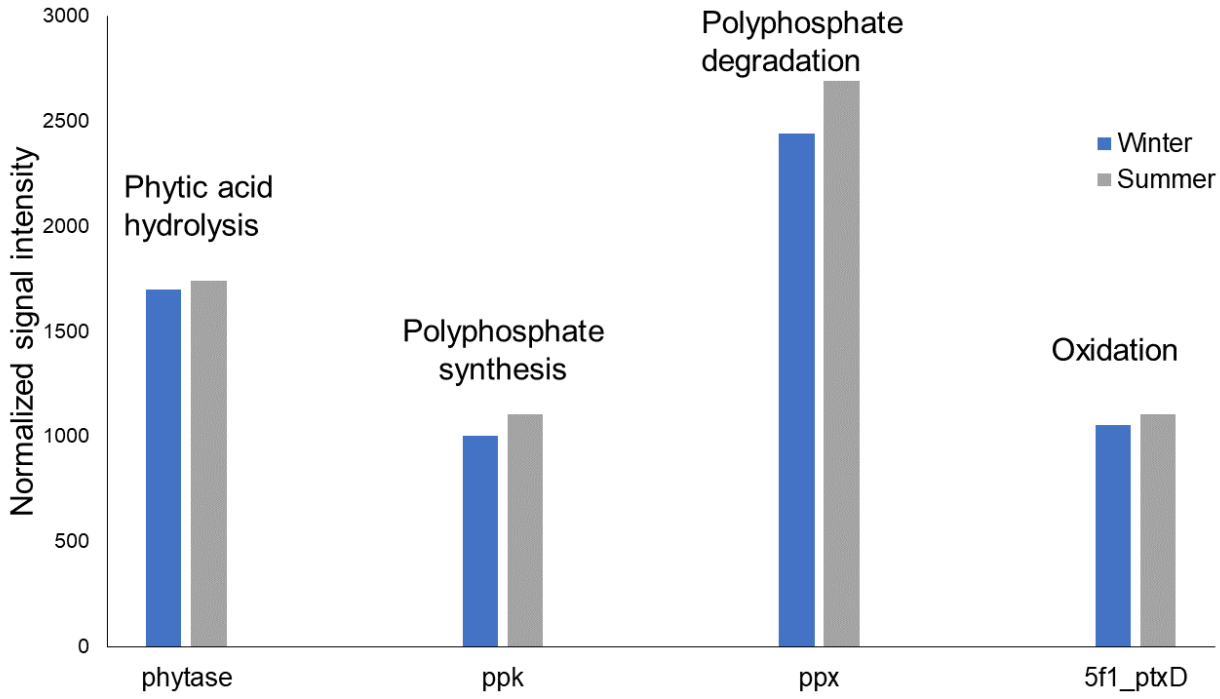

Figure 2.11 Normalized signal intensity of functional genes involved in the phosphorus cycle with processes indicated.

REFERENCES

Bittick, S. J., Sutula, M., & Fong, P. (2018). A tale of two algal blooms: Negative and predictable effects of two common bloom-forming macroalgae on seagrass and epiphytes. *Marine Environmental Research*. https://doi.org/10.1016/j.marenvres.2018.05.018

Boynton, W., & Kemp, W. (1985). Nutrient regeneration and oxygen consumption by sediments along an estuarine salinity gradient. *Marine Ecology Progress Series*, *23*, 45–55. https://doi.org/10.3354/meps023045

Cai, W. J., Hu, X., Huang, W. J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., … Gong, G. C. (2011). Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience*. https://doi.org/10.1038/ngeo1297

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., … Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The Isme Journal*, *6*, 1621. Retrieved from https://doi.org/10.1038/ismej.2012.8

Cloern, J. E. (2001). Our evolving conceptual model of the coastal\reutrophication problem. *Marine Ecology Progress Series*, *210*, 223–253. https://doi.org/10.3354/meps210223

Côté, I. M., Darling, E. S., & Brown, C. J. (2016). Interactions among ecosystem stressors and their importance in conservation. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1824), 20152592. https://doi.org/10.1098/rspb.2015.2592

Crain, C. M., Kroeker, K., & Halpern, B. S. (2008). Interactive and cumulative effects of

multiple human stressors in marine systems. *Ecology Letters*, *11*(12), 1304–1315. https://doi.org/10.1111/j.1461-0248.2008.01253.x

Cui, J., Shi, J., Zhang, J., Wang, L., Fan, S., Xu, Z., … He, P. (2018). Rapid expansion of Ulva blooms in the Yellow Sea, China through sexual reproduction and vegetative growth. *Marine Pollution Bulletin*, *130*, 223–228. https://doi.org/https://doi.org/10.1016/j.marpolbul.2018.03.036

Curd, E. E., Gold, Z., Kandlikar, G. S., Gomer, J., Ogden, M., Connell, T. O., … Meyer, R. S. (2018). Anacapa Toolkit : an environmental DNA toolkit for processing multilocus metabarcode datasets.

Dale, A. W., Sommer, S., Bohlen, L., Treude, T., Bertics, V. J., Bange, H. W., … Wallmann, K. (2011). Rates and regulation of nitrogen cycling in seasonally hypoxic sediments during winter (Boknis Eck, SW Baltic Sea): Sensitivity to environmental variables. *Estuarine, Coastal and Shelf Science*, *95*(1), 14–28. https://doi.org/https://doi.org/10.1016/j.ecss.2011.05.016

Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Steckbauer, A., Ramajo, L., … Mcculloch, M. (2013). Is Ocean Acidification an Open-Ocean Syndrome ? Understanding Anthropogenic Impacts on Seawater pH, 221–236. https://doi.org/10.1007/s12237-013-9594-3

Eckert, E. M., Salcher, M. M., Posch, T., Eugster, B., & Pernthaler, J. (2012). Rapid successions affect microbial N-acetyl-glucosamine uptake patterns during a lacustrine spring phytoplankton bloom. *Environmental Microbiology*, *14*(3), 794–806. https://doi.org/10.1111/j.1462-2920.2011.02639.x

Evensen, N. R., & Edmunds, P. J. (2018). Effect of elevated pCO2 on competition between the

scleractinian corals Galaxea fascicularis and Acropora hyacinthus. *Journal of Experimental Marine Biology and Ecology*, *500*(February 2017), 12–17. https://doi.org/10.1016/j.jembe.2017.12.002

Fong, P. (2008). Macroalgal-Dominated Ecosystems. In *Nitrogen in the Marine Environment*. https://doi.org/10.1016/B978-0-12-372522-6.00020-7

García -Robledo, E., Corzo, A., García de Lomas, J., & van Bergeijk, S. A. (2008). Biogeochemical effects of macroalgal decomposition on intertidal microbenthos: a microcosm experiment . *Marine Ecology Progress Series*, *356*, 139–151. Retrieved from https://www.int-res.com/abstracts/meps/v356/p139-151/

García-Robledo, E., & Corzo, A. (2011). Effects of macroalgal blooms on carbon and nitrogen biogeochemical cycling in photoautotrophic sediments: An experimental mesocosm. *Marine Pollution Bulletin*, *62*(7), 1550–1556. https://doi.org/https://doi.org/10.1016/j.marpolbul.2011.03.044

Gieswein, A., Hering, D., & Feld, C. K. (2017). Additive effects prevail: The response of biota to multiple stressors in an intensively monitored watershed. *Science of the Total Environment*, *593–594*, 27–35. https://doi.org/10.1016/j.scitotenv.2017.03.116

Gobler, C. J., DePasquale, E. L., Griffith, A. W., & Baumann, H. (2014). Hypoxia and Acidification Have Additive and Synergistic Negative Effects on the Growth, Survival, and Metamorphosis of Early Life Stage Bivalves. *PLOS ONE*, *9*(1), e83648. Retrieved from https://doi.org/10.1371/journal.pone.0083648

Grasshoff, K., Ehrhardt, M., Kremling K. (1999). Methods of Seawater Analysis.Weinheim, Germany:Wiley-VCH Verlag GmbH.

Green, L., Blumstein, D. T., & Fong, P. (2015). Macroalgal Mats in a Eutrophic Estuary Obscure

Visual Foraging Cues and Increase Variability in Prey Availability for Some Shorebirds. *Estuaries and Coasts*. https://doi.org/10.1007/s12237-014-9862-x

Green, L., & Fong, P. (2016). The good, the bad and the Ulva: the density dependent role of macroalgal subsidies in influencing diversity and trophic structure of an estuarine community. *Oikos*. https://doi.org/10.1111/oik.02860

Green, L., Sutula, M., & Fong, P. (2014). How much is too much? Identifying benchmarks of adverse effects of macroalgae on the macrofauna in intertidal flats. *Ecological Applications*. https://doi.org/10.1890/13-0524.1

Grillo-Puertas, M., Schurig-Briccio, L. A., Rodríguez-Montelongo, L., Rintoul, M. R., & Rapisarda, V. A. (2014). Copper tolerance mediated by polyphosphate degradation and low-affinity inorganic phosphate transport system in Escherichia coli. *BMC Microbiology*, *14*(1), 72. https://doi.org/10.1186/1471-2180-14-72

Hagens, M. (2015). *Impact of biogeochemical processes on pH dynamics in marine systems* (Vol. PhD thesis). Retrieved from http://dspace.library.uu.nl/handle/1874/313165

Hagens, M., Slomp, C. P., Meysman, F. J. R., Seitaj, D., Harlay, J., Borges, A. V., & Middelburg, J. J. (2015). Biogeochemical processes and buffering capacity concurrently affect acidification in a seasonally hypoxic coastal marine basin. *Biogeosciences*, *12*(5), 1561–1583. https://doi.org/10.5194/bg-12-1561-2015

Hardison, A. K., Canuel, E. A., Anderson, I. C., & Veuger, B. (2010). Fate of macroalgae in benthic systems: carbon and nitrogen cycling within the microbial community . *Marine Ecology Progress Series*, *414*, 41–55. Retrieved from https://www.int-res.com/abstracts/meps/v414/p41-55/

Hargrave, M. S., Foggo, A., Pessarrodona, A., & Smale, D. A. (2017). The effects of warming on

the ecophysiology of two co-existing kelp species with contrasting distributions. *Oecologia*, *183*(2), 531–543. https://doi.org/10.1007/s00442-016-3776-1

Harley, C. D. G., Anderson, K. M., Demes, K. W., Jorve, J. P., Kordas, R. L., Coyle, T. A., & Graham, M. H. (2012). EFfects Of Climate Change On Global Seaweed Communities. *Journal of Phycology*, *48*(5), 1064–1078. https://doi.org/10.1111/j.1529-8817.2012.01224.x

Herbert, R. A. (1999). Nitrogen cycling in coastal marine ecosystems. *FEMS Microbiology Reviews*, *23*(5), 563–590. https://doi.org/10.1111/j.1574-6976.1999.tb00414.x

Holzinger, A., Herburger, K., Kaplan, F., & Lewis, L. A. (2015). Desiccation tolerance in the chlorophyte green alga Ulva compressa: does cell wall architecture contribute to ecological success? *Planta*, *242*(2), 477–492. https://doi.org/10.1007/s00425-015-2292-6

Howarth, R., Chan, F., Conley, D. J., Garnier, J., Doney, S. C., Marino, R., & Billen, G. (2011). Coupled biogeochemical cycles: Eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. In *Frontiers in Ecology and the Environment* (Vol. 9, pp. 18–26). https://doi.org/10.1890/100008

Hu, X., Li, Q., Huang, W. J., Chen, B., Cai, W. J., Rabalais, N. N., & Eugene Turner, R. (2017). Effects of eutrophication and benthic respiration on water column carbonate chemistry in a traditional hypoxic zone in the Northern Gulf of Mexico. *Marine Chemistry*, *194*(November 2016), 33–42. https://doi.org/10.1016/j.marchem.2017.04.004

Jackson, M. C., Loewen, C. J. G., Vinebrooke, R. D., & Chimimba, C. T. (2016). Net effects of multiple stressors in freshwater ecosystems: A meta-analysis. *Global Change Biology*. https://doi.org/10.1111/gcb.13028

Jay, D. A., Smith, J. D. (1988) Residual Circulation in and Classification of Shallow, Stratified Estuaries. In: J. Dronkers & W. van Leussen (Eds.), Physical Processes in Estuaries (pp. 21-

41). Berlin, Heidelberg: Springer.

Joshi, S. R., Kukkadapu, R. K., Burdige, D. J., Bowden, M. E., Sparks, D. L., & Jaisi, D. P. (2015). Organic matter remineralization predominates phosphorus cycling in the mid-bay sediments in the chesapeake bay. *Environmental Science and Technology*, *49*(10), 5887–5896. https://doi.org/10.1021/es5059617

Kamer, K., & Fong, P. (2001). Nitrogen enrichment ameliorates the negative effects of reduced salinity on the green macroalga Enteromorpha intestinalis. *Marine Ecology Progress Series*. https://doi.org/10.3354/meps218087

Kamer, K., Fong, P., Kennison, R. L., & Schiff, K. (2004). The relative importance of sediment and water column supplies of nutrients to the growth and tissue nutrient content of the green macroalga Enteromorpha intestinalis along an estuarine resource gradient. *Aquatic Ecology*. https://doi.org/10.1023/B:AECO.0000021041.31385.19

Kemp, W. M., Boynton, W. R., Adolf, J. E., Boesch, D. F., Boicourt, W. C., Brush, G., … Stevenson, J. C. (2005). Eutrophication of Chesapeake Bay: Historical trends and ecological interactions. *Marine Ecology Progress Series*. https://doi.org/10.3354/meps303001

Kemp, W. M., Testa, J. M., Conley, D. J., Gilbert, D., & Hagy, J. D. (2009). Temporal responses of coastal hypoxia to nutrient loading and physical controls. *Biogeosciences*, *6*(12), 2985–3008. https://doi.org/10.5194/bg-6-2985-2009

Kennison, R. L., & Fong, P. (2014). Extreme Eutrophication in Shallow Estuaries and Lagoons of California Is Driven by a Unique Combination of Local Watershed Modifications That Trump Variability Associated with Wet and Dry Seasons. *Estuaries and Coasts*. https://doi.org/10.1007/s12237-013-9687-z

Kennison, R. L., Kamer, K., & Fong, P. (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*(3), 483–494. https://doi.org/10.1111/j.1529-8817.2011.00994.x

Kirchman, D. L. (2002). The ecology of Cytophaga–Flavobacteria in aquatic environments. *FEMS Microbiology Ecology*, *39*(2), 91–100. https://doi.org/10.1111/j.1574-6941.2002.tb00910.x

Klotz, M. G., Arp, D. J., Chain, P. S. G., El-Sheikh, A. F., Hauser, L. J., Hommes, N. G., … Ward, B. B. (2006). Complete genome sequence of the marine, chemolithoautotrophic, ammonia-oxidizing bacterium Nitrosococcus oceani ATCC 19707. *Applied and Environmental Microbiology*, *72*(9), 6299–6315. https://doi.org/10.1128/AEM.00463-06

Koch, M., Bowes, G., Ross, C., & Zhang, X.-H. (2013). Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology*, *19*(1), 103–132. https://doi.org/10.1111/j.1365-2486.2012.02791.x

Krause-Jensen, D., Duarte, C. M., Hendriks, I. E., Meire, L., Blicher, M. E., Marbà, N., & Sejr, M. K. (2015). Macroalgae contribute to nested mosaics of pH variability in a subarctic fjord. *Biogeosciences*, *12*(16), 4895–4911. https://doi.org/10.5194/bg-12-4895-2015

Kumari, P., Kumar, M., Reddy, C. R. K., & Jha, B. (2014). Nitrate and phosphate regimes induced lipidomic and biochemical changes in the intertidal macroalga ulva lactuca (ulvophyceae, chlorophyta). *Plant and Cell Physiology*, *55*(1), 52–63. https://doi.org/10.1093/pcp/pct156

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*, 357. Retrieved from https://doi.org/10.1038/nmeth.1923

Lapointe, B. E., Burkholder, J. M., & Van Alstyne, K. L. (2018). Harmful Macroalgal Blooms in

a Changing World: Causes, Impacts, and Management. *Harmful Algal Blooms*, 515–560. https://doi.org/10.1002/9781118994672.ch15

Laurent, A., Fennel, K., Cai, W. J., Huang, W. J., Barbero, L., & Wanninkhof, R. (2017). Eutrophication-induced acidification of coastal waters in the northern Gulf of Mexico: Insights into origin and processes from a coupled physical-biogeochemical model. *Geophysical Research Letters*, *44*(2), 946–956. https://doi.org/10.1002/2016GL071881

Lee, T. (2000) Phosphate starvation induction of acid phosphatase in Ulva lactuca L. (Ulvales, Chlorophyta). *Botanical Bulletin of Academia Sinica, 41*, 19–25.

Lewis, N. S., & DeWitt, T. H. (2017). Effect of Green Macroalgal Blooms on the Behavior, Growth, and Survival of Cockles (Clinocardium nuttallii) in Pacific NW Estuaries. *Marine Ecology Progress Series*, *582*, 105–120. https://doi.org/10.3354/meps12328

Li, H., Zhang, Y., Han, X., Shi, X., Rivkin, R. B., & Legendre, L. (2016). Growth responses of Ulva prolifera to inorganic and organic nutrients: Implications for macroalgal blooms in the southern Yellow Sea, China. *Scientific Reports*, *6*, 26498. Retrieved from https://doi.org/10.1038/srep26498

Liang, Y., He, Z., Wu, L., Deng, Y., Li, G., & Zhou, J. (2010). Development of a common oligonucleotide reference standard for microarray data normalization and comparison across different microbial communities. *Applied and Environmental Microbiology*, *76*(4), 1088–1094. https://doi.org/10.1128/AEM.02749-09

Lim, H. S., Diaz, R. J., Hong, J. S., & Schaffner, L. C. (2006). Hypoxia and benthic community recovery in Korean coastal waters. *Marine Pollution Bulletin*, *52*(11), 1517–1526. https://doi.org/10.1016/j.marpolbul.2006.05.013

Liu, Y., Deng, B., Du, J., Zhang, G., & Hou, L. (2019). Nutrient burial and environmental

changes in the Yangtze Delta in response to recent river basin human activities. *Environmental Pollution*, *249*, 225–235. https://doi.org/https://doi.org/10.1016/j.envpol.2019.03.030

Marchant, H. K., Holtappels, M., Lavik, G., Ahmerkamp, S., Winter, C., & Kuypers, M. M. M. (2016). Coupled nitrification–denitrification leads to extensive N loss in subtidal permeable sediments. *Limnology and Oceanography*, *61*(3), 1033–1048. https://doi.org/10.1002/lno.10271

McCrackin, M. L., Jones, H. P., Jones, P. C., & Moreno-Mateos, D. (2017). Recovery of lakes and coastal marine ecosystems from eutrophication: A global meta-analysis. *Limnology and Oceanography*, *62*(2), 507–518. https://doi.org/10.1002/lno.10441

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE*, *8*(4), e61217. Retrieved from https://doi.org/10.1371/journal.pone.0061217

Millero, F. J. (2007). The Marine Inorganic Carbon Cycle, (Table 1), 308–341.

Moore, T. N., & Cuker, B. E. (2018). Sedimentary Oxygen Demand and Orthophosphate Release : Sustaining Eutrophication in a Tributary of the Chesapeake Bay, *7*(3), 42–48. https://doi.org/10.11648/j.wros.20180703.13

Murray, C. J., Müller-Karulis, B., Carstensen, J., Conley, D. J., Gustafsson, B. G., & Andersen, J. H. (2019). Past, Present and Future Eutrophication Status of the Baltic Sea. *Frontiers in Marine Science* . Retrieved from https://www.frontiersin.org/article/10.3389/fmars.2019.00002

Nakamura, A., Kawano, N., Motomura, K., Kuroda, A., Sekiguchi, K., Miyado, M., … Miyado, K. (2018). Degradation of phosphate polymer polyP enhances lactic fermentation in mice.

Genes to Cells, *23*(10), 904–914. https://doi.org/10.1111/gtc.12639

Nędzarek, A., & Rakusa-Suszczewski, S. (2004). Decomposition of macroalgae and the release of nutrient. *Polar Bioscience*, *17*(December), 26–35.

Nezlin, N. P., Kamer, K., Hyde, J., & Stein, E. D. (2009). Dissolved oxygen dynamics in a eutrophic estuary, Upper Newport Bay, California. *Estuarine, Coastal and Shelf Science, 82*(1), 139-151. https://doi.org/10.1016/j.ecss.2009.01.004.

Nixon, S. W. (1995). Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia*, *41*(1), 199–219. https://doi.org/10.1080/00785236.1995.10422044

Nostrand, J. D. Van, Yin, H., Wu, L., Yuan, T., & Zhou, J. (2016). Microbial Environmental Genomics (MEG), *1399*, 183–196. https://doi.org/10.1007/978-1-4939-3369-3

Peach, K. E., Koch, M. S., Blackwelder, P. L., & Manfrino, C. (2017). Calcification and photophysiology responses to elevated pCO2 in six Halimeda species from contrasting irradiance environments on Little Cayman Island reefs. *Journal of Experimental Marine Biology and Ecology*, *486*, 114–126. https://doi.org/https://doi.org/10.1016/j.jembe.2016.09.008

Pinhassi, J., Sala, M. M., Havskum, H., Peters, F., Guadayol, Ò., Malits, A., & Marrasé, C. (2004). Changes in Bacterioplankton Composition under Different Phytoplankton Regimens. *Applied and Environmental Microbiology*, *70*(11), 6753 LP – 6766. https://doi.org/10.1128/AEM.70.11.6753-6766.2004

Pinnaka, A. K., & Tanuku, N. R. S. (2014). The Family *Cyclobacteriaceae*. In: E. Rosenberg et al. (eds.), The Prokaryotes – Other Major Lineages of Bacteria and the Archaea (pp. 551-575). Berlin Heidelberg: Springer-Verlag

Poore, A. G. B., Graba-Landry, A., Favret, M., Sheppard Brennand, H., Byrne, M., &

Dworjanyn, S. A. (2013). Direct and indirect effects of ocean acidification and warming on a marine plant-herbivore interaction. *Oecologia*, *173*(3), 1113–1124. https://doi.org/10.1007/s00442-013-2683-y

Rabalais, N. N., Turner, R. E., Díaz, R. J., & Justić, D. (2009). Global change and eutrophication of coastal waters. ICES Journal of Marine Science: Journal du Conseil, 66(7), 1528-1537. *Environment and Natural Resources Research*, *3*(2), p37. https://doi.org/10.5539/enrr.v3n2p37

Rasmussen, J. R., Dromph, K. M., Göke, C., & Krause-Jensen, D. (2015). Reduced Cover of Drifting Macroalgae Following Nutrient Reduction in Danish Coastal Waters. *Estuaries and Coasts*, *38*(5), 1664–1677. https://doi.org/10.1007/s12237-014-9904-4

Ruggiero, M. A., Gordon, D. P., Orrell, T. M., Bailly, N., Bourgoin, T., Brusca, R. C., … Kirk, P. M. (2015). A Higher Level Classification of All Living Organisms. *PLOS ONE*, *10*(4), e0119248. Retrieved from https://doi.org/10.1371/journal.pone.0119248

Russell, B. D., Thompson, J.-A. I., Falkenberg, L. J., & Connell, S. D. (2009). Synergistic effects of climate change and local stressors: CO2 and nutrient-driven change in subtidal rocky habitats. *Global Change Biology*, *15*(9), 2153–2162. https://doi.org/10.1111/j.1365-2486.2009.01886.x

Salcher, M. M., Posch, T., & Pernthaler, J. (2013). In situ substrate preferences of abundant bacterioplankton populations in a prealpine freshwater lake. *The ISME Journal*, *7*(5), 896–907. https://doi.org/10.1038/ismej.2012.162

Sangil, C., & Guzman, H. M. (2016). Macroalgal communities on multi-stressed coral reefs in the Caribbean : Long-term changes , spatial variations , and relationships with environmental variables. *Journal of Sea Research*, *117*, 7–19.

https://doi.org/10.1016/j.seares.2016.09.001

Sfriso, A., Pavoni, B., Marcomini, A., & Orio, A. A. (1992). Macroalgae, nutrient cycles, and pollutants in the Lagoon of Venice. *Estuaries*, *15*(4), 517. https://doi.org/10.2307/1352394

Strain, E. M. A., Thomson, R. J., Micheli, F., Mancuso, F. P., & Airoldi, L. (2014). Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. *Global Change Biology*, *20*(11), 3300–3312. https://doi.org/10.1111/gcb.12619

Sunda, W. G., & Cai, W.-J. (2012). Eutrophication Induced CO2-Acidification of Subsurface Coastal Waters: Interactive Effects of Temperature, Salinity, and Atmospheric PCO2. *Environmental Science & Technology*, *46*(19), 10651–10659. https://doi.org/10.1021/es300626f

Tango, P. J., & Batiuk, R. A. (2016). Chesapeake Bay recovery and factors affecting trends: Long-term monitoring, indicators, and insights. *Regional Studies in Marine Science*, *4*, 12–20. https://doi.org/https://doi.org/10.1016/j.rsma.2015.11.010

Testa, J. M., Blake Clark, J., Dennison, W. C., Caroline Donovan, E., Fisher, A. W., Ni, W., … Ziegler, G. (2017). Ecological Forecasting and the Science of Hypoxia in Chesapeake Bay. *BioScience*, *67*(7), 614–626. https://doi.org/10.1093/biosci/bix048

Thomas, F., Bordron, P., Eveillard, D., & Michel, G. (2017). Gene Expression Analysis of Zobellia galactanivorans during the Degradation of Algal Polysaccharides Reveals both Substrate-Specific and Shared Transcriptome-Wide Responses. *Frontiers in Microbiology*, *8*, 1808. https://doi.org/10.3389/fmicb.2017.01808

Valesini, F. J., Hallett, C. S., Hipsey, M. R., Kilminster, K. L., Huang, P., & Hennig, K. (2019). Chapter 7 - Peel-Harvey Estuary, Western Australia. In E. Wolanski, J. W. Day, M. Elliott,

& R. B. T.-C. and E. Ramachandran (Eds.) (pp. 103–120). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-814003-1.00007-1

Valiela, I., McClelland, J., Hauxwell, J., Behr, P. J., Hersh, D., & Foreman, K. (1997). Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography*, *42*(5part2), 1105–1118. https://doi.org/10.4319/lo.1997.42.5_part_2.1105

Wahl, M., Schneider Covachã, S., Saderne, V., Hiebenthal, C., Müller, J. D., Pansch, C., & Sawall, Y. (2018). Macroalgae may mitigate ocean acidification effects on mussel calcification by increasing pH and its fluctuations. *Limnology and Oceanography*, *63*(1), 3–21. https://doi.org/10.1002/lno.10608

Wallace, R. B., Baumann, H., Grear, J. S., Aller, R. C., & Gobler, C. J. (2014). Estuarine , Coastal and Shelf Science Coastal ocean acidi fi cation : The other eutrophication problem. *Estuarine, Coastal and Shelf Science*, *148*, 1–13. https://doi.org/10.1016/j.ecss.2014.05.027

Wasson, K., Jeppesen, R., Endris, C., Perry, D. C., Woolfolk, A., Beheshti, K., … Hughes, B. B. (2017a). Eutrophication decreases salt marsh resilience through proliferation of algal mats. *Biological Conservation*, *212*(June), 1–11. https://doi.org/10.1016/j.biocon.2017.05.019

Wasson, K., Jeppesen, R., Endris, C., Perry, D. C., Woolfolk, A., Beheshti, K., … Hughes, B. B. (2017b). Eutrophication decreases salt marsh resilience through proliferation of algal mats. *Biological Conservation*, *212*(January), 1–11. https://doi.org/10.1016/j.biocon.2017.05.019

Wolf-Gladrow, D. A., Zeebe, R. E., Klaas, C., Kortzinger, A., & Dickson, A. G. (2007). Total alkalinity: The explicit conservative expression and its application to biogeochemical processes. *Marine Chemistry*, *106*(1–2), 287–300. https://doi.org/10.1016/j.marchem.2007.01.006

Yang, C., Li, Y., Zhou, Y., Lei, X., Zheng, W., Tian, Y., … Zheng, T. (2016). A comprehensive insight into functional profiles of free-living microbial community responses to a toxic Akashiwo sanguinea bloom. *Scientific Reports*, *6*, 34645. Retrieved from https://doi.org/10.1038/srep34645

Zhai, W. D., Zhao, H. De, Zheng, N., & Xu, Y. (2012). Coastal acidification in summer bottom oxygen-depleted waters in northwestern-northern Bohai Sea from June to August in 2011. *Chinese Science Bulletin*, *57*(9), 1062–1068. https://doi.org/10.1007/s11434-011-4949-2

Zhang, F., Blasiak, L. C., Karolin, J. O., Powell, R. J., Geddes, C. D., & Hill, R. T. (2015). Phosphorus sequestration in the form of polyphosphate by microbial symbionts in marine sponges. *Proceedings of the National Academy of Sciences*, *112*(14), 4381 LP – 4386. https://doi.org/10.1073/pnas.1423768112

Zhang, X., & Vincent, A. C. J. (2019). Using cumulative-human-impact models to reveal global threat patterns for seahorses. *Conservation Biology*, *0*(ja). https://doi.org/10.1111/cobi.13325

Zhou, J., Richlen, M. L., Sehein, T. R., Kulis, D. M., Anderson, D. M., & Cai, Z. (2018). Microbial Community Structure and Associations During a Marine Dinoflagellate Bloom. *Frontiers in Microbiology*, *9*, 1201. https://doi.org/10.3389/fmicb.2018.01201

CHAPTER 3:

Tidal flushing stimulates microbially-mediated biogeochemical processes in macroalgal-dominated shallow coastal estuaries

ABSTRACT

Estuaries are productive and diverse ecosystems that link marine and terrestrial environments and are vulnerable to eutrophication from increased anthropogenic nutrients. While it is known that enhanced tidal flushing can reduce adverse effects of anthropogenic disturbances in larger, deeper estuarine ecosystems, this is unexplored for eutrophication in shallow coastal estuaries where macroalgae usually dominate. We simulated eutrophication as a macroalgal bloom in a mesocosm experiment, varied tidal flushing (flushed daily vs unflushed), and assessed the effects on water column and sediment biogeochemical processes and the sediment microbial community. While flushing did not ameliorate the negative effects of the macroalgal bloom, it caused transient differences in the rate of change in biogeochemical processes and promoted increased fluxes of nutrients from the sediment that were then "exported" during flushing. In the beginning, the macroalgal bloom induced basification and increased total alkalinity in the water column. During decomposition, acidification and the accumulation of nutrients in the sediment and water column occurred, which were driven by different microbial communities in each treatment. The flushed daily treatment was dominated by Bacteroidetes in the Marinilabiliaceae family, while the unflushed was dominated by Firmicutes in the Halobacteroidaceae family. Our results demonstrate macroalgal blooms are strong drivers of key biogeochemical processes across very different environmental contexts. Further, we suggest that macroalgal blooms can shift the microbial community structure to produce the same endpoint through different pathways.

INTRODUCTION

Eutrophication of coastal estuaries due to increased anthropogenic supplies of nutrients has been of critical global concern for decades (Ryther & Dunstan 1971; Nixon 1995; Lim *et al.* 2006; Testa & Kemp 2012; Moore & Cuker 2018). Excess nutrients fuel high rates of primary productivity that result in eutrophication, defined as a build-up of organic matter in an aquatic system (Nixon 1995); this organic matter is classically in the form of phytoplankton blooms (Cloern 2001; Testa & Kemp 2012). However, some studies have identified the importance of macroalgae in this process, particularly in shallow estuaries (Valiela *et al.* 1997; Fong 2008; McGlathery *et al.* 2012).

Cascading effects of nutrient-driven algal blooms, here defined as the sequential consequences of an initial stressor, include decreased water quality, habitat loss, depletion of oxygen (hypoxia/anoxia), pH fluctuations (basification/acidification), altered microbial activity, and changes in nutrient cycling (Long & Seitz 2009; Rabalais *et al.* 2009; Duarte *et al.* 2013; Moore & Cuker 2018; Moore *et al.* in prep). These effects all have potential for further cascades to ecosystem functions, including loss of biodiversity and trophic support (e.g., massive fish kills due to phytoplankton bloom-induced hypoxia (reviewed in Diaz & Rosenberg 2008), loss of shellfish due to acidification (Gobler *et al.* 2014), among many others), making research to understand mitigating these cascading effects over time complex but critical to conservation and management of these key coastal habitats.

In addition to phytoplankton and macroalgae, microbial communities are also an important player in eutrophic estuaries as they mediate many biochemical processes. Thus, microbial communities, especially in the sediment of shallow estuaries, are key drivers of the cascading effects of eutrophication. For example, as an algal bloom persists, oxygen from

photosynthesis fuels microbial aerobic respiration of organic matter (Hu *et al.* 2017; Duarte *et al.* 2013), which in turn can fuel ammonification, a process where organic nitrogen (N) released from the algae is microbially transformed to ammonium (NH_4^+) (Herbert *et al.* 1999). Once oxygen is depleted during bloom decomposition, organic matter is degraded via anaerobic microbial mineralization through processes such as sulfate reduction (Wolf-Gladrow *et al.* 2007), which produces hydrogen sulfide (H_2S), bicarbonate (HCO_3^-) ions, and increases total alkalinity (TA).

In bloom-induced anoxic conditions, microbially-mediated nutrient cycling is also altered (Herbert *et al.* 1999; Testa & Kemp 2012). For example, nitrifying bacteria lack oxygen needed to complete the aerobic process of nitrification, which is usually coupled with denitrification to remove N, and therefore NH_4^+ accumulates (Herbert 1999). Also, as heterotrophic bacteria remineralize organic matter, inorganic phosphorous (P, as orthophosphate PO_4^{3-}) is released (Joshi *et al.* 2015). As microbes mediate all of these processes, we can use community diversity combined with environmental data to understand these eutrophication-driven cascades and develop management strategies to reduce them.

Enhancement of tidal flushing (i.e., creating or enlarging the opening at the mouth of an estuary) is one management strategy proposed to reduce adverse effects of anthropogenic disturbances to estuarine ecosystems (Sinicrope *et al.* 1990; Zedler 2001; Jacobs *et al.* 2011; Dibble & Meyerson 2012; Lechêne *et al.* 2018). For example, in a marsh with restricted tidal flow due to construction of roads and bridges, fish had higher quantities of parasites compared to fish in that same marsh once tidal flushing was restored (Dibble & Meyerson 2012). In contrast, there is emerging evidence that enhanced flushing in systems with muted connectivity to the ocean or that lack natural flushing may have a cost to endangered or endemic species (Jacobs *et*

al. 2011). However, the rationale behind this management strategy is that enhanced tidal flushing should limit eutrophication of estuaries as it increases export of nutrients to the sea (Su *et al.* 2004), which reduces nutrients available for algal growth within the estuary, thereby decreasing an estuary's vulnerability to algal blooms.

Among the systems most impacted by eutrophication are shallow coastal estuaries as the depth results in strong linkages between sediment and water column processes (Cloern 2001; McGlathery *et al.* 2012). Eutrophication in these systems should co-occur both in the water column and sediment and potentially be influenced by tidal flushing, although this relationship has not been fully explored. Thus, more research is needed to understand the relationship between tidal flushing and eutrophication before enhanced tidal flushing is adopted as a management strategy for limiting the impacts of eutrophication.

Eutrophication of shallow estuaries is generally in the form of opportunistic red or green macroalgae, rather than phytoplankton (Fong & Kennison 2010), which respond to increased nutrient supplies with rapid growth (reviewed in Fong 2008; McGlathery *et al.* 2012). These macroalgae can form rafts that cover up to 70% of the surface area in shallow estuaries, and up to 100% of the sediment area during low tide (Green *et al.* 2014a; Moore *et al.* in prep), suggesting they can have a large impact to these ecosystems. As the bloom persists, nutrients (N and P) and carbon dioxide (CO_2) are utilized for photosynthesis and depleted from the water column. For phytoplankton, it has been documented that this uptake of CO_2 increases water column pH and has been termed 'bloom-induced basification' (Flynn *et al.* 2015), a cascading effect of eutrophication.

Conversely, as the bloom starts to decompose, nutrients and CO_2 are produced due to degradation of the algal organic matter and increased microbial respiration (Paytan &

McLaughlin 2007; Sunda & Cai 2012; Wallace *et al.* 2014). This release of nutrients stimulates new blooms (Howarth *et al.* 2011) while the production of CO_2 decreases water column pH causing acidification (Cai *et al.* 2011; Moore *et al.* in prep). In the process, photoautotrophic bacteria become dominated by heterotrophic communities and microbially-mediated biogeochemical processes are altered (Meyer-Reil & Koster 2000). These eutrophication-driven cascades from phytoplankton blooms have adverse impacts on ecosystem functioning; what remains a knowledge gap is whether this same process can be driven by macroalgal blooms.

One study identified cascading effects of eutrophication during a macroalgal bloom in a shallow estuary in Southern California, including pH fluctuations and altered nutrient cycling (Moore *et al.* in prep). In this study, differences among sites were attributed to variances in tidal flushing. Other studies have observed macroalgal blooms and subsequent cascading effects (Sfriso *et al.* 1992; García-Robledo *et al.* 2011; Krause-Jensen *et al.* 2015; Valença *et al.* 2016; Besterman & Pace 2018), but not while manipulating tidal flushing. The objective of our study was to identify if different flushing regimes (open/flushed daily vs closed/unflushed) affected the cascading effects of eutrophication in mesocosms simulating shallow coastal estuaries, including altering microbial communities. We hypothesized that daily tidal flushing would ameliorate the cascading effects of eutrophication, while no tidal flushing would enhance those cascades.

MATERIALS & METHODS

To accomplish the objectives of the study, mesocosm experiments were conducted to assess the effects of tidal flushing on eutrophication and the potential for subsequent cascading effects. In the mesocosm we simulated a shallow estuary with eutrophication in the form of a

macroalgal bloom, varied flushing regimes (open/flushed daily vs. closed/unflushed), and assessed changes in environmental parameters over time.

Mesocosm setup

The experiment was conducted during the summer of 2018 using sediment cores, seawater, and algae collected from an intertidal creek in Upper Newport Bay (33.6454° N, 117.8861° W), one of the largest remaining estuarine systems in Southern California. This site was chosen as eutrophication has been studied in this estuary and at this site over the last 20 years (Kamer & Fong 2001; Kennison *et al.* 2011; Kennison & Fong 2014; Moore *et al.* in prep). Upper Newport Bay has a highly-urbanized watershed, which leads to increased nutrient input and subsequent macroalgal blooms (Kennison *et al.* 2011).

To create each mesocosm, sediment was sampled with 20 push cores (acrylic liner, 16 cm length, 13 cm inner diameter) and to maintain intact sediment layers core liners were pushed 5 cm into the sediment. In the laboratory, a ~2 cm thick mat (50 g) of algae (*Ulva spp*.) and 1 liter of Upper Newport Bay water (hereafter, water) were added to the sediment surface. All sediment, algae, and water were collected from a site midway between the mouth and the head of the estuary (see Figure 1d in Kennison & Fong 2014) (see below for analysis of sediment characteristics). The water was collected during an inflowing tide, stored in the laboratory, and recollected as needed. Twenty total units were randomly assigned to one of 2 treatments;10 were used for the "flushed daily" treatment and the other 10 for the "unflushed" treatment. For the flushed treatment, the overlying water was poured off and replaced with water daily, while the unflushed treatment kept the same water throughout the experiment. Units were randomly placed in an outside water bath open to natural sunlight, maintained at 20 °C, which is typical for estuarine water in summer in California (Kennison *et al.* 2011), and exposed to an overhead fan

to simulate wind mixing and maintain temperature for the duration of the experiment. These conditions were achieved to simulate the natural estuarine environment. A 5 mL water sample of the initial water that was added to each mesocosm was collected and analyzed for pH, TA, PO_4^{3-}, and NH_4^+ (see methods below). The mesocosm ran for 3 weeks from late June-July 2018.

Mesocosm sampling

To visually track changes in the macroalgal bloom over time, 1 unit from each treatment was randomly selected, and each week pictures of the units were taken from directly overhead to avoid parallax. To determine final decomposition status of the macroalgal bloom, photos were taken of each unit during breakdown/week 3, and digital photos of algae on the sediment surface were used to calculate the percent cover of decomposing vs. alive algae. We projected 50 randomly chosen points on each photo using the CPCe 4.1 software and tallied green (alive) vs brown/white (decomposing) algae.

To assess changes in water quality conditions during the macroalgal bloom, pH was also measured at the same time of day weekly using an Oakton portable meter. Further, 5 mL of water was collected from the supernatant using a sterile syringe for analyses of NH_4^+, PO_4^{3-}, and TA. Water samples were immediately filtered with a Whatman 0.45 µM filter, and frozen at -30 °C until analysis. NH_4^+ and PO_4^{3-}, both inorganic nutrient species from recycled sources, were measured photometrically (Grasshoff *et al.* 1999). TA, used here to assess biogeochemical cycling (Wolf-Gladrow *et al.* 2007), was determined by acid titration (Dale *et al.* 2011). Daily fluxes of NH_4^+ and PO_4^{3-} were calculated based on changes in water column concentrations over time.

To assess sediment characteristics at the end of the experiment, sediment samples were collected for grain size, organic content, porewater, and microbial community (see below)

analyses. For grain size and organic content, sediment was first dried at 100 °C for 24 hours then grain size was determined using the hydrometer method (Bouyoucos 1962) and organic content as loss after ignition in a 400 °C muffle furnace. For porewater analysis, sediment from each unit was collected in 50 mL centrifuge tubes during breakdown. Samples were centrifuged for 20 mins at 10,000 rpms to separate the porewater from the sediment. After centrifugation, the overlying water was removed from each tube and analyzed as above for NH_4^+, PO_4^{3-}, and TA (n=10 for flushed, n=6 for unflushed due to sample/water loss).

All analyses were conducted in RStudio v. 3.5.1 using packages: *nlme, car*, and, *lsmeans*; all data that did not meet assumptions were log transformed. To test for significance differences between initial and final values for decomposing algal cover, porewater nutrients, TA, organic content, and grain size, we used a Welch's two sample t-test (Welch 1947). Next, to estimate effects on pH, NH_4^+, PO_4^{3-}, and TA over time, we used linear mixed-effect models (LME; Lindstrom & Bates 1988), with the interaction between time and treatment as fixed effects and the unit replicates as random effects. Finally, to identify significance of the model terms, we used function "Anova" followed by Tukey post hoc tests to explore where differences occurred.

Microbial community analysis

To assess the microbial community, using sterile gloves and spatulas, three 2 mL sediment samples were collected from the surface layer of each unit at the end of the experiment for metabarcoding. Sediment was collected from the surface layer as this is the interface where macroalgae and subsequent organic matter interacted with the sediment for the duration of the experiment. Pooled samples were extracted using the QIAGEN DNeasy PowerSoil Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Blank tubes were also extracted with each set of samples as a negative extraction control for a total of 5 controls.

Extracted DNA was then amplified in triplicate by polymerase chain reaction (PCR) using the 16S (515f and 806r; Caporaso *et al.* 2012) primers.

PCR amplification for each replicate and a PCR negative was performed in 15 µL reactions: 7.5 µL of QIAGEN Multiplex Taq PCR 2x Master Mix, was combined with 0.25 µL of each primer, 2 µL of template DNA and 5 µL of purified water. The reaction was then performed under the following thermocycler settings: 15 mins of initial denaturation at 95°C; 13 touchdown cycles of 30 s at 94°C, 30 s at 69.5°C and 60 s at 72°C; 35 amplification cycles of 30 s at 94°C, 30 s at 50°C and 60 s at 72°C; 10 min of final extension at 72°C; hold at 10°C. PCR amplification was conducted 3 times for each sample and successful amplification was visualized using gel electrophoresis in 2% agarose gels with 200 mL of 1x Tris-Acetate-EDTA buffer and stained with 6 µL of SyberSafe (Thermo Fisher, Waltham, MA, USA). Samples were cleaned using AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA), quantified on a Victor 3V Plate Reader, and indexed using dual i7 and i5 Illumina Nextera indices (Illumina, San Diego, CA, USA). The clean library was sent to the University of California, Santa Cruz Paleogenomics lab and sequenced on an Illumina MiSeq v3 2x300 platform. The raw sequence reads are available in the National Center for Biotechnology Information (NCBI) database under bioproject (ID pending).

We used the newly developed *Anacapa Toolkit* for taxonomy assignment using its default parameters (see version 1.1 supplemental in Curd *et al.* 2018). In brief, quality control was performed, and chimeras were removed. Then amplicon sequence variants (ASVs) were assigned using *DADA2* (Callahan *et al.* 2016), next taxonomy was assigned using *Bowtie 2* with the Bayesian Least Common Ancestor (BCLA) method (Langmead & Salzberg 2012).

Final results tables were generated by modifying the output tables of reads per site sample for each taxon. First, the NCBI-based classification was converted to follow recent revision (Ruggiero *et al.* 2015), which produced more complete taxonomic strings for estimating phylogenetic diversity at different taxonomic hierarchies. Samples were decontaminated by removing any taxa found in 3 or more reads in the PCR negatives, and extraction blanks. Lastly, taxa only present in one or two reads total across all samples were also removed.

The final ASV tables and metadata were converted to *Phyloseq* objects (McMurdie & Holmes 2013), which merges the taxonomy data with the measured variables for community analysis. After assessing the unrarefied data, sample "U7" was removed from the data set as an outlier (see S.1 'Taxon Accumulation Curve") making sample size for unflushed 9 and flushed 10 for subsequent analysis. Data were then normalized by rarefying samples to retain 2437 reads each, and a new object summarized to taxonomic family was created. OTU (operational taxonomic unit) tables were extracted to identify the most abundant (defined here as >1000 total OTU's) families between treatments and visualize differences. A dominance test, using function "dominant", was used to determine the most dominant family in each sample. Alpha (species) diversity of the microbial community families was assessed using the Shannon Index, and analyzed with a 1-factor ANOVA. Beta diversity, which accounts for treatment differences of the communities, was assessed with a principal coordinate analysis (PCoA) using the Bray-Curtis distance index and analyzed with a PERMANOVA. A constrained analysis of principal coordinates (CAP) was also conducted to observe how our measured variables contributed to the community structure between treatments; the model and variable significance was analyzed using function "anova". These analyses were conducted in RStudio v. 3.5.1 using packages: *dplyr*, *ranacapa, phyloseq*, *vegan*, *microbiome*, and, *ggplot2.*

RESULTS

In the unflushed treatment, *Ulva spp.* lost its color by week 2, while the *Ulva spp.* in the flushed treatment remained intact in week 2 (Fig. 3.1). However, the effects of flushing were reduced by week 3 as both treatments had greater cover of decomposing than alive macroalgae with no significant differences between treatments (unflushed: 98.8% ± 0.85 SE, flushed: 89.4% ± 4.5 SE, T-test, $p = 0.07$).

The initial conditions of the water added to each mesocosm were: pH (8.08), TA (3.84 mEq L^{-1}), PO_4^{3-} (2.62 µM), and NH_4^+ (0.04 µM). Average pH increased between week 1 and 2 in the mesocosms, but as the macroalgae started to decompose between week 2 and 3, average pH decreased regardless of flushing treatment (LME, $p = 0.001$ for time). TA increased over time in both treatments (Fig. 3.2B). However, increased TA was mediated by flushing as TA did not change significantly between weeks 2 and 3 in the flushed treatment, but rapidly increased in the unflushed treatment over this same period, resulting in a significant interaction (LME, $p = 0.005$ for time*treatment). PO_4^{3-} increased from week 1 to 3 in the unflushed treatment but remained statistically similar in the flushed treatment (Fig. 3.2C); as the accumulation of PO_4^{3-} was impacted by flushing, this resulted in a significant interaction (LME, $p < 0.0001$ for time*treatment).

Overall, NH_4^+ concentration increased during week 2 then decreased in week 3 (Fig. 3.2D, LME, $p < 0.0001$ for time) and was not affected by treatment. The daily fluxes for these nutrients showed large differences, however, with 0.06 mmol m^{-2} d^{-1} (± 0.02 SE) of NH_4^+ fluxing from the flushed treatments and only 0.0003 mmol m^{-2} d^{-1} (± 4.2 E^{-05} SE) of NH_4^+ from unflushed treatments. Similarly, 0.11 mmol m^{-2} d^{-1} (± 0.01 SE) of PO_4^{3-} was fluxing daily from the flushed while 0.012 mmol m^{-2} d^{-1} (± 0.003 SE) of PO_4^{3-} from the unflushed mesocosms.

Sediment porewater characteristics did not differ between flushing treatments (T-test, $p > 0.05$ for all comparisons); the grand mean for TA was 9.42 mEq L^{-1} (± 0.87 SE), for PO_4^{3-} 21.5 µM (± 4.01 SE), and NH_4^+ 23.84 µM (± 3.24 SE). Sediment organic content was also similar between treatments (T-test, $p > 0.05$); grand mean 4.47% (± 0.40 SE). Sand comprised the largest percentage of sediment grain size for both flushed (82.5% ± 1.91 SE) and unflushed (90. 3% ± 1.95 SE) and was different between treatments (T-test, $p = 0.01$), although this was likely due to random chance during collection.

We identified 234 families of bacteria and archaea in our samples, with 14 families comprising the most abundant (>1000 total reads) members of the community (Fig. 3.3). The family Marinilabiliaceae dominated the community in the flushed treatment while Halobacteroidaceae dominated the bacterial community in the unflushed treatment. There was no difference in the microbial community alpha diversity between treatments ($p > 0.5$). However, beta diversity was different between treatments ($p = 0.002$) with the flushed and unflushed replicates separating despite some overlap (Fig. 3.4). Porewater PO_4^{3-} and TA, water column NH_4^+, and pH were associated with the flushed community structure, while water column PO_4^{3-} and TA, porewater NH_4^+, grain size, and organic content were associated with the composition of the unflushed community (Fig. 3.5).

DISCUSSION

Alterations in Biogeochemical Processes

Our results demonstrate that the initial phase of decomposition of macroalgal blooms drives a series of short-term changes in biogeochemical processes, which may occur in shallow coastal estuaries that are either open or closed to tidal flushing. In the short term, pH increased,

likely due to the uptake of CO_2 during photosynthesis. Excess production of labile organic matter subsequently fueled ammonification, which produced a pulsed supply of NH_4^+ to the water column. Our findings support other mesocosm and field studies that have documented 'basification' (Björk *et al.* 2004; Middelboe & Hansen 2007; Krause-Jensen 2015; Wahl *et al.* 2018) and NH_4^+ fluxing (Viaroli *et al.* 1996; García -Robledo *et al.* 2011; Robertson & Savage 2018; Moore *et al.* in prep) as cascading effects of macroalgal blooms.

Due to the ability of *Ulva spp.* to utilize HCO_3^- dehydrated to CO_2 for photosynthesis (Björk *et al.* 2004), basification will be promoted in large blooms as the excess hydroxide (OH^-) from this reaction is transported outward and increases pH. For example, combined field and mesocosm experiments found *Ulva intestinalis* was capable of driving pH above 10 during growth phases with high rates of photosynthesis and thereby reduced the surrounding macroalgal diversity (Björk *et al.* 2004). Also, as *Ulva spp.* can store N in excess of growth requirements, ammonification onsets rapidly during decomposition due to the algae's high N:C ratio (reviewed in Herbert 1999). For example, one mesocosm experiment found ammonification rate increased from 4.4 μM m^{-2} d^{-1} to 25.3 μM m^{-2} d^{-1} in the presence of decomposing *Ulva spp.* (García -Robledo *et al.* 2011). While the responses in our study were not as dramatic as those found during rapid growth of *Ulva spp*., they still drove significant short-term changes in biogeochemical processes.

These short-term responses to eutrophication appear to have conflicting outcomes at the level of the ecosystem, as basification can mitigate ocean acidification (Peach *et al.* 2017; Wahl *et al.* 2018) but also can decrease biodiversity (Björk *et al.* 2004; Flynn *et al.* 2015). Further, while ammonification increases utilizable N supplies (Herbert 1999), it also sustains blooms (Nędzarek & Rakusa-Suszczewski 2004). It is possible that the flux of NH_4^+ from the sediment

to the water column in our mesocosms was stimulated by flushing maintaining a strong diffusive gradient and promoting nutrient release. The increased NH_4^+ did not maintain algal growth, however, because it fluxed to the water column and was removed during flushing. While limiting sustained algal blooms in the estuary, export of this high nutrient water has the potential to create eutrophication in the coastal ocean. In contrast, in the unflushed mesocosms the NH_4^+ flux was much lower and likely remained in the sediment due to decomposition processes of the algal organic matter. Overall, as these transient, dynamical changes in biogeochemical processes that occur during the initial decomposition of macroalgal blooms appear to have both costs and benefits that may be stimulated by flushing, it is critical to continue to explore their ecosystem impacts.

Our results also show that, as macroalgal blooms senescence, decomposition drives another suite of changes in biogeochemical processes that may also be similar in systems open or closed to tidal flushing, including decreases in pH. This finding supports other studies that have documented 'acidification' due to microbial aerobic respiration of algal organic matter (Sunda & Cai 2012; Melzner *et al.* 2013; Wallace *et al.* 2014; Moore *et al.* in prep), although these studies have mainly been on phytoplankton blooms. Here, we show blooms of *Ulva* can have these same effects, albeit the acidification was modest in our experiment.

The potential for *Ulva* to cause acidification is likely due to its high nutrient content, rapid microbial decomposition (Herbert 1999), and associated microbial mineralization of organic matter (García-Robledo *et al.* 2008). The pH decrease measured here was probably less than seen during phytoplankton blooms because phytoplankton organic matter is generally degraded aerobically in the water column (Sunda & Cai 2012; Wallace *et al.* 2014), which is likely different for macroalgal blooms, particularly in shallow estuaries, if degradation is

primarily in the sediment. For example, in the sediment anaerobic degradation would increase pH (Carmouze 1986) and in excess (i.e., during bloom decomposition) counterbalance pH decreases by aerobic degradation. However, we did measure a slight pH decrease, which suggests that the bloom may have altered other biogeochemical processes that also contributed to the acidification observed although not measured here (but see Moore *et al.* in prep).

Decomposition of the macroalgal bloom was also accompanied by increases in TA and PO_4^{3-}. This finding supports anaerobic degradation of the macroalgal bloom as increased TA has been linked to anaerobic mineralization of organic matter (Frankignoulle *et al.* 1996; Gaspar *et al.* 2018). This process likely explains the responses measured in our experiment as well, as labile organic matter from *Ulva* blooms stimulated remineralization processes. In addition, remineralization of algal organic P likely released PO_4^{3-} (Paytan & McLaughlin 2007); again, as *Ulva spp.* are nutrient-rich, this flux of PO_4^{3-} could reach levels higher than seen during phytoplankton blooms.

These cascading effects of the decomposition phase of macroalgal blooms also have differing effects at the ecosystem level as acidification is harmful to calcifying organisms (Russell 2009; Hofmann & Bischof 2014) but can promote primary productivity (Jansson *et al.*, 2012; Koch *et al.*, 2013). Further, increases in TA result in a higher buffering capacity (Carmouze 1986), which may alleviate atmospheric CO_2 stress, yet increases in PO_4^{3-} sustain eutrophication with a positive feedback loop (Rabalais *et al.* 2009; Moore & Cuker 2018). Ultimately our work suggests that macroalgal blooms have the potential to be the cause of, yet may also offer a partial solution to, global ecological changes to biogeochemical processes.

Differences in Microbial Diversity

A diverse and complex microbial community likely mediated the changes in biogeochemical processes that occurred during the decomposition phase of the macroalgal bloom in our experiment; however, tidal flushing influenced microbial community diversity and potentially the overall microbially-mediated changes in biogeochemical processes. For example, Bacteroidetes of the Marinilabiliaceae family, which dominated the community in the flushed mesocosms, have been found in marine sediments globally (Liu *et al.* 2015; Wu *et al.* 2016; Smith *et al.* 2017) and specifically in mud containing decayed algae (Ludwig *et al.* 2015). While Firmicutes of the Halobacteroidaceae family, which dominated the community in the mesocosms simulating closed estuaries, have also been isolated in marine sediment, they are only found when sediments are anoxic (Oren 2014).

Genera of the Marinilabiliaceae family are facultative anaerobes that switch between aerobic respiration to fermentation in the absence of oxygen (Yang *et al.* 2014). Therefore, the presence of Marinilabiliaceae suggests that aerobic respiration could have led to the subsequent decrease in pH in the flushed treatment. One study found Bacteroidetes to account for 60% of the bacterial community involved in phytoplankton bloom degradation (Smith *et al.* 2015), and a follow-up study found Marinilabiliaceae exclusively in the sediment coinciding with phytoplankton bloom deposition (Smith *et al.* 2017), although they did not measure pH. Further, increased flushing likely promoted microbial degradation activity supporting the higher fluxes of nutrients measured in the flushed treatment.

In contrast, the presence of Halobacteroidaceae, obligate anaerobes with fermentative or homoacetogenic metabolism (Oren 2014), implies that anaerobic processes were dominant and further explains why the decrease in pH measured may not have been as high as observed during

decomposition of phytoplankton blooms. The dominance of these halophilic bacteria also suggests that the lack of flushing caused the mesocosms to become hypersaline. Although we did not measure salinity, we did observe water loss due to evaporation, which is a byproduct of the lack of flushing that can cause closed estuaries to exceed 80 ppt in the summer (Fong 1986). Further, genera of Halobacteroidaceae were found to be highly abundant in a hypersaline eutrophic soda lake and primarily involved in C degradation (Vavourakis *et al.* 2018). Thus, changes in the microbial community were impacted by flushing even though the presence of excess macroalgal organic matter in the sediment ultimately drove the same net results.

Contrary to our expectations, flushing was not enough to ameliorate the negative effects of the macroalgal bloom; rather, tidal flushing caused some transient differences in the rate at which biogeochemical processes changed and promoted increased fluxes of nutrients from the sediment. In addition, when focusing on the two microbial community dominants, the dominance of obligate anaerobic bacteria confirmed that the unflushed sediment was anoxic, a well-known symptom of eutrophication (Diaz 2001; Testa & Kemp 2012), while the flushed systems may have remained oxygenated to a degree. Thus, macroalgal blooms and the subsequent decomposition processes, aerobic or anerobic, shift the structure of the sediment microbial community that mediates its decomposition. Our results suggest that increased tidal flushing may not be a universal panacea for eutrophication and may ultimately promote algal blooms with increased nutrient fluxing.

FIGURES

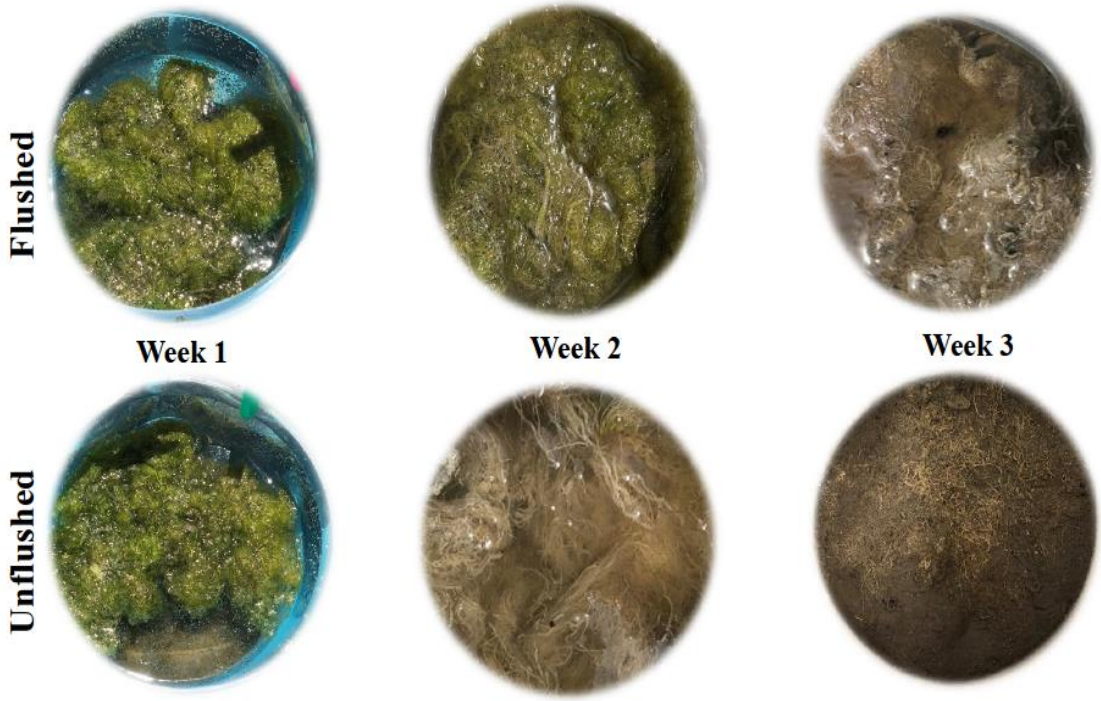

Figure 3.1. Observations of *Ulva spp.* over a time series in randomly selected flushed and unflushed units.

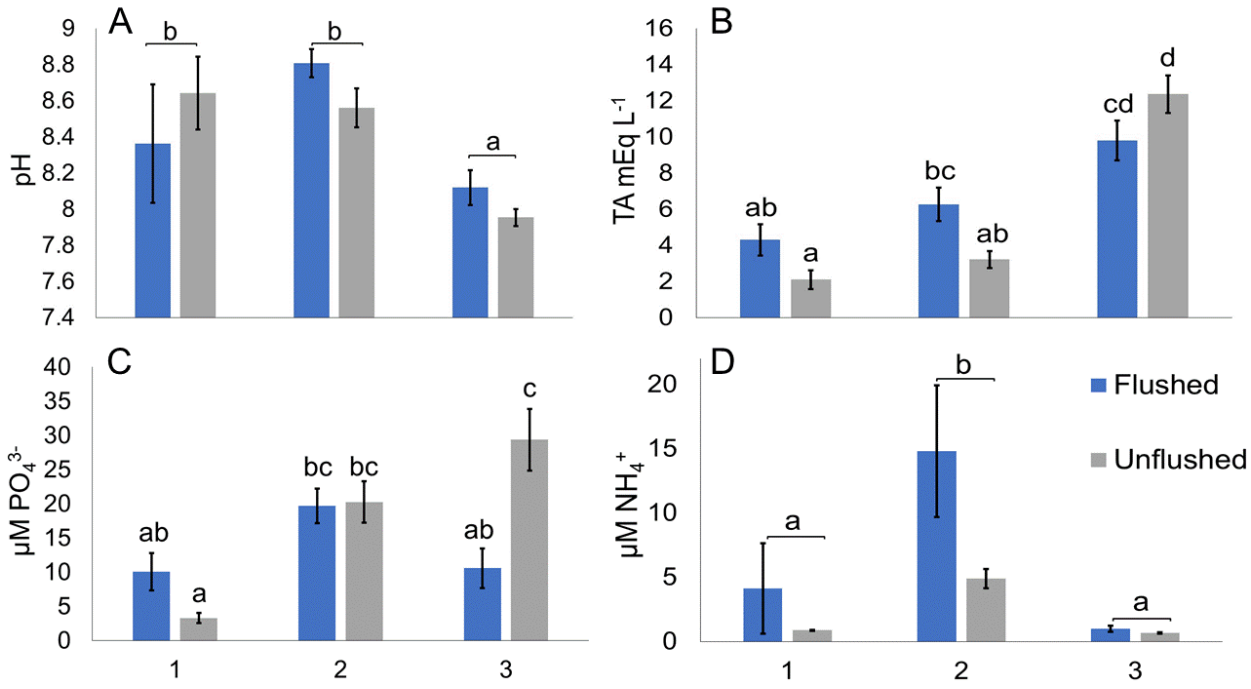

Figure 3.2. Weekly water column measurements. A) pH B) total alkalinity (TA) C) orthophosphate (PO_4^{3-}) D) ammonium (NH_4^+); with Tukey post hoc results. Bars are means ± SE.

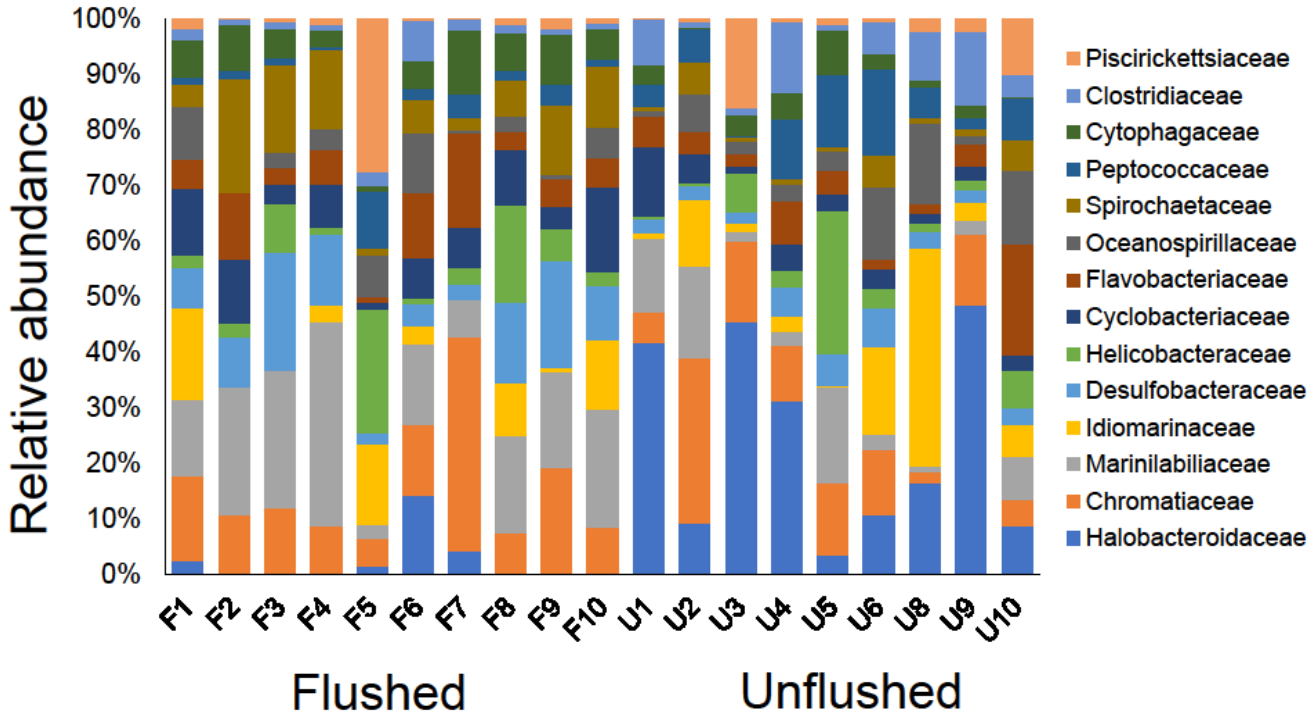

Figure 3.3. Most abundant (>1000 total OTU's) family's composition of the microbial community for each replicate.

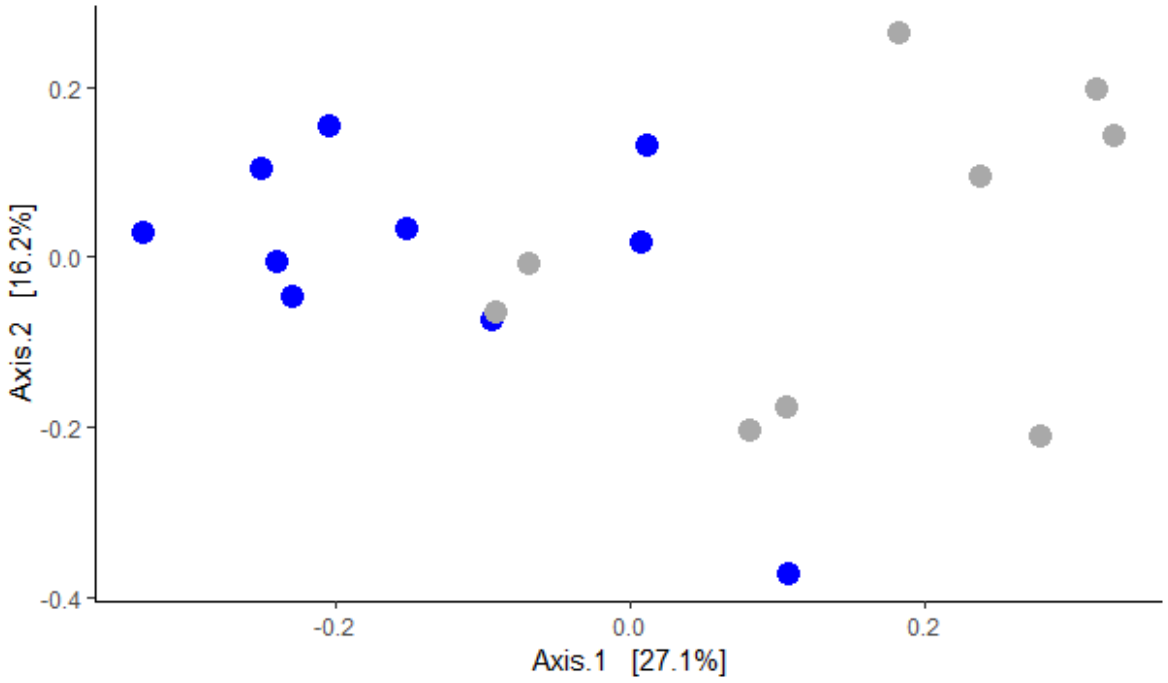

Figure 3.4. Principal Coordinate Analysis (PCoA) ordination of beta diversity of microbial families; flushed (blue), unflushed (grey). Unflushed n = 9.

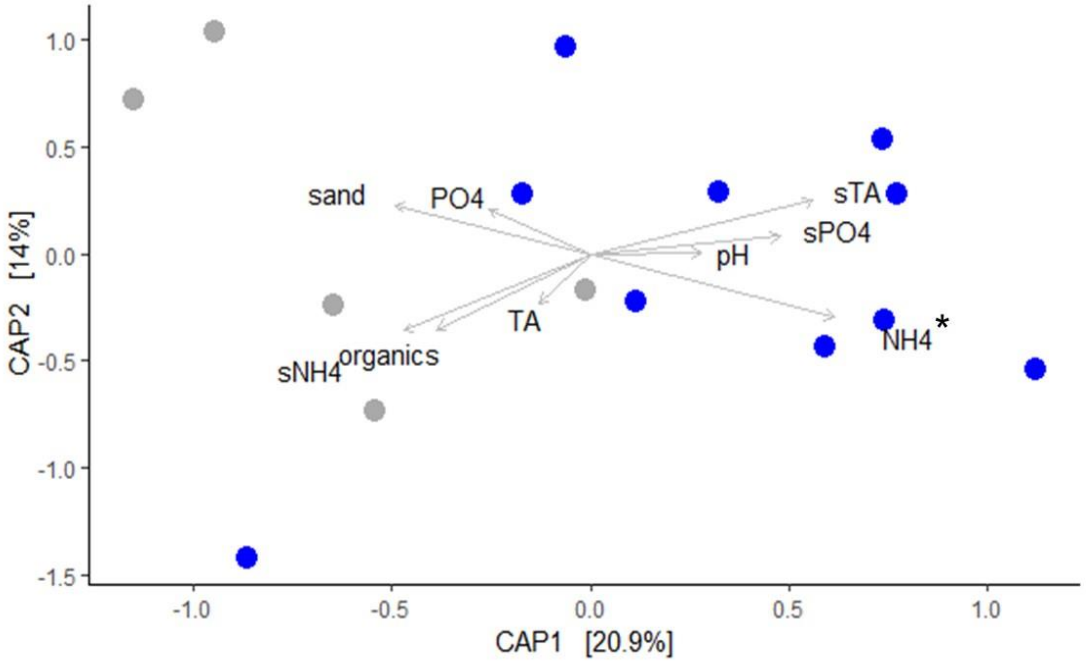

Figure 3.5. Constrained Analysis of Principal Coordinates (CAP) plot; flushed (blue), unflushed (grey). *indicates significance. Unflushed n = 5.

SUPPLEMENTARY MATERIALS

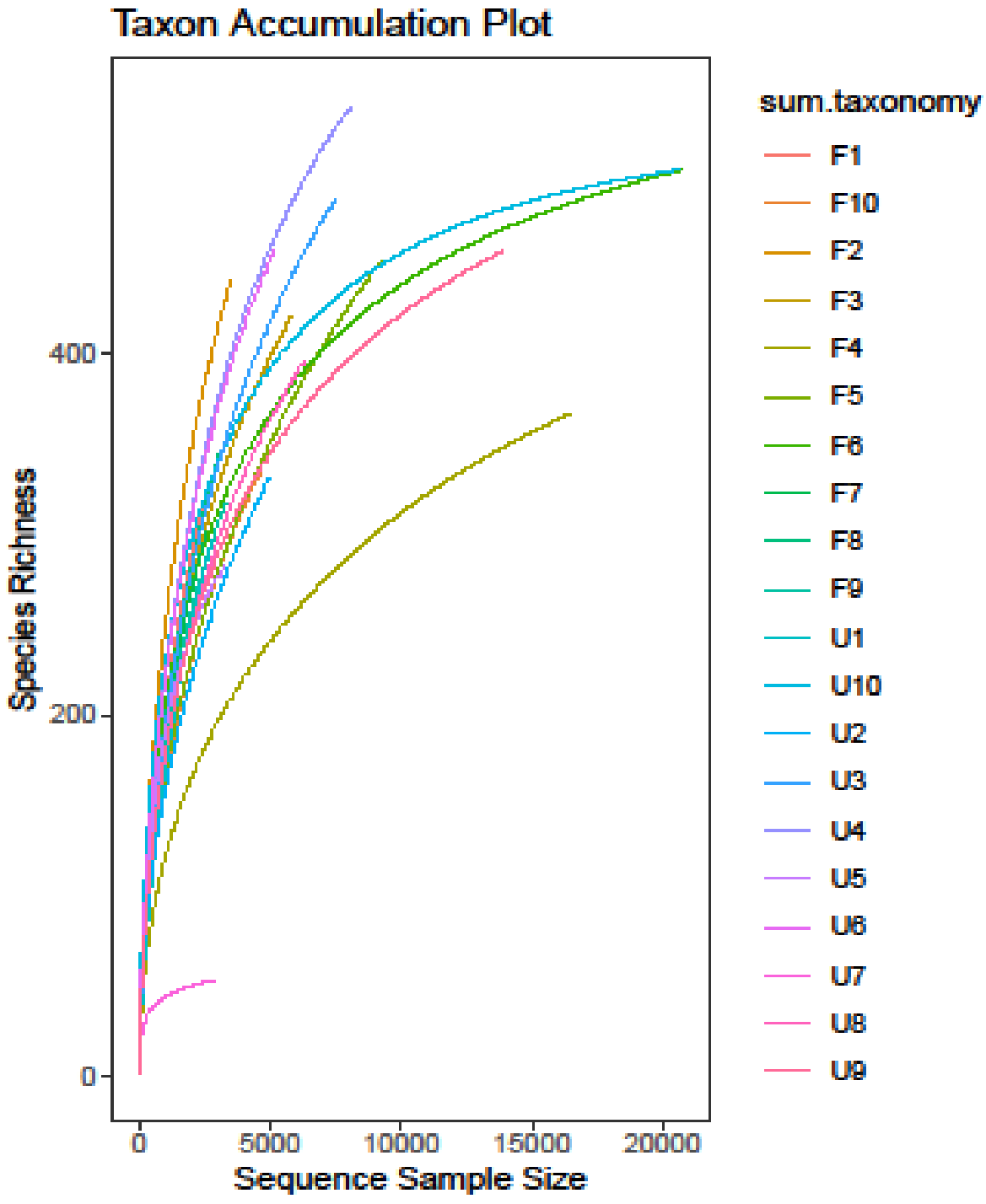

Figure S3.1. Taxon Accumulation Plot. Species richness for all samples. "U7" (lowest pink line) was removed for subsequent community analysis.

REFERENCES

Besterman, A.F. & Pace, M.L. (2018). Do Macroalgal Mats Impact Microphytobenthos on Mudflats? Evidence from a Meta-Analysis, Comparative Survey, and Large-Scale Manipulation. *Estuaries and Coasts*, 41, 2304–2316.

Björk, M., Axelsson, L. & Beer, S. (2004) Why is Ulva intestinalis the only macroalga inhabiting isolated rockpools along the Swedish Atlantic coast? *Mar Ecol Prog Ser*, 284, 109-116.

Bouyoucos, G.J. (1962) Hydrometer Method Improved for Making Particle Size Analysis of Soils. *Agron J*, 54, 464-465.

Cai, W.J., Hu, X., Huang, W.J., Murrell, M.C., Lehrter, J.C., Lohrenz, S.E., *et al.* (2011). Acidification of subsurface coastal waters enhanced by eutrophication. *Nat Geosci*, 11, 766--770.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods*, 13, 581–583.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., *et al.* (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *Isme J*, 6, 1621.

Carmouze, J.-P. (1986). Alkalinity as a useful measure in biogeochemical process studies (examples of aerobic photosynthesis and anaerobic mineralization of organic matter in a tropical lagoon). *Sci Total Environ*, 58, 187–193.

Cloern, J.E. (2001). Our evolving conceptual model of the coastal\reutrophication problem. *Mar Ecol Prog Ser*, 210, 223–253.

Curd, E.E., Gold, Z., Kandlikar, G.S., Gomer, J., Ogden, M., Connell, T.O., *et al.* (2018).

Anacapa Toolkit : an environmental DNA toolkit for processing multilocus metabarcode datasets.

Dale, A.W., Sommer, S., Bohlen, L., Treude, T., Bertics, V.J., Bange, H.W., *et al.* (2011). Rates and regulation of nitrogen cycling in seasonally hypoxic sediments during winter (Boknis Eck, SW Baltic Sea): Sensitivity to environmental variables. *Estuar Coast Shelf Sci*, 95, 14–28.

Diaz, R.J. (2001). Overview of Hypoxia around the World. *J Environ Qual*, 30, 275-281.

Diaz, R.J. & Rosenberg, R. (2008). Spreading Dead Zones and Consequences for Marine Ecosystems. *Science,* 321, 926-929.

Dibble, K.L. & Meyerson, L.A. (2012). Tidal Flushing Restores the Physiological Condition of Fish Residing in Degraded Salt Marshes. *PLoS One*, 7, e46161.

Duarte, C.M., Hendriks, I.E., Moore, T.S., Olsen, Y.S., Steckbauer, A., Ramajo, L., *et al.* (2013). Is Ocean Acidification an Open-Ocean Syndrome ? Understanding Anthropogenic Impacts on Seawater pH. *Estuaries and Coasts*, 36, 221–236.

Flynn, K.J., Clark, D.R., Mitra, A., Fabian, H., Hansen, P.J., Gilbert, P.M., *et al.* (2015). Ocean acidification with (de)eutrophication will alter future phytoplankton growth and succession. *Proc R Soc B Biol Sci*, 282, 2014-2604.

Fong, P. (1986). Monitoring and manipulation of phytoplankton dynamics in a Southern California estuary. Masters Thesis, San Diego State University.

Fong, P (2008). Macroalgal-dominated ecosystems. In: *Nitrogen in the Marine Environment*. [eds] E. J. Carpenter and D.G Capone. Academic Press, Cambridge, MA, USA. 917-948.

Fong, P. & Kennison, R. L. (2010). Phase shifts, alternative stable states, and the status of southern California lagoons. In: *Coastal Lagoons: Critical Habitats of Environmental*

Change. [eds] M. J. Kennish and H.W. Pearl. CrC Press, Boca Raton, FL, USA. 227-251.

Frankignoulle, M., Bourge, I. & Wollast, R. (1996). Atmospheric CO2 fluxes in a highly polluted estuary (the Scheldt). *Limnol Oceanogr*, 41, 365–369.

García -Robledo, E., Corzo, A., García de Lomas, J., & van Bergeijk, S. A. (2008). Biogeochemical effects of macroalgal decomposition on intertidal microbenthos: a microcosm experiment. *Mar Ecol Prog Ser*, 356, 139–151.

García-Robledo, E. & Corzo, A. (2011). Effects of macroalgal blooms on carbon and nitrogen biogeochemical cycling in photoautotrophic sediments: An experimental mesocosm. *Mar Pollut Bull*, 62, 1550–1556.

Gaspar, F.L., Pinheiro, B.R., Noriega, C.E.D., Araujo, M., Lefèvre, N. & Montes, M. de J.F. (2018). Alkalinity, inorganic carbon and CO2 flux variability during extreme rainfall years (2010-2011) in two polluted tropical estuaries NE Brazil . *Brazilian J Oceanogr*, 66, 115-130.

Gobler, C.J., DePasquale, E.L., Griffith, A.W. & Baumann, H. (2014). Hypoxia and Acidification Have Additive and Synergistic Negative Effects on the Growth, Survival, and Metamorphosis of Early Life Stage Bivalves. *PLoS One*, 9, e83648.

Grasshoff, K., Ehrhardt, M., Kremling K. (1999): *Methods of Seawater Analysis*. First Edition. Wiley-VCH Verlag GmbH, Weinheim, Germany. 1-419.

Green, L., Sutula, M. & Fong, P. (2014a). How much is too much? Identifying benchmarks of adverse effects of macroalgae on the macrobenthic community in intertidal flats. *Ecol Appl*, 24 300-314.

Herbert, R.A. (1999). Nitrogen cycling in coastal marine ecosystems. *FEMS Microbiol Rev*, 23, 563–590.

Hofmann, L.C. & Bischof, K. (2014). Ocean acidification effects on calcifying macroalgae, 22, 261–279.

Howarth, R., Chan, F., Conley, D.J., Garnier, J., Doney, S.C., Marino, R., *et al.* (2011). Coupled biogeochemical cycles: Eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. In: *Frontiers in Ecology and the Environment*. 18–26.

Hu, X., Li, Q., Huang, W.J., Chen, B., Cai, W.J., Rabalais, N.N., *et al.* (2017). Effects of eutrophication and benthic respiration on water column carbonate chemistry in a traditional hypoxic zone in the Northern Gulf of Mexico. *Mar Chem*, 194, 33-42.

Jacobs, D.K., Stein, E.D. & Longcore, T. (2011). Classification of California estuaries based on natural closure patterns: templates for restoration and management Modeling Rangewide Metapopulation Viability and Persistence of the Endangered Tidewater Goby: Implications for Long-term Conservation and Management, 1–60.

Jansson, M., Karlsson, J. & Jonsson, A. (2012). Carbon dioxide supersaturation promotes primary production in lakes. *Ecol Lett*, 15, 527–532.

Joshi, S.R., Kukkadapu, R.K., Burdige, D.J., Bowden, M.E., Sparks, D.L. & Jaisi, D.P. (2015). Organic matter remineralization predominates phosphorus cycling in the mid-bay sediments in the chesapeake bay. *Environ Sci Technol*, 49, 5887–5896.

Kamer, K. & Fong, P. (2001). Nitrogen enrichment ameliorates the negative effects of reduced salinity on the green macroalga Enteromorpha intestinalis. *Mar Ecol Prog Ser*, 218, 87-93.

Kennison, R.L. & Fong, P. (2014). Extreme Eutrophication in Shallow Estuaries and Lagoons of California Is Driven by a Unique Combination of Local Watershed Modifications That Trump Variability Associated with Wet and Dry Seasons. *Estuaries and Coasts*, 37, 164-179.

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

Koch, M., Bowes, G., Ross, C. & Zhang, X.-H. (2013). Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Glob Chang Biol*, 19, 103–132.

Krause-Jensen, D., Duarte, C.M., Hendriks, I.E., Meire, L., Blicher, M.E., Marbà, N., *et al.* (2015). Macroalgae contribute to nested mosaics of pH variability in a subarctic fjord. *Biogeosciences*, 12, 4895–4911.

Langmead, B. & Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat Methods*, 9, 357.

Lechêne, A., Boët, P., Laffaille, P. & Lobry, J. (2018). Nekton communities of tidally restored marshes: A whole-estuary approach. *Estuar Coast Shelf Sci*, 207, 368–382.

Lim, H.S., Diaz, R.J., Hong, J.S. & Schaffner, L.C. (2006). Hypoxia and benthic community recovery in Korean coastal waters. *Mar Pollut Bull*, 52, 1517–1526.

Lindstrom, M. J. & Bates, D. M. (1988). Newton—Raphson and EM Algorithms for Linear Mixed-Effects Models for Repeated-Measures Data. *J Am Stat Assoc*, 83, 1014-1022.

Liu, Q.Q., Li, J., Xiao, D., Lu, J.X., Chen, G.J. & Du, Z.J. (2015). Saccharicrinis marinus sp. Nov., isolated from marine sediment. *Int J Syst Evol Microbiol*, 65, 3427–3432.

Long, W.C. & Seitz, R.D. (2009). Hypoxia in chesapeake bay tributaries: Worsening effects on macrobenthic community structure in the York River. *Estuaries and Coasts*, 32, 287–297.

Ludwig, W., Euzéby, J. & Whitman, W.B. (2015). Marinilabiliaceae fam. nov. In: *Bergey's Manual of Systematics of Archaea and Bacteria*, [eds] W. B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos, B. Hedlund and S. Dedysh.

McGlathery, J.K., Sundback, K. & Fong, P. (2012). Estuarine benthic algae. In: *Estuarine Ecology*, Second Edition. [eds] J. W. Day, B. C. Crump, W. M. Kemp and A. Yáñez-Arancibia. John Wiley & Sons, Inc., Hoboken, NJ, USA. 203-234.

McMurdie, P.J. & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS One*, 8, e61217.

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M.A., Bange, H.W., *et al.* (2013). Future ocean acidification will be amplified by hypoxia in coastal habitats. *Mar Biol*, 160, 1875–1888.

Meyer-Reil, L.-A. & Köster, M. (2000). Eutrophication of Marine Waters: Effects on Benthic Microbial Communities. *Mar Pollut Bull*, 41, 255–263.

Middelboe, A.L. (2007). High pH in shallow-water macroalgal habitats . *Mar Ecol Prog Ser*, 338, 107–117.

Moore, T.N. & Cuker, B.E. (2018). Sedimentary Oxygen Demand and Orthophosphate Release : Sustaining Eutrophication in a Tributary of the Chesapeake Bay, 7, 42–48.

Nędzarek, A. & Rakusa-Suszczewski, S. (2004). Decomposition of macroalgae and the release of nutrient. *Polar Biosci*, 17, 26–35.

Nixon, S.W. (1995). Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia*, 41, 199–219.

Oren, A. (2014). The Order Halanaerobiales, and the Families Halanaerobiaceae and Halobacteroidaceae In: *The Prokaryotes: Firmicutes and Tenericutes*. [eds.] Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E. & Thompson, F. Springer Berlin Heidelberg, Berlin, Heidelberg. 153–177.

Paytan, A. & McLaughlin, K. (2007). The Oceanic Phosphorus Cycle. *Chem Rev*, 107, 563–576.

Peach, K.E., Koch, M.S., Blackwelder, P.L. & Manfrino, C. (2017). Calcification and photophysiology responses to elevated pCO2 in six Halimeda species from contrasting irradiance environments on Little Cayman Island reefs. *J Exp Mar Bio Ecol*, 486, 114–126.

Rabalais, N.N., Turner, R.E., Díaz, R.J. & Justić, D. (2009). Global change and eutrophication of coastal waters. *ICES J Mar Sci*, 66, 1528-1537.

Robertson, B.P. & Savage, C. (2018). Mud-entrained macroalgae utilise porewater and overlying water column nutrients to grow in a eutrophic intertidal estuary. *Biogeochemistry*, 139, 53–68.

Ruggiero, M.A., Gordon, D.P., Orrell, T.M., Bailly, N., Bourgoin, T., Brusca, R.C., *et al.* (2015). A Higher Level Classification of All Living Organisms. *PLoS One*, 10, e0119248.

Russell, B.D., thompson, J.-A.I., falkenberg, L.J. & connell, S.D. (2009). Synergistic effects of climate change and local stressors: CO2 and nutrient-driven change in subtidal rocky habitats. *Glob Chang Biol*, 15, 2153–2162.

Ryther, J.H. & Dunstan, W.M. (1971). Nitrogen, Phosphorus, and Eutrophication in the Coastal Marine Environment. *Science*, 171, 1008–1013.

Sfriso, A., Pavoni, B., Marcomini, A. & Orio, A.A. (1992). Macroalgae, nutrient cycles, and pollutants in the Lagoon of Venice. *Estuaries*, 15, 517–528.

Sinicrope, T.L., Hine, P.G., Warren, R.S. & Niering, W.A. (1990). Restoration of an impounded salt marsh in New England. *Estuaries*, 13, 25–30.

Smith M. W., Davis R. E., Youngblut N. D., Karna T., Herfort L., Whitaker R. J., … Simon H. M. (2015). Metagenomic evidence for reciprocal particle exchange between the mainstem estuary and lateral bay sediments of the lower Columbia River. *Front Microbiol*, 6, 1074–1090.

Smith, M.W., Herfort, L., Fortunato, C.S., Crump, B.C. & Simon, H.M. (2017). Microbial players and processes involved in phytoplankton bloom utilization in the water column of a fast-flowing, river-dominated estuary. *Microbiologyopen*, 6, 1–13.

Su, H.-M., Lin, H.-J. & Hung, J.-J. (2004). Effects of tidal flushing on phytoplankton in a eutrophic tropical lagoon in Taiwan. *Estuar Coast Shelf Sci*, 61, 739–750.

Sunda, W.G., & Cai, W.-J. (2012). Eutrophication induced CO2-acidification of subsurface coastal waters: Interactive effects of temperature, salinity, and atmospheric pCO2. *Environ Sci Techno*. 10, 651–659.

Testa, J. & Michael Kemp, W. (2012). *Hypoxia-induced shifts in nitrogen and phosphorus cycling in Chesapeake Bay*. *Limnol Oceanogr*, 57, 835-850.

Valença, A.P.M.C., Clemente, C.C.C., Neves, J.R., Silva, J.F., Bezerra, R.S., Botter-Carvalho, M.L., *et al.* (2016). Effects of algal mats on a tropical estuarine benthic system: sediment biogeochemistry and macrofauna. *Hydrobiologia*, 775, 197–211.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D. & Foreman, K. (1997). Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnol Oceanogr*, 42, 1105–1118.

Vavourakis, C. D., Andrei, A. S., Mehrshad, M., Ghai, R., Sorokin, D. Y., & Muyzer, G. (2018). A metagenomics roadmap to the uncultured genome diversity in hypersaline soda lake sediments. *Microbiome*, 6, 168.

Viaroli, P., Bartoli, M., Bondavalli, C., Christian, R.R., Giordani, G. & Naldi, M. (1996). Macrophyte communities and their impact on benthic fluxes of oxygen, sulphide and nutrients in shallow eutrophic environments. *Hydrobiologia*, 329, 105–119.

Wahl, M., Schneider Covachã, S., Saderne, V., Hiebenthal, C., Müller, J.D., Pansch, C., *et al.*

(2018). Macroalgae may mitigate ocean acidification effects on mussel calcification by increasing pH and its fluctuations. *Limnol Oceanogr*, 63, 3–21.

Wallace, R.B., Baumann, H., Grear, J.S., Aller, R.C. & Gobler, C.J. (2014). Estuarine , Coastal and Shelf Science Coastal ocean acidi fi cation : The other eutrophication problem. *Estuar Coast Shelf Sci*, 148, 1–13.

Welch, B. (1947). The Generalization of `Student's' Problem when Several Different Population Variances are Involved. *Biometrika*,34, 28-35.

Wolf-Gladrow, D.A., Zeebe, R.E., Klaas, C., Kortzinger, A. & Dickson, A.G. (2007). Total alkalinity: The explicit conservative expression and its application to biogeochemical processes. *Mar Chem*, 106, 287–300.

Wu W, Zhao J, Chen G, Du Z. (2016). Description of *Ancylomarina subtilis* gen. nov., sp. nov., isolated from coastal sediment, proposal of *Marinilabiliales* ord. nov. and transfer of *Marinilabiliaceae*, *Prolixibacteraceae* and *Marinifilaceae* to the order *Marinilabiliales*. *Int J Syst Evol Microbiol*, 66, 4243-4249.

Yang, S.H., Seo, H.S., Woo, J.H., Oh, H.M., Jang, H., Lee, J.H., *et al.* (2014). Carboxylicivirga gen. nov. in the family Marinilabiliaceae with two novel species, Carboxylicivirga mesophila sp. nov. and Carboxylicivirga taeanensis sp. nov., and reclassification of Cytophaga fermentans as Saccharicrinis fermentans gen. nov., comb. nov. *Int J Syst Evol Microbiol*, 64, 1351–1358.

Zedler, J.B. (2001). *Handbook for restoring tidal wetlands*. CRC Press, Boca Raton, FL, USA, 1-439.