UNIVERSITY OF CALIFORNIA,
IRVINE

Integrating microbial communities and associated top-down and bottom-up processes into intertidal and nearshore ecosystems

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Biological Sciences

by

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ABSTRACT OF THE DISSERTATION

Integrating microbial communities and associated top-down and bottom-up processes into intertidal and nearshore ecosystems

by

Lauren E. Lees

Doctor of Philosophy in Biological Sciences

University of California, Irvine, 2023

Professor Matthew E.S. Bracken, Chair

Rocky shores have long served as model systems for examining the drivers of community assembly and structure, and particularly the importance of top-down versus bottom-up control. Despite growing recognition of the importance of microbial communities in these habitats, including primary production, nutrient cycling, and contributions to trophic webs, microbes have rarely been incorporated into the traditional frameworks of intertidal community ecology. Epilithic biofilms on rocky shores consist of diverse assemblages of autotrophic and heterotrophic bacteria, algae, predatory protists, and more, but are often conceptualized and investigated as phototrophs in the context of the macroscopic community. As such, these communities are hypothesized to respond, e.g., to molluscan consumers or nutrient availability, in ways that are similar to larger macroalgae. However, as I show here, biofilm communities are actually composed of multiple, co-occurring trophic levels, and consumers and nutrients likely trigger changes in trophic dynamics within the biofilm. Bottom-up processes like nutrient concentrations determine the diversity and abundance of marine organisms, limiting their growth and survival. For example, kelp populations have been declining globally as temperatures rise

and nutrient availability declines. By understanding how these kelps, which act as both foundation species and conduits for nutrient inputs into marine systems, respond to variation in nutrient availability and identity, we can better understand how kelp populations may respond in the face of climate change.

To examine the role of top-down and bottom-up processes in structuring epibenthic intertidal communities, I used field manipulation experiments combined with molecular tools to characterize the prokaryotic and eukaryotic biofilm communities and assess how molluscan grazers, nutrient concentrations, and temperature structured these communities. I found that biofilm community composition was largely determined by temperature and nutrient concentrations, with increased nutrient concentrations increasing the impact that grazers had on these communities, suggesting that grazers may influence these communities more through nutrient facilitation or altering dispersal processes (Ch. 1). I took a comparative experimental approach to assessing how the strength of top-down and bottom-up processes in structuring biofilm communities changes across regions in California that experience difference ambient temperatures and nutrient concentrations. I found that microbial biofilm communities were largely structured by physical distance (differences between sites and regions), with local-scale top-down and bottom-up processes having relatively weak effects on these communities in comparison. While the effects were weaker, there was regional variation in the strength of grazers and nutrients structuring these communities: grazers and nutrients had stronger effects in northern California, whereas more localized processes like establishment stage and direct grazer access had larger effects in southern California (Ch. 2). Lastly, I investigated how natural variation in nitrogen availability impacted the uptake rate of different forms of nitrogen by two species of kelp in southern California during periods of low nitrogen availability. Initial

experiments included removal of biofilms from kelp blades to determine whether associated microbes might mediate nutrient uptake. The results of those experiments were inconclusive, but I subsequently found that both study species, *Macrocystis pyrifera* and *Eisenia arborea*, readily took up ammonium and nitrate, while urea uptake efficiency increased as ambient nitrogen availability decreased. Moreover, by calculating the expected rates of uptake of these nitrogen forms at various sites based on laboratory uptake experiments, I found that regenerated forms of nitrogen (ammonium and urea) contribute substantially to the nitrogen taken up by these kelps during periods of low nitrogen in the summer (Ch. 3).

INTRODUCTION

Microbes are ubiquitous in our environment and provide vital ecosystem services across scales of biological organization from organisms to the biosphere (Treseder et al. 2012; Crowther et al. 2019; Fuhrman 2009). While these functions are well recognized in open ocean systems (Worden et al. 2015; Moran et al. 2022), their roles in intertidal and coastal ecosystems remain understudied, though there is growing interest in their diversity and functions (Pfister, Gilbert, and Gibbons 2014). Although rocky shores have been model systems in understanding community assembly and structure (Connell 1972; Lubchenco 1978; Paine 1974), microbial communities have largely been ignored in this context, despite being noted for their contributions to the trophic web, primary production, and nutrient cycling (Golléty and Crowe 2013; Mandal et al. 2015). Macroecological concepts that have been well-studied in the intertidal zone, such as top-down and bottom-up control, can be applied to microbial communities to further understand the role of microbes in these ecosystems. Furthermore, variation across environmental gradients in cellular processes like nutrient uptake directly affect individual organisms, but these processes can scale up to impact larger communities like kelp forests (Beas-Luna et al. 2020; Smale 2020). Integrating microbial communities and bottom-up processes into macroecological frameworks and ecosystem dynamics can provide insight into communities' response to changing environments in the context of climate change.

Microbial communities have typically not been well-incorporated into traditional conceptual frameworks of community structure and dynamics (Prosser and Martiny 2020). The concepts of top-down and bottom-up control of communities have been explored for decades, yet microbial communities are rarely incorporated into such frameworks, particularly in rocky shore habitats, which have been model ecosystems for using field manipulation experiments to

understand top-down and bottom-up control (Connell 1972; Paine 1974; Lubchenco 1978; Nielsen 2001). While intertidal microbial biofilms have been increasingly recognized for their contributions to the food web and primary productivity, they are often viewed as a photosynthetic "organism" instead of the complex microbial community that they are (Kaehler and Froneman 2002; Thompson et al. 2000; Rindi and Benedetti-Cecchi 2023). These microbial biofilms consist of a diverse mix of organisms, including autotrophic and heterotrophic bacteria, algae, fungi, and protozoans, that are impacted by trophic interactions within the biofilm (Weitere et al. 2018) in addition to larger scale processes that can affect community assembly. Such larger scale processes that likely influence microbial community assemble include top-down and bottom-up factors.

Community composition on a macroscopic scale has been shown to be determined, in part, from the top-down by consumers and from the bottom-up by resource availability (Hunter and Price 1992; Meserve et al. 2003). These processes have been well studied in macroalgal assemblages, where consumer pressure from molluscan grazers affects communities from the top-down and nutrients affect communities from the bottom-up (Lubchenco 1978; LaScala-Gruenewald et al. 2016; Freidenburg et al. 2007). However, these grazers also alter bottom-up processes from the top-down by excreting nutrients that can facilitate macroalgal growth (Bracken 2004; Bracken, Dolecal, and Long 2014; Bracken et al. 2018). Given that microbial biofilms are functionally viewed primarily as contributors to primary productivity and food for grazers in the high intertidal zone, it is possible that these communities may respond in ways that are similar to macroalgal assemblages in response to grazers and nutrients. As such, most studies that have investigated the roles of grazing and nutrients in determining biofilm community composition only focus on the photosynthetic components of the biofilm (Kaehler and Froneman

2002; Nagarkar and Willlams 1999) although we know that heterotrophs contribute substantially to composition.

While we know that top-down and bottom-up processes play a large role in community assembly on rocky shores, the balance of these processes in determining community composition has been historically debated, with many studies now recognizing that the strength of these processes is context dependent. Large scale oceanographic processes that vary across gradients have been shown to alter the magnitude of top-down and bottom-up control (Menge et al. 1997; Sellers, Leung, and Torchin 2020). By performing comparative field experiments at regions with different environmental conditions, we can test how the magnitude of the processes changes with variations in temperature, upwelling frequency and strength, propagule pressure, and more (Menge et al. 2002). Comparative field experiments have been essential to understanding the complex processes in intertidal communities (Wernberg, Smale, and Thomsen 2012), but comparatively little of this work has included microorganisms.

Bottom-up processes, like nutrient availability, impact community composition by impacting the growth rates and survival of primary producers, which can cause compositional shifts in producer communities that may flow through the food web to affect higher trophic levels as well (Oksanen et al. 1981). Therefore, understanding the physiology of important primary producers can help us understand the drivers behind potential shifts in community structure as the climate changes. Kelps are important foundation species that have been in decline both globally and locally in southern California (Tegner et al. 1996; Krumhansl et al. 2016). While increasing temperatures have been a major driver of kelp declines (Smale 2020; Beas-Luna et al. 2020), nutrient concentrations decrease with higher temperatures (Zimmerman and Kremer 1984; Reed et al. 2016). For marine macroalgae, nitrogen (N) is typically the

nutrient limiting growth in nearshore coastal systems (Howarth and Marino 2006; Elser et al. 2007). Nitrate, an inorganic form of N, has been well studied, and it is generally considered to be the dominant N form used by kelp in southern California (Wheeler and North 1981; Sánchez-Barredo, Ladah, and Zertuche-González 2011; Gerard 1982a). However, more recent studies have begun to highlight the use of regenerated forms of N, such as ammonium and urea, by kelp. Ammonium can readily taken up by *Macrocystis pyrifera* (Haines and Wheeler 1978) and has been shown to sustain growth during low nitrate periods (Brzezinski et al. 2013). Additionally, urea was long ignored as a source of N for kelp despite phytoplankton regularly utilizing this source of N, but recent studies have indicated that urea can also be taken up by *Macrocystis* (Smith et al. 2018; 2021). Furthermore, there is the potential for microbial modification and facilitation of nitrogen uptake, particularly for urea (Bekheet and Syrett 1977). The use of alternative forms of nitrogen by kelp is of particular interest in southern California, as summer nitrate concentrations are relatively low, but *Macrocystis* maintains high growth rates (Wheeler and North 1981; Zimmerman and Kremer 1984). Ammonium has traditionally been thought to be in such low concentrations that its contribution to the total N used by kelps would be negligible compared to nitrate in southern California (Jackson 1977). Meanwhile, urea as a source of N is of particular interest due to its year-round availability due to local-scale imports from marine consumers (Regnault 1987) and its contribution to human-caused coastal nitrogen loading (Kudela, Lane, and Cochlan 2008). Modeling efforts have shown that regenerated forms of N (e.g. ammonium and urea) are present in concentrations comparable to that of nitrate on a local scale (Howard et al. 2014). Moreover, multiyear oceanographic monitoring studies have indicated that ammonium concentrations are increasing across years while nitrate concentrations are decreasing (Martiny et al. 2016). Given changing nutrient dynamics, understanding how

alternative forms of N are used by kelps in southern California coastal waters can help to predict how kelp populations may respond, which could provide insight into how these communities may change in the future (Beas-Luna et al. 2020).

Community composition is impacted by a variety of factors, including top-down and bottom-up processes. In my dissertation work, I examine how well-studied frameworks of community assembly can be applied to intertidal biofilm microbial communities and how cellular processes, like nutrient uptake by kelps, can change with variation in nutrient availability—a process that can provide insights into kelp survival and kelp forest community ecology. I used a combination of field manipulation experiments, genomic tools, and laboratory assays to answer the following questions: (1) How do top-down and bottom-up processes impact intertidal microbial biofilm communities on rocky shores? (2) Does the strength of top-down and bottom-up processes controlling biofilm communities vary across regions with different environmental conditions? And (3) How does the uptake of different N forms vary along a gradient of N availability, and what forms of N contribute the most to the N taken up by two species of kelp in southern California?

CHAPTER 1

Bottom-up factors alter the composition of rocky intertidal microbial communities and mediate top-down processes

ABSTRACT

Microbes mediate a variety of processes in ecosystems, but microbes and microbial communities have not been well-integrated into traditional models of community structure and dynamics. To assess how rocky intertidal microbial communities are structured by top-down and bottom-up processes, we performed a fully factorial field experiment within tide pools in the Bodega Marine Reserve, California, where we manipulated grazer density, nutrient concentrations, and temperature. Because most previous work had focused on the photosynthetic component of biofilms, we hypothesized that microbial communities would respond similarly to macroalgae: declining in diversity and abundance due to grazing and warming and increasing due to nutrients. We amplified and sequenced 16S and 18S rRNA genes to assess the diversity, community composition, and taxonomy of the biofilm communities in response to experimental treatments. We found that most of the variation in these communities was associated with elevated temperature and nutrient concentrations and that increasing nutrient concentrations enhanced the impacts of grazers on the microbial community. Grazers alone had little effect on prokaryotic community composition despite reducing alpha diversity, and they had no impact on eukaryotic communities at ambient nutrient concentrations but altered both prokaryotic and eukaryotic communities when nutrients were added. Biofilm communities did not respond to grazer and nutrient treatments as expected, as many of the taxa – both prokaryotic and eukaryotic – were heterotrophs. Intertidal biofilms are composed of a diverse assemblage of bacteria, microalgae, protists, and metazoans, and they respond in complex ways to top-down and bottom-up influences, likely due to trophic interactions within the microbial community.

INTRODUCTION

There is growing awareness that microbial communities are important for a variety of key processes across scales of biological organization (Balser et al. 2006; Treseder et al. 2012). From the organismal level to the biosphere, microbes are integral to system function (Crowther et al. 2019; Fuhrman 2009). While the roles of microbes are being explored more frequently and thoroughly *in situ*, integration of microbial and macroscopic organisms into our understanding of community dynamics and ecosystem functioning is still relatively rare, despite acknowledgment that microbes are essential components of communities and ecosystems.

Microbial communities have not typically been well-incorporated into traditional conceptual frameworks of community structure and dynamics (Prosser and Martiny 2020). Our understanding of communities, including perspectives on top-down and bottom-up control, is largely based on experiments and observations that include the macroscopic community members. Furthermore, when included, microbes—including phytoplankton, bacteria, and protists—are typically lumped together as a single group such as "phytoplankton" or "biofilm", without addressing the biodiversity and complexity associated with those groups. For example, most observations and experiments characterizing trophic interactions with biofilms have identified changes in the biofilms via morphology and/or chlorophyll content (Kaehler and Froneman 2002; Thompson et al. 2000) rather than molecular tools (but see, e.g., Russell et al. 2013; Tobias-Hünefeldt et al. 2021). These methods of characterizing biofilms do not allow analyses of community composition, and they tend to ignore heterotrophic taxa (e.g., by focusing on chlorophyll *a*), masking potential trophic interactions within the microbial community itself.

Intertidal and nearshore marine systems have long served as experimental models for understanding community structure and dynamics (Connell 1972; Lubchenco 1978; Paine 1974),

and recent work highlights the diversity of microbial taxa and their potential roles in these systems (Pfister, Gilbert, and Gibbons 2014; Pfister and Altabet 2019; Russell et al. 2013). However, despite recent advances in microbial ecology and the demonstrated importance of microbes in many communities and ecosystems (Malacrinò et al. 2021; Ourry et al. 2018) intertidal microbial assemblages are typically poorly characterized and seldom incorporated into our vision of how rocky shore communities and ecosystems function. At the macroscopic scale, community structure on rocky shores is regulated by both top-down and bottom-up processes (Bracken, Dolecal, and Long 2014; Menge 2000). Marine herbivores can mediate macroalgal diversity and abundance via both consumption (e.g., Kitching and Ebling, 1961; Lubchenco, 1978; Williams et al. 2013) and facilitation (Bracken, Dolecal, and Long 2014). Additionally, epilithic biofilms are recognized as important primary producers and food sources for intertidal grazers (Castenholz 1961; Thompson et al. 2000), especially as tidal elevation increases and macroalgal abundance decreases.

Studies investigating the role of grazers on intertidal biofilms have shown that experimental reductions in grazing by mollusks can increase microalgal biomass (Castenholz 1961; Thompson et al. 2000) and grazers may selectively consume nutrient-dense, loosely attached taxa, resulting in decreased diversity (Kaehler and Froneman 2002; LaScala-Gruenewald et al. 2016; Nagarkar et al. 2004). However, such studies have only investigated photosynthetic components of the biofilm, largely neglecting the complexity of biofilm communities. Furthermore, investigation of the role of bottom-up processes, alone or interactively with top-down processes, in structuring intertidal biofilm communities is even more rare (but see Chiu et al., 2008). Temperature and other environmental stressors have also been shown to alter the strength of top-down and bottom-up processes in structuring communities

(Menge et al. 2002; Williams, Bracken, and Jones 2013b) yet the omission of microbial diversity from these conceptual models limits our understanding of how these important components of the system respond to environmental stress.

We seek to open the 'black box' of microbial assemblages in a model system—rocky shore communities in the northeastern Pacific Ocean—to describe and experimentally evaluate the roles and responses of microbes in an intertidal system. To investigate the top-down and bottom-up effects of grazers, nutrients, and temperature on intertidal biofilm communities, we implemented a fully factorial field experiment in tide pools in northern California, USA. Grazer, temperature, and nutrient manipulations were applied at the tide pool level, while the effect of direct grazer access was tested using grazer exclusions within each pool.

Through this in-situ investigation of the effects of grazers, nutrients, and temperature on marine epilithic microbial communities we aimed to answer the following questions: (1) How do grazers alter these communities? (2) Do elevated temperatures and increased nutrient concentrations affect epilithic microbial diversity and community composition alone and in conjunction with grazer abundance? We expected direct grazer presence to decrease microbial diversity via top-down control, with increased temperature further intensifying this pattern by increasing grazers' metabolic and consumption rates (Russell et al. 2013). Conversely, we expected grazers to enhance microbial diversity via nutrient facilitation of photosynthetic microbes, and our experiment was designed to distinguish between these potential top-down and bottom-up roles. Further, experimentally increasing nutrient concentrations was expected to counteract both consumptive and facilitative effects of grazers, enhancing microbial diversity and abundance from the bottom-up.

METHODS

Location and Experimental Design

Field manipulations were conducted in high-intertidal natural tidepools at two spatially separated sites in the Bodega Marine Reserve, California, USA (38.328 N, 123.078 W). Each site included 16 experimental pools. One month prior to the start of our experiment, two $10cm^2$ x $10cm^2$ travertine tiles, which served as substrata for recruitment and growth, were attached to the rock in each pool. Tiles were fenced to limit access by grazers. Fences were composed of stainless-steel mesh that surrounded the tiles, but tops were open to avoid shading. Tiles were monitored every three days, and grazers that had entered the enclosures were removed. At the start of the experiment, the fence was removed from one of the established tiles, and two new tiles—one fenced and one open—were added, allowing for comparisons between established and naive communities in each tide pool.

Experimental manipulations

To evaluate the effects of grazing, nutrients, and temperature on the epilithic microbial community, we used a factorial design with 2 levels of each of the 3 factors (grazer abundance, nutrient addition, and warming), for a total of 8 treatment groups with $n = 4$ replicates each. Pools were paired based on volume, and each pool in each pair was randomly assigned to either a low-grazer-abundance or a high-grazer-abundance treatment. Grazers were counted and recorded in all pools, and grazers from each low-abundance pool were removed and added to the paired high-abundance pool every 3 days, and any grazers inside fences were removed. Temperature was increased using 60W heaters powered by two rechargeable 14 A-h gel cell batteries and a custom microcontroller housed in a waterproof box attached to the rock adjacent to each warmed pool. The controller and power supply were connected to a submerged heating

element in the pool. Controllers were programed to heat pools during low tide (Miller and Long 2015). Heaters increased maximum daily temperatures in pools by 1.42 ± 0.55 °C (mean ± 1 SD) throughout the experiment (paired *t*-test, $p < 0.001$).

Nutrients were added using perforated, threaded PVC end caps anchored to the bottom of tide pools. Each cap was filled with a nutrient-enriched (1.0 g NH_4Cl, 3.3 g K_3PO_4, and 0.1 g $NaNO_3$ in 63 ml H_2O) 3% agar solution that released phosphate (PO_4^{3-}), nitrate (NO_3^-), and ammonium (NH_4^+) at a rate of 2.67 ± 1.05, 1.16 ± 0.33, and 5.46 ± 0.41 mmol L^{-1} hr^{-1} (mean ± SD), respectively. Effectiveness was assessed by measuring nutrient concentrations in pools over time (Appendix A: supplemental methods), and dispensers were replaced every two weeks.

Sample processing, DNA extraction, and genetic analysis

After experimental conditions were maintained for 5 weeks, tiles were removed from the pools and immediately processed. One quarter (25 cm^2) of the tile was scraped with a sterile razor blade to collect the epilithic biofilm. The collected biomass was stored in a sterile 5 mL centrifuge tube with 1620 µL of lysis buffer (23.4 mg mL^{-1} NaCl, 257 mg mL^{-1} sucrose, 50 mmol L^{-1} Tris-HCl, 20 mmol L^{-1} EDTA).

To extract microbial DNA, samples were subject to bead-beating at 4800 Hz 4 times for 30s to improve cell lysis followed by the DNA extraction protocol outlined in Larkin et al. (2020). DNA was purified using a Zymo genomic DNA Clean and Concentrator kit (Zymo Research Corp., Irvine, CA). DNA concentration was assessed using a Qubit dsDNA HS Assay and a Qubit fluorometer (ThermoFisher, Waltham, MA) and then diluted to 2 ng μL^{-1}.

The V4 region of the 16S rRNA gene was amplified using a two-step polymerase chain reaction (PCR) with an intermediate purification with the 515F-C, 926R primer pair to characterize prokaryotic community composition (Needham and Fuhrman 2016; Appendix A: supplemental

menthods). The V9 region of the 18S rRNA gene was amplified using PCR with the 1391F-EUKB primer pair (Stoeck et al. 2010), Appendix A: supplemental methods) to characterize eukaryotic microbial community composition and target broadly diverse taxonomic groups, prioritizing the capture of overall eukaryotic richness (Choi and Park 2020).

Purified libraries were sequenced in 2 separate Illumina MiSeq PE runs at the UCI Genomic High Throughput Facility. Amplicon sequences were processed using QIIME2 (Bolyen et al. 2018) and denoised using the QIIME2-dada2 plugin. Sequences were clustered into ASVs (amplicon sequence variants) using dada2. Taxonomy of the prokaryotic ASVs was assigned using the Ribosomal Database Project (RDP) Naïve Bayesian classifier and the SILVA138 database. Chloroplast, mitochondria, eukaryote, and unannotated ASVs were removed from the prokaryotic ASV table, and the prokaryotic ASV table was rarefied to the minimum sequence depth of 4500 for alpha and beta diversity analyses. Eukaryotic taxonomy was assigned using the RDP Naïve Bayesian classifier and the PR2 database after initial taxonomic assignment of ASVs using the SILVA138 database for identification and removal of prokaryotic ASVs. Further filtration of eukaryotic ASVs included removal of ASVs that were only assigned to Kingdom level, and the eukaryotic ASV table was rarefied to a minimum sequence depth of 6502 for alpha and beta diversity analyses.

Statistical Analyses

All statistical analyses were completed in R version 3.6.2 (R Core Team, 2019), using the *vegan* (Oksanen et al. 2020) and *phyloseq* (McMurdie and Holmes 2013) packages for community analysis. The Shannon-Wiener diversity index, species richness, and Pielou's evenness index were calculated for each sample based on ASVs, and assumptions of normality were tested using the Shapiro-Wilkes Test. Non-Gaussian datasets were assessed via generalized

linear models with the same model design but fit to a Gamma distribution with a log link. To assess which ASVs were driving alpha diversity differences, relative abundances of ASVs were calculated using BiodiversityR package (Kindt and Coe 2005).

Differences in community composition were assessed using a PERMANOVA on a Bray-Curtis dissimilarity matrix generated from rarefied ASV tables, including all possible two-way interactions between the main effects of grazer, nutrient, and temperature treatments with site as an additional main effect. Fencing and establishment stage were nested within main effects. To assess which ASVs significantly differed between treatment groups, differential abundance analyses were performed using DESEq2 version 1.26.0 on the rarefied dataset to reduce the rate of false discovery and account for a positive within-ASV mean-variance relationship (Warton, Wright, and Wang 2012; Weiss et al. 2017). To assess which ASVs were the most different between treatment groups, the 10 ASVs with the largest absolute log2FoldChange were extracted from those that were significantly different (adjusted p-value using Benjamini-Hochberg false discovery rate correction < 0.05) between grazer, nutrient, and temperature treatments.

Results

Grazer effects

Grazer addition decreased prokaryotic ASV diversity compared to grazer removal pools. This decrease in the Shannon Index was related to a decrease in evenness (Table 1.1). In grazer addition pools, prokaryotic ASVs assigned to the cyanobacterial genus *Pleurocapsa* (10.6%) and heterotrophic *Granulosicoccus* (6.0%) had high relative abundances followed by Rhodobacteraceae with 3.0% relative abundance in grazer addition pools. ASVs in removal pools were more evenly distributed. The most abundant ASVs were assigned to *Pleurocapsa*, *Schizothrix*, *Granulosicoccus*, and Rhodobacteraceae, with relative abundances of 2.7-5.2%.

In contrast to prokaryotic communities, eukaryotic communities were more even (i.e., higher Pielou's *J*) in grazer addition pools compared to grazer removal pools, and there was a significant interaction between grazer and temperature treatment. Grazer removal resulted in a decrease in evenness under ambient temperatures, but evenness was unaffected under heated conditions (Table 1.1). This difference in evenness in grazer treatments was related to a relative dominance of the most abundant ASV assigned as Ulvophyceae (25.5%) in grazer removal pools. However, the top two most abundant ASVs in grazer addition pools were also assigned as Ulvophyceae, with further potential taxonomic differences between these ASVs remaining unknown. While we saw grazer effects on diversity at the pool level, there was no difference in the fencing effect between grazer treatments, or other pool-level experimental treatments, in prokaryotic or eukaryotic communities, indicating that grazers impact diversity at the pool scale rather than through direct access to the biofilm (Appendix A: Table S3).

There was a significant difference in prokaryotic community composition between grazer addition and grazer removal pools (PERMANOVA: $p = 0.007$, Appendix A: Table S4; Fig 1.1). Additionally, there was a significant interaction between grazer and nutrient treatments in which there was a larger shift in community composition due to grazer treatment when nutrients were increased compared to ambient nutrients for both prokaryotic and eukaryotic communities (PERMANOVA: $p<0.05$, Appendix A: Table S4; Fig.1. 2). However, there was no interaction between temperature and grazer treatments on composition for either community. Overall, the significant effects of grazers alone and interactively accounted for 3.29% and 2.59% of the variance in prokaryotic and eukaryotic communities, respectively (Fig. 1.1).

Differential abundance analysis amongst prokaryotic communities revealed an increase in multiple ASVs assigned to cyanobacteria in grazer addition pools compared to removal pools,

along with ASVs assigned to Rhodobacteraceae and Bacteroidia. Of the top ten ASVs with the largest difference between treatments, only one, assigned to Flavobacteraceae, was more abundant in herbivore removal pools (Fig. 1.3a). While there was no significant difference in community composition amongst eukaryotic communities, all of the top ten eukaryotic ASVs that were the most different between grazer addition and removal treatments were more abundant in nutrient addition treatments and had similar magnitudes of change. This included ASVs identified as diatoms (*Amphora*, *Navicula*, Raphid-Pennate, and Bacilariophyta), Alveolates, Heteroamobea, Maxilopoda, and Placidiales (Fig. 1.3b).

Temperature effects

We observed higher ASV richness in warmed eukaryotic communities (Table 1.1). Warming affected prokaryotic diversity, alone and interactively with grazers, only on established tiles (warmed pools had higher Shannon Index, especially when grazers were added; Appendix A: Table S1, Fig. S1). Temperature treatment significantly changed prokaryotic and eukaryotic community composition and explained 6.07% and 6.03% of the variance in these respective communities, alone and interactively (PERMANOVA: $p < 0.01$, Fig. 1.1). There was a significant interactive effect of temperature and nutrient treatments on prokaryotic and eukaryotic community composition, in which there was a decreased impact of nutrient addition when temperature was increased for both communities. Prokaryotic communities also responded to temperature treatment more strongly when nutrients were not experimentally increased, though the interactive effects of temperature and nutrient treatment were poorly captured via two-dimension NMDS for the prokaryotic communities (PERMANOVA: $p < 0.001$, pairwise PERMANOVA, Fig. 1.2). Differential abundance analysis showed that, of the top ten differentially abundant ASVs, most were more abundant in ambient temperature pools compared

to heated pools (Fig. 1.3a). For eukaryotic communities, temperature treatment had the largest response of diatoms, with 8 of the top 10 most different ASVs being diatoms. These diatoms were more abundant in ambient temperature pools compared to heated pools (Fig. 1.3b).

Nutrient effects

Nutrient treatment significantly altered community composition for both prokaryotic and eukaryotic communities (PERMANOVA: $p < 0.003$, Fig. 1.2) but did not affect Shannon diversity, despite increased ASV richness in ambient nutrient pools for eukaryotic communities (Table 1.1). Overall, the effects of nutrient treatments, alone and interactively with temperature and grazer treatment, accounted for 6.78% of the variance in prokaryotic communities and 6.20% of the variance in eukaryotic communities. While the effect of nutrient treatments on prokaryotic communities was similar alone and interactively, the amount of variance explained by nutrient treatments in eukaryotic communities was larger interactively with temperature (Fig. 1.1g).

The most differentially abundant prokaryotic ASVs between nutrient treatments varied in directionality. For example, ASVs assigned to the family Flavobacteraceae were more abundant in ambient nutrient pools, while Alphaproteobacteria were more abundant in nutrient addition pools. Cyanobacterial ASVs also differed in their directionality (Fig. 1.3a). Eukaryotic differential analyses revealed that that the most divergent ASVs included an increase in heterotrophic maxilopods and an uncharacterized opisthokont as well as autotrophic diatom ASVs in nutrient addition pools compared to ambient pools. Archeplastidia ASVs did not respond homogeneously, with 2 different genera of green algae being more abundant in opposing treatments (Fig. 1.3b).

Taxonomy characteristics

Xenococcaceae, a family of cyanobacteria, was the most abundant family overall for the prokaryotic ASVs, followed closely by Rhodobacteraceae, a functionally diverse heterotopic family, with overall proportions of 23.6% and 21.4%, respectively. Xenococcaceae was the most abundant family in grazer addition pools followed by Rhodobacteraceae, while the positions were reversed in grazer removal pools, but only in those that experienced increased nutrient concentrations. Additionally, Granulosicoccus and Phormidesmiaceae were abundant in higher proportions in grazer addition pools and Saprospiraceae and Synechococcales Incertae Sedis were more abundant in grazer removal pools (Fig 1.4).

The most abundant classes assigned from eukaryotic ASVs were autotrophic with Ulvophyceae (50.9%), Bacilariophyta (20.5%), and Phaeophyceae (10.3%), followed by heterotrophic Arthropoda (9.3%) and Heteroblosea (2.9%). When relative abundance was broken down into treatment groups, we saw a dominance of Ulvophyceae at all tide pool level treatments except heated grazer-addition pools and heated increased-nutrient pools with grazers removed, where Bacilariophyta was the most abundant class. Additionally, we saw a decrease in the relative abundance of Phaeophyceae in heated treatments compared to ambient temperature pools with the same grazer and nutrient treatment (Fig. 1.4).

Discussion

Top-down effects of grazers and bottom-up effects of nutrients altered prokaryotic and eukaryotic microbial communities, with grazers having a larger effect in structuring prokaryotes. However, in both cases, the bottom-up effects of nutrients and the environmental impacts of temperature, both alone and interactively, explained more variance in these communities compared to grazer treatments. We saw complex interactions between grazers, nutrients, and temperature structuring communities in ways that differed from many of our original predictions.

Previous studies of the effects of top-down and bottom-up processes on intertidal epilithic communities have largely focused on microphytobenthic and macroalgal communities (Bracken, Dolecal, and Long 2014; Kaehler and Froneman 2002; Nagarkar et al. 2004), yet our analyses show that these microscopic communities consist of a diverse mix of bacteria, algae, fungi, protozoans, and metazoans which are characterized by comparably diverse methods of metabolism and resource acquisition and would not be likely to respond to changing environmental conditions homogeneously. This likely translates into complex functional diversity and trophic interactions within the biofilm that are then affected by top-down and bottom-up processes outside the biofilm. Although the major function of marine biofilms is frequently assumed to be primary productivity, we detected large proportions of heterotrophs. As such, these biofilms responded to treatments in ways that were not consistent with purely autotrophic communities.

While increased grazer abundance was associated with reduced prokaryotic diversity, a pattern driven by higher evenness in grazer removal pools, we saw the opposite pattern in eukaryotic communities, with increased evenness in herbivore addition tide pools, although this pattern was not strong enough to affect the Shannon index (Table 1.1). Previous studies of molluscan grazers highlight their roles as passive-selective grazers, more easily removing some taxa than others (Kaehler and Froneman 2002; Nicotri 1977). While we found no evidence that direct consumption was driving changes in alpha diversity (i.e., differences between fenced and unfenced tiles), an increase in grazer abundance in tide pools likely translated to increased consumption within the tidepool, which could impact dispersal.

In grazer-addition pools, we saw an increase in the dominance of a specific ASV identified as *Pleurocapsa*, a nitrogen-fixing cyanobacterial genus that can be abundant on rocky

shores (Ortega-Morales, Santiago-Garcia, and López-Cortés 2005; Rippka et al. 2005). However, higher evenness of eukaryotic communities in grazer-addition pools was associated with an increase in the proportion of an ASV from the green algal class Ulvophyceae, which resulted in lower evenness in grazer removal pools. This increased dominance of a green algal ASV could be related to increased consumption of easily removed and fast-growing green algae at the tide-pool level, allowing for the proliferation of this alga when grazers were removed.

Despite contrasting effects of grazers on prokaryotic and eukaryotic diversity, grazers had a relatively minor main effect on community composition compared with nutrient and temperature treatments. However, grazer effects on both prokaryotic and eukaryotic communities were enhanced when nutrients were added. Increased nutrient concentrations can decrease top-down effects in marine systems (Firstater et al. 2012; Sellers et al. 2021), despite ecological theory suggesting that increased nutrients—and therefore increased primary productivity—should support higher herbivore densities and strengthen top-down impacts (Oksanen et al. 1981). Yet, these patterns are typically related to direct consumptive effects of herbivores, and we saw no difference between fenced and open tiles, indicating that compositional differences due to grazers were an effect of abundance in the tide pool rather than actual consumption of the biofilm. The tide-pool-level effects of grazers are consistent with facilitation of microbial communities via nutrient recycling. Additionally, previous studies typically quantify intertidal biofilm area and photosynthetic biofilm components rather than assessing the entire microbial community, including micrograzers (Weitere et al. 2018). We saw increases in various heterotrophic and predatory microbial ASVs—including heteroamoebae, heterotrophic bacteria, and amoebas—as well as autotrophic ASVs associated with cyanobacteria and diatoms (Bacillariophyta, raphid-pennate, *Amphora*, and *Achnanthes*) when nutrients and grazer

abundance were increased. Molluscan grazers have been shown to alter behavior when exposed to lower quality food, resulting in increased consumption to compensate for low nutrient quality (Fink and Von Elert 2006). Increased nutrient availability could have thereby decreased the direct consumptive effects of molluscan grazers, while simultaneously increasing nutrient availability via recycling (Bracken, Dolecal, and Long 2014) and allowing proliferation of certain micrograzers and micropytobenthos.

Temperature played a large role in structuring prokaryotic communities, and the interactive effects of temperature and nutrients impacted eukaryotic community composition. Increased temperature has been shown to increase photosynthetic biomass and alter diatom and prokaryotic communities in marine biofilms (Chiu et al. 2005; Misic and Covazzi Harriague 2019). Increasing temperature can increase growth rates of taxa that are tolerant of temperature changes while also acting as a stressor for other taxa. In prokaryotic communities, experimental warming alone accounted for the largest amount of variance, but in both heated and ambient temperature communities, nutrient treatment had a further effect. However, we observed no effect of nutrient additions on eukaryotic community composition in heated pools. The lack of a nutrient effect when pools were heated could be indicative of increased temperature acting as a physical stressor rather than facilitating growth.

Overall, most of the variation in prokaryotic and eukaryotic assemblages was associated with bottom-up processes and abiotic conditions, with the impact of macrobiotic consumers being further influenced from the bottom-up. Our experimental treatments accounted for roughly 20% of the variance in these communities, with other underlying environmental factors—such as salinity, pH, dissolved oxygen, and settlement substrate— also likely playing a role in their structure (Guo et al. 2017). Although they were within close proximity to one another, the site of

our experiment also played a role in community composition, indicating that factors varying across space—even over small scales—impact these communities. Along with changes in abiotic factors, we expect the results of this field experiment to be context-dependent, with the strength of interactions between top-down and bottom-up processes changing as environmental conditions change.

While we saw intriguing responses from a variety of taxa that differed between treatments, given the level of taxonomic identification, we cannot say how these changes in composition might alter the functionality of rocky intertidal biofilms. Variability in the function of closely related microbes makes it difficult to infer functional roles at the scale examined in this study. By looking at functional traits, future studies could further understand how microbial assemblages interact with larger scale processes and how function changes with changes in the environment. However, assessing microbial community composition and how top-down and bottom-up processes structure those communities is the first step in understanding the roles these assemblages play in the larger ecosystem.

Despite intertidal biofilms providing some functions in high intertidal communities similar to those provided by macroalgae lower on the shore (e.g., primary productivity and food), they are a complex community that responds differently to top-down and bottom-up processes. These microbial assemblages are affected by internal trophic interactions (i.e., heterotrophy), and these interactions are likely altered by changes in top-down and bottom-up processes and environmental conditions. Experiments on rocky shores have played a foundational role in shaping our understanding of how both bottom-up and top-down processes structure a community. By investigating how microbes fit into these well-studied communities, we can not only gain insights into how these systems function, but also infer how microbes are likely to act

in other systems. Integrating microbial processes into ecological frameworks can allow us to organize and interpret their roles and improve predictive models as we attempt to understand how ecosystems will be impacted by global change (Otwell et al. 2018; Treseder et al. 2012).

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TABLES

Table 1.1 Differences in alpha diversity associated with experimental manipulations based on linear models.

	Prokaryotes			Eukaryotes		
	Estimate	t value	p	Estimate	t value	p
(a) Shannon Index						
Grazer Removal	**0.295 ± 0.149**	**1.980**	**0.050**	-0.312 ± 0.160	- 1.948	0.054
Increased Nutrients	-0.069 ± 0.148	- 0.466	0.642	-0.22 ± 0.158	- 1.386	0.168
Increased Temperature	0.182 ± 0.148	1.233	0.220	0.096 ± 0.158	0.606	0.545
Site B	0.144 ± 0.085	1.685	0.095	**0.265 ± 0.092**	**2.891**	**0.005**
Grazers x Nutrients	0.099 ± 0.170	0.578	0.564	0.047 ± 0.183	0.258	0.797
Grazers x Temperature	-0.282 ± 0.171	- 1.651	0.101	0.303 ± 0.183	1.650	0.102
Nutrients x Temperature	0.003 ± 0.171	0.018	0.986	-0.143 ± 0.183	0.777	0.439
(b) Richness						
Grazer Removal	0.140 ± 0.099	1.421	0.158	0.048 ± 0.113	0.421	0.675
Increased Nutrients	-0.049 ± 0.098	- 0.501	0.617	**-0.263 ± 0.112**	**- 2.351**	**0.020**
Increased Temperature	0.005 ± 0.098	0.052	0.959	**0.272 ± 0.112**	**2.432**	**0.017**
Site B	**0.215 ± 0.057**	**3.810**	**0.0002**	0.118 ± 0.065	1.821	0.071
Grazers x Nutrients	0.041 ± 0.113	0.366	0.715	0.254 ± 0.129	1.963	0.052
Grazers x Temperature	-0.134 ± 0.113	- 1.185	0.238	0.106 ± 0.129	-0.817	0.416
Nutrients x Temperature	0.011 ± 0.113	0.095	0.924	0.125 ± 0.129	- 0.969	0.334
(c) Evenness						
Grazer Removal	0.048 ± 0.026	1.893	0.061	**-0.083 ± 0.031**	**- 2.702**	**0.008**
Increased Nutrients	-0.014 ± 0.025	- 0.496	0.578	-0.026 ± 0.030	- 0.846	0.399
Increased Temperature	0.045 ± 0.025	1.783	0.077	-0.01 ± 0.030	- 0.314	0.754
Site B	-0.004 ± 0.015	- 0.299	0.770	**0.052 ± 0.018**	**2.968**	**0.004**
Grazers x Nutrients	0.021 ± 0.029	0.681	0.480	-0.018 ± 0.035	- 0.506	0.614

Grazers x Temperature	-0.048 ± 0.029	- 1.621	0.104	**0.085 ± 0.035**	**2.413**	**0.017**
Nutrients x Temperature	0.005 ± 0.029	0.112	0.877	-0.016 ± 0.035	- 0.454	0.651

FIGURES

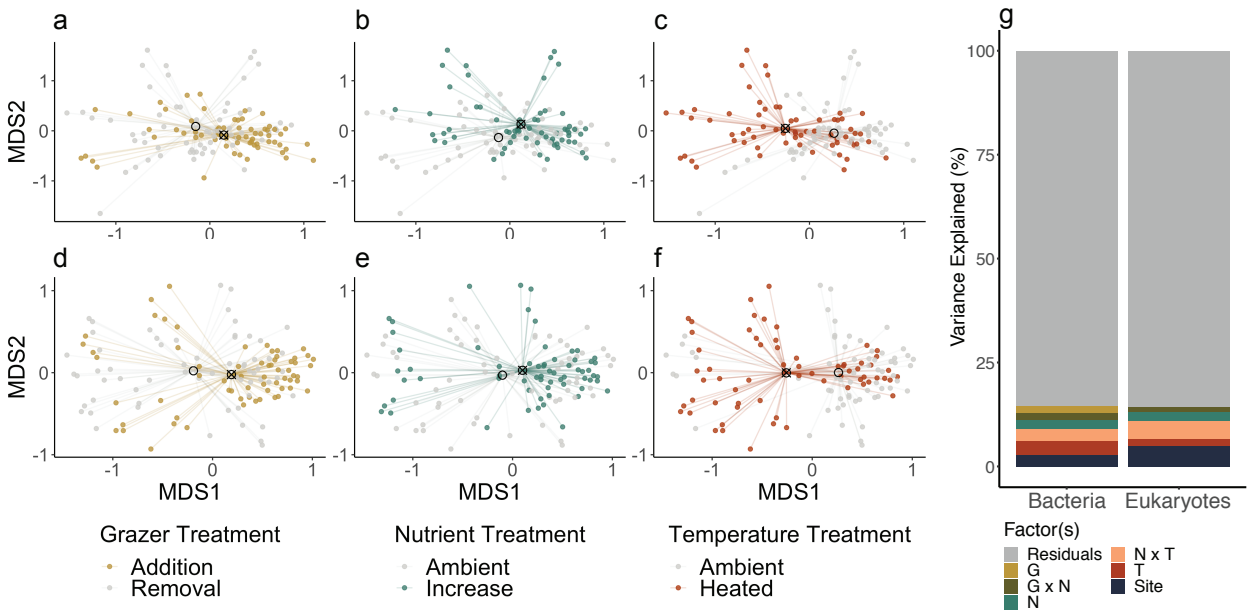

Figure 1.1 Microbial communities varied between grazer (G), nutrient (N), and temperature treatments (T). Non-metric multidimensional scaling (NMDS) ordination depicting differences in treatment groups in prokaryotic (a-c) and eukaryotic (d-f) communities. (g) The percentage of variance explained in a PERMANOVA for prokaryotic and eukaryotic communities.

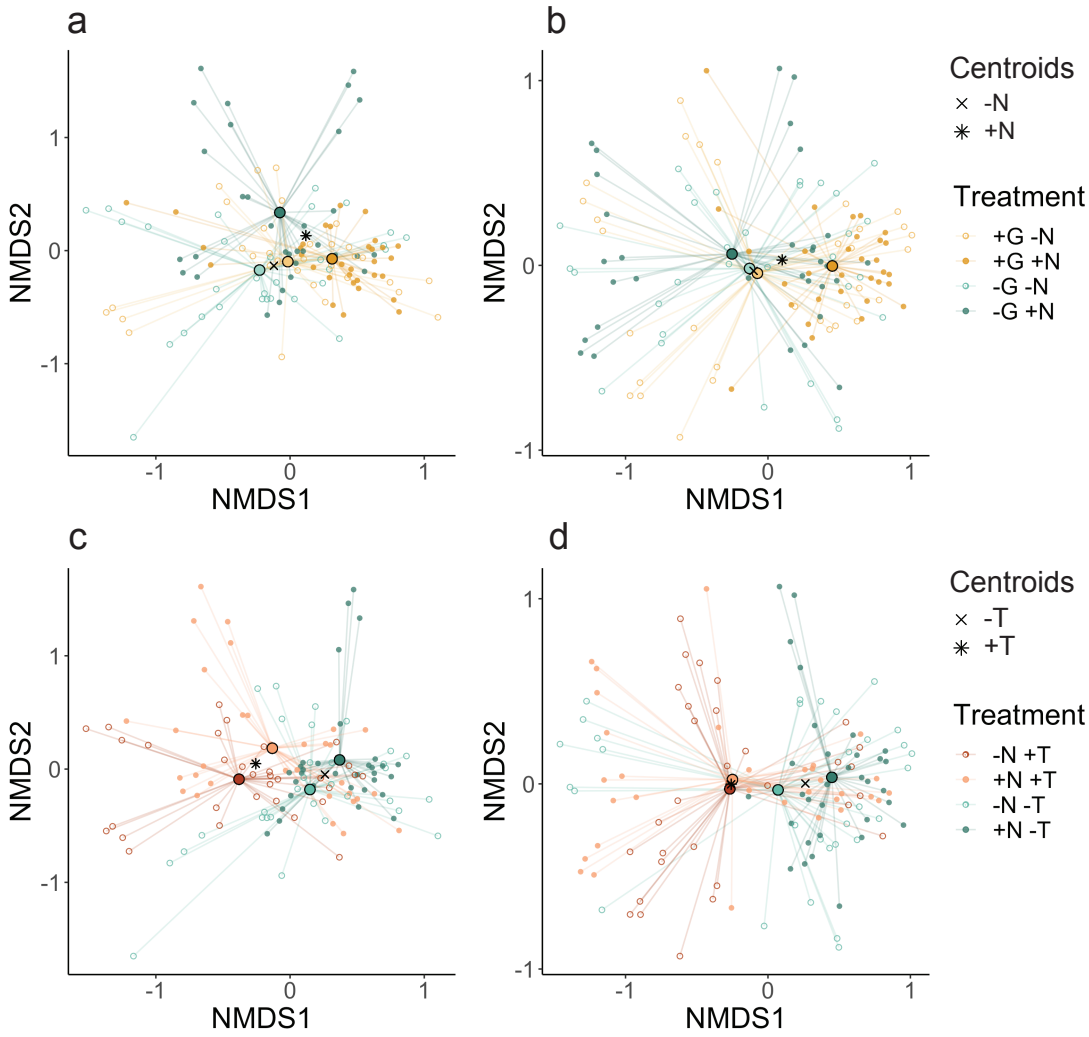

Figure 1.2 Interactive effects of grazers (G), nutrients (N), and temperature (T) on prokaryotic (a&c) and eukaryotic communities (b&d). (a-b) Grazer effects on community composition were greater when nutrient concentrations were experimentally increased. (c-d) Differences in the effects of nutrient treatments on community composition were reduced when temperature was increased.

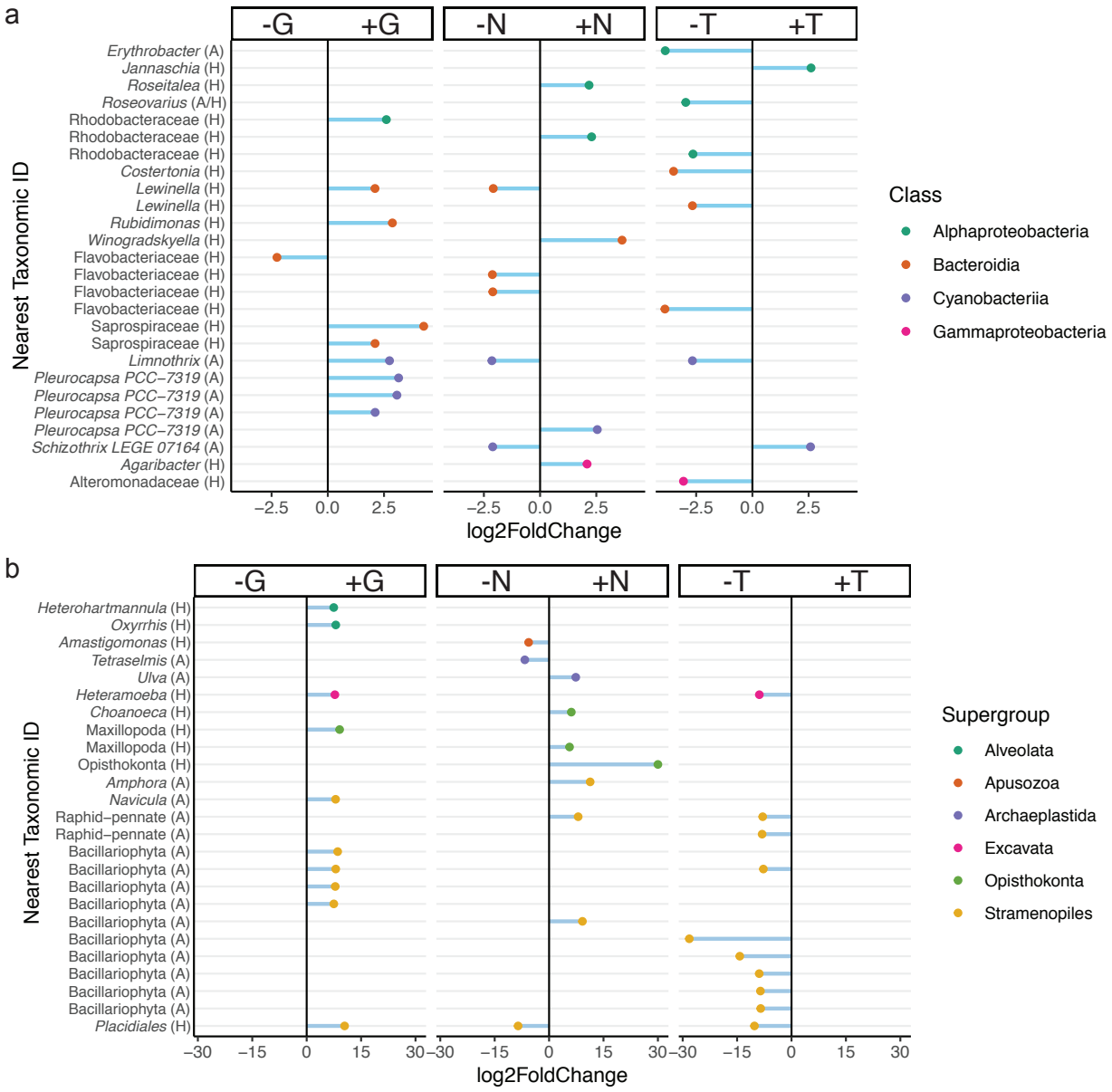

Figure 1.3 The top ten most differentially abundant ASVs between grazer addition and removal (+/- G), nutrient addition and ambient nutrients (+/- N), and heated and ambient temperature (+/- T) tide pools. Prokaryotic (a) and eukaryotic (b). ASVs were assigned to their nearest taxonomic ID based on database assignment and were assigned as autotrophic (A) or heterotrophic (H) based on relevant literature (Appendix A: Table S5).

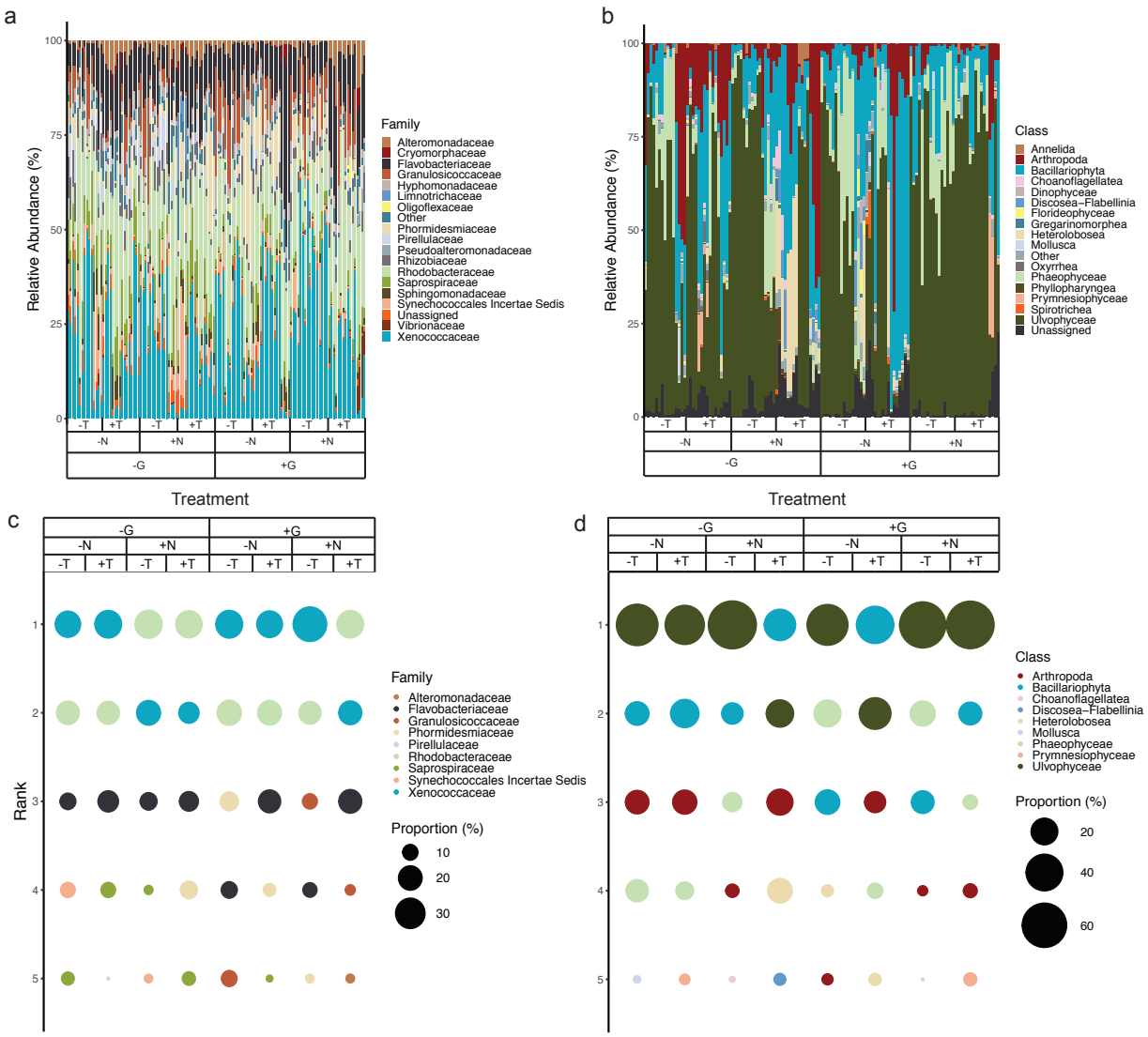

Figure 1.4 Taxonomic composition of the microbial communities in experimental tidepools. The relative abundance of prokaryotic families (a) and eukaryotic classes (b) from each tile in experimental tide pools, ordered by grazer (G), nutrient (N), and temperature (T) treatment. Prokaryotic families shown are in abundance of >0.999%, while eukaryotic classes >5% relative abundance are shown. Rank abundance plots of the top 5 most abundant prokaryotic families (c) and eukaryotic classes (d) organized by treatment groups.

CHAPTER 2

Regional variation in top-down and bottom-up processes is secondary to spatial distance in determining intertidal microbial community composition

ABSTRACT

Top-down and bottom-up processes play a vital role in structuring communities along rocky shores. However, the strength of these factors as drivers of community assembly varies across space and time, changing with a variety of biotic and abiotic factors. Comparative field experiments have been crucial in understanding the balance between top-down and bottom-up processes in community assembly. While rocky shores have been model systems to examine these processes, the community dynamics of epilithic microbial biofilms have largely been ignored despite their contributions to primary productivity and trophic webs in the high intertidal. In this study, we compared the impact of molluscan grazers and nutrient availability on prokaryotic and eukaryotic biofilm communities across two regions in California, USA, by deploying simultaneous field manipulation experiments in northern and southern California. We found that top-down and bottom-up processes played larger roles in microbial community assembly in northern California, while localized processes had larger impacts in southern California. Despite this regional variation in the strength of top-down and bottom-up processes, these effects were minimal in comparison to the effects of physical distance, including local and regional scales, indicating that changes in environmental conditions across spatial scales play larger roles in the community assembly of intertidal microbial biofilms. Our results highlight the importance of applying macroecological theory to microbial communities, given the unique aspects of microbial communities that can mediate the impacts of well-studied processes in community ecology.

INTRODUCTION

The balance between top-down and bottom-up processes in structuring communities has been a topic well-studied in ecology, with communities being influenced from the top-down by

consumers and from the bottom-up by resources (Hunter and Price 1992; Meserve et al. 2003). In marine systems, the rocky intertidal zone is a model system that has been historically important in studying how these processes influence community structure (Menge 2000; Nielsen 2001). Importantly, work on rocky shores has demonstrated that the dominant forces structuring intertidal communities are context dependent, changing across locations and seasons (Menge et al. 2002; Sellers et al. 2021; Thompson, Norton, and Hawkins 2004). Additionally, top-down and bottom-up factors may indirectly influence one another, as ecological theory suggests that increased nutrient availability can alter plant-grazer interactions (Oksanen et al. 1981).

Despite the potential for nutrients to enhance macroalgal abundance and quality and therefore increase grazing rates, effects are not equal across species. Increased nutrient availability can increase the abundance of dominant algal species while grazers decrease species richness by consuming more ephemeral and/or rare species, resulting in reduced evenness in macroalgal assemblages (Hillebrand 2003, Nielsen and Navarrete 2004). However, the magnitudes of nutrient and grazer effects can vary with larger-scale oceanographic factors as well (Freidenburg et al. 2007; Menge et al. 2002). In upwelling ecosystems, nutrient addition can alter algal communities, especially in nutrient-limited microhabitats (Bracken and Nielsen 2004) or when grazer abundances are reduced (Nielsen 2003; Wootton et al. 1996). Similarly, macroalgal assemblages at sites with relatively low levels of upwelling and nutrient availability are largely structured from the top-down, with nutrient facilitation of macroalgae being apparent only when grazing is experimentally limited (Guerry, Menge, and Dunmore 2009). Environmental conditions can also affect the balance of top-down and bottom-up control by altering disturbance and dispersal dynamics (Nielsen 2001; Nielsen and Navarrete 2004).

Our understanding of how top-down and bottom-up forces shape communities is largely based on visible changes to macroscopic organisms. However, emerging evidence suggests that microbial assemblages, including epilithic microbial biofilms, are essential components of rocky shore communities, where they play vital roles in primary production and nutrient cycling (Golléty and Crowe 2013; Mandal et al. 2015). Whereas researchers have often focused on the primary producers in these assemblages, they are comprised of a complex assemblage of micrograzers, bacteria, periphyton, and other organisms, including both autotrophs and heterotrophs (Weitere et al. 2018). Thus, the response of these communities to consumption by molluscan grazers and exposure to increased nutrients is likely to be more complex than macroalgal assemblages, as trophic interactions occur on the microscopic scale in addition to the grazing associated with larger molluscan grazers. Although these epilithic biofilms are themselves complex communities nested within larger rocky-shore communities, grazing and nutrient facilitation of the biofilms may resemble effects observed on the macroscopic scale. For example, molluscan grazers decrease the evenness of macroalgal assemblages by consuming more palatable species (Nielsen 2001), and, similarly, molluscan grazers may consume more loosely attached cyanobacteria in biofilms compared with hardier diatoms (Kaehler and Froneman 2002). Furthermore, an increase in nutrients flowing through the food chain may present as increases in heterotrophic bacteria and protists while altering the composition of photosynthetic microbes, a process that may be exaggerated by decreased consumption by molluscan grazers, which have also been shown to alter micrograzer activity directly (Wootton et al. 1996).

Comparative experiments across geographic scales can be important tools in assessing the generality of top-down and bottom-up controls over multiple gradients (Menge et al. 2002).

Similar designs may allow assessment of the roles of top-down (consumer) and bottom-up (nutrient) effects on epilithic biofilm assemblages on rocky shores. Epilithic biofilms can vary greatly across small geographic scales, suggesting biofilm communities are structured by even small changes in their environmental conditions (Narváez-Zapata, Rodríguez-Ávila, and Ortega-Morales 2005). Despite this potential for variation at local scales, assessing the strength of large-scale processes (grazer abundance and nutrient availability) at different sites can give further insight into how these communities are structured and what environmental variables may affect these processes.

To examine the how bottom-up and top-down processes structure intertidal biofilm communities across different locations, we deployed two simultaneous field experiments in northern and southern California, USA where we manipulated grazer abundance and nutrient concentrations in rocky tide pools. Our goals were to understand how top-down and bottom-up processes influence these communities and how these effects vary in locations that differ in ambient temperature and nutrient conditions. We aimed to answer the following questions: (1) Do the strength of top-down and bottom-up processes in structuring intertidal biofilm communities vary across different regions in California and (2) what factors consistently contribute to structuring these microbial communities across regions. While we expected the regions to have distinctly different communities given the distance and different substratum, we also expected the strength of grazer and nutrient treatments to vary. We hypothesized that nutrient addition would have a larger effect on communities in southern California, given the lower ambient nutrient levels compared to northern California. As molluscan grazers may mitigate the impact of increased nutrients, we expected that grazer removals would increase the impact of increased nutrients, particularly amongst eukaryotic micrograzers, as a higher trophic

level has been removed. Furthermore, we expected that consumptive effect of grazers could be greater in southern California as there is typically less macroalgal cover – and therefore food for grazers – in southern California high-intertidal pools (Bracken et al. 2018).

MATERIALS AND METHODS

Location and experimental design

Field manipulations were conducted in high intertidal tidepools at two regions in California, USA: the Bodega Marine Reserve (BMR) on the Sonoma County coast (38.31°N, 123.07°W) and Corona del Mar State Beach (CDM) on the Orange County Coast (33.59°N, 117.87°W). Temperature of experimental tide pools were monitored with TidBiT temperature dataloggers (Onset, Bourne, Massachusetts, USA) while water samples were taken to assess differences in nutrient availability between regions. Tide pools in CDM experienced average maximum daily temperatures roughly 4°C warmer than the BMR, while nutrient concentrations were approximately 4 to 9 times higher in the BMR compared to CDM (Fig. 2.1). As such, we characterized the BMR as a high nutrient, low temperature region and CDM as a low nutrient, high temperature region. Within regions, we identified two spatially separated sites comprised of 16 experimental pools each. One month prior to the start of our experiment, two $10cm^2$ x $10cm^2$ travertine tiles, which served as substrata for recruitment and growth, were attached to the rock in each pool. Tiles were fenced to limit access by grazers. Fences were composed of stainless-steel mesh that surrounded the tiles, but tops were open to avoid shading. Tiles were monitored every three days, and grazers that had entered the enclosures were removed. At the start of the experiment, the fence was removed from one of the established tiles, and two new tiles—one fenced and one open—were added, allowing for comparisons between established and naive communities in each tide pool.

Experimental manipulations

To examine the effects of nutrients and grazers on epilithic microbial communities in northern and southern California, we used a factorial design with 2 levels of 2 factors (grazer abundance and nutrient addition), for a total of 4 treatment groups with n = 8 replicates at Corona del Mar. The experiment in Bodega Marine Reserve included experimental heating as an additional factor for a total of 8 treatment groups, but experimentally warmed pools were excluded from these analyses to allow for direct comparisons with Corona del Mar, leaving n = 4 replicates for each nutrient and grazer treatment group. Pools were paired in each region based on volume, and each pair of pools was randomly assigned to low-grazer- or high-grazer-abundance treatments. Grazers were counted and recorded in all pools, and grazers from low-abundance pools were removed and added to high-abundance pools every 3 days. At this time, any grazers inside the fences were also removed.

Nutrients were added using perforated, threaded PVC end caps anchored to the bottom of tide pools. Each cap was filled with a nutrient-enriched (1.0 g NH_4Cl, 3.3 g K_3PO_4, and 0.1 g $NaNO_3$ in 63 ml H_2O) 3% agar solution that released phosphate (PO_4^{3-}), nitrate (NO_3^-), and ammonium (NH_4^+) at a rate of 2.67 ± 1.05, 1.16 ± 0.33, and 5.46 ± 0.41 mmol L^{-1} hr^{-1} (mean ± SD), respectively. Effectiveness was assessed by measuring nutrient concentrations in pools over time (Appendix B: supplemental methods), and dispensers were replaced every two weeks.

Sample processing, DNA extraction, and genetic analysis

After experimental conditions were maintained for 5 weeks, tiles were removed from the pools and immediately processed. One quarter (25 cm^2) of the tile was scraped with a sterile razor blade to collect the epilithic biofilm. The collected biomass was stored in a sterile 5 mL

centrifuge tube with 1620 µL of lysis buffer (23.4 mg mL^{-1} NaCl, 257 mg mL^{-1} sucrose, 50 mmol L^{-1} Tris-HCl, 20 mmol L^{-1} EDTA).

To extract microbial DNA, samples were subject to bead-beating at 4800 Hz 4 times for 30s to improve cell lysis followed by the DNA extraction protocol outlined in Larkin et al. (2020). DNA was purified using a Zymo genomic DNA Clean and Concentrator kit (Zymo Research Corp., Irvine, CA). DNA concentration was assessed using a Qubit dsDNA HS Assay and a Qubit fluorometer (ThermoFisher, Waltham, MA) and then diluted to 2 ng μL^{-1}.

The V4 region of the 16S rRNA gene was amplified using a two-step polymerase chain reaction (PCR) with an intermediate purification with the 515F-C, 926R primer pair to characterize prokaryotic community composition (Needham & Fuhrman 2016; Appendix B: supplemental methods). The V9 region of the 18S rRNA gene was amplified using PCR with the 1391F-EUKB primer pair (Stoeck et al., 2010, Appendix B: supplemental methods) to characterize eukaryotic microbial community composition and target broadly diverse taxonomic groups, prioritizing the capture of overall eukaryotic richness (Choi and Park 2020).

Purified libraries were sequenced in 4 separate Illumina MiSeq PE runs at the UCI Genomic High Throughput Facility. Amplicon sequences were processed using QIIME2 (Bolyen et al. 2018) and denoised using the QIIME2-dada2 plugin. Sequences were clustered into ASVs (amplicon sequence variants) using dada2. Taxonomy of the prokaryotic ASVs was assigned using the Ribosomal Database Project (RDP) Naïve Bayesian classifier and the SILVA138 database (Wang et al. 2007). Chloroplast, mitochondria, eukaryote, and unannotated ASVs were removed from the prokaryotic ASV table, and the prokaryotic ASV table was rarefied to the minimum sequence depth of 4742 for alpha and beta diversity analyses. One prokaryotic sample from Corona del Mar was removed due to its low sequencing depth with 1636 reads. Eukaryotic

taxonomy was assigned using the RDP Naïve Bayesian classifier and the PR2 database after initial taxonomic assignment of ASVs using the SILVA138 database for identification and removal of prokaryotic ASVs. Further filtration of eukaryotic ASVs included removal of ASVs that were only assigned to Kingdom level, and the eukaryotic ASV table was rarefied to a minimum sequence depth of 2636 for alpha and beta diversity analyses.

Statistical analysis

Statistical analyses were performed in R version 3.6.2 using *vegan* and *phyloseq* packages for community analysis. Alpha diversity metrics (Shannon-Wiener diversity index, species richness, and Pielou's evenness index) were calculated for each sample based on ASVs, and assumptions of normality were tested using the Shapiro-Wilkes Test. Effects of fencing and establishment stage were examined by calculating the difference in alpha diversity metrics between fenced and unfenced tiles paired by establishment stage within each tidepool. The impact of tidepool level treatment by region was then assessed via linear model with the alpha diversity difference as the response variable as predicted by a three-way interaction between region, nutrient treatment, and grazer treatment for established and naïve samples. Nutrient and grazer treatment effects on each alpha diversity metric were assessed with linear models examining the main and interactive effects of experimental treatments and region on each alpha diversity metric. These analyses were completed for prokaryotic communities and eukaryotic communities independently.

Differences in community composition were tested using PERMANOVAs on a Bray-Curtis dissimilarity matrix generated from rarefied prokaryotic and eukaryotic ASV tables which assessed all possible two-way interactions between region, grazer, and nutrient treatments, with fencing and establishment stage nested within grazer and nutrient treatments and within region

site as an additional main effect. The percentage of variance explained was calculated by dividing statistically significant estimates by the sum of estimates given by the PERMANOVA output in R (Finks et al. 2021). The directionality of significant interactive effects was characterized using NMDS visualizations and pairwise PERMANOVAs using '*pairwiseAdonis*' (Arbizu 2020). To assess if shared ASVs between the two regions responded similarly to experimental treatments, this PERMANOVA was repeated using the same model structure on a Bray-Curtis dissimilarity matrix generated from ASVs found in both regions. To further investigate experimental treatments driving changes in community structure within each region, separate PERMANOVAs were run within each region assessing interactions between the main effects of grazer and nutrient treatments with site as an additional main effect. The effects of fencing and establishment stage were nested within main effects.

To identify ASVs that drove differences in community composition, differential abundance analyses were performed for each significant main effect based on PERMANOVA results using DESeq2 (Love et al., 2014). Differential abundance analyses were performed on the rarefied dataset in order to reduce the false discovery rate (Warton et al. 2012; Weiss et al. 2017). To assess which ASVs were the most different between treatment groups, the 10 ASVs with the largest absolute log2FoldChange were extracted from those that were highly significantly different (adjusted p-value using Benjamini-Hochberg false discovery rate correction < 0.001) between treatments of interest. To characterize broad taxonomic differences between regions and treatments of interest, the core prokaryotic and eukaryotic microbiomes were characterized using the "core" function of the *microbiome* package (Lahti et al., 2017) where core members were defined as ASVs present in at least 75% of samples.

RESULTS

Location accounted for most of the variation in community composition, with region explaining 18.63% and 21.828% and site explaining 4.644% and 6.139% of variance in prokaryotic (16S) and eukaryotic (18S) communities, respectively (PERMANOVA $p < 0.001$, Table 2.1, Fig. 2.2). Differences in prokaryotic communities between regions were driven by an increase in the frequency of Granulosicoccaceae, a family of heterotrophic Gammaproteobacteria, and the cyanobacterial family, Xenococcaceae in the Bodega Marine Reserve (BMR) while Nostocaceae and other Flavobacteria families were more frequent in Corona del Mar (CDM) (differential abundance analysis, $p < 0.001$, Fig. 2.3). While Xenococcaceae and Granulosicoccus were significantly more frequent in the BMR, they were also present in most samples from CDM, but in lower frequencies. As such, both Xenococcaceae and Granulosicoccus were part of the shared core community between regions, along with Rhodobacteraceae, Pirellulaceae, Rhizobiaceae, and Synechococcales, with Rhodobacteraceae being notably more dominant in CDM. Within each region, core members in the BMR included Flavobacteraceae, Pirellulaceae, and Altermonadaceae while those at CDM included Saprospiraceae, Sphingomonadaceae, Nostocaceae, and Phormidesmiaceae (Fig. 2.4, Table 2.2). Eukaryotic core members shared between the regions included, Phaeophyceae, Raphid-Pennate diatoms, Maxillopod, Ulvales, and Monosigidae Group A. CDM's core eukaryotic community also included a variety of other photosynthetic classes in lower average abundance (Fig. 2.4, Table 2.2). Differential abundance analysis revealed that Bacillariophyta, Phaeophyceae, and Ulvophyceae were significantly more frequent in Bodega, while a variety of photosynthetic and heterotrophic Families were more frequent in Corona del Mar (differential abundance analysis, $p < 0.001$, Fig. 2.3)

While grazer and nutrient treatments significantly affected community composition overall (PERMANOVA, $p < 0.05$, Table 2.1), these effects also differed by region (PERMANOVA $p = 0.001$, $p < 0.034$ for grazer × region and nutrient × region, respectively; Table 2.1). Grazer treatment, alone and interactively with region, accounted for 3.7% and 3.5% of the variance in prokaryotic and eukaryotic communities, respectively (Table 2.1, Fig. 2.2a). Grazers had a larger effect on prokaryotic and eukaryotic communities in BMR compared to CDM (Table 2.3), though the difference in prokaryotic communities in BMR was partially due to differences in betadisperson between grazer treatments (betadispersion analysis, $p = 0.003$, Fig. 2.5). Of the most differentially abundant ASVs between grazer treatments, all ASVs but one across a variety of taxa were more frequent in grazer removal pools in BMR while the response varied more in CDM. In CDM, *Glaciecola* and *Algimonas* were more frequent in grazer addition pools. Amongst both regions, ASVs assigned as Rhodobacteraceae were more frequent in grazer removal pools (Fig 5b). The eukaryotic ASVs that differed the most between grazer treatments were largely more frequent in removal pools compared to addition pools in both regions and mostly assigned as autotrophic taxa. Most Stramenopile ASVs, which were largely assigned as a type of diatom, and maxilopod ASVs were more frequent in grazer removal pools. Green algal ASVs (*Ulva*, Ulvales relatives, and Ulotrichales) varied in their directionality of change within both regions. One amoebozoa was more frequent in grazer addition pools, but only in the BMR (Fig. 2.5d).

The effect of nutrient treatment on community composition was larger in BMR than CDM and accounted for less of the variance overall than grazer treatment (Table 2.3). Nutrient treatment, alone and interactively with region and grazer treatment accounted for 2.63% and 2.53% of the variance in prokaryotic and eukaryotic communities (PERMANOVA $p < 0.05$,

Table 2.1, Fig. 2.6). Within each region, nutrients, alone and interactively, accounted for nearly double the variance in prokaryotic community composition in BMR (6.19%) compared for CDM (3.48%, Fig. 2.2a). The varying effect of nutrients by region was stronger in eukaryotic assemblages, with nutrient treatment explaining 7.00% and 2.13% of the variance in BMR and CDM eukaryotic assemblages, respectively. In both regions, the most differential ASVs between nutrient treatments were largely more frequent in ambient nutrient pools compared to pools with experimentally increased nutrient concentrations. All but two of the cyanobacterial ASVs, all Bacteroidota ASVs, and all Proteobacteria ASVs, with the exception of *Glaciecola* at CDM, were more frequent under ambient nutrient conditions (Fig. 2.6). The most differential eukaryotic ASVs in BMR also were largely more frequent in ambient nutrient conditions, but eukaryotic ASVs at CDM varied in their directionality. With the exception of three green algal ASVs, the most differentially abundant ASVs were more frequent in increased nutrient treatments at CDM. These ASVs were assigned to a variety of taxa, with heterotrophs (Alveolata and Maxilopoda) as well as autotrophs increasing in frequency when nutrients were added (Fig. 2.6). The interactive effect of nutrients and grazers, while significant for both prokaryotic and eukaryotic assemblages, explained less than 1% of the variance in either community (Table 2.1).

Within-tidepool level treatments (establishment stage and fencing) significantly affected community composition overall, but this pattern varied when investigated within regions (PERMANOVA, $p < 0.05$, Table 2.2). Overall, establishment stage accounted for 2.16% and 1.76% of the variance in prokaryotic and eukaryotic communities, respectively (Table 2.1, Fig. 2.2a). However, establishment stage only significantly impacted eukaryotic communities from BMR and had no significant impact on prokaryotes. At CDM, establishment stage significantly affected both prokaryotes and eukaryotes, accounting for 3.07% and 3.85% the variance in the

communities, respectively (Table 2.2, Fig. 2.2a). Fencing significantly altered community composition at CDM but not in BMR, explaining similar percentages of variance in prokaryotic and eukaryotic communities (Table 2.2, Fig. 2.2). Although fencing alone altered community structure at CDM, the effect of fencing did not change based on grazer treatment.

To assess if these effects were acting on members that existed in both regions in a similar manner described above, we identified 1,193 shared ASVs of the 19,711 and 10,710 prokaryotic ASVs from CDM and BMR, respectively. These ASVs were largely characterized by Rhodobacteraceae (13% of ASVs), Flavobacteraceae (12%), and Rhizobiales (4.6%). The shared prokaryotic core community (found in 75% of samples) consisted of Rhodobacteraceae and Rhizobiaceeae ASVs (Appendix B: Fig. S1a). Both regions shared 416 eukaryotic ASVs from the 1,532 and 1,608 ASVs from CDM and BMR, respectively. These shared ASVs were largely Metazoa (17%), Ciliophora (17%), Ochrophyta (14%), and Chlorophyta (10%), however the core community was characterized by Ulvales relatives, Sarcinochrysidaceae, Phaeophyceae, Raphid-pennate diatoms, and Monosigidae Group A (Appendix B: Fig. S1b). PERMANOVAs examining the effects of the experimental treatments on only these shared ASVs revealed similar results to the PERMANOVA, with geographic space (region and site) accounting for most of the variation and grazer and nutrient treatments having minor roles in structuring these communities which also differ with region in both prokaryotic and eukaryotic communities ($p < 0.001$, Appendix B: Table S1).

There was no difference in the effect of fencing on alpha diversity metrics between any grazer or nutrient treatment (linear models, $p > 0.05$). Rather the difference in diversity between fenced and unfenced tiles only differed by region amongst naïve communities, with CDM having more positive values for the Shannon-Weiner diversity index, ASV richness, and evenness than

the BMR (linear models, $p < 0.001$, Appendix B: Table S2). Additionally, there were minimal effects of grazers that varied by region and no significant effects of nutrients on alpha diversity. In BMR, the prokaryotic Shannon index was higher in grazer removal pools compared to addition pools (linear model, $p = 0.002$), but there was no difference at CDM. Similarly, there were moderate interactive effects of grazer treatments and region on prokaryotic richness and evenness, with removal pools being slightly more even and rich in BMR while there was no difference at CDM (linear model, $p = 0.002$, $p = 0.04$, respectively for evenness and richness). For all prokaryotic alpha diversity metrics investigated, CDM was more diverse than BMR (linear models, $p < 0.001$). This pattern was only seen in prokaryotic communities and not in eukaryotic communities.

DISCUSSION

Although our analysis revealed that biofilm communities on rocky shores primarily vary spatially (i.e., with site and region), this comparative experiment also indicates that the strength of top-down and bottom-up processes in structuring these communities, though minor relative to spatial effects, differed between northern and southern California. Experimental grazer and nutrient manipulations played a larger role in structuring communities in northern California, whereas more localized processes like establishment stage and fencing played larger roles at our southern California sites. Although grazer treatment explained more of the variation in BMR, it still significantly impacted prokaryotic and eukaryotic assemblages at CDM, indicating that grazers affect these microbial communities despite the differences due to location and other covarying physical factors.

Region overwhelmingly accounted for more of the variance in biofilm community composition compared to our manipulated experimental factors. While these experimental

regions are over 500 miles apart, sites within each region that were essentially adjacent to one another were also compositionally different. Given the strength of these spatial factors in structuring this community, it appears that the well-known top-down and bottom-up up processes that typically play a strong role in structuring macroscopic intertidal communities may be less important in structuring microbial biofilm assemblages. By existing in a biofilm, microorganisms are more protected from external abiotic and biotic stressors and disturbances (Rode, Singh, and Drescher 2020), largely due to the extracellular polymeric substance (EPS) matrix that is formed by the microbes within the biofilm (Flemming, Neu, and Wozniak 2007). The EPS has been shown to increase the resistance of biofilms to chemical influences as well as grazing (DePas et al. 2014; Gill et al. 2022; Pinto et al. 2020), although some molluscan grazers' pedal mucus has been shown to decrease biofilm cover, indicating that there may be chemical components of this mucus that could increase the biofilms' susceptibility to grazing (Arboleda-Baena et al. 2022). Marine biofilms have also been shown to be relatively physiologically resistant to the influence of increased nutrients (Rindi and Benedetti-Cecchi 2023). As the stability of these epilithic microbial communities increases with biofilm formation, it is possible that early successional settlement dynamics that vary across spatial scales play a larger role in the assembly of a community that is then minimally influenced by external top-down and bottom-up factors.

While grazer and nutrient effects had relatively small impacts on these biofilm communities, we saw that these effects had a larger role at our northern California sites compared to those in southern California. Southern California rocky shores are subjected to stressful conditions in the summer, with tide pools experiencing relatively high maximum temperatures and low nutrient waters. However, biofilm development can allow microorganisms to be more resistant to the negative effects of thermal stress and oligotrophic conditions. In

response to these conditions, biofilm members can alter gene expression that increases resistance to environmental stressors which may not translate to compositional shifts (Yin et al. 2019). While the magnitude of these effects varied between regions, grazer abundance significantly altered both prokaryotic and eukaryotic communities across regions and was characterized by similar increases in abundance of eukaryotic photosynthetic taxa when grazers were removed. However, the prokaryotic response to grazer treatments appears to be more consumptive in the BMR while the most differentially abundant taxa at CDM varied in their directionality, indicating that in some cases, increased grazer abundance was facilitative. Interestingly, the ASVs that responded strongly to grazer treatment across regions were largely not photosynthetic, especially at CDM, indicating that heterotrophs, particularly Rhodobacteraceae, were being influenced by our grazer manipulations. It has been shown that grazers can increase photosynthetic microbial abundance by removing canopy and increasing light availability and access to nutrients (Skov et al. 2010). Recent studies have also indicated that grazer gut microbiomes and pedal mucus microbiomes are distinct from the epilithic biofilm communities they exist in (Arboleda-Baena et al. 2022; Panova et al. 2022), indicating that the effects that grazers have on biofilm communities are likely less dispersal related, but more likely impacts from the consumption of the biofilm and the nutrients they excrete.

Similarly to grazer effects, nutrient treatment had a significantly larger effect on microbial communities in northern California. This is contrary to our hypothesis, as we expected added nutrients to have greater effects in southern California, where ambient nutrient concentrations are typically lower, particularly in the summer. This regional difference in the impact of nutrients may be partially related to increased ability of the biofilm to resist change in response to stressors, with CDM potentially experiencing more frequent perturbations like

temperature spikes. Additionally, previous studies have also shown that nutrient limited environments are largely structured from the top-down (Guerry et al. 2009), though periodic increases in nutrient availability can temporarily weaken herbivory (Sellers et al. 2021). Despite these patterns being seen in phototrophic communities, nutrient effects on microbial communities in CDM were stronger when grazer abundance was increased. It is possible that grazers increased turnover allowing for nutrient facilitation of various taxa and proliferation of other heterotrophic organisms (Nielsen and Navarrete 2004; Skov et al. 2010). Surprisingly, of the ASVs that responded strongly to nutrient treatments, few cyanobacteria were more abundant when nutrients were added in both regions, but photosynthetic eukaryotes increased in opposing directions across regions, with most being more abundant under ambient conditions in the BMR while they mostly increased in abundance with nutrient addition at CDM. The increased abundance of photosynthetic organisms in ambient nutrient conditions in the BMR may indicate that nutrient addition treatments acted more as stressors for the community rather than facilitation. Additionally, regional variation in phototroph response could be due to differences in the overall community structures, with CDM prokaryotic communities having less cyanobacteria and largely being comprised by heterotrophic Rhodobacteraceae while the eukaryotic community had less diatoms and more green algae to respond to nutrient additions.

Communities from CDM were also impacted more by localized treatments like fencing and establishment stage, though not interactively with tide-pool-level treatments as expected. CDM may be more influenced by these localized treatments in part due to the differences in productivity between northern and southern California. Northern California has highly productive coastal waters compared to southern California, resulting in increased propagule pressure and planktonic diversity. Thus, early establishment processes may play a larger role in

community assembly at CDM, as assembly history and species pools have larger effects when interspecific interactions are weaker in structuring the community (Cornell and Harrison 2014). The influence of assembly history can be further impacted by regional variation in a variety of factors such as nutrient supply, productivity, and disturbance frequency (Chase 2003; Kardol, Souza, and Classen 2013). Alone, establishment stage significantly impacted both prokaryotic and eukaryotic CDM communities, but only eukaryotic communities in the BMR. This difference in northern California may result in more rapid prokaryotic community assembly compared to eukaryotic communities given differences cell size and, therefore, growth rate (Nielsen 2006; Massana and Logares 2013). Fencing effects were only seen at CDM, but not in conjunction with grazer addition and removal, indicating that fences may not have effectively limited direct grazing access, and/or that the fences impacted another unforeseen process. Biofilm communities are notably sensitive to the material used as substrate, but the importance of the substrate in structuring the community has been shown to lessen as the community matures (Tobias-Hünefeldt et al. 2021). Biofilms are known to form on many different materials, including metals (Tuck et al. 2022), indicating that there may have been a distinct biofilm community on the metal fences that then interacted with the tile community.

Additionally, the compositional differences between experimental treatments did not necessarily translate to differences in diversity, with the exception of fencing increasing both prokaryotic and overall diversity at CDM. As we qualitatively describe southern California as being more stressful, given the increased temperatures and decreased nutrient concentrations, the higher diversity is slightly unexpected as one would expect higher stress to select for a more homogeneous community. However, given the increased stress, we may also see increases in positive interactions—such as biofilm formation—that can reduce abiotic and biotic stressors

(Stachowicz 2001). Additionally, stochastic assembly processes, which we hypothesize are playing a larger role at CDM based on our results, are dominant in higher diversity communities (Xun et al. 2019). Particularly in fenced tiles where direct grazing access should be limited, we potentially see stochastic processes like founder effects and early colonization processes lead to diverse biofilm communities resistant to further change.

Given how distinct the prokaryotic and eukaryotic communities were between CDM and the BMR, characterizing the shared community can give us insight to important members of intertidal biofilms that remain consistent across large spatial scales. As such, we saw similar compositional results in response to our experimental factors between the shared community and the total community, with regional variation in the strength of top-down and bottom-up processes, indicating that the patterns driving differences between regions were not only because the microbes at each location were largely different. Moreover, the shared prokaryotic community was largely heterotrophic and consisted mainly of members of the Rhodobacteraceae, which are known to play important roles in the initial formation of marine biofilms and secrete various secondary metabolites known for their diverse functions in marine ecosystems (Elifantz et al. 2013; Kviatkovski and Minz 2015; Henriksen et al. 2022). While characterization of rocky biofilm communities can begin to provide us with insight into the roles of these biofilms in the context of the macroscopic community, more work should be done to assess how these communities vary in their functionality.

Our results indicate that although top-down and bottom-up processes are critical to structuring macrobiota communities, these processes play a weaker role in microbial assembly along rocky shores compared to spatial separation, with dissimilarity increasing with distance. Although distance, and potentially associated factors that vary across physical space, accounted

for most of the variance in these communities, the strength of top-down and bottom-up processes varied by region, with grazers and nutrients having stronger impacts in northern California. Meanwhile, more localized processes played a stronger role in community structure in southern California. The magnitude and balance of factors influencing macrobiotic community assembly are known to vary with various abiotic and oceanographic factors (Menge et al., 2002), and although the strength of these processes was weak amongst microbial communities, regional differences still further influenced the impacts of top-down and bottom-up factors. Our results highlight how unique aspects of microbial communities may mediate the impacts of well-studied community dynamics, and that evaluating macroecological concepts and theories in microbial communities can provide insights into the generality of these processes across scales.

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TABLES

Table 2.1: Percent variance of community composition explained by significant experimental factors based on PERMANOVA results.

	Variance explained (%)	
Effect	***Prokaryotes***	***Eukaryotes***
Region	18.628	21.828
Site	4.644	6.139
Grazers	2.056	1.868
Nutrients	0.869	0.718
Region × Grazers	1.740	1.720
Region × Nutrients	0.831	0.841
Grazers × Nutrients	0.976	1.005
Fencing	0.993	0.922
Stage	2.202	1.784
Residuals	67.060	63.727

Table 2.2: Average relative abundance of core ASVs agglomerated to the Family taxonomic level (or the closest available taxonomic level) across all samples.

Family	**Average relative abundance (± SE)**
Prokaryotes	
Corona del Mar	
Rhodobacteraceae	45.73 ± 1.86
Saprospiraceae	20.47 ± 2.10
Xenococcaceae	9.50 ± 1.37
Synechococcales Incertae Sedis	8.50 ± 1.43
Rhizobiaceae	4.59 ± 0.42
Sphingomonadaceae	4.21 ± 0.36
Nostocaceae	3.70 ± 0.51
Phormidesmiaceae	1.79 ± 0.32
Bodega Marine Reserve	
Rhodobacteraceae	28.66 ± 1.94
Xenococcaceae	24.15 ± 2.09
Flavobacteriaceae	14.15 ± 1.15
Granulosicoccaceae	11.13 ± 1.27
Synechococcales Incertae Sedis	10.37 ± 1.37
Rhizobiaceae	4.94 ± 0.42
Pirellulaceae	4.34 ± 0.68
Alteromonadaceae	2.26 ± 0.40

Eukaryotes	
Corona del Mar	
Maxillopoda	34.82 ± 2.74
Ulvales-relatives	33.98 ± 2.71
Sarcinochrysidaceae	12.70 ± 1.76
Cladophoralea	8.05 ± 1.38
Phaeophyceae	7.20 ± 1.92
Raphid-pennate	1.00 ± 0.39
Chlorarachnida	0.87 ± 0.11
Monosigidae GroupA	0.59 ± 0.09
Rhizochromulinales	0.47 ± 0.07
Neobodonid	0.25 ± 0.05
Rhodomelaceae	0.08 ± 0.01
Bodega Marine Reserve	
Ulvales-relatives	33.51 ± 2.71
Maxillopoda	25.21 ± 3.16
Raphid-pennate	18.92 ± 1.81
Phaeophyceae	15.78 ± 2.06
Monosigidae Group A	3.99 ± 1.30
Malacostraca	2.60 ± 0.70

Table 2.3: Percent variance of community composition explained by significant experimental factors based of PERMANOVA results within each region. Asterisks indicate that there was a significant interactive effect of region with the indicated factor. based on previous analysis.

	% variance explained			
	Prokaryotes		***Eukaryotes***	
Effect	***BMR***	***CDM***	***BMR***	***CDM***
Site	5.147	6.018	11.780	5.831
Grazers*	6.193	4.081	6.214	3.967
Nutrients*	3.463	1.595	4.272	*NS*
Grazer × nutrients	3.127	1.986	2.958	2.234
Stage	*NS*	5.136	3.174	4.035
Fencing	*NS*	2.461	*NS*	2.167
Grazer × stage	*NS*	1.647	*NS*	*NS*

FIGURES

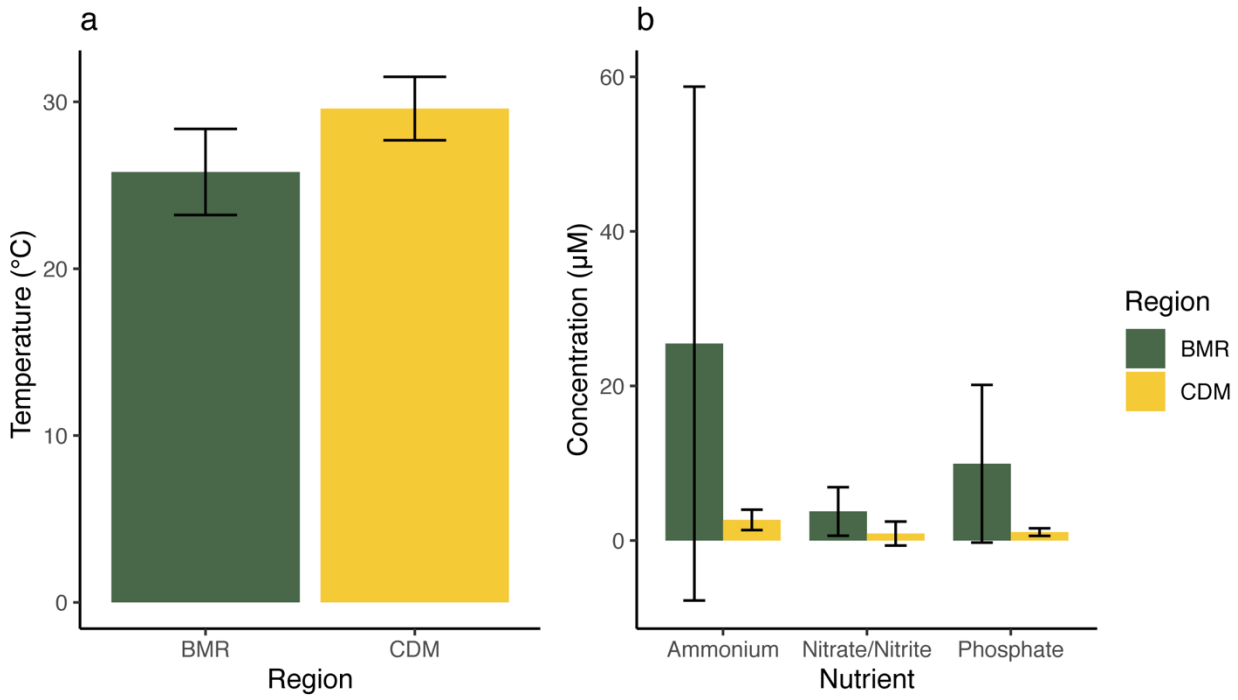

Figure 2.1: Differences in the ambient (a) temperature of tide pools (n = 16, 31 for BMR and CDM, respectively) and (b) nutrient concentrations of tide pools (n = 32 for each region) between experimental regions.

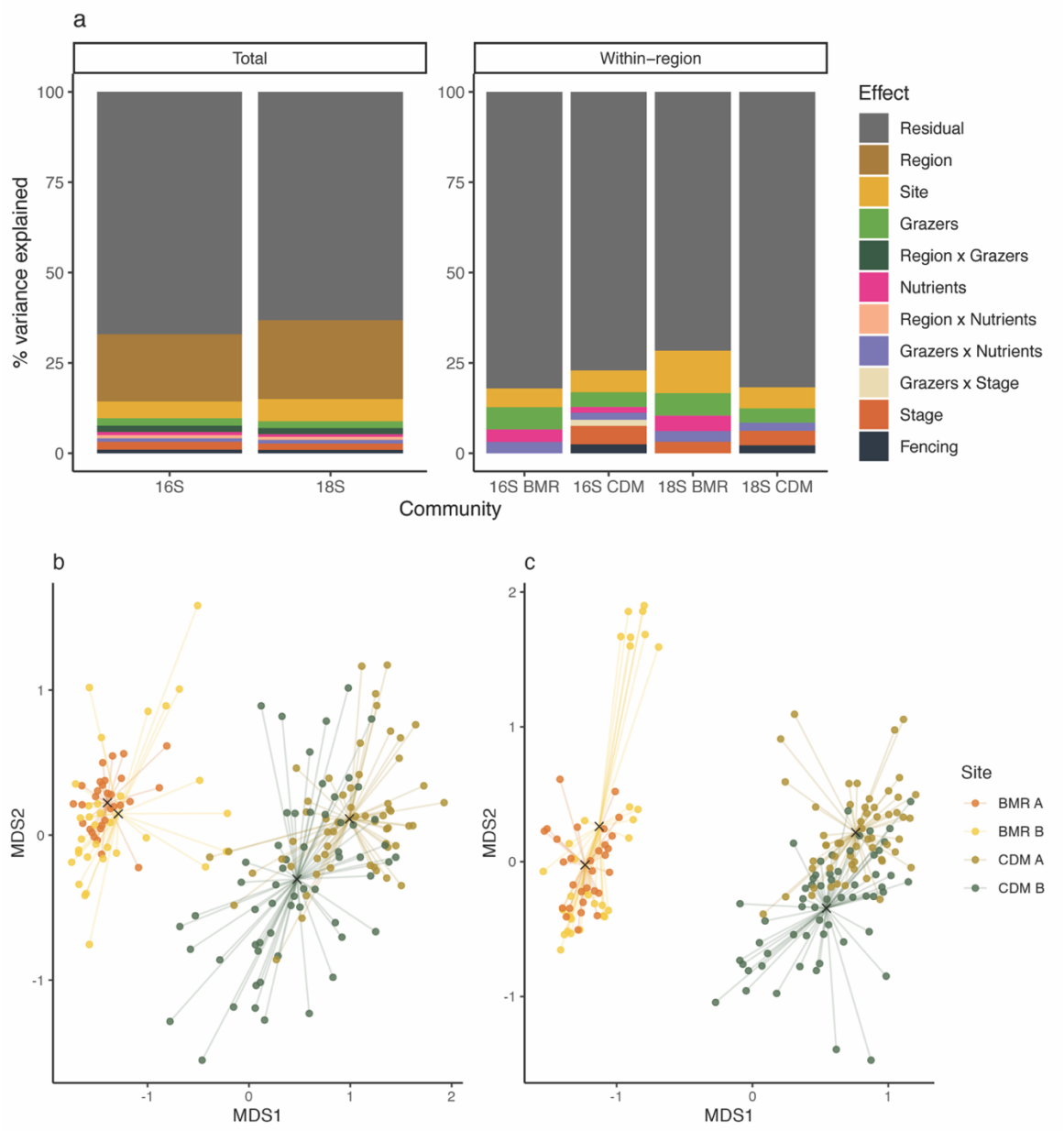

Figure 2.2: Significant factors affecting biofilm microbial communities. (a) Percent variance explained my significant factors influencing prokaryotic (16S) and eukaryotic (18S) communities based on PERMANOVAs on the total dataset and within each region. (b-c) NMDS plots showing differences in (b) prokaryotic and (c) eukaryotic communities by sites within each region. Centroids are marked with a black ‘x’.

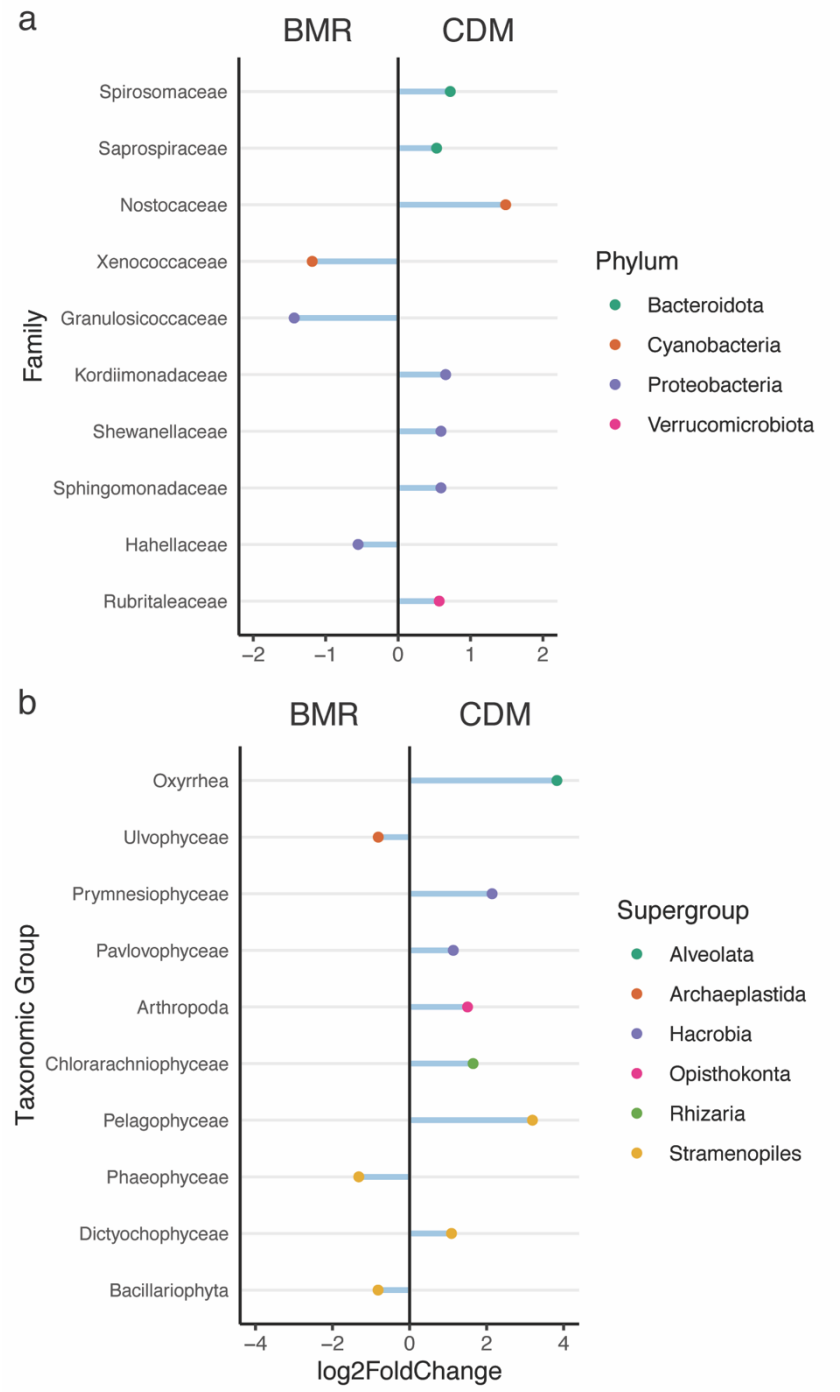

Figure 2.3: The most differentially abundant (a) prokaryotic Families and (b) eukaryotic Families or next closest taxonomic group. Points to the left of the middle axis are were significantly more abundant in the BMR while points to the right were significantly more abundant in CDM.

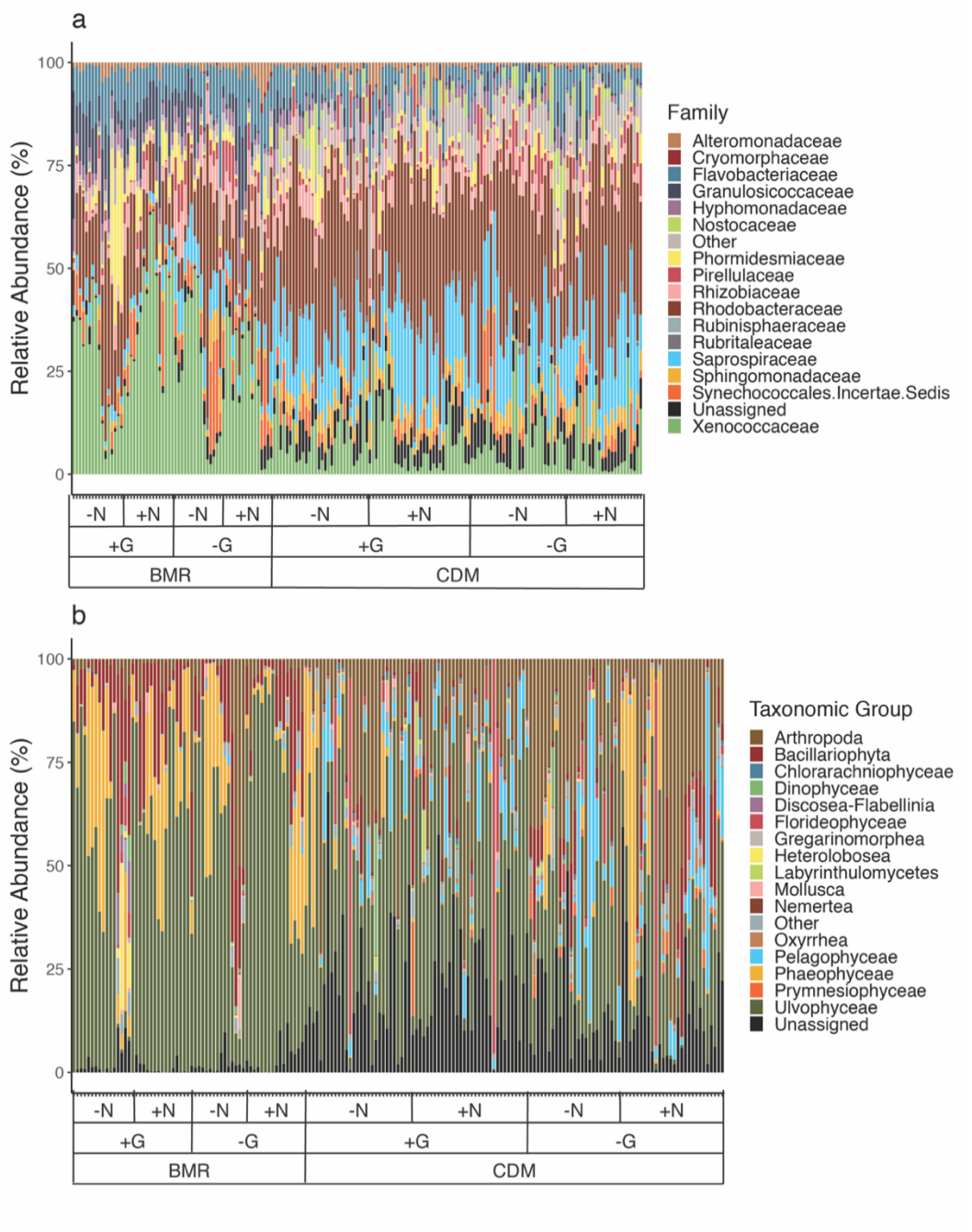

Figure 2.4: Relative abundance of (a) prokaryotic and (b) eukaryotic microbial communities agglomerated to the Family or next closest taxonomic level. Each bar is a single sample with sample ordered and bracketed by region (BMR or CDM), grazer removal (-G) or addition (+G), and nutrient addition (+N) or ambient nutrient conditions (-N)

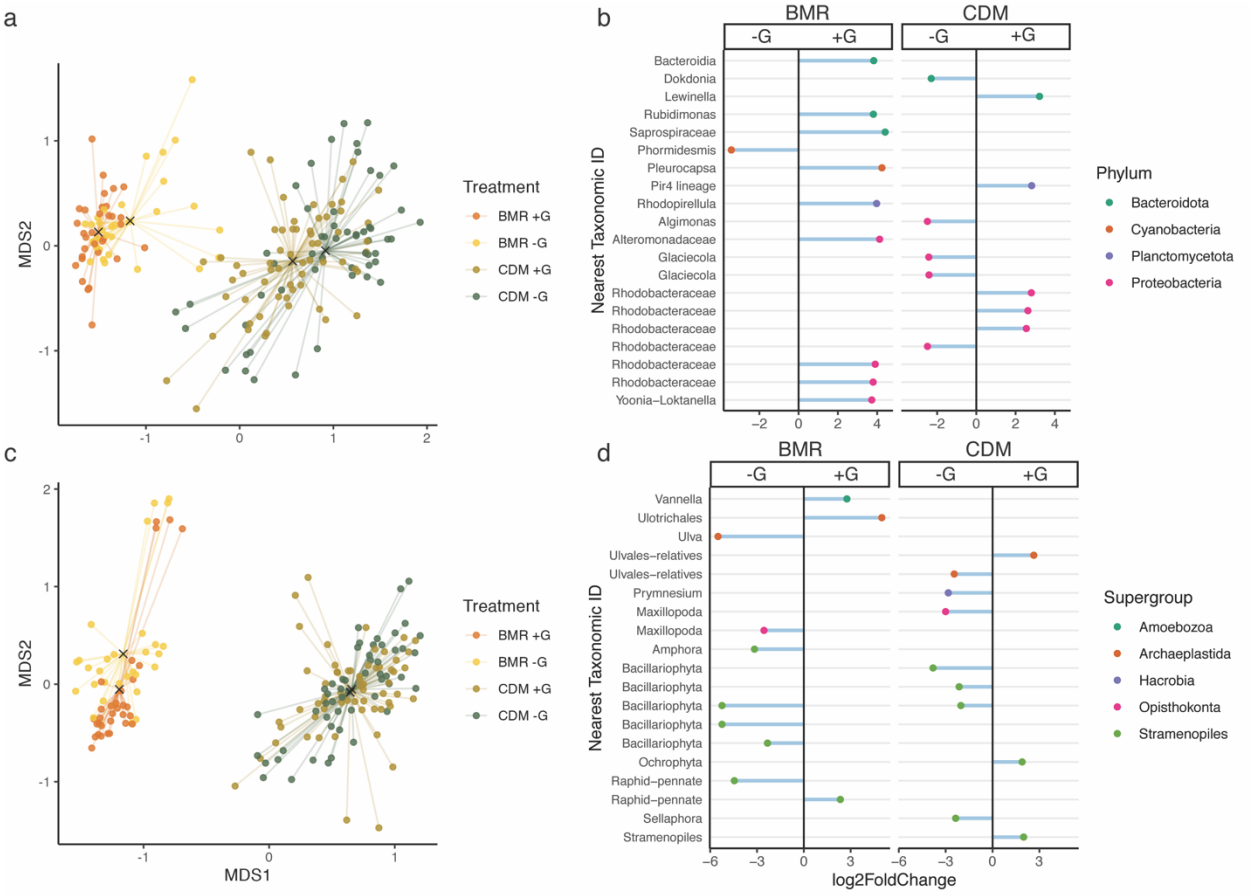

Figure 2.5: Regional variation in the effects of grazers on biofilm (a-b) prokaryotic and (c-d) eukaryotic community composition. (a & c) NMDS plots showing the difference in community composition between grazer addition (+G) and removal (-G) across different regions. Each point is a single sample with centroids marked by a black 'x.' (b & d) ASVs assigned to their nearest taxonomic ID that were the most differentially abundant between grazer treatments within each region. Negative log2FoldChange values were significantly more abundant when grazers were removed while positive values were more abundant when grazers were added.

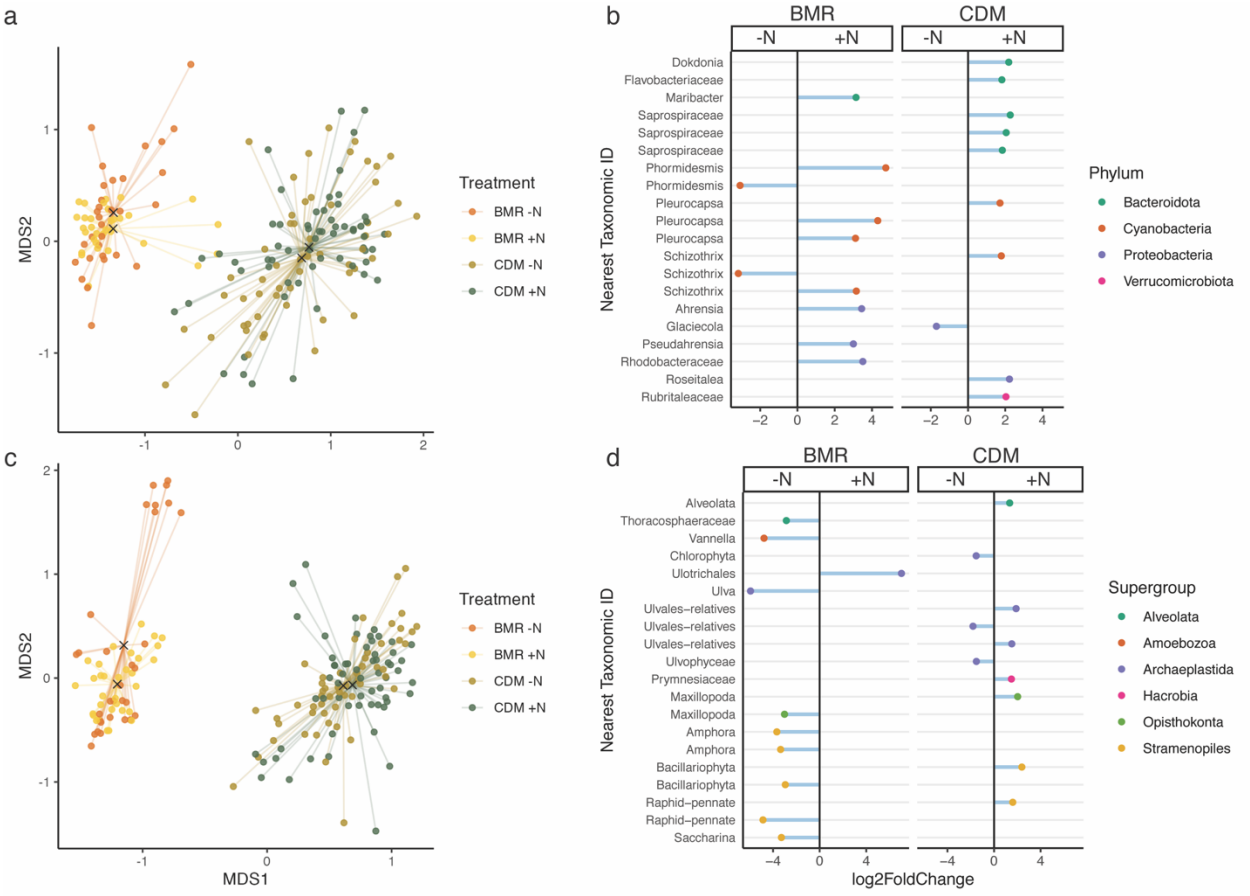

Figure 2.6: Regional variation in the effects of nutrients on biofilm (a-b) prokaryotic and (c-d) eukaryotic community composition. (a & c) NMDS plots showing the difference in community composition between nutrient addition (+N) and ambient nutrient concentrations (-N) across different regions. Each point is a single sample with centroids marked by a black 'x.' (b & d) ASVs assigned to their nearest taxonomic ID that were the most differentially abundant between nutrient treatments within each region. Negative log2FoldChange values were significantly more abundant when nutrient concentrations were not manipulated while positive values were more abundant when nutrients were added.

CHAPTER 3

Kelps may compensate for low nitrate availability by using regenerated forms of nitrogen, including urea and ammonium

ABSTRACT

Nitrate, the form of nitrogen often associated with kelp growth, is typically low in summer during periods of high macroalgal growth. More ephemeral, regenerated forms, such as ammonium and urea, are much less studied as sources of nitrogen for kelp, despite the relatively high concentrations found in the Southern California Bight. To assess how nitrogen uptake by kelps varies by species and nitrogen form in southern California, USA, we measured uptake rates of nitrate, ammonium, and urea by *Macrocystis pyrifera* and *Eisenia arborea* individuals from 4 regions characterized by differences in nitrogen availability, Orange County, San Pedro, eastern Santa Catalina Island, and western Santa Catalina Island, during the summers of 2021 and 2022. Seawater samples collected at each location showed that overall nitrogen availability was low, but ammonium and urea were often more abundant than nitrate. We also quantified the internal % nitrogen of each kelp blade collected, which was positively associated with ambient environmental nitrogen concentrations at the time of collection. We found that both kelp species readily took up nitrate, ammonium, and urea, with *Macrocystis* taking up nitrate and ammonium more efficiently than *Eisenia*. Urea uptake efficiency for both species increased as internal percent nitrogen decreased. Our results indicate that lesser studied, more ephemeral forms of nitrogen can readily be taken up by these kelps, with possible upregulation of urea uptake as nitrogen availability declines.

INTRODUCTION

Kelps provide vital ecosystem services globally, including enhancing primary production (Mann 1973), increasing biodiversity (Graham 2004), regulating water flow and coastal erosion (Gaylord et al. 2012), and supporting fisheries (Bertocci et al. 2015). As the climate changes, kelp populations are facing increases in multiple stressors, resulting in declines around the world (Krumhansl et al. 2016; Smale et al. 2019). In southern California, kelp populations have experienced long-term declines (Tegner et al. 1996), with recent studies highlighting associated large structural and functional changes in kelp forest communities (Beas-Luna et al. 2020). Amidst declining kelp populations and subsequent shifts in community dynamics, understanding factors influencing kelp growth and productivity are essential for managing and restoring these habitats.

Kelp declines in southern California have been linked, in part, to increasing temperatures (Beas-Luna et al. 2020), and increased water temperatures are often associated with decreased nutrients (Zimmerman and Kremer 1984; Reed et al. 2016). Nutrient supply is a major factor affecting algal growth and productivity, and nitrogen is typically the most common nutrient associated with macroalgal growth. Work on nutrients affecting kelp growth has typically focused on nitrate (Gerard 1982a; Sánchez-Barredo, Ladah, and Zertuche-González 2011; Wheeler and North 1981), which is brought into shallow coastal waters through upwelling and seasonal overturn, but evidence is emerging of the importance of other, regenerated forms, including ammonium and urea, as nitrogen sources for kelp. Ammonium can be readily taken up and assimilated by *Macrocystis pyrifera* (Haines and Wheeler 1978) and can sustain *Macrocystis* growth when nitrate concentrations are low (Brzezinski et al. 2013). Emerging research also indicates that *Macrocystis* can take up urea during periods of low nitrogen availability (Smith et

al. 2018; 2021). The use of urea as a source of nitrogen for macroalgae is of particular interest, not only due to its year-round availability due to local-scale inputs from marine consumers (Regnault 1987) and its contribution to coastal nitrogen loading (Kudela, Lane, and Cochlan 2008), but also energetically. As an uncharged molecule, it has the potential to be a low cost, high reward source of nitrogen, providing two atoms of N per molecule through diffusion, although its uptake in macroalgae has been shown to follow saturating kinetics, indicating active transport (Phillips and Hurd 2004).

In southern California, nitrate concentrations are low during summer months when upwelling is infrequent, but *Macrocystis* maintains growth, with natural population growth rates saturating at relatively low nitrate concentrations (Wheeler and North 1981; Zimmerman and Kremer 1984). While nitrate availability in southern California varies seasonally, oceanographic time series have shown that nitrate concentrations have significantly declined over multiple years, while ammonium concentrations have increased (Martiny et al. 2016). With chronically low nitrate concentrations in the summer and declining nitrate concentrations in coastal waters overall, there remains a clear gap in our understanding of how kelps use alternative forms of nitrogen and their potential to mitigate stress associated with low nitrogen availability. Previous studies focused on nitrate uptake and assimilation, assuming that concentrations of ammonium and other regenerated forms of nitrogen were too low and/or ephemeral to substantially affect kelp growth in southern California (Wheeler and North 1981). However, in addition to relatively high concentrations of ammonium and organic nitrogen associated with human activity along southern California's highly urbanized coastline (Howard et al. 2014), high concentrations of these alternative, regenerated nitrogen forms can occur due to pulses from consumer waste and

sediment efflux in close proximity to kelp beds (Bray, Purcell, and Miller 1986; Bray et al. 1988).

Macrocystis pyrifera (Linnaeus) C. Agardh (hereafter, *Macrocystis*) and *Eisenia arborea* J.E. Areschoug (hereafter, *Eisenia*) are southern California kelp species that frequently occur along the same shorelines but occupy separate niches. *Eisenia* typically inhabits the low intertidal and shallow subtidal zones, whereas *Macrocystis* is found at greater depths of up to 25 meters (Graham et al. 2010). While nutrient uptake, storage, and limitation in *Macrocystis* have been investigated for decades, *Eisenia*'s nutrient dynamics remain understudied, despite its ability to survive in warm, nutrient-poor waters (Hernández-Carmona, Robledo, and Serviere-Zaragoza 2001; Matson and Edwards 2006), conditions that can induce stress in other kelp species. Although *Eisenia* populations persist in stressful conditions, studies of its nitrogen use and storage have focused on nitrate (Sánchez-Barredo, Ladah, and Zertuche-González 2011), and *Eisenia*'s ability to take up and assimilate regenerated forms of nitrogen remains unknown. It has been hypothesized that *Macrocystis* can tolerate nutrient-poor surface waters in part because individuals grow from the bottom to the surface, potentially spanning the thermocline/nutricline, though nutrient input from episodic thermocline motion has not been shown to sustain maximal growth (Gerard 1982b; Zimmerman and Kremer 1984). In contrast, *Eisenia* does not have access to a vertical gradient in nutrient availability, as it typically lives in well-mixed shallow waters. Given *Eisenia*'s proximity to the shore, it could also encounter pulses of regenerated nitrogen forms (i.e., ammonium, urea) from freshwater runoff and consumer waste.

In this study, we seek to understand the importance of regenerated nitrogen for *Macrocystis* and *Eisenia* in southern California during low nutrient conditions by measuring the uptake rates of different nitrogen (N) forms by answering the following questions: (1) Does

uptake efficiency of nitrate, ammonium, and urea by *Macrocystis* and *Eisenia* change with N availability? And (2) What forms of N are contributing the most to the nitrogen taken up by each species across various sites in southern California? We predict that uptake efficiency of regenerated forms of N will increase as N availability declines. We predict that this pattern may be especially apparent for urea, as it may be more energetically costly to assimilate. We also predict that more urbanized, mainland sites will be characterized by higher concentrations of urea and ammonium, given their exposure to wastewater outfalls and freshwater runoff, and regenerated N forms will be more important for kelps' N uptake and budgets at those locations.

MATERIALS AND METHODS

Collection sites and methods

We collected *Macrocystis pyrifera* and *Eisenia arborea* blades from four regions in southern California, USA: Orange County (Shaw's Cove: 33°32.73'N, 117°47.89'W), San Pedro (White Point: 33°42.87'N, 118°18.85'W; Point Fermin: 33°42.36'N, 118°17.33'W), east Santa Catalina Island (Big Fisherman's Cove: 33°26.70'N, 118°29.05'W; Bird Rock: 33°27.06'N; 118°29.23'W), and west Santa Catalina Island (Little Harbor: 33°23.12'N, 118°28.50'W). All collections were conducted per State of California Department of Fish and Wildlife Scientific Collecting Permit S-190310011-20037-001. Catalina Island collections and experiments took place in June and July of 2021, while mainland collections and experiments took place in June, July, and August of 2022. In San Pedro and east Catalina, *Macrocystis* and *Eisenia* did not coexist at the same site during sampling events and were therefore collected from separate sites as close as possible to each other within the region (Fig. 3.1). We collected six blades from 4 individuals of each species of kelp in each region. Blades collected from *Macrocystis* were randomly collected from the top 3 m of adult plants. These collections were repeated once more

at each site, for a total of two collection events per site, with the exception of White Point, which could only be sampled once. Blades were cut from the stipe, stored in individual mesh bags, and placed into a cooler with seawater from the collection site for transport to the laboratory. Each blade was then tagged with an individual blade ID and weighed before being placed into a flowing seawater system to recover from wounding for at least 24 hours. Blades were kept in flowing seawater prior to use in nitrogen (N) uptake trials and subject to 12 hour light cycles using 2 Luxx Clone LED Lights (Hawthorne Gardening, Vancouver, Washington, USA) in Catalina trials and 4 T5 10,000K high-output fluorescent lamps in mainland trials. Blades were kept in laboratory seawater systems for no longer than 60 hours prior to uptake trials.

Ambient nitrogen (N) availability and internal N content

To quantify ambient N availability, 500 mL of seawater was collected during each kelp collection at each site, kept on ice for transport, and filtered within 2 hours of collection. Filtered seawater samples were aliquoted into 50 mL tubes and frozen for later nitrate (NO_3^-) , ammonium (NH_4^+), and urea quantification. Nitrate concentrations were quantified with a QuickChem FIA 8500 autoanalyzer (Lachat Instruments, Loveland, Colorado, USA; detection limit: 0.014 μmol L^{-1} NO_3^-). NH_4^+ and urea concentrations were quantified spectrophotometrically using methods adapted from Solórzano (1969) and Goeyens et al. (1998), respectively.

To quantify internal N concentrations, all kelp blades were immediately frozen after uptake trials were completed. Blades were then dried in an oven at 65 °C to constant mass, ground to a fine powder using a mixer mill, and analyzed for %N (Thermo Flash 2000 Elemental Analyzer, CE Elantech, Inc., Lakewood, New Jersey, USA).

Nitrogen (N) uptake experiments

To measure the form-specific N uptake rates of *Macrocystis* and *Eisenia* in different regions of southern California, N uptake was measured at four different initial concentrations of either nitrate, ammonium, or urea. N uptake of kelps was measured over 1 hour by placing blades into 8 chambers containing artificial seawater with target concentrations of 2, 10, 20, and 30 μM of the nitrogen form, adapting methods from Bracken et al. (2011) and Benes & Bracken (2016). Two blades from each kelp individual were used in each trial. During the trials, high water flow was maintained by using magnetic stir bars and stir plates, which provided sufficient velocities to maximize uptake (Hurd, Harrison, and Druehl 1996). Chambers were kept at ~15 °C by placing them in a circulating chilled water bath. All trials took place outside in natural sunlight (>1000 μmol photons m^{-2} s^{-1}). In 2021, 1 L chambers were used, but these were replaced with 3 L chambers in 2022 to increase the volume and amount of N in chambers. There were no differences in uptake efficiency associated with chamber volume based on uptake trials performed using blades collected from the same individuals at Shaw's Cove (linear model: initial concentration x method interaction: $p > 0.05$, Appendix C: Fig. S1; see analytical details below). Kelp blades were rinsed with deionized water to remove epiphytes and residual N and placed into chambers. The target form of N was then added to each chamber to achieve the desired initial N concentrations. After a one-minute mixing period, water (10 mL for ammonium and nitrate; 35 mL for urea) was collected from each chamber every 15 minutes for two hours (n = 9 samples per chamber) in 2021 and one hour (n = 5 samples per chamber) for 2022. The duration of the trials was shortened after 2021, because our data from the earlier trials revealed that uptake remained relatively linear for 60 minutes, allowing accurate calculation of uptake rates over that time interval. Therefore, no time points after 60 minutes were used in further analyses. Water samples from Catalina Island were immediately frozen for later analysis, while samples

from the mainland were immediately analyzed due to laboratory proximity. N concentrations of each sample were quantified using the methods described above for environmental N concentrations.

The rate of uptake (μmol N h^{-1}) for each chamber was found by calculating the change in amount of N (μmol) from the initial sample to the 60-minute timepoint and dividing by time (h). The rate of uptake was divided by the dry mass of the kelp blade in the respective chamber to assess biomass-specific uptake rates (μmol N h^{-1} g^{-1}). The relationship between dry-mass-specific N uptake rate (μmol N h^{-1} g^{-1}) and initial concentration (μM N) was fit to a linear model to assess the efficiency of uptake. Uptake model fits (linear models versus Michaelis-Menten models) were compared for each site, kelp species, and N form to account for variation by site and species. There were significant linear relationships between mass-specific uptake rates and initial N concentrations ($p < 0.05$), while few Michaelis-Menten models were significant. When both linear and Michaelis-Menten models were significant, AIC values of each model were compared, and the model with the lower AIC was considered a better fit. Linear uptake models provided better fits for these relationships compared to Michaelis-Menten models of uptake kinetics in every case, as saturating rates of N uptake were not reached despite experimental concentrations of N far exceeding environmental concentrations.

Data analyses

Micromolar urea concentrations were multiplied by two to calculate the nitrogen (N) concentration from urea, as urea contains two amino groups ($CO[NH_2]_2$). To determine if some forms of N contributed more than others to the total observed N availability, the percentage of the total environmental N from ammonium, urea, and nitrate was calculated for each sampling event to assess whether N availability varied by form and site, alone and interactively.

Differences in environmental N concentrations between sites and regions were assessed with one-way ANOVAs examining N concentrations ($[NO_3^-]$ + $[NH_4^+]$ + [N from urea]), $[NO_3^-]$, $[NH_4^+]$, and [N from urea] from each site. To assess if internal %N was associated with environmental nitrogen concentrations, separate linear models were fit on a log-log scale for each form of N independently, as well as for total environmental N. These analyses evaluated whether environmental N concentrations were associated with the average internal %N for each kelp from a collection event. As we had greater sampling depth for blade internal %N, internal %N was used as a proxy for each blade's recent N history in subsequent models.

To assess whether kelp species and nitrogen availability at the time of collection impacted the efficiency of N uptake (slope of the relationship between initial N concentrations and N uptake rates), general linear models were fit examining the main and interactive effects of initial N concentration, species, and internal %N on N uptake rate for each form of N studied. Kelp species and %N were added into the linear models sequentially and tested against the simple linear regression between uptake rate and initial N concentration to find the most parsimonious model for each form of N. Differences between methods used in 2021 and 2022 were investigated within each species using a similar model structure looking for significant effects of method on the slope between N uptake rates and initial N concentration. To compare each species' expected rates of uptake for each form of N across sites, we calculated the rate of uptake at the average observed environmental N concentration based on the site- and species-specific linear model between uptake rate and initial N concentration for each N form. If the linear model included an intercept different from zero, an intercept of zero was forced, as there can be no N uptake when N concentration is zero. These expected uptake rates for each form of N were then pooled to assess which form of N contributed the most to overall N uptake. To

assess if one form contributed more to the overall N uptake of the kelps studied, we ran a two-way ANOVA testing if the calculated uptake rates varied by N form and kelp species.

RESULTS

There were significant positive linear relationships between N uptake rate and initial N concentration for all forms of nitrogen studied (linear regression, $p < 0.001$, $R^2 > 0.15$), and both *Macrocystis* and *Eisenia* took up all forms of N studied. The most parsimonious model examining N uptake from urea as a function of initial N concentration included the interactive effect of internal %N ($p < 0.001$, $R^2 = 0.268$), but not kelp species. Urea uptake efficiency (i.e., the slope of the relationship between initial concentration and uptake) increased as total internal percent N decreased ($p = 0.008$, Fig. 3.2). This pattern was consistent overall (i.e., there was no 'species × initial concentration' interaction), but it was primarily associated with *Macrocystis* rather than *Eisenia* based on within-species patterns. There was no difference in the efficiency of urea uptake between *Macrocystis* and *Eisenia*. The most parsimonious models assessing the relationships between species, internal %N, and uptake efficiency of ammonium and nitrate included species as an interactive effect (i.e., uptake efficiency differed between species: $p < 0.001$, $R^2 = 0.59$; $p < 0.001$, $R^2 = 0.54$, respectively for ammonium and nitrate), but not internal %N ($p = 0.880$, $R^2 = 0.60$; $p = 0.479$, $R^2 = 0.$ 0.42, respectively for ammonium and nitrate). Ammonium and nitrate uptake efficiency differed between kelp species ($p < 0.001$); *Macrocystis* took up both forms of N more efficiently than *Eisenia* (Fig. 3.3). There was a significant positive, saturating (linear on a log-log plot) relationship between internal %N and total environmental N concentrations ($p = 0.013$, $R^2 = 0.322$, Appendix C: Fig. S2). We found positive, saturating relationships between %N and concentrations of urea ($p= 0.027$, $R^2=0.254$) and ammonium ($p= 0.002$, $R^2=0.475$), but not concentrations of nitrate ($p=0.642$).

Total environmental nitrogen concentrations at collection sites averaged 5.14 ± 0.702 μM N and ranged from 1.14 μM N to 12.64 μM N. Overall, ammonium contributed more to the total observed N availability compared to urea and nitrate, regardless of site ($p < 0.012$, Fig. 3.1b). There was a significant difference in total environmental N concentration between regions (ANOVA, $F_{3,11}= 3.74$, $p = 0.045$), driven by higher total N concentrations in Orange County (7.63 ± 1.69 μM N, n=4) and San Pedro (6.35 ± 0.503 μM N, n=3) compared to east (3.54 ± 0.826 μM N, n=4) and west Catalina Island (1.58 ± 0.789 μM N). When environmental N concentrations were examined within each N form, we found significant differences between regions in urea concentrations (ANOVA, $F_{3,11}= 3.69$, $p = 0.047$), with San Pedro (2.53 ± 0.788 μM N from urea, n=3) having higher urea concentrations than west Catalina (0.859 ± 0.216 μM N from urea, n=4). There were marginal differences in ammonium concentrations (ANOVA, $F_{3,11}= 3.206$, $p = 0.067$), with Orange County having the highest observed concentrations of ammonium (4.23 ± 0.875 μM NH_4^+, n=4). There were no significant differences between nitrate concentrations of our study regions (mean = 1.15 ± 0.318 NO_3^-, n=15) with all samples being less than 2 μM NO_3^- except one sample from Orange County with 4.95 μM NO_3^-.

Based on the calculated rate of uptake by kelps at each site associated with observed environmental N concentrations, ammonium was taken up more than nitrate and urea (ANOVA, $F_{2,18}= 5.58$, $p=0.013$, Fig. 3.4), with no significant differences between kelp species. East and west Catalina *Macrocystis* and east Catalina *Eisenia* took up urea more than nitrate, whereas kelps from other sites took up nitrate more, though the difference in the calculated uptake rates of N from urea and nitrate were frequently very small (Fig. 3.4).

DISCUSSION

We had predicted that kelps' use of regenerated forms of nitrogen (N) – ammonium and urea – would increase as N availability declined. This prediction was supported, as urea uptake efficiency increased as internal nitrogen content decreased for both species of kelp, with no differences in uptake between species. This apparent upregulation of N uptake with decreasing N availability was not seen for ammonium or nitrate, but *Macrocystis* took up both ammonium and nitrate more efficiently than *Eisenia*, a pattern not seen in urea trials. Based on calculated uptake rates and observed environmental N concentrations, regenerated forms of N, like ammonium and urea, contribute substantially to the N taken up by kelps in southern California during seasonally low N conditions. Moreover, our results suggest that these kelps increase urea uptake as overall N concentrations decline.

Decreased N availability, as approximated by kelp blade internal percent N, was associated with increased efficiency of urea uptake, indicating possible upregulation of urea uptake as N stress increases. The role of urea and the regulation of its uptake and breakdown has been well-studied in phytoplankton and bacteria but remains broadly understudied in macroalgal lineages. Eukaryotic phytoplankton, particularly diatoms, reared in low N conditions have shown decreased urea uptake rates when ammonium and nitrate are more available (Lund 1987; Lomas 2004; Solomon et al. 2010). Similarly, increased ammonium availability has been related to decreased urea uptake in green and red macroalgae (Tyler, McGlathery, and Macko 2005; Ross et al. 2018), highlighting ammonium availability as a potential environmental cue that regulates urea uptake across eukaryotic algae. We used internal percent N as a measure of recent nitrogen history, but also found a significant positive relationship between percent N and water-column

ammonium concentrations at the time of collection, highlighting the potential for reduction of urea uptake efficiency associated with higher water-column ammonium concentrations.

While potential mechanisms of urea uptake regulation remain speculative in macroalgae, the main mechanism of urea uptake regulation in phytoplankton is modification of transporter activity, where ammonium represses urea active transport (Berg et al. 2008; Solomon et al. 2010). There is also evidence that seaweed-associated bacteria also contribute to urea use in macroalgae, with antibiotic treated algae exhibiting substantially reduced urea-degrading enzyme activity (Bekheet and Syrett 1977). The observed reduction in the uptake efficiency of urea with increasing nitrogen availability may be associated with regulation of uptake by the kelp but also by kelp-associated microbes. While much of the classic macroalgal nutrient dynamic literature neglects the contribution of kelp-associated microbes to kelps' physiological processes, this field of research is rapidly expanding.

Macroalgal internal N content mainly consists of stored N reserves and amino acids and varies with nutrient conditions recently experienced by the algae (Wheeler and North 1981; Lyngby 1990). In *Macrocystis*, %N declines when individuals are exposed to low N environments, as they deplete N reserves to maintain growth. The internal N concentrations seen in those low-N individuals were similar to those observed in our study (Gerard 1982a). Likewise, the %N values we measured in field-collected *Eisenia* were consistent with those experimentally starved of nitrate (Sánchez-Barredo, Ladah, and Zertuche-González 2011). Although we saw significant positive relationships between ambient N availability and internal %N, blades from certain locations exhibited higher %N than expected given the low N concentrations observed, particularly *Macrocystis* collected from east Catalina Island (Appendix C: Fig. S2e). Blades present near the top of a mature frond are actively growing and may therefore have increased

%N values due to increased protein synthesis (Gerard 1982a). As the blades used in this study were collected from this portion of the frond, it is possible that a recent pulse of N availability led to an increase in N reserves and growth rates, leading to increased internal %N. However, these blades also exhibited some of the highest rates of urea uptake, supporting the idea that mechanisms of urea uptake regulation are more likely associated with ambient ammonium concentrations rather than internal N content.

There were also differences between the ammonium and nitrate uptake rates of *Macrocystis* and *Eisenia*, with *Macrocystis* exhibiting more efficient uptake of these forms. Morphologically, *Macrocystis* blades are thinner compared to the thick, leathery blades of *Eisenia*, likely related to increased wave exposure on the shallow, subtidal, moderately wave exposed shores where *Eisenia* occurs (Roberson and Coyer 2004). This difference in blade morphology likely translates to higher surface-area-to-volume ratios in *Macrocystis* blades, enhancing uptake rates.

While urea uptake rates were relatively high, observed environmental concentrations of urea may limit the uptake of urea at our study locations, particularly in comparison to ammonium along the California coast. Ammonium contributed the most to our estimates of total N uptake, especially along the mainland; urea contributed relatively similar amounts of N to ammonium at Catalina sites. However, regenerated forms of nitrogen (ammonium and urea) have increased relative to nitrate along the highly urbanized southern California coast (Howard et al. 2014; Martiny et al. 2016), and kelp populations exposed to more freshwater runoff may experience even higher urea concentrations. Although these regenerated N forms are associated with anthropogenic sources, they are also produced by consumers within kelp beds (Bray, Purcell, and Miller 1986; Bray et al. 1988). By providing habitat for consumers, canopy-forming kelps may

enhance N regeneration by animals, facilitating kelp growth during periods of low nitrate availability.

We have shown that regenerated forms of N contribute to the N taken up by kelps during seasonally low N conditions, with urea contributing relatively more to total N uptake up at more isolated sites further from densely populated areas. As kelp populations continue to face multiple stressors, including decreased nutrient concentrations and increased ocean stratification, understanding nutrient uptake dynamics becomes increasingly important to protect and manage these populations. As coastal nitrate concentrations decline and anthropogenic N inputs – including regenerated forms such as ammonium and urea - increase, elucidating the use of these N forms, not only by kelps but by their associated microbiomes and the phytoplankton assemblages within kelp beds, will give us a better understanding of how these communities function from a microscopic-to-macroscopic scale.

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FIGURES

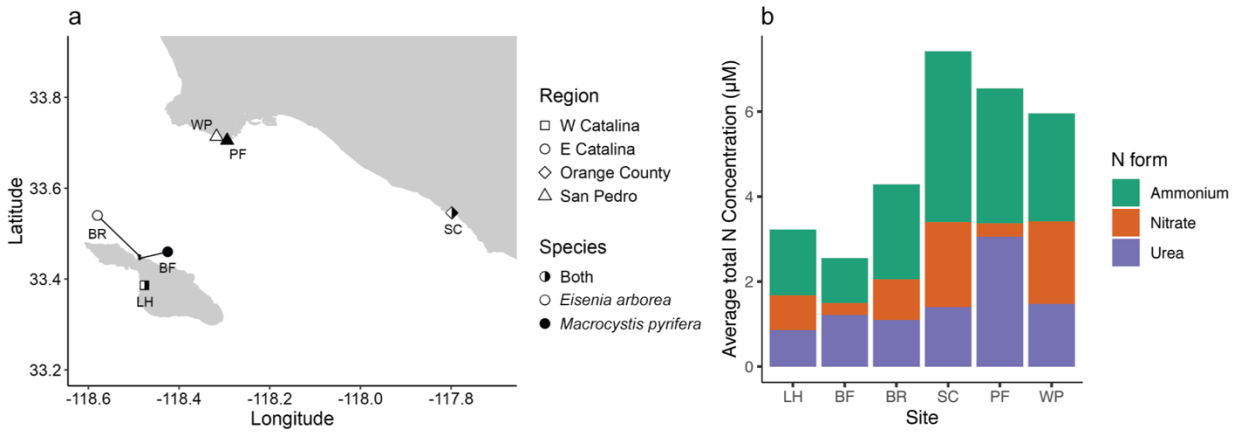

Figure 3.1 Sampling sites and the average available N available at each site. (a) Location of sampling sites in southern California for two species of kelp, organized into four regions. (b) Average total N concentration of three forms of N collected from each sampling site. Sites are abbreviated as follows: Little Harbor (LH), Big Fisherman's Cove (BF), Bird Rock (BR), Shaw's Cove (SC), Point Fermin (PF), White Point (WP).

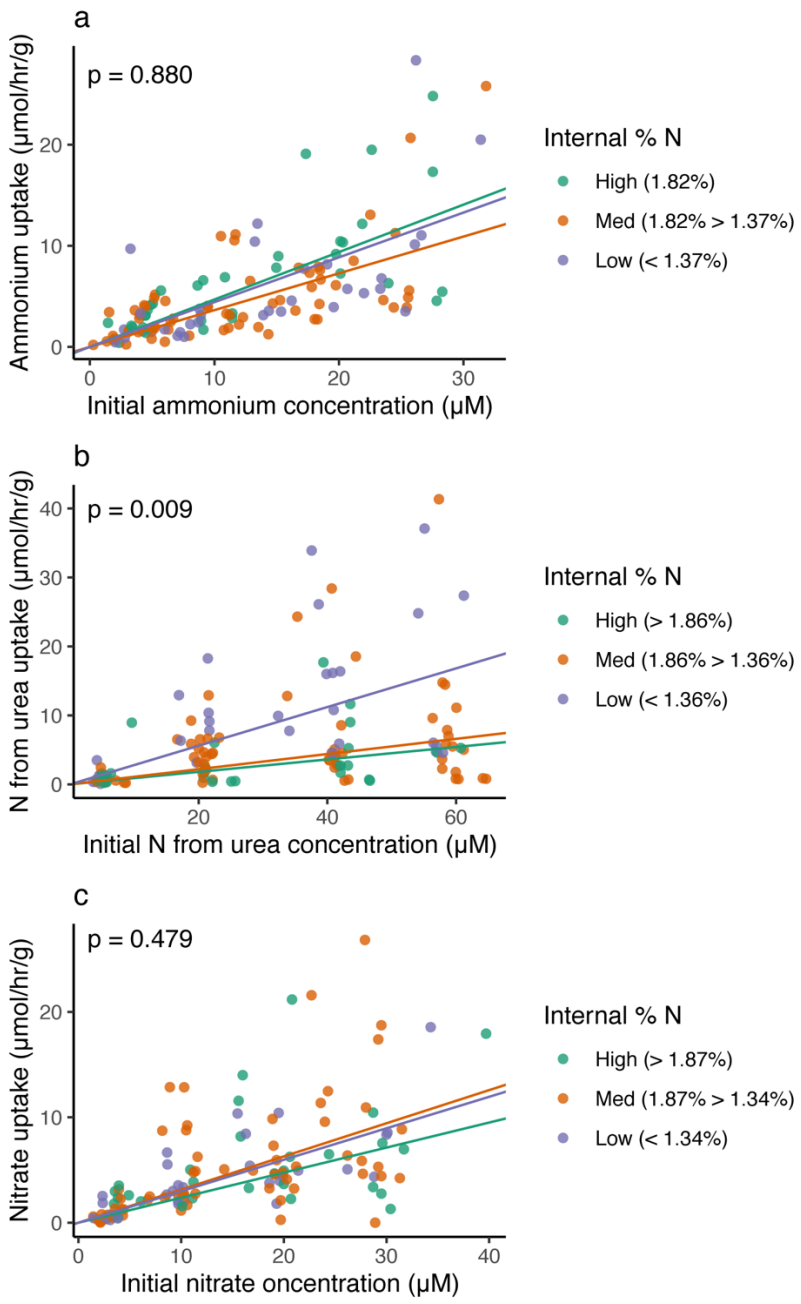

Figure 3.2 Relationships between internal %N and efficiency of N uptake for (a) ammonium, (b) urea, and (c) nitrate. Significant linear relationships between N uptake rate and initial N concentration for high, medium, or low values of %N illustrate the efficiency of uptake for each form of N. P-values indicate whether N uptake was related to internal % N for each form of N.

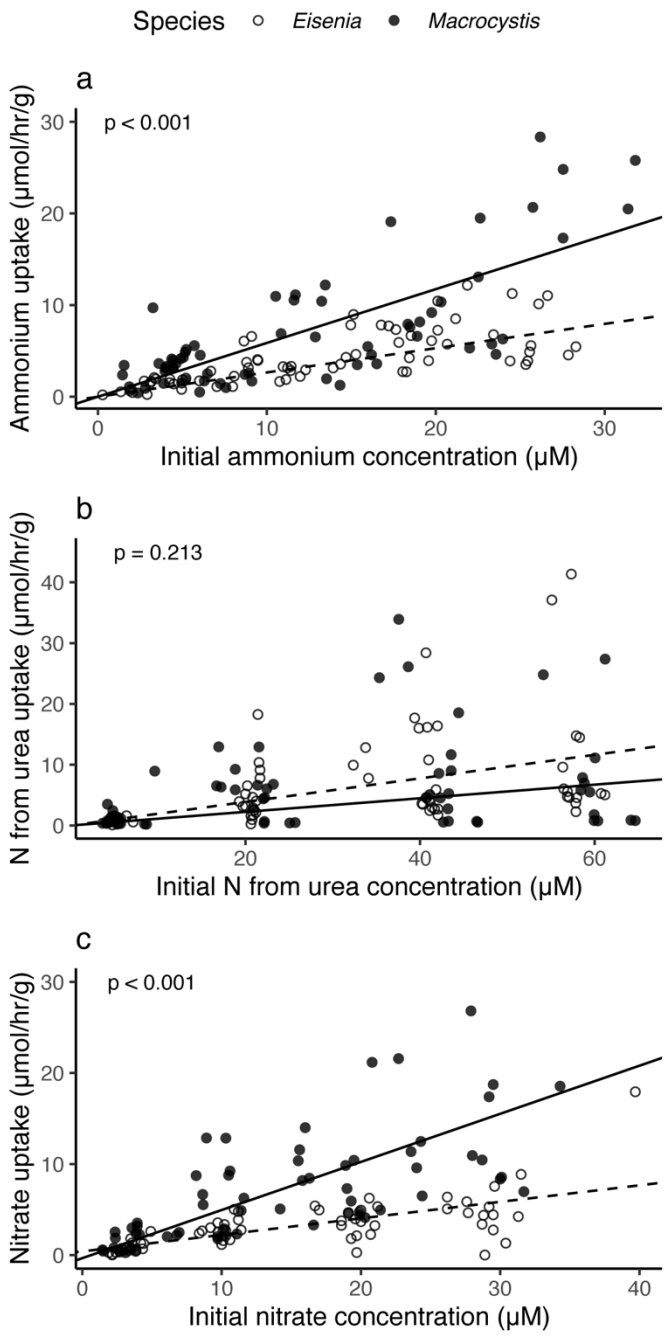

Figure 3.3 Efficiency of (a) ammonium, (b) urea, and (c) nitrate by the kelps *Macrocystis pyrifera* and *Eisenia arborea*. Significant linear relationships between N uptake rate and initial N concentration for each species illustrate the efficiency of uptake for each N form. P-values indicate whether N uptake efficiency differed between *Macrocystis* and *Eisenia*.

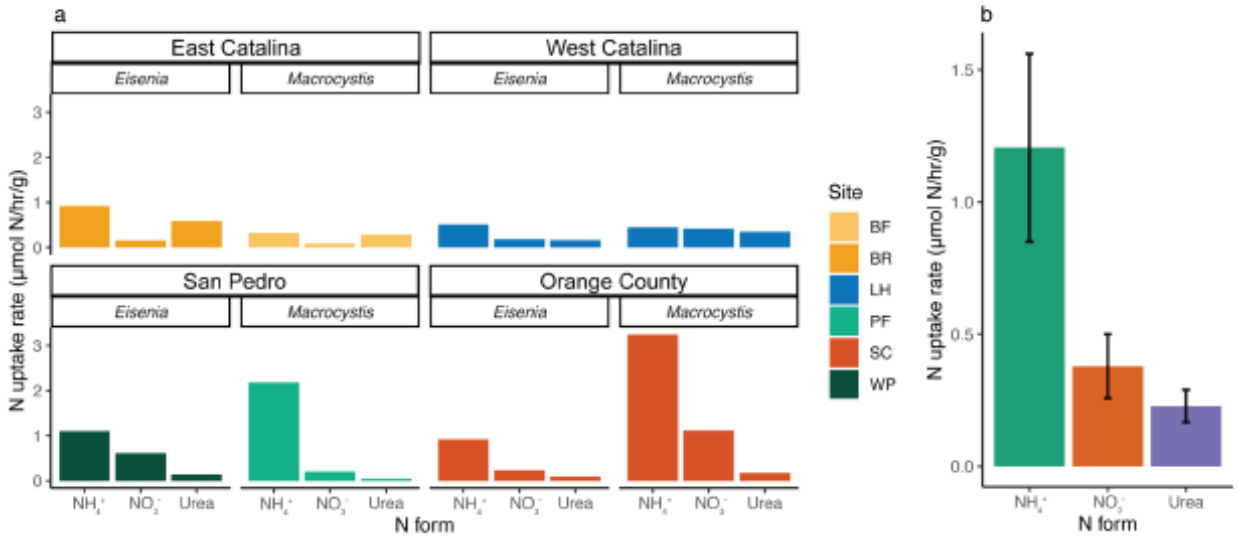

Figure 3.4 Calculated rates of nitrogen (N) uptake. (a) Expected N uptake rates based on average environmental nitrogen concentrations and uptake equations for each species, region, and site: Little Harbor (LH), Big Fisherman's Cove (BF), Bird Rock (BR), Shaw's Cove (SC), Point Fermin (PF), White Point (WP). (b) Overall average expected N uptake rates.

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APPENDIX A: Supplemental Information – Chapter 1

SUPPLEMENTAL METHODS

Nutrient Dispenser Efficacy

Based on water samples collected at 0, 3, and 10 days after deployment, nutrient dispensers enhanced PO_4^{3-} (repeated-measures ANOVA: Nutrients: $F_{1,11} = 48.6$, $P < 0.001$; Time * Nutrients: $F_{2,22} = 5.8$, Greenhouse-Geisser-adjusted $P = 0.027$) and NO_3^- concentrations (Nutrients: $F_{1,11} = 21.5$, $P < 0.001$; Time * Nutrients: $F_{2,22} = 4.5$, G-G-adjusted $P = 0.031$), and caused transient, but inconsistent elevation of NH_4^+ concentrations (Nutrients: $F_{1,11} = 1.8$, $P = 0.203$; Time * Nutrients: $F_{2,22} = 0.6$, G-G-adjusted $P = 0.532$).

PCR Methods

The V4 region of 16S rRNA gene was amplified by using 4 uL of diluted DNA was added to 20 uL reactions (1 uL of each primer, 10 uL of Accustart PCR Supermix 2x reaget (Quantabio; Beverly, MA), and 4.0 uL sterile water). PCR conditions were as follows: 94ºC for 3 min, followed by 26 cycles of 94ºC for 30s, 55ºC for 30s, 68ºC for 40s, then held at 25ºC and removed to undergo purification. The PCR products were purified using a 1:1 ration of AMPure beads eluted to 20 uL with sterile water. 4 uL of purified DNA, 1 uL each of unique i5 and i7 Nextera (Illumina Inc., San Diego, CA) Indices, and 4 uL sterile water was used in a second PCR with the following conditions: 12 cycles of 94ºC for 30s, 55ºC for 30s, and 68ºC for 40s, followed by a final extension at 68ºC for 10 min.

The V9 region of the 18S rRNA gene was amplified using 4 uL of diluted DNA was added to 20 uL reactions (1 uL of each primer, 10 uL of Accustart PCR Supermix 2x reagent, and 4.0 uL sterile water). PCR conditions were as follows: 95ºC for 5 min, followed by 28 cycles of 94ºC for 30s, 57ºC for 45s, 72ºC for 60s, followed by extension at 72ºC for 10 min then held

at 25ºC and removed to undergo purification using the method described above. Purified DNA was barcoded with unique i5 and i7 Nextera Indices, and underwent a second PCR as outlined above. Both 16S and 18S libraries were pooled separately based on band brightness and cleaned using a 1:1 ratio of AMPure beads, eluted to 55 uL.

Statistical Analyses

Species richness was log-transformed to meet assumptions of normality in the 16S dataset. The impact of fencing on alpha diversity on naïve and established tiles was tested by calculating the difference in the Shannon Index between fenced and unfenced tiles (fencing effect). The differences in fencing effect between tide pool level treatments were tested using a linear model with grazer, nutrient, and temperature treatments, tested with all possible two-way interactions, with site as an additional fixed effect within naïve and established tiles. As the only difference detected in Shannon Index between pool-level treatments of naïve or established communities was between temperature treatments and grazer temperature interactions (Appendix A: Table S1), further differences in alpha diversity based on tide pool level treatments were assessed via linear models in which the main effects of herbivore, temperature, and nutrient treatments were tested alone and crossed by all possible two-way interactions with site as a single fixed effect.

As fencing and establishment stage had no effect on community composition, they were removed from the PERMANOVA final model (Appendix A: Table S2). Multivariate homogeneity of variance between treatments was tested using the "*betadisper*" function from the "*vegan*" package (Oksanen et al. 2020) which revealed no significant differences in dispersion between grazer, nutrient, or temperature treatments ($p < 0.05$). The percentage of variance explained was calculated by dividing statistically significant estimates by the sum of estimates

given by the PERMANOVA output in R. The directionality of significant interactive effects was characterized using NMDS visualizations and pairwise PERMANOVAs using '*pairwiseAdonis*' (Martinez Arbizu 2020).

SUPPLEMENTAL RESULTS

Site effects

18S ASV diversity differed between sites, a pattern driven by differences in evenness (Table 1). While there was no difference in the Shannon index of 16S ASVs between sites, though there was a difference in ASV richness (Table 1). Additionally, 16S and 18S communities were significantly different between sites (PERMANOVA: $p = 0.001$, Appendix A: Table S4), but they also had significantly different group dispersions (Beta-dispersion analysis, $P < 0.01$).

TABLES

Table S1: Differences between tidepool level experimental factors amongst tiles of different age

Factor	Estimate	Std. Error	t value	Pr(>|t|)
16S				
Naïve				
Grazer Removal	0.215	0.214	1.006	0.319
Nutrient Increase	-0.242	0.214	-1.131	0.263
Warming	-0.09	0.214	-0.422	0.674
Grazers x Nutrients	0.183	0.247	0.743	0.460
Grazers x Temperature	0.077	0.247	0.314	0.755
Nutrients x Temperature	0.125	0.247	0.505	0.615
Established				
Grazer Removal	0.359	0.204	1.761	0.084
Nutrient Increase	0.092	0.199	0.461	0.647
Warming	**0.442**	**0.199**	**2.218**	**0.031**
Grazers x Nutrients	0.025	0.231	0.109	0.913
Grazers x Temperature	**-0.63**	**0.231**	**-2.725**	**0.009**
Nutrients x Temperature	-0.112	0.231	-0.484	0.630
18S				
Naïve				
Grazer Removal	-0.343	0.211	-1.63	0.109
Nutrient Increase	-0.053	0.211	-0.25	0.804

Warming	0.057	0.211	0.27	0.788
Grazers x Nutrients	-0.066	0.243	-0.27	0.788
Grazers x Temperature	0.287	0.243	1.181	0.242
Nutrients x Temperature	-0.291	0.243	-1.196	0.237
Established				
Grazer Removal	-0.299	0.255	-1.173	0.246
Nutrient Increase	-0.414	0.249	-1.659	0.103
Warming	0.108	0.249	0.434	0.666
Grazers x Nutrients	0.173	0.29	0.597	0.553
Grazers x Temperature	0.33	0.29	1.141	0.259
Nutrients x Temperature	0.027	0.29	0.092	0.927

Table S2: Results of PERMANOVA model that includes tile age and fencing

Community and Factors	**df**	**SS**	**MS**	**Pseudo-F**	**R^2**	**P**
(a) Bacteria						
Grazers	**1**	**0.698**	**0.698**	**2.372**	**0.017**	**0.009**
Nutrients	**1**	**0.98**	**0.98**	**3.33**	**0.024**	**0.002**
Temperature	**1**	**1.319**	**1.319**	**4.485**	**0.033**	**0.001**
Site	**1**	**1.12**	**1.12**	**3.805**	**0.028**	**0.002**
Grazers/Age	2	0.587	0.293	0.997	0.014	0.449
Nutrients/Age	1	0.197	0.197	0.669	0.005	0.861
Temperature/Age	1	0.26	0.26	0.885	0.006	0.572
Grazers/Fencing	2	0.313	0.157	0.533	0.008	0.996
Nutrients/Fencing	1	0.146	0.146	0.495	0.004	0.971
Temperature/Fencing	1	0.093	0.093	0.315	0.002	1
Grazers x Nutrients	**1**	**0.612**	**0.612**	**2.079**	**0.015**	**0.017**
Grazers x Temperature	1	0.41	0.41	1.393	0.01	0.128
Nutrients x Temperature	**1**	**1.112**	**1.112**	**3.779**	**0.027**	**0.001**
Residuals	111	32.656	0.294	NA	0.806	NA
(b) Eukaryotes						
Grazers	1	0.487	0.487	1.702	0.012	0.107
Nutrients	**1**	**0.818**	**0.818**	**2.859**	**0.021**	**0.012**
Temperature	**1**	**0.75**	**0.75**	**2.622**	**0.019**	**0.015**
Site	**1**	**1.886**	**1.886**	**6.597**	**0.048**	**0.001**
Grazers x Nutrients	**1**	**0.538**	**0.538**	**1.88**	**0.014**	**0.043**
Grazers x Temperature	1	0.391	0.391	1.369	0.01	0.175
Nutrients x Temperature	**1**	**1.584**	**1.584**	**5.54**	**0.04**	**0.001**
Grazers/Fencing	2	0.449	0.224	0.785	0.011	0.705
Nutrients/Fencing	1	0.119	0.119	0.415	0.003	0.977
Temperature/Fencing	1	0.133	0.133	0.464	0.003	0.95
Grazers/Age	2	0.44	0.22	0.769	0.011	0.759
Nutrients/Age	1	0.155	0.155	0.544	0.004	0.892
Temperature/Age	1	0.106	0.106	0.37	0.003	0.982

Residuals	111	31.74	0.286	NA	0.802	NA

Table S3: Differences between tidepool level experimental factors of the fencing effect on the Shannon Index (fenced – unfenced)

Factor	Estimate	Std. Error	t value	Pr(>|t|)
(a) 16S				
Naïve				
Grazer removal	0.095	0.22	0.432	0.670
Heat	0.282	0.22	1.284	0.211
Nutrients	0.356	0.22	1.619	0.118
Site	0.226	0.128	1.762	0.091
Grazers x Temperature	0.038	0.253	0.152	0.881
Grazers x Nutrients	-0.131	0.253	-0.517	0.610
Temperature x Nutrients	-0.407	0.253	-1.608	0.121
Established				
Grazer removal	-0.14	0.274	-0.508	0.616
Heat	0.521	0.274	1.899	0.071
Nutrients	-0.073	0.26	-0.282	0.781
Site	-0.014	0.155	-0.094	0.926
Grazers x Temperature	-0.198	0.307	-0.644	0.526
Grazers x Nutrients	0.375	0.309	1.214	0.238
Temperature x Nutrients	-0.367	0.309	-1.187	0.248
(b) 18S				
Naïve				
Grazer removal	0.203	0.211	0.962	0.346
Heat	0.03	0.211	0.143	0.888
Nutrients	-0.004	0.211	-0.02	0.984
Site	-0.074	0.123	-0.603	0.552
Grazers x Temperature	-0.133	0.242	-0.548	0.589
Grazers x Nutrients	0.119	0.242	0.49	0.628
Temperature x Nutrients	0.128	0.242	0.528	0.602
Established				
Grazer removal	-0.059	0.284	-0.208	0.837
Heat	0.131	0.284	0.461	0.649
Nutrients	-0.48	0.269	-1.787	0.088
Site	-0.068	0.16	-0.423	0.676
Grazers x Temperature	-0.435	0.318	-1.369	0.185
Grazers x Nutrients	0.568	0.32	1.775	0.090
Temperature x Nutrients	0.421	0.32	1.317	0.201

Table S4. PERMANOVA results

Community and factor	df	SS	MS	Pseudo-F	R^2	P	% Variance Explained
(a) Bacteria							
Grazers	**1**	**0.698**	**0.698**	**2.426**	**0.017**	**0.007**	**1.741**
Nutrients	**1**	**0.980**	**0.980**	**3.405**	**0.024**	**0.002**	**2.443**
Temperature	**1**	**1.319**	**1.319**	**4.586**	**0.033**	**0.001**	**3.291**
Site	**1**	**1.120**	**1.120**	**3.891**	**0.028**	**0.001**	**2.792**
Grazers x Nutrients	**1**	**0.622**	**0.622**	**2.162**	**0.015**	**0.011**	**1.551**
Nutrients x Temperature	**1**	**1.116**	**1.116**	**3.879**	**0.028**	**0.001**	**2.784**
Grazers x Temperature	1	0.411	0.411	1.429	0.010	0.106	N/A
Residuals	119	34.237	0.288		0.845		85.397
(b) Eukaryotes							
Grazers		0.487	0.487	1.747	0.012	0.076	N/A
Nutrients	**1**	**0.818**	**0.818**	**2.936**	**0.021**	**0.003**	**2.112**
Temperature	**1**	**0.750**	**0.750**	**2.692**	**0.019**	**0.006**	**1.936**
Site	**1**	**1.886**	**1.886**	**6.773**	**0.048**	**0.001**	**4.872**
Grazers x Nutrients	**1**	**0.538**	**0.538**	**1.930**	**0.014**	**0.048**	**1.389**
Nutrients x Temperature	**1**	**1.584**	**1.584**	**5.688**	**0.040**	**0.001**	**4.091**
Grazers x Temperature	1	0.391	0.391	1.405	0.010	0.147	N/A
Residuals	119	33.141	0.278		0.837		85.600

Table S5: References used for trophic designations in Figure 1.3 with A indicating that the nearest taxonomic ID is autotrophic and H indicating heterotrophic.

Nearest Taxonomic ID	Trophic method	Reference
a) Prokaryotes		
Erythrobacter	A	Yurkov et al. 1994 https://pubmed.ncbi.nlm.nih.gov/7520734/
Jannaschia	H	Wagner-Döbler et al. 2003 https://doi.org/10.1099/ijs.0.02377-0
Roseitalea	H	Hyeon et al. 2017 https://doi.org/10.1099/ijsem.0.001633
Roseovarius	A/H	Labrenz et al. 1999 https://doi.org/10.1099/00207713-49-1-137
Rhodobacteraceae	H	Pujalte et al. 2014 https://oceanrep.geomar.de/id/eprint/40065/

Costertonia	H	Kwon et al. 2006 https://doi.org/10.1099/ijs.0.64168-0
Lewinella	H	Sly and Fegan 2011 https://doi.org/10.1002/9781118960608.gbm00358
Rubidimonas	H	Yoon et al. 2012 https://doi.org/10.1007/s10482-011-9653-3
Winogradskyella	H	Nedashkovskaya and Kim 2016 https://doi.org/10.1002/9781118960608.gbm00350.pub2
Flavobacteriaceae	H	Bernardet 2011 https://doi.org/10.1002/9781118960608.fbm00069
Saprospiraceae	H	McIlroy and Nielsen 2014 https://doi.org/10.1007/978-3-642-38954-2_138
Limnothrix	A	Sfriso et al. 2014 https://doi.org/10.1007/s10811-013-0095-3
Pleurocapsa	A	Rippka et al. 2001 https://doi.org/10.1002/9781118960608.gbm00431
Schizothrix	A	Garcia-Pichel et al. 2001 https://doi.org/10.1128/AEM.67.4.1902-1910.2001
Agaribacter	H	Teramoto and Nishijima 2014 https://doi.org/10.1099/ijs.0.061150-0
Alteromonadaceae	H	Ivanova et al. 2004 https://doi.org/10.1099/ijs.0.02997-0
b) Eukaryotes		
Heterohartmannula	H	Liu et al. 2017 https://doi.org/10.1016/j.ejop.2017.04.007
Oxyrrhis	H	Roberts et al. 2011 https://doi.org/10.1093/plankt/fbq118
Amastigomonas	H	Molina and Nerad 1991 https://doi.org/10.1016/S0932-4739(11)80257-9
Tetraselmis	A	Craigie et al. 1966 https://doi.org/10.1139/b66-140
Ulva	A	Bracken and Williams 2013 https://doi.org/10.1890/12-2182.1
Heteramoeba	H	Droop 1962 https://doi.org/10.1007/BF00422044
Choanoeca	H	Leadbeater 1977 https://doi.org/10.1017/S0025315400021767
Maxillopoda	H	Newman 1992 https://doi.org/10.1111/j.1463-6395.1992.tb01100.x
Opisthokonta	H	Medina et al. 2004 https://doi.org/10.1017/S1473550403001551
Amphora	A	Stepanek and Kociolek 2014 https://doi.org/10.1016/j.protis.2014.02.002
Navicula	A	Doghri et al. 2017 https://doi.org/10.1007/s10811-016-0943-z

Raphid-pennate	A	Ashworth et al. 2017 https://doi.org/10.1016/j.protis.2016.11.001
Bacillariophyta	A	Round and Crawford 1981 https://doi.org/10.1098/rspb.1981.0004
Placidiales	H	Moriya et al. 2002 https://doi.org/10.1078/1434-4610-00093

FIGURES

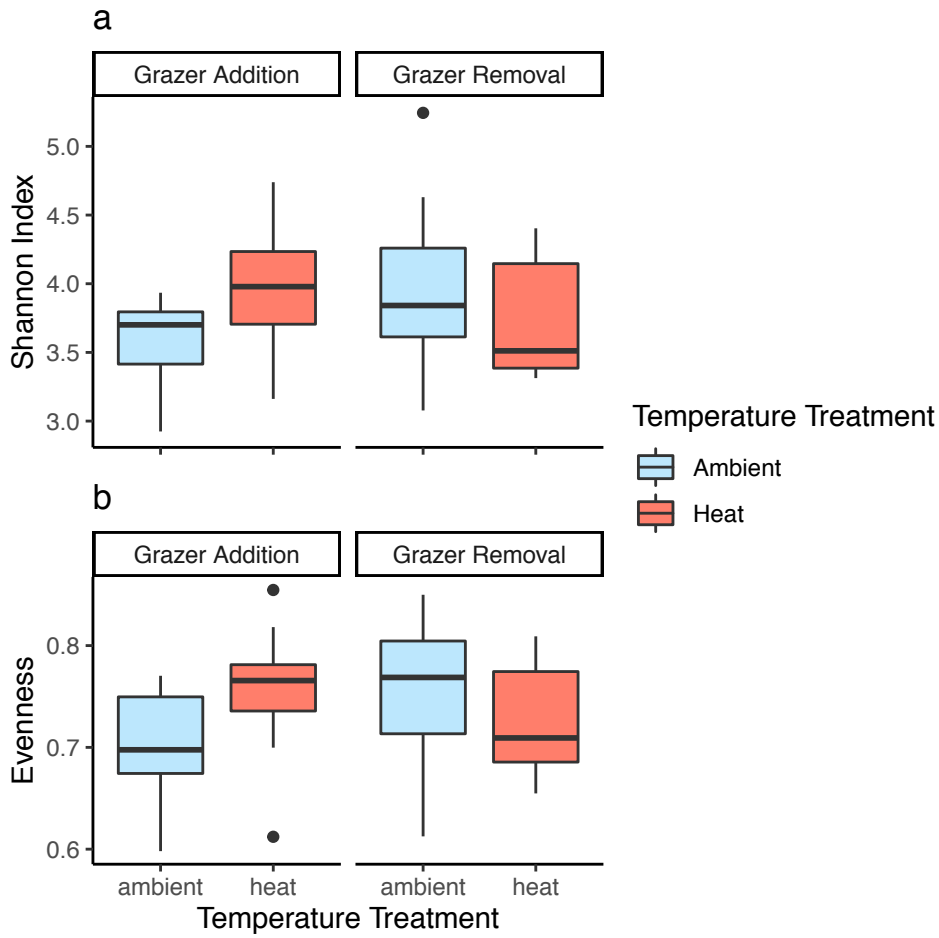

Figure S1: Differences in the Shannon Index (a) and evenness (b) metrics due to temperature and grazer treatment amongst established tiles.

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APPENDIX B: Supplemental Information – Chapter 2

SUPPLEMENTAL METHODS

Nutrient Dispenser Efficacy

Based on water samples collected at 0, 3, and 10 days after deployment, nutrient dispensers enhanced PO_4^{3-} (repeated-measures ANOVA: Nutrients: $F_{1,11} = 48.6$, $P < 0.001$; Time * Nutrients: $F_{2,22} = 5.8$, Greenhouse-Geisser-adjusted $P = 0.027$) and NO_3^- concentrations (Nutrients: $F_{1,11} = 21.5$, $P < 0.001$; Time * Nutrients: $F_{2,22} = 4.5$, G-G-adjusted $P = 0.031$), and caused transient, but inconsistent elevation of NH_4^+ concentrations (Nutrients: $F_{1,11} = 1.8$, $P = 0.203$; Time * Nutrients: $F_{2,22} = 0.6$, G-G-adjusted $P = 0.532$).

PCR Methods

The V4 region of 16S rRNA gene was amplified by using 4 uL of diluted DNA was added to 20 uL reactions (1 uL of each primer, 10 uL of Accustart PCR Supermix 2x reaget (Quantabio; Beverly, MA), and 4.0 uL sterile water). PCR conditions were as follows: 94ºC for 3 min, followed by 26 cycles of 94ºC for 30s, 55ºC for 30s, 68ºC for 40s, then held at 25ºC and removed to undergo purification. The PCR products were purified using a 1:1 ration of AMPure beads eluted to 20 uL with sterile water. 4 uL of purified DNA, 1 uL each of unique i5 and i7 Nextera (Illumina Inc., San Diego, CA) Indices, and 4 uL sterile water was used in a second PCR with the following conditions: 12 cycles of 94ºC for 30s, 55ºC for 30s, and 68ºC for 40s, followed by a final extension at 68ºC for 10 min.

The V9 region of the 18S rRNA gene was amplified using 4 uL of diluted DNA was added to 20 uL reactions (1 uL of each primer, 10 uL of Accustart PCR Supermix 2x reagent, and 4.0 uL

sterile water). PCR conditions were as follows: 95ºC for 5 min, followed by 28 cycles of 94ºC for 30s, 57ºC for 45s, 72ºC for 60s, followed by extension at 72ºC for 10 min then held at 25ºC and removed to undergo purification using the method described above. Purified DNA was barcoded with unique i5 and i7 Nextera Indices, and underwent a second PCR as outlined above. Both 16S and 18S libraries were pooled separately based on band brightness and cleaned using a 1:1 ratio of AMPure beads, eluted to 55 uL.

TABLES

Table S1: Differences in the shared community composition of BMR and CDM based of PERMANOVA results

Factor	**Df**	**SumOfSqs**	**R2**	**F**	**Pr(>F)**	**% Variance**
Prokaryotes						
Region	1	11.262	0.18004	45.2835	0.001	18.24
Site	2	3.043	0.04864	6.1173	0.001	4.93
Stage	1	1.583	0.02531	6.3655	0.001	2.53
Grazers	1	1.386	0.02215	5.5718	0.001	2.24
Region × Grazers	1	1.034	0.01654	4.1594	0.001	1.68
Fencing	1	0.707	0.0113	2.8422	0.001	1.14
Grazers × Nutrients	1	0.649	0.01038	2.6111	0.012	1.05
Nutrients	1	0.575	0.0092	2.3134	0.005	0.93
Region × Nutrients	1	0.519	0.0083	2.0883	0.029	0.84
Grazers × Stage	1	0.458	0.00733	1.8434	0.044	0.74
Grazers × Fencing	1	0.27	0.00432	1.0871	0.273	*NS*
Nutrients × Fencing	1	0.084	0.00134	0.3383	1	*NS*
Nutrients × Stage	1	0.235	0.00375	0.943	0.445	*NS*
Fencing × Stage	1	0.21	0.00336	0.8446	0.592	*NS*
Residual	163	40.538	0.64804	-	-	65.64
Eukaryotes						
Region	1	12.421	0.1863	45.8724	0.001	19.00
Site	2	3.516	0.05273	6.4919	0.001	5.38
Stage	1	1.144	0.01716	4.2247	0.001	1.75
Herb	1	1.137	0.01706	4.2004	0.001	1.74
Region × Grazers	1	1.074	0.01611	3.9656	0.001	1.64
Fencing	1	0.768	0.01152	2.8355	0.004	1.17
Grazers × Nutrients	1	0.64	0.0096	2.3649	0.014	0.98

Region × Nutrients	1	0.572	0.00857	2.1108	0.025	0.87
Nutrients	1	0.514	0.00771	1.8987	0.039	0.79
Grazers × Fencing	1	0.323	0.00484	1.1925	0.237	*NS*
Nutrients × Fencing	1	0.199	0.00299	0.7354	0.724	*NS*
Grazers × Stage	1	0.386	0.00579	1.4257	0.112	*NS*
Nutrients × Stage	1	0.231	0.00347	0.8543	0.574	*NS*
Fencing × Stage	1	0.152	0.00228	0.5621	0.932	*NS*
Residual	161	43.596	0.65387	-	-	66.68

Table S2: Results of linear models examining the differences in the fencing effect on alpha diversity metrics by experimental treatments and regions. Results are only shown for naïve prokaryotic communities, as no other communities had significant differences in the fencing effect

Factor	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Shannon-Weiner Diversity Index				
Grazer removal	0.224	0.250	0.894	0.374
Nutrient addition	0.211	0.256	0.826	0.411
Region (CDM)	1.069	0.256	4.178	<0.001
Grazer x Nutrients	-0.234	0.358	-0.653	0.516
Region x Grazers	-0.536	0.376	-1.427	0.157
Region x Nutrients	-0.362	0.362	-1.000	0.320
Evenness				
Grazer removal	0.030	0.021	1.449	0.151
Nutrient addition	0.027	0.021	1.250	0.215
Region (CDM)	0.080	0.021	3.752	<0.001
Grazer x Nutrients	-0.008	0.025	-0.325	0.746
Region x Grazer	-0.033	0.025	-1.307	0.195
Region x Nutrients	-0.018	0.025	-0.733	0.466
Richness				
Grazer removal	21.020	50.230	0.418	0.677
Nutrient addition	17.040	51.400	0.332	0.741
Region (CDM)	254.130	51.400	4.944	<0.001
Grazer x Nutrients	-62.370	71.870	-0.868	0.388
Region x Grazers	-141.940	75.520	-1.879	0.064
Region x Nutrients	-111.710	72.700	-1.537	0.128

FIGURES

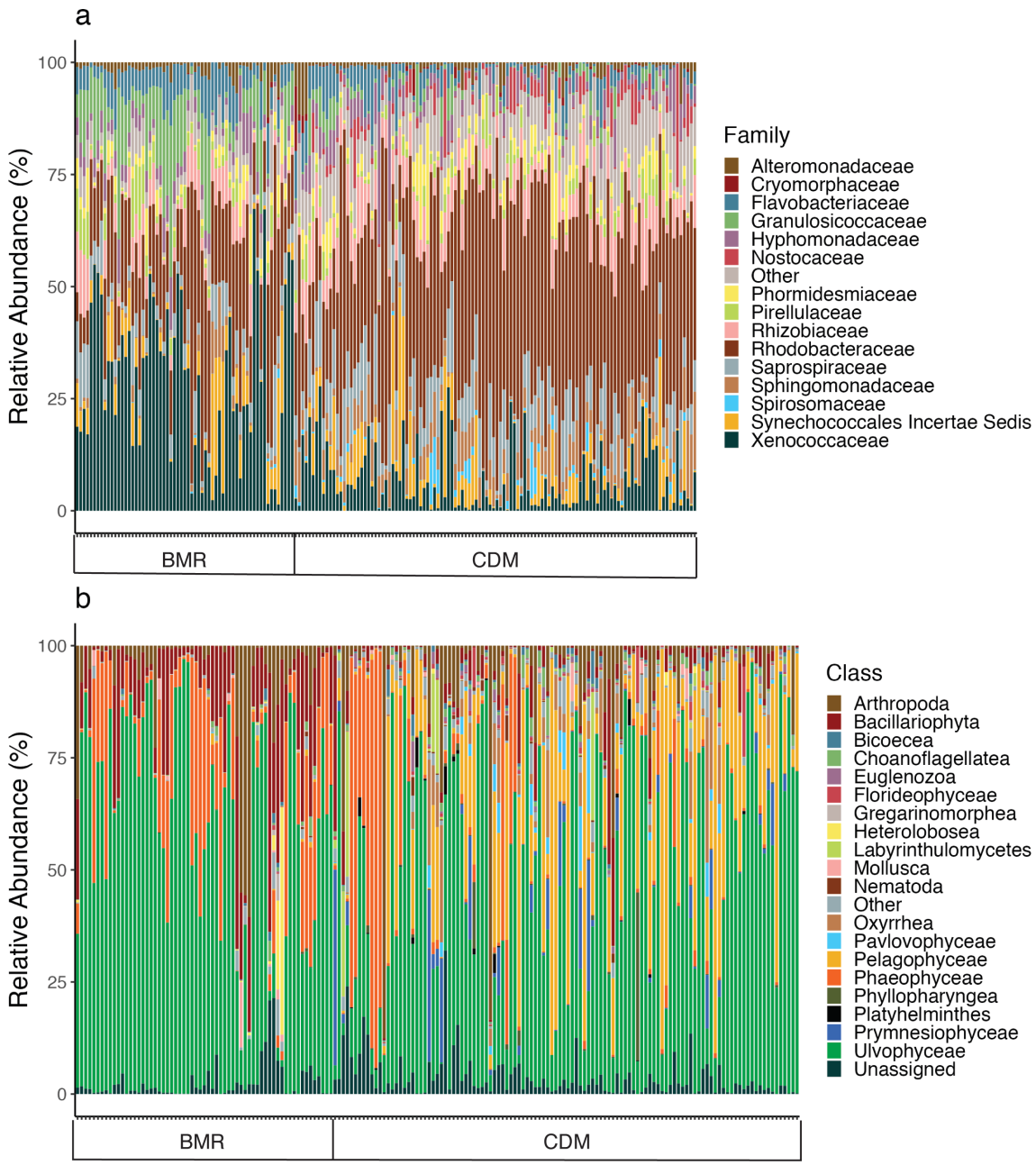

Figure S1: Relative abundance (%) of ASVs shared across the BMR and CDM. (a) Shared prokaryotic shared ASVs were agglomerated to the Family taxonomic level while (b) shared eukaryotic ASVs were agglomerated to the Class taxonomic level. Each bar is a single sample sorted by region.

APPENDIX C: Supplemental Information – Chapter 3

FIGURES

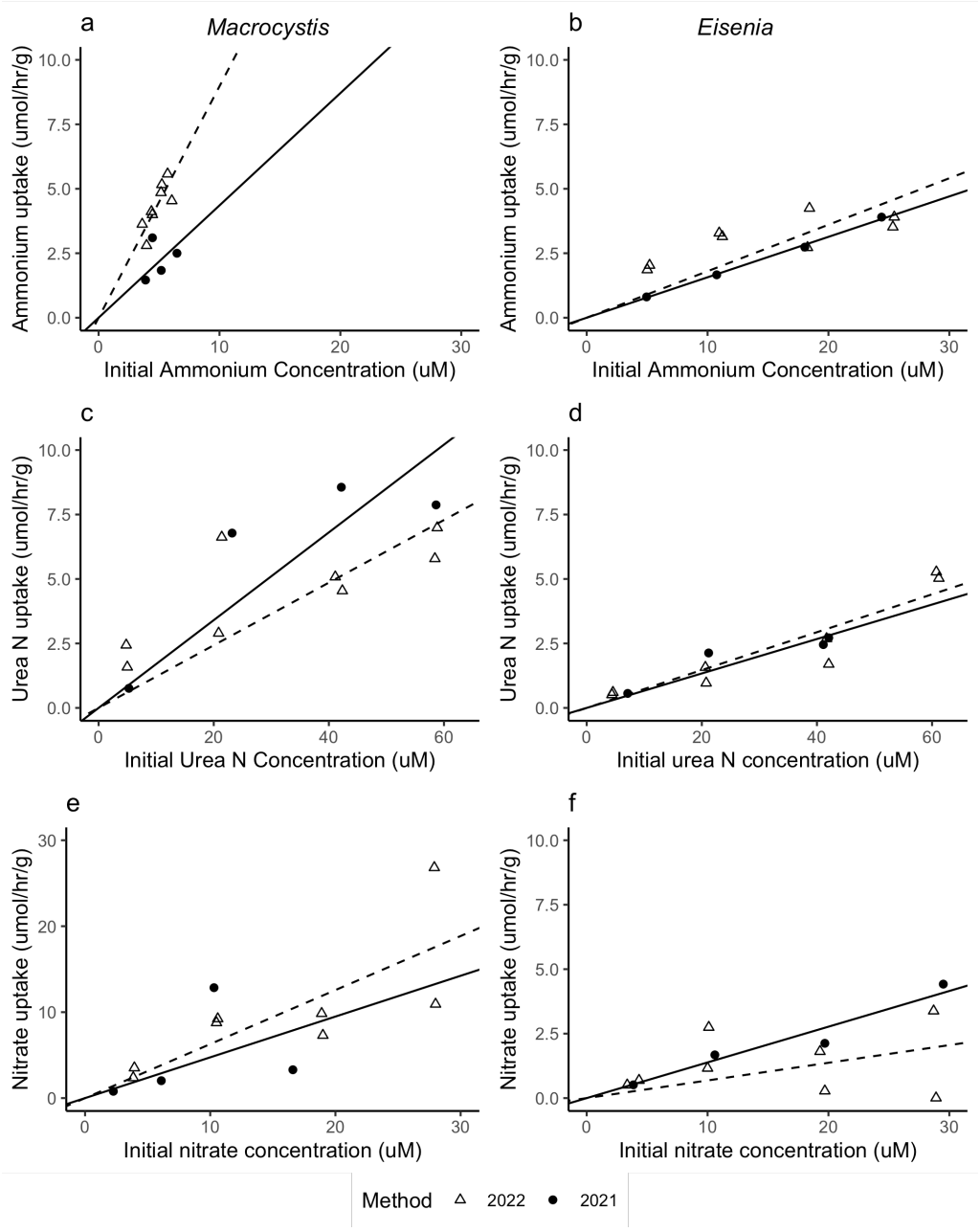

Figure S1. Comparisons of measured uptake efficiency between methods used in 2021 and 2022. There were no significant differences in the slopes between methods for (a-b) ammonium, (c-d) urea, or (e-f) nitrate for either (a, c, e) *Macrocystis* or (b, d, f) *Eisenia* (linear model, $p > 0.05$).

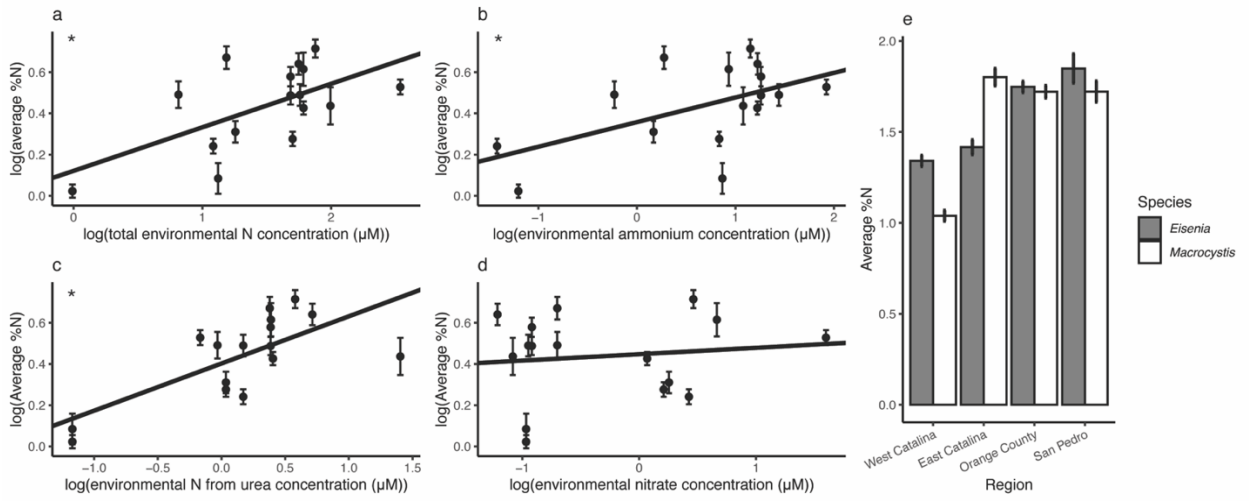

Figure S2. (a-d) Relationships between average internal percent N for each collection event and N measured from environmental seawater samples. There were significant saturating relationships between %N and (a) total environmental N, (c) ammonium, and (d) urea concentrations, while there was no relationship between %N and (b) nitrate concentrations. (e) Average %N for each species of kelp across southern California regions..