ALGAE AFFECT CORAL REEF MICROBIAL COMMUNITIES AT MULTIPLE SPATIAL SCALES, WITH CONSEQUENCES FOR CORAL-ALGAL REGIME SHIFTS

by

AMY A. BRIGGS

(Under the Direction of Craig W. Osenberg)

ABSTRACT

Microbes affect many important ecological processes that operate at different scales, influencing everything from the health of individual macroorganisms and the interactions between competing species, to the global carbon cycle. However, many aspects of their spatial ecology are poorly understood, particularly how some environmental drivers can affect microbial communities at multiple spatial scales. This understanding is necessary to explain variation in natural microbial communities, and their effects on the functions of, and spatial patterning in, ecosystems.

One system in which microbes play an outsized role is coral reefs, which are experiencing increasing disturbances that stimulate the proliferation of algae. This dissertation investigates how algae can affect microbial communities on reefs, focusing on the responses of prokaryotic microbes in the microbiomes of corals, as well as several genera of toxic dinoflagellates (eukaryotic microalgae) that are associated with ciguatera poisoning in humans.

I investigated how local algal contact and regional (site-level) algal density affected coral microbiomes. Local and regional effects of algae interacted antagonistically to affect microbiome diversity. Coral microbiomes became more similar to macroalgal microbiomes with increasing regional macroalgal density and with algal contact.

Because microbes mediate many of the negative effects that algae have on corals, I then used theoretical models to investigate how positive feedbacks between algae and microbes with harmful effects on corals can influence regime shifts—abrupt transitions in reefs from coral to algal-dominated states. My results illustrate that algae-associated microbes increase the likelihood of regime shifts in reefs, and that sufficiently strong single positive feedbacks, or multiple combined positive feedbacks, only produce algal-dominated reefs.

Other microbes that may contribute to regime shifts in reefs include toxic epiphytes that grow on macroalgae and reduce herbivory on it (e.g., ciguatoxic dinoflagellates). I found that local effects of algae (patch area and species composition) could not explain most of the heterogeneity in ciguatoxic dinoflagellate densities, suggesting that high regional algal densities might have affected dinoflagellates. Future research disentangling local and regional effects of algae on these dinoflagellates will help determine whether increases in algae in reefs could amplify ciguatera poisoning risk and facilitate regime shifts from coral- to algal-dominance.

INDEX WORDS: microbial ecology, coral reefs, microbiomes, dinoflagellates, algae, alternative stable states

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DEDICATION

This dissertation is dedicated to all my family, friends, and mentors who have supported and cheered for me along my academic journey, and to the many people I spoke to in Kiribati and French Polynesia about ciguatera poisoning, who helped me realize the importance of studying microbes in coral reefs.

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CHAPTER 1

INTRODUCTION: MICROBES AND THEIR EFFECTS ON ECOSYSTEMS

Overview of microbes and their important ecological functions

Microbes, single-celled microscopic organisms, are ubiquitous components of Earth's biosphere, occupying every ecosystem, including the most extreme environments where life is found (Pikuta et al. 2007). Microbes fall into five main groups, bacteria, archea, fungi, protozoa (e.g., *Amoeba*), and algae (including many types of phytoplankton). Each of these groups has differing characteristics, but they each contribute to many critical ecosystem processes. Here, I briefly review some of what is known about microbes' importance to ecological systems and identify several major gaps in our understanding of their ecology and effects on ecosystems. I then address these gaps in the following chapters using a series of empirical and theoretical studies.

At the largest spatial scales, microbes play an important role in biogeochemical cycles (Falkowski et al. 2008, Averill et al. 2014), including the movement of energy (carbon) and nutrients (e.g., nitrogen) across ecosystem boundaries. Prokaryotic microbes (bacteria and archea) are estimated to contain approximately half of the carbon stored in living organisms, and are one of the largest living pools of macronutrients like nitrogen and phosphorus (Whitman et al. 1998). Additionally, photosynthetic microbes like phytoplankton form the base of the food web in many marine and aquatic ecosystems, and are responsible for approximately half of global net primary production (Field et al. 1998). Sinking organic material produced by phytoplankton exports nutrients and energy to the deep ocean (the "biological pump"), which

affects the ocean's uptake of atmospheric CO_2 (Falkowski 1997). The ability of many microbes such as some bacteria, archea, and fungi to fix nitrogen (N) from atmospheric sources (i.e., N_2), or to convert various inorganic species of N (e.g., ammonia, nitrite) to more bioavailable forms (e.g., nitrate) is also critical for the growth of eukaryotes and other organisms, especially in ecosystems in which the supply of N is otherwise limited. For example, nitrogen-fixing cyanobacteria are a major source of N to open ocean habitats (Montoya et al. 2004, Zehr 2011). Thus, these microbes likely support a large fraction of productivity in these systems (Capone et al. 1997, Montoya et al. 2004). Similarly, nitrogen-fixing symbionts can increase the productivity of their host plants in terrestrial systems, influencing plant community productivity, succession, diversity, and composition (Vitousek and Walker 1989, Spehn et al. 2002, Van Der Heijden et al. 2006, van der Heijden et al. 2008).

Microbial breakdown of organic materials is also crucial for nutrient cycling (Falkowski et al. 2008, Averill et al. 2014). In marine systems, heterotrophic microbes such as bacteria and protists recycle a large portion of energy produced by photosynthetic taxa such as phytoplankton, reducing the loss of nutrients and carbon in the water column—a function that has been named "the microbial loop" (Azam et al. 1983, Pomeroy et al. 2007). In terrestrial systems, rates of microbial decomposition in soils can affect whether an ecosystem is a net source of carbon to the atmosphere or a carbon sink (Bardgett et al. 2008, Trivedi et al. 2013).

At a smaller spatial scale, microbes also directly affect the health of individual macroorganisms. Many microbes have consistent associations with specific macroscopic taxa (e.g., Ainsworth et al. 2015, Akorli et al. 2019), and some of these relationships are evolutionarily conserved, suggesting long histories of coevolution (Moran and Wernegreen 2000, Pollock et al. 2018). These microbial associates can affect the phenotype and fitness of the

macroscopic individuals (reviewed in Lynch and Hsiao 2019). Many microbes help macroorganisms acquire nutrients from sources that would otherwise be chemically unavailable to them, such as the endosymbiotic bacteria that assist some species of polychaete worms from the family Siboglinidae to digest whale carcass bones (Verna et al. 2010), or microbes that fix nitrogen for hosts in exchange for simple carbohydrates (e.g., nitrogen-fixing rhizobia in the root nodules of legumes; MacLean et al. 2007). Similarly, many bacterial endosymbionts are important for the production of enzymes or essential vitamins for host macrobes, like bacteria that allow aphids to subsist on phloem from plants, which has low concentrations of certain essential amino acids (Akman Gündüz and Douglas 2009). However, other microbes act as pathogens, with negative consequences for the health of infected hosts (Ryan 2013; more details about effects of microbial pathogens below).

Macroorganisms typically host a whole consortium of microbial associates, which are collectively known as their microbiome. Like individual microbial symbionts or pathogens, the microbiome of a macroscopic host can have an aggregate influence on its health and survival, modifying its physiology and growth (Shin et al. 2011, Glasl et al. 2016), as well as resistance to disease (Rosshart et al. 2017) and environmental stressors (Levin et al. 2017, Ziegler et al. 2017, Rosado et al. 2019). Microbiomes also produce chemical cues that influence macroorganism behavior. For example, chemical signatures of specific microbial communities are used by macroorganisms to detect appropriate habitat for behaviors such as oviposition (Ponnusamy et al. 2008) or settlement (Negri et al. 2001, Webster et al. 2004), and can allow social animals (e.g., bees and ants) to distinguish group members from outsiders (Teseo et al. 2019, Vernier et al. 2020).

These effects of microbes on individual hosts can scale up to host population dynamics and affect their interactions with other species (Jackrel et al. 2020), which can influence the composition of ecological communities. For example, microbes can mediate coexistence between macroscopic competitors, which can alter community diversity (Bever et al. 1997, Van Der Heijden et al. 2006, Kandlikar et al. 2019). Depending on the direction and strength of the interaction between microbes and macrobes, the effects can be stabilizing or destabilizing for ecological communities.

Stabilizing and destabilizing effects of microbes on macroscopic communities

One mechanism through which microbes may promote diversity is by inducing negative frequency dependence (Kandlikar et al. 2019). As a macroscopic taxon becomes increasingly abundant, microbes that are harmful to it can build up in the local environment, reducing its growth. This allows other macrobes that are less affected by the microbes to increase in abundance, thereby enhancing community diversity or facilitating succession (Van der Putten et al. 1993) to a new, less susceptible community (similar to the Janzen-Connell hypothesis; reviewed in Mordecai 2011). Microbes are also thought to promote coexistence of macroscopic species by facilitating resource partitioning in macroscopic hosts (Bever et al. 2010, Luo et al. 2018). Host taxa with different microbial symbionts may be able to access different resources. For example, trees with different mycorrhizal fungal associates may be able to access different forms of soil N, which could reduce competition between the tree species (Luo et al. 2018).

However, microbes can also have destabilizing effects on communities, especially when they create positive feedbacks. For example, in pathogen spillover, if a common macrobe hosts generalist microbial pathogens that have more negative effects on heterospecific macrobes than on the more tolerant host, then increases in the tolerant host could reduce the growth of

susceptible macrobes, eventually leading to their competitive exclusion. Similarly, mutualistic relationships between macrobes and microbes, like a plant with beneficial soil microbial associates, can facilitate dominance of the macrobe (McGuire 2007) when other macrobes have a weaker positive response to the microbes (e.g., Bever et al. 2010).

Theoretical explorations of the stabilizing vs. destabilizing effects of microbes on ecological communities (through negative vs. positive feedbacks) have primarily focused on their effects on competitive exclusion vs. coexistence (Umbanhowar and McCann 2005, Miki et al. 2010, Kandlikar et al. 2019). However, positive feedbacks are a key component of systems that exhibit abrupt transitions between alternative states, i.e., regime shifts (Jacob and Monod 1961, Soulé 2003, Kéfi et al. 2016). Strong positive feedbacks can produce alternative stable states that exist under the same set of environmental conditions (Scheffer et al. 2001, Kéfi et al. 2016). In systems exhibiting alternative stable states, starting conditions (or perturbations to a new starting condition) influence which stable state the system equilibrates to. Thus, systems with alternative stable states are susceptible to regime shifts caused by disturbances. Because of this susceptibility to rapid transitions to alternative stable states, which are typically difficult to reverse (Scheffer and Carpenter 2003), and because alternative stable states typically support very different ecological communities and ecosystem services (Crépin et al. 2011), predicting which ecosystems have alternative stable states, and which ecological processes produce alternative states and drive transitions between states is of great interest and value to managers and researchers.

By modifying interactions between macroscopic taxa, microbes may contribute to positive feedbacks that can produce alternative ecological communities that can exist under the same environment. As a consequence, microbes may make some ecological communities

susceptible to abrupt shifts in their composition. Regime shifts have been explored for a variety of ecosystems such as arid landscapes that can transition from vegetated to unvegetated states (Schlesinger et al. 1990, Van Nes and Scheffer 2005), and alternative stable states have been investigated in microbial communities themselves (Lahti et al. 2019, Amor et al. 2020, Bardgett and Caruso 2020). However, the effects of microbes on regime shifts in macroscopic communities not been investigated, despite the increasingly recognized role of microbes in many important ecosystem functions.

This dissertation addresses how microbes influence regime shifts in macroscopic communities in Chapter 3. There, we develop metrics to quantitatively compare the effects of microbial feedbacks on ecological systems exhibiting alternative stable states and model the effects of microbes on regime shifts in coral reefs—an ecosystem in which microbes modify the interactions between corals and algae, and that can exhibit alternative coral vs. algal-dominated states (Mumby et al. 2007, Schmitt et al. 2019).

The spatial ecology of microbes

In addition to our limited understanding of microbes' contributions to regime shifts in ecological systems, our understanding of their spatial ecology—both the spatial scale at which they vary and the spatial scales of their effects on macroscopic communities—is not well-resolved (Ladau and Eloe-Fadrosh 2019). Microbes tend to differ from macrobes in several key ways that might cause them to exhibit slightly different patterns of spatial ecology (Nemergut et al. 2013), such as their high abundances, ability to enter dormancy for long periods (Lennon and Jones 2011), high dispersal ability (Finlay 2002), and capacity for rapid evolution and exchange of genetic information between taxa (Ochman et al. 2000). Microbes may also exhibit different

biogeographic patterns and processes compared to macrobes, although this is debated (Meyer et al. 2018.)

Complicating this understanding, microbial communities frequently exhibit disparate responses when their relationship to an environmental factor is evaluated at multiple spatial scales (Franklin and Mills 2003, Martiny et al. 2011). For example, total organic carbon (TOC) in soil can explain variation in soil microbial communities among plots within wetlands, but cannot explain heterogeneity in microbial communities among wetlands (Peralta et al. 2012). Similarly, as mentioned in previous sections, microbes can affect macroorganisms at multiple spatial scales, affecting individuals, groups of individuals in a localized area on a landscape, and entire populations or ecosystems. However, most studies investigate the responses and effects of microbial communities at a single spatial scale. Discrepancies in the relationship between microbial communities and an environmental driver (or response) that occur when that relationship is evaluated at different spatial scales might arise because the dominant processes driving these relationships vary with scale. For example, local plant diversity is enhanced next to organic farms (compared to conventional farms) due to reduced exposure to detrimental herbicides (a localized effect of farming type; Rundlöf et al. 2009). However, local plant diversity is also enhanced by increased regional prevalence of organic farms, which increases the regional pool of plants species and enables seed dispersal from locations of higher diversity (areas next to organic farms) to areas with lower diversity (areas near conventional farms; Rundlöf et al. 2009). Microbial communities likely exhibit similar characteristics, because their community composition is influenced by abiotic filtering, which can happen at both larger, regional scales, as well as smaller local scales due to microhabitat differences, and is also

influenced by both long and short-distance dispersal (Cadotte and Fukami 2005, Bowker et al. 2006, Martiny et al. 2011, Nemergut et al. 2013).

Focusing on the effects of ecological factors at a single spatial scale makes it difficult to identify important mechanisms operating at other spatial scales that produce heterogeneity in patterns of microbial community composition and function at the focal scale. Additionally, ignoring the effects of factors that operate at multiple spatial scales likely reduces the precision of our estimates of the effects of these factors at single, focal spatial scales, and could even confound our ability to detect the effects of a factor at one or more spatial scales if its effects at those scales interact. Furthermore, the spatial extent of different processes (or effects) causing positive and negative feedbacks in a system can influence spatial patterning on a landscape (Klausmeier 1999, van de Koppel et al. 2005, Rietkerk and van de Koppel 2008). Thus, identifying the scales at which different important ecological processes and feedbacks operate can be used to explain spatial patterns, such as patchy distributions of monodominant stands within forested landscapes (e.g., McGuire 2007). Due to our comparatively limited understanding of microbial spatial ecology (relative to our understanding in macro-communities), there is a need for studies identifying the scales at which microbial communities vary, which environmental factors drive that variation, and the cross-scale effects of microbes on macroscopic communities.

To address these gaps in our understanding of the spatial ecology of microbes, I conducted studies of micro- and macro-scopic communities in coral reefs in the Pacific Ocean, focusing on two different groups of microbes that vary dramatically in form and ecological niche: prokaryotes (e.g., bacteria), and dinoflagellates, eukaryotic microalgae that are common components of both benthic and pelagic phytoplankton communities in marine and some

freshwater systems, and which include several genera that can form blooms that are harmful to human and wildlife health.

Coral reefs are increasingly experiencing disturbances, such as nutrient enrichment, climate change, and overfishing of herbivores, that favor the growth of fleshy macroalgae and filamentous turf algae (Goldberg and Wilkinson 2004, Hughes et al. 2007). Macroalgae and turf algae (hereafter, combined into 'algae') affect prokaryotic microbes in the water column and in the microbial communities associated with corals (i.e., their microbiomes), increasing the relative abundance of opportunistic heterotrophs and potential pathogens (Vega Thurber et al. 2012a, Haas et al. 2016). Coral microbiomes can influence coral health and physiology, so factors (like algae) that alter the coral microbiome can influence coral growth and survival (Vermeij et al. 2009, Morrow et al. 2013, Zaneveld et al. 2016). Algae also provides a preferred substrate for several genera of benthic dinoflagellates that produce bioaccumulating neurotoxins that cause ciguatera poisoning in people who consume contaminated seafood (Yasumoto et al. 1979, Grzebyk et al. 1994, Parsons et al. 2011). However, no previous studies have explicitly addressed the spatial scale(s) at which algae affects these two types of microbial communities (coral microbiomes and benthic dinoflagellates).

In Chapter 2, I investigate how the prokaryotic microbiomes of corals respond to algae at local (< 1 m^2) and site-level (100-10,000 m^2) scales. Then, in Chapter 4, I investigate how algae influences the density and distribution of toxic benthic dinoflagellates in the genera *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum* at algal patch (< 1 m^2) and site-level (100-10,000 m^2) scales. Finally, in Chapter 5, I synthesize my findings from Chapters 2-4. I discuss how the effects of algae on these microbial groups might affect the dynamics and spatial distributions of coral and algae on reefs, such as whether that could facilitate algal growth, and consequently,

accelerate the decline of corals on reefs. Finally, I discuss potentially fruitful avenues for future research investigating drivers of toxic benthic dinoflagellates, and microbial contributions to regime shifts in other ecosystem types.

This dissertation develops new methods for quantitatively evaluating the contribution of specific ecological processes to alternative stable states, and demonstrates the potential for microbes to enhance the likelihood of regime shifts in coral reef ecosystems. The research also expands our understanding of the spatial scales of variation in natural marine microbial communities and reveals factors that explain that variation. The combined results from each of these sections provides a framework for future work considering how microbes contribute to spatial patterning in ecosystems, including how they might influence patterns of coral- vs. algal-dominance in coral reefs, as well as the risk of ciguatera poisoning in humans who consume reef resources.

CHAPTER 2

LOCAL VS. SITE-LEVEL EFFECTS OF ALGAE ON CORAL MICROBIAL COMMUNITIES [1]

[1] AA Briggs, AL Brown, and CW Osenberg. 2021. *Royal Society Open Science.* 8: 210035. https://doi.org/10.1098/rsos.210035. Reprinted here with permission of the publisher.

Abstract

Microbes influence ecological processes, including the dynamics and health of macro-organisms and their interactions with other species. In coral reefs, microbes mediate negative effects of algae on corals when corals are in contact with algae. However, it is unknown whether these effects extend to larger spatial scales, such as at sites with high algal densities. We investigated how local algal contact and site-level macroalgal cover influenced coral microbial communities in a field study at two islands in French Polynesia, Mo'orea and Mangareva. At 5 sites at each island, we sampled prokaryotic microbial communities (microbiomes) associated with corals, macroalgae, turf algae, and water, with coral samples taken from individuals that were isolated from or in contact with turf or macroalgae. Algal contact and macroalgal cover had antagonistic effects on coral microbiome alpha and beta diversity. Additionally, coral microbiomes shifted and became more similar to macroalgal microbiomes at sites with high macroalgal cover and with algal contact, although the microbial taxa that changed varied by island. Our results indicate that coral microbiomes can be affected by algae outside of the coral's immediate vicinity, and local- and site-level effects of algae can obscure each other's effects when both scales are not considered.

Introduction

Coral reefs around the world are increasingly faced with disturbances such as bleaching events, hurricanes, disease outbreaks, overfishing, and eutrophication, which can permit the rapid proliferation of fleshy algae (Hughes 1994, McCook 1999, Nyström et al. 2000, Diaz-Pulido and McCook 2002, Goldberg and Wilkinson 2004, Hughes et al. 2007). These increases in algae have important consequences for corals, which compete with algae for space, but are much more slow-growing, and thus take longer to recover when their populations decline. Besides pre-

empting space on reefs, algae have a variety of other negative effects on corals, including reducing coral recruitment, growth, and survival, often mediated through direct mechanisms such as overgrowth, abrasion, and allelopathy (McCook et al. 2001, Rasher and Hay 2010, Haas et al. 2011, Vieira et al. 2016). However, indirect effects of algae mediated through microbes are increasingly recognized as an important avenue through which algae can harm corals and potentially reduce their abundance (Smith et al. 2006, Barott and Rohwer 2012).

Corals typically have consistent associations with certain microbial taxa (Ainsworth et al. 2015, Chu and Vollmer 2016, Pollock et al. 2018), many of which influence coral physiology, including internal nutrient cycling, production of enzymes and essential vitamins, as well as resistance to pathogen invasion (reviewed in Peixoto et al. 2017). Algae can influence the microbial communities associated with corals, i.e., their microbiomes (Vega Thurber et al. 2012a, Morrow et al. 2013, Zaneveld et al. 2016). This effect on coral microbiomes could occur because algae leak dissolved organic carbon (DOC), allelochemicals, and other secondary metabolites that can alter the growth and composition of microbes on the surface of the algae and in the surrounding seawater (Morrow et al. 2011, Haas et al. 2011, Egan et al. 2013), and these changes may subsequently affect the coral microbiome. In particular, algae often facilitate the growth of copiotrophs, fast-growing heterotrophic taxa that do best under high nutrient environments, as well as potential pathogens (Haas et al. 2011, 2016, Nelson et al. 2013). Since alterations to the coral microbiome can affect the coral's sensitivity to abiotic stressors (Levin et al. 2017, Ziegler et al. 2017, Rosado et al. 2019), its susceptibility to disease (Nugues et al. 2004, Kuntz et al. 2005, Kline et al. 2006, Barott and Rohwer 2012), and its physiological performance and survival (Glasl et al. 2016), microbiome shifts associated with algae could have significant consequences for corals.

As algae increase in abundance on many reefs around the world, it is important to understand the spatial scale at which algae affect coral. However, the observational and experimental studies that have demonstrated effects of algae on coral microbiomes all focus on corals that were in close proximity (e.g., < 1 m) or in contact with algae: i.e., a "local" effect (Vega Thurber et al. 2012a, Morrow et al. 2013, Zaneveld et al. 2016). Although there are no studies that document effects of algae on corals at larger "regional" scales (e.g., at sites that are each 1,000-10,000 m^2), Haas et al. (2016) found that water column DOC, microbial density, and microbial community composition were correlated with the regional percent cover of algae (a proxy for regional algal biomass or density). Similarly, Kelly et al. (2014) found shifts in near-benthos water microbial communities with changes in benthic community composition (in 20 m^2 areas). If these larger-scale effects of algae or benthic communities on water microbial communities also manifest on corals, then algae may alter coral microbiomes at both local and regional scales. However, dilution and benthic boundary layers might prevent effects at larger (i.e., regional) spatial scales. Furthermore, studies of corals in contact with algae suggest that changes in coral microbiomes disappear at distances > 5 cm from the zone of interaction between the algal and coral (Barott et al. 2011, Pratte et al. 2018, Brown et al. 2019). Unfortunately, no studies have directly evaluated if coral microbial communities change in response to regional algal density, or if the possible effects of algae at local and regional scales interact with one another.

Given current knowledge, there are several plausible hypotheses about the effects of local and regional algae on coral microbiomes. The first hypothesis is that only local algae have an effect and that there is no additional effect of regional algal density on the coral microbiome (Fig. 2.1a), possibly because of dilution or boundary layers, which would limit contact of benthic

corals with microbes in the overlying waters. If, on the other hand, algae also have effects that increase with the regional density of algae, these regional and local effects could combine additively (Fig. 2.1b). Alternatively, local and regional effects could interact with one another (Figs. 2.1c,d). A synergistic interaction would occur if regional effects of algae are more pronounced when a coral is also in contact with algae (Fig. 2.1c). In contrast, an antagonistic interaction would occur if regional effects of algae are most pronounced in the absence of algal contact (Fig. 2.1d). Such antagonisms could arise if the coral microbiome response is saturated by the local effects of algae, causing an absence of any regional algal effect in the presence of algal contact. As a result, the response of corals not in contact with algae would converge on the response of corals experiencing algal contact as the regional abundance of algae increases (Fig. 2.1d). Of course, more complex interactions also are possible.

Effects of algae on the coral microbiome could also be assessed by comparing the coral microbiome of a coral exposed to algae to the pristine state of the coral (not exposed to algae) and the microbial assemblage of the algae. Exposure of a coral to algae might cause the coral microbiome to become intermediate in composition between that found on isolated corals vs. on algae (Fig. S2.1a). Alternatively, exposure to algae could act as a stressor that facilitates the development of a new microbiome, distinct from the ones typically found on either corals or algae (a deterministic shift; Fig S2,1b). A third possibility is that exposure to algae could disrupt the coral microbiome and allow idiosyncratic shifts in its composition that vary widely among similarly exposed corals (a stochastic shift; Fig S2.1c).

To determine whether algae affect coral microbiomes at multiple spatial scales and if coral microbiomes change in predictable ways in response to algae, we conducted a field study at two islands in French Polynesia, Mo'orea and Mangareva. At each island we surveyed benthic

communities and collected microbial samples at five sites that fell along a gradient of macroalgal cover (5 % - 51 %; Fig. S2). We collected microbial samples from individual coral heads of the dominant coral species at each island, stratifying our sampling across corals that were either in contact with algae (i.e., touching) or not in contact with algae (i.e., at least 20 cm from the nearest alga). Because algal functional groups leak different types and amounts of chemical compounds (Paul and Hay 1986, Haas et al. 2011), and typically have different biomasses on reefs, which could modify their effects on microbes, we sampled corals in contact with turf algae and corals in contact with the dominant species of macroalgae at each island. Additionally, to aid in the interpretation of coral microbiome responses, we also sampled microbes from turf, macroalgae, and the water column.

We sequenced the 16S rRNA gene to characterize the prokaryotic microbial communities in our samples, and compared the microbial community composition, alpha diversity, beta diversity, and the relative abundance of specific microbial taxa among groups to evaluate the local and regional effects of algae. Each of these metrics provided different insights into how the microbial communities changed for each sample type. We also compared the responses between the islands to evaluate if the resulting patterns were robust across reefs separated by ~1600 km and characterized by different communities, abiotic conditions, and levels of human impact. Contrary to previous hypotheses, we found that algae affected multiple aspects of coral microbiomes at both local and regional scales, and that the effects of algae at one scale modified its effects at the other scale. Here, we describe those patterns, potential mechanisms driving them, and their implications for our interpretation of previous studies exploring the effects of algae at only local scales.

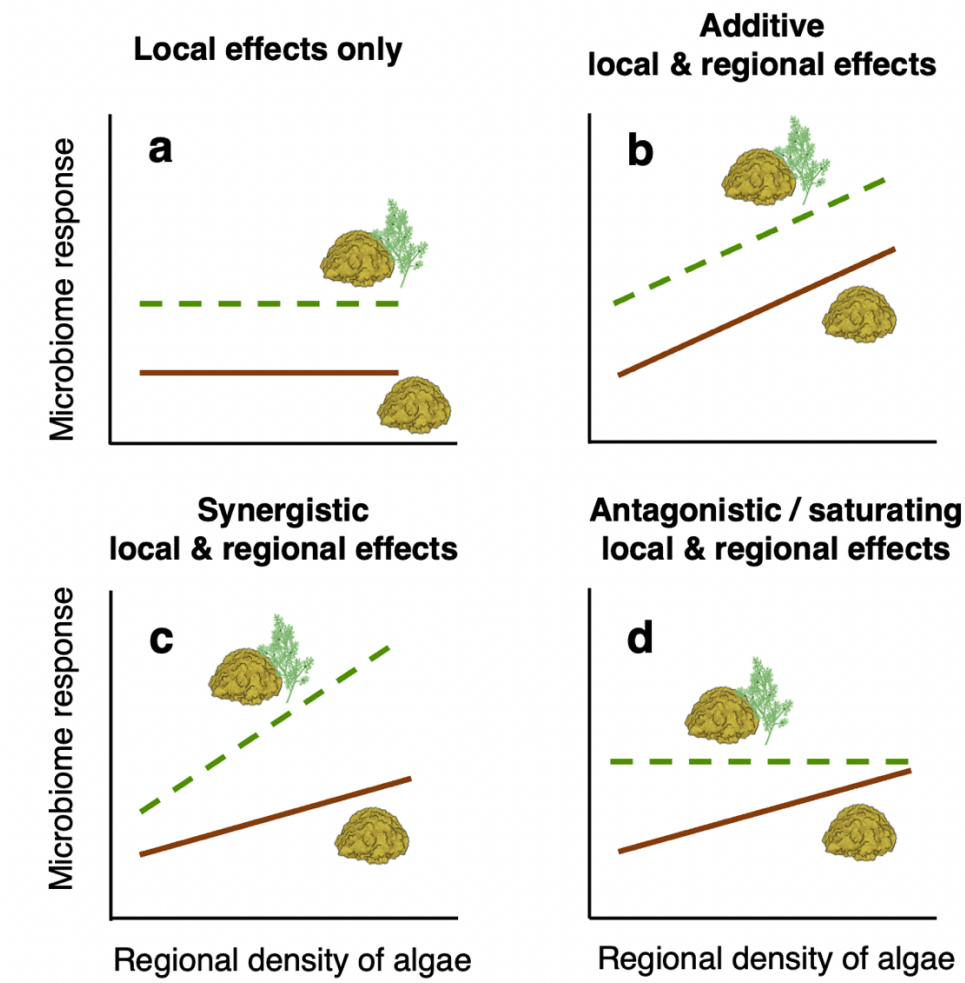

Fig. 2.1) Hypothetical responses of coral microbiomes to algae at local and regional scales. Local effects are indicated by differences between the dashed green line (contact with algae) vs. the solid brown line (no contact). Regional effects are depicted by the slopes of these lines: i.e., the response of the microbiome to increasing regional density of algae. a) Local effects of algae without any regional effects; b) additive local and regional effects; c) synergistic local and regional effects; and d) antagonistic local and regional effects.

Methods

Study locations

Field collections and surveys were conducted in reef sites around the islands of Mo'orea and Mangareva, French Polynesia (South Pacific) from December 26, 2017 to January 21, 2018. (All fieldwork and collections were approved by the French Polynesian government.) Mo'orea is located in the Society Islands archipelago, which is located ~1600 km to the northwest of Mangareva (Gambier Islands; Fig. S2.2). Mo'orea has a larger human population (~17,000) than Mangareva (~1,400) and regularly exchanges people and goods with the nearby (17 km), densely populated island of Tahiti (ISPF 2012). Therefore, at least some of its reefs likely experience more anthropogenic influence than Mangareva.

Benthic surveys

At each island, we selected five back reef or fringing reef sites with an average depth of 1 - 2.25 m that encompassed a gradient of coral to algal dominance (Fig. S2.2, GPS coordinates in Table S2.1). At each site, we haphazardly placed four 25 m transects (except in Mo'orea, where one site had five transects) parallel to shore, with each transect at least 10 m away from any other. We characterized the benthic community composition at each site by visually estimating the percent cover of all living and non-living substrates within ten 0.25 m^2 quadrats that were placed in pairs on each side of the transect (shore-side and ocean-side) at five fixed positions. We recorded the cover of any benthic organism (identified at the genus-level) or non-living substrate (e.g., sand, rubble, newly exposed reef carbonate) that occupied at least 1 % of the quadrat area. From these quadrats, we determined the mean site-level cover of turf, macroalgae, coral, and other benthic functional groups. These site-level means were used as regional-scale predictors in our analyses.

Field microbial sampling

We identified the most widely distributed, abundant coral and macroalgal taxa at each island (i.e., the dominant taxa) for microbial sampling: the coral *Montipora aequituberculata* and the macroalgae *Dictyota bartayresiana* at Mangareva, and the massive *Porites* species complex (including species *Porites lobata* and *Porites lutea* that are not visually distinguishable in the field; Forsman et al. 2009, 2015) and *Turbinaria ornata* at Mo'orea. At each site on both islands, we also sampled turf algae, i.e., short lawns (< 1 cm) of primarily filamentous algae and cyanobacteria intermixed with small juvenile forms of macroalgae. Along each transect, we identified one coral individual of the dominant taxon in each of three local interaction types: 1) No contact, greater than 20 cm from any algae; 2) in contact with turf; and 3) in contact with the dominant macroalgae for that island. Using a 4 mm biopsy punch, we collected a standardized amount of tissue from each coral. For corals with macroalgae contact, tissue was collected in the area where macroalgae made contact with coral; for corals with turf contact, tissue was collected 1-2 mm from the interaction zone with the turf; and for the no-contact corals, tissue was collected at least 20 cm from any algal contact. (Previous studies have found that changes in coral microbiomes due to algal contact generally disappear more than 5 cm from the zone of contact (Barott et al. 2011, Pratte et al. 2018). At sites with only four transects, we sampled a fifth coral in each local interaction type to increase replication (n = 5). For the coral specimens in contact with algae, we also collected a sample of the macroalgae or turf that was near the zone of contact. Macroalgae were gently placed in a sterile 50 mL falcon tube underwater and sealed, while turf was sampled with a 50 mL sterile syringe that we used to suck water off the surface of the turf. We also collected 1-2 water column samples per transect (collected at least 1 m from the benthos) using a sterile 50 mL syringe (n = 5 per site). Immediately after collection, samples

were put on ice until they could be refrigerated and processed. In the laboratory, the falcon tubes containing macroalgae were shaken for 20 seconds and then the seawater in each tube was collected by a sterile syringe. Each syringe for turf, macroalgae, and water samples was passed through separate sterile Sterivex filters (0.22 μm, © Millipore). After processing, all samples were preserved in 1 ml RNA*later* and refrigerated until they could be taken to a laboratory and frozen for storage at -20 °C until DNA extraction.

Molecular methods (DNA extraction, PCR, and bioinformatics processing)

DNA was extracted from samples using Qiagen DNeasy PowerSoil kits per the package instructions (Qiagen, Germantown, MD) with bead-beating for 15 minutes. For the filter samples (macroalgae, turf, and water), we opened the filter cartridge over a sterile petri dish and cut the filter into fine segments using a sterilized scalpel. Then we added the filter to the bead tube and added twice the volume of Solution C1 (120 μl instead of 60 μl). Following DNA extraction, we used the guidelines from the Earth Microbiome Project 16S Illumina Amplicon Sequencing Protocol for library preparation (Caporaso et al. 2011), except we added 2 μl of DNA for each sample. Additionally, we used 50 μM mPNA clamps for the library preparation of algal samples to prevent the amplification of chloroplasts, which would overwhelm the downstream sequences (Lundberg et al. 2013). We used the primer set with barcoded forward primer 515FB (Parada et al. 2016) and reverse primer 806RB (Apprill et al. 2015). The V4 region of the 16S rRNA gene was amplified in triplicate for each sample using the Phusion High Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA). PCRs used the following protocol. First, samples were denatured at 94 ºC for 3 minutes. Next, samples underwent denaturation for 45 s at 94 ºC (or for 10 s at 78 ºC for the algal turf and macroalgal samples, as part of the mPNA clamps procedure; Lundberg et al. 2013), followed by primer annealing for 1 min at 50 ºC, and extension for 1 min

30 s at 72 ºC, repeated 35 times. The final elongation step was at 72 ºC for 10 minutes. Samples were then held at 4 ºC or refrigerated. Negative PCR controls were examined by gel electrophoresis on ethidium bromide-stained, 1% agarose gels to determine if there was contamination. Triplicate PCR products were combined and purified using MinElute PCR Purification Kit (Qiagen). Cleaned and concentrated PCR product (amplicon library) concentrations were measured on a Nanodrop 1000 or a Denovix DS-11 FX+ (Denovix,Wilmington, DE). A total of 240 ng of each amplicon library was pooled for sequencing on an Illumina MiSeq with paired 150-bp reads (v.2 cycle format) at the University of Florida Interdisciplinary Center for Biotechnology Research.

Barcodes and primers were removed using cutadapt v.1.8.1 (Martin 2011). We then completed bioinformatics in R v. 3.2.0. We used the DADA2 v. 1.16 (Callahan et al. 2016) pipeline for quality filtering, error estimation, merging of reads, dereplication, removal of chimeras and selection of amplicon sequence variants, i.e., ASVs (filtering parameters are provided in Appendix 2.1). ASVs are similar to OTUs (Operational Taxonomic Units), however, they provide exact sequences instead of sequences grouped together based on a similarity threshold (Callahan et al. 2016). We used the Silva reference database (v. 132) in DADA2 to assign taxonomy to the ASVs to the genus level (Yilmaz et al. 2014).

The sequence table, taxa table, and metadata table were then imported into the *phyloseq* R package (McMurdie and Holmes 2013). We removed all chloroplast and mitochondrial DNA sequences and filtered out samples with a read depth less than 100 before analyzing the prokaryotic microbial communities in each sample. Community analyses and calculation of diversity metrics were performed on ASV- and family-level data in R using the *phyloseq* (McMurdie and Holmes 2013) and *vegan* (Oksanen et al. 2020) packages.

Diversity responses

We quantified alpha diversity using the first and second-order Hill numbers (D^1, Shannon diversity and D^2, Simpson diversity). These metrics provides the effective number of taxa, i.e., the number of equally abundant taxa needed to produce a community of equivalent diversity as the observed system (Hill 1973, Jost 2006), although each Hill number weights the contributions of rare and abundant taxa to alpha diversity differently. Shannon diversity has similar sensitivity to rare and abundant taxa, while Simpson diversity is influenced more by abundant taxa (Jost 2006, Alberdi and Gilbert 2019). We used these Hill numbers to evaluate alpha diversity because they are relatively robust to low sample sizes (Chao and Shen 2003), variation in sampling depth, DNA amplification biases, and rare taxa, which are all common in microbial data (Lemos et al. 2011, Haegeman et al. 2013, He et al. 2013). Additionally, when used in combination, Hill numbers provide information on the distribution of taxon abundances, with declines from D^1 to D^2 indicating heterogeneity, i.e., unevenness, in abundances (Chao et al. 2014). We did not calculate the zero-order Hill number (D^0, richness), as we had a small proportion of samples with low sequencing depth (Fig. S2.4).

Beta diversity, i.e., the amount of variability in the microbial communities among individual samples in a group, was quantified using the dispersion of samples in multivariate space. High beta dispersion is an indicator that the microbial communities in a group of samples have been destabilized (Moeller et al. 2013, Zaneveld et al. 2017), which could occur in response to a stressor such as algal contact. Using the relative abundance of microbial taxa (not rarefied), we calculated pairwise weighted Bray-Curtis dissimilarity among samples, and then used this dissimilarity matrix to estimate the distance from a sample to its group centroid. These distances were calculated using the *betadisper* function in the *vegan* package in R, with groups based on

substrate type (coral, water, turf, macroalgae) and site (1-5 at each island), or site and local algal contact (no algal contact, turf contact, or macroalgal contact) for the within-coral comparisons. For three instances in which there was only one sample in a group (Table S2.2), we omitted that group from analyses.

We fit linear mixed effects models to the alpha diversity data (Shannon and Simpson diversity) using the *lmer()* function in the R package *lme4* (Bates et al. 2015). We used beta regression, which is appropriate for analyses of continuous proportions (Ferrari and Cribari-Neto 2004, Douma and Weedon 2019), to analyze the beta dispersion data. The coral samples contained several dispersions equal to zero, which cannot be fit with beta regression, so we used the rescaling equation in Appendix 3 of (Douma and Weedon 2019) to add a small constant to all of the dispersions to remove the zero values. We fit the beta regression models with a logit link function using the *glmmTMB* package in R (Brooks et al. 2017). To compare our alpha and beta diversity responses among substrate types (i.e., coral, turf, macroalgae, water) and islands, we fit models that included island, substrate type, and their interaction as fixed effects, and site as a random effect. To identify the effect of macroalgal cover on the microbiomes of each substrate and if that effect differed by island, we fit separate models to each substrate type. Models for the algae and water microbiome samples included the fixed effects of island, site-level macroalgal cover (i.e., a regional effect of algae), and their interaction, and site as a random effect. Models for the coral samples included algal contact (no algal contact, contact with turf, or contact with macroalgae) as an additional fixed effect. To test the hypothesis that algae have local and regional effects that interactively influence coral microbiomes, and to determine if that interaction was consistent between islands, we fit models that contained a three-way interaction of algal contact x macroalgal cover x island to the coral diversity responses. When the three-way

interaction was not significant, we then tested a model that contained algal contact x macroalgal cover and island. P-values for the fixed effects were estimated using a Wald Chi-square Type III test, calculated using the *car* package in R (Fox and Weisberg 2011). Model residuals were checked for patterns that indicated violation of model assumptions.

We focused our analyses on the site-level effects of macroalgae, rather than other regional descriptions of algal communities (e.g., turf cover or total algal cover) for several reasons. First, previous studies have found that macroalgae release more labile forms of DOC and these exudates increase densities of bacterioplankton more than coral exudates (Nelson et al. 2013, Haas et al. 2016). Turf algae also release large amounts of labile DOC (Nelson et al. 2013) but have substantially lower biomass per unit area than macroalgae. Additionally, turf cover was lower and less variable at our sites (Fig. S2.3). Thus, when scaled up to the site-level, this could result in smaller absolute effects of turf on seawater DOC and microbial growth than macroalgae, making it a less informative regional predictor. We evaluated the contribution of macroalgae to variation in the macroscopic benthic community composition among sites (relative to other benthic functional groups) using Principal Component Analysis (PCA). We performed PCA on the centered and variance-standardized mean percent cover of the main benthic functional groups (coral, macroalgae, turf, crustose coralline and peyssonnelid algae, non-living substrate, and "other") at each site.

Community composition

Due to the highly skewed nature of our data, which contained many rare ASVs and a few highly abundant taxa, we compared microbial community composition of our samples in ordination space using non-metric multidimensional scaling (NMDS), based on the weighted Bray-Curtis dissimilarity matrix, calculated from the relative abundance of microbial taxa within

each sample at the ASV level; we also provide results at the family level, primarily in Appendix 2. We calculated the stress of each NMDS to evaluate how well it described the community composition. Each NMDS had stress less than 0.2 indicating adequate model fit to the data (Legendre and Legendre 2012). Differences in composition were tested using PERMANOVA (999 iterations), with the *adonis* function in *vegan* (Oksanen et al. 2020), fitting models containing substrate type, island, and their interaction. Because that analysis demonstrated that community composition differed between the islands (see the *Variation among substrates and islands* section of the *Results*), we fit separate models to the Bray-Curtis dissimilarities of each substrate type at each island and used the *adonis2* function to perform a PERMANOVA to test the effects of the continuous predictor, site-level macroalgal cover. Coral models also included local algal contact and its interaction with site-level macroalgal cover as predictors. We visualized the changes in coral and macroalgal communities with site-level macroalgal cover using separate NMDS ordinations for each island. For each island, we binned site-level macroalgal cover into three categories: low (0-19 %), medium (20-35), and high (> 35 %), and created a separate ordination plot for the samples in each bin.

Identifying specific taxa that change:

Finally, we used separate beta-binomial regression models for each island to identify which coral microbial families responded to site- or local-level effects of algae, implemented using the *corncob* package in R. This approach accounts for variation in sampling depth among samples, can handle zero counts, and is robust to other common issues in microbial data, such as overdispersion of abundances (Martin et al. 2020). For each island, we modeled the effects of algae on the mean relative abundance of microbial families at each scale (local contact or site-level macroalgal cover), while controlling for the effects of algae at the other (non-focal) scale,

and overdispersion differences between groups (i.e., differences in within-group variation). These analyses controlled for a false discovery rate (FDR) of 0.05. Separate models were fit for each island because of the large differences in the composition of the coral microbiomes between islands.

To evaluate whether the families that changed in response to algae were major constituents of the coral microbiome, and if the families that increased with algae potentially came from sources such as algae or the water column, we characterized the most common microbial families associated with each substrate type. We determined which microbial families were present in at least 30 % of samples and had a median relative abundance > 1 % across all samples of a substrate type from each island (which we categorized as the dominant microbial taxa for that island-substrate combination). We then compared these dominant microbial families to the families that were identified by the corncob analysis of the coral microbial communities. If a family that increased in corals with algal contact or site-level macroalgal cover was a dominant family in the turf or macroalgae samples, this suggested that algae might transfer propagules of this taxa to the coral, and/or stimulated its growth within the coral community. If water-associated taxa increased in corals with algal contact or site-level macroalgae, this could suggest that algae disrupted the coral microbiome, allowing opportunistic invasion of microbial taxa from the surrounding environment.

Results

Site and island-level patterns in benthic communities

Benthic community composition varied by site and island (Figs. S2.2, S2.3). Non-living substrates (e.g., sand, rubble) were present at all of the sites, but were more common in Mo'orea (site means ranged from 3-55% cover) than in Mangareva (2-30%), primarily because reefs in

Mangareva were larger or more contiguous. Mean site-level macroalgal cover ranged from 5-44% at Mo'orea and 19-51% at Mangareva, while coral cover ranged from 5-36 % at both islands (Fig. S2.3). Turf algae ranged from 8-23 % and 15-32 % at Mangareva and Mo'orea, respectively, and cyanobacteria cover was generally low (0-2 %). PCA indicated that sites were largely separated by variation in macroalgae and non-living substrate on the first principal component (PC1, which explained 45 % of the variance, Fig. S2.4). Macroalgae had the highest loading on PC1, indicating that it contributed the most to variation on this axis (Table S2.3). Coral and turf algae largely distinguished sites on the second component (PC2, which explained 24 % of the variance; Fig. S2.4, Table S2.3). Thus, macroalgal cover seemed to be an appropriate variable to distinguish the benthic communities of our sites while also allowing us to directly test our hypotheses about the effects of algae on reef microbes.

Sequence characteristics

We had 8,239,519 total reads across all samples after quality filtering and removing samples with low read depth (fewer than 100 reads per sample). Samples with low read depth were distributed across all sites, islands, and sample types, although they were particularly prevalent among the coral samples and sites 2 and 5 at Mo'orea. After filtering and accounting for a handful of additional samples that were lost due to human or equipment error, we had 186 samples, generally with 2-5 samples per site and local algal contact or substrate type group (Table S2.2). Sequence data are available in the NCBI SRA database under BioProject PRJNA681520. Post-filtering reads per sample ranged from 101 – 204,285 reads, with a median of 31,859 and a mean of 44,298. Sampling depth for each substrate type is summarized in Fig. S2.4 and Table S2.4). Post-filtering ASVs per sample ranged from 7 – 2,316, with a mean of

625 and a median of 590. ASV counts were heavily skewed, with most ASVs being rare (< 100 reads across all the samples).

Variation among substrate types and islands

Diversity responses. Microbial community alpha diversity (measured as Shannon diversity, i.e., D^1, the Hill number for q = 1, and Simpson diversity, i.e., D^2, the Hill number for q = 2) and beta diversity (measured by beta dispersion) differed by substrate type (coral, macroalgae, turf, and water; for coral we used samples from all local contact categories), and island, with a significant interaction between island and substrate type (Fig. S2.6a,b,c, Table S2.5). Corals had approximately one half to one fourteenth of the alpha diversity observed on macroalgae, turf, and water (Fig. S2.6a,b), although the magnitude of the difference between coral and the other substrate types was generally larger at Mo'orea, and when alpha diversity was equally weighted by the contributions of both rare and dominant taxa (i.e., Shannon diversity). Beta dispersion was the lowest in the water samples (i.e., these samples were the most homogeneous), with similar dispersion in the water samples at both islands (Fig. S2.6c). In contrast, the coral and algae samples had demonstrably higher dispersion, indicating a high degree of variability in the microbial communities of individual samples. These differences in dispersion between water and the other substrate types depended on island. At Mangareva, dispersion in the coral and turf samples was approximately twice the dispersion in water samples; macroalgae exhibited intermediate dispersion. At Mo'orea, coral, macroalgae, and turf had similar dispersion, which was 1.6-1.8 times the dispersion among water samples.

Community composition. NMDS ordination indicated that the microbiome composition of samples tended to cluster by substrate type, with some separation between islands within a substrate type (Fig. 2.3). Generally, macroalgae, turf, and water samples were more similar to

one another than to coral samples. Coral samples generally showed the greatest variability in microbiome composition (reflected in the spread of points in Fig. 2.3, with coral samples from Mangareva exhibiting the greatest variation). Some of this variation in the coral microbiomes might have been a result of aggregating coral samples across the three algal contact groups: e.g., some coral samples (especially from sites at Mangareva with high macroalgal cover) were close to algae in ordination space (see further analyses below). PERMANOVA results indicated there was a significant interaction between the effects of substrate type and island on ASV- and family-level microbial community composition ($p = 0.001, 0.001$, $R^2 = 0.09, 0.10$, respectively; Table S2.8), largely due to the greater differentiation in the coral microbiomes between the two islands compared to the other substrate types.

Dominant families. Although there was a large amount of overlap in the families found across all substrate types, the identity and relative abundance of the dominant microbial families generally differed among substrates, and to a lesser extent, islands within a substrate (Appendix 2.3, Fig. S2.9-S2.10). More dominant microbial families were shared between macroalgae, turf, and water than were shared with corals. Within a substrate type, dominant families differed the most between islands in the coral samples. *Montipora* corals from Mangareva contained a total of 416 microbial families, but had 10 dominants: Alteromonadaceae, Colwelliaceae, Endozoicomonadaceae, Flavobacteriaceae, Midichloriaceae, Pseudoaltermonadaceae, Rhodobacteriaceae, Saccharosprillaceae, Saprospiraceae, and Vibrionaceae. Community dominance was distributed relatively evenly among these families, with Pseudoaltermonadaceae as the most common family (Fig. S2.9). In contrast, massive *Porites* corals from Mo'orea were overwhelmingly dominated by Endozoicomonadaceae, which comprised on average 65 % of the microbial reads observed in these samples (with a median of 77 %). Vibrionaceae was the only

other family meeting the dominance criteria in Mo'orea corals (out of 420 families). Dominant families in the algae and water samples are described in Appendix 2 and Fig. S2.10.

Variation associated with site-level macroalgal cover and algal contact

Diversity responses. Within the coral samples, there was a significant interaction between local algal contact and site-level macroalgal cover for both Shannon diversity and Simpson diversity ($p = 0.016$, $p = 0.001$, Table S2.6). Shannon (D^1) and Simpson diversity (D^2) of coral microbiomes increased with site-level macroalgal cover in corals that were *not* in local contact with algae (Fig. 2.2a-d). In contrast, Simpson and Shannon diversity slightly declined with site-level macroalgal cover in corals that were in contact with macroalgae or turf (Fig. 2.2a,e). Thus, algal contact caused alpha diversity to increase in corals when site-level macroalgae cover was low (below ~ 30%) and decrease when site-level cover was high (above ~ 40% cover), when both considering rare and dominant taxa (Shannon diversity), vs. primarily dominant taxa (Simpson diversity). In addition, both alpha diversity metrics differed between islands ($p = 0.002$, < 0.001, respectively), with corals from Mangareva having higher microbial alpha diversity than corals from Mo'orea. In contrast to coral microbiomes, the alpha diversity of microbial communities associated with macroalgae, turf, and water were unresponsive to variation in site-level macroalgal cover (Fig. S2.7, Table S2.7).

Beta dispersion, or variability in the microbial communities among individual samples, generally responded similarly to alpha diversity (Tables S2.6, S2.7). For the coral samples, there was a significant interaction between local algal contact and site-level macroalgal cover ($p = 0.028$). Beta dispersion trended lower in corals from Mo'orea ($p = 0.054$), but increased with site-level macroalgae cover for corals that were not in contact with algae at both islands (Fig. 2.2e,f). Dispersion declined with site-level macroalgae in corals contacting turf and macroalgae.

In contrast to the coral samples, microbial beta dispersion in macroalgae, turf, and water column samples did not respond to site-level macroalgal cover (Fig. S2.7, Table S2.7).

Community composition. Coral microbiome composition responded differently to site-level macroalgal cover and local algal contact at each island (Tables S2.9). At Mangareva, coral microbiomes shifted with macroalgal cover at (PERMANOVA: $p = 0.002$, $R^2 = 0.07$) when compared at the ASV-level. However, family-level analyses of microbiomes from Mangareva showed interactive effects of algal contact and macroalgal cover ($p = 0.03$, $R^2 = 0.07$). In contrast, macroalgal cover and algal contact were not associated with significant changes in microbiome composition in corals from Mo'orea (Table S2.9).

The microbiomes of macroalgae (ASV-level) were associated with site-level macroalgal cover at both islands. However, family-level analyses of microbiomes of macroalgae only varied with macroalgal cover at Mo'orea (Table S2.9). Turf microbiomes (ASV- and family-level) varied with macroalgal cover at Mo'orea, but not at Mangareva (Table S2.9). In contrast, water microbiomes did not vary with macroalgal cover at either island when compared at the ASV- or family-level (Table S2.9).

Viewed in the same NMDS ordination space, the microbiome composition of coral samples tended to become more similar to the macroalgae samples as site-level macroalgae increased at Mangareva (Fig. 2.4a-c). This was especially obvious for corals not in contact with algae (orange circles) along the primary axis distinguishing the coral from the macroalgae samples (NMDS1; Fig. 2.4d). However, this effect was much weaker at Mo'orea, which generally had lower macroalgal cover and fewer samples (Fig. 2.4e-g). To test these changes in the similarity of coral vs. macroalgae microbiomes, we fit linear mixed effect models with site as a random factor and macroalgal cover and sample-contact type (either macroalgae, coral-no

contact, coral-turf contact, or coral-macroalgae contact) as fixed-effect predictors of NMDS1 in the coral and macroalgae samples at each island. Mangareva samples demonstrated a significant macroalgal cover x sample-contact type interaction, ($p = 0.024$; Table S2.11), with the position of coral samples on NMDS1 becoming more negative (and, thus, closer to the macroalgae samples) as macroalgal cover increased, for corals with no algal contact and macroalgal-contact (Fig. 2.4d). Corals with turf contact did not move closer to macroalgae samples on NMDS1 as macroalgal cover increased. In contrast, at Mo'orea, macroalgal cover was not an important predictor of the position of coral and macroalgae samples on NMDS1, although the sample-contact types did significantly differ ($p < 0.001$; Table S2.11). Corals with no algal contact were the farthest from the macroalgae samples on NMDS1, while corals with turf or macroalgae contact were closer on average (Fig. 2.4h). (However, these results must be interpreted cautiously, as NMDS ordination distances are not independent.)

Responses of specific microbial families. We used corncob analysis to identify which microbial families changed in coral samples in response to algal contact and site-level macroalgal cover at each island, and to estimate the magnitude of those effects (Figs. 2.5,6). We also compared the identified families to the dominant microbial families found on corals and other substrates (Fig. 2.S9, 2.S10) to explore potential mechanisms through which coral microbiomes changed in response to site-level algae, such as through the transfer of common algal microbes and their subsequent increase in the coral microbiome.

Within the coral microbial communities, the majority (85 %, or 34/40) of microbial families that were identified by the corncob analysis as changing in response to algae, did so at only one scale (either in response to algal contact, or in response to site-level macroalgal cover). Only 6/40 families changed in response to both factors. Additionally, the families that responded

to algae at each scale differed between islands. Within an island, most families (67 and 75 % at Mangareva and Mo'orea, respectively) exhibited the same directional response to contact with turf vs. macroalgae, although approximately one third (5 out of 15) of the responses for one algal contact group were not significant (as inferred from their 95 % CI intervals). Four of the 6 families that responded to algae at both scales responded in the same direction at both scales.

Most of the families identified in the corncob analysis as showing responses to algae were not dominant members of the corals' microbiomes. The notable exceptions were Endozoicomonadaceae and Saprospiraceae, which declined with algal contact and site-level macroalgal cover, respectively (Fig. 2.5a, 2.6a). However, 33 % of the families that increased with either algal contact group (turf or macroalgae) or with macroalgal cover were a dominant family in either the turf or macroalgae microbiomes for that island, e.g., Cellvibrionaceae, Cyclobacteriaceae, Rubritalaceae, Nostocaceae, and Hyphomonadaceae (Fig. 5,6), suggesting that algae were a source of these microbes. Additionally, several oligotrophic taxa that were dominants in the water samples declined with algal contact at Mo'orea (e.g., SAR11 SAR86, and Cyanobiaceae, the latter of which contained sequences from the genera *Synechococcus and Prochlorococcus*).

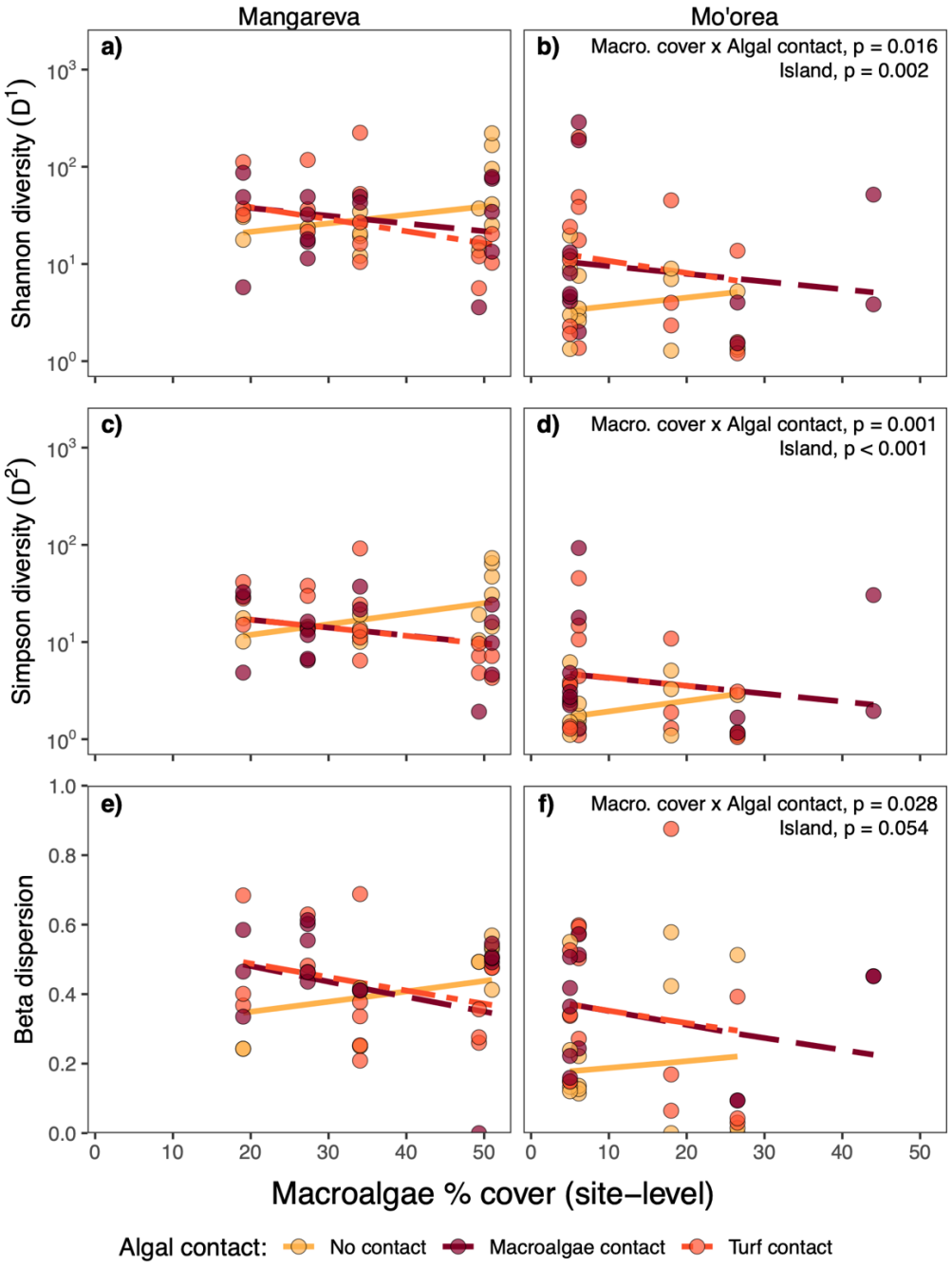

Fig. 2.2) Coral microbial community (ASV-level) alpha and beta diversity vs. site-level percent cover of macroalgae (macro. cover), for corals from each island (columns) and algal contact group (colors). a, b) Hill number for q = 1, i.e., Shannon diversity. c, d) Hill number for q = 2, i.e., Simpson diversity. e, f) Beta dispersion. Points represent the observed values for individual coral samples, with predicted regression lines based on the estimated model coefficients back-

transformed to the original scale. Statistically significant predictors are indicated for each response variable. (Significant main effects are not shown if the interaction is significant; results are summarized in Table S2.6). The y-axis for a-d is on a $\log_{10}$ scale.

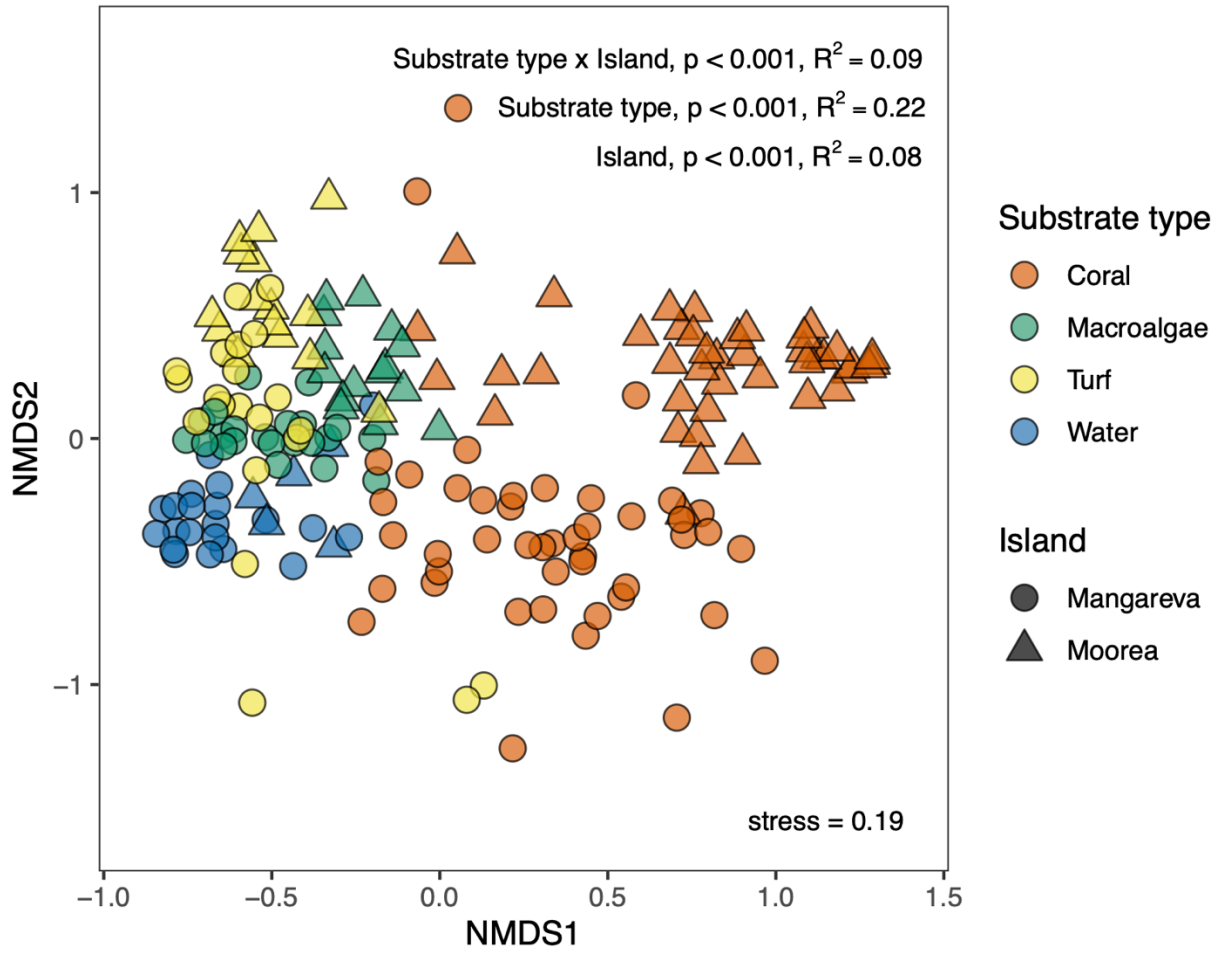

Fig. 2.3) NMDS ordination based on weighted Bray Curtis dissimilarity of microbial community composition (ASV-level) associated with different substrate types (colors) from two islands (shapes). Coral samples include corals from all three algal contact groups (in contact with macroalgae or turf, or not in contact with algae). PERMANOVA results are shown, with additional details in Table S2.8.

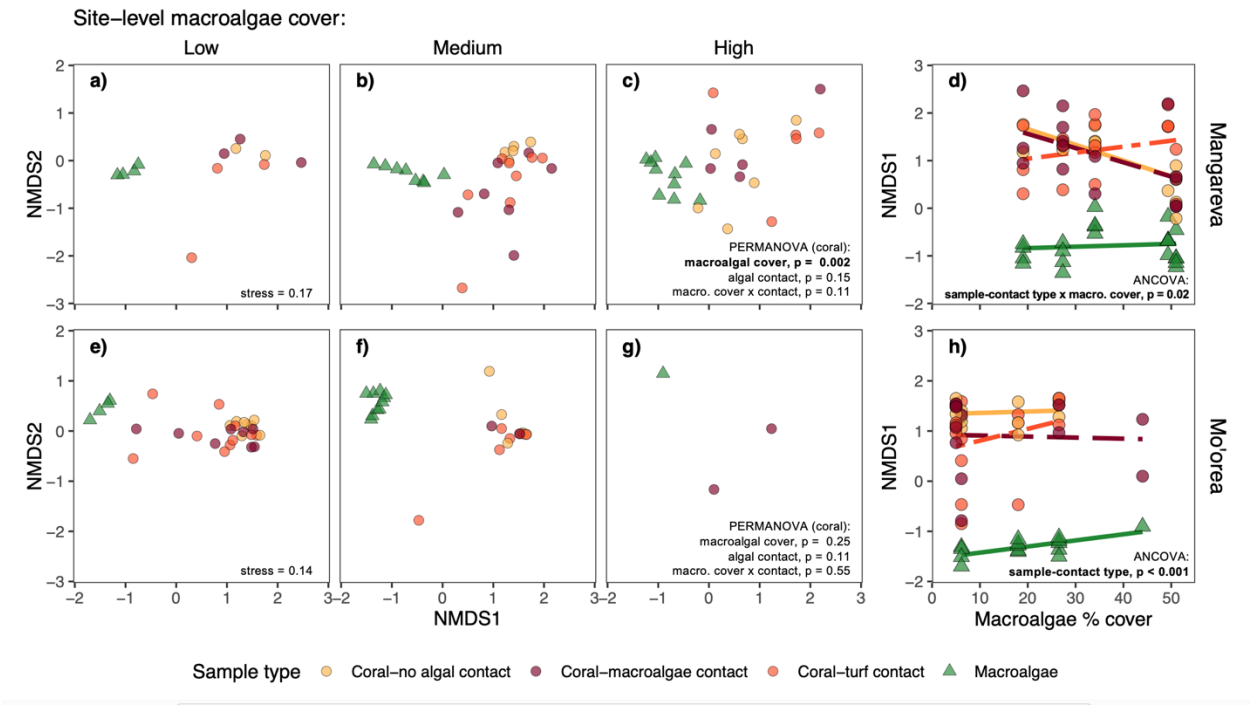

Fig. 2.4) NMDS ordination based on Bray-Curtis dissimilarity of ASV-level microbial communities associated with macroalgae (green triangles) and coral (orange, red circles) samples from Mangareva (a,b,c) and Mo'orea (e,f,g), compared across different levels of site-level macroalgal cover: Low (0-20 %), Medium (20- 35 %), and High (> 35 %). Local algal contact groups for the coral samples are indicated by the color of each circle. Macroalgal bins are used here for visualization purposes only; PERMANOVA used continuous macroalgal cover values (p-values on graph, full results in Table S2.9, S2.10). d,h) The position of samples along NMDS1 (the major axis distinguishing coral from macroalgae samples) vs. site-level macroalgal cover (p-values for significant predictors on graph, full results in Table S2.11).

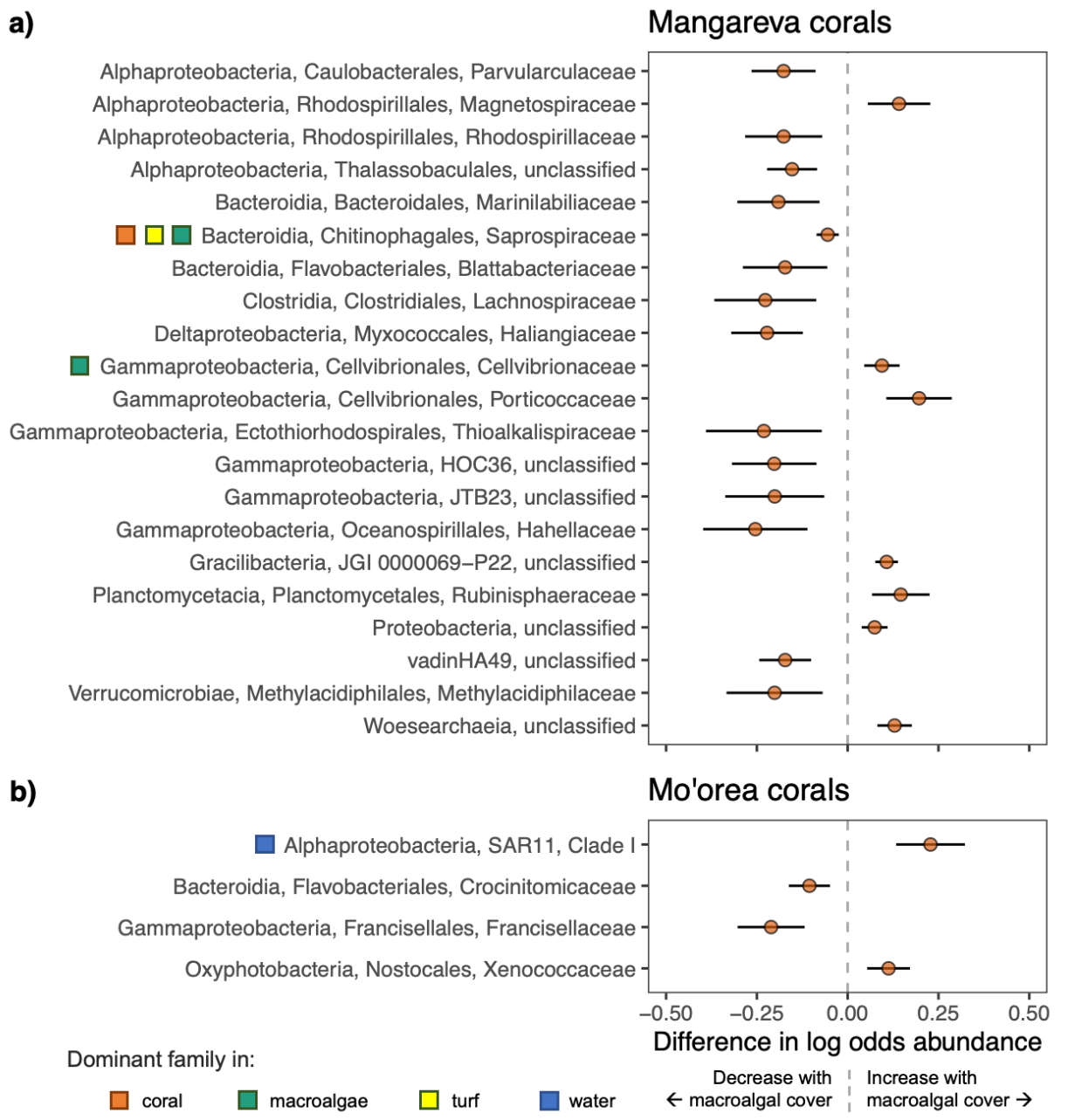

Fig. 2.5) Microbial families in the coral samples that had changes in abundance associated with site-level macroalgal cover, controlling for the effect of local algal contact, at a) Mangareva and b) Mo‘orea. Positive numbers indicate taxa that increased with site-level macroalgal cover, negative numbers indicate taxa that declined with macroalgal cover. Points represent coefficient estimates for each taxon; error bars represent 95 % CI. Squares identify microbial taxa that were

a dominant family in one or more of the substrate types (coral, turf, macroalgae, or water, indicated by the square color). Taxa are organized alphabetically by class, order, family.

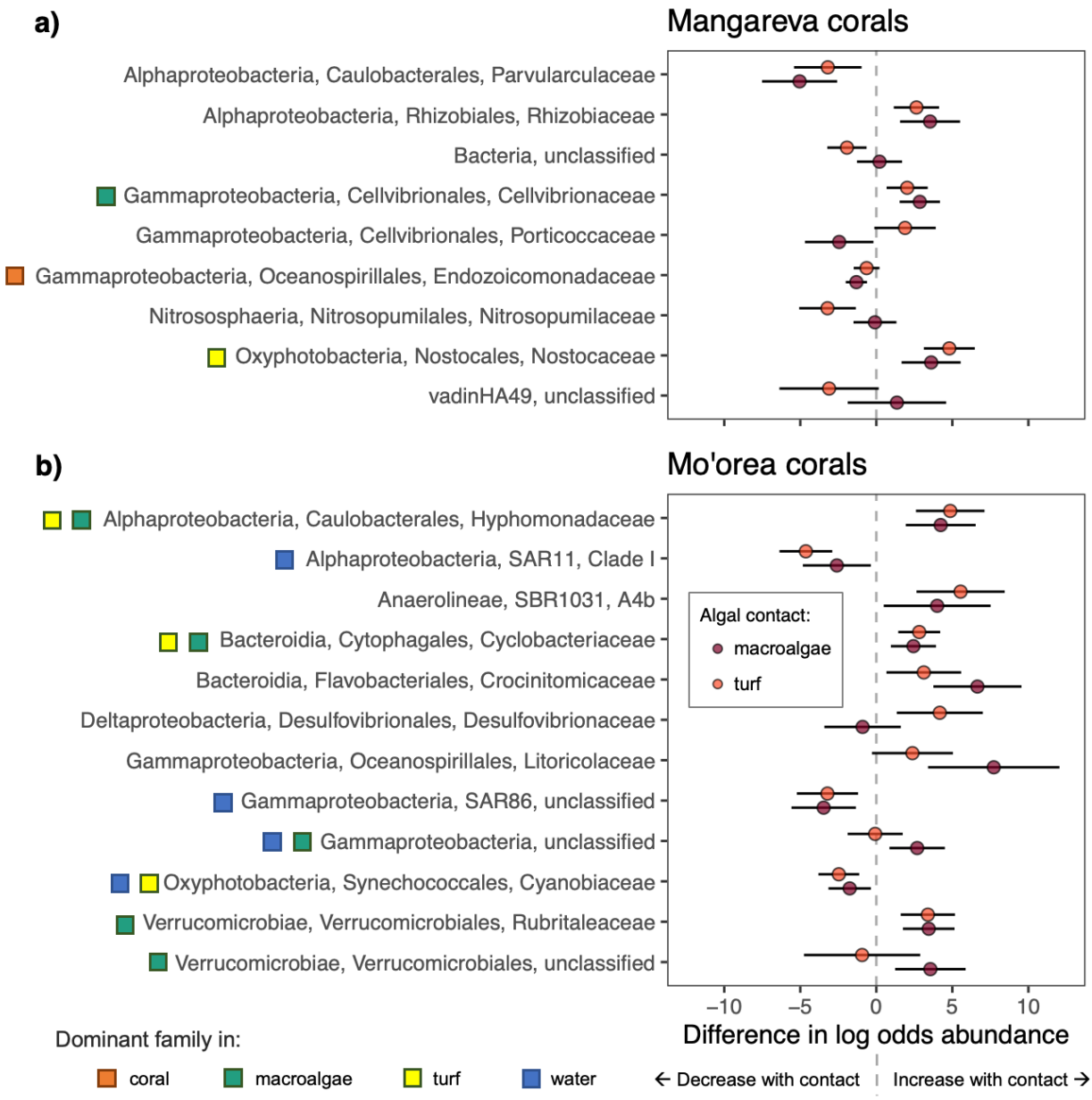

Fig. 2.6) Microbial families at each island that changed in response to local algal contact, controlling for the effect of site-level macroalgal cover. Changes in taxa are shown for coral in contact with macroalgae or turf (indicated by the color of the points) relative to coral samples not in contact with algae. Positive numbers indicate a family increased with algal contact, negative numbers indicate a family declined with algal contact. Points represent coefficient estimates for each taxon; error bars represent 95 % CI. Squares identify microbial taxa that were a dominant

family in one or more of the substrate types at that island (coral, turf, macroalgae, or water, indicated by the square color). Taxa are organized alphabetically by class, order, family.

Discussion

Previous studies have observed within-species variation in the microbiomes of corals from different geographic locations (Morrow et al. 2012, Dunphy et al. 2019). Our results suggest that site-level macroalgal cover could help explain those differences. Coral microbiomes showed shifts in alpha and beta diversity with site-level macroalgal cover, with the direction and magnitude of these changes depending on local algal contact. Specifically, when corals were in contact with algae, the alpha and beta diversity of their microbial communities declined with site-level macroalgal cover (for alpha vs. beta diversity, respectively). In contrast, alpha and beta diversity increased with site-level macroalgal cover in corals that were *not* in contact with algae. This statistical interaction was more extreme than a simple antagonism: it caused the direction of the effects of algal contact to switch from negative to positive at different levels of site-level macroalgal cover (Fig. 2.2). These results indicate that corals free from local competitive interactions with algae could still be affected by algae present elsewhere at a reef site. They also reveal that it is difficult to predict how coral microbiomes will respond to competitive interactions with algae without also considering algal densities at a larger (e.g., site-level) spatial scale, which could contribute to why studies have reported increases (Zaneveld et al. 2016, 2017), decreases (Vega Thurber et al. 2012a, Morrow et al. 2017), and no effect (Clements et al. 2020) of algal contact on coral microbiome alpha and beta diversity.

The interactive effects of site-level macroalgal cover and local algal contact on coral microbiome alpha diversity (Fig. 2.2a-d) could be the net effect of several processes that potentially increase the establishment of new microbes in the coral microbiomes and/or alter the growth of specific microbes within the microbiome. For example, if the densities of microbes in the water column increase as site-level algae increases (as in Haas et al. 2016), this could

increase the rate at which microbes colonize corals. If this immigration rate was sufficiently high, it could overwhelm the ability of corals to regulate their microbiome, leading to the addition of new taxa and thereby increasing the alpha diversity of the coral microbiome. Conversely, algae could change abiotic conditions (e.g., increase dissolved organic material or allelochemicals, or reduce pH, O_2, etc.), and this could increase the growth of some microbial taxa (particularly copiotrophs and potential pathogens), and decrease the growth of others (Paul et al. 2006, Nelson et al. 2013, Haas et al. 2016). When these changes in abiotic conditions are large enough, such as when corals are in contact with algae at sites with high algal cover, fast-growing, algae-tolerant microbes might be able to proliferate and displace taxa associated with the healthy coral microbiome, thereby reducing microbiome alpha diversity. Therefore, the antagonistic effects of local and regional algae on coral microbiome alpha diversity are likely a balance between diversity-decreasing processes associated with altered microbial growth and diversity-*increasing* processes associated with enhanced colonization. Furthermore, because increased site-level macroalgal cover generally did not increase alpha diversity for corals contacting algae (Fig. 2.2a-d), our results suggest that algal contact alone saturates the diversity-increasing process associated with colonization.

Changes in coral microbiome beta diversity mirrored those observed for alpha diversity (Fig. 2.2a-d vs. 2.2e,f). This correspondence between alpha and beta diversity suggests that the taxa that caused increases in alpha diversity were variable among individual corals from the same site and algal contact group. Moreover, as site-level macroalgal cover increased, some coral microbiomes shifted towards the microbial community composition of the algae samples, while other coral microbiomes did not respond or shifted towards an entirely different community configuration, accentuating the among-coral variability (Fig. 2.4). Local algal

contact also tended to make coral microbial communities more variable (Fig. 2.2e,f), with some coral microbiomes becoming more like the algal microbial communities (Fig. 2.4). However, algal contact and site-level macroalgal cover exhibited antagonistic effects, much like they did with alpha diversity (Fig. 2.2e,f). This antagonistic effect of local algal contact and high site-level macroalgal cover on beta diversity could occur through a similar mechanism as we proposed for alpha diversity. Specifically, increases in microbial densities due to algae might facilitate opportunistic colonization of the coral microbiome by new taxa, with successful colonists differing among individual corals (thereby increasing beta diversity). However, large shifts in abiotic conditions created by algal contact *and* high site-level algal cover could reduce the number of microbial taxa capable of persisting in the coral microbiome, causing the microbiomes of individual corals to become more similar to one another. Independent of the mechanism, our results support the conclusion that algae tend to shift coral microbiomes to be more similar to algal microbiomes (Morrow et al. 2013), although a coral's microbiome response to algae can be highly variable (Zaneveld et al. 2016, 2017).

Together, our results suggest a new model of algal effects not anticipated in Fig. 2.1. Assume that we can convert the exposure of corals to algae by combining the local and regional effects into a single aggregate measure of exposure. Our results then suggest a unimodal (hump-shaped) relationship between coral microbiome diversity and the coral's exposure to algae (Fig. 2.7). The initial increasing part of this curve could represent the range over which enhanced microbial colonization due to increasing algal exposure dominates the external processes driving microbiome diversity. Corals with no algal contact but with low to high regional algal cover could fall within this range. In contrast, the declining part of the curve could represent the algal exposure over which fast-growing taxa proliferate and begin to take over the coral, such as for

corals with algal contact and/or from sites with very high macroalgal cover. This relationship could generate the antagonistic patterns of interaction we observed between local and regional effects. The slopes of the increasing and decreasing portions of this curve and the location of the peak could depend on a coral taxon's innate ability to modulate its microbiome and/or resist invasion of new microbial taxa, as well as site-specific conditions such as water flow, nutrient levels, and other factors that might influence microbial dispersal and colonization as well as species sorting processes within the microbiome (e.g., growth, competition, and survival).

Specific microbiome changes

Although there was variability in the microbial community composition of corals, some taxa changed predictably with site-level macroalgal cover or algal contact. Generally, site-level macroalgal cover caused smaller changes in the relative abundances of specific microbes compared to the effects of contact with algae at both islands (Fig. 2.5 vs. Fig. 2.6). Additionally, fewer of the microbial taxa that increased with site-level macroalgal cover were common in the algae samples (~ 11 %), whereas approximately half of the families that increased in a least one algal contact group were dominant families in the turf or macroalgae samples. One explanation for this pattern is that direct algal contact might transfer more microbes to corals than are transferred via the water column, or more greatly change abiotic conditions and/or increase the concentration of the algal by-products that influence microbial growth. This could occur because algae-associated microbes and by-products are likely diluted in the water column relative to the interaction zones between corals and algae. Additionally, biotic and abiotic filtering in the water column could further reduce densities of algae-associated taxa before they colonize corals. Together, these processes could reduce the site-level effects of algae relative to the direct local effects of algal contact.

Several families that declined in response to either local or site-level algae were potentially beneficial symbionts that have been suggested to have coevolved with corals, including Hahellaceae and Endozoicomonadaceae (order Oceanospirillales), and Haliangiaceae (order Myxococcales) (Pollock et al. 2018). Hahellaceae and Endozoicomonadaceae are believed to be important for nutrient acquisition and cycling within corals (Neave 2016), and have previously been found to decline in stressed corals (McDevitt-Irwin et al. 2017, Brown et al. 2019). Haliangiaceae is in an order of predatory bacteria that is correlated with disease resistance in corals (Rosales et al. 2019). Interestingly however, Endozoicomonadaceae declined in *Montipora* from Mangareva but not in *Porites* from Mo'orea, in which it was the most abundant microbial family (Fig. S2.9). This could indicate a tighter relationship between the coral host and this microbial taxa in *Porites* compared to *Montipora*, making it more resistant to change. Other families of potential importance to the coral holobiont that declined with regional algal cover included Thalasobacculales and Rhodospirillaceae, which are positively associated with certain species of Symbiodinaceae (Quigley et al. 2020). Rhodospirillaceae is considered oligotrophic, and a previous study found that water column Rhodospirillaceae was associated with site-level coral cover (Haas et al. 2016). Therefore, Rhodospirillaceae might decline in the water column as increasing regional algal cover increases water column DOC (Haas et al. 2016), which could subsequently reduce its incorporation into the microbiome of corals. Several other oligotrophic taxa that were dominants in the water samples (SAR11 Clade I, SAR86, and Cyanobiaceae) also declined with algal contact (Fig. 2.6), suggesting a similar mechanism related to DOC produced by algae.

As these potentially beneficial taxa declined in the coral microbiomes in response to local algal contact or increasing regional macroalgal cover, other taxa increased. Increasing taxa

included five of the most abundant microbial families found in the algae samples (Cellvibrionaceae, Cyclobacteriaceae, Rubritalaceae, Nostocaceae, and Hyphomonadaceae), suggesting algae acted as a source for these taxa. Other families that increased with local or site-level algae included taxa that are enriched in corals stressed by disease or environmental factors, including several associated with stony coral tissue loss syndrome (Rubritalaceae, Cyclobacteriaceae, and Rhizobiaceae: (Meyer et al. 2019, Rosales et al. 2020), and one associated with black band disease and elevated water temperature (Desulfovibrionaceae: (Frias-Lopez et al. 2002, Viehman et al. 2006, Rice et al. 2019). Desulfovibrionaceae and Rhizobiaceae are common in marine sediments (Rosales et al. 2020) . Since both turf and macroalgae readily capture sediments (Airoldi and Virgilio 1998, Nugues and Roberts 2003, Stamski and Field 2006), algae may encourage the introduction of sediment-associated microbes to coral microbiomes.

Our results indicate that functionally and taxonomically distinct types of algae can have qualitatively similar effects on coral microbial communities. Both macroalgae and turf had local effects on coral microbiomes, shifting their alpha and beta diversity and the abundance of many microbial families in similar directions. Site-level macroalgal cover also influenced diversity and composition of microbiomes. However, results were not consistent between islands for all microbiome responses. For example, site-level macroalgal cover had weaker effects on microbiome composition at Mo'orea relative to Mangareva (Fig. 2.4). This difference could have been caused by the smaller range of site-level macroalgal cover at Mo'orea (and lower statistical power due to the loss of many samples from the highest macroalgal cover site). Additionally, differences in the coral and algae species at both islands could have influenced the magnitude of the response. Some research suggests that microbial communities in *Montipora*

vary more across environmental gradients than microbial communities in *Porites* (Tong et al. 2020). Similarly, previous work has found that *Dictyota* has stronger local effects on coral microbiomes than other fleshy algae (Zaneveld et al. 2016). Thus, we might have expected that the *Montipora* corals sampled at Mangareva would show larger effects of local and regional algae than *Porites* corals at Mo'orea, especially for corals in contact with macroalgae. However, since both macroalgae and coral species differed at each island, we cannot disentangle how the identities of each of these competitors affected the magnitude of the observed responses. Despite the lack of consistency in the response of microbiome composition between islands, alpha and beta diversity showed similar patterns between islands (Fig. 2.2), suggesting that the interactive effect of local and site-level algae on these diversity metrics (e.g., Fig. 2.7) might be robust across many coral and algae combinations.

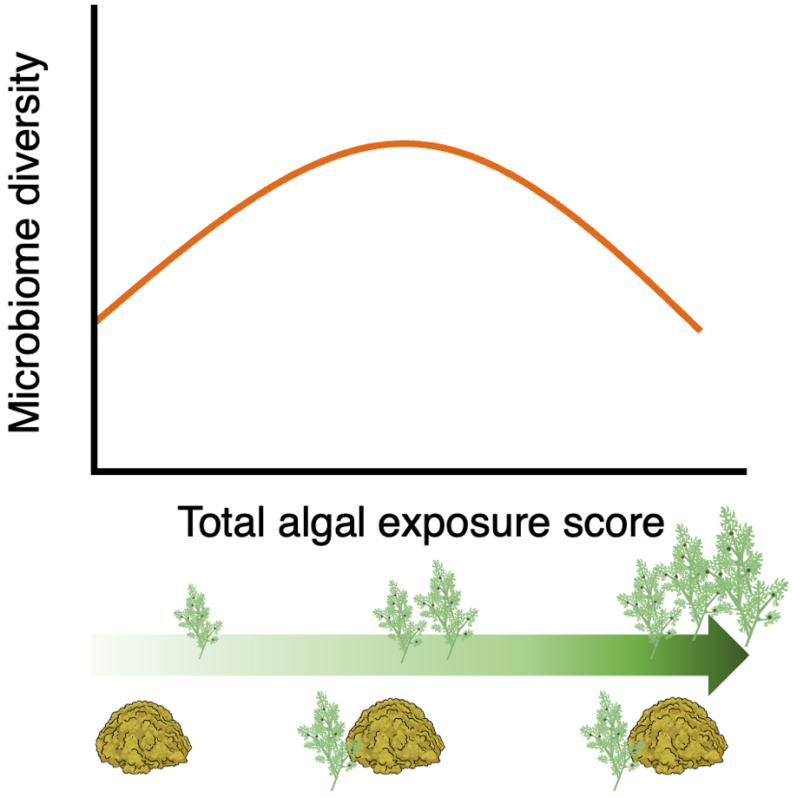

Fig. 2.7) Hypothetical response of microbiome diversity (alpha or beta) to the overall exposure of corals to algae at both local (e.g., contact) and regional (e.g., site-level algal cover) spatial scales (total algal exposure score). Corals at sites with low macroalgal cover and not in contact with algae have the lowest exposure, while corals in contact with algae at sites with high algal cover have the highest exposure score.

Conclusions

Overall, our results indicate that algae alter coral microbial communities at both local and site-level scales, altering their community composition, alpha and beta diversity, and disrupting important microbial associations with putatively beneficial symbionts, while simultaneously promoting disease and stress-associated taxa. Despite these changes, none of the corals that we sampled were visibly diseased or unhealthy. The greater-than-antagonistic effects of local and regional algae on coral microbiome alpha and beta diversity agree with other multi-stressor studies on corals (McDevitt-Irwin et al. 2019, Maher et al. 2019), which suggests that coral microbiomes might be constrained in the amount to which they can change without resulting in serious negative health consequences for the host. Thus, although shifts in microbiome community characteristics like alpha and beta diversity may be indicative of stress, it may be difficult to use these responses as direct estimates of the total amount of stress that a coral is experiencing, particularly when multiple stressors are simultaneously affecting the coral.

Many of the microbes that we observed changing are known to have ecological and physiological importance to the coral. As a result, our work suggests that algae could alter coral microbiome function, and therefore modify the resilience of corals to environmental stressors. Both local algal contact and increased regional algal cover appeared to make coral microbial communities more similar to the communities on algae, and algal contact increased the abundance of many dominant algae-associated microbes in corals, while simultaneously reducing the abundance of several beneficial microbial symbionts. These changes could reduce the beneficial functions performed by the coral microbiome, making corals more vulnerable to competition with algae and potentially less resilient to additional environmental stressors. Theoretical and empirical work suggests that coral reefs might exhibit bistability of coral vs.

algal-dominated states partially mediated by the strength of coral-algal competition (Mumby et al. 2007, Leemput et al. 2017, Schmitt et al. 2019). Consequently, the microbiome shifts that we observed could result in stronger feedbacks between corals and algae than expected, reducing the size of the perturbation needed to shift a reef to an algal dominated state and increasing the likelihood of bistability in coral algal-systems, making it more difficult to return to a coral-dominated state once a reef switches to algal dominance. It is important, however, to keep in mind that our work was observational; therefore, the patterns that we documented could have been caused not by algae, but by factors that were correlated with algal cover or algal contact. Future experimental work is needed to examine causation, unravel the interactions between local and regional algae on microbial community functions, and determine whether there are specific thresholds of regional algal cover or coral microbial community change that result in predictable shifts in coral health.

Acknowledgements

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Appendix 2

Hypothetical responses of coral microbiomes to algal contact and regional algae density

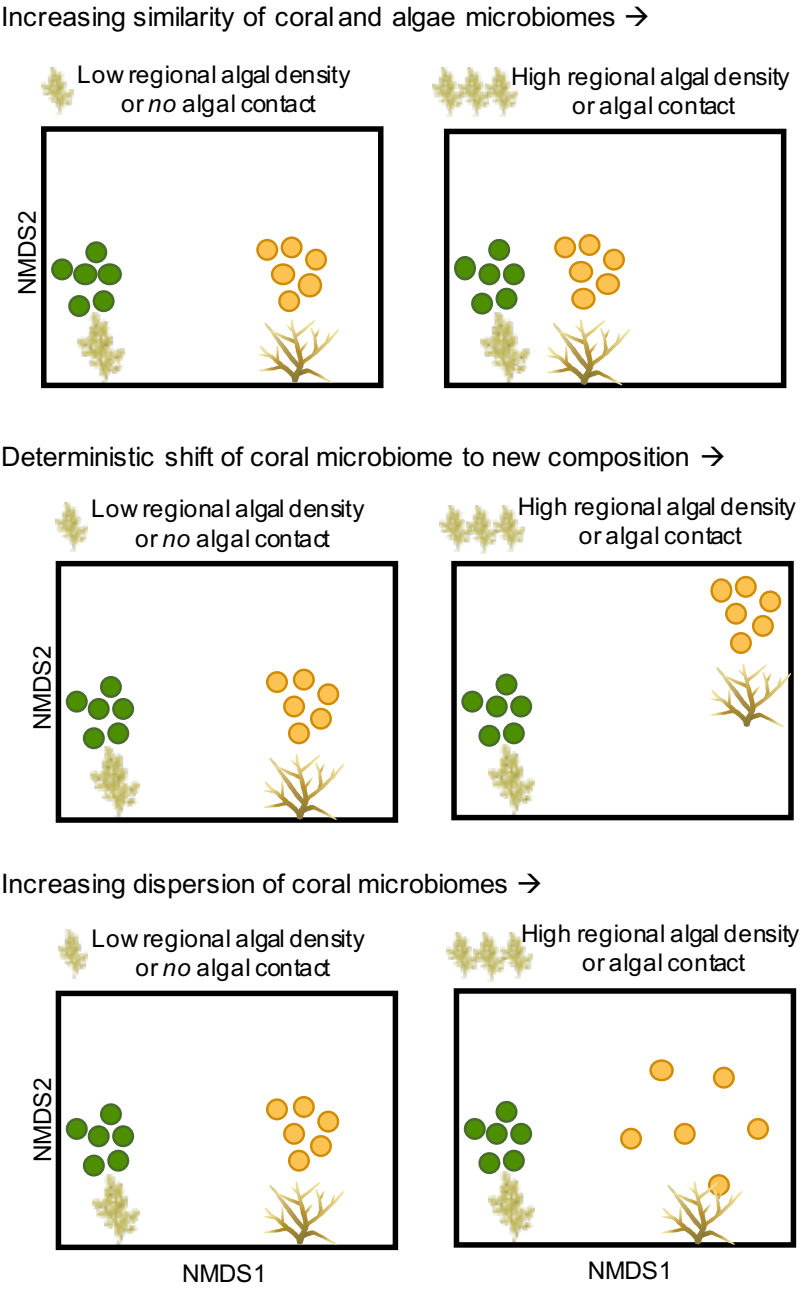

Fig. S2.1) Three hypothetical responses of coral microbial community composition in response to local contact and regional algal density. Points indicate individual coral (orange) or algae (green) samples.

Study site locations

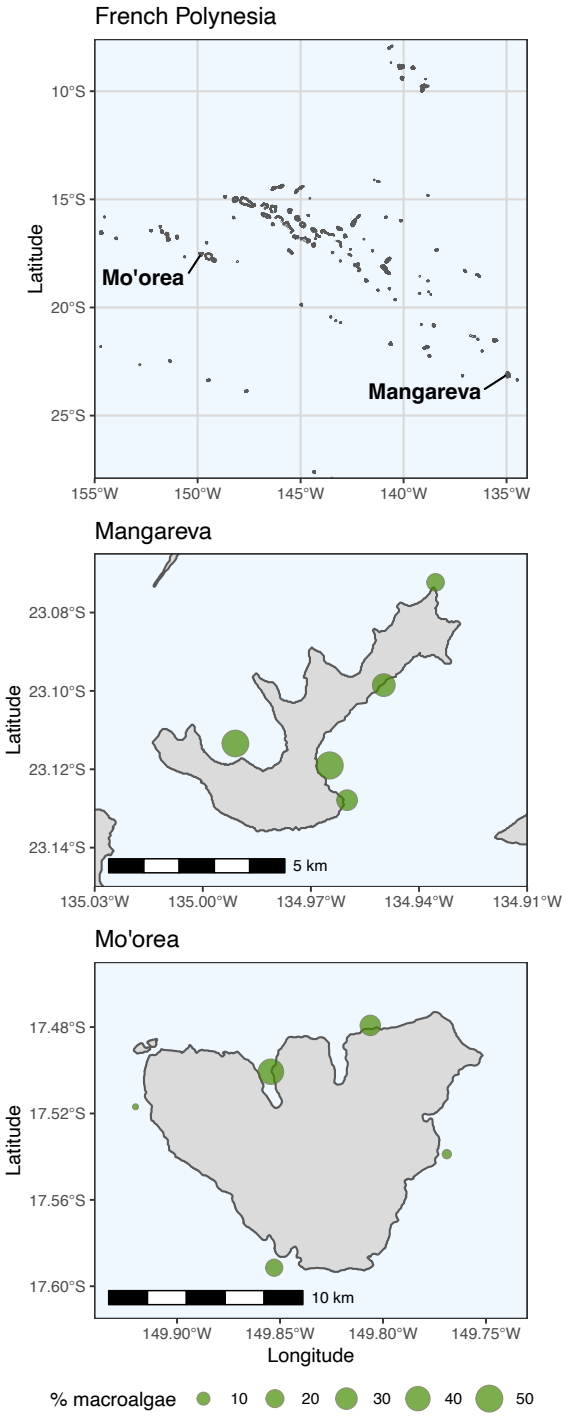

Fig. S2.2) Maps of the study locations. Green points represent the study sites at each island, with the size of the point indicating the mean percent cover of macroalgae at that site.

Table S2.1) GPS coordinates of the study sites, given in decimal degrees.

Island	Site	Latitude	Longitude
Moorea	S1	-17.539156	-149.769194
	S2	-17.591420	-149.852826
	S3	-17.479221	-149.805810
	S4	-17.516918	-149.920157
	S5	-17.500680	-149.854430
Mangareva	S6	-23.127884	-134.959954
	S7	-23.072238	-134.935416
	S8	-23.098522	-134.949773
	S9	-23.119028	-134.964790
	S10	-23.113417	-134.990953

Bioinformatics processing - Appendix 2.1

The bioinformatics filtering parameters we used in the DADA2 v. 1.16 (Callahan et al. 2016) pipeline were: filterAndTrim(fnFs, filtFs, fnRs, filtRs, truncLen=c(150,150),maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE,compress=TRUE, multithread=TRUE).

References

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016 DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. (doi:10.1038/nmeth.3869)

Sample size summary

Table S2.2) Sample size summary for each site and substrate type group (algae and water samples) or site and local algal contact group (coral samples) after removing samples that had low read depth or were lost due to equipment error.

Island	Site	Substrate type	Local contact	n
Moorea	S1	Coral	Coral-macroalgae contact	3
Moorea	S1	Coral	Coral-no contact	4
Moorea	S1	Coral	Coral-turf contact	5
Moorea	S1	Macroalgae	NA	4
Moorea	S1	Turf	NA	5
Moorea	S1	Water	NA	0
Moorea	S2	Coral	Coral-macroalgae contact	0
Moorea	S2	Coral	Coral-no contact	3
Moorea	S2	Coral	Coral-turf contact	3
Moorea	S2	Macroalgae	NA	5
Moorea	S2	Turf	NA	5
Moorea	S2	Water	NA	1
Moorea	S3	Coral	Coral-macroalgae contact	2
Moorea	S3	Coral	Coral-no contact	3
Moorea	S3	Coral	Coral-turf contact	3
Moorea	S3	Macroalgae	NA	5

Moorea	S3	Turf	NA	3
Moorea	S3	Water	NA	2
Moorea	S4	Coral	Coral-macroalgae contact	5
Moorea	S4	Coral	Coral-no contact	4
Moorea	S4	Coral	Coral-turf contact	5
Moorea	S4	Macroalgae	NA	0
Moorea	S4	Turf	NA	0
Moorea	S4	Water	NA	0
Moorea	S5	Coral	Coral-macroalgae contact	2
Moorea	S5	Coral	Coral-no contact	0
Moorea	S5	Coral	Coral-turf contact	0
Moorea	S5	Macroalgae	NA	1
Moorea	S5	Turf	NA	2
Moorea	S5	Water	NA	2
Mangareva	S6	Coral	Coral-macroalgae contact	5
Mangareva	S6	Coral	Coral-no contact	1
Mangareva	S6	Coral	Coral-turf contact	3
Mangareva	S6	Macroalgae	NA	4
Mangareva	S6	Turf	NA	4
Mangareva	S6	Water	NA	5
Mangareva	S7	Coral	Coral-macroalgae contact	3
Mangareva	S7	Coral	Coral-no contact	2
Mangareva	S7	Coral	Coral-turf contact	3

Mangareva	S7	Macroalgae	NA	4
Mangareva	S7	Turf	NA	4
Mangareva	S7	Water	NA	5
Mangareva	S8	Coral	Coral-macroalgae contact	2
Mangareva	S8	Coral	Coral-no contact	4
Mangareva	S8	Coral	Coral-turf contact	5
Mangareva	S8	Macroalgae	NA	4
Mangareva	S8	Turf	NA	5
Mangareva	S8	Water	NA	3
Mangareva	S9	Coral	Coral-macroalgae contact	4
Mangareva	S9	Coral	Coral-no contact	5
Mangareva	S9	Coral	Coral-turf contact	2
Mangareva	S9	Macroalgae	NA	5
Mangareva	S9	Turf	NA	5
Mangareva	S9	Water	NA	4
Mangareva	S10	Coral	Coral-macroalgae contact	1
Mangareva	S10	Coral	Coral-no contact	2
Mangareva	S10	Coral	Coral-turf contact	3
Mangareva	S10	Macroalgae	NA	5
Mangareva	S10	Turf	NA	3
Mangareva	S10	Water	NA	4

Benthic community composition

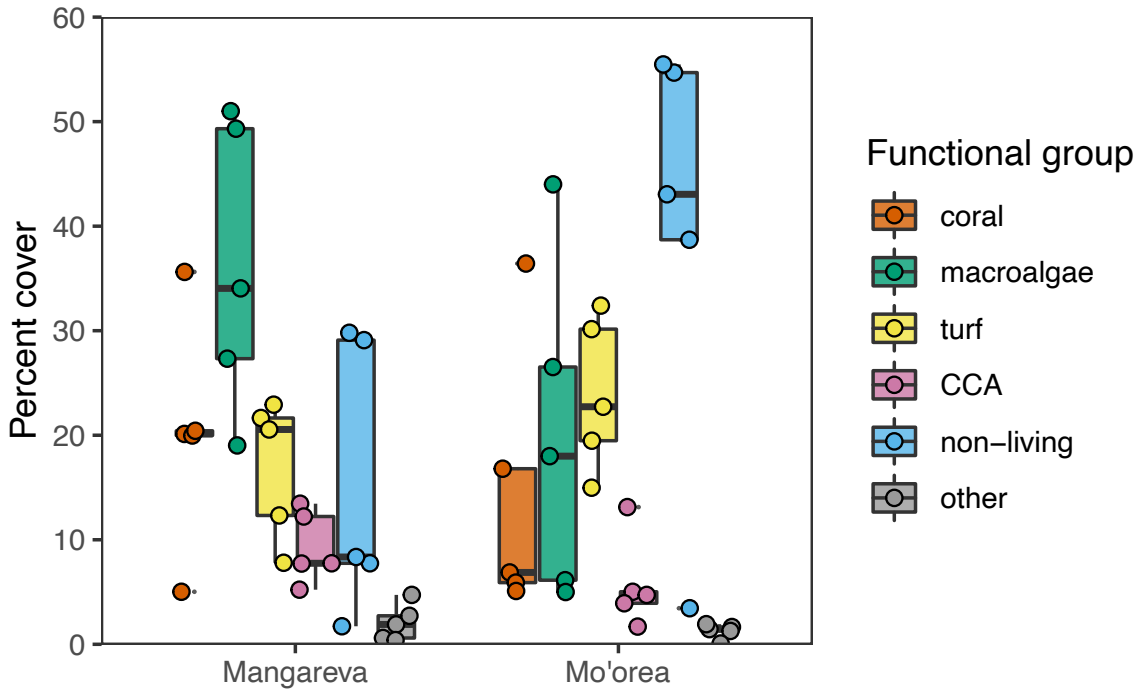

Fig. S2.3) Percent cover of main benthic functional groups for each island, based on site means (n = 5). Tukey's boxplots show the interquartile range and median relative abundance, with error bars (whiskers) indicating the 25th and 75th percentiles ± 1.5 x the IQR. Points indicate site means. Abbreviations: CCA = crustose coralline and peyssonnelid algae, non-living = sand, rubble, and bare pavement (exposed reef carbonate). "Other" includes all remaining functional groups, such as cyanobacteria, fire corals, soft corals, and all non-coral invertebrates.

Appendix 2.2 PCA methods

Principal components analysis (PCA) was conducted on centered and variance-standardized site-level benthic community composition (i.e., mean site-level percent cover of main benthic functional groups). Results indicated that sites were largely separated by variation in macroalgae and non-living substrate on the first principal component (PC1, which explained 45 % of the variance), and coral and turf algae on the second component (PC2, which explained 24 % of the variance; Fig. S2.4, Table S2.3).

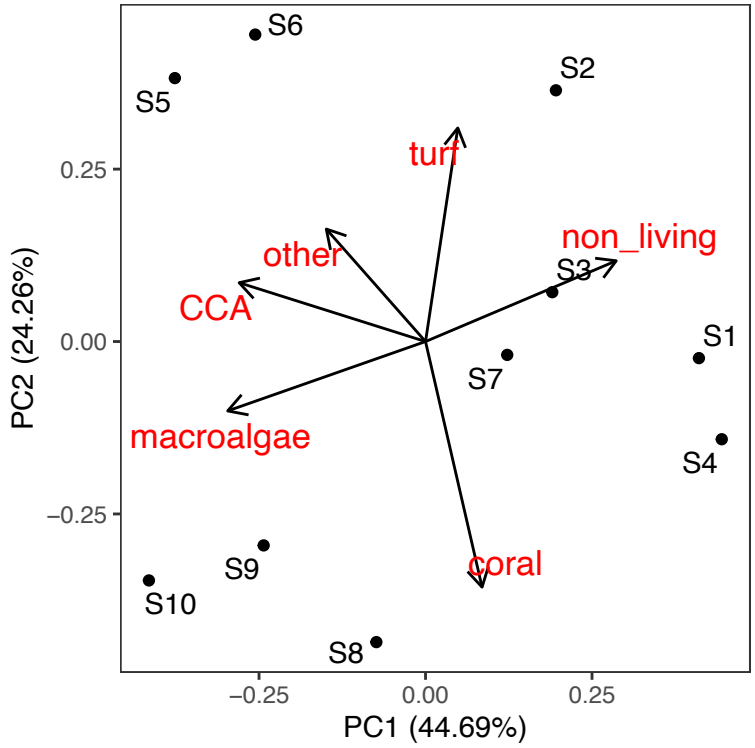

Fig. S2.4) Plot of principal components analysis of benthic community composition by site (points). Data was centered and standardized by variance before analysis.

Table S2.3) Loadings for the contributions of each benthic functional group to each of the first two PCA axes.

Functional group	**PC1**	**PC2**
coral	0.160	-0.672
macroalgae	-0.561	-0.190
turf	0.092	0.585
CCA	-0.529	0.161
non-living	0.541	0.221
other	-0.281	0.308

Sample read depth

Table S2.4) Summary of the read depth per sample for each substrate type.

Substrate type	mean	std. dev.	median	minimum	maximum
Coral	20116.92	23344.53	10070	101	101351
Macroalgae	56648.03	26549.47	65357	13132	100787
Turf	46072.64	34460.39	33480.5	115	153265
Water	105176.88	51462.96	117245	6149	204285

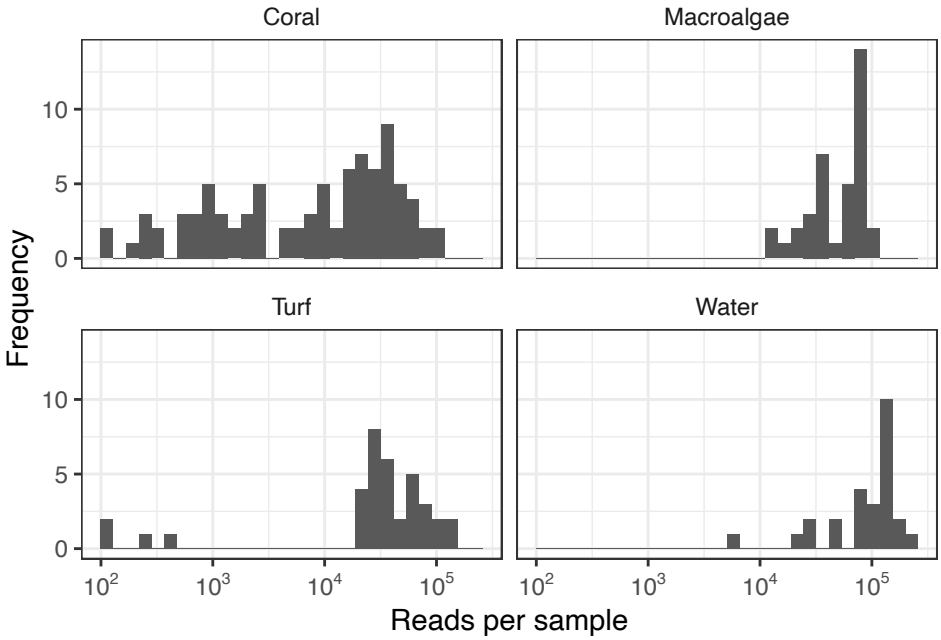

Fig. S2.4) Distribution of sample read depth (reads per sample).

Sample rarefaction curves

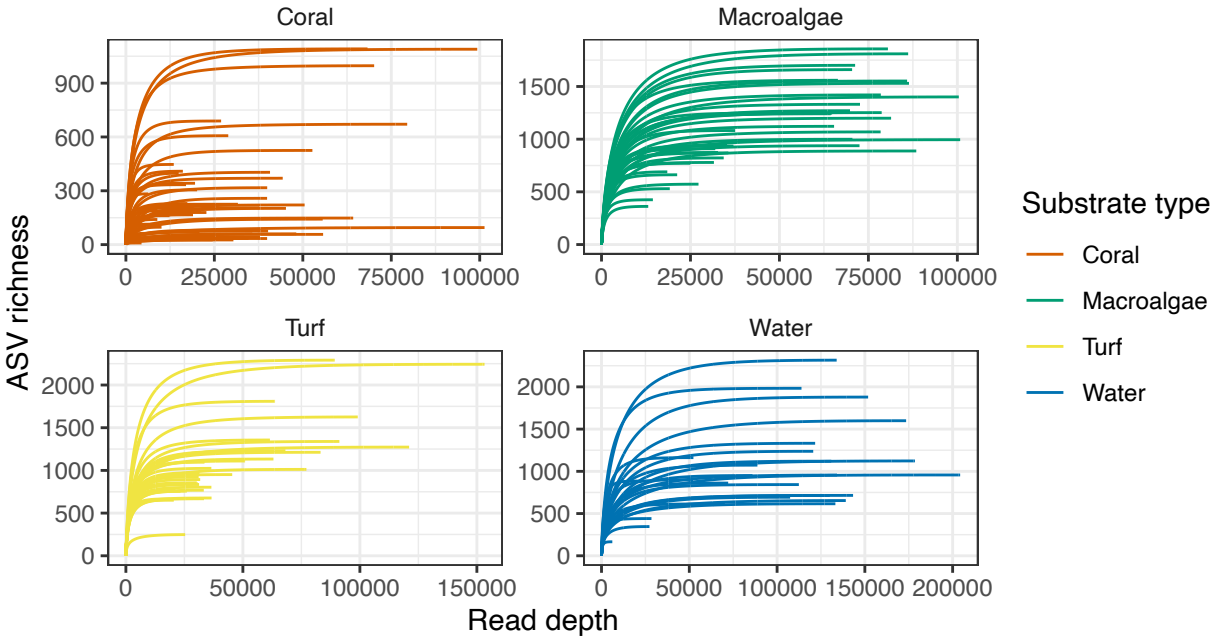

Fig S2.5) Rarefaction curves for each sample showing ASV richness for a given read depth.

Microbial diversity by substrate type and island

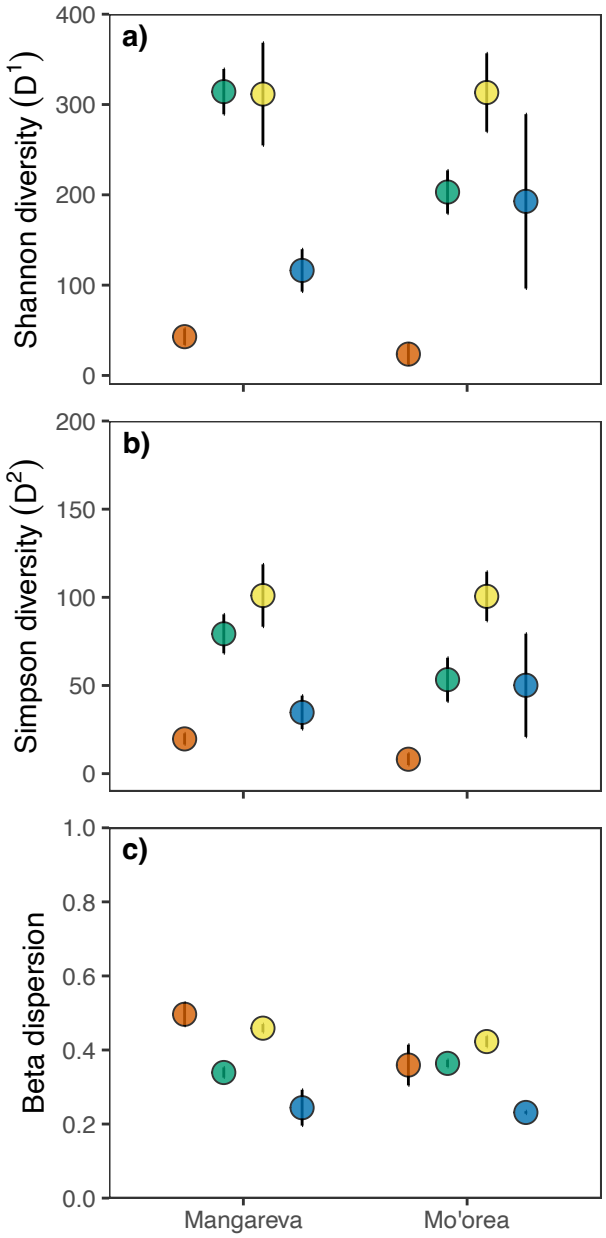

Fig. S2.6) Comparison of alpha and beta diversity of microbial communities (ASV-level) associated with different substrate types (colors) and islands (x-axis). **a)** Shannon diversity (D^1, i.e., the Hill number for q = 1). Coral estimates include both the coral samples in contact with algae and the corals not in contact.) **b)** Simpson diversity (D^2, i.e., the Hill number for q = 2). **c)** Beta dispersion, based on the weighted Bray Curtis dissimilarity of samples within each substrate

type at an island. Points represent group means, calculated from the means at each site (n = 5), and error bars represent SE. Statistical results are given in Table S5.

Table S2.5) Summary of statistical comparisons of microbial diversity (ASV-level) among substrate types (coral, macroalgae, turf, or water) and islands (Mangareva or Mo‘orea) using the data summarized in Fig. S3. P-values are estimates using Wald Chi-squared tests with Type III sums of squares. Models were fit to log-transformed Shannon diversity and Simpson diversity (the Hill numbers for q = 1 and q = 2, respectively).

Response	**Predictor**	**df**	X^2	**p-value**
Shannon diversity (D^1)	**island**	1	32.118	**< 0.001**
	substrate type	3	98.731	**< 0.001**
	substrate type x island	3	26.936	**< 0.001**
Simpson diversity (D^2)	**island**	1	73.295	**< 0.001**
	substrate type	3	64.231	**< 0.001**
	substrate type x island	3	36.990	**< 0.001**
Beta dispersion	**island**	1	16.360	**< 0.001**
	substrate type	3	40.230	**< 0.001**
	substrate type x island	3	11.861	**0.008**

Predictors of coral microbial diversity

Table S2.6) Statistical summary of coral microbiome diversity (ASV-level) responses to local algal contact, site-level macroalgal cover, and island, using the data summarized in Fig. 2.2 (main text). P-values are estimated using Wald Chi-squared tests with Type III sums of squares. Significant predictors are indicated in bold. Models were fit to log-transformed Shannon diversity and Simpson diversity (the Hill numbers for q = 1 and q = 2, respectively).

Response	Predictor	df	X^2	p-value
ASV-level analyses				
Shannon diversity (D^1)	**island**	**1**	32.439	**0.002**
	algal contact	**2**	9.210	**0.007**
	macroalgae cover	1	9.858	0.324
	macro. cover x contact	**2**	0.974	**0.016**
Simpson diversity (D^2)	**island**	1	14.225	**< 0.001**
	algal contact	2	11.583	**0.003**
	macroalgae cover	1	1.572	0.210
	macro. cover x contact	2	13.560	**0.001**
Beta dispersion	**island**	**1**	**6.9968**	**0.008**
	algal contact	**2**	**11.9979**	**0.002**
	macroalgae cover	1	1.085	0.298
	macro. cover x contact	**2**	**7.318**	**0.026**

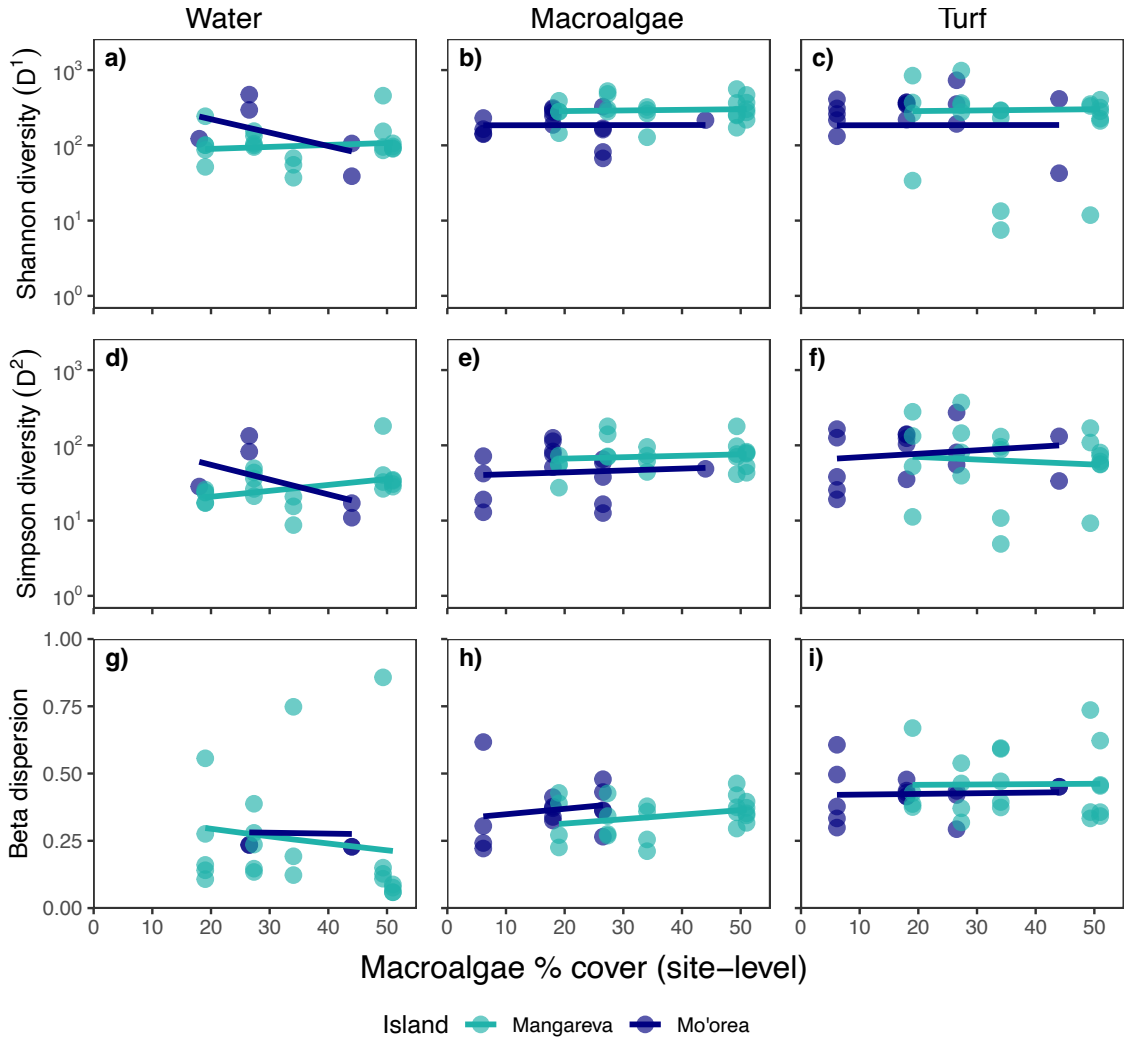

Fig. S2.7) Diversity responses of microbial ASVs in water, macroalgae, and turf samples (columns) vs. site-level macroalgal cover, with predicted regression lines for each island (indicated by color). **a, b, c)** Shannon diversity (D^1, i.e., the Hill number for q = 1). **d, e, f)** Simpson diversity (D^2, i.e., the Hill number for q = 2). **g, h, i)** Beta dispersion of microbial communities within a site and substrate type. Beta dispersion was not calculated when there was only 1 sample of a substrate type at a site. Statistical results are provided in Table S2.7.

Table S2.7) Statistical summary of microbial community (ASV-level) diversity responses in algae and water samples to mean site-level macroalgae cover and island, using the data summarized in Fig. S2.4. P-values are estimated using Wald Chi-squared tests with Type III SS. Significant predictors are indicated in bold.

Response	Predictor	df	X^2	p-value
Macroalgae samples				
Shannon diversity (D^1)				
	island	1	0.050	0.822
	macroalgae cover	1	0.145	0.704
	macro. cover x island	1	0.008	0.930
Simpson diversity (D^2)	island	1	0.312	0.576
	macroalgae cover	1	0.068	0.795
	macro. cover x island	1	0.003	0.956
Beta dispersion	island	1	0.461	0.497
	macroalgae cover	1	1.600	0.206
	macro. cover x island	1	0.011	0.916
Turf samples				
Shannon diversity (D^1)	island	1	0.050	0.822
	macroalgae cover	1	0.145	0.704
	macro. cover x island	1	0.008	0.930
Simpson diversity (D^2)	island	1	0.097	0.756
	macroalgae cover	1	0.176	0.675
	macro. cover x island	1	0.415	0.519
Beta dispersion	island	1	2.000	0.668
	macroalgae cover	1	0.197	0.944
	macro. cover x island	1	1.473	0.964
Water samples				
Shannon diversity (D^1)	island	1	1.781	0.182
	macroalgae cover	1	0.084	0.772

	macro. cover x island	1	1.324	0.250
Simpson diversity (D^2)	island	1	2.049	0.152
	macroalgae cover	1	0.552	0.458
	macro. cover x island	1	1.869	0.172
Beta dispersion	island	1	0.027	0.869
	macroalgae cover	1	0.968	0.325
	macro. cover x island	1	0.061	0.805

Microbial community composition by substrate type and island

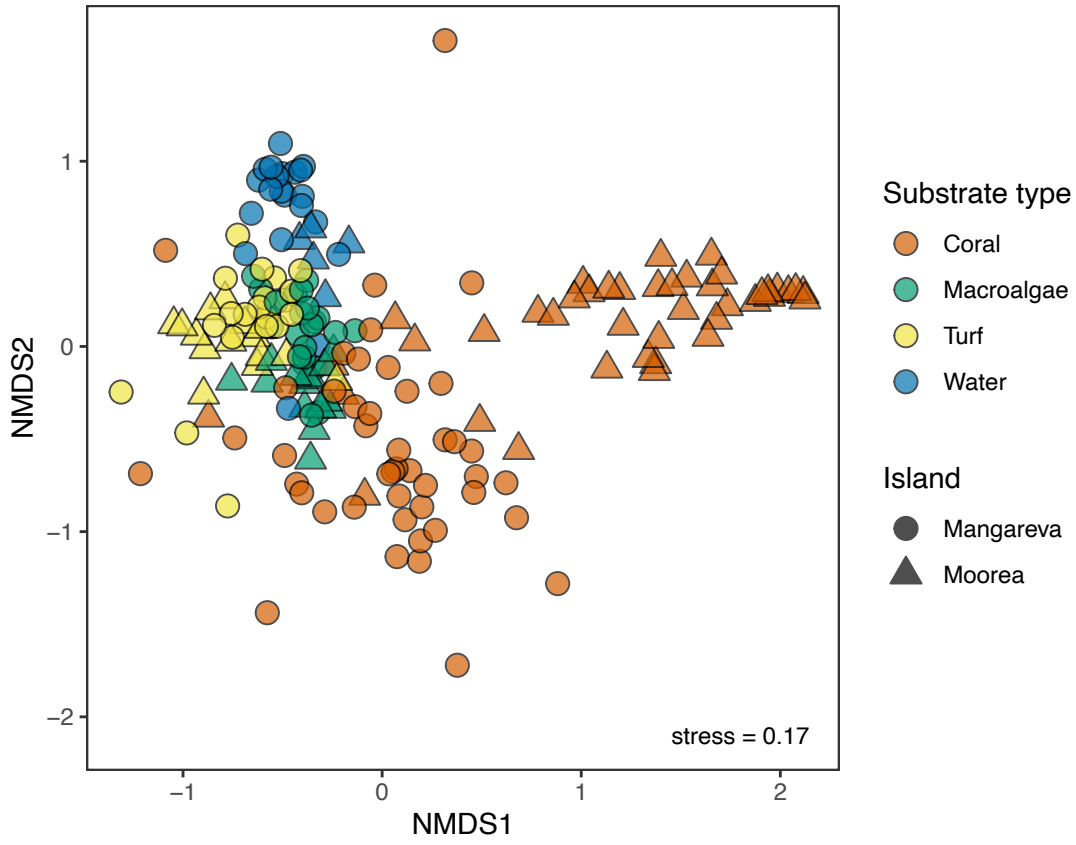

Fig. S2.8) NMDS ordination based on weighted Bray Curtis dissimilarity of microbial community composition (family-level) associated with different substrate types (colors) from two islands (shapes). Coral samples include corals from all three algal contact groups (in contact with macroalgae or turf, or not in contact with algae). PERMANOVA results are provided in Table S2.8. Figure 2.3 in the main paper provides a comparable presentation based on ASV-level composition.

Table S2.8) PERMANOVA results comparing the microbial community composition (ASV- and family-level) among substrate types and islands, using data summarized in Fig. 2.3 (main text) for ASV-level analysis, and Fig. S2.6 (supplement) for family-level analysis. Significant predictors are highlighted in bold.

Predictor	df	R^2	F	p-value
ASV-level analysis				
island	1	0.085	24.806	**0.001**
substrate type	3	0.219	21.432	**0.001**
substrate type x island	3	0.090	8.766	**0.001**
Residual	178	0.607		
Total	185	1.000		
Family-level analysis				
island	1	0.107	38.794	**0.001**
substrate type	3	0.299	36.056	**0.001**
substrate type x island	3	0.102	12.315	**0.001**
Residual	178	0.492		
Total	185	1.000		

Predictors of microbial community composition within substrate type

Table S2.9) PERMANOVA results for the association of coral microbial community composition at each island to site-level macroalgal cover and algal contact, analyzed at the ASV- and family-level. The ASV-level results complement Fig. 2.4a-c,e-g (main text), although macroalgal cover was a continuous predictor in the PERMANOVA, but visualized categorically (low, medium, and high cover) in Fig. 2.4.

Response	Predictor	df	R^2	F	p-value
Coral (ASV-level)					
Mangareva	**macroalgae cover**	1	0.071	3.349	**0.002**
	algal contact	2	0.050	1.187	0.149
	macro. cover x algal contact	2	0.054	1.264	0.113
	Residual	39	0.825		
	Total	44	1.000		
Mo'orea	macroalgae cover	1	0.028	1.181	0.250
	algal contact	2	0.068	1.425	0.109
	macro. cover x algal contact	2	0.042	0.883	0.554
	Residual	36	0.861		
	Total	41	1.000		
Coral (Family-level)					
Mangareva	**macroalgae cover**	1	0.059	2.795	**0.006**
	algal contact	2	0.052	1.244	0.150
	macro. cover x algal contact	2	0.068	1.604	**0.031**
	Residual	39	0.821		
	Total	44	1.000		
Mo'orea	macroalgae cover	1	0.015	0.623	0.614
	algal contact	2	0.073	1.514	0.173
	macro. cover x algal contact	2	0.038	0.783	0.539
	Residual	36	0.873		
	Total	41	1.000		

Table S2.10) PERMANOVA results for the association of algae and water microbial community composition at each island to site-level macroalgal cover, analyzed at the ASV- and family-level.

Response	Predictor	df	R^2	F	p-value
Macroalgae (ASV-level)					
Mangareva	**macroalgae cover**	1	0.080	1.737	**0.019**
	Residual	20	0.920		
	Total	21	1.000		
Mo'orea	**macroalgae cover**	1	0.109	1.596	**0.034**
	Residual	13	0.891		
	Total	14	1.000		
Macroalgae (Family-level)					
Mangareva	macroalgae cover	1	0.069	1.492	0.169
	Residual	20	0.931		
	Total	21	1.000		
Mo'orea	**macroalgae cover**	1	0.146	2.219	**0.022**
	Residual	13	0.854		
	Total	14	1.000		
Turf (ASV-level)					
Mangareva	macroalgae cover	1	0.048	0.957	0.518
	Residual	19	0.952		
	Total	20	1.000		
Mo'orea	**macroalgae cover**	1	0.144	2.195	**0.003**
	Residual	13	0.856		
	Total	14	1.000		
Turf (Family-level)					
Mangareva	macroalgae cover	1	0.038	0.754	0.672
	Residual	19	0.962		

	Total	20	1.000		
Mo'orea	**macroalgae cover**	1	0.203	3.307	**0.002**
	Residual	13	0.797		
	Total	14	1.000		
Water (ASV-level)					
Mangareva	macroalgae cover	1	0.067	1.359	0.197
	Residual	19	0.933		
	Total	20	1.000		
Mo'orea	macroalgae cover	1	0.366	1.733	0.200
	Residual	3	0.634		
	Total	4	1.000		
Water (Family-level)					
Mangareva	macroalgae cover	1	0.053	1.063	0.360
	Residual	19	0.947		
	Total	20	1.000		
Mo'orea	macroalgae cover	1	0.398	1.980	0.167
	Residual	3	0.602		
	Total	4	1.000		

Similarity of coral vs. algae microbiomes

Table S2.11). Statistical summary of mixed effect models testing for differences in the position of coral and macroalgae samples along NMDS1 at each island with site-level macroalgal cover and sample-contact type (either macroalgae, coral-no algal contact, coral-turf contact, or coral-macroalgae contact). Models use data summarized in Fig. 2.4d,h (ASV-level community data, main text). P-values are estimated using Wald Chi-squared tests with Type III SS. Significant predictors are indicated in bold.

Response	Predictor	df	F	p-value
Mangareva				
NMDS1	**sample-contact type**	3	45.641	**< 0.001**
	macroalgae cover	1	2.788	0.095
	sample-contact type x macro. cover	3	9.447	**0.024**
Mo'orea				
NMDS1	**sample-contact type**	3	44.766	**< 0.001**
	macroalgae cover	1	0.025	0.873
	sample-contact type x macro. cover	3	1.833	0.608

Dominant microbial families in each substrate type – Appendix 2.3

Pooling islands, macroalgae, turf, and water samples hosted more unique families (519, 509, 572, respectively) than corals (490), although there was some overlap in the families found across all substrates types. The dominant microbial families, which we defined as families present in at least 30 % of samples that also had a median relative abundance greater than 1 %, tended to differ by substrate type and island (Fig. S2.9,2.10). Vibrionaceae, Rhodobacteraceae, Rubritaleaceae, Cellvibrionaceae, Alteromonadaceae, and Saccharospirillaceae were especially common in macroalgae samples (Fig. S2.10a). Turf samples had many of the same dominant families as macroalgae, with Rhodobacteraceae, Saprospiraceae, and Nostocaceae among their most abundant families. Water column samples were dominated by oligotrophic taxa such as the family Cyanobiaceae, SAR11, SAR86, SAR 116, Marinimicrobia (SAR 406), Flavobacteriaceae, Cryomorphaceae, and Actinomarinaceae (Fig. S2.10c). There were many shared dominant families between islands within the algae and water samples. Corals shared fewer dominant families (Fig. S2.9).

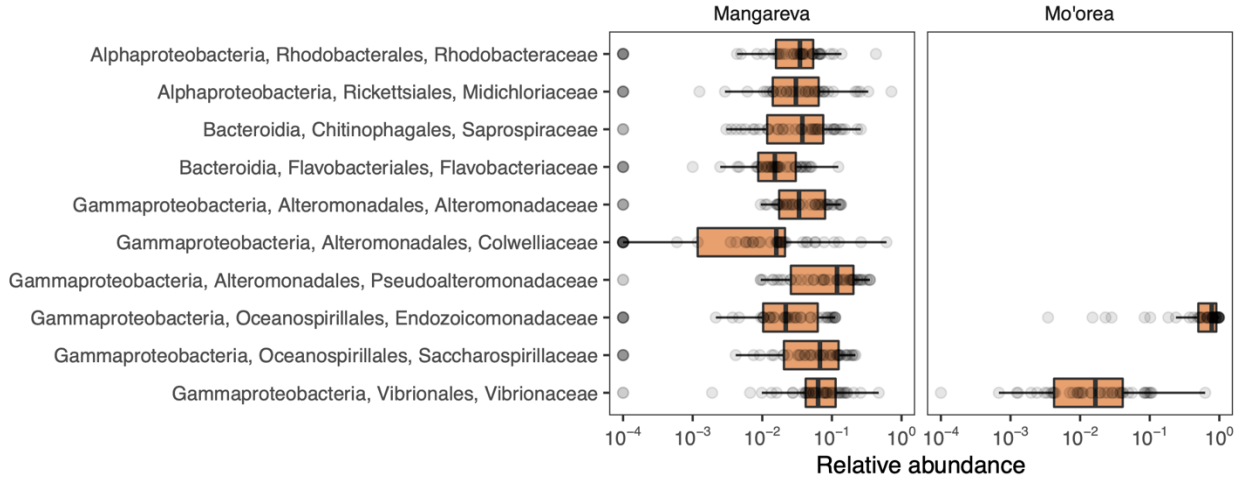

Fig. S2.9) Relative abundance of dominant microbial families in coral samples at each island. Taxa names are given as Class, Order, Family. Non-dominant taxa at each island, i.e., taxa that did not have a prevalence > 30 % and a median relative abundance > 1 %, are lumped into the "Other" category. Boxplots show the interquartile range and median relative abundance, error bars (whiskers) indicate the 25th and 75th percentiles $\pm$ 1.5 x the IQR (Tukey's boxplots). Points indicate the relative abundance of taxa in individual samples. A constant (+ 0.0001) was added to relative abundances so zeroes could be plotted on the $\log_{10}$ scale.

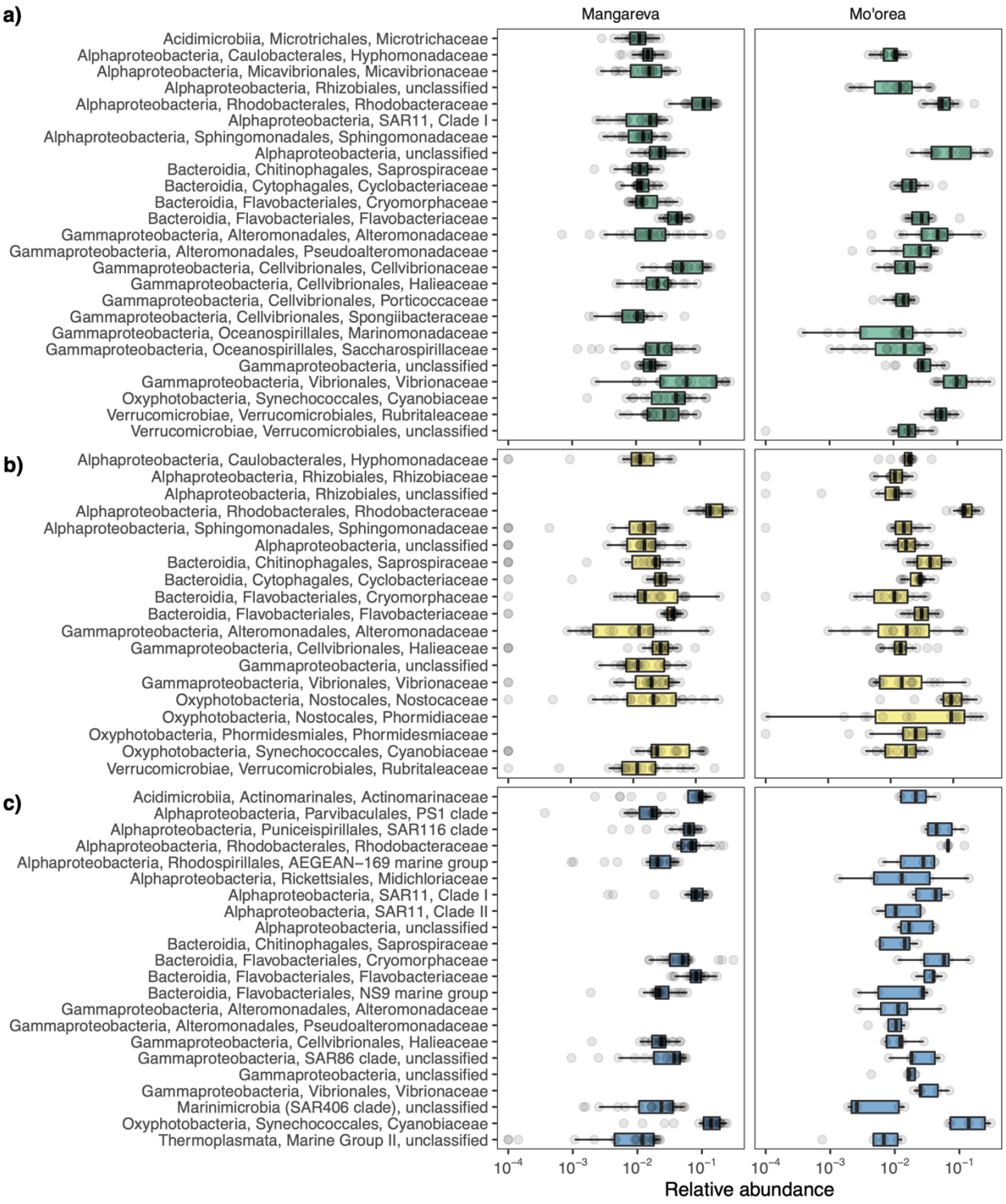

Fig. S2.10) Relative abundance of dominant microbial families in each substrate type at each island (columns): **a)** macroalgae (green), **b)** turf (yellow), and **c)** water (blue). Taxa names are given as Class, Order, Family. Non-dominant taxa at each island, i.e., taxa that did not have a

prevalence > 30 % and a median relative abundance > 1 %, are combined in the “Other” category. Boxplot formatting is the same as in Fig. S2.9.

CHAPTER 3

MICROBES AFFECT THE LIKELIHOOD OF REGIME SHIFTS AND ALTERNATIVE STABLE STATES IN CORAL REEFS [2]

[2] AA Briggs, SA McKinley, and CW Osenberg. To be submitted to *American Naturalist*.

Abstract

Regime shifts, which occur when an ecological system undergoes a large and abrupt change due to a small disturbance or change in environmental conditions, can have strong effects on the structure, functioning, and services provided by an ecosystem. One form of regime shifts occurs when a system has alternative stable states that exist under the same environmental conditions: i.e., the system is bistable. Alternative stable states require strong positive feedbacks, so researchers typically focus on single, strong interactions when investigating the drivers of regime shifts in specific systems. However, multiple weak interactions can combine to form strong feedbacks, and thus, may play an underappreciated role in regime shifts. Microorganisms influence numerous ecosystem processes and can modify the interactions between macroorganisms in a variety of systems. However, their contributions to alternative states in ecological communities have not been theoretically explored.

Here, we develop a conceptual model to quantify the effect of an ecological process on the occurrence and characteristics of regime shifts that arise from bistability. We then apply these methods to model the effects of microbes on regime shifts in coral reefs, many of which are currently undergoing dramatic transitions from coral- to algae-dominated states, and may exhibit bistability under certain conditions. Incorporating algae-associated microbes and their negative effects on corals made regime shifts more likely in reefs by facilitating bistability, decreasing the environmental tipping point for a regime shift, and reducing the resilience of coral-dominated alternative stable states to disturbances. Including additional positive feedbacks, e.g., a Type II functional response for herbivore foraging, exacerbated the microbial effect, while extreme feedback strengths (of single or combined feedbacks) produced solely algal-dominated reef states. These results illustrate that microbial feedbacks are likely essential to understanding

regimes shifts in coral reef systems, and that their omission from models could lead to unexpected deviations between expected and observed ecosystem dynamics. Given the important effects of microbes in other ecological systems, our findings suggest that microbially-mediated feedbacks could have significant implications for the management of many, if not all, ecosystems.

Introduction

Ecological systems can experience rapid and dramatic transitions in their population, community, or ecosystem states in response to a perturbation or a relatively small change in conditions. These abrupt changes, or "regime shifts," contrast with typical ecosystem change, which occurs gradually as environmental conditions or external drivers change. Outbreaks of pests or pathogens (Holling 1975, Casti 1982, Soukhovolsky et al. 2005, Martinson et al. 2012), transitions in arid landscapes from vegetated to unvegetated states (Schlesinger et al. 1990, Van Nes and Scheffer 2005), and shifts from clear to turbid lakes (Hosper 1989, Scheffer et al. 1993), are all examples of regime shifts. Regime shifts frequently have strong effects on the structure of, and services provided by, ecosystems, as one regime typically has a diminished capacity to perform important ecosystem functions or to support diverse communities (Crépin et al. 2011). These negative consequences have led many researchers to refer to the abrupt transition from one state to another less desirable one as a "catastrophic shift" (Scheffer et al. 2001), and has inspired research into the causes of these types of shifts in many systems (deYoung et al. 2008, Staver et al. 2011, Xu et al. 2015).

Systems with regime shifts must include a positive feedback (Jacob and Monod 1961, Thomas 1981, Soulé 2003), i.e., when an increase in one ecosystem component has an effect on another ecosystem component that indirectly feeds back to further benefit the first component

(DeAngelis et al. 1986, Wilson and Agnew 1992). Furthermore, the strength of the positive feedback determines the general form of the regime shift. Weaker positive feedbacks can generate a "phase shift", a continuous, but threshold-like change from one ecological state to another in response to a change in an external driver (Petraitis and Dudgeon 2004). In contrast, strong positive feedbacks can produce alternative stable states, i.e., two (or more) stable equilibria that exist under the same set of environmental conditions or parameter values (Scheffer et al. 2001, Kéfi et al. 2016). Alternative stable states are self-reinforcing; thus, a shift from one state to another can only be reversed only by relaxing the external driver more than was needed to shift the system in the first place (a phenomenon called hysteresis), or by a large perturbation that pushes the system state into a region in which it is now attracted to an alternate state (reviewed in Scheffer and Carpenter 2003). Since systems with alternative stable states require a more concerted management effort to restore the system after a catastrophic shift compared to a system with a phase shift, there is an incentive to understand drivers of alternative stable states in ecosystems in order to anticipate, prevent, and/or reverse this type of regime shift.

In complex systems, multiple weak interactions can compound into a sufficiently strong positive feedback to create alternative stable states (Kéfi et al. 2016, Leemput et al. 2017, Tenenbaum et al. 2020). Multiple interacting feedback loops are common in ecosystems, and biological systems in general (Chang et al. 2010, Kéfi et al. 2016). Fields like cellular biology have recognized the importance of interacting feedbacks in regime shifts (Verdugo et al. 2013, Tenenbaum et al. 2020), but historically, most ecological research on regime shifts has focused on single, strong feedbacks between the most conspicuous actors in a system. This tendency to overlook how multiple feedbacks might interact in naturally complex systems could reduce the likelihood of detecting (or even suspecting) regime shifts in systems where they have occurred

(or have the potential to occur). More recently, ecologists have begun to investigate the effects of multiple feedbacks on regime shifts (Muthukrishnan et al. 2016, Leemput et al. 2017), although this work is still limited, and their effects on regime shifts are frequently evaluated semi-qualitatively.

One ecosystem component that has previously been overlooked in many studies of regime shifts has been microbes, despite their crucial role in many ecosystem processes such as organic material breakdown, nutrient cycling (Falkowski et al. 2008, Averill et al. 2014), disease dynamics (Parris and Cornelius 2004, Mordecai 2011), and their ability to modify interactions between macroscopic species (Nugues et al. 2004, McFall-Ngai et al. 2013). Empirical and theoretical work suggests that microbial community structure might exhibit regime shifts (Lahti et al. 2019, Amor et al. 2020, Bardgett and Caruso 2020), which could influence the health of macroscopic hosts in host-microbiome contexts (Guittar et al. 2021) and cause ecosystem-level shifts in abiotic regimes, such as shifts from oxic to anoxic environments (Bush et al. 2017). However, the ability of microbes to influence regime shifts in macroscopic ecological communities has not been addressed.

Microbes play an important ecological role in coral reefs (Nugues et al. 2004, Barott and Rohwer 2012), which can exhibit regime shifts from coral to algal-dominated states, possibly as a result of bistability in at least some reef systems (Mumby et al. 2007, Schmitt et al. 2019). These dynamics arise from the competition between corals and fleshy and filamentous algae (hereafter, 'algae') for space. Algae have a variety of negative effects on corals, and because they have much faster growth rates, they can take over space, resulting in a shift in reefs from coral- to algal-dominance if their growth is not kept in check by forces such as herbivory. Because of the connection between algal mortality and herbivory, most research on regime shifts

in reefs has focused on the effects of herbivores, and how factors that affect herbivory—primarily fishing (Mumby et al. 2007, Mumby 2009, Fung et al. 2011), but also anti-herbivory defenses in algae (Briggs et al. 2018), or positive associations between herbivores and corals (Blackwood et al. 2010, Muthukrishnan et al. 2016, Leemput et al. 2017)—can drive shifts in reefs from coral to algal-dominated states.

However, microbes mediate many of the competitive effects of algae on corals. At localized spatial scales (e.g., near areas of coral-algal contact), algae-associated microbes can produce anoxic conditions or secrete allelochemicals, which can slow coral growth by killing or harming coral tissue (Smith et al. 2006, Paul et al. 2011). They also can reduce survival of coral recruits (Vermeij et al. 2009, Bulleri et al. 2018), and increase the prevalence of disease in juvenile and adult corals (Nugues et al. 2004, Kuntz et al. 2005, Kline et al. 2006, Barott and Rohwer 2012, Zaneveld et al. 2016). At larger (i.e., reef-level) spatial scales, increased densities of algae are associated with increased production of dissolved organic carbon (DOC) and increased densities of prokaryotic microbes in seawater, with increased representation of copiotrophs (heterotrophs with fast growth in high nutrient environments) and taxa with virulence-associated genes (Nelson et al. 2013, Haas et al. 2016). Algae-associated microbes can also have negative effects on coral microbiomes, which can have important consequences for coral health and survival (Vega Thurber et al. 2012a, Morrow et al. 2013, Zaneveld et al. 2016, Brown et al. 2019). Recent work has demonstrated that shifts in coral microbiomes are associated with both site-level algal densities and algal contact (Briggs et al. 2021). Thus, there are several positive associations between algae and microbes that suggest that microbes could have an increasingly negative effect on corals as the density of algae on a reef increases, creating a positive feedback that could facilitate algal growth and hasten coral declines. Consequently,

microbes could enable regime shifts from coral to algal-dominated states without involving herbivory directly. Shifts in reefs to algal-dominance can result in significant declines in important functions performed by corals, such the construction of biogenic habitat, which supports much of the surrounding reef community (Pratchett et al. 2018) and protects coastal human populations from wave and storm damage (Wilkinson 2008, Ferrario et al. 2014). Therefore, since reefs are increasingly affected by a variety of disturbances that facilitate algae and microbial growth (e.g., nutrient enrichment, sedimentation, overfishing, climate change, etc. (Goldberg and Wilkinson 2004, Hughes et al. 2007, Ziegler et al. 2016, McDevitt-Irwin et al. 2017), there is a growing need to understand if and how microbes might affect shifts in reefs to algal-dominated states.

To evaluate the contribution of specific ecological feedbacks (and potential interactions between them) to regime shifts and the formation of alternative stable states, we need a framework that allows us to quantify the effect of new processes relative to a baseline condition. Here we propose a conceptual approach for quantifying attributes of systems that exhibit alternative stable states, which allows us to make explicit comparisons of regime shifts (or lack thereof) under different feedbacks strengths and combinations of feedbacks. We then use this framework to evaluate the effects of the proposed microbe-algae positive feedback on alternative stable states in coral reefs, and how this feedback may interact with another feedback that has typically been included (but not systematically evaluated) in prior models of coral reef regime shifts—the herbivore functional response. This approach allows us to explore how these feedbacks influence the likelihood of regime shifts in reefs (e.g., the range of environmental conditions over which bistability occurs and the relative resilience of the system to perturbations in coral and algal cover), the timing of shifts (e.g., the minimum amount of fishing pressure

under which a regime shift could occur), and the magnitude of a regime shift (i.e., the difference in coral cover between alternative stable states).

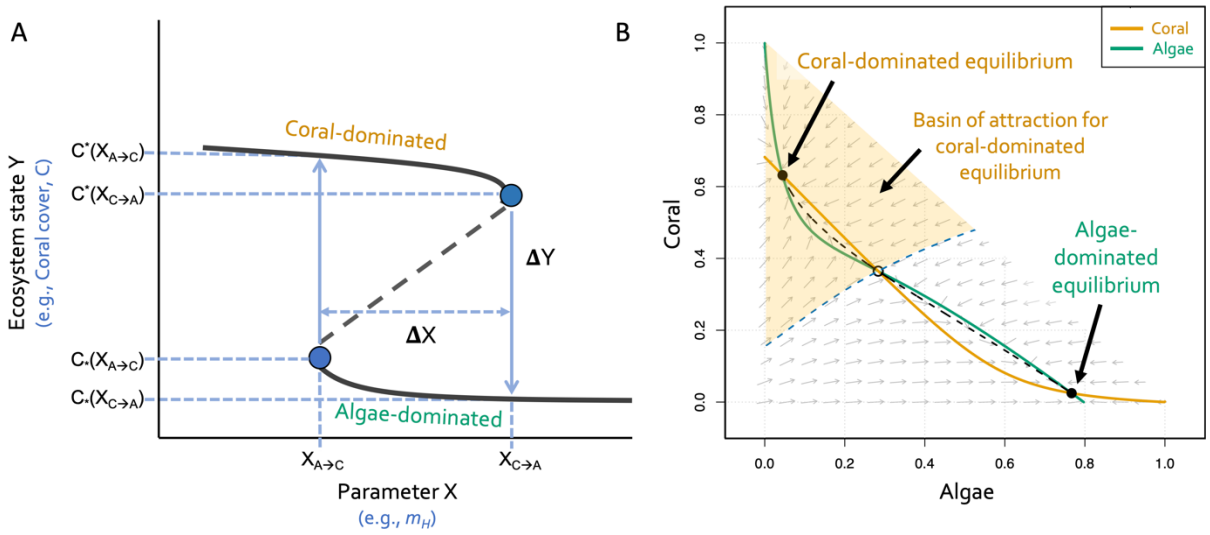

Fig. 3.1 (a) Bistability diagram showing how a model parameter (X), such as herbivore mortality (m_H), could influence ecosystem state (Y), e.g., equilibrium coral cover (C^*or C_*), indicated by a solid black line for the stable equilibria and a dashed black line for the unstable equilibria. Characteristics of this relationship can be extracted and used to define two metrics of the effects of a feedback on bistability (although this approach can be generalized to systems with > 2 stable equilibria, I concentrate on the case with two stable equilibria). 1) The "**range of bistability**" gives the range of the parameter X that produces bistability (i.e., $\Delta X = X_{C\to A} - X_{A\to C}$). 2) The "**magnitude of the regime shift**" gives the state change as the system passes an environmental tipping point (i.e., $\Delta Y = C^*(X_{C\to A}) - C_*(X_{C\to A})$). **(b)** Phase plane diagram of the proportional cover of coral (C) vs. algae (A), with solid lines showing the zero-growth isoclines for coral (orange line) and algae (green line). Filled circles represent stable equilibria for the system, while the open circle represents an unstable equilibrium. Arrows indicate the trajectory of the system for any set of initial conditions (C and A values), but do not indicate the rate of change along a given trajectory. The blue dashed line shows the unstable manifold, which separates the **basin of attraction** for the coral vs. the algae-dominated stable equilibria (i.e., the starting conditions for C and A that lead to

each equilibrium). Starting conditions in the shaded region (yellow) to the left of the manifold go to the coral-dominated equilibrium, and conditions to the right of the manifold go to the algal-dominated equilibrium. This gives rise to the third metric: 3) The "**size of the basin of attraction**" for an equilibrium state can be estimated as the proportion of starting conditions that result in that equilibrium. This metric is positively correlated with the system's resilience, i.e., how easily it could be shifted to an alternative state by a perturbation (e.g., by a disturbance that decreases C). A larger basin of attraction indicates the system is resilient to a larger proportion of possible perturbations.

Methods

A conceptual model to quantify bistability.

Regime shifts in systems exhibiting alternative stable states can be initiated in two different ways – 1) by crossing an environmental threshold (e.g., fishing mortality) that leads to a regime shift; and 2) by a disturbance to the state variables in a system (e.g., a change in the amount of coral or algae) that pushes the system into the basin of attraction of an alternative state. Therefore, to accurately describe the behavior of a system that has alternative stable states, we need separate metrics to quantify when the system is susceptible to regime shifts produced by each of these causes. With this aim, we start by developing a framework to quantify: 1) the range of environmental conditions or system parameters needed for the system to exhibit bistability; 2) how much the system will change as it passes a tipping point; and 3) how susceptible or likely the system is to experience a discontinuous regime shift under a specific set of environmental conditions given a perturbation to the state variables. We introduce our method in the context of coral reefs (and coral- vs. algal-dominated states), and then apply this method to an analysis of coral reef dynamics using numerical simulations of a model of coral-algae competition as influenced by herbivores and microbes.

Given a model system that incorporates ecological processes or feedbacks that may contribute to alternative stable states, we start by evaluating the system dynamics over a range of parameter values. For example, in the context of coral reefs, the intensity of fishing mortality on herbivores can greatly influence the occurrence of bistability (Mumby et al. 2007, Mumby 2009, Blackwood et al. 2010), so our focal parameter might be fishing mortality. To quantify how that process (e.g., herbivore morality) affects the regime shift, we propose evaluating several metrics of bistability (Fig. 3.1). First, we can quantify the **"range of bistability,"** or the range of

environmental conditions (represented by a parameter, X, such as fishing mortality) over which the system is bistable (ΔX in Fig. 3.1a). A system that is not bistable has $\Delta X = 0$. The range of X over which bistability occurs indicates the range of conditions over which the system is susceptible to a regime shift due to a perturbation in coral or algal cover. The absolute value of ΔX indicates the scope of hysteresis, or how different the system's parameters need to be to return to its original state following a "catastrophic shift", i.e., a regime shift caused by passing an environmental threshold or tipping point (Scheffer et al. 2001, $X_{C \to A}$ in Fig. 3.1a). Next, we can quantify the "**magnitude of the shift**" in the ecosystem state (e.g., coral cover) from one equilibrium to another at the environmental tipping point for a regime shift (ΔY in Fig. 3.1a): i.e., the magnitude of the catastrophic shift. Finally, for each environmental condition (i.e., parameter set), we can quantify the "**size of the basin of attraction**" for one ecosystem state (e.g., the coral-dominated state). The size of this basin gives the proportion of all possible starting conditions that result in that equilibrium state (the shaded region relative to the total area in Fig. 3.1b). Basin size provides a crude measure of the resilience of the system, i.e., how susceptible the system is to being pushed to an alternative stable state by a perturbation (e.g., a reduction in coral cover). A system with a small basin of attraction for its current equilibrium can only return to that state following a relatively small perturbation – larger perturbations will lead to a new state. A system with a large basin of attraction will return to its current equilibrium for a much broader range of perturbations.

Next, we can compare these three metrics of bistability in the presence vs. absence of a feedback in the system, or across different values of a parameter that modulates the strength of that feedback (e.g., the feedback between algal cover and microbial density). For example, a feedback that has no effect on the range of bistability of a system can be dismissed as playing a

key role in the regime shift. In contrast, the introduction of a new feedback (e.g., a microbe-algae feedback) might greatly expand the range of conditions (e.g., fishing mortalities) under which the system is bistable. This approach can also be used to evaluate the effects of combined feedbacks and their interaction: e.g., by crossing the presence/absence of one feedback with the presence/absence of another.

We next develop a model of coral-algae dynamics that incorporates possible feedbacks involving microbial responses to algae and the effects of microbes on corals, and apply these metrics of bistability to assess the possible importance of microbes in driving regimes shifts in coral reefs.

An Application: Microbe-Algae-Coral Interactions

We used a set of ordinary differential equations based on a previous theoretical model used to study coral reef regime shifts (Mumby 2009, Leemput et al. 2017), which has many similarities to earlier dynamical models of regime shifts in coral reefs (Mumby et al. 2007, Blackwood et al. 2010, Fung et al. 2011). These equations model the proportion of benthic habitat occupied by coral (C) or algae (A); all other available habitat is open space (S). Thus,

$$1 = A + C + S. \quad (1)$$

Algae and coral propagules are imported from outside the system and settle on open space at rates i_A and i_C respectively. Coral and algae expand into open space at rates b_A and b_C. Corals die at a constant rate (μ_C), while algae dies through consumption by herbivores (H), whose per capita feeding rate is described by a non-linear, Type II functional response (Holling 1959), defined by an attack rate (a) and handling time (h; Equation 2). Note that when $h = 0$, the feeding rate increases linearly with algae density (a Type I functional response). The recruitment and growth of coral into open space is reduced by the presence of algae, as

determined by the competitive effect of algae, α. Corals have no effect on algal growth or settlement. Thus, competition is asymmetric. Because corals and algae cannot overgrow one another, the strength of the coral-algae interaction is weakened relative to other possible competition scenarios, allowing us to focus on the contributions of other feedbacks to bistability. Taken together, we therefore have:

$$\frac{dC}{dt} = (i_C + b_C C)S(1 - \alpha A) - \mu_C C, \quad (2)$$

$$\frac{dA}{dt} = (i_A + b_A A)S - \frac{aHA}{ahA+1}. \quad (3)$$

The baseline model (from Mumby 2009, Leemput et al. 2017) also assumes herbivores exhibit logistic growth, with their carrying capacity (K_H) and intrinsic growth rate (r_H), determined by a suite of factors and not exclusively on the density of their food, A. Herbivore abundance is expressed as a proportion of their carrying capacity, such that $H = N/K_H$. Additionally, like in most other coral reef regime shift models (Mumby et al. 2007, Mumby 2009, Blackwood et al. 2010, 2011, Fung et al. 2011, Muthukrishnan et al. 2016, Leemput et al. 2017), herbivores experience an additional mortality rate (m_H) due to fishing:

$$\frac{dH}{dt} = r_H(1 - H)H - m_H H \ . \quad (4)$$

Fishing mortality, m_H, is used here (and by previous researchers) to investigate the effects of changing this external driver (which can be modified through fisheries regulations and enforcement) on the occurrence of alternative stable states.

Because the dynamics of herbivores are independent of the other state variables in the system, we take the additional step of assuming that herbivores are at their equilibrium density, so that we can ignore their dynamics and express H (in Equation 3) as the proportion of the herbivore equilibrium relative to its carrying capacity (or similarly, set $K_H = 1$):

$$H = 1 - \frac{m_H}{r_H}\,. \tag{5}$$

(Note though, that r_H must be $\geq\ m_H$, or else the herbivore population will go extinct.)

We then modified the baseline model (Equations 1-4) to incorporate effects of microbes. First, we replaced the baseline competitive effect of algae on coral, $(1 - \alpha A)$ in Equation 2, with $e^{-(\alpha A)}$. At low to intermediate algal densities (e.g., < 40 % cover) the negative exponential term ($e^{-(\alpha A)}$) approximates the linear decline in coral recruitment and expansion (produced by the term $1 - \alpha A$) that was used by Leemput et al. (2017) (Appendix 3, Fig. S3.1). The use of the negative exponential term (rather than the linear term) precludes this proportion from becoming negative (e.g., if the effect of algae is large). When $\alpha = 0$, algae have no direct effects on coral recruitment or expansion. $e^{-(\alpha A)}$ can be thought of as the proportion of open space that is available for growth and settlement of coral given the presence of algae.

To incorporate the effect of algae-associated microbes on the expansion of coral into open space, we incorporate an additional term $e^{-(\gamma MA)}$, in which M is the density of microbes per unit of algae, and γ is the per microbe effect on coral expansion and recruitment. Thus, Equation 2 is replaced with Equation 6:

$$\frac{dC}{dt} = (i_C + b_C C)S\left(e^{-(\alpha A + \gamma MA)}\right) - \mu_C C\,. \tag{6}$$

The competition term $\left(e^{-(\alpha A+\gamma MA)}\right)$ combines the direct effect of algae (αA) with the effect of the reef-scale abundance of algae-associated microbes (γMA). When $\gamma = 0$, microbes have no effect. The relationship between this competition term and algal cover means that microbes and algae can prevent coral growth and recruitment into bare space when the microbial effect (γ) and algal cover (A) are high, e.g., when $\gamma = 10$ and $A > 0.4$ (Appendix 3, Fig. S3.1).

Microbes have fast dynamics relative to macro-organisms. Therefore, we assume that microbes rapidly equilibrate to the cover of algae, and that the reef-wide cover of algae (A)

determines the density of microbes. Based on empirical results from Haas et al. (2016), we model the equilibrium density of microbes per unit algae (M^*) as an exponential function of algal cover:

$$M^* = ce^{zA}, \quad (7)$$

where z describes the rate of increase in microbial density and c represents the baseline density of microbes per unit algae in the absence of any other algae on the reef. Thus, when $z = 0$, the density of microbes per unit algae is constant and does not respond to reef-scale algae, but the total abundance of microbes on the reef still increases with A, simply because there is more algal substrate for the microbes.

Implementation

We used the deSolve package (Soetaert et al. 2010) in R (R Core Team 2016) to simulate the dynamics of our system. For most combinations of parameter values and starting conditions, the system equilibrated within 200 years. However, a few rare scenarios (approximately 0.1 % of all unique parameter combinations) exhibited slow transients—meta-stable states that lasted for a long time before the system moved toward a true equilibrium. These slow transients generally occurred when the starting values of coral and algae were either near or when they approached a saddle point (a type of unstable equilibrium) or when the coral-algae state-space had a region in which the coral and algae zero-growth isoclines were close to each other but did not intersect, causing very slow transient dynamics. To ensure that all simulations equilibrated, we simulated dynamics for 6000 years. For each parameter set, simulations were run using a range of starting values for the proportional cover of coral and algae (each with values varied between 0 and 1 in increments of 0.1, which always summed to ≤ 1). For each set of parameter values, the resulting equilibrium value(s) for coral (C^*) and algae (A^*) were extracted and used to

determine the number of stable equilibria. In the presence of multiple stable states, a single set of parameter values yielded different equilibria depending on the starting conditions (e.g., Fig 3.1b). When there were two stable equilibria, we calculated the three metrics of bistability in our system (Fig. 3.1a: see below). Parameter values, units, and references for the range of values used in simulations are in Appendix 3, Table S3.1.

Evaluating regime shifts

To quantify how the incorporation of the microbially-mediated effects on coral altered the behavior of our system, we first evaluated the three metrics of bistability outlined in Box 1, over a range of fishing mortality, m_H (0 to 1.5 yr $^{-1}$ in increments of 0.05): 1) the range of fishing mortality that led to bistability (Δm_H, i.e., ΔX in Fig 3.1a); 2) the magnitude of the shift in the coral equilibrium from the coral- to the algal-dominated state (ΔC^*, i.e., ΔY in Fig. 3.1a); and 3) the size of the basin of attraction for the coral-dominated state, i.e., the proportion of all possible starting conditions for C and A that resulted in the high coral equilibrium (Fig. 3.1b). The frequency distribution of the equilibrium coral cover (C^*) across all simulations was bimodal, with all equilibria falling in the range of 0 to 0.3 and 0.45 to 0.7 (Fig. S3.2). Based on these distributions, coral cover < 0.35 was designated the algae-dominated (i.e., low coral cover) state, while coral cover $\geq$ 0.4 was designated the coral-dominated (i.e., high coral cover) state.

We then examined the relationship between each of these three metrics of bistability and the strength of the microbial feedback, as indicated by the parameters γ (the effect of microbes on coral-algae competition) and z (the effect of algae on microbial density). We varied γ from 0 to 10 [0, 0.001, 0.01, 0.1, 1, 10]. When $\gamma = 0$ (Equation 6), algae-associated microbes did not influence the competitive effect of algae on coral. We further explored how the strength of the microbial response to reef-scale algae influenced coral by varying the parameter z, the rate of

increase of microbial densities on algae as reef-scale algal cover increases. When $z > 0$, the density of microbes on algae increases with algal cover, which amplifies the strength of the algae-microbe positive feedback for a given value of γ. When $z = 0$, microbial densities on algae remain constant, reducing the strength of the algae-microbe feedback, but not eliminating it (so long as $\gamma > 0$). Because varying z has a similar effect as varying γ (Fig S3.1), we considered only two values of z: 0 and 4.6. We chose $z = 4.6$, which corresponds with a two-order of magnitude increase in microbial density as reef-scale algal cover increases from 0 to 100 percent, based on the observations by Haas et al. (2016) in multiple ocean basins (Appendix 3.1).

Previous regime shift models have typically incorporated a Type II functional response for herbivores (e.g., Mumby et al. 2007, Blackwood et al. 2010, Fung et al. 2011), which influences the per capita efficiency of herbivores at removing algae. When herbivores exhibit a Type II response, their per capita consumption of algae increases but at a decelerating rate as algal density increases (due to a non-zero handling time, h, needed to consume algae). This causes a reduction in the algal mortality rate at higher algal densities, creating a positive feedback between algae density and net algal growth. However, when herbivores exhibit a Type I functional response ($h = 0$), the herbivore-imposed mortality rate on algae is independent of algal density, making bistability unlikely under the baseline model (Leemput et al. 2017). Because the effect of the functional response on regime shift characteristics has not been systematically evaluated in prior studies, and because little empirical work has focused on quantifying the functional response of reef herbivores, we sought to explore the role of handling time and its possible interaction with the microbial feedback. Thus, we also varied the herbivore handling time for algae, h, from 0 (no feedback) to 10 yr^{-1} (strong positive feedback) in increments of 1.

Results

In the absence of both the microbial feedback ($\gamma = 0$) and the herbivore feedback ($h = 0$), the system lacked bistability, but exhibited a phase-shift from coral to algal dominance at intermediate-high levels of fishing mortality on herbivores (m_H, Fig. 3.2). However, in the presence of the microbial feedback in which algae-associated microbes had constant densities ($z = 0$) but reduced coral expansion ($\gamma = 1$), bistability occurred over a narrow range of intermediate levels of fishing mortality without the herbivore feedback (Fig. 3.2a). Furthermore, when algae stimulated microbial growth ($z = 4.6$), a smaller microbial effect on coral was needed to produce bistability: e.g., bistability arose at $\gamma = 0.1$ when $z = 4.6$ (Fig. 3.2b), but at $\gamma = 1$ when $z = 0$ (Fig. 3.2a). The herbivore feedback ($h > 0$) in the absence of a microbial feedback also produced bistability at intermediate levels of fishing mortality (Fig. 3.2). Thus, both of the positive feedbacks that we tested (herbivore or microbial) could induce bistability in the system in the absence of the other feedback.

Furthermore, the strength of the positive feedback(s) operating in the system (modulated by parameters z, h, and γ) influenced both the environmental tipping point for a regime shift, and the range of environmental conditions (m_H) over which the system exhibited bistability. Thus, feedback strength influenced the conditions under which the system was susceptible to perturbation-induced regime shifts. As the strength of the positive feedback applied to the system increased (either for a single feedback acting in isolation or for multiple feedbacks occurring together), algal growth was increasingly favored. Consequently, the amount of herbivory required to maintain a single coral-dominated equilibrium increased, meaning that the maximum fishing mortality rate that the system could tolerate without exhibiting bistability declined (Figs. S3.3, 3.2). This reduction in fishing mortality permitting a single coral-

dominated system was associated with the formation of alternative coral- vs. algal-dominated stable states at intermediate fishing mortality rates (Figs. S3.3, 3.2). The minimum fishing mortality producing bistability continued to decline as feedback strength increased (e.g., as γ increased; Fig. S3.3), allowing the range of fishing mortalities producing bistability (Δm_H) to initially increase with feedback strength (Fig. 3.3a,b). However, the maximum fishing mortality producing bistability (i.e., the environmental tipping point for a catastrophic shift) also declined with increasing positive feedback strength, as lower fishing mortalities were needed to push the system to a single, algae-dominated state (Fig. S3.4). Eventually, the positive feedback facilitating algae was strong enough that bistability occurred without any fishing mortality (e.g., when $h \geq 5$, or when $h = 2$ and γ =1; Figs. 2, S3.3). After this point, further increases in feedback strength only acted to reduce the range of bistability and expand the range of conditions over which a single algal-dominated system occurred (Fig. 3.3a,b, S3.3). Finally, at extreme feedback strengths (e.g., γ = 10 and $h > 0$), bistability was entirely absent and only an algal-dominated equilibrium was possible (Fig. 3.2, S3.3).

Because the range of conditions over which the system exhibited bistability responded non-linearly to increases in the strength of a single positive feedback, combining multiple positive feedbacks produced complex interactions. For example, the range of fishing mortality rates over which bistability occurred (Δm_H) exhibited a unimodal (hump-shaped) relationship with γ (the microbial feedback) at lower herbivore handling times (e.g. $h \leq 3$), and a monotonically decreasing relationship with γ at higher values of *h* (Fig. 3.3a,b). When the microbial feedback was strengthened by allowing microbial densities to increase with algae (i.e., when *z* = 4.6), the peak range of bistability was further increased for low-to-intermediate values of *h*, and this peak in occurred at lower values of γ until $h > 3$ Fig. 3.3b). The range of

bistability, Δm_H, was maximized when $\gamma = 0.1,\ h = 3,$ and $z = 4.6$, and under these conditions, bistability occurred when fishing mortality was between 0.05 and 0.65.

Similarly, Δm_H initially increased with h and then decreased for all levels of γ, except when $\gamma = 10$ (at which point bistability was not possible and only a single algal-dominated equilibrium existed, except when h and $z = 0$). Thus, combining the microbial feedbacks with the herbivore feedback could either exacerbate or reduce the amount of bistability exhibited by the system (and thus, affect the range of conditions over which the system was susceptible to experiencing a regime shift due to a perturbation in coral or algal cover), depending on the strengths of each of the positive feedbacks. In general though, the range of fishing mortalities over which bistability occurred (and thus, the range of conditions over which a perturbation-induced regime shift was possible) was greatest at intermediate values of h, γ, and z.

However, the strength of the microbial and the herbivore feedbacks had limited effects on the magnitude of the change in equilibrium coral cover at the environmental tipping point for a catastrophic shift (ΔC^*), except in the transitions to or from bistability (Fig. 3.3 c,d). (Note that in the absence of bistability, we set $\Delta C^* = 0$.) Equilibrium coral cover (C^*) was usually approximately 55 - 70 % or 0 - 5 % in the coral- vs. the algae-dominated states, respectively, and ΔC^* was typically ~0.6. Herbivore handling time, h, primarily influenced ΔC^*, by producing bistability in the absence of the microbial feedback when $h > 0$. However, when the microbial feedback produced bistability in the absence of the herbivore feedback, there was a less pronounced regime shift at the environmental tipping point. As the microbial feedback increased in strength (e.g., either through increasing γ and/or z), ΔC^* increased until it approached ~0.6, the value observed in most scenarios exhibiting bistability.

Stronger microbial or herbivore feedbacks generally reduced the size of the basin of attraction for the coral-dominated state (Fig. 3.3e,f), making it possible to shift the system from coral to algal-dominance with a smaller perturbation. Combining multiple positive feedbacks further reduced basin size, until it was equal to zero when the system only had a single algal-dominated equilibrium. Basin size also declined with increasing fishing mortality (Fig. S3.5). This indicates that when microbes had stronger effects on corals (or when herbivores had high handling times), the system was less resilient to perturbations in coral cover, and this reduction in resilience was amplified by increasing fishing mortality (until the system could no longer support corals).

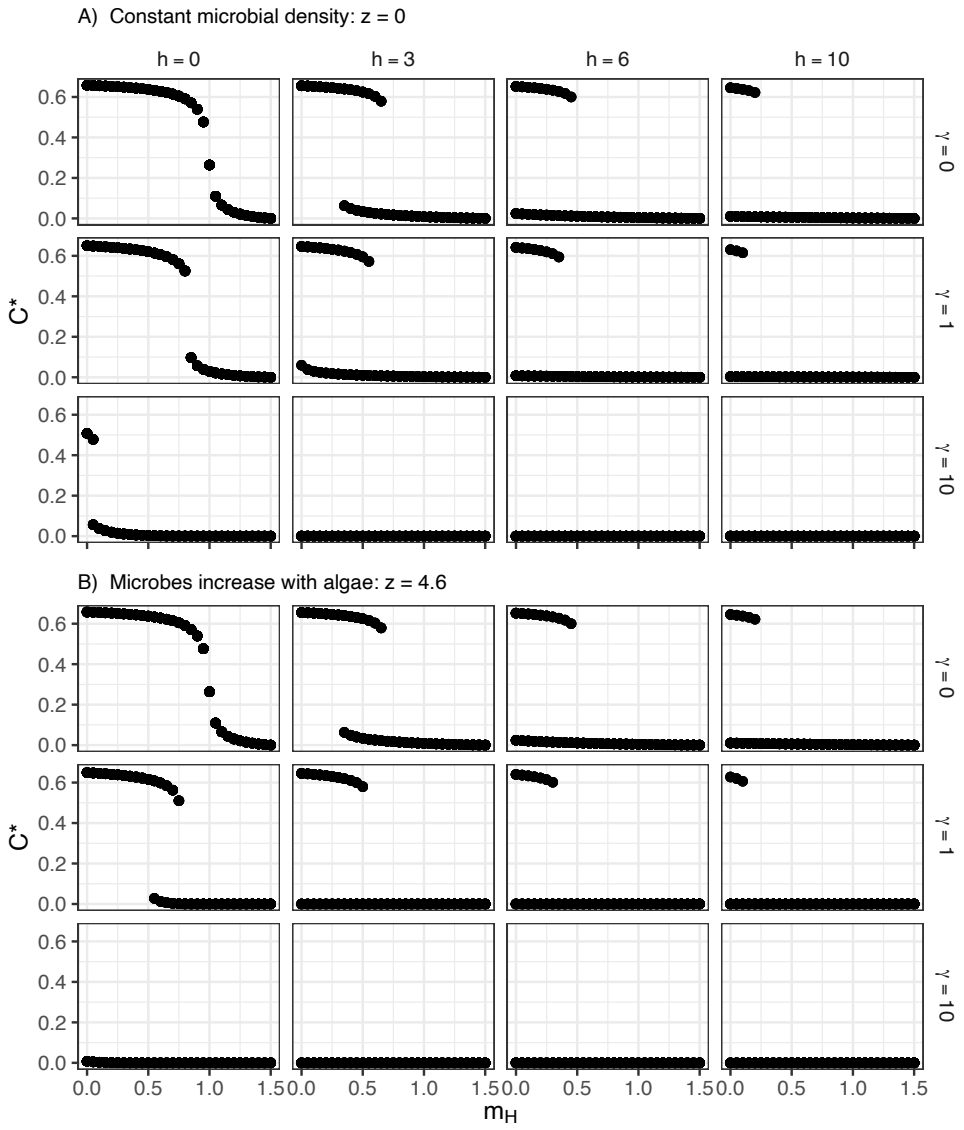

Fig. 3.2 Hysteresis plots of equilibrium coral cover (C^*) at different rates of fishing-related mortality of herbivores (m_H), shown for different herbivore handling times (h) and harmful effects of microbes on coral growth and recruitment (γ) when **a)** the density of microbes per unit algae does not increase with reef-scale algal cover (i.e., when $z = 0$), and **b)** when the density of microbes increases with algal cover (i.e., when $z = 4.6$). To make patterns easier to see, results are shown for a subset of h and γ values used in simulations.

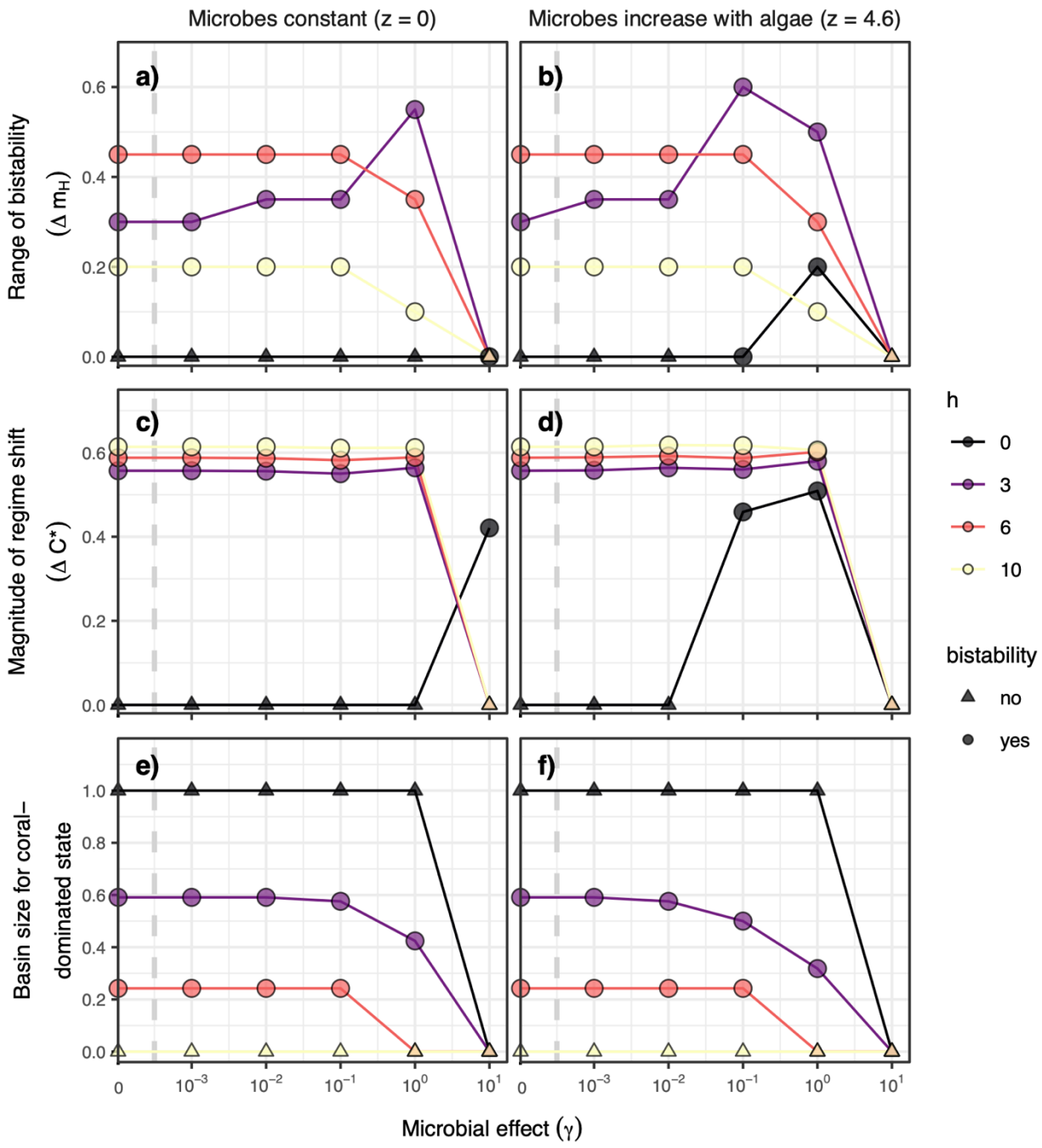

Fig. 3.3 Regime shift responses to changes in the negative effect of microbes on coral growth and recruitment (γ; varied on the x-axis); the exponential increase of microbial densities with reef-scale algae (z; varied by column); and herbivore handling time (h; indicated by color). **(a, b)** The range of fishing mortality rates (Δm_H) that produce bistability. Triangles indicate points when bistability does not occur, while circles indicate bistability. **(c, d)** The magnitude of the

regime shift from the coral- to the algal-dominated state (ΔC^*) at the environmental tipping point from the coral-dominated to algae-dominated state. **(e, f)** The size of the basin of attraction for the coral-dominated state, shown for one level of fishing mortality ($m_H = 0.4$). (See this relationship under more levels of m_H in Appendix 3 Fig. S3.5.) To make patterns easier to see, we only show results for a subset of h values used in simulations for **a-f**. The vertical dashed line indicates a break in the $\log_{10}$ scale on the x-axis, with the points at the far left of the graph representing $\gamma = 0$. Values for all other parameters were: $\alpha = 0.5, r_H = 1.5, i_C = .01, b_C = .3, \mu = .1\ , i_A = .05, b_A = .8, a = 1, c = 1.2$.

Discussion

Both of the positive feedbacks that we evaluated—an empirically-supported positive association between algae and microbes that have negative effects on coral growth and recruitment, and a feedback associated with the commonly-assumed form of the herbivore functional response—made regime shifts more likely. Specifically, as the strength of the feedback or the combined feedbacks increased (modulated by the parameters z, h, and γ), the range of fishing mortality rates (Δm_H) producing bistability initially increased (Fig. 3.3a,b), making a regime shift initiated by a perturbation in coral or algal cover possible over a broader range of environmental conditions. The size of the basin of attraction for the coral-dominated state also declined as feedback strength increased (Fig. 3.3e,f), meaning the system could be shifted from coral- to algal-dominance by a smaller disturbance to coral or algal cover. Basin size was also reduced by increasing fishing mortality (Fig. S3.5), indicating that when microbes had stronger effects on corals (or when herbivores had higher handling times), the system was less resilient to perturbations in coral cover, and this reduction in resilience was worsened by increases in fishing pressure.

However, as the feedback strength increased, it also lowered the environmental tipping point for the catastrophic shift from a coral-dominated state to an algal-dominated state occurred at lower fishing mortalities (Fig. 3.2, S3.4). Above a threshold feedback strength, this decrease in the tipping point reduced the range of conditions producing bistability (Δm_H), as an increasing proportion of the higher m_H values produced only a single, algae-dominated equilibrium (and an alternative coral-dominated equilibrium was no longer possible; Fig. 3.2). Eventually, the feedback (or combined feedbacks) was strong enough to preclude the existence of a coral-dominated state under any amount of fishing mortality.

Despite these effects on the susceptibility of the system to regime shifts, microbial and herbivore feedbacks generally had weak effects on the magnitude of regime shifts (i.e., the difference in coral cover between the coral- vs. the algae-dominated stable states at the environmental tipping point for the regime shift; Fig. 3.1a). The positive feedbacks had the largest influence on the magnitude of regime shifts when they produced bistability under conditions that it would otherwise not occur, such as when other feedbacks were weak (e.g., when γ =10 and h and $z = 0$), or when they prevented bistability because the feedbacks were strong enough to prevent coral dominance (Fig. 3.3c,d).

These results suggest that factors that exacerbate the negative effects of algae-associated microbes on corals could increase the likelihood of regime shifts in reefs to algal-dominated states. For example, harmful or opportunistic microbial taxa increase on reefs with nutrient and sediment inputs, such as from terrestrial runoff and wastewater effluent (Ziegler et al. 2016) and from atmospheric deposition of dust (Westrich et al. 2016). Nutrient inputs could stimulate microbial growth, exacerbating the response of microbes to DOC produced by algae (effectively increasing z). This effect would result in higher microbial densities (M), thereby enhancing the reduction in coral growth and recruitment due to algae-associated microbes (γMA). Similarly, the introduction of coral pathogens to a reef, such as through sewage effluent (Sutherland et al. 2011), atmospheric deposition (Garrison et al. 2003, Archer et al. 2020), or ship ballast water (Aguirre-Macedo et al. 2008, Cohen 2010) could amplify the magnitude of the negative effect of microbes on coral growth and recruitment (i.e., increase γ) without a concurrent increase in microbial densities. Climate-change could also amplify harmful microbial impacts on corals, stimulating the growth and virulence of opportunistic taxa (Toren et al. 1998, Boyett et al. 2007) and contributing to shifts in coral microbiomes (Tracy et al. 2015, McDevitt-Irwin et al. 2017,

Morrow et al. 2018). These changes can reduce the physiological performance (Glasl et al. 2016), and consequently, growth of corals, as well reduce their resilience to other environmental stressors, including increased densities of algae (Zaneveld et al. 2016) and exposure to disease (Kuntz et al. 2005, Kline et al. 2006, Zaneveld et al. 2016).

Reefs are increasingly exposed to disturbances that facilitate the growth and proliferation of algae (e.g., overfishing, coral bleaching, outbreaks of coral-eating sea stars, hurricanes, etc.; (Goldberg and Wilkinson 2004, Hughes et al. 2007), as well as the aforementioned factors that promote microbial growth and their harmful effects on corals (e.g., nutrients inputs, pathogen introductions, and temperature increases). Given these changes, our results suggest that reefs could be increasingly susceptible to regime shifts. Since corals are primary habitat-builders in reefs, supporting diverse fish and invertebrate communities (Pratchett et al. 2018) and absorbing significant wave energy (Ferrario et al. 2014), regime shifts in reefs from coral- to algal-dominated states could have huge ramifications for reef ecosystems, as well as the hundreds of millions of people worldwide who depend on coral reefs for food, employment, or wave and storm protection for their homes (Wilkinson 2008, Ferrario et al. 2014). Therefore, explicitly considering how microbial feedbacks could hasten coral declines and facilitate difficult-to-reverse algal take-overs of reef habitats will be critical for effective management of reef ecosystems.

The microbe and herbivore positive feedbacks in our model, (which were modulated by the parameters h , z, and γ), exhibited complex interactions. Sometimes combining these feedbacks facilitated regime shifts—increasing the range of fishing-related mortality rates for herbivores that produced alternative stable states and increasing the magnitude of the shift in coral cover from the coral- to algal-dominated state at the environmental tipping point. At other

times, combining positive feedbacks impeded regime shifts. Previous studies and reviews have focused on how strengthening the net positive feedback in a system, such as through combining multiple positive feedback loops, can facilitate regime shifts and the formation of alternative stable states (Kéfi et al. 2016, Leemput et al. 2017). Our work supports these conclusions, but also illustrates how intensifying the strength of a net positive feedback (either by strengthening a single, strong positive feedback or by combining multiple weaker positive feedbacks) can have a unimodal effect on bistability.

Furthermore, the interactions between the two positive feedbacks that we evaluated demonstrates the critical importance of considering multiple feedbacks when investigating regime shifts in reefs and in other systems in which multiple feedbacks are plausible. Previous research in coral reefs has predominately focused on the effects of herbivory on regime shifts (Mumby et al. 2007, Mumby 2009, Blackwood et al. 2010, 2018, Muthukrishnan et al. 2016, Leemput et al. 2017, Briggs et al. 2018), which has contributed to the widely held belief among reef scientists that the maintenance of herbivore populations (e.g., through effective fishing management policies) is crucial for maintaining reefs as coral-dominated ecosystems. Our work demonstrates that overlooking the additional effects of microbes could prevent managers from anticipating when a regime shift might occur in a reef, and could prevent the restoration of the coral-dominated state once a shift occurs, even after a significant restoration of herbivores (if factors that exacerbate microbial effects on corals continue to persist).

However, our results also illustrate the importance of herbivore foraging behavior to the dynamics of coral and algae, and the presence of alternative stable states in reefs. Herbivore handling time, h, strongly influenced bistability in our model, because handling time regulated the strength of the algae-herbivore positive feedback that promoted regime shifts to algae-

dominated states under elevated herbivore mortality rates. Handling time did this by determining the degree to which individual herbivore feeding could be saturated by algae. When h is zero, per capita feeding does not saturate, but as h increases, herbivore feeding is saturated at lower and lower algal densities, facilitating algal expansion. Despite the important effects of the herbivore functional response on the ability of herbivores to regulate algae, estimates of functional response parameters used in previous regime shift models were based on data from studies with inappropriate designs to characterize a functional response relationship. We found only one empirical study that directly investigated this relationship for a reef herbivore, a surgeonfish, *Acanthurus chirurgus* (Capitani et al. 2021). However, functional responses could vary among herbivore feeding guilds, especially among roving fish browsers that consume algae with few structural or chemical defenses (which therefore might require negligible handling time for consumption and digestion), vs. more resident grazers like urchins or other roving herbivores that consume more heavily defended algae. Therefore, a better characterization of functional response relationships for a variety of herbivore taxa and food types is needed to accurately describe herbivory processes in reefs, and to make reliable predictions of when abrupt transitions may occur in specific reef locations with different herbivore and algal assemblages.

Our work shows that although corals and algae can coexist in the presence of microbes with harmful effects on corals, microbes influence whether coral can become a dominant component of the reef benthic community and whether the system exhibits more complex nonlinear dynamics, such as abrupt transitions and alternative stable states. This approach expands on previous theoretical work that has focused on how microbes can influence the coexistence of macroscopic competitors, such as soil microbes that alter the growth of competing plant species (Bever et al. 1997, Umbanhowar and McCann 2005, Miki et al. 2010, Kandlikar et

al. 2019). Our model captures many of the processes that influence coexistence in these previous models: species with differential growth rates and susceptibility to predation that compete for space, with new individuals imported from outside the system (through dispersal, seed banks, etc.). However, by tracking how initial conditions influence community outcomes, and how multiple positive feedbacks (including feedbacks mediated by microbes) influence bistability of ecosystem states, our approach provides a framework for investigating how historical contingencies (e.g., starting conditions) can alter community assembly, and how the strength of specific ecological interactions modifies how resilient a community is to disturbances (i.e., can it be pushed to an alternative stable state?). This framework and the associated methods for quantifying bistability will assist the study of regime shifts and alternative stable states in ecological systems, which historically have been difficult to predict (Scheffer et al. 2012). Despite this difficulty, quantitative reviews have found that management that uses information about regime shifts is demonstrably more successful (Kelly et al. 2015). Therefore, making predictions about regime shifts and their drivers more robust will improve our ability to develop effective strategies to prevent losses in ecosystem services or declines in human well-being associated with catastrophic shifts, and to restore degraded systems to their original states after a shift occurs. Since microbes are key actors in virtually every ecosystem, efforts to integrate their influence into studies of regime shifts is therefore a critical next step to improve our predictions of when and how regime shifts will occur in a variety of systems.

Acknowledgements

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We thank D. Gokhale for assistance with the modeling code, and A. Park and R. Holdo for feedback about the model and analyses.

Appendix 3

Table S3.1) Parameters and state variables used in models, their units (when applicable), their default value used in simulations, and the range of values for each parameter from the empirical literature or from previous models of coral reef regime shifts. Parameters that were varied to explore their effects on the equilibrium dynamics of the system are highlighted in **bold** text.

Parameter	Description	default	units	literature range	Ref. for range	notes
State variables						
S	proportion of benthos that is bare space	NA	none	[0, 1]		
A	proportion of benthos that is algae	NA	none	[0, 1]		
C	proportion of benthos that is coral	NA	none	[0, 1]		
H	herbivores; proportion of carrying capacity (N/K)	NA	none	[0, 1]		
M	prokaryotic microbe density per unit algae	NA	cells / unit algae	[*c*, inf]		
Parameters						
i_C	import rate of propagules (coral)	0.01	yr -1	[0.00002, 0.01]	Fung et al. 2011	empirically derived for broadcast spawning corals
				[0.0004, 0.5]	Fung et al. 2011	empirically derived for brooding corals
				[0, 0.05]	Leemput et al. 2017	no justification given for range
				[0, 0.05]	Briggs et al. 2018	no justification given for range; their default: 0.001
i_A	import rate of propagules (algae)	0.05	yr -1	0	Fung et al. 2011	no known empirical estimates in coral reef systems, Fung et al. assumed macroalgal recruitment was negligible
				0.05	Leemput et al. 2017	no justification given for value
				[0, 0.05]	Briggs et al. 2018	no justification given for range; their default: 0.0001

Parameter	Description	default	units	literature range	Ref. for range	notes
b_C	expansion rate of corals into bare space	0.3	yr -1	[0.02, 0.2]	Fung et al. 2011	empirically derived, combining ranges for "pristine" and "non-pristine" reefs
				0.3	Leemput et al. 2017	no justification given for value
				[0, 0.2]	Briggs et al. 2018	no justification given for range; their default: 0.1
b_A	expansion rate of algae into bare space	0.8	yr -1	[0.05, 1.6]	Fung et al. 2011	empirically derived, combining ranges for "pristine" and "non-pristine" reefs
				0.8	Leemput et al. 2017	no justification given for value
				[0, 1.2]	Briggs et al. 2018	no justification given for range; their default: 0.5
μ_C	mortality rate of corals	0.1	yr -1	[0.02 , 0.3]	Fung et al. 2011	empirically derived
				0.1	Leemput et al. 2017	no justification given for range
				[0. 0.1]	Briggs et al. 2018	no justification given for range; their default: 0.05
α	competition coefficient (negative effect of algae on coral expansion & recruitment, not including the effects of microbes)	0.5	none	[0.2 , 0.9]	Fung et al. 2011	empirically derived
				0.5	Leemput et al. 2017	no justification given for value

Parameter	Description	default	units	literature range	Ref. for range	notes
$\boldsymbol{\gamma}$	effect of algae-associated microbes on coral expansion & recruitment	**[0, 10]**	none	none	introduced for this study	
r_H	intrinsic rate of increase for herbivores	1.5	yr -1	[0 , 1.5]	O'Farell 2011	empirically derived
				1	Leemput et al. 2017	no justification given for value
m_H	additional herbivore mortality (e.g., due to fishing)	[0, 1.5]	yr -1	[0, 0.6]	McIlwain & Taylor 2009	empirically observed range [0.2, 0.6], with an assumed minimum mortality of 0 in a perfectly isolated or protected reef
a	herbivore attack rate of algae	1	yr -1	[0.05, 15]	Fung et al. 2011	empirically derived
				1	Leemput et al. 2017	no justification given for value
$\boldsymbol{h}$	herbivore handling time for algae	**[0 , 10]**	yr -1	[0 , 5]	Leemput et al. 2017	no justification given for value
c	baseline microbial density per unit algae	1	x 100 cells cm^{-2}	$[10^0, 10^5]$	Egan et al. 2013	empirically observed
$\boldsymbol{z}$	exponential rate of increase of microbial densities with reef-scale algal cover	**0 or 4.6**	none	[0, 5]	derived for this study (see Appendix 3.1)	empirically derived from Haas et al. 2016

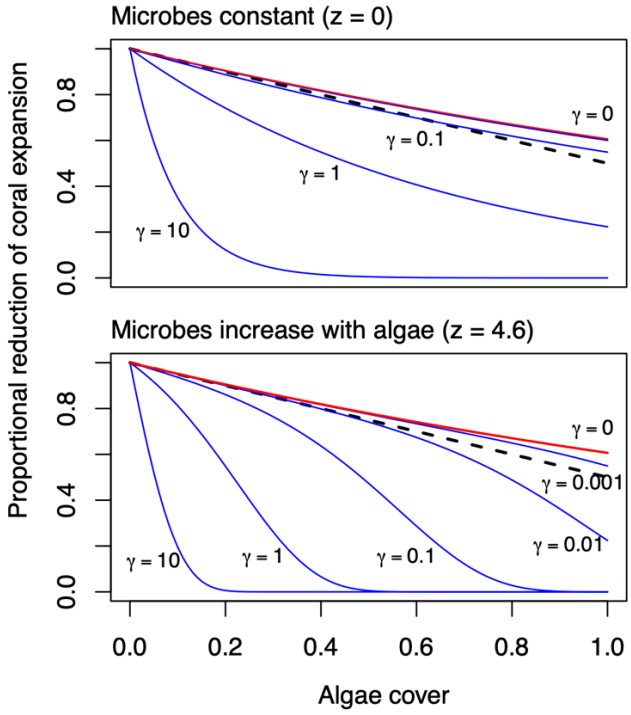

Fig. S3.1 The relationship between the proportion cover of algae and the proportional reduction in coral expansion and recruitment into bare space for different values of the parameters z, which determines the relationship between microbial densities and algal cover, and γ, which determines the magnitude of the microbial effect on coral. The dashed black line indicates the linear relationship used by Leemput et al. (2017), $(1 - \alpha A)$, which approximates the non-linear relationship used here ($e^{-(\alpha A)}$) when microbes have no effect ($\gamma = 0$; solid red line). In those cases, all reduction in coral expansion is due to the direct effects of algae only. The blue lines represent the non-linear relationship used in our model when microbes have a negative effect on corals (i.e., $\gamma > 0$). γ decreases from 10 to 0.001 by orders of ten, moving from the left to right. (However, when $z = 0$, the lines for $\gamma = 0.01, 0.001$ are not distinguishable from $\gamma = 0$.) Thus, microbes reduce coral expansion to a larger degree as algal cover increases. Algae reduces coral growth more rapidly at higher values of γ, and this reduction is further accelerated when microbes increase in density with algae ($z = 4.6$).

Appendix 3.1: Empirical derivation of parameter values used in models

z, *the rate at which microbial densities increase with site-level algae*

Haas et al. (2016) observed an increase in the density of microbial cells in the water column as site-level algal cover increased at reef sites in each of the three ocean basins that they evaluated. They found that microbes increased from approximately 10^5 to 10^7 cells mL^{-1} of seawater as algal cover increased from 0 to 100 % cover. Using these observations and our equation for microbial densities (M):

$$M = ce^{zA}$$

we can solve for the z that would produce this relative change in density for this change in algal cover (A). If $M_1 = 10^7$ at 100 % algal cover ($A = 1$) and $M_0 = 10^5$ at 0 % cover ($A = 0$), then:

$$\frac{M_1}{M_0} = \frac{ce^{z1}}{ce^{z0}} = \frac{e^z}{e^0}$$

$$\frac{10^7}{10^5} = \frac{e^z}{e^0}$$

$$10^2 = e^z$$

$$\ln(100) = \ln(e^z)$$

$$4.6 = z$$

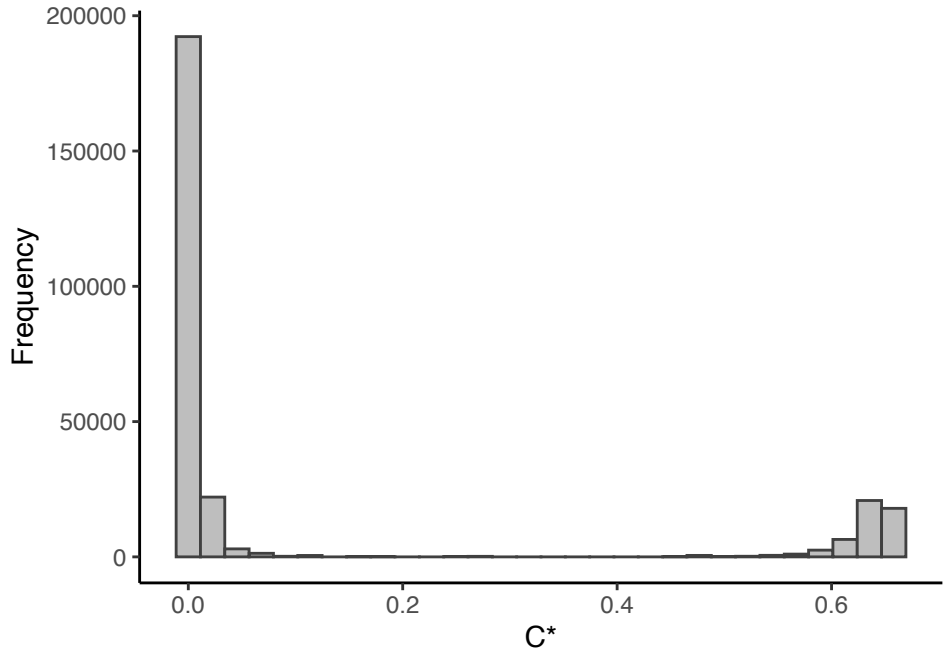

Fig. S3.2 Histogram of frequency distribution of the equilibrium coral cover (C*) for simulations of each combination of parameters (h, γ, z, and m_H) and starting conditions for coral and algae cover. Data is bimodal, falling in the range of 0 to 0.3 or 0.4 to 0.7. These distributions were used to determine the cutoffs for the high- vs. low-coral states, with coral cover between 0 and 0.35 representing the low-coral, algae-dominated state, and coral cover greater than or equal to 0.4 representing the high-coral, coral-dominated state.

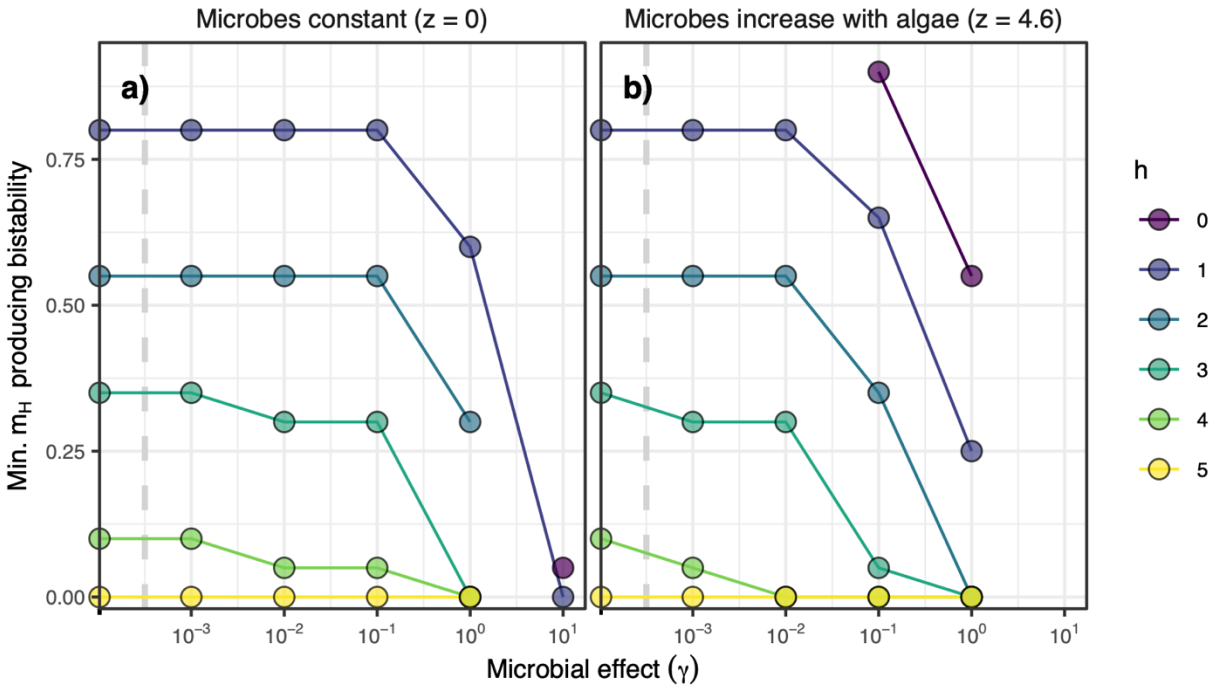

Fig. S3.3 Minimum fishing mortality required for bistability to occur, for different herbivore handling times (h), harmful effects of microbes on coral growth and recruitment (γ), and responses of microbial densities to algae (z). This response indicates the environmental threshold for the amount of fishing that will make the system susceptible to regime shifts caused by perturbations to coral or algal cover. Results are shown for h = 0 to 5 yr $^{-1}$, because the minimum m_H producing bistability is 0 for all values of $h \geq 5$. The vertical dashed line indicates a break in the $\log_{10}$ scale, with the points at the far left of the graph representing $\gamma = 0$.

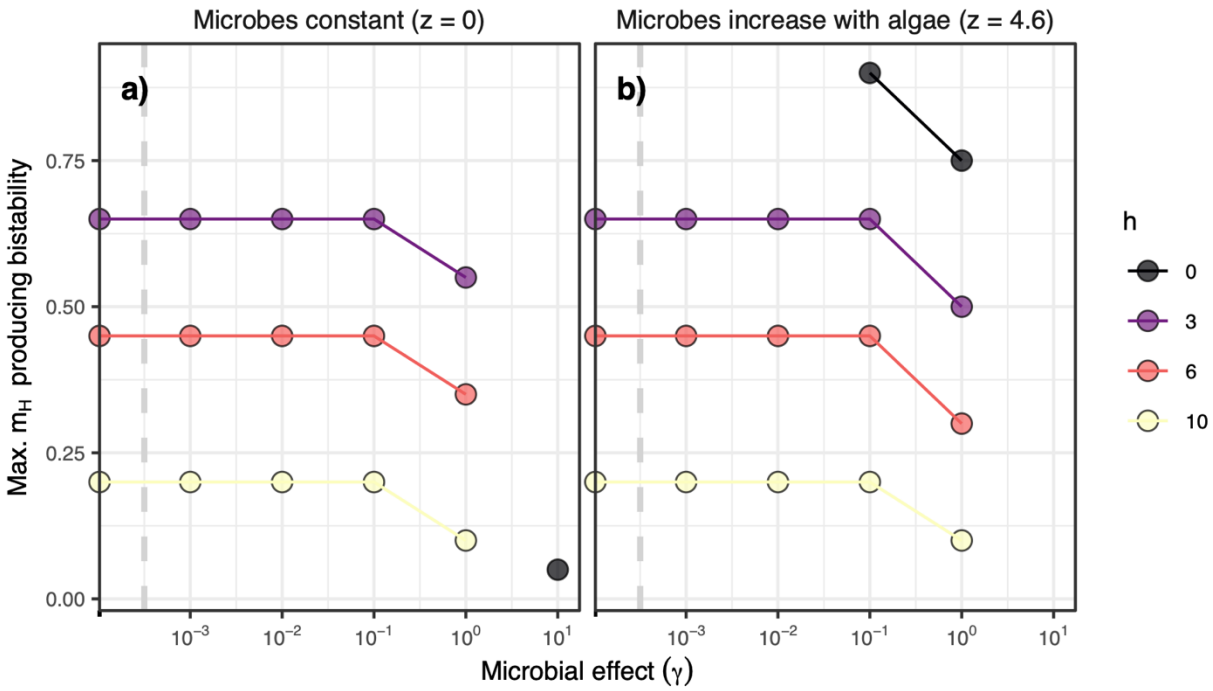

Fig. S3.4 Maximum fishing mortality producing bistability (i.e., the environmental tipping point for a catastrophic shift from coral- to algal-dominance) for different herbivore handling times (h), harmful effects of microbes on coral growth and recruitment (γ), and responses of microbial densities to algae (z). Results are shown for a subset of h values used in simulations to make patterns easier to see. The vertical dashed line indicates a break in the $\log_{10}$ scale, with the points at the far left of the graph representing $\gamma = 0$.

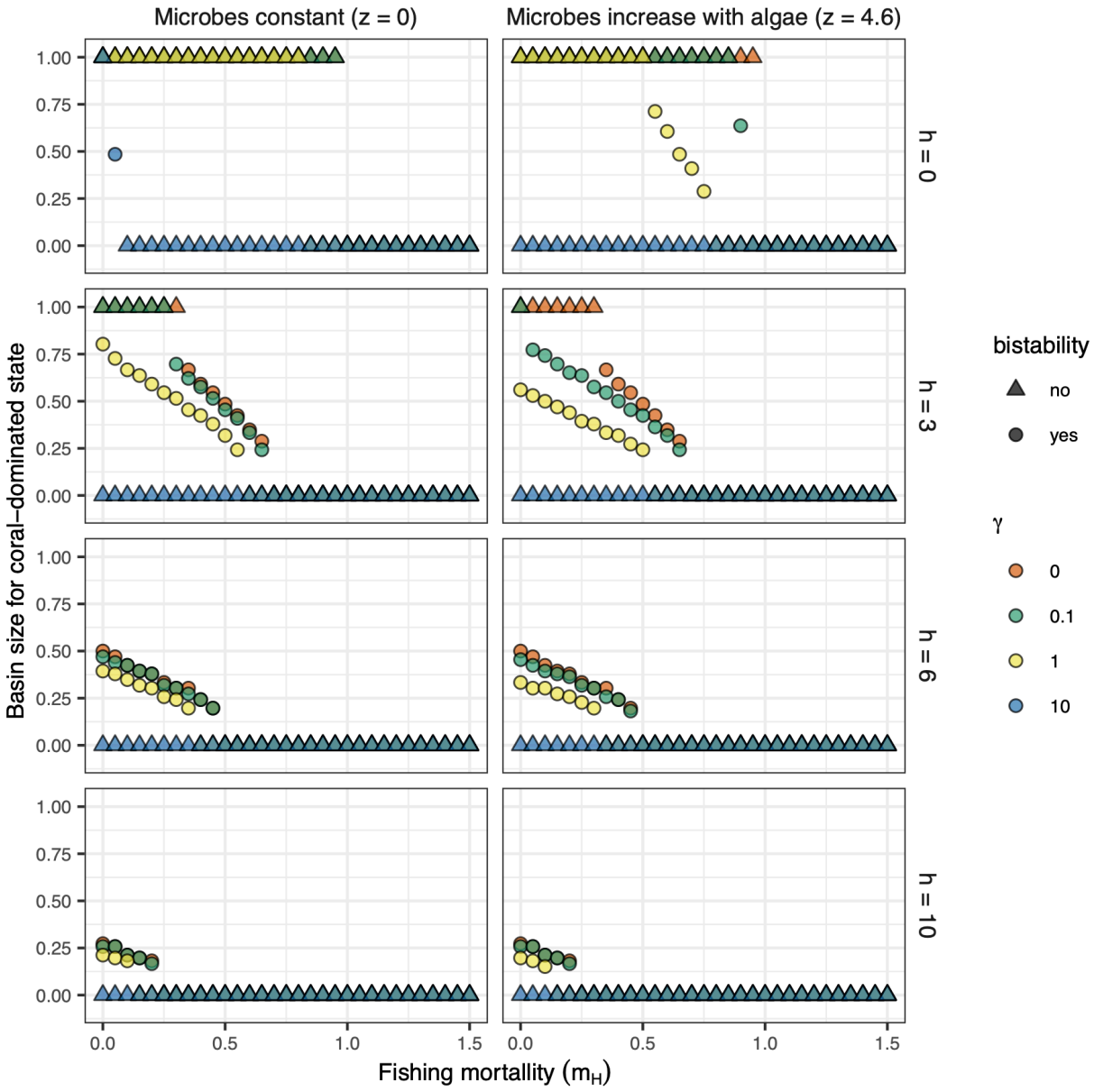

Fig. S3.5 The size of the basin of attraction for the coral-dominated state as the microbial feedback (γ, z) and fishing mortality (m_H) varied. To make patterns easier to see, we only show results for a subset of levels of h and γ. Points shapes indicate whether the system exhibited bistability for that combination of parameter values (triangles indicate only one stable equilibrium existed, circles indicate bistability was present).

CHAPTER 4

THE EFFECTS OF MACROALGAL HOST SPECIES AND PATCH SIZE ON CIGUATOXIC DINOFLAGELLATES IN A PACIFIC CORAL REEF [3]

[3] AA Briggs, J Freeze, and CW Osenberg. To be submitted to *Harmful Algae*.

Abstract

Some benthic dinoflagellates produce toxins that have negative consequences for humans and aquatic life. Some of these dinoflagellates are noted for having patchy distributions at fine spatial scales within marine environments. However, the drivers of these patterns are still poorly understood. We evaluated the effects of several biotic factors that varied across microhabitats within a coral reef in Moorea, French Polynesia, on the densities of three toxin-producing genera of benthic dinoflagellates, *Gambierdiscus*, *Prorocentrum*, and *Ostreopsis*, which are implicated in ciguatera poisoning in humans. Because these dinoflagellates frequently associate with macroalgae, we investigated the effects of the size and species composition of the macroalgal patches on the dinoflagellate densities within the patch. Using artificial sampling substrates deployed in either open, algae-free patches, or patches of macroalgae of five different areal (and biomass) size classes and composed of either *Sargassum*, *Turbinaria*, *Dictyota*, *Amansia*, or a mixture of these species, we determined that patch size was not a significant predictor of dinoflagellate densities. The macroalgae dominating a patch was a significant predictor of *Ostreopsis* densities, with lower densities in patches without algae and in patches dominated by *Dictyota*. *Prorocentrum* did not exhibit significant variation among patches dominated by different species of macroalgae, although its mean density was ~ 34 % higher in the no-algae patches. *Gambierdiscus*, which had very low densities in all patches, was not observed in many samples, and its presence in patches did not vary among patch types. Overall, the relatively high densities of dinoflagellates found in the no-algae patches suggest that site-level factors may be the primary driver of their densities, with smaller-scale factors like patch composition or other microhabitat differences adding only small additional variation in their densities.

Introduction

Ciguatera poisoning (CP) is the most common marine toxin-related illness reported in humans (Friedman 2008, Friedman et al. 2017). It is caused by neurotoxins produced by several dinoflagellate species, including species within the genera *Gambierdiscus* and *Fukuyoa*, which are found primarily in near-shore reefs in the tropics and subtropics (Litaker et al. 2010). Other toxin-producing dinoflagellates like *Prorocentrum* and *Ostreopsis* also have been implicated in ciguatera-like poisonings and other intoxication syndromes (Yasumoto et al. 1987, Tosteson 1995, Van Dolah 2000, Gallitelli et al. 2005, Aligizaki et al. 2011, Parsons et al. 2012). Toxins produced by these dinoflagellates bioaccumulate in the tissues of consumers that ingest the dinoflagellates, and can be trophically transferred through the food web, resulting in elevated concentrations in some species of fish and large invertebrates (Lehane and Lewis 2000, Darius et al. 2018). Humans can then become sick with ciguatera poisoning after consuming seafood contaminated with these toxins. However, despite the large number of people affected by ciguatera poisoning, many aspects of the ecology of the causative dinoflagellates are still poorly understood.

Although ciguatoxic dinoflagellate genera can be found free-living in the water column (Yasumoto et al. 1977, Aligizaki and Nikolaidis 2006), they are more generally considered to be associated with benthic substrates, including non-living substrates like sand or rubble (Grzebyk et al. 1994, Aligizaki and Nikolaidis 2006), and algal substrates, including upright macroalgae as well as filamentous turf algae (Yasumoto et al. 1979, Grzebyk et al. 1994, Aligizaki and Nikolaidis 2006, Parsons et al. 2011). Algae sometimes host very high densities of these dinoflagellates (Yasumoto et al. 1979, Grzebyk et al. 1994, Parsons et al. 2011), and free-swimming dinoflagellate cells will attach themselves to algal thalli with mucilage (Nakahara et

al. 1996, Rains and Parsons 2015). This suggests that these dinoflagellates may derive some benefits from associating with algae or use it as an indicator of a favorable microhabitat. A variety of factors that promote algal growth are increasingly affecting many of the reef ecosystems in which ciguatoxic dinoflagellates are commonly found, including climate change, overfishing of reef herbivores, and increased nutrient inputs (Goldberg and Wilkinson 2004, Hughes et al. 2007). Given the associations between ciguatoxic dinoflagellates and macroalgae, it is therefore imperative to understand how increases in macroalgae associated with these changes could influence the densities of ciguatoxic dinoflagellates on reefs, and thus affect the risk of ciguatera poisoning in human populations that consume reef resources.

Macroalgae could benefit ciguatoxic dinoflagellate populations through several mechanisms. First, macroalgae could provide physical structure for dinoflagellates to inhabit, potentially providing protection from high light, which *Gambierdiscus* is known to be sensitive to (Morton et al. 1992, Xu et al. 2016), spatial refugia from consumers, which may reduce dinoflagellate densities (Villareal and Morton 2002, Loeffler et al. 2015), and protection from dislodgement from their substrate by strong water motion (Nakahara et al. 1996). Macroalgae also influence seawater chemistry, leaking nutrients and other chemical compounds that could influence dinoflagellate growth (Bomber et al. 1989, Grzebyk et al. 1994, Parsons et al. 2011, Rains 2015). For example, macroalgae leak dissolved organic carbon and other chemical compounds (Nelson et al. 2013, Haas et al. 2016) that directly stimulate the growth of epiphytic dinoflagellates or stimulate bacteria that promote dinoflagellate growth (Sakami 1999). In contrast, some macroalgae produce high concentrations of allelopathic compounds (Paul and Hay 1986), which might create a less favorable microenvironment for epiphytic dinoflagellates and reduce their growth and local densities (Ben Gharbia et al. 2017). Since macroalgae species

have varying morphologies and produce different types and amounts of chemical compounds (Paul and Hay 1986, Rasher et al. 2011, Nelson et al. 2013), the direction and magnitude of their effects on dinoflagellates likely vary.

Previous studies have found that certain algae promote higher growth rates or densities of ciguatoxic dinoflagellates (Parsons et al. 2011, Rains and Parsons 2015). However, the algal taxa that support the most growth or highest densities of dinoflagellates vary widely among studies (Parsons et al. 2012). For example, Yasumoto et al. (1979) found relatively high densities of *Gambierdiscus* on *Turbinaria ornata* in reefs at Tahiti compared to other macroalgae (including *Halimeda*, *Sargassum*, and *Ulva*), while (Nakahara et al. 1996) found at the same reef site that *Gambierdiscus* was associated with *Jania* sp. but not *Turbinaria*. In the Caribbean, several studies have reported that *Dictyota* hosted high densities of *Gambierdiscus* (Carlson et al. 1984, Ballantine et al. 1988), while the opposite pattern was observed in the Pacific (Nakahara et al. 1996, Parsons et al. 2011). Previous authors have hypothesized that these contrasting results among studies are the result of differences in the associations between dinoflagellate taxa and macroalgal genera from different geographic regions (e.g., in Caribbean vs. Pacific reefs) and variation among dinoflagellate species or strains in their responses to specific macroalgal hosts (Rains and Parsons 2015). However, dinoflagellate densities can be patchy, demonstrating spatial aggregation (Richlen and Lobel 2011) and dramatic variations in densities among the same species of algae collected at the same sampling site and time period (Lobel et al. 1988, Ballantine et al. 1988), suggesting other mechanisms may contribute to the heterogeneity observed in field studies.

First, inconsistencies in the effects of different algal taxa among studies could be related to different methods that were used to estimate dinoflagellate densities, which can give different

results for the same macroalgal species (Lobel et al. 1988). The most common of these methods standardizes dinoflagellate cell counts by the wet weight of algae that was sampled. However, this makes comparisons difficult among macroalgal taxa with disparate morphologies, especially for taxa that differ in their surface area to mass ratios: e.g., structurally defended, dense taxa with thick thalli like *Turbinaria* vs. thin and flat taxa like *Padina* or *Dictyota* (Yasumoto et al. 1979, Lobel et al. 1988).

Another potential explanation for some of the heterogeneity observed in previous studies is that they did not consider the amount of macroalgae surrounding the sample from which dinoflagellate densities were estimated (i.e., macroalgal patch biomass or size). Algal biomass likely affects the amount of chemical compounds leaked into the environment by algae, and thus, could modify the magnitude of any beneficial or negative effects of macroalgae comprising the patch on densities of ciguatoxic dinoflagellates within the patch. Previous studies in aquatic systems have found that higher biomasses of macrophytes produce larger effects on phytoplankton growth and photosynthetic activity (Korner and Nicklisch 2002). In marine systems, the metabolic activity of large patches of macroalgae produces greater changes in local seawater chemistry, e.g., pH, O_2, pCO_2, compared to small patches (Wahl et al. 2017). However, in addition to affecting the amount of chemical compounds released into an area, patch size and biomass can also influence how long those compounds are locally retained within a patch. Larger, denser patches of macroalgae reduce flow and slow the dilution of compounds produced by macroalgae into the water column (Mork 1996, Hurd 2000) to a greater degree than smaller patches. Consequently, concentrations of secondary metabolites and dissolved organic matter could increase more within larger patches relative to smaller patches, which could influence dinoflagellates and other organisms living within the patch. Additionally, larger algal patches

could enhance dinoflagellate densities by providing more structure for the dinoflagellates to occupy and more protection from dislodgement by water motion or consumption by macro-herbivores, which tend to avoid areas with dense patches of macroalgae (Hoey and Bellwood 2011). Higher densities of ciguatoxic dinoflagellate have been observed in benthic substrates protected from grazers (Loeffler et al. 2015). Thus, patch size could modulate the magnitude of any beneficial or negative effects of macroalgae comprising the patch on densities of ciguatoxic dinoflagellates within the patch.

To investigate the local scale (within-patch) effects of macroalgal biomass and species composition on the densities of ciguatoxic dinoflagellates, we sampled dinoflagellates using artificial substrates with standardized surface areas, deployed in naturally occurring patches of macroalgae of different size classes and species composition at a field site in Moorea, French Polynesia. We deployed sampling substrates either in open, algae-free patches, or patches of macroalgae of five different area (and biomass) size classes dominated by either *Sargassum, Turbinaria, Dictyota, Amansia,* or a mixture of these macroalgal species. We quantified densities of *Gambierdiscus, Ostreopsis, and Prorocentrum* cells found on each substrate. We tested the hypotheses that 1) ciguatoxic dinoflagellate densities vary among patch types and that these differences vary according to dinoflagellate genus, and 2) patch size will produce different effects depending on whether the patch type if preferred or non-preferred: specifically dinoflagellate density will increase with patch size for the patch types hosting the highest dinoflagellate densities, but will decrease with patch size for non-preferred patch types (e.g., patches dominated by macroalgae that produce many allelochemicals, such as *Dictyota*; Paul and Hay 1986).

Methods

Study site

Sampling for dinoflagellates was conducted at a backreef site on the North Shore of Moorea, French Polynesia, in an area to the west of Cook's Bay (-17.478029 °, -149.839498 °), during the austral winter (July, 2015). This site had a depth of 1-4 m and contained numerous patches of macroalgae that varied in size and species composition.

Dinoflagellate sampling using artificial substrates

Dinoflagellates were sampled using artificial substrates, consisting of an 11 x 18 cm rectangle of fiberglass screen attached to a subsurface float (Fig. 4.1) deployed in the center of patches of macroalgae. This method provides estimates of dinoflagellate densities that strongly correlate with densities found on surrounding natural substrates (Tester et al. 2014, Fernández-Zabala et al. 2019). Patches were selected if > 90% of their biomass was composed of one of the four most common species of macroalgae in this section of the Moorean backreef (*Turbinaria ornata, Sargassum pacificum, Amansia rhodantha, Dictyota bartayresiana*), or a mixed assemblage of all four of these species. For each type of macroalgae, 5 patches of varying sizes, roughly falling into 5 separate size classes were sampled using this method. Patch size classes were: $\leq$ 400 cm^2, 401 – 900 cm^2, 901 - 1600 cm^2, 1601 – 3600 cm^2, and > 3600 cm^2. Additionally, a control screen was deployed in the center of sand or rubble patches, with at least 3 m to the nearest patch of macroalgae, to estimate ambient densities of dinoflagellates in the absence of algae. Three of these algae-free patches were sampled in each experimental round.

After patches were selected, they were picked through to look for non-dominant species, and the subsurface float setup was deployed in the center of patch, without the dinoflagellate collection screen. Patches were then left to settle and re-equilibrate for 1-2 days, after which the

dinoflagellate collection screens were attached. Screens were deployed for 24 hours to allow dinoflagellates to colonize their surfaces, based on previous studies that found that this deployment duration provided accurate estimates of dinoflagellate densities (Tester et al. 2014, Fernández-Zabala et al. 2019). After the deployment period, screens were carefully collected in 1 L Ziplock bags and sealed underwater. Bags were transported back to the boat and put on ice until they could be processed in the lab. This sampling procedure was conducted two times, each time on a new set of patches, each macroalgal species (or mixed assemblage) across a gradient of patch sizes. A few screens were lost overnight in the field or their sampling bags leaked, so these samples were removed from future analyses.

Patch characteristics

Patch area was calculated using the formula for the area of an ellipse, based on measurements of the widest points on the two major axes of each patch. Patch biomass density was sampled by collecting all the macroalgae in a 20 x 20 cm quadrat positioned near the sampling device in the center of the patch. To account for heterogeneity in biomass density in larger patch size classes, multiple samples were collected from quadrats that were placed haphazardly within the patch. One quadrat was used to sample biomass in the patches $\leq$ 900 cm^2, two quadrats were used in the 901 - 1600 cm^2 size class, three in the 1601 – 3600 cm^2 size class, and four in the largest size class. The wet mass of macroalgae within the quadrat was measured after it was spun in a salad spinner for 30 seconds to remove excess water. Total patch biomass was estimated by multiplying the area of the patch by the wet mass of macroalgae per unit area (using the average macroalgae wet mass in patches with multiple biomass samples).

Dinoflagellate sample processing & density estimates

The screens were returned to the lab and processed within 20 hrs of collection. Each

Ziplock containing a screen was gently kneaded and shaken for 1 minute to loosen the dinoflagellates, and then the contents of the bag were poured onto a stack of sieves (212 μm on top of 20 μm), and the screens were rinsed with filtered seawater. The filtrate from the 20 μm sieve was collected with a pipette and placed into a 15 ml falcon tube (filled with a consistent volume of filtered seawater and preserved with formalin (with a final concentration of 10%). Both sieves were cleaned thoroughly with filtered (2 μm) seawater between samples to avoid cross-contamination.

The falcon tubes were transported to the University of Georgia, USA, where they were shaken, and allowed to settle overnight to help separate the cells and other organic matter from the fine sediment. The thin layer of greenish or light brown organic material that collected on top of the denser white sediment was collected using a pipette and placed in a 2 mL capped vial. This procedure was performed 3 times for each sample, adding organic material to the same 2 mL vial each time. Once this process was complete, the final, concentrated samples were brought to a final volume of 2 mL.

Cell counts

Counts of *Gambierdiscus, Ostreopsis,* and *Prorocentrum* cells were performed on 2-6 separate 20 μl sub-samples from the 2 mL vials for each screen, after its contents had been homogenized by vigorously inverting the vial 10 times. (Cell counts were performed on at least 3 sub-samples for all samples except for two that exhibited evaporation.) Thus, cell counts were generally based on 3 % - 5 % of each sample. Generally, more sub-samples were taken from samples in which *Gambierdiscus* was not observed, i.e., sub-sampling effort was increased to increase the likelihood of detecting this rare taxon in the sample. Cell counts were performed on slides of each sub-sample using an Olympus BX50 compound microscope at 200 x

magnification (and using 400 x magnification to confirm identifications). To estimate the total cell abundance of each sample, cell counts of individual sub-samples were added together and then multiplied by a scaling factor based on the proportional volume of the total 2 mL sample that was viewed for cell counts. Cell densities were then calculated for each sample by dividing the estimated cell abundance by the surface area of the sampling screens (258.4 cm^2, calculated using the equations in Tester et al. 2014). Cells were identified to genus based on size, shape, and thecal plate morphology, which were compared to line drawings, photomicrographs, and species descriptions (Fukuyo 1981, Faust and Gulledge 2002, Richlen and Lobel 2011).

Statistical analyses

We fit generalized linear models with either patch area or patch biomass, patch algal host species, the interaction between algal species and patch area or biomass, and temporal block as fixed-effect predictors for dinoflagellate densities. Because patch biomass and patch area were highly correlated and had similar relationships with dinoflagellate densities (Fig. S1, Appendix 1), we only report the results for the models with patch area as a predictor. We did not include interactions between temporal block with the other treatments because we had limited observations with which to evaluate these interactions, and no *a priori* reason to expect the effect of biomass or macroalgal species to change with block. The baseline, no-algae samples were left out of analyses containing the predictor patch area, as they were collected from reef locations in which the area covered by algae always equaled zero. Because our analyses that included either patch area or biomass as predictors indicated that they did not drive significant variation in dinoflagellates, we evaluated effects of patch type by including no-algae samples in the analysis and pooling samples from all patch sizes within each algal host.

We modeled cell densities of *Prorocentrum* and *Ostreopsis* with a negative binomial

distribution, as their counts per screen were overdispersed relative to a Poisson distribution. *Gambierdiscus* was very rare; therefore, we used a logistic regression to model its presence/absence. When there were significant differences among groups (patch type), we conducted post-hoc tests to determine which groups drove differences.

We used the *MASS* package (Venables and Ripley 2002) in R 4.0.3 (R Core Team 2020) to fit the negative binomial models. The P-value for each predictor was estimated using a likelihood ratio test, performed by the *MASS* package for the negative binomial models and the *stats* package (R Core Team 2020) for the binomial models. Post-hoc comparisons of groups with Tukey's corrected P-values were performed with the *emmeans* package (Lenth 2020). Model predictions were graphically compared to the data to verify that model distributions approximated the observed data.

Results

We were able to sample algal patches across a gradient of sizes for all algal taxa except for *Dictyota*, which was primarily found in smaller patches, unless it was in a mixed species patch (Fig. S4.1, Appendix 4.1). Neither patch area nor the interaction between patch area and algal species was significantly associated with the densities of any of the dinoflagellate genera ($P > 0.3$, Table 4.1, Fig. 4.2). Thus, we dropped patch area from subsequent analyses and compared dinoflagellate densities among the different patch host algae (pooling patch sizes) and the algae-free (i.e., no-algae) patches.

Ostreopsis showed significant variation among patch types ($P = 0.045$, Table 4.2). On average, patches dominated by *Turbinaria ornata* had the highest densities of *Ostreopsis*, with *Sargassum* and mixed algae patches also exhibiting relatively high densities (Fig. 4.3a). The lowest densities of *Ostreopsis* were in the *Dictyota* patches, followed by the no-algae patches.

The no-algae patches had densities of *Ostreopsis* that were on average 50 % lower compared to the algal patches, however, the mixed algae patches had similar densities to the other host alga types, indicating there was no unique effect of a mixed patch compared to the combined effects of each host alga comprising the mixed patch. There was no significant difference in the densities of *Ostreopsis* between temporal blocks (Table 4.2). Post-hoc analyses of the patch types indicated that only *Turbinaria* and *Dictyota* were significantly different (*df* = inf., z-ratio = -2.942, P = 0.038). Because we did see a significant difference between the patch type in the analyses that included the no-algae patches (Table 4.2), but not in the analyses that excluded these samples and included patch area as a predictor (Table 4.1), it suggested that the no-algae group, with its low average cell densities, could have contributed to the significant difference between the patch alga groups. However, a post-hoc analysis of the no-algae vs. the algae samples did not detect a significant difference between these groups (*df* =1, deviance = 2.47, P = 0.12).

Prorocentrum showed little variation among patches with algae, and patches with algae had slightly lower densities than did the no-algae patches (Fig. 4.3b). The no-algae patches supported the highest average density of *Prorocentrum* (338 cells 100 cm^{-2} $\pm$ 156 SE), compared to an average of 224 cells 100 cm^{-2} $\pm$ 14 SE for the algae patches (a 34 % decrease). However, overall, there was no significant difference among the patch types (Table 4.2). There was a significant difference between temporal blocks (P = 0.009), with higher densities during the second block.

Gambierdiscus had much lower densities than *Ostreopsis* and *Prorocentrum* in all sample types and was not observed in 45 % of the samples. Therefore, we evaluated whether the presence or absence of *Gambierdiscus* was affected by patch size or patch type. The likelihood

that *Gambierdiscus* was present in a patch was not correlated with patch size or patch type (Tables 4.1, 4.2), although there was a non-significant trend in which *Gambierdiscus* was more likely to be found in smaller patches of mixed algae and *Sargassum*, but larger patches of *Dictyota*, *Amansia*, and *Turbinaria* (Fig. S4.2).

Tables

Table 4.1) Summary of statistical tests of factors influencing dinoflagellate cell densities (*Ostreopsis*, *Prorocentrum*) or presence of cells (*Gambierdiscus*) on artificial substrates. Significant predictors are highlighted in **bold**. These analyses did not include samples from the no-algae patches, which had a patch area equal to zero.

predictor	**df**	**deviance**	**P-value**
Gambierdiscus - presence/absence			
temporal block	1	0.367	0.545
patch area	1	0.067	0.796
algal species	4	4.994	0.288
patch area x algal species	4	8.148	0.086
Ostreopsis - count response			
temporal block	1	0.952	0.329
patch area	1	0.090	0.765
algal species	4	8.880	0.064
patch area x algal species	4	6.913	0.141
Prorocentrum - count response			
temporal block	**1**	**4.783**	**0.029**
patch area	1	0.921	0.337
algal species	4	1.123	0.891
patch area x algal species	4	2.399	0.663

Table 4.2) Summary of statistical tests of the effect of patch type (no-algae, or dominated by *Turbinaria*, *Sargassum*, *Dictyota*, *Amansia*, or mixed algae) on dinoflagellate cell densities (*Ostreopsis*, *Prorocentrum*) or presence of cells (*Gambierdiscus*) on the artificial substrates. These analyses included the samples from the no-algae patches. Significant predictors are highlighted in **bold**.

predictor	**df**	**deviance**	**P-value**
Gambierdiscus - presence/absence			
temporal block	1	0.496	0.481
patch type	5	4.931	0.424
Ostreopsis - count response			
temporal block	1	0.987	0.321
patch type	**5**	**11.315**	**0.045**
Prorocentrum - count response			
temporal block	**1**	**6.902**	**0.009**
patch type	5	3.820	0.576

Figures

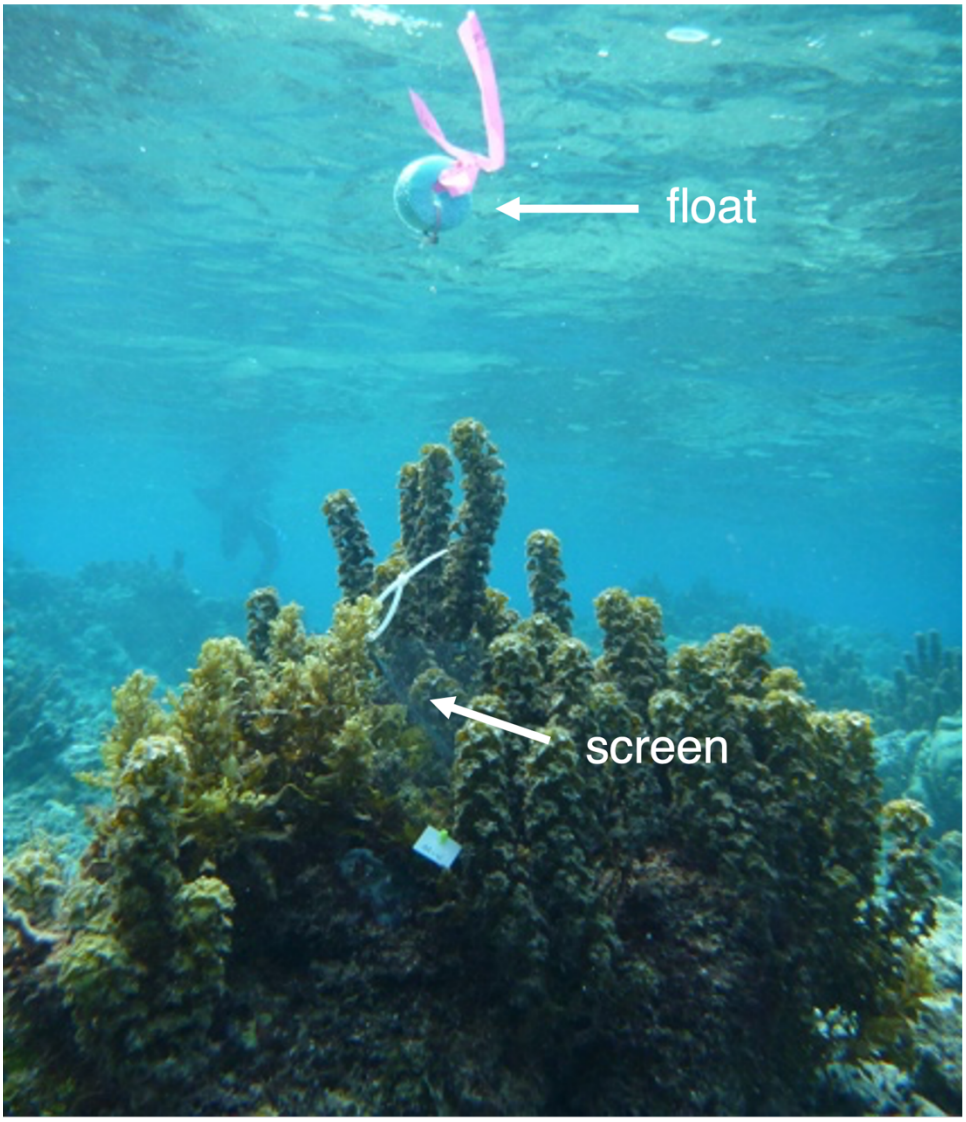

Fig. 4.1) Dinoflagellate collection screen deployed with a subsurface float in a mixed algae patch.

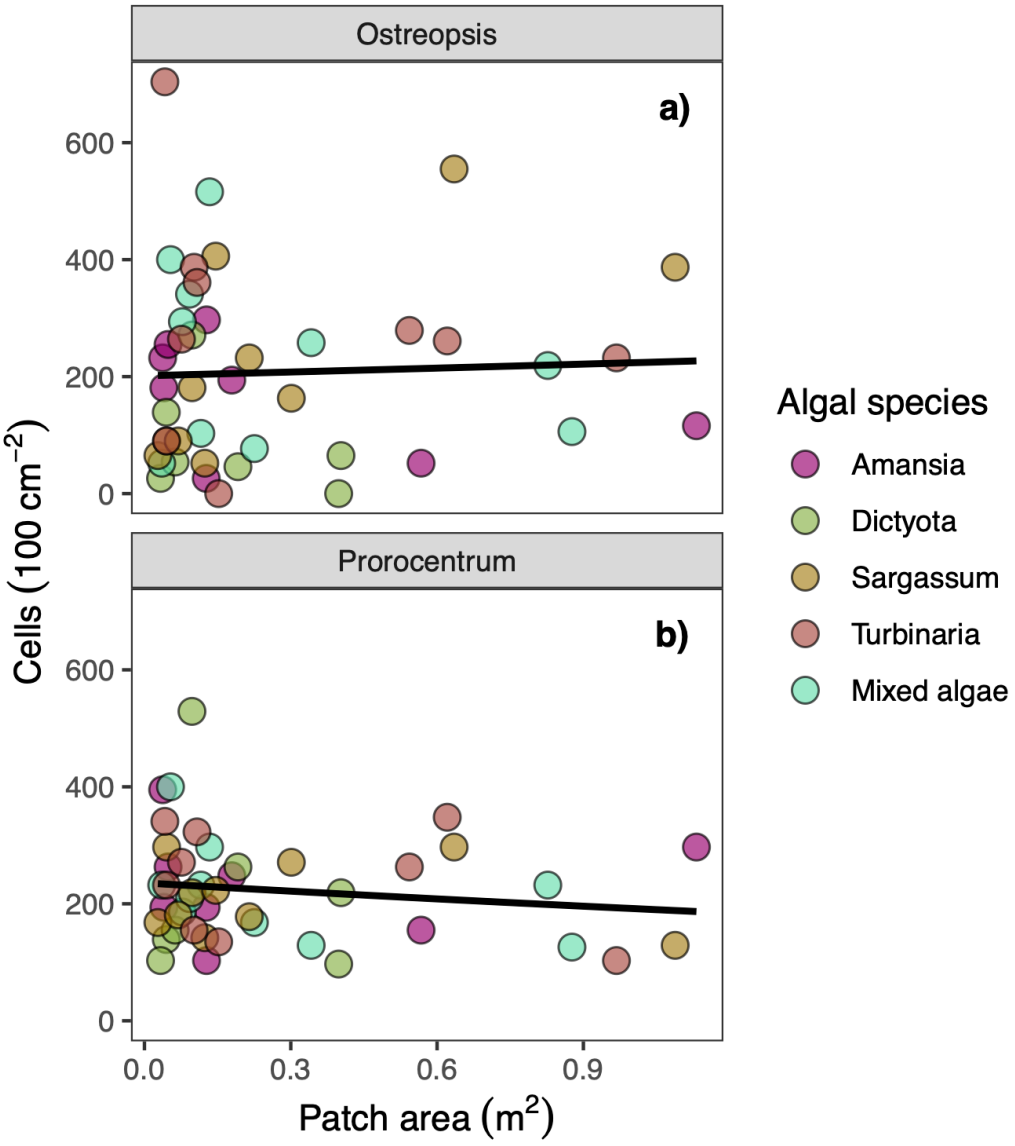

Fig. 4.2) Densities of *Ostreopsis* **(a)** and *Prorocentrum* **(b)** cells on artificial substrate sampling screens deployed in patches of algae of different sizes and species composition (indicated by color). *Gambierdiscus* densities are not shown since many samples had zeroes cells; therefore, this genus could not be analyzed with a count regression. The overall negative binomial regression line for each dinoflagellate genus is shown in black.

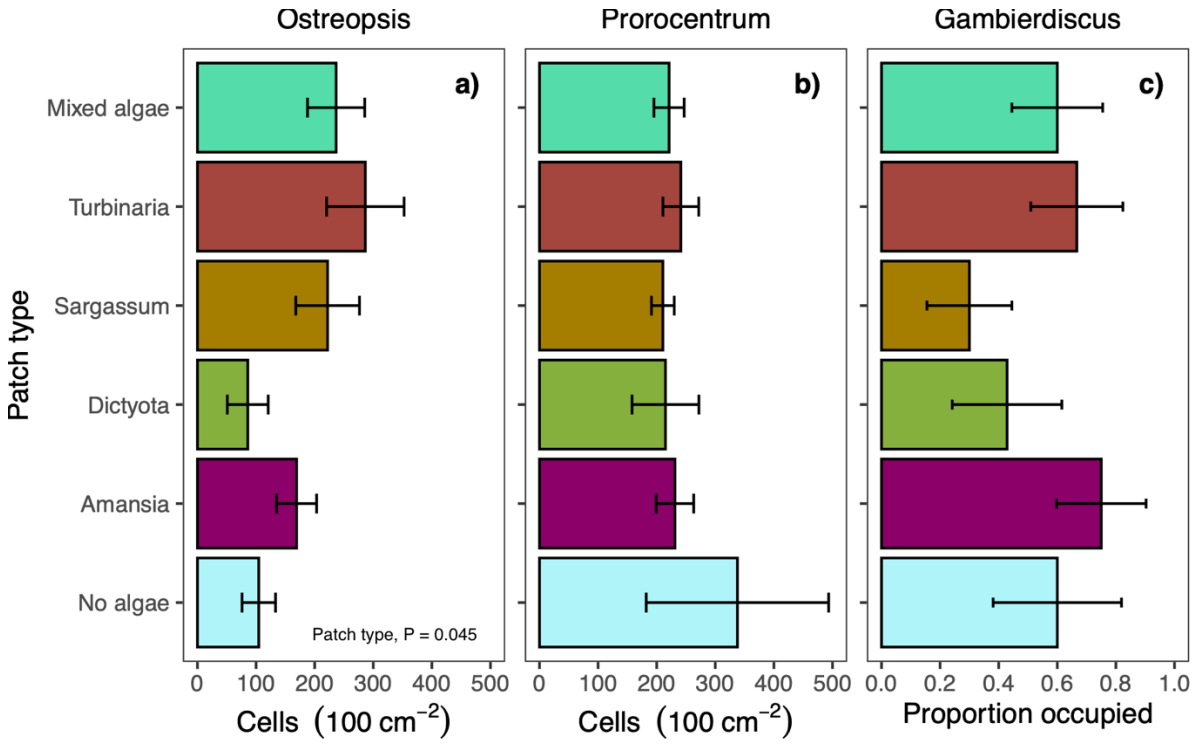

Fig. 4.3) Densities of dinoflagellate cells on artificial substrate sampling screens deployed in patches of algae of different host species composition (pooling across the patch sizes), or in sand or rubble patches at least 3 m to the closest alga ("no-algae" patches). Bars represent means ± SE (n = 5 for the no-algae patches, and 7-10 for the algae patches). Panels are separated by dinoflagellate genera. *Gambierdiscus* is shown as the proportion of occupied patches, with SE calculated for a binomial distribution.

Discussion

We observed a large degree of heterogeneity in the densities of *Ostreopsis*, *Prorocentrum*, and *Gambierdiscus* among the algal patches sampled during this study. This heterogeneity, however, was not well explained by the size of each patch (i.e., its areal coverage) nor by the algal species that dominated the patch (Table 4.1 and 4.2).

However, the patch type did have a demonstrable influence on the densities of *Ostreopsis* (Fig. 4.3a), due largely to the lower density of *Ostreopsis* in the no-algae and *Dictyota* samples compared to the other algal patch types. This result suggests that even though *Ostreopsis* is not an obligate epiphyte, it tends to associate with algae or microhabitats containing algae more than it does with algae-free microhabitats. Previous studies have noted that *Ostreopsis* can be found on algal substrates, in the water column, and on non-living benthic substrates like sand; however these studies were not able to discern differences in density to variation in the way densities were calculated across substrate types (Aligizaki and Nikolaidis 2006). In contrast, because the sampling screens we used provided us with a consistent method with which to calculate densities, we can directly compare cell densities and infer specific habitat preferences. Our results agree with Yong et al. (2018), who found that *Ostreopsis* was not strongly associated with reef areas dominated by sand or rubble (the primary substrate type in our no-algae patches). Thus, *Ostreopsis* may gain some benefits from associating with algae rather than occupying a primarily planktonic niche. The exception to its increased association with algae occurred in patches of *Dictyota*, which produces many allelochemicals (Paul and Hay 1986) and has negative effects on other photosynthetic marine taxa (Rasher et al. 2011), including *Gambierdiscus* (Rains and Parsons 2015). Therefore, *Dictyota* patches may reduce the local density of *Ostreopsis* and/or deter it from settling out of the water column onto the sampling substrates.

We did not detect any significant differences among patch types for the other two dinoflagellate genera. *Prorocentrum* exhibited a non-significant trend in which its densities were 50 % higher on average in the no-algae patches (Fig. 4.3b). This pattern agrees with Yong et al. (2018), who observed higher densities of *Prorocentrum* in reef areas dominated by sand or rubble, suggesting that *Prorocentrum* may be deterred from occurring in algal patches. The qualitative agreement of our results (although not significant) with Yong et al. (2018) suggests that we may have lacked the statistical power needed to detect real differences among the patch types. However, it could also indicate that *Prorocentrum* has only weak preferences for specific substrate types and that variation in its density is driven more by other factors.

Indeed, the weak effects of algal species that we observed for all the dinoflagellate genera seem at odds with the numerous pieces of evidence suggesting ciguatoxic dinoflagellates benefit from associating with some species of algae, exhibiting increased growth when supplied with water containing exudates from these algae (Grzebyk et al. 1994, Rains and Parsons 2015), and attaching themselves to algal surfaces when provided with the opportunity (Rains and Parsons 2015) especially under certain environmental conditions, such as increased water motion (Nakahara et al. 1996). Additionally, blooms of ciguatoxic dinoflagellates and outbreaks of ciguatera poisoning have occurred on reefs following disturbances that lead to rapid increases in algal cover (Ruff 1989, Kohler and Kohler 1992, Lehane and Lewis 2000). Given this evidence, there are a few possible explanations for why we detected limited or negligible effects of algal patch type and size. First, many of the studies that have demonstrated beneficial effects of algae were conducted in laboratory conditions, where low (or frequently absent) flow may have concentrated algal exudates, and hence, enhanced their effects. In contrast, the exudates of algae in natural reef environments may be diluted rapidly enough to minimize or prevent their effects

on dinoflagellates. Alternatively, algae might have larger, "regional" scale effects on ciguatoxic dinoflagellates, and these effects might confound the local effects of the algal patch.

For example, our study was conducted at a single site, with generally high cover of algae, which allowed us to sample a range of algal species and patch sizes. Since our study and others have shown that all three of these dinoflagellate taxa can be found in both the water column and associated with benthic substrates like algae (Yasumoto et al. 1979, Aligizaki and Nikolaidis 2006), it seems likely that some dispersal of dinoflagellates between patches types occurs. Therefore, patches may influence each other, with some patches acting as sources of dinoflagellates to other patches, which might act as ecological sinks for their populations. This dispersal could obscure some of the differences that might otherwise be apparent in dinoflagellate densities among patches due to differences in the quality of the habitat provided by the algae comprising each patch. Furthermore, it could mean that the site-level density of algae could affect dinoflagellate densities within patches. Thus, there could be an interaction between the site-level (regional) effects of algae, and patch-level (local) effects. If the site-level effect of algal density is a prominent driver of local densities of dinoflagellates, then a study might fail to detect an effect of the algal patch at reefs with high site-level algal densities (like in our study), whereas a study at a site with low algal densities might be able to detect a patch effect. Furthermore, this could explain observations of ciguatera outbreaks following disturbances that produce a regional proliferation of algae.

It also is possible, however, that other environmental factors (and not algal density) drive the densities of ciguatoxic dinoflagellates, and any associations between algal density and these drivers are confounded. For example, differences in microbial communities, or abiotic factors such as water motion (Richlen and Lobel 2011) and temperature (Xu et al. 2016) could drive

temporal variation in densities of ciguatoxic dinoflagellates within a site, or spatial variation among sites. Furthermore, these same factors might also drive small-scale (among-patch) variation in dinoflagellates within a site due to microhabitat differences. Future work disentangling the effects of local vs. regional factors (such as the ones mentioned above) could provide insights into the high spatial heterogeneity commonly observed in ciguatoxic dinoflagellates.

Ciguatera can have substantial negative effects on coastal populations that rely on the marine environment for dietary protein (Lewis and Ruff 1993, Rongo and van Woesik 2012). Therefore, identifying easy-to monitor indicators of ciguatera risk (such as algal density on a reef) would be of immense value to these populations. Further research will be needed to determine if such indicators exist, and if the current increases in disturbances that stimulate rapid increases in algae on reefs (Goldberg and Wilkinson 2004, Hughes et al. 2007) could promote blooms of ciguatoxic dinoflagellates, and thus, increase the incidence of ciguatera poisoning.

Acknowledgements

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Appendix 4

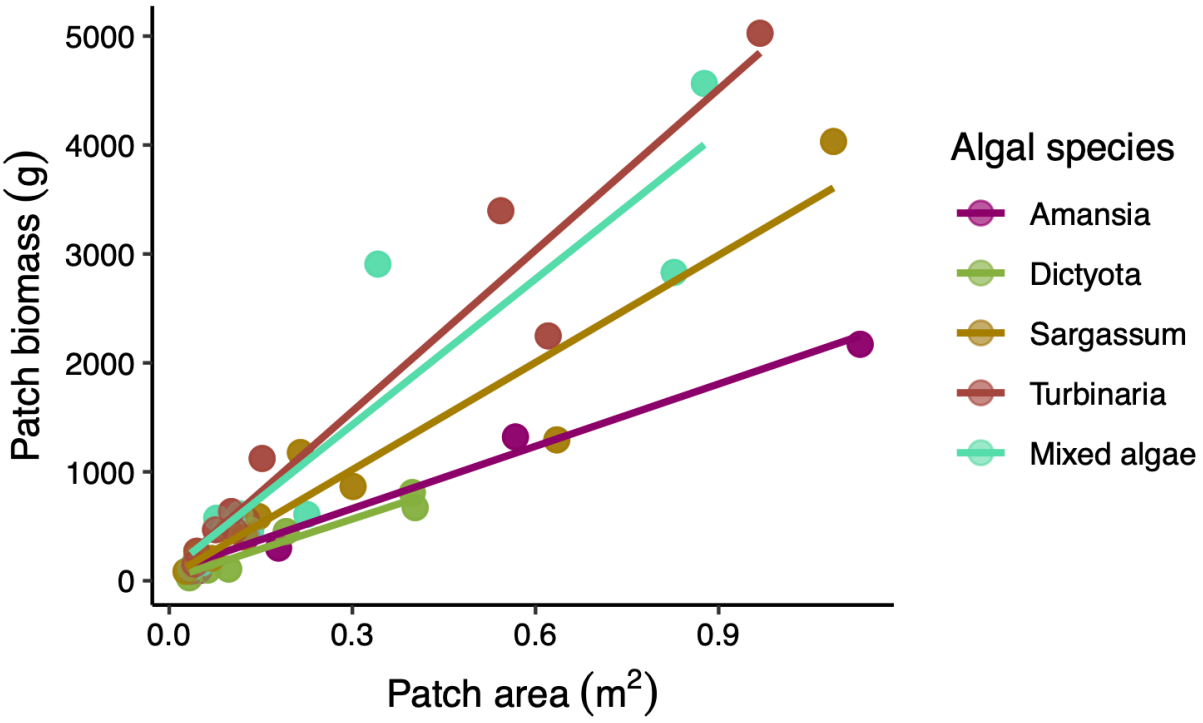

Fig. S4.1) Patch area vs. biomass of algae in the patch, shown for patches dominated by five different types of macroalgae (indicated by color). Lines indicate a linear regression fit for each algal type. Algae with thicker, denser thalli (e.g., *Turbinaria*) have more rapid increases in biomass with patch area compared to thinner, more structurally simple algae (e.g., *Dictyota*).

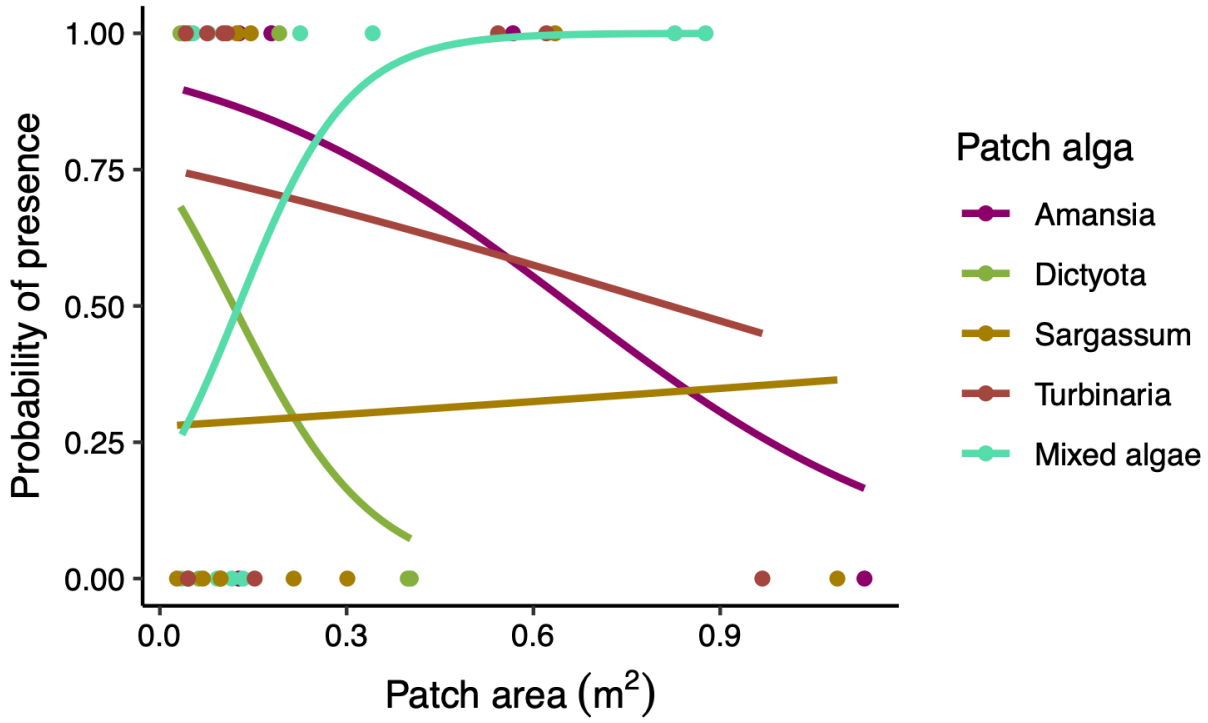

Fig. S4.2) The probability that *Gambierdiscus* was present in a patch, given the area and the algae composing that patch. Points indicated the observed data for a patch (presence or absence), and lines represent the predicted probabilities based on a logistic regression. The fitted lines should be interpreted with caution, as none of the predictors or their interactions were significant in likelihood ratio tests. See Table 4.1 in the main text.

CHAPTER 5

CONCLUSION

Summary

Microbial ecology is increasingly recognizing the importance of spatial scale in driving patterns of microbial distributions and their effects on ecosystems (Ladau and Eloe-Fadrosh 2019). However, less research has been conducted into the spatial ecology of microbes relative to macroorganisms. Of the studies that have investigated it, there is some disagreement as to the relative importance of factors operating at local vs. regional spatial scales in driving microbial community structure. Some researchers have contended that local spatial scales are the most important drivers of microbial communities (Peralta et al. 2012), at least when considering biotic factors (Cohen et al. 2016). In some sub-fields, like coral reef research, inherent assumptions that biotic interactions between macroorganisms (like algae) and microbes occur at highly localized spatial scales has meant that all but a few studies have focused on the effects of algae on microbes at small ($< 1\ m^2$) spatial scales, frequently sampling reef substrates within 5 cm of algae (although more frequently in contact with it; Barott et al. 2011, Pratte et al. 2018). Furthermore, at least one study has concluded that algae can only affect coral microbiomes through direct physical contact (Clements et al. 2020).

In Chapter 2 of this dissertation, I describe mechanisms through which the density of algae at larger spatial scales (at the level of a site) could influence microbial communities associated with corals (coral microbiomes). In an empirical study of five reef sites at two

different islands in the Pacific, Moorea and Mangareva, I found that site-level macroalgal density (measured at the mean percent cover of macroalgae) had a greater-than-antagonistic interaction with algal contact, affecting coral microbiome alpha and beta diversity (Fig. 2.2). Coral microbiomes also showed increases in algae-associated microbial families with algal contact and site-level algae (Figs. 2.6, 2.7), and increases in disease- or stress-associated microbes, with concurrent decreases in putatively beneficial microbial families. Thus, coral microbiomes likely respond to algae, even when not directly adjacent to algae. However, the interaction between site-level algae and algal contact means that the effect of algae at one scale can obscure its effect at the other scale if both are not taken into consideration. This result could explain why studies that have only considered the local effects of algae have observed conflicting patterns in the response of coral microbiome alpha and beta diversity to algae (Vega Thurber et al. 2012b, Zaneveld et al. 2016, Morrow et al. 2017, Clements et al. 2020).

My results demonstrate that a biotic factor (algae) can have both highly localized and larger site-level effects on microbial communities. In the framework used by Langenheder and Lindström (2019) to describe the dependence of microbial community assembly processes on different spatial scales, my study likely encompassed small to intermediate spatial scales. At small to intermediate scales, mass effects due to the dispersal of microbes likely influences communities, as well as localized species sorting. Thus, microbes from algal patches within a reef may be able to disperse to algae-free patches, affecting those communities, while simultaneous changes to environmental conditions caused by algae, such as increased dissolved organic carbon (Haas et al. 2011, 2016), might increasingly favor certain microbes, like the fast-growing heterotrophs that benefit from increased availability of labile carbohydrates produced by algae (Haas et al. 2011, Nelson et al. 2013). However, at larger spatial scales, variation in

abiotic factors like temperature or pH might influence microbial communities than biotic factors like algae (Cohen et al. 2016, Langenheder and Lindström 2019).

Changes to coral microbiomes by algae are often deleterious and can have substantial effects on coral health and survival (Glasl et al. 2016, Zaneveld et al. 2016). Since I found that algae can affect coral microbiomes over site-level spatial scales, a relevant next question was: how might the effects of algae on microbes at a site influence the competition between corals and algae for space at that site? To address this question, I used ordinary differential equation models to explore how the strength of a positive relationship between algae and microbes with deleterious effects on corals could influence the likelihood of a regime shift, an abrupt transition from a coral- to algal-dominated reef. I found that incorporating algae-associated microbes in models increased the range of conditions over which the system exhibited alternative stable states –alternative coral vs. algal-dominated reef states that the system equilibrates to depending on the starting conditions. Microbes also decreased the environmental tipping point (fishing mortality) at which the system transitioned from coral to algal dominance, and decreased the resilience of the coral-dominated states to disturbances. Furthermore, if microbes increased in density with algae, then these effects were exacerbated. If the positive-feedback between microbes and algae was increased enough, then only an algal-dominated system was possible. These results and the known role of microbes in mediating competitive interactions between corals and algae (Nugues et al. 2004, Kuntz et al. 2005, Kline et al. 2006, Vermeij et al. 2009, Zaneveld et al. 2016, Bulleri et al. 2018) indicate that microbes likely play a critical role in reef dynamics. Additionally, ignoring the influence of microbes on coral-algal interactions in either models exploring regime shifts or in management strategies implemented to conserve coral reefs

could result in researchers and managers failing to anticipate regime shifts in situations in which they might occur.

Since factors that affect herbivory are a major factor influencing regime shifts in reefs (Fung et al. 2011, Blackwood et al. 2018, Schmitt et al. 2019), microbes that affect herbivory also have the potential to influence regime shifts in coral reefs. One group of microbes that may have this effect are toxic epiphytes, such as some dinoflagellates, cyanobacteria, and other microalgae that grow on the surface of macroalgae or filamentous turf algal assemblages. Some of these microalgal epiphytes produce toxins that enter the food web after the epiphytes are consumed by grazers or detritivores, and then often biomagnify up the food chain. We know about phycotoxins like these primarily because of their deleterious effects on higher trophic levels, including humans, which can become ill after eating contaminated aquatic organisms. Toxic microalgae are found in freshwater and marine ecosystems around the globe, and have significant negative effects on a variety of aquatic and terrestrial species (Landsberg 2002, Glibert et al. 2005).

In Chapter 4, I investigated the relationship between macroalgae in reefs and several genera of toxic dinoflagellates. The dinoflagellates, *Gambierdiscus, Prorocentrum,* and *Ostreopsis*, occur in reefs in the tropics and subtropics (Litaker et al. 2010), and are implicated in ciguatera poisoning (Yasumoto et al. 1987, Tosteson 1995, Van Dolah 2000, Gallitelli et al. 2005, Aligizaki et al. 2011, Parsons et al. 2012), the most common human illness caused by marine toxins (Friedman 2008, Friedman et al. 2017). These dinoflagellates frequently associate with algae (Yasumoto et al. 1979, Grzebyk et al. 1994, Parsons et al. 2011), however, previous studies had found mixed results as to which algal taxa promote higher dinoflagellate densities compared to other algae (e.g., Yasumoto et al. 1979, Nakahara et al. 1996, Parsons et al. 2011,

Rains and Parsons 2015). Using artificial substrates with standardized surface areas that allowed me to make comparisons among algal taxa with differing morphologies, I investigated whether the size (area) or the species composition of a patch of macroalgae influenced the density of ciguatoxic dinoflagellates that it supported. Patch area did not affect the densities of any of the three dinoflagellate genera (Fig. 4.2). Additionally, all genera exhibited considerable heterogeneity among samples, even within patches of the same species of macroalgae. Only *Ostreopsis* exhibited significant variation among patch types, with the highest densities in *Turbinaria* patches, and the lowest densities in *Dictyota* patches, followed by the algae-free patches (Fig. 4.3). The limited variation in dinoflagellate densities explained by patch area and patch type indicates that dinoflagellate densities are likely driven by other factors. Since this study was conducted at a single site with high site-level algal cover, and because these dinoflagellates can occur in the water column or on algal surfaces and other reef substrates (Yasumoto et al. 1979, Aligizaki and Nikolaidis 2006), this suggests that potentially the effects of site-level algal cover could have interacted with the local effects of the algal patch, confounding their effects.

However, other local factors could have contributed to the heterogeneity in dinoflagellate densities. For example, prokaryotic microbes like some bacteria can influence the growth of ciguatoxic dinoflagellates (Tosteson et al. 1989, Sakami 1999, Wang et al. 2018). Thus, perhaps variation in microbial communities contributed to variation in dinoflagellate densities among patches.

Future directions

In the future, I will evaluate whether ciguatoxic dinoflagellates show an interaction between small-scale, local effects of algae as well as "regional" effects of site-level algal

densities (similar to the prokaryotic microbes in the coral microbiomes that I investigated in Chapter 2), using samples that I collected while conducting the field study in Chapter 2. I have formalin-preserved dinoflagellate cells as well as eDNA samples, the latter of which will give me a more sensitive method to detect rare toxic dinoflagellate cells, as well as identify prokaryotic microbial communities associated with the dinoflagellates. This work will enable me to test whether algal density is an important driver of ciguatoxic dinoflagellates, and whether local variation in in benthic dinoflagellate communities is associated with shift in prokaryote communities. If neither site-level nor local (patch-level) algal densities have strong effects on dinoflagellates, this suggests other factors, such as microhabitat variation in abiotic conditions or the presence of grazers might drive variation in dinoflagellate densities.

Ciguatoxic dinoflagellates contribute to ciguatera poisonings that can have considerable socioeconomic and health impacts on human populations, especially in the tropics (Lewis and Ruff 1993, Rongo and van Woesik 2012). However, much less is known about their effects on reef organisms. Ciguatoxins are potent in both fish and mammal tissues in lab studies (Lewis 1992), and cause developmental abnormalities in fish embryos (Edmunds et al. 1999, Yan et al. 2017). Therefore, ciguatoxic dinoflagellates could negatively affect herbivorous fishes that consume algae hosting dinoflagellates, which could indirectly benefit algal populations. Based on the results of Chapter 3, if ciguatoxic dinoflagellates negatively affect reef herbivores, they may facilitate regime shifts in reefs, depending on the strength and mechanism of their effects on herbivores (e.g., if they affect herbivore survival vs. grazing rate).

In a future study I will undertake during my postdoctoral position, I will use laboratory and field studies to evaluate whether vertebrate and invertebrate herbivores can detect and avoid these toxic dinoflagellates, and also quantify the effects of toxin consumption on the growth,

survival, physiology, reproductive potential, and feeding behavior of herbivores. I will use the results of these experiments to parameterize an ordinary differential equation model based on the one I implemented in Chapter 3, to evaluate how ciguatoxic dinoflagellates (or other toxic epiphytes on algae) could influence the dynamics of corals, algae, and herbivores in reefs. Using this model, I will investigate if these dinoflagellates might contribute to positive feedbacks that promote regime shifts to algal-dominated reefs with increased risk of ciguatera poisoning.

Conclusion

Microbes drive many important ecological processes that operate at different scales, influencing everything from the health of individual macroorganisms, the interactions between competing species (Umbanhowar and McCann 2005, Bever et al. 2010, Kandlikar et al. 2019), to the global carbon cycle (Bardgett et al. 2008, Trivedi et al. 2013). In turn, their dynamics and distributions are driven by a variety of ecological processes, such as abiotic conditions, the distribution of potential hosts or symbionts, surrounding microbial communities, and dispersal. However, the spatial scales at which microbes vary and the factors driving that variation are still poorly described in many systems. Consequently, it frequently remains difficult to accurately predict the magnitude of the effects of microbes on ecosystems, and the spatial patterning produced by their effects.

I identified the scales at which one biotic factor, algae, affects the composition of microbial communities associated with corals. I then theoretically demonstrated how positive associations between algae and microbes could alter reef dynamics, increasing the likelihood of regime shifts in reefs from coral to algal-dominated states. However, other ecological feedbacks affecting coral and algal dynamics operating at different spatial scales from the algae-microbe feedback could produces spatial patterning in reefs, such as producing landscape mosaics of

coral vs. algal dominated reef patches. Many ecosystems exhibit similar characteristics to the coral reef system I investigated, containing sessile producer or habitat-building species like plants whose dynamics can be modified by microbial associates. Thus, my work suggests that microbes could contribute to regime shifts in some of these other ecosystems as well. The framework that I developed for quantitatively evaluating the effects of microbial feedbacks on regime shifts can be applied to these other ecosystems, which will further enhance our understanding of how microbes influence community dynamics across environments and ecosystem types.

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