CYANOBACTERIAL COMMUNITY DISTRIBUTION IN THE LAGOON OF MOOREA, FRENCH POLYNESIA

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Abstract. Cyanobacteria are important contributors to nutrient cycling and primary productivity in coral reef environments, and their abundance has been increasing in recent decades. In the lagoon surrounding Moorea, French Polynesia, benthic cyanobacterial mats (BCMs) are common residents of reef communities, but they are largely unstudied. In this study, I surveyed the fringing and barrier reef in and around Paopao Bay to investigate environmental factors that affect the ranges of *Lyngbya*, *Anabaena*, and plurispecific mats of *Leptolyngbya* and *Hydrocoleum*. Plurispecific mats were transplanted to three areas of the fringing reef with differing water quality, and their growth was measured over twelve days. Turbidity and substrate had the largest effects on community composition, and the three BCM types studied were largely partitioned by these two variables, though their ranges often overlapped. These results suggest that cyanobacterial taxa will vary in their response to anthropogenic eutrophication and global change.

Key words: cyanobacteria, niche theory, eutrophication, French Polynesia, Moorea

INTRODUCTION

Cyanobacteria were among the first organisms on Earth to perform photosynthesis, and their modern descendants are key to global nutrient cycling in diverse environments (Cardona 2015, Sánchez-Baracaldo 2015, Michaud 2012). In aquatic environments, especially marine systems, cyanobacteria are important contributors to carbon and nitrogen fixation (Zehr 2011, Karlson 2015). For both benthic and pelagic cyanobacteria, these contributions have high spatial and temporal variation, in part due to massive 'blooms' of proliferating bacteria (Huisman 2018, Bouma-Gregson 2019). Due to anthropogenic eutrophication and climate change, these blooms have become more common, larger, and longer-lasting in the past few decades than ever before (Paerl 2009, Brocke 2015a, Visser 2016, Ullah 2018). Because of their growing threat to ecological stability, fisheries, and recreation, the dynamic role of cyanobacteria is under increasing scientific scrutiny (Huisman 2018).

The high primary productivity of coral reef environments is in part facilitated by cyanobacterial nitrogen fixation (Charpy-Roubaud 2001). In addition to this biogeochemical role, colonial benthic cyanobacterial mats (BCMs) inhibit coral recruitment (Kuffner 2006), act as coral pathogens (Carlton 1995, Meyer 2015), and often produce toxic compounds (Nagle 1999, Sneed 2017). Global change is likely to increase the impacts of cyanobacteria. For example, a 40-year study of Caribbean reefs by de Bakker et al. (2017) found BCM blooms were becoming more frequent and larger, suggesting that cyanobacteria are favored in the eutrophic, warming conditions which currently endanger corals in tropical reef environments (Hoegh-Guldberg 2007).

Isolated volcanic islands represent a unique opportunity for studying the ecological dynamics of BCMs on account of their small size and relatively low species diversity. In French Polynesia, there have been a number of studies on BCMs, especially in the Tuamotu atolls (Mao Che 2001, Abed 2003, Palińska 2015). The island of Moorea, a windward member of the Society Islands, is an ideal site to study the factors accounting for spatial variation in BCMs in lagoon and reef ecosystems, due to its isolation, small size, and concentrated agricultural industry. The island is roughly two million years old, formed by a hot spot in the Pacific plate (Dymond 1975). A barrier reef surrounds the island, except for a number of channels to the ocean, forming a lagoon in which the ocean mixes with the discharge of a number of watersheds. The island's north coastline is dominated by two inlets, the Opunohu and Paopao bays. The Paopao Bay is surrounded by three of the largest municipalities on the island, and fed by

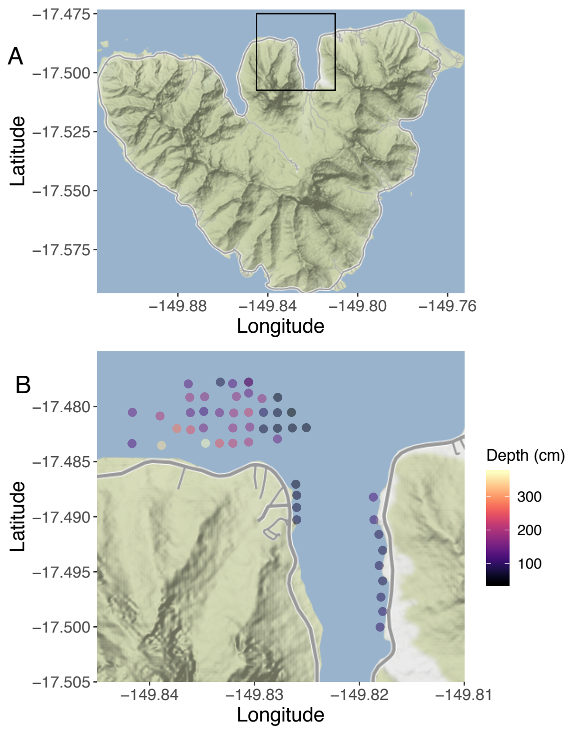

FIG. 1. A. Relief map of Moorea, French Polynesia, with the Opunohu Bay at left and Paopao Bay at right. B. Close-up of study area denoted by black square above, with survey sites and their depths indicated with dots.

the Paopao River, whose watershed includes some of the most heavily cultivated land on the island (Nixon 1995, Shea 2011, Clausing 2016).

A study by Zubia et al. (2019) investigated the taxonomic composition of BCMs in the Moorea lagoon and differences in compositions between healthy and degraded reef habitats. These results suggest that human activity, and associated impacts such as increased nutrients in runoff water, is related to distribution and abundance of BCMs. To the best of the author's knowledge, BCM distribution has not been the focus of a study on Moorea.

This study aimed to understand the factors that account for spatial variation in BCMs in lagoon and reef ecosystems of Moorea. The research included a survey of three morphotypes of BCMs: *Lyngbya*, *Anabaena*, and a plurispecific mat composed of *Leptolyngbya* and *Hydrocoleum*, spanning areas of low and high human habitat interference. I predicted that anthropogenic eutrophication, measured by water clarity, would affect their distribution and be one of the factors by which the ranges of these BCMs vary. In addition, I investigated the relationship between the growth of plurispecific mats and water clarity using transplant experiments. A causal relationship between the plurispecific mats' abundance and anthropogenic eutrophication would suggest that, independent of other climatic changes, the mats will become more common as the island's population increases and the amount of human effluent increases.

METHODS

Study site

Experiments were performed during October and November 2019 in the area surrounding the UC Gump Research Station on Paopao Bay, Moorea, French Polynesia. The waters of the Paopao Bay mix with the ocean as they drain northward. The fringing and barrier reef in this area was chosen as the focus for surveys to study the distribution of BCMs in the island's reefs.

Field Identification

Contemporary genomic identification approaches were not available for use, necessitating grouping BCMs based on consistent morphology, although the composition of these BCMs might not reflect their being the same species or same groups of species.

Lyngbya were identified based on their black, tufty appearance. Plurispecific mats have a crimson exterior and beige-brown underside; photosynthetic activity can create oxygen bubbles which pull spindle-like 'spires' of cells upward. *Anabaena* forms tufts of bright green tissue, often growing epiphytically on macroalgae, and also formed tufts which are pulled upward by photosynthetically-derived oxygen bubbles.

BCM Abundance Survey

In the barrier reef northeast of Paopao Bay, I surveyed 25 square meter plots at the on a grid formed of roughly 140 m line segments (five seconds of latitude or longitude) (see Figure 1.B.). In Paopao Bay, sites were spaced regularly every four seconds of latitude on the west fringing reef and every five seconds on the east fringing reef. For each of the fifty plots, the number, size, and substrate of each BCM in the area was recorded, along with depth and coral cover measurements for the area. I spent between five and twenty minutes at each site. BCMs were identified in the field using macroscopic features (Appendix: Figure A1). I binned sites into one of four turbidity levels based on the distance from which I could see a survey flag.

Site Diversity Analysis

Shannon indices were calculated for each site as a measure of their alpha diversity (Spellerberg 2003). Beta diversity was a calculated for each site as the number of taxa in that site divided by the average number of taxa per site minus one (Whittaker 1960).

In-Situ *BCM Growth Measurements*

In order to determine baseline growth expectations for the BCMs under consideration, I recorded the lengths of ten plurispecific mats on the fringing reef northeast of Paopao Bay (Hereafter "north fringing", 17°29′4.5″ S, 149°49′44.5″ W) and ten *Lyngbya* mats in the fringing reef north of the UC Gump Research Station (Hereafter "fringe corner", 17°29′13.9″ S, 149°49′32.4″ W) every two to three days for a period of ten days between November 11 and 23, 2019.

Plurispecific Mat Transplants

I harvested plurispecific mats on November 9, 2019 from a site (17°28′50″ S, 149°49′40″ W) on the barrier reef north of the UC Gump Research Station and incubated in a water bath filled with continually circulating seawater for two days. Following this, they were transported to three study sites and implanted into sandy soil. These sites were the aforementioned north fringing and fringe corner sites, as well as a site near a stream mouth south of the Station (Hereafter "stream mouth", 17°29′28.6″ S, 149°49′34.6″ W). They were measured along their longest axis every two to three days for a period of ten days November 11 and 23, 2019.

Statistical Analysis

All analyses were run in R version 3.6.1 (R Core Team 2019). Packages were downloaded from the CRAN repository (CRAN 2019).

I analyzed survey distribution survey data using a canonical correlation analysis in the *vegan* package to group similar communities and identify which variables contribute most to the segregation of these grouped communities. With the same package, I calculated the Shannon diversity of the sites. Generalized linear models with a Poisson distribution were fit to survey data to establish the diversity contributions of environmental variables.

Using the *lmerTest* package in R, I fit a linear mixed-effects model to BCM growth data to determine whether populations were significantly different in their growth rate based on treatment.

OpenStreetMap data was used to produce maps, along with the *ggmap* and *tmaptools* packages (OpenStreetMap 2017). Plots were generated using the *ggplot2* (Wickham 2016), *grid*, *egg*, *gtable*, and *viridis* packages. Dataframes were manipulated using the *tidyverse* package (Wickham 2017).

Alpha was set equal to 0.05 for all analyses.

Data availability

All raw data can be found at: https://github.com/lucaskampman/moorea.

TABLE 1. p values (<0.05 in bold) for each environmental variable collected in this survey, generated using a generalized mixed linear model using a Poisson distribution.

Genus/Morphotype	*Lyngbya*	*Leptolyngbya/Hydrocoleum*	*Anabaena*
Depth (plot mean)	**< 2e-16**	0.0732	0.5051
Distance to channel	**< 2e-16**	**1.51e07**	**< 2e-16**
Percentage coral heads in plot	0.8127	**4.51e-14**	**< 2e-16**
Presence of *Padina*	**5.52e-05**	**< 2e-16**	**< 2e-16**
Presence of *Sargassum*	**0.0354**	**1.81e-08**	**2.13e-05**
Presence of *Turbinaria*	0.0710	**0.0097**	**0.0108**
Presence of *Halimeda*	0.0396	**5.02e-11**	**0.0004**

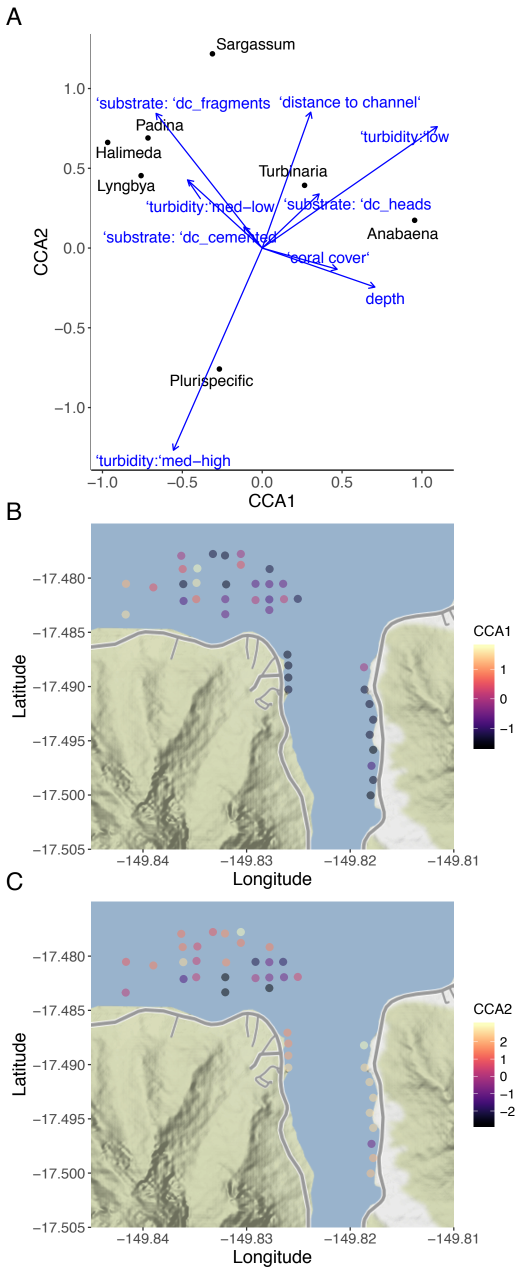

FIG. 2. A. Canonical correlation analysis of survey data indicates most significant environmental variables are turbidity and substrate. ("dc" refers to dead coral.) B. CCA1 roughly corresponds to a measure from the southeast to the northeast. C. CCA2 in both the fringing and barrier sites may indicate their closeness to mixing ocean waters in the fringing and barrier reefs, represented by the channel and the open ocean, respectively.

RESULTS

BCM Abundance Survey

Survey data were analyzed using a canonical correlation analysis (CCA), which reduces multidimensional community and environmental data to two axes with the highest spread, CCA1 and CCA2 (Figure 2). CCA1 generally increased as one moved from the southeast of the study area to the northwest, roughly corresponding to distance from the Paopao watershed, while CCA2 appeared to increase with proximity to the algal ridge for sites on the barrier reef or proximity to the channel for sites in Paopao Bay. This analysis indicated that the greatest contributing environmental variables to a community's composition were turbidity and substrate, though many variables also co-varied with these. Turbid waters were dominated by plurispecific mats, while less turbid environments are split between *Lyngbya* and *Anabaena* based on substrate and depth.

Generalized linear models were run to examine relationships between BCM abundance and environmental variables (Figure 3). These models indicated that many of these variables—depth, distance to the channel, percentage of coral heads, and the presence of *Padina, Sargassum, Turbinaria,* and *Halimeda*—are statistically significant (Table 1).

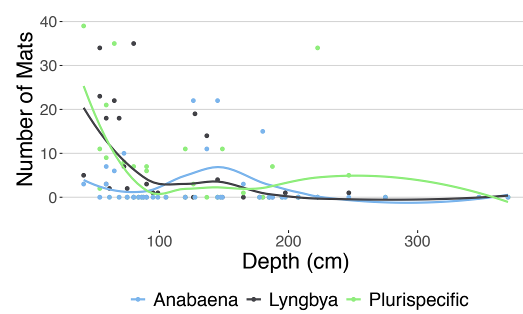

FIG. 3. BCM abundance versus plot depth with locally estimated scatterplot smoothing best-fit curves for each morphotype. *Lyngbya* was commonly found in shallow waters, whereas *Anabaena* was more common in medium-depth waters. Plurispecific mats were present in either very shallow or very deep water.

Site Diversity Analysis

There was a cluster of high alpha and beta diversity values at the east end of the barrier reef (the area closest to Paopao Bay), but little spatial structure elsewhere (Figure A2).

In-Situ *BCM Growth Measurements*

Over 12 days, the sizes of *Lynbgbya* and plurispecific mats were monitored in the fringing reef north of the Gump station. Plurispecific mats appeared to grow healthily during this time period, but *Lyngbya* mats generally did not grow substantially and had high mortality (Figure A3).

Plurispecific Mat Transplants

Transplanted plurispecific mats did not grow at significantly different rates from one another: when compared to the stream outlet site, the interaction of the time elapsed with the fringe corner (p = 0.80) and north fringe (p = 0.24) site treatments did not significantly differ, but the *in-situ* population outgroup did (p = 1.6e-05) (Figure 4).

DISCUSSION

The survey's results emphasize the importance of water clarity in determining community structure, in addition to traditional zonation of fringing and barrier reef types. Though the two axes of the CCA in Figure 2 did not strictly adhere to a single environmental variable, their apparent connection to water quality demonstrates that contact with either turbid water near coastal watersheds or relatively clear oceanic water coming over the algal ridge has a large effect on the abundance of different BCMs. The high diversity indices found at the eastern end of the barrier (Figure A2) may be explained by the mixing of these waters there. Canonical reef zonation also played a significant role: Figure 3 indicates that *Lyngbya* and *Anabaena* partition based on a number of factors that vary from the channel to the algal ridge, including depth, substrate, and the presence of various brown algae taxa.

The growth data (Figure 4) suggest that there was a methodological problem with the transplants, as their growth was significantly slower than their *in-situ* counterparts, raising the possibility that their substrate was poorly chosen or their time in a water table lowered their viability. Moreover, the uniformity across sites suggests they were not as different as originally thought. Future studies with these organisms may require more experimentation with cultivation methods.

In light of global trends in tropical reefs such as a rise in the abundance of BCMs (Ford 2018) and a decrease in coral health (Hoegh-Guldberg 2007), this research suggests BCMs on Moorea may have divergent fates in the coming decades. The survey's substrate data showed that all three morphotypes grew frequently on dead coral and epiphytically on *Turbinaria* and other macroalgae, suggesting they may fare well in the aftermath of coral bleaching events. However, the plurispecific mats' apparent preference for turbid waters may prove an advantage if the island's population continues to rise and the amount of urban and agricultural runoff in Moorea's lagoon increases. It is conceivable that diversity among the island's BCMs might decrease as those varieties best adapted to warm, turbid, eutrophic water crowd out the others.

To reinforce this study's findings, future researchers should consider expanding the study area to include other parts of the island, especially Opunohu Bay, and include more BCM taxa as study organisms. Zubia et al. 2019 showed that the lagoon channel is rich in BCMs, whose ecology could be studied easily with SCUBA equipment.

Though research has established a global trend toward increasing BCM abundance, the

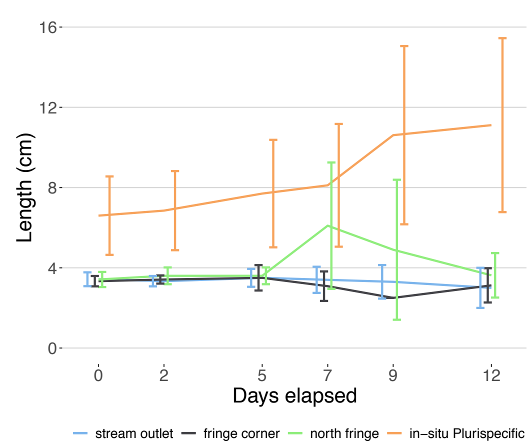

FIG. 4. Lengths of transplanted plurispecific mats at three sites over twelve days, with *in-situ* plurispecific mat lengths as a comparison. The growth rates of the different transplant sites were not significantly different. Error bars indicate mean ±1 standard deviation.

details of this shift are poorly understood. This study suggests its effects will not be uniform across all BCM taxa, and may even endanger historically critical players in reef ecosystems. More broadly, considering the key ecological role cyanobacteria play in nitrogen and carbon cycling, this change could have large and difficult-to-predict effects on global nutrient cycling and productivity generally.

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LITERATURE CITED

Abed, R. M. M., Golubic, S., Garcia-Pichel, F., Camoin, G. F., & Sprachta, S. (2003). Characterization of microbialite-forming cyanobacteria in a tropical lagoon: Tikehau Atoll, Tuamotu, French Polynesia. Journal of Phycology, 39(5), 862–873. https://doi.org/10.1046/j.1529-8817.2003.02180.x

de Bakker, D. M., van Duyl, F. C., Bak, R. P. M., Nugues, M. M., Nieuwland, G., & Meesters, E. H. (2017). 40 Years of benthic community change on the Caribbean reefs of Curaçao and Bonaire: the rise of slimy cyanobacterial mats. Coral Reefs, 36(2), 355–367. https://doi.org/10.1007/s00338-016-1534-9

Bouma-Gregson, K., Olm, M. R., Probst, A. J., Anantharaman, K., Power, M. E., & Banfield, J. F. (2019). Impacts of microbial assemblage and environmental conditions on the distribution of anatoxin-a producing cyanobacteria within a river network. ISME Journal, 13(6), 1618–1634. https://doi.org/10.1038/s41396-019-0374-3

Brocke, H. J., Polerecky, L., De Beer, D., Weber, M., Claudet, J., & Nugues, M. M. (2015a). Organic matter degradation drives benthic cyanobacterial mat abundance on caribbean coral reefs. PLoS ONE, 10(5), 1–19. https://doi.org/10.1371/journal.pone.0125445

Brocke, H. J., Wenzhoefer, F., De Beer, D., Mueller, B., Van Duyl, F. C., & Nugues, M. M. (2015b). High dissolved organic carbon release by benthic cyanobacterial mats in a Caribbean reef ecosystem. Scientific Reports, 5, 1–7. https://doi.org/10.1038/srep08852

Brocke, H. J., Piltz, B., Herz, N., Abed, R. M. M., Palinska, K. A., John, U., … Nugues, M. M. (2018). Nitrogen fixation and diversity of benthic cyanobacterial mats on coral reefs in Curaçao. Coral Reefs, 37(3), 861–874. https://doi.org/10.1007/s00338-018-1713-y

Cardona, T., Murray, J. W., & Rutherford, A. W. (2015). Origin and evolution of water oxidation before the last common ancestor of the cyanobacteria. Molecular Biology and Evolution, 32(5), 1310–1328. https://doi.org/10.1093/molbev/msv024

Charpy-Roubaud, C., Charpy, L., & Larkum, A. (2001). Atmospheric dinitrogen fixation by benthic communities of Tikehau lagoon (Tuamotu Archipelago, French Polynesia) and its contribution to benthic primary production. Marine Biology, 139(5), 991–997. https://doi.org/10.1007/s002270100636

Clausing, R. J., & Fong, P. (2016). Environmental variability drives rapid and dramatic changes in nutrient limitation of tropical macroalgae with different ecological strategies. *Coral Reefs*, *35*(2), 669–680. https://doi.org/10.1007/s00338-016-1403-6

CRAN: The comprehensive R archive network (2019). Institute for Statistics and Mathematics, Vienna, Austria. https://cran.r-project.org

Dymond, J. (1972). K-Ar ages of Tahiti and Moorea, Society Islands, and implications for the hot-spot model. Geology, 3(5), 236–240.

Ford, A. K., Bejarano, S., Nugues, M. M., Visser, P. M., Albert, S., & Ferse, S. C. A. (2018). Reefs under siege-the rise, putative drivers, and consequences of benthic cyanobacterial mats. *Frontiers in Marine Science*, *5*(FEB), 1–15. https://doi.org/10.3389/fmars.2018.00018

Hoegh-Guldberg, O., & Multae, C. (2007). Coral reefs under rapid climate change and ocean acidification. Science, 1737 (December 2007), 1737–1743. https://doi.org/10.1126/science.1152509

Karlson, A. M. L., Duberg, J., Motwani, N. H., Hogfors, H., Garbaras, A., Klawonn, I., … Elmgren, R. (2015). Nitrogen fixation by cyanobacteria stimulates production in Baltic food webs, 44. https://doi.org/10.1007/s13280-015-0660-x

Kuffner, I. B., Walters, L. J., Becerro, M. A., Paul, V. J., Ritson-Williams, R., & Beach, K. S. (2006). Inhibition of coral recruitment by macroalgae and cyanobacteria. Marine Ecology Progress Series, 323, 107–117. https://doi.org/10.3354/meps323107

Mao Che, L., Andréfouët, S., Bothorel, V., Guezennec, M., Rougeaux, H., Guezennec, J., … Caumette, P. (2001). Physical, chemical, and microbiological characteristics of microbial mats (kopara) in the South Pacific atolls of French Polynesia. Canadian Journal of Microbiology, 47(11), 994–1012. https://doi.org/10.1139/cjm-47-11-994

Meyer, J. L., Gunasekera, S. P., Scott, R. M., Paul, V. J., & Teplitski, M. (2015). Microbiome shifts and the inhibition of quorum sensing by Black Band Disease cyanobacteria, 10(5), 1204–1216. https://doi.org/10.1038/ismej.2015.184

Nagle, D. G., & Paul, V. J. (1999). Production of secondary metabolites by filamentous tropical marine cyanobacteria: ecological functions of the compounds. Journal of Phycology, 35(6), 1412–1421. https://doi.org/10.1046/j.1529-8817.1999.3561412.x

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

OpenStreetMap contributors (2017). Planet dump retrieved from https://planet.osm.org. https://www.openstreetmap.org/

Palińska, K. A., Abed, R. M. M., Charpy, L., Langlade, M. J., Beltrán-Magos, Y., & Golubic, S. (2015). Morphological, genetic and physiological characterization of Hydrocoleum, the most common benthic cyanobacterium in tropical oceans. European Journal of Phycology, 50(2), 139–154. https://doi.org/10.1080/09670262.2015.1010239

Richert, L., Golubic, S., Le Guédès, R., Hervé, A., & Payri, C. (2006). Cyanobacterial populations that build “kopara” microbial mats in Rangiroa, Tuamotu Archipelago, French Polynesia. European Journal of Phycology, 41(3), 259–279. https://doi.org/10.1080/09670260600804868

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://R-project.org

Sánchez-Baracaldo, P. (2015). Origin of marine planktonic cyanobacteria. Scientific Reports, 5, 14–17. https://doi.org/10.1038/srep17418

Shea, A. G. (2011). Coral health and disease: a comparison of Cook's and Opunohu Bays in Moorea, French Polynesia.

Sneed, J. M., Meickle, T., Engene, N., Reed, S., Gunasekera, S., & Paul, V. J. (2017). Bloom dynamics and chemical defenses of benthic cyanobacteria in the Indian River Lagoon, Florida. Harmful Algae, 69, 75–82. https://doi.org/10.1016/j.hal.2017.10.002

Spellerberg, I. A. N. F., & Fedor, P. J. (2003). Tribute to Claude Shannon (1916-2001). *Global Ecology and Biogeography*, 177–179. https://doi.org/10.1046/j.1466-822X.2003.00015.x

Wickham, H. (2016). ggplot2: elegant graphics for data analysis. Springer-Verlag New York.

Wickham, H. (2017). tidyverse: easily install and load the 'Tidyverse'. R package version 1.2.1. https://CRAN.R-project.org/package=tidyverse

Whittaker, R. H. (1960). Vegetation of the Siskiyou Mountains, Oregon and California. *Ecological Monographs*, *30*(4), 407–407. https://doi.org/10.2307/1948435

Zubia, M., Vieira, C., Palinska, K. A., Roué, M., Gaertner, J.-C., Zloch, I., … Golubic, S. (2019). Benthic cyanobacteria on coral reefs of Moorea Island (French Polynesia): diversity response to habitat quality. Hydrobiologia, 8. https://doi.org/10.1007/s10750-019-04029-8

APPENDIX A

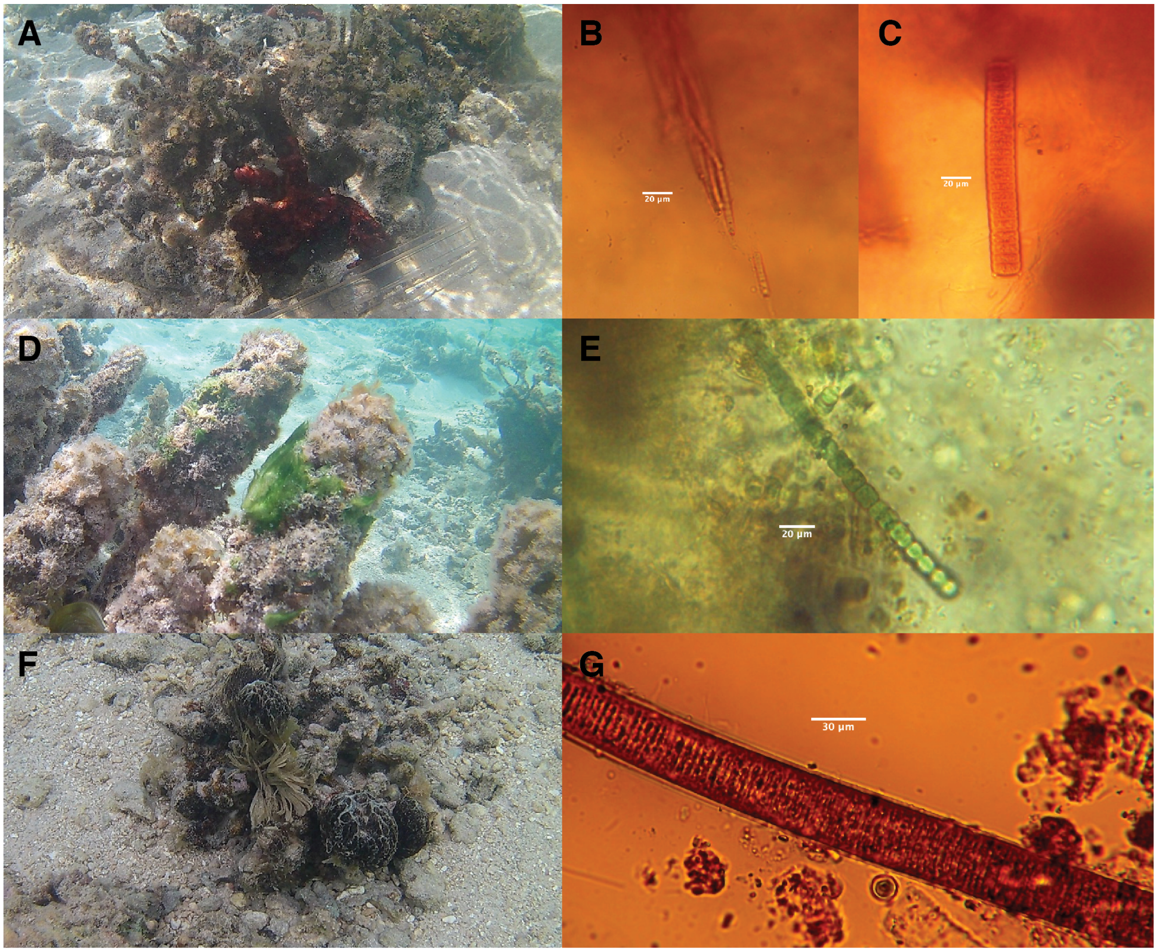

FIG A1. The study morphotypes, *in-situ* at left and under 400x magnification at right. A. crimson plurispecific mat. B. *Leptolyngbya*, one component of the mats, recognizable by its terminal gas vesicles. C. *Hydrocoleum*, recognizable by its lack of a sheath. D. *Anabaena* growing epiphytically on *Turbinaria* as bright green tufts. E. A fiber of *Anabaena* showing its beady microscopic morphology. F. *Lyngbya*, which grows as black-brown tufts. G. *Lyngbya* under the microscope is recognizable by the presence of a thick sheath. (Zubia 2019)

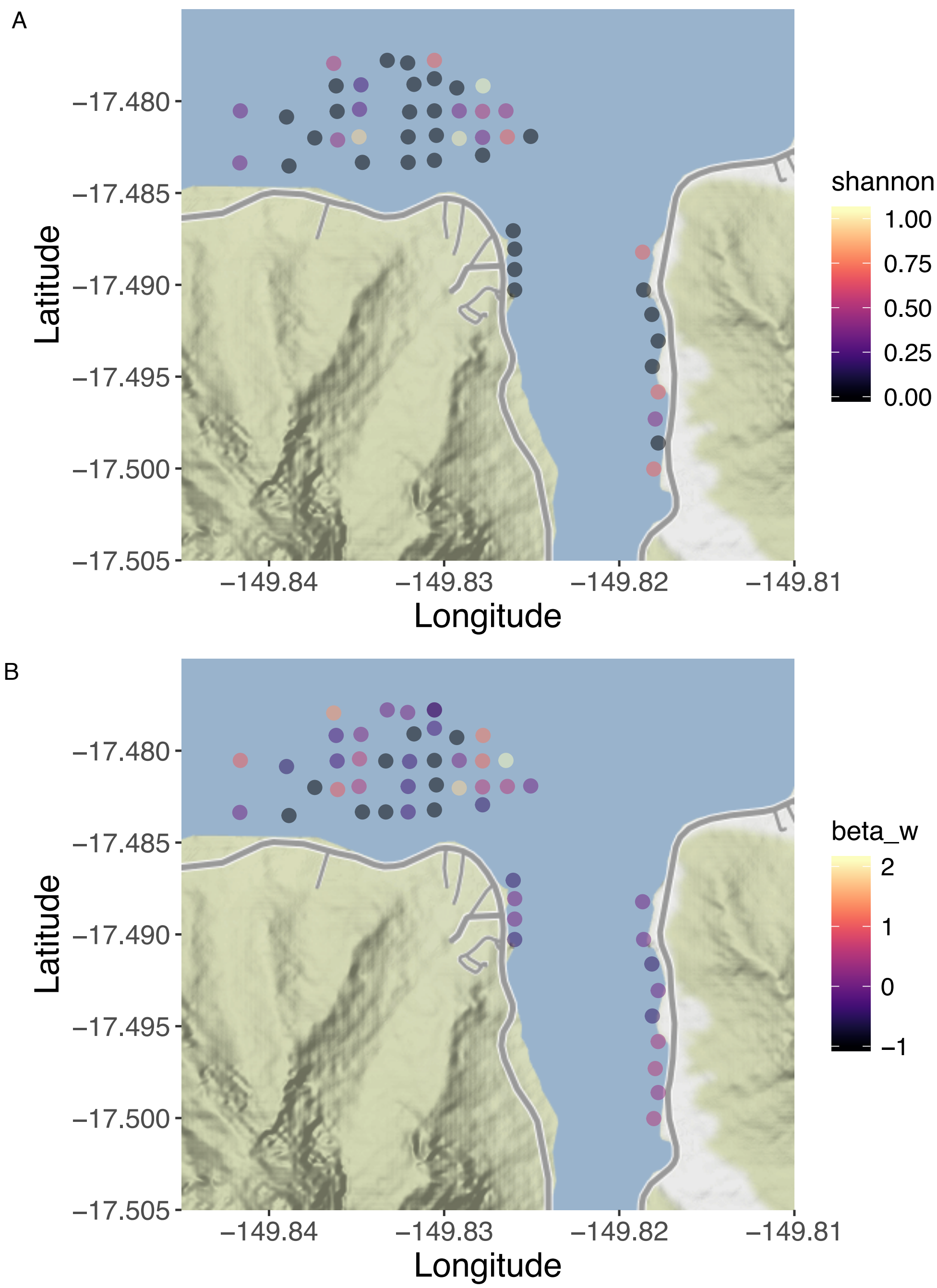

FIG. A2. A. Alpha (Shannon) and B. beta (Whittaker) diversity of survey sites. Both indices have a cluster of high values at the eastern end of the barrier reef northwest of Paopao Bay, but otherwise have little spatial structure.

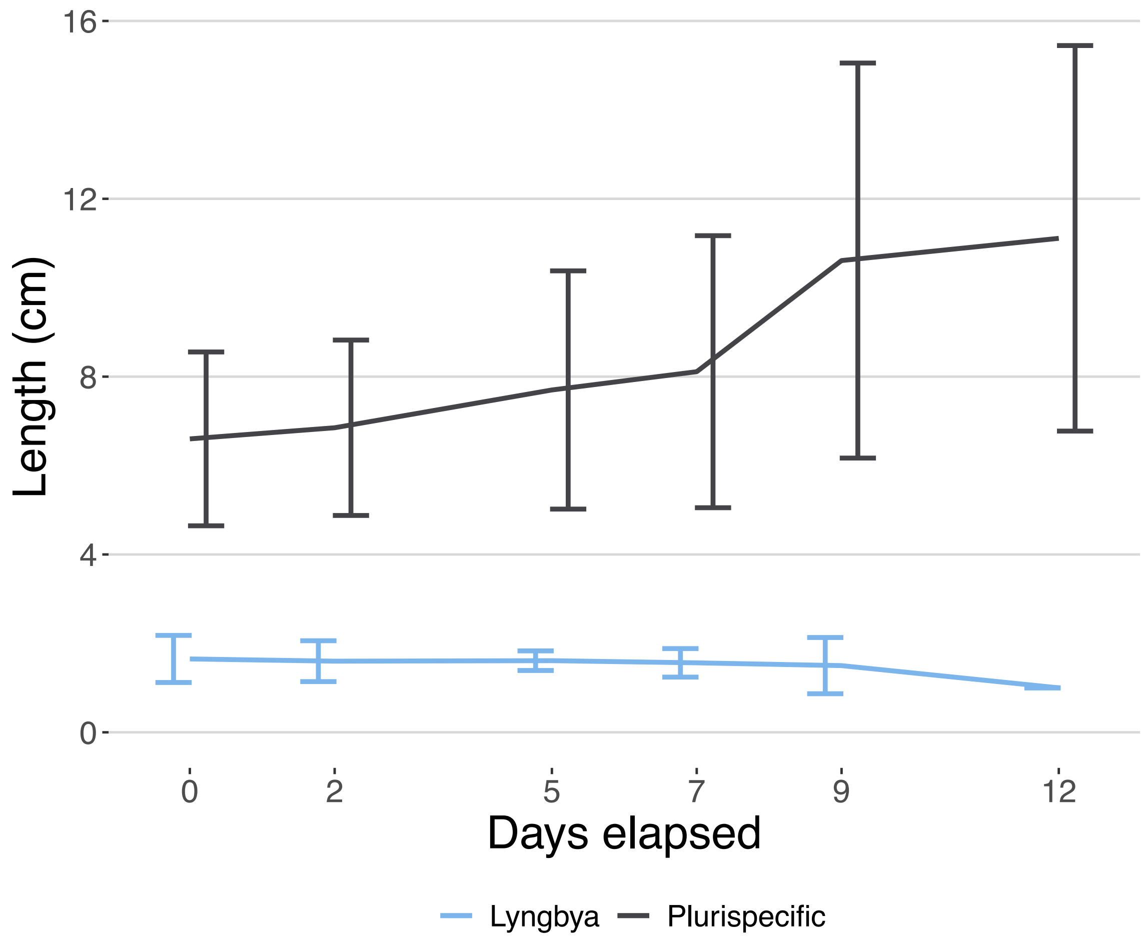

FIG. A3. *In-situ* measurements of the lengths of ten *Lyngbya* BCMs and ten plurispecific BCMs. Error bars indicate mean ±1 standard deviation.