



# Microbial ecology of coral-dominated reefs in the Federated States of Micronesia

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**ABSTRACT:** Microorganisms are central to the functioning of coral reef ecosystems, but their dynamics are unstudied on most reefs. We examined the microbial ecology of shallow reefs within the Federated States of Micronesia. We surveyed 20 reefs surrounding 7 islands and atolls (Yap, Woleai, Olimarao, Kosrae, Kapingamarangi, Nukuoro, and Pohnpei), spanning 875 053 km<sup>2</sup>. On the reefs, we found consistently higher coral coverage (mean  $\pm$  SD = 36.9  $\pm$  22.2%; max 77%) compared to macroalgae coverage (15.2  $\pm$  15.5%; max 58%), and low abundances of fish. Reef waters had low inorganic nutrient concentrations and were dominated by *Synechococcus*, *Prochlorococcus*, and SAR11 bacteria. The richness of bacterial and archaeal communities was significantly related to interactions between island/atoll and depth. High coral coverage on reefs was linked to higher relative abundances of *Flavobacteriaceae*, *Leisingera*, *Owenweeksia*, *Vibrio*, and the OM27 clade, as well as other heterotrophic bacterial groups, consistent with communities residing in waters near corals and within coral mucus. Microbial community structure at reef depth was significantly correlated with geographic distance, suggesting that island biogeography influences reef microbial communities. Reefs at Kosrae Island, which hosted the highest coral abundance and diversity, were unique compared to other locations; seawater from Kosrae reefs had the lowest organic carbon (59.8–67.9  $\mu$ M), highest organic nitrogen (4.5–5.3  $\mu$ M), and harbored consistent microbial communities (>85% similar), which were dominated by heterotrophic cells. This study suggests that the reef-water microbial ecology on Micronesian reefs is influenced by the density and diversity of corals as well as other biogeographical features.

**KEY WORDS:** Coral reef · Microbiology · Micronesia · Oligotrophic · Cyanobacteria · SSU rRNA gene

## 1. INTRODUCTION

Tropical coral reefs are among the most biodiverse and economically important ecosystems in the ocean (Moberg & Folke 1999, Hughes et al. 2017). Most tropical coral reefs occur in oligotrophic waters, and reef-associated microorganisms and their biogeo-

chemical processes are thought central to maintaining the high biomass and diversity associated with coral reefs (Sorokin 1973a). Microorganisms engage in symbiotic relationships with reef organisms, including corals and sponges, by providing carbon, nitrogen, and other critical substrates to their hosts (Wilkinson & Fay 1979, Wilkinson 1983, Muscatine

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1990). Additionally, picoplankton (planktonic bacteria and archaea) play central roles in coral reef ecosystems by fixing carbon, cycling dissolved nutrients and organic matter, and serving as prey for abundant corals, sponges, and other reef filter feeders (Sorokin 1973b, Ducklow 1990, Houlbrèque et al. 2004, McNally et al. 2017).

Advances in microbial taxonomic identification methods have uncovered a rich diversity of planktonic bacteria and archaea in reef waters and have provided new insights into coupling between the water column and reef benthos. Several studies have identified distinct microbial communities overlying reefs that can be related to the physical structure of the reef (Nelson et al. 2011) or biological composition of the reef benthos (Dinsdale et al. 2008, Kelly et al. 2014, Haas et al. 2016, Weber et al. 2019, 2020). As coral reefs transition from coral to macroalgal dominance, their microbial communities also change, shifting to communities characterized by increased heterotrophy, virulence, and prevalence of pathogens (Kelly et al. 2014, Haas et al. 2016). These changes have been attributed to differences in the amount and composition of neutral sugars released in exudates from corals and algae (Haas et al. 2013, Nelson et al. 2013, Cárdenas et al. 2018), thus suggesting a direct link between benthic reef organisms and reef-water microorganisms. Further, a multi-sampling effort of Great Barrier Reef waters showed associations between reef-water microbial communities and water temperature and state of eutrophication (Glasl et al. 2019). Other reef-water microbiology studies have provided evidence of relationships between microbial communities and reef biogeography, suggesting that neutral community assembly processes also contribute to microbial community composition (Jeffries et al. 2015, Salerno et al. 2016).

Due to climate change and other anthropogenic impacts, coral reefs are among the most globally threatened ecosystems on Earth (Pandolfi et al. 2003, Hughes et al. 2017, 2018). Despite the likely importance of reef-water microorganisms and biogeochemical connections to the benthos, there are limited field-based measurements of nutrient and microbial parameters on reefs. Much of this knowledge for the Pacific is centered on higher-nutrient reefs near zones of oceanic upwelling (Dinsdale et al. 2008, Kelly et al. 2014) as well as an embayment in Mo'orea (Nelson et al. 2011). Recent studies have investigated oligotrophic reefs within a larger geographic area, thus providing additional context for understanding reef-water microbial ecology (Jeffries et al. 2015, Haas et al. 2016, Salerno et al. 2016, Weber et al. 2020).

The reefs comprising the Federated States of Micronesia (hereafter referred to as Micronesia) constitute one of the largest reef systems in the world. They are not located near nutrient upwelling zones, and their microbial biogeochemistry has not been previously investigated. Micronesia is composed of 4 states (Yap, Chuuk, Pohnpei, and Kosrae), consists of 607 islands, and houses over 14 000 km<sup>2</sup> of coral reefs, which surround a variety of islands and atolls covering 3 000 000 km<sup>2</sup> of the North Pacific (Andrefouet et al. 2006). Island residents utilize the natural resources of these reefs for food, their livelihood, and traditional cultural practices. While storms (typhoons in 1990 in Pohnpei and 2004 in Chuuk and Yap) and coral bleaching (1998, 2004, 2013–2017) have caused some mortality on reefs, these reefs are further threatened by human population growth, overharvesting, and habitat destruction (Abraham et al. 2004, Goldberg et al. 2008).

The purpose of this study was to examine the microbial ecology of coral reefs within the islands and atolls of Micronesia, in relation to benthic cover, select fish densities, biogeography, and the environmental conditions on the reefs. Over a 3 wk period in 2012, we examined 20 coral reefs spanning across 7 islands and atolls within Micronesia, with specific reefs opportunistically sampled alongside other unrelated cruise objectives. We hypothesized that the microbial ecology in the seawater overlying the reefs would vary according to the benthic composition of the reef as well as biogeographic features. By conducting reef surveys and water collections at these reefs, we found that most of the reefs harbored similar microbial communities, including an abundance of picocyanobacteria and oligotrophic-dwelling microbial taxa. Biogeographical distance-related differences as well as select nutrients also played a role in structuring differences within the reef seawater microbial communities. Lastly, we found that microbial diversity was positively correlated with coral coverage and that the reefs of Kosrae Island harbored high coral cover and high coral diversity, and displayed unique microbial biogeochemical properties, suggesting a link between the coral communities and pelagic reef-water microbial functioning.

## 2. MATERIALS AND METHODS

### 2.1. Cruise overview and sites

During 21 October to 10 November 2012, we studied 20 coral reefs spread over 7 islands and atolls in

Micronesia with varying human populations (from uninhabited to 32 178 residents). Two to 6 reef sites were examined per island or atoll (Fig. 1, Table 1; see Table S1 in the Supplement at [www.int-res.com/articles/suppl/a086p115\\_supp.xlsx](http://www.int-res.com/articles/suppl/a086p115_supp.xlsx)), and geographic distances between sites spanned from 0.15 (intra-island/atoll) to 2775 km (between islands/atolls), covering an area of 875 053 km<sup>2</sup>. The reefs ranged in depth from 6 to 13 m, and included forereef, fringing, and lagoon reef habitats (Table 1). At each site, benthic and fish surveys were conducted using SCUBA, physicochemical seawater properties were measured, and surface (1 m) and reef depth seawater (taken within 1 m of the benthos) were sampled for specific microbial and biogeochemical measurements. Additionally, a deep off-reef (4261 m depth) location was sampled at the surface (1 m depth) for physicochemical seawater properties as well as microbial abundances and community composition.

## 2.2. Reef surveys

Two 25 m tape measures were deployed along depth contours and run from an appropriate spot near the anchor point into the oncoming current. The second transect began approximately 3 m from the end of the first transect and continued in the same direction. Substrate characteristics were measured using the point-intercept method, whereby the substrate underlying the tape measure was classified and recorded at 50 cm intervals. On the island of Yap, only coral coverage was quantified. Corals within 50 cm on either side of the tape measure (25 × 1 m belt) were examined, and all coral taxa, down to genus level, were recorded.

We surveyed herbivorous and corallivorous fish populations at each site. Standard underwater visual surveys of herbivorous species (parrotfish, surgeonfish, and rabbitfish) and coral-feeding species (butterflyfish) were conducted along the same belt transects as surveyed for coral abundance. A diver swam along each transect at a constant speed (~15 min per transect), recording fish visible within 2–3 m to either side of the central line. Transect width (4 or 6 m) was dependent on water clarity.

## 2.3. Seawater physicochemical analysis and water sample collections

Physicochemical properties of the seawater, including temperature, salinity, and dissolved oxygen, were measured at the sites using casts with an EXO water

quality sonde (YSI). Cast data were averaged at surface and reef depths over 0.5 m. Seawater samples were collected from surface and reef depths at each site, except for Yap, where only 1 sample of surface seawater was collected. Samples were collected for the following analyses: total organic carbon and nitrogen concentrations (60 ml), inorganic nutrient concentrations (100 ml), cell abundances (1 ml), and microbial biomass for molecular analyses (4 l). Seawater for analysis of phytoplankton pigments (4 l) was only collected from surface waters. Surface samples were obtained by holding bottles under the water, and reef depth samples were collected using Niskin bottle casts. All samples were collected into triple-rinsed vials and stored on ice for up to 4 h before processing.

## 2.4. Organic and inorganic nutrients, phytoplankton pigments, and cell abundances

Seawater collected for inorganic nutrient analyses was frozen at –20°C, and concentrations of dissolved inorganic nutrients (ammonium, nitrate + nitrite, nitrite, phosphate, and silicate) were measured using a continuous segmented flow system including a Technicon AutoAnalyzer II (SEAL Analytical) and an Alpkem RFA 300 Rapid Flow Analyzer at Oregon State University. Nitrate + nitrite and nitrite were measured according to Armstrong et al. (1967), with detection limits of 0.07 and 0.01 µM, respectively. Phosphate was measured using an adjusted molybdenum blue method (Bernhardt & Wilhelms 1967), with a detection limit of 0.01 µM. The majority (23 out of 34 total measurements) of ammonium measurements were conducted in the field on unfrozen samples using the ortho-phthaldialdehyde fluorescence method (Holmes et al. 1999, Taylor et al. 2007), with a detection limit of 0.02 µM (Table S1). Auto-analyzer measurements were used to measure ammonium for the remaining (11/34) samples using the indophenol blue method (US Environmental Protection Agency 1983), with a detection limit of 0.02 µM. Seawater samples collected for total organic carbon (TOC) and total organic nitrogen (TON) analyses were acidified with phosphoric acid and stored at 4°C until analysis using a Shimadzu TOC-V<sub>CSH</sub> TOC analyzer (Hansell & Carlson 2001), where total non-purgeable particulate and dissolved organic carbon (TOC) and total particulate and dissolved inorganic and organic nitrogen (total nitrogen, TN) were resolved. TON concentrations were calculated by subtracting nitrate + nitrite and ammonium concentrations from TN concentrations. For analysis of

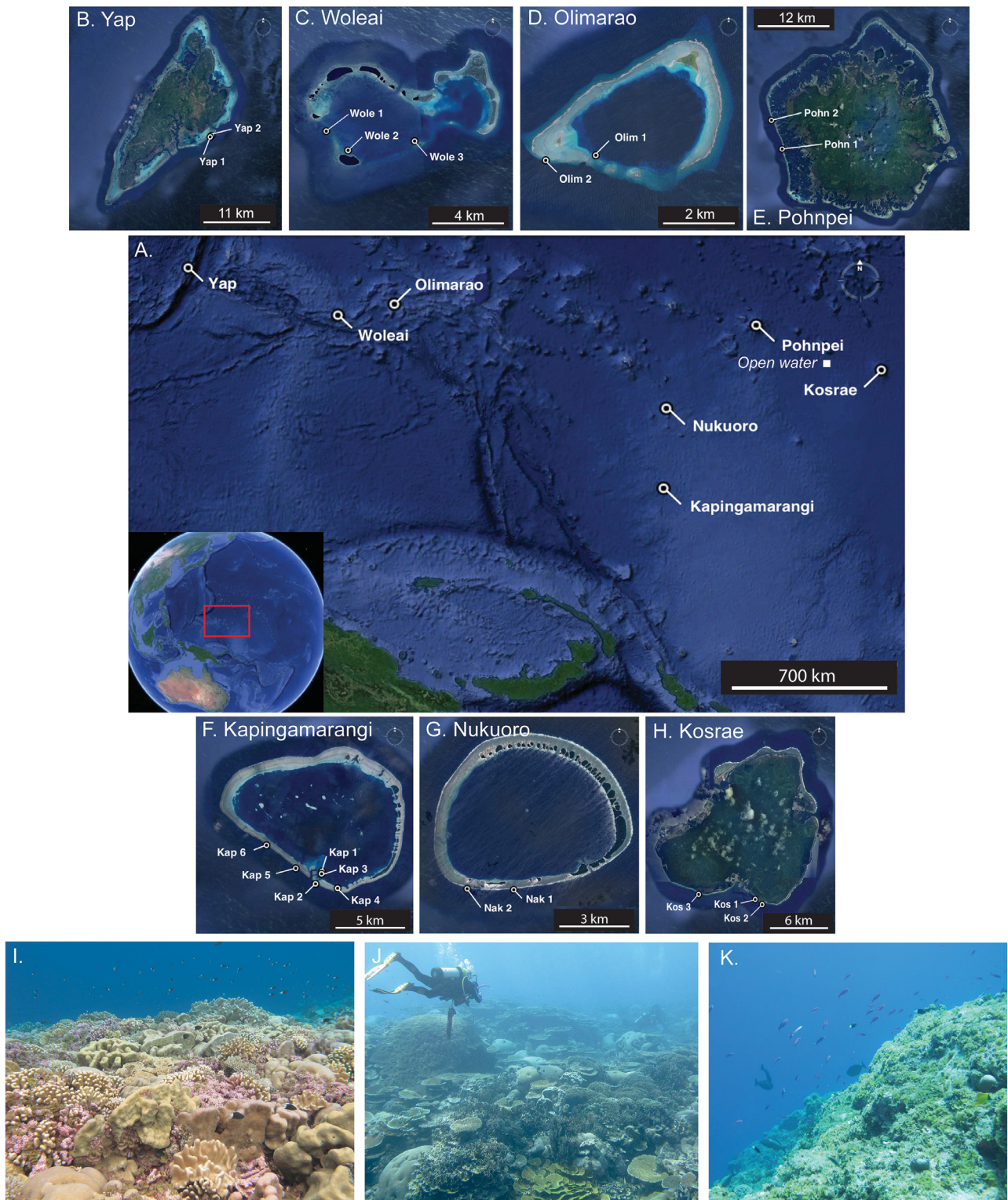


Fig. 1. Study sites in Micronesia. (A) Overview of the islands; inset displays the global location. Close-ups of field sites at each island or atoll: (B) Yap, (C) Woleai, (D) Olimarao, (E) Pohnpei, (F) Kapingamarangi, (G) Nukuoro, and (H) Kosrae. Representative photographs of coral reefs from highly coral-dominated (I) Nukuoro atoll and (J) Kosrae Island, and from (K) the macroalgal-dominated reef of Olimarao. Map images courtesy of Google Earth (2016) under the academic print distribution guidelines, including data from SIO, NOAA, US Navy, NGA, and GEBCO and images from the US Geological Survey, Cnes/Spot Image, and 2001 DigitalGlobe

Table 1. Reef sites examined in this study. NA: not applicable

Site name	Location	Human population <sup>a</sup>	Reef depth sampled (m) <sup>b</sup>	Reef type	Latitude (°N)	Longitude (°E)
Yap 1	Yap Island	11241	NA	Forereef	9.507	138.175
Yap 2	Yap Island	11241	NA	Forereef	9.508	138.177
Wole 1	Woleai Atoll	978	10	Forereef	7.339	143.830
Wole 2	Woleai Atoll	978	6	Lagoon	7.330	143.840
Wole 3	Woleai Atoll	978	6	Forereef	7.334	143.872
Olim 1	Olimarao Atoll	None	6	Forereef	7.682	145.860
Olim 2	Olimarao Atoll	None	10	Forereef	7.681	145.850
Kos 1	Kosrae Island	7686	13	Fringing	5.263	162.984
Kos 2	Kosrae Island	7686	10	Fringing	5.259	162.990
Kos 3	Kosrae Island	7686	10	Fringing	5.266	162.935
Open water	NA	NA	NA	Offshore	6.158	160.567
Kap 1	Kapingamarangi Atoll	474	8	Lagoon	1.037	154.766
Kap 2	Kapingamarangi Atoll	474	10	Forereef	1.029	154.762
Kap 3	Kapingamarangi Atoll	474	12	Lagoon	1.035	154.766
Kap 4	Kapingamarangi Atoll	474	NA	Lagoon	1.027	154.775
Kap 5	Kapingamarangi Atoll	474	12	Forereef	1.038	154.751
Kap 6	Kapingamarangi Atoll	474	10	Forereef	1.051	154.734
Nuk 1	Nukuoro Atoll	362	10	Forereef	3.821	154.936
Nuk 2	Nukuoro Atoll	362	10	Forereef	3.821	154.920
Poh 1	Pohnpei Island	32178	6	Fringing	6.863	158.107
Poh 2	Pohnpei Island	32178	10	Fringing	6.905	158.090

<sup>a</sup>Federated States of Micronesia: 2000 Population and Housing Census Report. FSM National Government, Department of Economic Affairs; May 2002 (FSM National Government 2002)

<sup>b</sup>Samples were collected at the surface (1 m) as well as the designated reef depth

phytoplankton pigments, 4 l of seawater were filtered onto GF/F filters (Whatman) and frozen in liquid nitrogen until pigment extraction. Further processing and analysis of phytoplankton pigments were conducted using high-performance liquid chromatography at the Horn Point Laboratory of the University of Maryland Center for Environmental Science. Seawater samples (1 ml) collected in duplicate for microbial cell enumeration were preserved with paraformaldehyde (1% final concentration), fixed for 20 min in the dark, and frozen in liquid nitrogen. Abundances of *Synechococcus*, *Prochlorococcus*, picoeukaryotes, and unpigmented cells (a proxy for heterotrophic bacteria and archaea) were enumerated using flow cytometry at the University of Hawaii, similar to a previous methodological description (Apprill & Rappé 2011). Fluorescence spectra were transformed into cell abundances using FlowJo software (v. 6.4.7).

## 2.5. Picoplankton diversity and community structure

For molecular analysis of picoplankton communities, duplicate samples (2 l) of seawater were filtered onto 25 mm, 0.22 µm Supor polyethersulfone membrane

filters (Pall) using peristalsis. Microbial biomass samples were frozen in liquid nitrogen until analysis. Genomic DNA was extracted from the filters by first bead beating at maximum speed with a benchtop vortexer and 24-tub vortex adapter (10 min) with sucrose-EDTA lysis buffer (0.75 M sucrose, 20 mM EDTA, 400 mM NaCl, 50 mM Tris) and 100 µl of 10% sodium dodecyl sulfate, followed by a Proteinase K digestion for 4 h at 55°C, and then purification with the DNeasy kit purification (Qiagen) (Santoro et al. 2010). The hypervariable IV region of small subunit (SSU) ribosomal RNA genes from bacteria and archaea was amplified using PCR with 515F (5'-GTG CCA GCM GCC GCG GTA A-3') (Caporaso et al. 2011) and 806RB (5'-GGA CTA CNV GGG TWT CTA AT-3') (Apprill et al. 2015) primers. The forward primer did not include the modification shown to increase recovery of *Thaumarchaea* (Parada et al. 2016). The primers were each equipped with a unique 8 bp barcode, 10 bp pad, and 2 bp link, and were designed after Kozich et al. (2013). Reactions (25 µl) were conducted in triplicate, and included 1.25 U of GoTaq Flexi DNA Polymerase (Promega), 5× colorless GoTaq Flexi Buffer, 2.5 mM MgCl<sub>2</sub>, 200 µM dNTP mix (Promega), 200 nM of each barcoded primer, as well as 1 to 4 ng of genomic template (generally 1 µl). For

the reactions, an initial denaturation step was conducted at 95°C for 2 min, followed by 27–38 cycles of 95°C for 20 s, 55°C for 15 s, and 72°C for 5 min, concluding with an extension step at 72°C for 10 min on a thermocycler (Bio-Rad Laboratories). Reaction products (5 µl) were screened on a 1% agarose/tris-borate-EDTA gel using the HyperLadder 50 bp DNA ladder (Bioline) to validate the size of the amplified product and general PCR performance. The number of PCR cycles varied by sample in order to produce similar minimal PCR product yields across samples, and this was evaluated by visualizing the agarose gel. Triplicate amplicons were pooled for each sample and purified using the QIAquick Purification Kit (Qiagen) and quantified using a Qubit 2.0 fluorometer with the dsDNA High Sensitivity Assay (Invitrogen). Amplicons were pooled into equimolar ratios and shipped to the University of Illinois W.M. Keck Center for Comparative and Functional Genomics, where they were sequenced using paired-end 250 bp MiSeq (Illumina). Control samples included sterile water (negative control) and a mock community sample (positive control) that was obtained through BEI Resources, NIAID, and NIH as part of the Human Microbiome Project: Genomic DNA from Microbial Mock Community B (even, low concentration), v5.1L, HM-782D. Results from these controls were as expected and the data are not presented.

Sequence data were analyzed using *mothur* v.1.33.3 (Schloss et al. 2009) and included construction of contigs from paired ends, quality filtering, alignment to the SSU rRNA gene, and chimera detection using UCHIME (Edgar et al. 2011). Samples contained 5147–55382 quality sequences (mean 26389). Sequences were grouped using minimum entropy decomposition (MED), which allocated sequences into 496 MED nodes (also referred to as MEDs; range of 267–441 MED nodes per sample) representing homogeneous operational taxonomic units based on Shannon entropy decomposition (Eren et al. 2015). Taxonomy was assigned to the sequences using the *k*-nearest-neighbor consensus algorithm in *mothur* using the Silva database (v119) (Quast et al. 2013). Sequences were not rarefied, due to the relatively even nature of the dataset and challenges associated with this practice (McMurdie & Holmes 2014, Weiss et al. 2017). Statistical comparisons of microbial community sequence data were conducted using Primer-E software (v. 7.0.9) using non-rarefied sequence data. In order to compare sequences from each sample, we transformed the MED counts into relative abundances, then performed a square-root transformation of the MED relative abundances, and finally, the data

were compared using Bray-Curtis dissimilarity (Bray & Curtis 1957). The Bray-Curtis dissimilarity data were then compared using CLUSTER, or hierarchical cluster analysis, using the group average mode as well as non-metric multi-dimensional scaling (nMDS) analysis with 1000 iterations, with vectors overlaid using Pearson correlation analysis. Permutational multivariate ANOVAs (PERMANOVAs) (Anderson 2001) were conducted using group average clustering methods, and Monte Carlo corrections were applied. Permutational analysis of multivariate dispersions (PERMDISP) permutation tests (Anderson 2006) were used to assess if within-group dispersion was responsible for any observed differences, as these differences can influence PERMANOVA results (Anderson & Walsh 2013). We used the Bio-Env + Stepwise (BEST) analysis (Clarke & Warwick 2001) in Primer to examine relationships between environmental parameters, benthic cover, and fish abundances (all square-root transformed), and the microbial community composition, conducted using the Spearman rank correlations. The BEST analysis searches for high rank correlations between the compared data, and reports the correlation coefficient ( $\rho$ ) and significance (*p*) value. RELATE analysis, a nonparametric form of the Mantel test conducted using the Spearman rank correlation method (Clarke & Warwick 2001), was used to examine relationships between microbial community composition and geographic distances between sites, and data are reported as the correlation coefficient ( $\rho$ ) and significance (*p*) value. Similarity and percentages routine (SIMPER) analysis (Clarke & Warwick 2001) was used to calculate the contribution of each microbial MED node to the dissimilarity between the island and atolls. SIMPER analysis was conducted using the relative abundance-based microbial composition data, and data are reported as specific MEDs that contributed to similarity within the group.

To further investigate relationships between specific microbial taxa and reef composition, differential abundance of all MED nodes was tested using the R (v 3.6.2) package 'corncob' (v 0.1.0), which employed beta-binomial regression models and accounted for varying sequencing depth and within-sample correlations between taxa (Martin et al. 2020). Specifically, 3 tests were performed to understand how abundances of the MED nodes changed with (1) increasing percent coral cover, (2) increasing percent macroalgal cover, and (3) between Olimarao (low coral cover reefs) and Kosrae (high coral cover reefs). Only reef depth microbial communities were used in this differential abundance analysis. Models examining

increasing coral or macroalgal cover examined data from all sites with benthic coverage data (16 sites: Kap 1, 2, 3, 5, and 6; Kos 1, 2, and 3; Nuk 1 and 2; Olim 1 and 2; Poh 1 and 2; Wole 1 and 3), and these data included 496 MED nodes. The model examining just Olimarao and Kosrae utilized data from 5 sites (Kos 1, 2, and 3 and Olim 1 and 2) and also considered 496 MED nodes. All MED node sequence counts per sample were input into the 'corn-cob' program, which modeled relative abundances of MED nodes. Differential abundance was modeled as a function of either (1) percent coral cover, (2) percent macroalgal cover, or (3) island/atoll (Kosrae vs. Olimarao). The parametric Wald test was used to test the hypothesis that the relative abundance of a given MED node changed significantly with respect to the variable (percent coral cover, percent algal cover, site category), and the Benjamini-Hochberg false discovery rate (FDR) correction was applied to account for multiple comparisons, with the cutoff at 0.05.

Sequence data are available at NCBI's Sequence Read Archive under BioProject IDs PRJNA636810 and PRJNA27914, and representative sequences for each MED are provided in Table S2.

## 2.6. Statistical analyses

Statistical comparisons of reef and environmental data were conducted using SigmaPlot v14 (Systat Software). For *t*-tests and regressions, the data were verified as normal prior to analyses using Shapiro-Wilk normality tests and Brown-Forsythe equal variance tests. If normalization failed, Mann-Whitney rank sum tests were used. Two-way ANOVA tests were applied to either raw or square root transformed (for normalization) data in order to test the impact of site, depth, and site × depth interactions on reef-water nutrient and microbial parameters.

## 3. RESULTS

### 3.1. High coral cover and low fish abundance on reefs

Across the islands and atolls of Micronesia, hard coral primarily dominated reefs (Fig. 1I,J), with average coral cover ranging from 7 to 73% (mean ± SD: 36.9 ± 22.3%) on reefs per island or atoll, with the highest coverage observed on Kosrae Island reefs

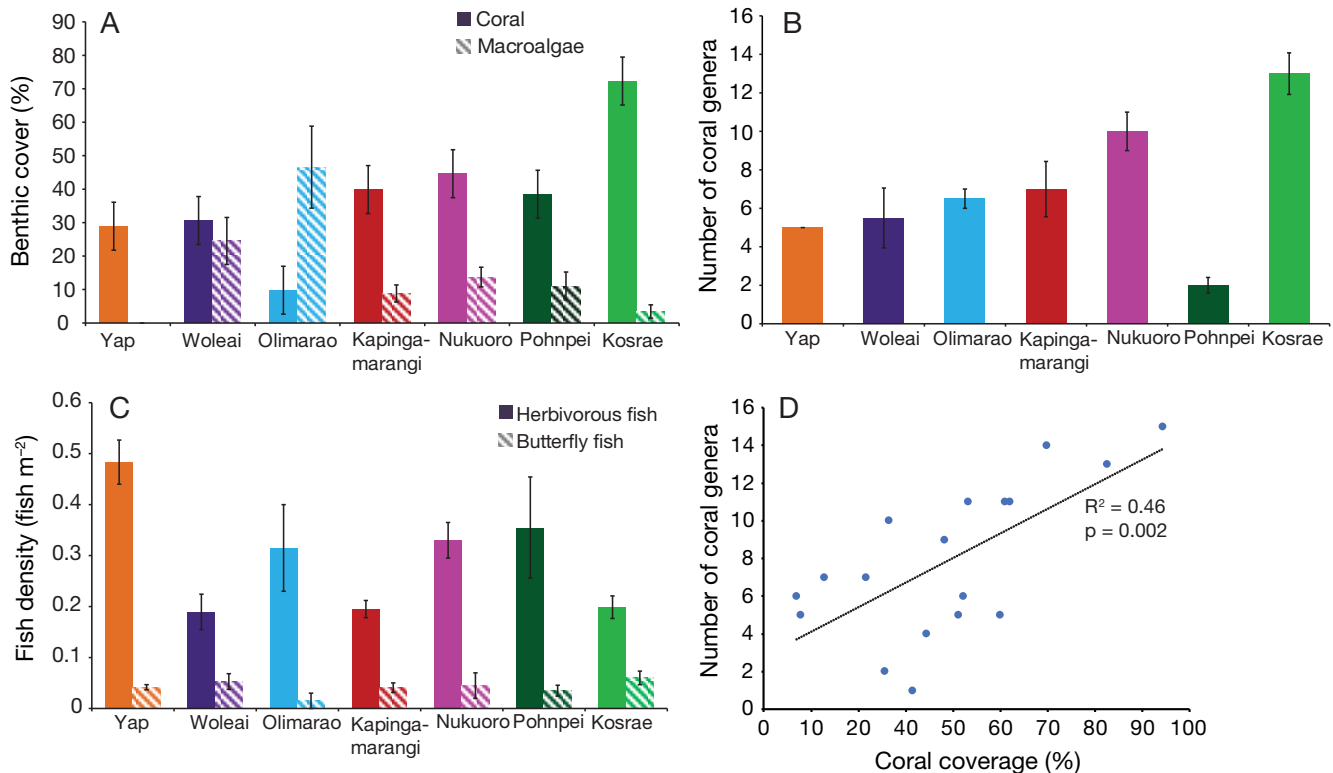


Fig. 2. Macro-organism abundance and diversity on reefs of each island or atoll. (A) Average abundance of coral and macroalgae, (B) average coral richness (by genera), and (C) density of herbivorous and butterflyfish and (D) relationship between coral genera and coral coverage on the reefs. Error bars represent standard deviation over 2–6 reef sites per island or atoll. Differently colored bars indicate site groupings (1–6 sites per island/atoll; see Table 1)

(variability in coverage presented in Fig. 2A). Macroalgal cover was 10% or less on Kapingamarangi, Nukuoro, Pohnpei, and Kosrae reefs, and higher on Woleai and Olimarao at 25 and 50% cover, respectively, with a mean coverage of  $15.2 \pm 15.5\%$  across all island or atoll reefs (macroalgae were not quantified on Yap) (Figs. 1K & 2A). Coral diversity was assessed by richness of genera, and this ranged from 2 to 13 genera per island or atoll and was highest on Kosrae and lowest on Pohnpei (Fig. 2B; Table S3). Across the Micronesian reefs, there was a significant relationship between coral coverage and richness of coral genera (regression analysis,  $R^2 = 0.46$ ,  $p = 0.002$ ; Fig. 2D). Average abundances of herbivorous fish on the reefs ranged from 0.19 to 0.48 fish  $m^{-2}$  (mean:  $0.26 \pm 0.14$ ), with the highest abundance on Yap and lowest abundances on Woleai, Kapingamarangi, and Kosrae (Fig. 2C). Corallivorous fish (butterflyfishes) abundances ranged from an average of 0.02 to 0.06 fish  $m^{-2}$  (mean:  $0.04 \pm 0.02$ ), with lowest abundance on Olimarao reefs, which also had the lowest coral cover (Fig. 2C). Significant relationships were also observed between fish abundances and the benthic community. Specifically, regression analysis showed a relationship between increasing butterfly fish abundance and increasing coral cover ( $R^2 = 0.163$ ,  $p = 0.09$ ). In contrast, butterfly fish abundance decreased with increasing macroalgae ( $R^2 = 0.40$ ,  $p = 0.006$ ). A significant positive relationship existed between coral richness and herbivore density ( $R^2 = 0.179$ ,  $p = 0.08$ ). There were no significant relationships observed between hard coral cover and herbivore abundance; coral richness and butterfly fish abundance; or herbivore density and macroalgae abundance ( $R^2 = 0.009$ – $0.02$ ,  $p > 0.1$ ).

### 3.2. Consistent physicochemical parameters across reefs

Temperature at the reefs ranged from 29.1 to 31.8°C (mean  $\pm$  SD:  $30.1 \pm 0.6^\circ\text{C}$ ) and was highest at the southernmost reefs of Kapingamarangi atoll (Table S1). Salinity was similar throughout the reefs (34.1–34.7; mean:  $34.4 \pm 0.14$ ), and oxygen was saturated (near 100%) at all sites (Table S1).

### 3.3. Nutrient concentrations indicate oligotrophic conditions, with low TOC at high coral cover reefs

Inorganic nutrient concentrations were consistently low across the reef sites. Nitrate + nitrite varied from detection limits (0.07  $\mu\text{M}$ ) to 2.0  $\mu\text{M}$  (mean  $\pm$  SD:  $0.58$

$\pm 0.67 \mu\text{M}$ ), reached the highest concentrations at the Nukuoro and Pohnpei reef depths (Fig. 3A), and significantly related to island/atoll ( $p = 0.004$ , Table 2). Ammonium concentrations ranged from the detection limit (0.02  $\mu\text{M}$ ) to 0.23  $\mu\text{M}$  (mean:  $0.07 \pm 0.05 \mu\text{M}$ ) (Fig. 3B). Phosphate varied from 0.05 to 0.20  $\mu\text{M}$  (mean:  $0.11 \pm 0.04 \mu\text{M}$ ) across the reefs (Fig. 3C), and was significantly related to island/atoll ( $p = 0.001$ , Table 2). Concentrations of silicate ranged from 0.80 to 1.86  $\mu\text{M}$  (mean:  $1.10 \pm 0.20 \mu\text{M}$ ) at the reef sites and were highest in Pohnpei island surface waters (Fig. 3D). Across the reefs, surface and reef depth inorganic nutrient concentrations were not significantly different according to depth category or island/atoll  $\times$  depth interactions ( $p > 0.1$ ; Table 2).

TOC concentrations in the reef waters ranged from 59.8–104.4  $\mu\text{M}$  (mean:  $72.2 \pm 8.7 \mu\text{M}$ ) (Fig. 3E). The highest concentration of TOC was measured in the surface waters of Kap 3 (Fig. 1F), a lagoon in the Kapingamarangi atoll. The 3 Kosrae Island reefs, which had the highest abundance and diversity of hard coral among all islands, consistently had concentrations of TOC below the mean for the study, ranging from 59.8 to 67.9  $\mu\text{M}$  (Fig. 3E). However, when comparing all islands and atolls, there was no trend between TOC concentrations and coral abundance (linear regression,  $R^2 = 0.140$ ,  $p > 0.1$ ). TON concentrations were variable across the reef locations, ranging from 1.4 to 5.5  $\mu\text{M}$  (mean:  $4.0 \pm 1.1 \mu\text{M}$ ), with the lowest concentrations measured in the reef depth waters of Nukuoro (Nuk 1) and consistently high concentrations in the surface and reef depth waters of Kosrae (4.5–5.3  $\mu\text{M}$ ; mean:  $5.0 \pm 0.26 \mu\text{M}$ ; Fig. 3F). We found no significant relationship between TON and coral abundance ( $R^2 = 0.04$ ,  $p > 0.1$ ).

### 3.4. Abundant picocyanobacteria in reef waters

Abundances of specific microbial groups measured using flow cytometry were relatively consistent across the reefs. *Prochlorococcus* ranged from non-detectable (Poh 2, surface) to 85 400 cells  $\text{ml}^{-1}$  (Kap 6, reef depth) with a mean  $\pm$  SD of  $35\,400 \pm 20\,400$  cells  $\text{ml}^{-1}$  for all samples (Fig. 4A). Throughout all of the reef sites, *Prochlorococcus* were present at significantly higher concentrations at the reef depth compared to the surface waters (reef depth:  $44\,300 \pm 19\,100$  cells  $\text{ml}^{-1}$ ; surface:  $28\,100 \pm 19\,600$  cells  $\text{ml}^{-1}$ ;  $p = 0.078$ , Table 2). *Synechococcus* concentrations were variable across the reefs, ranging from  $660 \pm 19$  cells  $\text{ml}^{-1}$  (Kos 3, surface) to  $175\,000 \pm 120$  cells  $\text{ml}^{-1}$  (Poh 2, surface) with a mean of  $28\,000 \pm 39\,400$  cells  $\text{ml}^{-1}$  for all



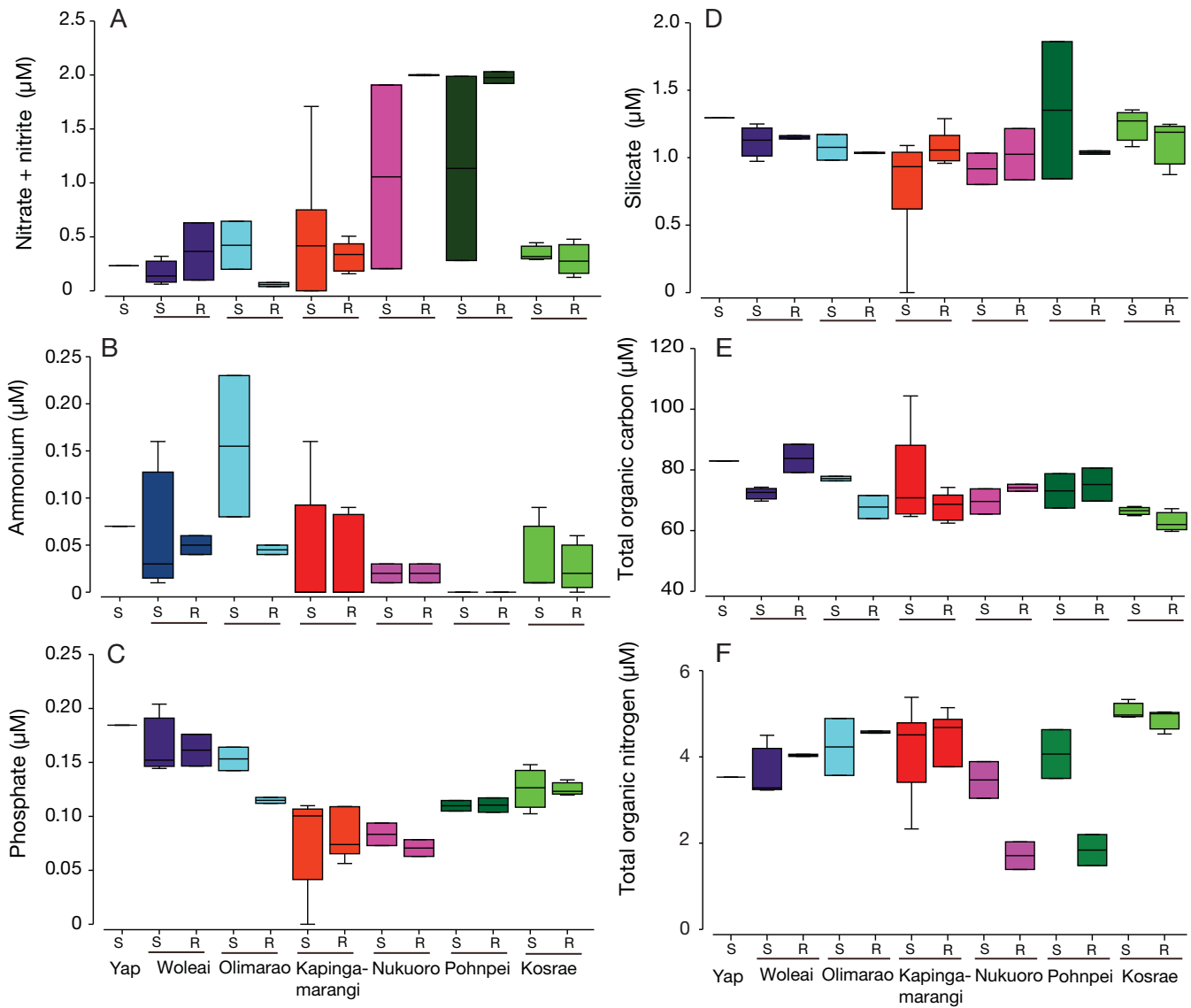


Fig. 3. Inorganic nutrient concentrations in Micronesian reef waters. Concentrations of (A) nitrate + nitrite, (B) ammonium, (C) phosphate, (D) silicate, (E) total organic carbon, and (F) total organic nitrogen grouped by surface (S) and reef depth (R) waters per island or atoll (1–6 sites; see Table 1). Colors designate the site groupings, the black bar across the boxplot represents the median value and the whiskers extend to values at 1.5 times the interquartile range (delimited by the box)

samples (Fig. 4B). Picoeukaryotes were generally low in abundance at the reefs, ranging from  $160 \pm 28$  cells  $\text{ml}^{-1}$  (Kap 2, surface) to  $7800 \pm 850$  cells  $\text{ml}^{-1}$  (Poh 2, surface) with a mean of  $1300 \pm 1400$  cells  $\text{ml}^{-1}$  across the reefs (Fig. 4C). Unpigmented cells, interpreted as heterotrophic bacteria and archaea, displayed variability throughout the reef sites, ranging from  $76\,900 \pm 22\,70$  cells  $\text{ml}^{-1}$  (Kap 2, surface) to  $1\,121\,000 \pm 16\,100$  cells  $\text{ml}^{-1}$  (lagoon at Kap 3, surface), with mean concentrations of  $437\,000 \pm 193\,000$  cells  $\text{ml}^{-1}$  throughout the reefs (Fig. 4D). Concentrations of all prokaryotes (unpigmented cells + *Synechococcus* + *Prochlorococcus*) generally followed the same patterns per site and depth as the unpigmented cells

(Fig. 4E). Across all of the reefs, abundances of *Synechococcus*, picoeukaryotes, and unpigmented cells were not related to island/atoll, depth category, or island/atoll  $\times$  depth interactions (Table 2).

In order to estimate potential metabolic contributions of the picoplankton to the reefs, we used the flow cytometry measurements to compare the ratio of heterotrophic to autotrophic, prokaryotic cells (unpigmented cells / [*Prochlorococcus* + *Synechococcus*]). This comparison showed that on average, the reefs contained 9:1 heterotrophic to autotrophic cells (Fig. 4F). The Kosrae reefs had the highest ratio of heterotrophic to autotrophic cells at both surface and reef depths (Fig. 4F). Across all reefs, the ratio of het-

Table 2. Two-way ANOVA of reef seawater nutrient and cell abundance parameters, by island and depth interactions ( $p < 0.1$  are **bolded**). NA: not applicable

Parameter	Island (df = 5)		Depth (df = 1)		Island × Depth (df = 5)	
	F	p	F	p	F	p
NO <sub>3</sub> <sup>-</sup> + NO <sub>2</sub> <sup>-</sup>	<b>5.00</b>	<b>0.004</b>	0.576	>0.1	1.22	>0.1
NH <sub>4</sub> <sup>+</sup>	0.97	>0.1	0.74	>0.1	2.14	>0.1
PO <sub>4</sub> <sup>3-</sup>	<b>11.96</b>	<b>0.001</b>	1.99	>0.1	0.51	>0.1
TOC	1.58	>0.1	0.03	>0.1	1.23	>0.1
TON	2.13	>0.1	1.68	>0.1	1.04	>0.1
Silicate	0.84	>0.1	0.13	>0.1	0.65	>0.1
<i>Prochlorococcus</i>	1.30	>0.1	<b>3.46</b>	<b>0.078</b>	0.65	>0.1
<i>Synechococcus</i>	1.91	>0.1	0.81	>0.1	0.56	>0.1
Unpigmented picoplankton	0.98	>0.1	0.31	>0.1	0.26	>0.1
Picoeukaryotes	1.78	>0.1	0.10	>0.1	1.23	>0.1
Chlorophyll <i>a</i>	1.20	>0.1	NA	NA	NA	NA
Heterotrophic: autotrophic prokaryotes	3.10	<b>0.033</b>	0.30	>0.1	0.139	>0.1
Microbial richness	1.30	>0.1	0.28	>0.1	<b>3.75</b>	<b>0.015</b>

erotropic to autotrophic cells did not differ significantly according to island/atoll, depth category, or island/atoll × depth interactions (Table 2).

Total chlorophyll was only measured in the surface waters, and concentrations ranged between 0.001 and 0.219 µg l<sup>-1</sup> (mean 0.099 ± 0.055 µg l<sup>-1</sup>) at the reef sites, with the lowest concentration at the open water site and highest at Woleai atoll (Wole 3) (Fig. 4G). Pigment concentration data were used to estimate the proportion of the pigmented cells corresponding to micro-, nano-, and picoplankton communities. For all reef waters, picoplankton were responsible for the majority of the chlorophyll biomass (range of 54–82%, mean of 71 ± 8.7% of chlorophyll biomass), followed by nanoplankton (range of 3–33%, mean of 17 ± 6.1%) and then microplankton (range of 5–32%, mean of 13 ± 8.1%) (Table S1; individual pigment concentrations are also presented).

### 3.5. Reef depth microbial richness related to benthic reef community

Richness of reef-water microorganisms, as assessed by number of MED nodes from SSU rRNA gene amplicons of bacteria and archaea, was generally consistent throughout the reef waters, with values ranging from 267 to 441 MED nodes with an average of 393 ± 33 MED nodes (Fig. 5A). Nukuoro (n = 2) and Kosrae (n = 3) reefs showed the highest microbial richness (391–430 and 377–434 MED nodes, respectively) compared to the other reefs (Fig. 5A). The 2-way ANOVA revealed an island/atoll × depth interaction for microbial richness ( $p = 0.015$ , Table 2).

Linear regression analysis indicated that reef depth microbial richness was positively correlated with percentage of hard coral coverage ( $R^2 = 0.33$ ,  $p = 0.013$ ; Fig. 5B) as well as negatively related to percent macroalgal coverage ( $R^2 = 0.30$ ,  $p = 0.023$ ; Fig. 5C). These trends between microbial alpha diversity at reef depth and coral and macroalgal cover were also examined for Shannon and Simpson's diversity indices, and the relationships were not significant ( $R^2 = 0.04$ – $0.10$ ;  $p > 0.1$ ). No relationships were detected between coral diversity or densities of other reef life (herbivores or butterflyfish) and microbial richness ( $R^2 = 0.05$ ;  $p > 0.1$ ). There was a relationship between increasing number of MED nodes and increasing silicate concentrations ( $R^2 = 0.113$ ,  $p = 0.056$ ), but no relationships were detected between microbial diversity and concentrations of phosphate, nitrate+nitrite, ammonium, TON, or TOC ( $R^2 = 0.008$ – $0.022$ ,  $p > 0.1$ ).

### 3.6. Reef seawater microbial communities are related to biogeographical and environmental factors

Comparison of microbial community composition using the Bray-Curtis dissimilarity index demonstrated that communities were 65–85% similar, with the exception of surface waters from a Pohnpei reef (Poh 2), which was only 42% similar to the other reef waters (Fig. 6A). Reefs that were most similar in microbial composition included the Kosrae Island sites, which clustered together with 85% similarity, and the Kapingamarangi atoll sites with 75% similarity, except for 1 benthic sample (Kap 2, Fig. 6A). PERMANOVA of the effect of island/atoll and depth

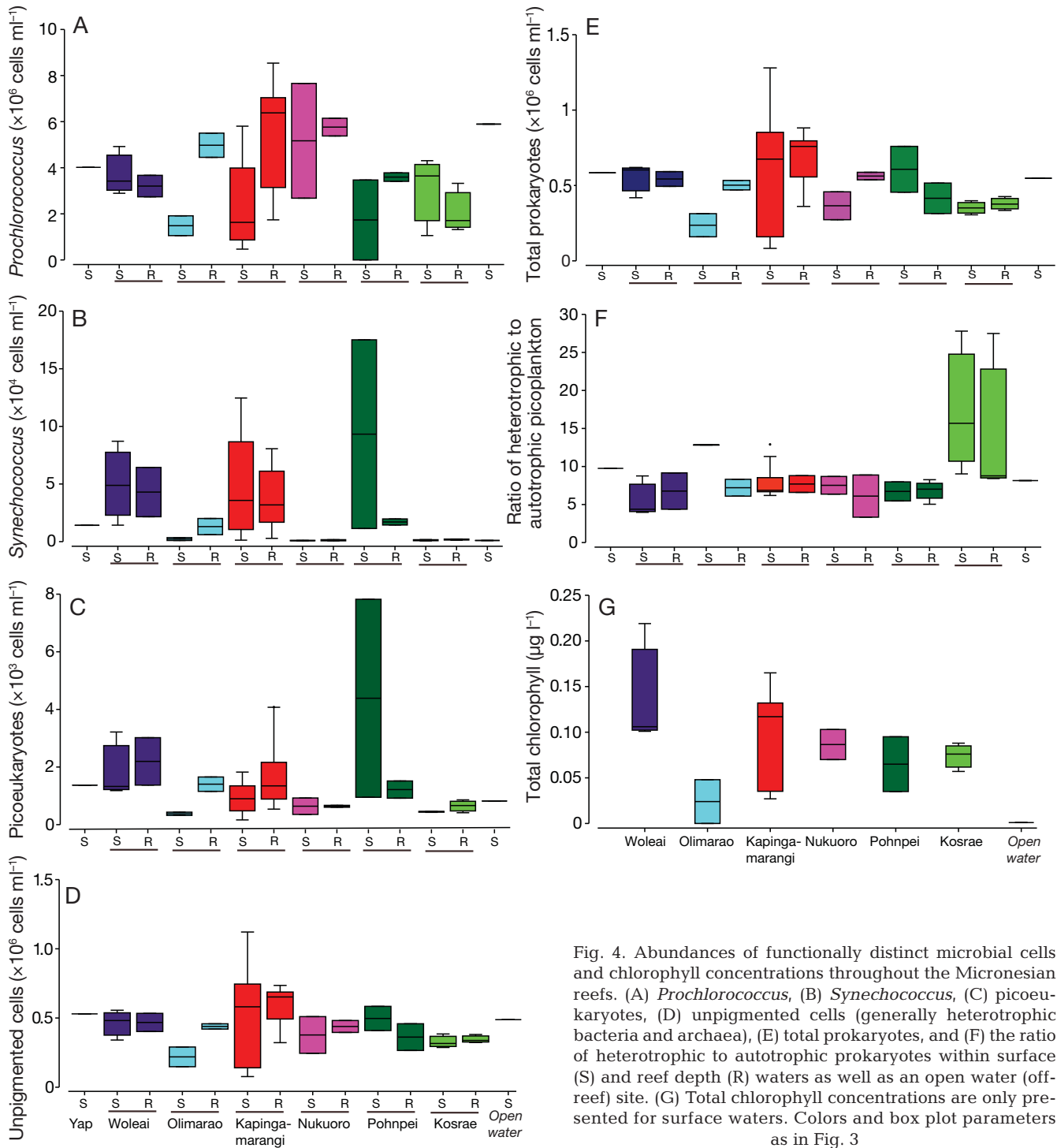


Fig. 4. Abundances of functionally distinct microbial cells and chlorophyll concentrations throughout the Micronesian reefs. (A) *Prochlorococcus*, (B) *Synechococcus*, (C) picoeukaryotes, (D) unpigmented cells (generally heterotrophic bacteria and archaea), (E) total prokaryotes, and (F) the ratio of heterotrophic to autotrophic prokaryotes within surface (S) and reef depth (R) waters as well as an open water (off-reef) site. (G) Total chlorophyll concentrations are only presented for surface waters. Colors and box plot parameters as in Fig. 3

on the microbial community composition showed a significant relationship between island/atoll in reef depth (pseudo- $F = 2.9127$ , Monte Carlo  $p = 0.002$ ) as well as surface waters (pseudo- $F = 2.6749$ , Monte Carlo  $p = 0.002$ , Table S4). Sampling depth, without consideration of island/atoll, did not significantly influence microbial community composition (pseudo-

$F = 0.90961$ , Monte Carlo  $p = 0.427$ ). PERMANOVA pairwise comparisons suggested that the island/atoll difference was mostly due to differences in surface and reef depth microbial communities at Kapingamarangi atoll compared to Kosrae, Nukuoro, Olimarao, and Woleai as well as differences between Kosrae and Woleai sites ( $p < 0.1$ ; Table S4). PERM-

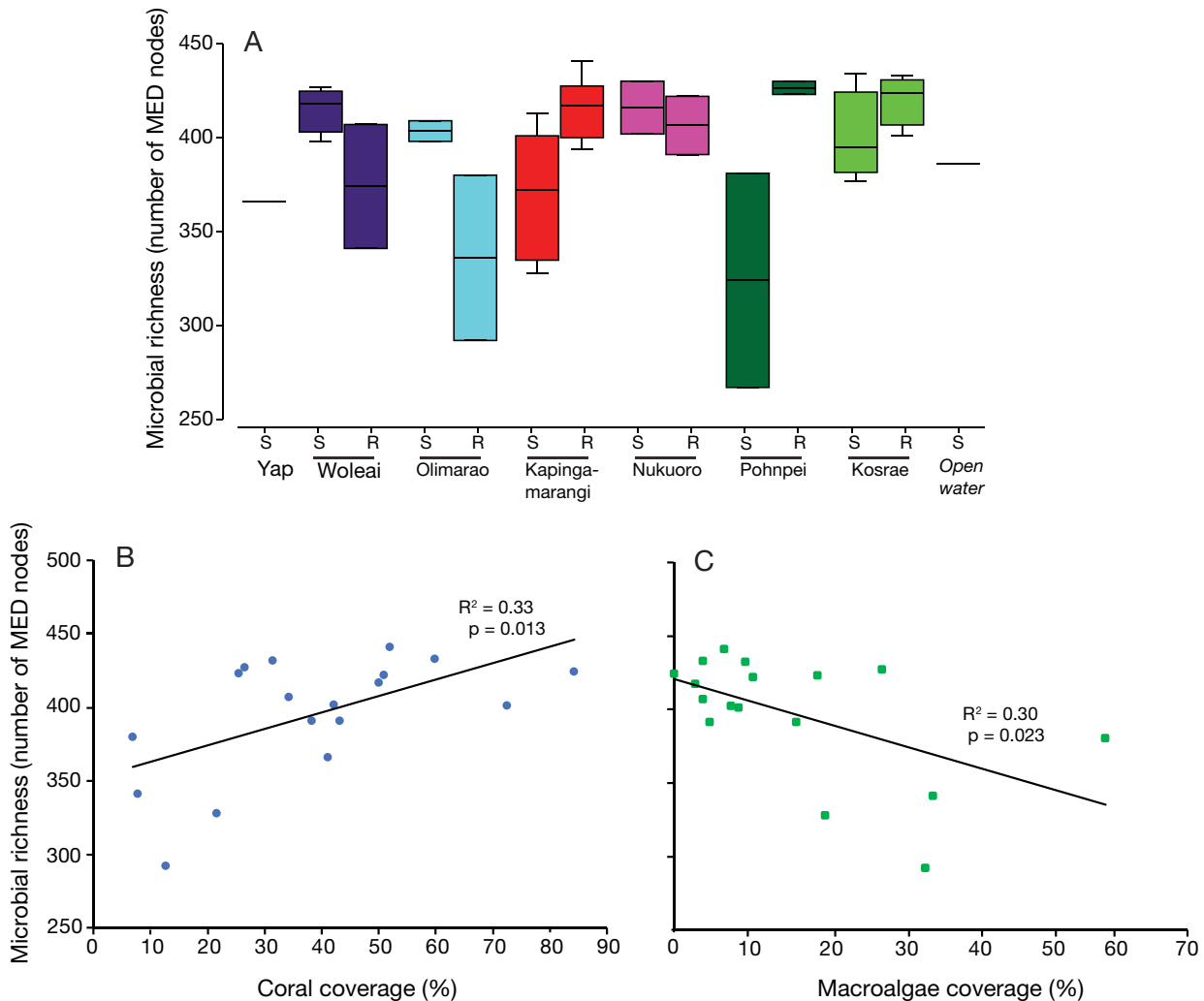


Fig. 5. (A) Reef seawater microbial richness for each island and atoll according to depth. Microbial richness at the reef depth was significantly related to the abundance of (B) coral and (C) macroalgae comprising the reefs. Microbial richness is represented by number of MED nodes obtained from SSU rRNA gene amplicons of bacteria and archaea. In panel (A), 'S' represents surface samples and 'R' represent reef depth samples. Only surface waters were collected for Yap and the open-water site. Colors and box plot parameters as in Fig. 3

DISP also demonstrated that these relationships were not related to the degree of beta dispersion ( $p > 0.1$ ; Table S4). Additionally, there were differences in reef depth community composition between Kapingamarangi and Pohnpei as well as Kosrae and Pohnpei (PERMANOVA  $p < 0.1$  with PERMDISP  $p > 0.1$ ; Table S4). For the open-water site, surface water microbial community composition was only significantly different from reef-water microbial communities at Kapingamarangi atoll (PERMANOVA  $p = 0.025$  with PERMDISP  $p > 0.1$ ; Table S4).

To further examine the relationship between microbial community composition and reef and environmental features, an nMDS analysis of the microbial community composition (Bray-Curtis dissimilarity) was conducted and overlaid with vectors represent-

ing other reef and environmental features (Fig. 6B). This analysis showed that the Poh2 surface water microbial community was distinct from the other reef-water microbial communities, as evidenced by the symbol that is plotted outside of the predictor variable circle (Fig. 6B). Kapingamarangi atoll reef-water microbial communities, which were closest to the equator, correlated with temperature, silicate concentrations, as well as abundances of unpigmented picoplankton, *Synechococcus*, and picoeukaryotes (Fig. 6B). Kosrae reef microbial communities were also correlated with these same factors (temperature, silicate concentrations, and abundances of unpigmented picoplankton, *Synechococcus*, and picoeukaryotes) as well as with *Prochlorococcus* abundance, concentrations of TOC and TON, and ratios of

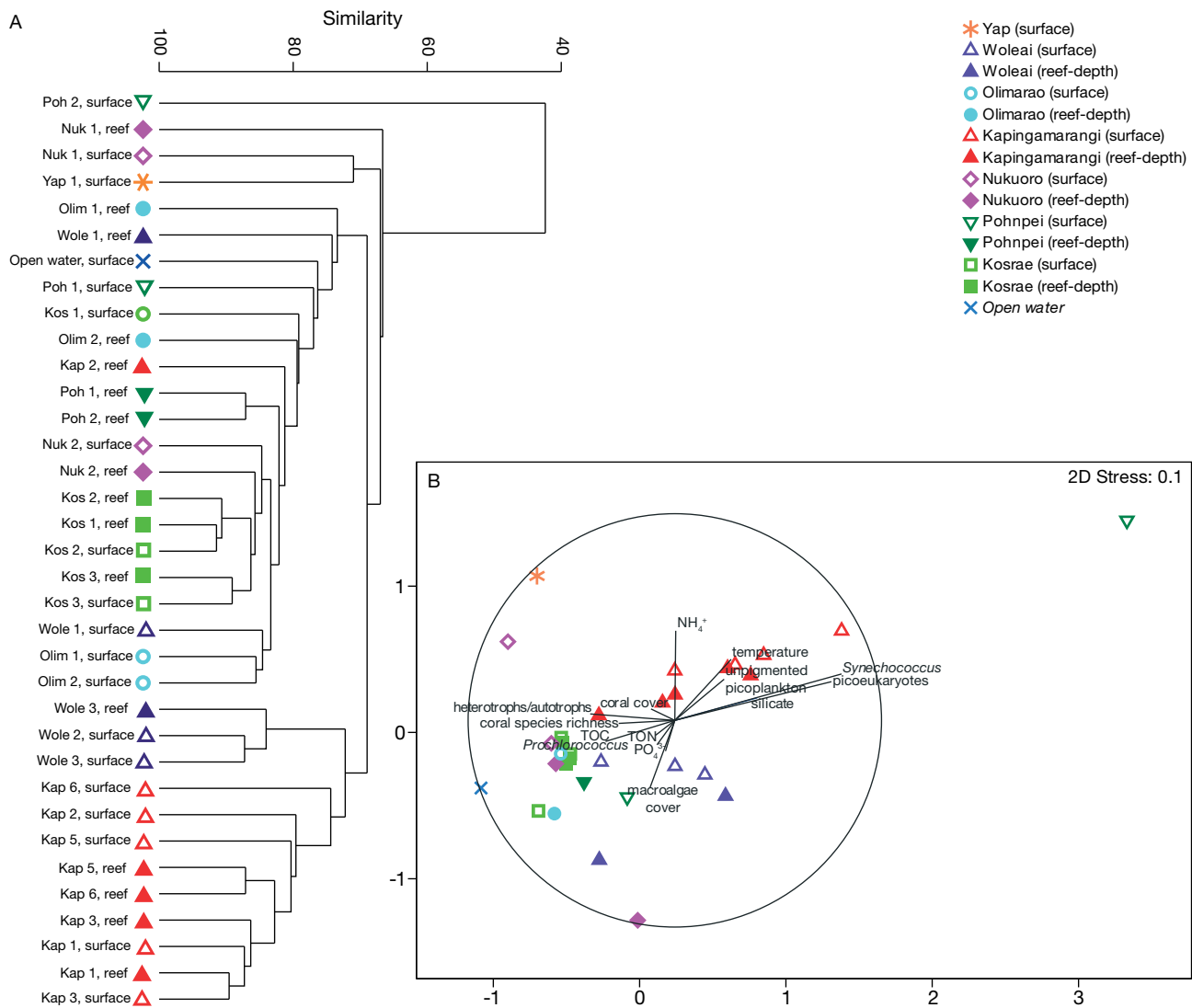


Fig. 6. Comparison of reef-water microbial communities and relationship to environmental factors. (A) Cluster dendrogram of reef-water bacterial and archaeal communities, according to island or atoll and sampling depth. (B) Nonmetric multidimensional scaling (nMDS) analysis of reef-water bacterial and archaeal communities, according to island or atoll and depth, with vector overlays representing multiple correlations between ordination axes and environmental variables. Clustering was conducted using group average linkages on Bray-Curtis dissimilarity indices computed from the square-root transformed relative abundances of small subunit rRNA gene amplicons grouped into minimum entropy decomposition nodes. Only surface water from 1 Yap site was collected. TOC (TON): total organic carbon (nitrogen)

heterotrophic to autotrophic microorganisms (Fig. 6B). Comparison of the environmental parameters to microbial community composition in surface waters using BEST analysis showed significant relationships between community composition and ammonium and silicate concentrations, as well as latitude ( $\rho = 0.546$ ,  $p = 0.045$ ). Reef depth microbial community composition was related to latitude and longitude ( $\rho = 0.519$ ,  $p < 0.02$ ).

RELATE analysis was used to further investigate the relationship between geographical distance and

the composition of the microbial community. For reef depth microbial communities, there was a significant relationship with geographical distance ( $\rho = 0.253$ ,  $p = 0.032$ ). In contrast, RELATE analysis showed no significant relationship between surface water microbial community composition and geographic distance between the sites ( $\rho = 0.092$ ,  $p = 0.181$ ). We also conducted the RELATE analysis to examine relationships between geographic distance and microbial community composition over a smaller spatial scale at the Kosrae and Kapingamarangi sites. Reef water

from both of these locations was sampled at least 3 or more times at different intra-island sites surrounding each island or atoll (within island/atoll distances: 0.15–5.36 km; between islands/atoll distances: 1021–1028 km). For both surface and reef depth waters, geographic distance had a significant impact on the microbial community structure (i.e. intra-island sites had more similar microbes than sites between Kosrae and Kapingamarangi) (reef depth:  $\rho = 0.507$ ,  $p = 0.033$ ; surface:  $\rho = 0.807$ ,  $p = 0.002$ ).

### 3.7. Reef waters are dominated by picocyanobacteria and bacteria common to oligotrophic regions

Reef-water bacterial and archaeal composition, as assessed using SSU rRNA gene amplicons, showed a high abundance of picocyanobacteria in all reef waters. Picocyanobacterial taxa mostly consisted of *Synechococcus* and *Prochlorococcus* (35–50% collective relative abundance; Fig. 7). The relative sequence abundances of *Synechococcus* were at least 3-fold greater than *Prochlorococcus* (Fig. 7), whereas cellular concentrations identified using flow cytometry showed *Synechococcus* to be about twice as abundant as *Prochlorococcus* (Fig. 4A,B). Sequences

associated with the SAR11 clade regularly made up 20–30% of the microbial community in the reef waters. *Rhodospirillales*, *Oceanospirillales*, *Acidimicrobiales*, *Rickettsiales*, and *Rhodobacterales* affiliated sequences were consistently represented in all samples (Fig. 7). Of these major taxonomic groups, the open water (non-reef) site displayed a similar profile, but with fewer *Synechococcus* and *Thermoplasmales* (*Euryarchaeota*) compared to the reef sites (Fig. 7). Generally, taxonomic profiles of microbial communities were similar between the surface and reef depth seawater at each site.

We used SIMPER analysis to identify specific MED nodes that contributed to island- or atoll-based microbial community similarity. The identified taxa were generally similar between the islands and atolls, and included members of the *Prochlorococcus* (uncultured and MIT9313), *Synechococcus* CC9902, SAR11 (clades I, Ia, Ib, and II), SAR86, SAR116, AEGEAN-169 marine group, as well as sequences affiliated with the 'Candidatus Actinomarina' (Table S5). Despite the similarities, relative abundances of microbial taxonomic groups were distinctly shifted at one of the Nukuoro sites (Nuk 1), which contained fewer cyanobacteria (20%) compared to the other reefs (40–50% cyanobacteria)

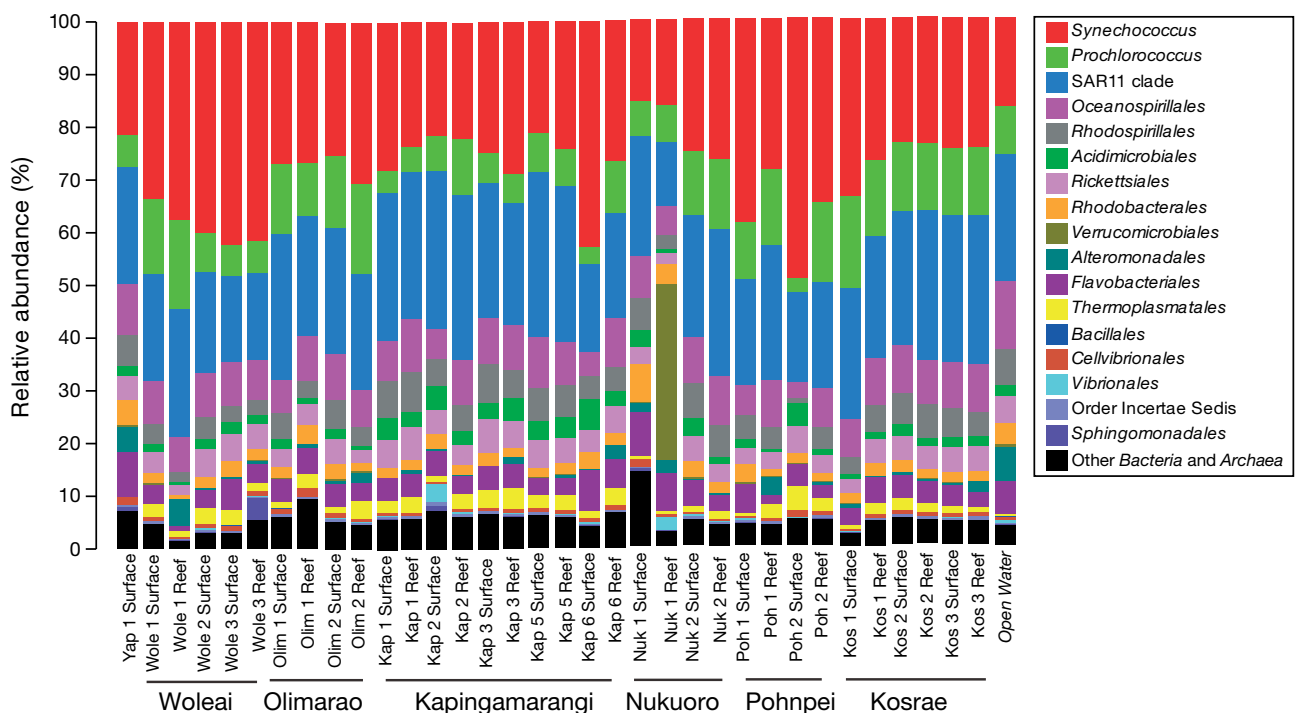


Fig. 7. Relative abundances of the most prevalent taxonomic groups of bacteria and archaea present within the reef waters, as examined using small subunit rRNA gene amplicons. Taxa are generally represented by family, with the more abundant genera and clades specifically noted (e.g. *Synechococcus*, *Prochlorococcus*, SAR11 clade, etc.)

(Fig. 7). The Nuk 1 reef depth microbial community also harbored elevated relative abundances of *Verrucomicrobiales* (primarily *Rubritalea*) (35%) compared to the other sites (<1%) (Fig. 7).

### 3.8. Microbial species indicative of coral- and macroalgal-dominated reefs

In order to further discern possible contributions of the benthic reef features to the overlying reef depth microbial community, we used differential abundance analyses implemented in the R package 'corncob' to identify specific MED nodes (MEDs) that were significantly differentially enriched in relative abundance on the coral- and macroalgal-dominated reefs from 16 sites. Coral-dominated reefs were positively enriched in 9 MEDs that were associated with primarily copiotrophic lineages of bacteria, including unclassified *Flavobacteriaceae*, *Leisingera*, *Owenweeksia*, *Vibrio*, OM27 clade, *Desulfuromonadales*, and the NS4 marine group (Fig. 8A). In contrast, macroalgal-rich reefs were only positively enriched with 3 MEDs, including an unclassified *Alphaproteobacteria* as well as 2 *Prochlorococcus* MEDs (Fig. 8B).

A differential abundance analysis comparison of reef depth waters was conducted to specifically compare MEDs from the coral-dominated reefs from Kosrae (mean coral cover 72%; n = 3 sites) to the most macroalgal-dominated sites of Olimarao (mean macroalgal cover 46%; n = 2 sites). This analysis showed that 74 MEDs contributed to differences between these microbial communities. Microbial taxa differentially abundant on the Kosrae reefs (i.e. the most positive estimate values) included *Marinobacterium*, *Owenweeksia*, an unclassified *Gammaproteobacteria*, NS5 marine group, SAR11 clades I, Ib, II, and III, an unclassified *Cryomorphaceae*, *Desulfuromonadales* group PB19, SAR116, SAR86, AEGEAN-169, '*Candidatus Actinomarina*', *Marinicella*, and the SAR92

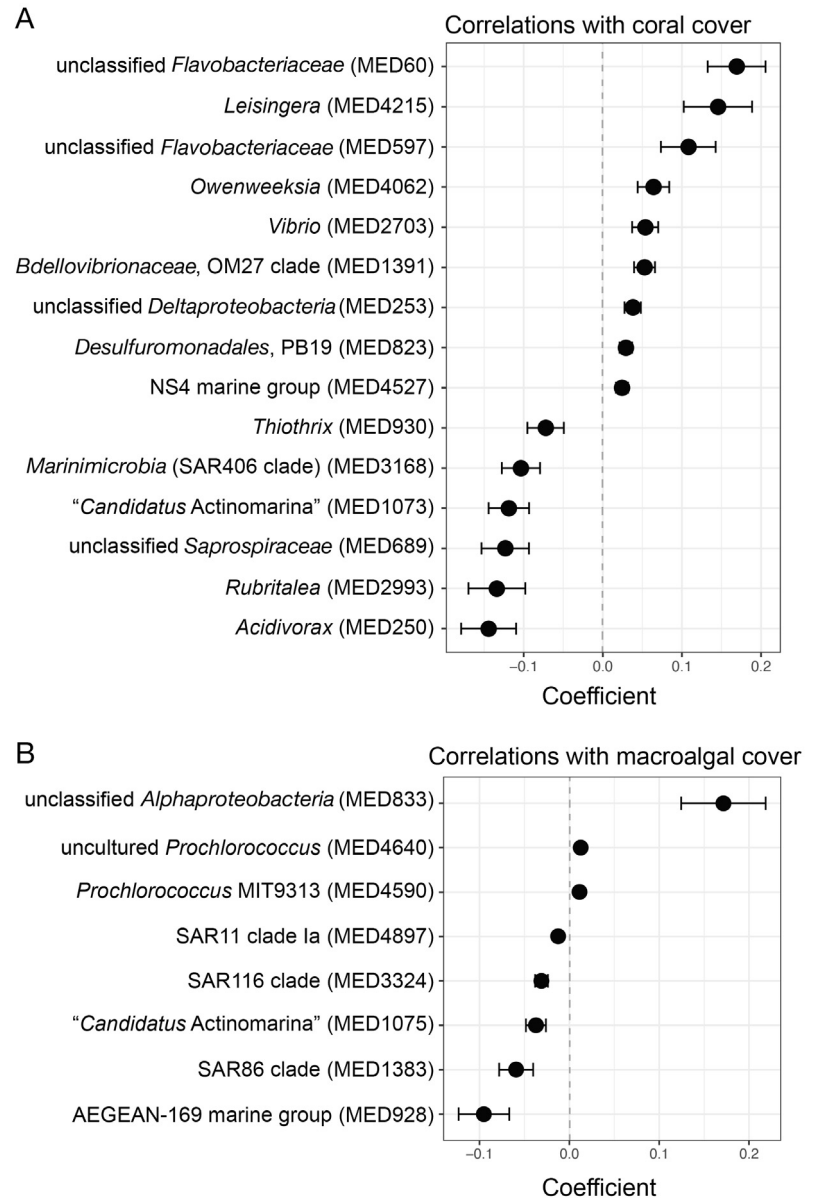


Fig. 8. Differentially abundant microbial taxa (minimum entropy decomposition, MED) as a function of (A) coral coverage and (B) macroalgal coverage, assessed using reef depth microbial communities with the analysis in the R package 'corncob' (v 0.1.0). The relative abundance of each MED node was modeled as a function of the percentage of coral and macroalgal coverage, and the coefficient indicates the change in MED node relative abundance, with the positive coefficients most related to coral (A) and macroalgal (B) coverage

clade (Table S6). For the Olimarao reefs, differentially abundant associated microbial taxa (with the most negative estimate values) included unclassified *Rhodobacteraceae*, NS9 marine group, OM6 clade, *Synechococcus*, *Alcanivorax*, *Roseibacillus*, several Marine Group II and III *Euryarchaeota* groups, SAR11 clades I, Ib, and II, *Pirellula*, unclassified *Dadabacteriales*, and the SAR324 clade (Table S6).

#### 4. DISCUSSION

This is the first study to examine the microbial ecology of the reef waters within Micronesia. Overall, we found high similarity in the composition of the microbial communities as well as in the concentrations of nutrients and organic matter between surface and reef depth waters, and ammonium and silicate concentrations were linked to microbial community composition in surface waters. Microbial richness was significantly related to island/atoll and depth category interactions and was positively correlated with coral cover. Reefs with high coral cover were enriched in distinct copiotrophic and coral-associated microbial lineages, suggesting that the reef macro-organismal community is tightly linked to the microbial ecology in the overlying waters. Further, biogeographic distance-related factors were significantly related to the composition of the reef depth microbial community. We also noted unique features at the coral-dominated Kosrae reefs that provide additional insights into reef community microbial biogeochemistry.

##### 4.1. Context of Micronesian reefs and nutrient and organic matter concentrations

The reefs surveyed in Micronesia all harbored generally high abundances of coral. Kosrae reefs hosted the highest abundances (up to 77% coverage) of coral, consistent with previous observations (Richards 2015). More generally across the islands and atolls, coral and macroalgal coverages were in line with previous observations on other Micronesian reefs including Pohnpei, Chuuk, and Yap Islands (Abraham et al. 2004, Goldberg et al. 2008). Comparing Micronesian reefs more globally, the coverage of coral we observed in Micronesia is similar to that of the northern Line Islands (Sandin et al. 2008) but much higher than many other global reefs, including the Great Barrier Reef (mean 13.8%) (De'ath et al. 2012) and Caribbean reefs (<10%) (Gardner et al. 2003). Thus, our study provides a reference for microbial and nutrient parameters on reefs with high coral cover. We found that the densities of herbivorous fish were generally low, consistent with previous observations on Micronesian reefs (Mumby et al. 2013). The Micronesian reef fisheries are not sustainably managed (Houk et al. 2012, Rhodes et al. 2015), and fish densities could be related to the presence of human settlements on the islands as well as foreign fishing interests. Herbivorous fish abundances were highest on the most populated islands of Pohnpei and

Yap (Table 1), suggesting that local management practices that protect herbivorous fish are having a measurable impact. Corallivorous fish were lowest on the macroalgal-dominated Olimarao atoll, reflective of the fewer corals available for feeding. Overall, our results reflect the microbial and nutrient parameters of these Micronesian reefs with high coral coverage and low abundances of fish.

The concentrations of inorganic nutrients measured on the Micronesian reefs were within the ranges reported for other reef locations and suggest a rapid turnover of the nutrient pools by microorganisms and other reef biota. Concentrations of phosphate and inorganic nitrogen were slightly higher in Micronesian seawater compared to measurements collected from reef seawater across Cuba and the Florida Keys (Weber et al. 2020), yet similar to concentrations in coastal Hawaii (Apprill & Rappé 2011, Yeo et al. 2013) and the Line Islands (Sandin et al. 2008, Kelly et al. 2014), which potentially reflect reef or larger ocean basin-related differences. TOC concentrations in the present study averaged 72  $\mu\text{M}$ , and were lower than concentrations measured on Cuban and Florida Keys reefs (75–130  $\mu\text{M}$ ) (Weber et al. 2020) and slightly lower than Hawaiian reefs (73–79  $\mu\text{M}$ ) (Apprill & Rappé 2011). Concentrations of TON (mean 4  $\mu\text{M}$ ) were similar to the offshore Cuban reefs, and much lower than nearshore reefs (>10  $\mu\text{M}$ ) in the Florida Keys (Weber et al. 2020).

##### 4.2. Context of Micronesian reef-water microbiology

Abundances of photosynthetic and heterotrophic (unpigmented) microbial cells varied throughout the reefs, which could be related to the quality and quantity of inorganic nutrients and organic matter in the reef waters as well as subtle differences in physicochemical properties. *Prochlorococcus* and *Synechococcus* abundances in Micronesian reef waters were typical of forereef-type and coastal locations measured in Cuba and Florida reefs (Weber et al. 2020), suggesting that photosynthetic metabolisms are consistently important features on coral reefs. Throughout the Micronesian reefs, *Prochlorococcus* were significantly elevated at the reef depths compared to the surface waters, which was seen at one site in a previous study in St. John, US Virgin Islands (Weber & Apprill 2020). This trend may be related to the decreased light levels at depth that lower photoinhibition in *Prochlorococcus* cells and/or presence of reef-specific metabolites that are re-



leased as exudates from benthic organisms like corals and macroalgae. Concentrations of unpigmented cells (heterotrophic bacteria and archaea) in Micronesian waters were on average 438 600 cells ml<sup>-1</sup>, which is about 30% of abundances measured on Cuban and Florida reefs (average ~1 600 000 cells ml<sup>-1</sup>) (Weber et al. 2020), but are more in line with concentrations reported from the Line Islands as well as an embayment in Mo'orea (Dinsdale et al. 2008, Nelson et al. 2011). One possible explanation for these differences in unpigmented cell abundances is the high abundance of corals and filter feeders on the Micronesian/Line Island/Mo'orea reefs, which may be ingesting these cells, thus driving down their abundances. Indeed, differential bacterioplankton abundances on the reefs of Mo'orea were attributed to this feeding hypothesis (Nelson et al. 2011). Additionally, a mesocosm-based experiment showed evidence for removal of specific bacteria from the microbial community over time, possibly due to predation by corals or coral-associated epibionts (McNally et al. 2017). These heterotrophic cell differences between the reefs may also be related to the differential benthic composition of Pacific and Caribbean reefs, which may affect the labile dissolved organic carbon (DOC) pool and heterotrophic cell growth (e.g. Nelson et al. 2013). On the Micronesian reefs, ratios of heterotrophic cells were relatively consistent except for Kosrae reefs, where ratios were much higher due to lower abundances of *Prochlorococcus* and *Synechococcus*. Kosrae reefs were also highest in coral coverage and diversity, and this removal of picocyanobacterial cells could be attributed to ingestion (McNally et al. 2017) or availability of labile DOC.

The amplicon sequencing data showed that the Micronesian reef microbial communities harbored consistently high alpha diversity. Similar methods were used on Floridian and Cuban reefs by Weber et al. (2020), thus providing an opportunity to directly compare findings. Microbial richness in Micronesia (340–425 MED nodes) was most similar to that of the Cuban Jardines de la Reina offshore reefs (320 MED nodes), and higher than coastal and nearshore reefs in Cuba and Florida (mean 140–280 MED nodes). This finding suggests that proximity to land, and therefore relationships to run-off of nutrients or pollutants, or tidal influences could be a factor contributing to decreased microbial diversity. We also identified significant relationships between coral and macroalgal coverage and number of MEDs associated with reef depth waters. These same relationships were not found when using Shannon and Simpson's alpha diversity indices, indicating that this

trend is due to numbers of differential sequences, and does not account for community evenness. To our knowledge, this is the first study to report a statistical relationship between microbial species diversity and coral/macroalgal coverage on reefs, which is consistent with the finding that protected reefs harbor higher microbial diversity than less protected locations (Weber et al. 2020). Due to the similarities in the most abundant members of the microbial community between sites, this benthic community-based difference in microbial richness is most likely driven by community members belonging to rarer MED nodes (containing fewer sequences compared to the abundant community members). Indeed, we identified *Leisingera*, *Owenweeksia*, and *Vibrio* sequences enriched on the coral-abundant reefs as well as unclassified sequences belonging to the *Flavobacteriaceae*, OM27 clade, PB19 clade of *Desulfuromonadales*, and the NS4 marine group. Marine isolates of *Leisingera* can grow on betaine (Vandecastelaere et al. 2008), which is produced by many corals (Hill et al. 2010). *Owenweeksia* and the OM27 clade are enriched in the near-coral seawater environment (Weber et al. 2019), and *Vibrio* are the most frequently recovered sequences from corals (Huggett & Apprill 2019). Thus, corals and their metabolic products appear to be linked to the composition of the overlying reef-water microbial community.

Microbial communities throughout the Micronesian reefs were 65–85% similar in composition and dominated by oligotrophic-dwelling microbial taxa, including *Synechococcus*, *Prochlorococcus*, SAR11 clades (I, Ia, Ib, and II), SAR86, SAR116, AEGEAN-169, and '*Candidatus Actinomarina*', among others. These communities are similar in composition to other reports from the Caribbean (Weber et al. 2020) and the Line Island and Polynesian reefs in the Pacific (Nelson et al. 2011, Kelly et al. 2014, Haas et al. 2016). However, the reef-water microbial taxa found in all of these prior studies, as well as this study, are also similar in SSU rRNA gene sequence taxonomy to taxa sampled in the surface waters of the open ocean (e.g. Brown et al. 2009, Giovannoni & Vergin 2012). It is not yet clear how microbial taxa residing within coral reef habitats function in relation to their open ocean counterparts, and comparing genomes from these dominant cells recovered from open ocean and reef environments could help inform this question. Notably, surface water microbial community composition was related to ammonium and silicate concentrations, which may indicate their relationship or co-variation with these nutrients. Measurements from the only off-reef (open water)

site suggest that the non-reef waters harbor relatively fewer sequences of *Synechococcus* and the *Euryarchaeota* family *Thermoplasmatales*, compared to the reef sites. Elevated growth and representation of *Synechococcus* in coastal environments compared to open ocean is well studied (Partensky et al. 1999), including in reef environments (Nelson et al. 2011). The elevated abundance of *Thermoplasmatales* in reef environments could be related to reef organisms (Kellogg 2004).

#### 4.3. Reef depth microbial community structure exhibits biogeographic patterns

Planktonic marine microorganisms are thought to exhibit biogeographic patterns, which are driven by the local environmental conditions and historical events such as limitations in dispersal (reviewed by Martiny et al. 2006). Here we observed biogeographical partitioning in the microbial communities, suggested by the island/atoll-based differences in microbial community structure as well as correlation between reef depth seawater microbial community similarity and geographic distance. Biogeographical patterns in reef depth microorganisms were previously shown for the eastern Pacific, including the Hawaiian Archipelago, Johnston Atoll, and American Samoan reefs (Salerno et al. 2016). A surface-water-based biogeographical study of Indian Ocean reefs crossed over distinct marine biogeographic provinces, and suggested that some of the observed reef biogeography was driven by primary production and thermohaline properties of the water (Jeffries et al. 2015). Interestingly, in our study we did not detect a correlation between surface reef-water microbial community structure and geographic distances, suggesting that currents may erode biogeographic patterns in surface waters. In contrast, the significant correlation between reef depth seawater microbial community similarity and geographic distance between reefs indicates that geographic or island-based factors observed in this study are more influential on microbial growth at reef depth. This may be due to more localized pulses of nutrients and physical dynamics contained within the reef benthic boundary layer at each reef (Shashar et al. 1996, Stocking et al. 2016). Microbial dispersal-based limitations could also play a role in these observed biogeographic patterns (Martiny et al. 2006, 2011), but, due to the shallow nature of these reefs, it seems unlikely that dispersion alone would cause the observed differences in surface and reef depth biogeography.

#### 4.4. Unique microbial community features in Kosrae may be linked to the dense and diverse coral communities

There is a growing body of work examining the connections between benthic substrates on reefs and the overlying microbial ecology, and this study adds to that knowledge. Previous investigations have found linkages between the benthic flora and fauna on reefs and the pelagic microbial communities and their associated functional genes that overlie the reefs (Kelly et al. 2014, Haas et al. 2016, Weber et al. 2019, Glasl et al. 2020). Here we identified microbial and organic carbon and nitrogen features that are unique to the Kosrae reefs ( $n = 3$  sites), which harbored the most dense and diverse coral communities in our study. Indeed, concentrations of TOC at Kosrae reefs were the lowest measured in our study (59.8–67.9  $\mu\text{M}$ ). In contrast, the concentrations of TOC measured on the macroalgal-dominated reef of Olimarao (mean 45% macroalgae cover) were considerably higher (on average) than Kosrae, ranging from 63.9 to 77.9  $\mu\text{M}$ . Concentrations of organic nitrogen at Kosrae were among the highest in the study (4.5–5.3  $\mu\text{M}$ ), indicating possible production of dissolved organic nitrogen such as by diazotrophs. We also observed elevated ratios of heterotrophic compared to autotrophic cells on Kosrae, compared to other Micronesian islands and atolls. Interestingly, our findings for Kosrae and Olimarao are not consistent with descriptions of ‘microbialized’ reefs, which suggests that algal-dominant reefs have lower standing stocks of DOC and higher abundances of heterotrophic microbial metabolisms compared to reefs with higher coral coverage (Haas et al. 2016). In contrast, we found a higher ratio of heterotrophs compared to autotrophs, potentially responsible for the low organic carbon concentrations, on reefs with low algal cover. Although the concentrations of organic carbon (DOC vs. TOC) are not directly comparable between the studies, our observations of enhanced organic carbon with increased macroalgal cover in this study suggest that there may be variation in the microbial response to benthic community composition, and additional replication across coral and macroalgal-sites in Micronesia are needed to further verify this trend.

The reef-water microbial community at Kosrae was also highly diverse (425 MED nodes), and more consistent between reef sites (85% similar), compared to the other islands and atolls we measured in our study. These features may be related to the high coral abundance and diversity at these sites. Further, we identified a suite of microorganisms differentially

enriched on these reefs (compared to those of the macroalgal-rich reefs of Olimarao), which are not typical of surface oligotrophic waters, including *Marinobacterium*, *Owenweeksia*, *Cryomorphaceae*, the PB19 group of *Desulfuromonadales*, and SAR11 clade III. This comparison also showed that typical oligotrophic bacteria were also differentially enriched in both Kosrae and Olimarao reefs, and the drivers of these relationships are less clear. The 'coral ecosphere' was recently described as the near-coral reef-water environment that supports a unique microbial community compared to just 1 m off the reef, and growth of microorganisms in this coral ecosphere environment may be influenced by coral and reef-produced metabolites (Weber et al. 2019). Similarly, other studies show that exposure to coral and macroalgal exudates influence microbial community composition and growth (Nelson et al. 2013, Cárdenas et al. 2018). In fact, high growth rates of SAR11 cells ( $1.2 \text{ cells d}^{-1}$ ) were observed experimentally in the presence of corals (McNally et al. 2017). Further investigation of the relationship between microbial diversity and growth and coral exudate quality and quantity is needed to better understand the factors contributing to microbial patterns associated with coral-abundant reefs.

There is interest in developing and using reef-water microbial communities, as well as other reef-associated parameters, as predictive indicators of reef health and water quality (Glasl et al. 2017, 2018). Further exploration of the relationship between microbial richness and benthic cover on globally distributed reefs could help us understand the utility of this metric for predicting reef health. Additionally, microorganisms can be useful indicators of reefs which require further study. For example, microbial abundance and microbial community features were unique at the Poh 2 and Nuk 1 sites, yet no other environmental factors measured in this study explained their distinct microbial communities. Overall, additional survey studies of geographically distinct reefs are needed to develop a microbial diagnostic indicator concept for reefs.

#### 4.5. Considerations

This study is the first to report on the microbial ecology of Micronesian reefs and provides insights into how nutrient concentrations, benthic composition, and biogeographic features related to the microbial community. Additionally, we describe particularly unique microbial and organic matter-based

features on Kosrae Island reefs, which may be attributed to the high coral abundance and diversity at these reefs. We expect that the benthic coverage and fish abundances of these reefs likely changed between when this study took place (2012) and the time of publication, due to the coral bleaching events that occurred between 2013 and 2017. During this time period, staghorn *Acropora* and other corals may have died, thus impacting coral coverage and diversity (Raymundo et al. 2019). As such, there is now an opportunity to return to these reefs and examine how the reef microbial biogeochemistry and ecology has changed during this period of potential bleaching in the coral communities, thus providing new insights for developing microbial communities as indicators of reef health.

Our study reported on limited geographic coverage throughout the islands and sampled across different types of reefs (lagoon, forereef, and fringing), which can influence reef-water microbial ecology (Apprill & Rappé 2011, Nelson et al. 2011). Sampling of reef types was inconsistent between atolls and islands, and therefore did not allow for direct comparison between lagoon, forereef, and fringing reef microbial biogeochemistry. Future efforts could target sampling of these reef types per geographic region, to better constrain the factors contributing to variability between reefs. Additionally, our sampling scheme consisted of observations collected at a single point in time and do not cover seasonal trends (McCliment et al. 2012) or the diel or daily dynamics uncovered in recent reef studies (Kelly et al. 2014, Weber & Apprill 2020). Nonetheless, this study offers new insights into the Micronesian reefs at an important time when understanding reef health and functioning is of critical importance to predicting the fate of and designing strategies to protect these essential ecosystems.

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