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Near-benthic coral reef picoplankton vary at fine scales decoupled from benthic cover

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ABSTRACT: Seawater microorganisms impact ecological and biogeochemical cycling on coral reefs and are sensitive indicators of ecosystem status. Microbialization, a shift towards trophic collapse and resultant high microbial biomass, is a global concern on coral reefs. Indeed, macroorganisms can influence microbial processes and community composition on reefs, which is best understood as increased macroalgae resulting in copiotrophic microbial growth and oxygen reduction. Whether or not smaller-scale changes in macroorganisms influence the overlying seawater microbial communities is largely unknown. Here, we assessed seawater microorganisms across 3 coral reefs to understand their connection to reef site and within-reef benthic characteristics. At 3 coral reefs in St. John, US Virgin Islands, we collected 60 ml seawater samples 2 cm above the seafloor, spaced 2 m apart in a grid pattern, and assessed bacterial and archaeal communities via sequencing of small subunit ribosomal RNA genes. Benthic cover within 1 m of each sample was determined at 10 cm resolution through photogrammetry. Our results reveal that overall reef site overwhelmingly shapes microbial community structure, while within-reef benthic cover surrounding sample locations has minimal influence. However, ecospheres as areas that reflect the small-scale effects of benthic cover directly under each sample, significantly explain as much as 12.1% of within-reef microbial variation and may even outweigh variation attributable to reef site alone. These findings provide new insights into fine-scale spatial variability in reef seawater microbiomes that are crucial for the use of microorganisms as indicators of microbialization and coral reef health.

KEY WORDS: Coral reef communities · Seawater · Microbial · Picoplankton · Spatial variation · Benthic cover

1. INTRODUCTION

Coral reefs have experienced a tripling of disease prevalence over the past 25 yr (Burke et al. 2023) and widespread loss of function due to coastal development and a changing climate (Rosenberg et al. 2022). This has contributed to microbialization of coral reef ecosystems, an overabundance of copiotrophic microbes that are less efficient at degrading carbohydrates and potentially pathogenic to corals and reef organisms (Haas et al. 2016). Detecting microbialization is crucial for coral reef management, necessitating an improved understanding of the factors that structure microbial communities and the scales at which these associations are apparent.

Here, we quantify the role of biogeography at the scale of individual reef sites (site effects), benthic cover within a reef (e.g. algal dominance), and direct benthic interactions (e.g. organismal effects) as key factors underpinning picoplankton community composition. The link between benthic cover and seawater microbial composition, coastal benthic–pelagic coupling, constitutes a fundamental aspect of coral reef ecosystems, as resources from dominant benthic organismal classes shape overlying microbial communities (Griffiths et al. 2017). Dissolved organic carbon

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(DOC), in particular, is often depleted above coral reefs and is a reflection of the differentiation of reef-associated planktonic microorganisms from their open ocean counterparts (Nelson et al. 2011). Globally, coral reef ecosystems have undergone a widespread shift from coral dominance to steady states of algal dominance (Fung et al. 2011); coinciding with this change in benthic cover is a cascading trophic decline of bacterioplankton in the form of microbialization (Haas et al. 2016). Shifts in the competitive landscape of coral reefs are also reflected in the microbiomes of corals themselves, which shift to resemble those of highly competitive macroalgae at interaction zones (Morrow et al. 2013). Changes to the benthic community can directly impact picoplankton communities, as the former provides exudates (Ferrier-Pagès et al. 2000) including DOC and inorganic nutrients that directly promote the growth of seawater microorganisms (Haas et al. 2013). For example, the exudates produced by algae are distinct from those produced by coral and alter the genetic diversity of reef-associated picoplankton (Nelson et al. 2013). Exometabolites produced by benthic organisms vary greatly, reflecting the diversity of coral reefs themselves (Weber et al. 2022). Importantly, picoplankton respond to these conditions and benthic cover differently, and on different timescales, than other reef microbial communities such as biofilms (Remple et al. 2021).

Distinct reef organisms significantly influence picoplankton structure as a component of, or in addition to, the cumulative effects observed in response to benthic cover. Heterotrophy is a primary mechanism for these effects, with sponges acting as a major consumer of autotrophic and heterotrophic picoplankton above reefs (Lesser 2006, Lesser & Slattery 2020), converting biomass originating from coral-derived DOC to detritus as part of the sponge loop (Rix et al. 2018). Corals also contribute to heterotrophy in oligotrophic waters (Patten et al. 2011), and the effect of this process on picoplankton composition is directly mediated by both local temperatures (Meunier et al. 2019) and hydrodynamics (Ribes & Atkinson 2007). The effects of coral heterotrophy on picoplankton can also be selective, with studies of *Porites astreoides* demonstrating preferential removal of *Synechococcus,* SAR11, and *Rhodobacteraceae* cells (McNally et al. 2017).

Reef organisms can also alter picoplankton structure through enrichment, resulting in so-called aura-biomes (Walsh et al. 2017) or ecospheres (Weber et al. 2019), where altered microbial composition is evident in seawater within centimeters of an organism's surface. First discovered in 2017 with large-volume water samples (20 l) collected above different reef organisms (Walsh et al. 2017), these effects were also detected in a later study using smaller seawater volumes (60 ml) and are hypothesized to be facilitated by the formation of boundary layers near the coral colony surface (Weber et al. 2019). In contrast to heterotrophy, where cells are preferentially removed from the water column, microbial ecospheres can occur through 2 mechanisms: shedding of cells from the coral microbiome or selective growth on coral exudates. Regular shedding of coral surface mucus layers may elevate the abundance of coral symbionts, such as *Endozoicomonas*, in near-coral waters (Weber et al. 2019) and may play a major role in the localized spread of coral disease on reefs (Williams et al. 2021). In combination with shedding, exudates released by benthic organisms act to encourage the growth of picoplankton and alter microbial community structure on the reef. Sponges have a demonstrated affinity for enriching certain *Archaea* near their surface (Polónia et al. 2016), macroalgal exudates enrich *Vibrionaceae* and *Pseudoalteromonadaceae* (Nelson et al. 2013), crustose coralline algae facilitate bacterioplankton known to produce antimicrobial compounds (Quinlan et al. 2019), and thermally stressed corals enrich pathogenic bacterioplankton through an increased flux of dissolved organic matter (Sparagon et al. 2024). Collectively, the mechanisms of heterotrophy, shedding, and enrichment via exudates allow benthic reef organisms to shape picoplankton communities in their immediate vicinity and exert a significant effect on both reef microbial ecology and biochemistry (Patten et al. 2011). Recent trans-Pacific surveys have identified high variability in coral microbiomes within individual reefs, as well as similarity between surface reef water and water above coral colonies for both free-living (0.2–3 µm) and particle- or eukaryote-associated (3–20 µm) plankton (Galand et al. 2023).

Local conditions unique to each reef site (site effects) as well as regional patterns (biogeography) combine with within-reef benthic and organismal effects to drive picoplankton structure. Regional differences are regularly observed in reef picoplankton samples as a function of geographic distance, in addition to baseline differences between reef sites (Ma et al. 2022). Abiotic factors that contribute to patterns between reef sites can include differences in ocean circulation (Nelson et al. 2011), water velocity (Ribes & Atkinson 2007), sediment resuspension, light availability (e.g. resulting from turbidity), pH, and temperature. Light consistently drives diel variations in autotrophic picoplankton (Kelly et al. 2019, Becker et al. 2020). Seasonal variability of microbial commu-

nities on coral reefs is especially prevalent (Nuryadi et al. 2018). Temperature stress can interact with light and wave action to influence total alkalinity and within-reef net calcification rates (Zhang et al. 2012), processes that influence reef microbial communities (Meron et al. 2012). Experimentation outside of coral reefs has demonstrated synergistic effects of nutrient pollution, sediment resuspension, and water column mixing, increasing algal biomass and autotrophic picoplankton (Rhew et al. 1999). Biotic factors beyond the benthic community, such as herbivore abundance, may also interact with these abiotic factors. For example, in studies simulating overfishing on coral reefs, herbivore exclusion and nutrient pollution combine to compromise the coral microbiome (Zaneveld et al. 2016). Further, by affecting coral health, these disturbances are likely linked to picoplankton structure through differential exometabolite production (Weber et al. 2022), as well as through the impact of an altered coral microbiome on the surrounding water column via organismal effects (Weber et al. 2019).

The sensitivity of reef microbial communities to ambient conditions suggests that they may be useful indicators of reef health conditions. However, 1 unexplored aspect of these communities is if microorganisms can reflect reef condition at fine spatial scales. While satellite sea surface temperature data at the kilometer scale are often used to study reef health (Fordyce et al. 2019, Sutherland et al. 2023), these data do not resolve within-reef patterns. Great progress has been made to characterize coral reefs at fine spatial scales with the advent of distributed temperature sensors detecting submeter thermal variation in rugose reef habitats (Reid et al. 2019, Sinnett et al. 2020) and the widespread use of orthomosaics to assess fine-scale benthic cover (Roach et al. 2021). However, spatial variation of microbial communities within reef environments remains considerably less resolved. Previous work in the Pacific reported significant within-reef and between-reef effects of algal cover on the microbiome of corals but did not investigate these effects on reef picoplankton (Briggs et al. 2021). As pressure mounts to incorporate reef seawater microbial data into predictive models of reef health (Terzin et al. 2024), a critical first step is the identification of picoplankton variability at small spatial scales and their relationship to both benthic cover and distinct reef organisms that might serve as visual indicators of reef condition. In the Caribbean, the highest spatial resolution studies examined near-reef picoplankton communities every 1 m along 10 m transects sampled 5 cm distance from the benthos and found microbial communities to be highly similar within distinct reefs and not related to general benthic cover (Ma et al. 2022). To our knowledge, no studies in the Caribbean have systematically addressed the relationship of spatial and underlying organismal influences on overlying reef picoplankton.

Our study aims to quantitatively compare the influence of reef site (site effects), within-reef benthic cover, and sample-specific organismal influences on picoplankton communities at fine spatial resolution. Using water samples collected 2 cm above the benthos, we assessed picoplankton community differences across 3 St. John, US Virgin Islands, reef sites, examining picoplankton variability attributable to the reef site itself, benthic cover within each reef, or the benthic cover directly under a sample collection point. Benthic cover was manually classified every 10 cm in a 1 m^2 region surrounding each water sample, and its role in shaping microbial communities was compared to that of the organism or benthic cover located 2 cm directly beneath the sample syringe. Existing microbialization literature links algal dominance on reefs to an overabundance of copiotrophs in the picoplankton, while additional work has identified corals and other discrete reef organisms that directly influence picoplankton structure heterotrophy, exudates, and mucus shedding (McNally et al. 2017). Based on these factors, we hypothesized that locally dominant benthic cover would strongly predict reef picoplankton community structure in near-benthos samples. Here, we provide the first quantitative comparison of site effects (reef site), within-reef benthic cover, and sample-specific organismal effects in reef seawater microbiomes, as well as identify residual variability not explained by these factors.

2. MATERIALS AND METHODS

2.1. Sample collection

In late October and early November 2022, we selected 3 reef sites, Tektite, Booby Rock, and Salt Pond (note: Salt Pond is a coral reef and not a salt pond or hypersaline site), spanning 1.5 km along the south coast of St. John, US Virgin Islands, for benthic cover and microbial characterization. All reef sites used are oligotrophic coral reefs, and while nutrient concentrations were not taken in this study, previous reports of Tektite and Booby Rock document low nutrient concentrations (<0.2 µM nitrate + nitrite, <90 µM total organic carbon) (Becker et al. 2024). Within each reef site, we identified a 10 × 10 m area with maximum

benthic diversity. Starting from the bottom left corner, we laid out a 10 m transect across the benthos. At 2 m intervals along this transect, we collected 60 ml water samples using capped sterile syringes, drawing seawater from 2 cm above the bottom at a rate of approximately 12 ml s^{-1} and recapping syringes after collection. We recorded the organism and type of benthic cover (e.g. turf algae, rock, sand, macroalgae, coral) 2 cm directly under each sampling point during collection. Care was taken that divers did not swim over areas not yet sampled. After collecting samples within a transect, we shifted the transect tape 2 m to the right, creating a rectangular grid of samples in a transect-by-transect manner. To mark the location of each sample, we used 50 cm of flagging tape attached to an 85 g fishing weight, using blue tape for the first sample on the first transect, yellow tape for intermediate samples, and red tape for the last sample on the last transect. Scale markers for photogrammetry of the sample area were distributed as five 20 × 20 cm aluminum georectification tags and approximately ten 20 cm long PVC pipes throughout the grid area. Imagery for an orthomosaic of the sample area was captured using 2 GoPro Hero7 cameras spaced 60 cm apart on a PVC pipe and set to capture 1 image per second while being swum approximately 2 m off the seafloor by a diver in a lawnmower-style pattern. In total, we collected 30 seawater samples covering an 80 m^2 area (max. depth 10 m) at the Tektite reef on 28 October 2022 at approximately 15:45 h, 36 from a 100 m^2 area at the Booby Rock reef (max. depth 13 m) on 31 October 2022 at approximately 14:00 h, and 30 from an 80 m^2 area at the Salt Pond reef (max. depth 6 m) on 2 November 2022 at approximately 15:00 h. Due to the intense sample scheme used, as well as logistical constraints, it was not possible to sample multiple reef sites on the same day. All samples proceeded to extraction, but not all samples successfully amplified under the conditions described below in Section 2.2. Samples that did not amplify, or that fell below quality thresholds in post-processing, were discarded from final analysis (Data S1; all data, S1–S8, are available in an Open Science Framework repository at https://osf.io/w495h/, see also 'Data archive').

2.2. Sample preservation, sequencing, and data processing

After collection, all capped syringes were immediately placed on ice and transported to the Virgin Islands Ecological Research Station (VIERS). At VIERS, the water from each syringe was passed through a sterile 24 mm diameter 0.2 µm Supor filter (Pall) loaded into an acid-washed Swinnex (Millepore) filter holder. Syringes were not reused between reef sites, and fresh 0.2 µm filters were used for each sample. Filters were placed into labeled cryovials and preserved in liquid nitrogen vapors in a dry shipper for transportation to Woods Hole Oceanographic Institution for extraction and amplification. DNA was extracted from the filters using the QIAGEN DNEasy PowerBiofilm Kit and manufacturer's protocols (Ma et al. 2022), wherein the filter was added directly to the bead-beating tube for each sample. Partial small subunit ribosomal RNA genes were amplified from bacterial and archaeal communities in the DNA using 515FY (Parada et al. 2016) and 806RB (Apprill et al. 2015) dual indexed primers with 20 µl reactions consisting of 5 µl of GoTaq Flexi 5× buffer, 2.5 µl of 25 mM magnesium chloride, 1 µl of 10 mM deoxynucleoside triphosphate, 1 µl each of forward and reverse primers, and 1 µl of DNA template, with sterile water as the remaining volume. Amplification of each reaction consisted of 2 min at 95°C initialization followed by 32 cycles of 20 s at 95°C denaturation, 15 s at 55°C annealing, and 5 min at 72°C elongation followed by a final 72°C elongation for 5 min. All PCR reactions were performed without grouping or pooling by reef site. The amplified PCR product was visualized using gel electrophoresis, purified using the QIAGEN MinElute Kit, quantified using PicoGreen (Invitrogen), and diluted to a final concentration of 1.04 ng $µl^{-1}$ prior to pooling of samples into a single library. Samples were sequenced at the University of Illinois with Illumina MiSeq 250 base pair paired-end sequencing, and data were processed using DADA2 in the statistical analysis software R (Data S1). The Silva nr99 v138.1 (Quast et al. 2013) pretrained reference database was used for taxonomic assignment. Amplicon sequence variants (ASVs) (Callahan et al. 2017), labeled as chloroplast or mitochondria or assigned with kingdom unknown, were removed. A total of 5 contaminant ASVs were identified using extraction and PCR negative controls and the 'decontam' R package version 1.18.0 (Davis et al. 2018) and subsequently removed; these ASVs matched groups (e.g. *Burkholderiaceae*) previously identified as common laboratory contaminants recovered during next-generation sequencing (Laurence et al. 2014). Representative sequences of putative contaminant ASVs are available in Data S5. Raw sequence data are accessible in the NCBI Sequence Read Archive under submission PRJNA1036262. Taxonomies of ASVs (see Figs. 3 & 4) were further verified by importing sequences aligned using SINA v1.2.12 and comparing alignments

to the SILVA database (v138) (Pruesse et al. 2007, Quast et al. 2013), with alignments further verified using the ARB software package (Ludwig et al. 2004).

2.3. Orthomosaic processing

Imagery of each reef site was separately processed in Agisoft Metashape Pro to produce a high-resolution 3-dimensional (3D) model of the entire sampled area. For each model, 20 cm PVC pipes and 20 cm square aluminum scale markers placed throughout the reef were used to establish scale and calibrate the model output. 3D models of each reef site (Tektite, Booby Rock, Salt Pond) were then used to generate an output 2-dimensional photo planar orthomosaic of the entire gridded sample area (Data S2–S4). These scaled orthomosaics were imported into Adobe Photoshop, and a 1 m^2 area centered on each sample marker was exported as individual images for sample-specific benthic cover analysis in CoralNet (Chen et al. 2021). Within CoralNet, a regular grid of 100 points was overlaid onto each image, equating to benthic annotation every 10 cm within the 1 m^2 area surrounding each microbial sampling point. Benthic annotation points that fell over a scale marker or area unable to be reconstructed by Agisoft Metashape Pro were labeled as UNKNOWN. Otherwise, each point falling over benthic habitat was classified as either live hard coral (HARD), live soft coral (SOFT), live sponge (SPNG), bare sand (SAND), dead hard coral (DEAD), bare rubble (RUBL), peyssonnelid algal crust (PEYS), or a combined class of turf or macroalgae (ALGAL_COVER). All benthic annotations were manually confirmed across all images. Proportional benthic cover for each image associated with a seawater sample was exported from CoralNet and paired with microbial sequence data recovered from that sample.

2.4. Statistical analysis

Statistical analysis was carried out to address the factors structuring microbial community composition in a stepwise fashion, proceeding from the largest spatial explanatory measures (reef site) to the smallest (organism or benthic cover 2 cm directly under sample syringe). After subsampling to an even sequencing depth using the 'Phyloseq' function rarefy_even_depth(), sequence count data were transformed to relative abundance, where appropriate. Distance-based tests of the microbial community or benthic cover used Bray-Curtis dissimilarity, whereas physical distance between sampling points within each reef site was calculated as Euclidian distance. For all analyses, plots were generated using the R package 'ggplot2' version 3.4.2 (Wickham 2016), combined with functionalities of the 'phyloseq' version 1.42.0 (McMurdie & Holmes 2013), 'microViz' version 0.10.10 (Barnett et al. 2021), and 'MicrobiotaProcess' version 1.10.3 (Xu et al. 2023) packages.

Analysis proceeded with an initial test for differences in beta dispersion and permutational multivariate ANOVA (PERMANOVA) for overall microbial dissimilarity across reef sites, the goal of these tests being to establish if site effects were present in the microbial data and necessary in later statistical tests. The function adonis2() was used from the R package 'vegan' for PERMANOVAs, as was the function betadisper() for tests of significant beta dispersion. The same tests were used to determine if reef sites significantly differed in their overall benthic composition using data from orthomosaic analysis. The R package 'corncob' (Martin et al. 2020) was used to determine what microbial ASVs, if any, significantly differed in abundance between the 3 reef sites using the function differentialTest().

Within each reef site, Mantel tests with Spearman rank tests were used to analyse correlation between within-reef dissimilarity in benthic cover and dissimilarity in the overlying seawater microbiome. Mantel tests, using the function mantel() from the 'vegan' package, were also used to assess if physical (Euclidian) distance between samples within each reef site significantly correlated to microbial dissimilarity among these samples. The 'corncob' function differentialTest(), with a control for reef site (site effects), was used to determine ASVs that significantly differed in abundance with changes in local benthic cover within 1 m^2 surrounding each sample (orthomosaic data). The function cor_heatmap() from the R package 'microViz' (Barnett et al. 2021) was used to plot Pearson correlations of ASVs significantly associated with the dominant benthic cover class (e.g. highest percent cover) within the 1 m^2 annotated area around each sample. Dominant benthic cover and cover directly under samples were both represented in R as categorical variables.

Further analysis investigated the influence of organismal ecospheres on microbial composition. 'Corncob' was used to test if differing benthic cover or organisms 2 cm under water samples produced differential abundance among microbial ASVs. The response of log-transformed microbial richness and Shannon diversity to organisms or cover under the syringe (log(richness) ~ SampleCover) were assessed using a linear mixed effect model (lmer) with a ran-

dom intercept for reef site (1|Site). Random intercepts in linear models control for differences between reef sites by allowing the intercept (baseline level) of the outcome variable (e.g. picoplankton richness) to vary randomly across different reef sites and were employed as follows:

$$\text{lmer}[\log(\text{richness}) \sim \text{SampleCover} + (1|\text{Site})] \quad (1)$$

Fixed effects were assessed for significance using a type 2 ANOVA performed using the R package 'car' version 3.1-2 (Fox & Weisberg 2019). Strength of association between benthic cover directly underlying samples and the dominant class observed in benthic cover data from orthomosaics was assessed by calculating Cramer's V (Acock & Stavig 1979).

To compare the effects of reef site, within-reef benthic cover, and sample cover, a PERMANOVA was performed comparing the significance of these terms to overall microbial dissimilarity among samples. For this test, local benthic cover was summarized as the dominant benthic class within the 1 m^2 area surrounding each sample. Strength of association between benthic cover directly underlying samples and the dominant class observed in benthic cover data from orthomosaics was assessed by calculating Cramer's V. PERMANOVAs were structured to assess the significance of terms sequentially, in order of spatial scale, as follows:

$$\text{MicrobialDissimilarity} \sim \text{Site} + \text{DominantCover} + \text{SampleCover} \quad (2)$$

as well as marginally by adjusting the by term from by = term to by = margin.

Sequential tests allowed for variation in microbiome communities to be attributed in order of spatial scale, beginning with the reef site a sample originated from (Site), followed by the dominant benthic cover within 1 m^2 surrounding the sample (DominantCover), and ending with the organism or cover 2 cm below the syringe during sampling (SampleCover). An additional marginal PERMANOVA test was run that included effects for reef site and the organism or benthic cover under each sample, as well as percent cover for all benthic classes in a 1 m^2 area centered on the sample collection point; this PERMANOVA took the form listed in Eq. (3), below. Sequential PERMANOVA tests were arranged to account for site effects prior to testing for effects of within-site characteristics such as benthic percent cover or organism directly under the sample location. Preliminary tests using a restricted marginal PERMANOVA design implementing the parameter strata = site indicated no difference in significance among terms compared to marginal tests run without this parameter.

$$\text{MicrobialDissimilarity} \sim \text{Site} + \text{HARD} + \text{SPNG} + \text{PEYS} + \text{ALGAE} + \text{SAND} + \text{SOFT} + \text{SampleCover} \quad (3)$$

3. RESULTS

3.1. Sequencing results

A total of 66 seawater samples persisted through amplification, sequencing, and quality controls: 14 at Booby Rock, 26 at Tektite, and 26 at Salt Pond. Read depth across these samples averaged (±SD) 95 913 ± 16 517. At full read depth, 24 533 ASVs were detected after removal of 5 suspected contaminant ASVs. Where appropriate, sequence data were rarefied to an even read depth of 65 996, with no loss of samples and 21 899 ASVs present after subsampling.

3.2. Influence of benthic cover on picoplankton composition

A total of 9600 benthic classifications were manually generated across all images and used to assess benthic cover at each reef site. A smaller subset of 6600 benthic classifications corresponding to samples that passed sequencing quality controls was used in analyses linking picoplankton and benthic composition. Orthomosaic data (Fig. 1A,B) indicated that algal cover (turf algae mixed with macroalgae) was the dominant benthic class at all 3 reef sites (Fig. 2A), with an average percent cover of 47.8 ± 13.8% at Tektite, 37.2 ± 14.3% at Booby Rock, and 73.8 ± 13.8% at the most degraded reef, Salt Pond. The second most dominant benthic classes were 28.0 ± 21.1% uncolonized sand at Tektite, 17.9 ± 11.2% peyssonnelid algal crust (PEYS) at Booby Rock, and 8.8 ± 8.7% uncolonized sand at Salt Pond. By comparison, living hard coral cover averaged 5.9 ± 7.9% at Tektite, 6.0 ± 4.5% at Booby Rock, and 1.0 ± 1.4% at Salt Pond. PERMANOVAs indicated that benthic communities differed significantly between reef sites, significantly explaining 39% of variation in benthic composition (df = 2, R^2 = 0.39, pseudo-F = 20.31, Pr(>F) = 0.001). Dispersion of benthic communities also differed significantly among reef sites (df = 2, pseudo-F = 3.92, Pr(>F) = 0.026).

The influence of dominant benthic cover within 1 m^2 centered on each sample, on picoplankton Bray-

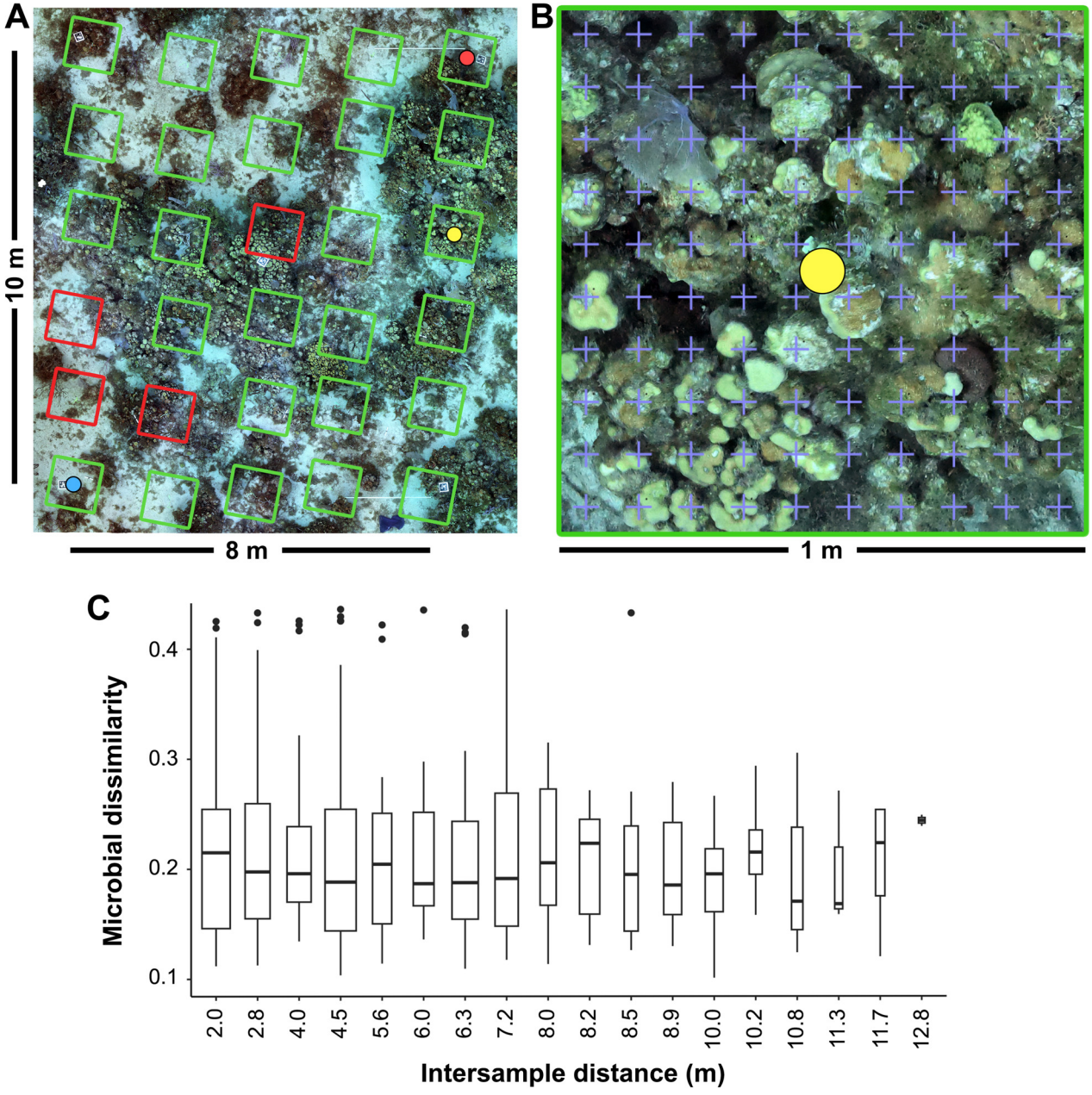

Fig. 1. (A) Overview of data collected at the Tektite reef site, where picoplankton samples and orthomosaic data were collected across an 80 m^2 area. 1 m^2 boxes centered on sample locations indicate areas annotated for benthic cover. Green boxes indicate samples successfully sequenced and red boxes indicate samples that did not pass quality controls and were removed from microbial analysis. At each reef, all annotated areas (red and green boxes) were used to establish reef-level benthic cover. (B) Example view of 100 benthic annotation points (purple crosses) within a box, centered on sample location (e.g. yellow dot). (C) Microbial community dissimilarity versus intersample physical distance at Tektite. Distance between samples averaged 5.9 m across 26 total samples at this site. Boxplots indicate first and third quartiles of microbial dissimilarity, horizonatal lines indicate medians, whiskers indicate minimums and maximums and outliers are indicated by dots

Curtis dissimilarity, was assessed using a PERMANOVA that tested for the significance of these effects, conditioned on the reef from which samples originated. This test indicated that the differences in dominant benthic class between samples did not significantly explain picoplankton dissimilarity. Benthic class within a 1 m^2 area centered on the sample collection point did not explain picoplankton community structure after taking site effects into consideration (df = 3, R^2 = 0.03, pseudo-F = 0.71, Pr(>F) = 0.84). Linear mixed effect models with a random intercept for reef site determined no significant effect of dom-

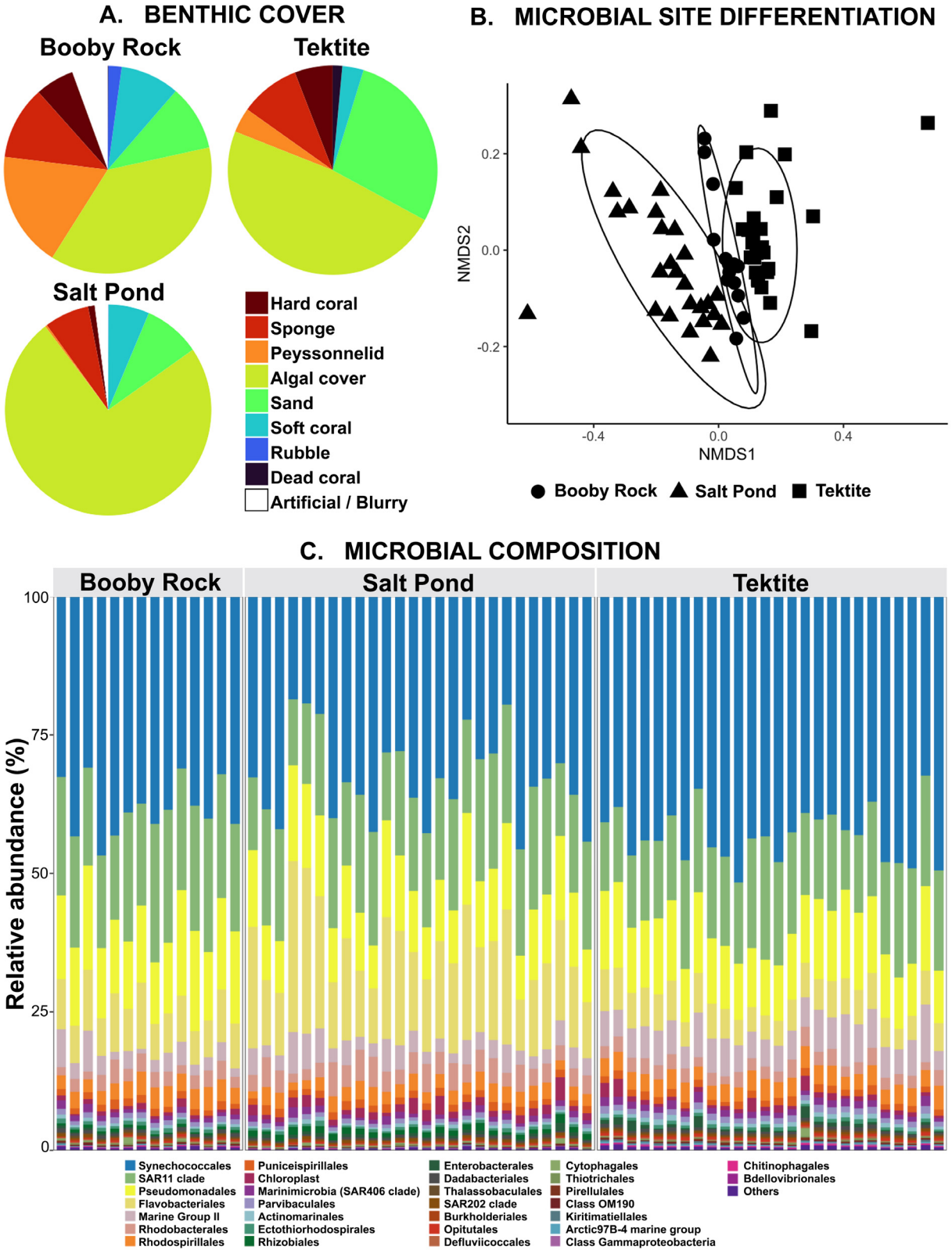

Fig. 2. (A) Summarized orthomosaic benthic cover data for all 3 reef sites. (B) Principal coordinates analysis of picoplankton Bray-Curtis dissimilarity across reef sites. (C) Relative abundances of the 30 most abundant bacterial and archaeal orders in seawater samples, with less abundant orders grouped into 'Others'

inant benthic class on either picoplankton log richness or diversity (Shannon). Whereas richness, diversity, and dominant benthic class represent summary statistics of multidimensional community data, Mantel tests comparing the full scope of picoplankton Bray-Curtis dissimilarity and benthic Bray-Curtis dissimilarity also determined that no significant relationship existed between these data at the Tektite ($r = -0.09$, $p = 0.83$), Booby Rock ($r = -0.19$, $p = 0.86$), or Salt Pond ($r = -0.05$, $p = 0.62$) reef sites. Similarly, site-specific Mantel tests determined that picoplankton sample composition, as well as benthic cover surrounding each sample (Data S1), had no significant association with physical (Euclidian) distance between samples that averaged 5.9, 5.5, and 5.6 m at Tektite ($r = -0.02$, $p = 0.58$), Booby Rock ($r = 0.23$, $p = 0.06$), and Salt Pond ($r = 0.05$, $p = 0.25$), respectively (Fig. 1C).

3.3. Reefs differ in baseline picoplankton community structure

Unconstrained principal coordinates analysis using Bray-Curtis dissimilarity showed that picoplankton communities differed by reef site (Fig. 2B). PERMANOVAs confirmed this result, with reef site explaining 27.9% of picoplankton dissimilarity in a single-term test (Table 1, Test 1). A secondary PERMANOVA randomly selecting an equal number of seawater samples from each reef determined that these site differences could be reliably detected with as few as three 60 ml samples per reef site. Though each reef was microbially distinct, beta dispersion did not significantly differ between Booby Rock, Tektite, and Salt Pond (df = 2, pseudo-$F = 2.67$, Pr(>F) = 0.076).

To control for baseline differences in reef picoplankton, we employed random intercepts for reef site in subsequent linear models and included reef site as a factor in all PERMANOVA tests. 'Corncob' analysis of differential picoplankton abundance identified 239 ASVs that significantly differed across the 3 reef sites and were investigated after agglomerating to order (Data S6). Among these site-differentiating groups, Tektite exhibited 48.6% higher average abundance of Marine Group II *Archaea* than Salt Pond and 61.5% higher average abundance than Booby Rock. *Flavobacteriales* were 67.9% higher in average abundance at Salt Pond compared to Booby Rock and 98.7% compared to Tektite. Average SAR11 abundance at Booby Rock was 15.2% higher than at Salt Pond and 24.9% higher than at Tektite. *Synechococcales* was most abundant at Tektite, occurring at 10.9% higher average abundance compared to Booby Rock and 27.8% compared to Salt Pond (Fig. 2C).

3.4. Specific picoplankton ASVs associate with dominant benthic classes

Analysis for differential abundance determined that 6 picoplankton ASVs significantly associated with differences in dominant benthic class within a 1 m^2 area of each seawater sample (Fig. 3, Data S7). A highly prevalent ASV313 classified as *Margulisbacte-*

Table 1. Results of PERMANOVAs performed on picoplankton communities (transformed as Bray-Curtis dissimilarity), with test number corresponding to separate analyses. *$p < 0.05$

Test no.	Picoplankton variability ~ (in response to)	Test type	df	R^2	Pseudo-F	Pr(>F)
1	Site	Single term	2	0.279	12.232	0.001*
2	Site +	Marginal	2	0.171	7.833	0.001*
	Dominant benthic cover (1 m^2 sample area) +	Marginal	3	0.029	0.882	0.578
	Cover 2 cm under sample	Marginal	7	0.114	1.489	0.021*
3	Site +	Sequential	2	0.279	12.781	0.001*
	Dominant benthic cover (1 m^2 sample area) +	Sequential	3	0.026	0.801	0.717
	Cover 2 cm under sample	Sequential	7	0.114	1.489	0.024*
4	Site +	Marginal	2	0.092	4.192	0.001*
	Hard coral (% cover in 1 m^2 sample area) +	Marginal	1	0.009	0.796	0.596
	Sponge (% cover in 1 m^2 sample area) +	Marginal	1	0.013	1.196	0.268
	Peyssonnelid (% cover in 1 m^2 sample area) +	Marginal	1	0.010	0.940	0.470
	Algae (% cover in 1 m^2 sample area) +	Marginal	1	0.007	0.627	0.766
	Sand (% cover in 1 m^2 sample area) +	Marginal	1	0.010	0.929	0.438
	Soft coral (% cover in 1 m^2 sample area) +	Marginal	1	0.011	1.048	0.379
	Cover 2 cm under sample	Marginal	7	0.121	1.576	0.013*

ria (candidate division ZB3) occurred at an average abundance of 0.025%, was positively associated with areas where sponge and algae were the dominant benthic cover, and was negatively associated with areas dominated by hard coral or PEYS. ASV1078, classified as a *Thalassobaculum* sp., was most abundant in sponge-dominated areas and least abundant over sandy benthos, averaging 0.002% abundance across all samples. In contrast, ASV930, classified as *Vibrio* sp., was most abundant in seawater from areas of high algal dominance but also generally occurred in low abundance, with an average of 0.003% across all samples. No ASV found to associate with specific dominant benthic classes exceeded an average abundance of 0.025%.

3.5. Distinct reef organisms influence picoplankton community composition

Picoplankton diversity, richness, and specific ASVs varied significantly depending on the type of organism or benthic cover located immediately (2 cm) below the sample syringe. Sequential PERMANOVAs determined that cover immediately below the sample significantly explained 11.4% of variation in picoplankton after accounting for the effects of reef site and benthic cover surrounding each sample (Table 1). Linear mixed effect models with a random intercept for reef site determined that only certain benthic cover classes underlying pelagic microbes produced significant effects on picoplankton diversity, including Shannon diversity and richness. Picoplankton diversity and richness were both significantly elevated in the seawater collected over macroalgae, seagrass, sponges, and substrates colonized by turf algae (Fig. 4A). Analysis for differential abundance indicated 9 total ASVs indicative of the organism or benthic cover directly under a seawater sample (Fig. 4B, Data S8). These ASVs averaged 0.18% relative abundance with an average prevalence of 89.39%, and they included members of the *Proteobacteria*, *Cyanobacteria*, *Marinimicrobia* (SAR406 clade), *Bacteroidota*, and *Actinobacteriota*. For example, water samples collected 2 cm over sand were depleted in ASV122, classified as the SAR116 clade, but enriched in ASV228, classified as an Sva0996 marine group *Actinobacteria*. ASV63, a *Synechococcus* sp., was reliably depleted in samples collected over sand but enriched over soft coral and turf algae. The mean relative abundance of these ASVs across all samples was 0.18%,

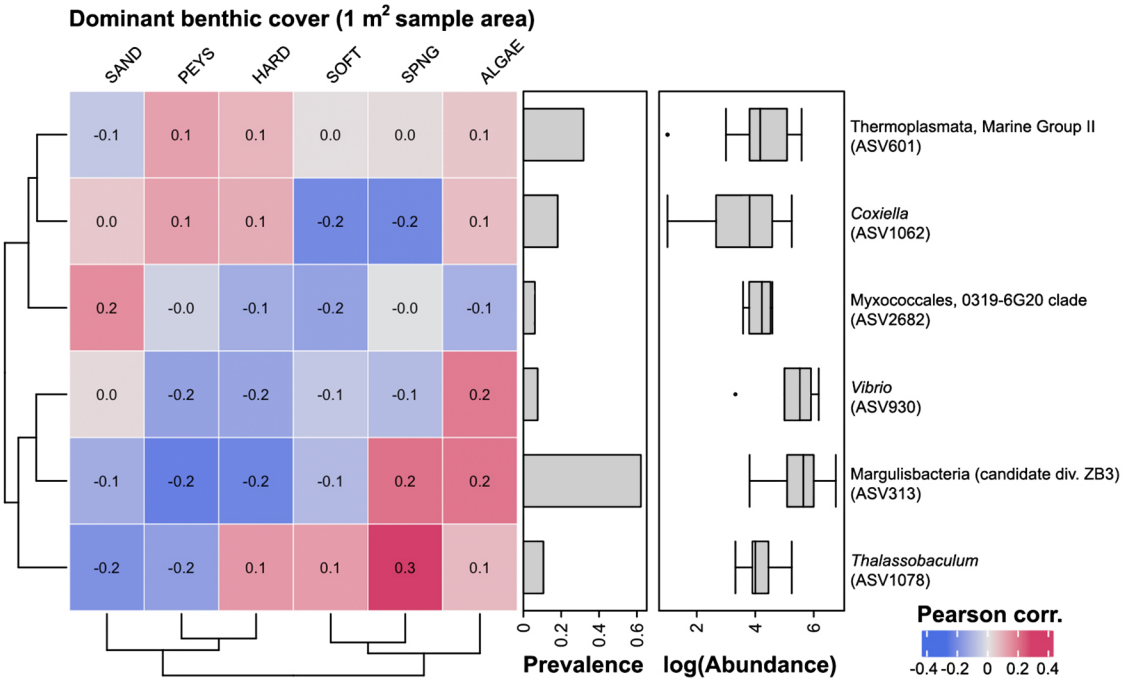

Fig. 3. Heatmap showing picoplankton amplicon sequence variants (ASVs) differing significantly by dominant local benthic cover within 1 m^2 of a sample, controlling for reef site and colored by Pearson correlation coefficient to each benthic cover class. See Section 2.3 for definitions of benthic habitats. Prevalence of these groups across all samples and reefs is also indicated, followed by log-transformed ASV abundance

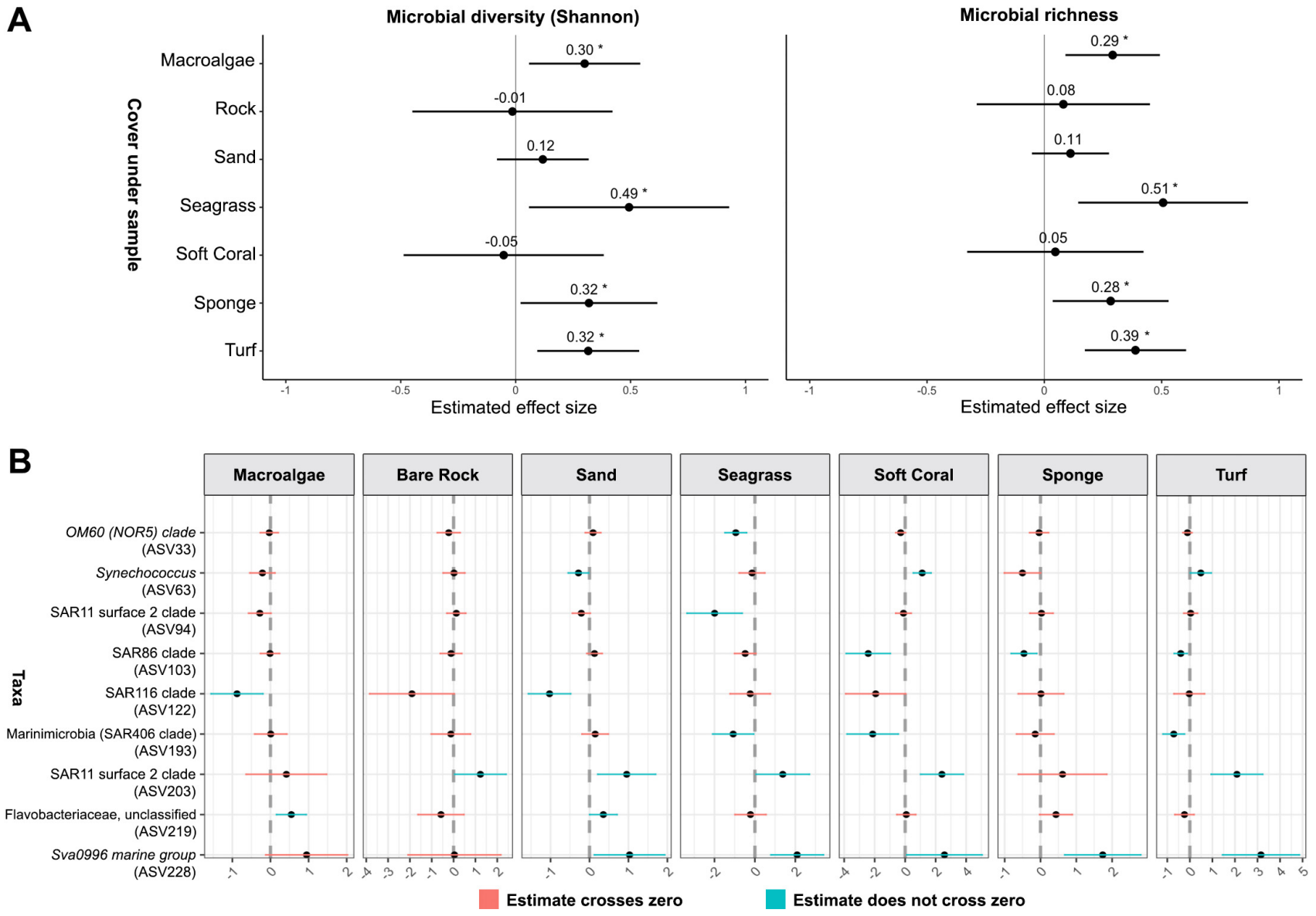

Fig. 4. (A) Effect sizes of benthic cover 2 cm below water sample locations and their influence on picoplankton diversity and richness. Effect sizes are generated from a linear mixed effect model with a random intercept for reef site, with significance indicated by an asterisk. An effect size greater than zero indicates an expected positive effect on amplicon sequence variant (ASV) abundance. (B) Nine ASVs identified as differentially abundant with benthic organism 2 cm below a sample

suggesting organismal effects may be most evident in low-abundance ASVs.

3.6. A majority of picoplankton variability remains unexplained by site, benthic, or organismal effects

PERMANOVAs allowed comparison of the marginal effects of reef site, the dominant benthic class (e.g. percent cover of each class) underlying pelagic microbes, and the organism or benthic cover 2 cm directly underneath each water sample collected. From a marginal PERMANOVA including reef site, within-reef dominant benthic cover, and the benthic cover under each sample, site effects significantly explained 17.1% of microbial dissimilarity, dominant cover was insignificant, and cover directly under the sample significantly explained 11.4% (Table 1, Test 2). An additional sequential PERMANOVA test of these effects, accounting for reef site, sample-specific dominant benthic cover, and sample-specific organismal effects in order of spatial scale, provided revised R^2 estimates of 27.9% for reef site and 11.4% for organism or benthic cover under the sample, with no significant effect of locally dominant benthic cover (Table 1, Test 3). A final marginal PERMANOVA accounting for reef site, the percent cover of each benthic class within 1 m^2 of the sample, and the organism or cover directly under the sample reinforced the result that picoplankton communities significantly varied with reef site (9.2%) and the organism or cover under each sample (12.1%) but were not influenced by any local benthic class in the 1 m^2 area (Table 1, Test 4). Among these PERMANOVA tests, as much as 57.9% of picoplankton variability remained unattributed to the variables measured (reef site, within-reef benthic cover, organismal effects). Furthermore, we found that samples collected over the same benthic cover (e.g. sand) were consistent within reefs and showed significant site specificity. For example, a PERMANOVA test indicated significant site-level differences in picoplankton from seawater collected over sandy reef areas, despite the apparent benthic similarity of these samples (df = 2, R^2 = 0.399, pseudo-F = 4.97, Pr(>F) = 0.001). Samples collected over sand at Tektite featured 2852 ASVs not found over sand at any other reef site, followed by 1462 and 978 ASVs spe-

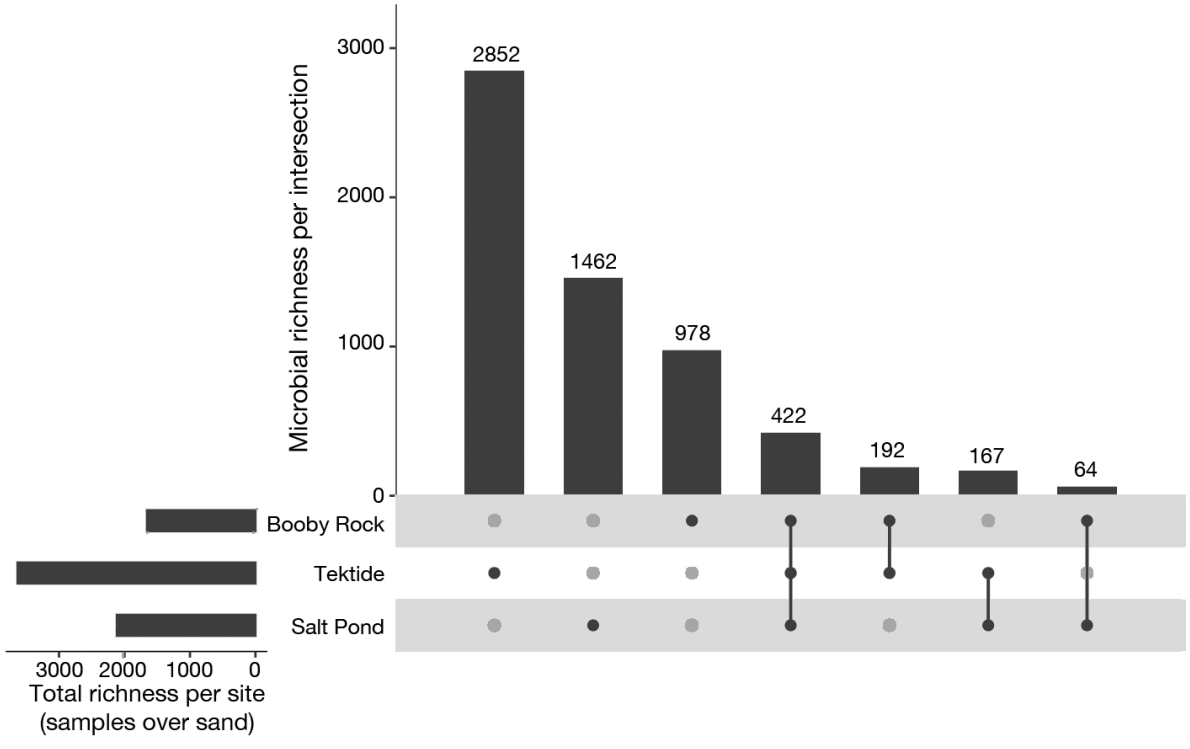

Fig. 5. Reef site specificity observed in seawater samples collected over sand at 3 different reefs. Bars show the total number of amplicon sequence variants (ASVs) observed in each subset, and membership in these subsets is indicated by darkened dots, with joint membership across multiple sites indicated by dots joined by a darkened line. Of all ASVs detected over sand at the Tektite location, 2852 were specific to sand at Tektite and did not occur over sand at any other reef; 422 ASVs were shared among sand-associated samples at all 3 reef sites

cific to sand at Salt Pond and Booby Rock, respectively (Fig. 5). A total of 422 ASVs were shared among all 3 reef sites for samples collected over sand.

4. DISCUSSION

In this study, we addressed the association of picoplankton communities to coral reef benthic communities at multiple spatial scales, comparing picoplankton variability as a function of reef site, benthic cover, and the distinct reef organism or benthic cover underlying each sample. Reef organisms have previously been shown to influence the structure of picoplankton communities (Walsh et al. 2017, Weber et al. 2019), but how these interactions vary across an individual reef, with benthic cover comprising many organisms, or affect intersite patterns in picoplankton composition is largely unknown. In this study, we determined that reef site explained as much as 27.9% of picoplankton variability. By comparison, benthic cover in a 1 m^2 area around each sample had no significant effect on overall picoplankton composition but did influence a limited set of 6 ASVs such as ASV930, a *Vibrio* sp. found here to be associated with algal cover. Furthermore, we confirm that organismal ecospheres are significant drivers of picoplankton structure on Caribbean coral reefs, explaining as much as 11.4% of variation after accounting for effects of reef site and dominant benthic cover (Table 1, Test 3).

4.1. Effect of reef site on near-benthos reef microorganisms

This study showed a clear impact of reef site on the near-benthos collected seawater communities. The 3 reef sites sampled in this study, all residing in Virgin Islands National Park, represent a spectrum of coral reef health. As the most degraded reef site, Salt Pond contained just 1% hard coral cover compared to 5.9% at Tektite and 6.0% at Booby Rock. Circulation of seawater likely differs across these reef sites, with separate embayments containing Salt Pond and Tektite likely leading to increased residence time of seawater compared to Booby Rock, a rocky outcropping approximately 500 m offshore. Nutrient samples were not taken at the time of sampling, but previous reports of Booby Rock and Tektite show similar and consistent nutrient environments (Becker et al. 2024). Reef sites in the study area are known to vary in benthic composition (Edmunds & Bruno 1996), and the connection between overall reef condition and microbial content is well established in the literature (Kelly et al. 2014, Bourne et al. 2016, Glasl et al. 2019). Using 26 samples at Tektite, 14 at Booby Rock, and 26 at Salt Pond, we had sufficient sample density to detect baseline reef differences and demonstrate high within-site variability of reef picoplankton communities. For example, the shallowest and most degraded reef site in this study, Salt Pond, contained the most ASVs from *Flavobacteriales*, which have previously been reported as indicative of low-quality reef environments (Kelly et al. 2014) and macroalgal exudates (Nelson et al. 2013). *Chitinophagales*, a group sensitive to warming in reef environments (Sun et al. 2022), was also at its lowest abundance in samples from Salt Pond. Differences in picoplankton composition by region and specific reef sites are well documented on Caribbean (Ma et al. 2022) and Pacific coral reefs, with up to 31% of microbial dissimilarity attributed to environmental differences (Galand et al. 2023). Potential drivers of this variability are numerous and may include land-based inputs (Tsai et al. 2010), seawater stratification (Thomas et al. 2010), or site-level differences in temperature (Chen et al. 2014, Wei et al. 2022) and varying degrees of benthic–pelagic coupling (e.g. through heterotrophy) (Ribes et al. 2003, McNally et al. 2017, Lesser & Slattery 2020). Relatively large-scale processes such as these establish the conditions on which within-reef picoplankton structure occurs. Contextualizing reef-specific and within-reef picoplankton variability is key to detecting ecosystem change on coral reefs.

4.2. Comparisons of inter- and intrareef picoplankton variability

While not used yet in a predictive context, reef microbiomes are often suggested as indicators of reef health and function, and it is critical that we build an understanding of the scale at which they vary. Multiple studies have identified broad geographic trends in reef microbiome structure and function (Kelly et al. 2014, Ma et al. 2022). Likewise, the relationship between reef-level coral or algal cover and microbial composition has been shown in some locations (Haas et al. 2013, Kelly et al. 2014), suggesting that seawater samples may be useful at detecting disturbance at reef scales (Glasl et al. 2019) and a valuable diagnostic tool in the future. Whereas ample evidence supports reef-level characterization of reef microbiomes, these same site effects pose a significant challenge to the use of water sampling to describe within-reef patterns, such as the detection of disease indicators in coral-associated seawater (Becker et al. 2022), by con-

founding characteristics of distinct reefs with those of the organisms sampled within or between these reefs. Site effects are a common issue in picoplankton studies (Galand et al. 2023) and can be addressed through increased sample density, as we have attempted to demonstrate here.

Previous work in the Caribbean did not identify within-reef (transect to transect) seawater picoplankton differences (Ma et al. 2022), though the benthos underlying each sample was not examined. Detailed characterization of benthic cover associated with each sample was applied here, which allowed this study to capture intrareef variation of reef-associated seawater microbiomes and account for site effects when interpreting these data. We found no association between these data when considering differences in benthic cover within 1 m of sample collection points. We did identify associations between specific ASVs and samples taken from areas dominated by particular benthic classes. For example, picoplankton samples in reef areas dominated by algae were, as a whole, not significantly different from samples in areas dominated by coral. However, these samples from algae-dominated reef areas were enriched in specific ASVs, such as ASV930 classified as *Vibrio*, which has been previously detected in the macroalgae boundary layer (Nelson et al. 2023). Similarly, a highly prevalent ASV313 (*Margulisbacteria* [candidate division ZB3]) was positively associated with areas dominated by sponges or algae and negatively associated with areas dominated by sand, PEYS, hard coral, or soft coral. ASV-specific associations were present for all benthic classes (see Fig. 3) but did not result in broad community dissimilarity across samples of different benthic composition. While benthic cover is a critical assessment in our understanding of coral reef function, these results suggest that in this study, it is a poor predictor of picoplankton community composition.

By contrast, the organism or benthic cover directly under each sample had a significant effect on the picoplankton recovered. Previous research has identified that benthic organisms can impact picoplankton communities immediately adjacent to their surface, but the relative scale of this effect versus that of the local benthic community and the reef site itself has not been rigorously compared. We determined that the organism or benthic cover class underlying each sample had a significant effect, akin to ecospheres previously identified in other work (Weber et al. 2019). Similar to previous work, we found enrichment of *Flavobacteriales* related to macroalgae (Nelson et al. 2013, 2023), but ecospheres associated with the other benthic organisms are less studied. In models that included reef site and sample-specific dominant benthic cover, the organism or cover 2 cm directly under each sample explained as much as 11.4% of picoplankton variability compared to 25.2% explained by reef site (Table 1, Test 3). Interestingly, in a model that included reef site as well as the percent cover of each benthic class, the organism or cover under each sample explained 12.1% of picoplankton variability compared to 9.2% explained by reef site (Table 1, Test 4). These results indicate a significant outsized effect of distinct reef organisms on nearby picoplankton that occurs in parallel with baseline reef site differences.

Importantly, we also observed high reef site-level specificity within picoplankton associated with particular benthic organisms or cover directly under the sample. For example, sand-associated samples from Tektite, Booby Rock, and Salt Pond contained 77.9, 58.5, and 68.2%, respectively, of ASVs that were entirely reef site specific, with 443 ASVs shared across sand-associated samples in all 3 reefs (Fig. 5). While the use of ASVs as a method of characterizing 16S rRNA genes from the bacterial genome poses a risk of artificially inflating sample differences (Schloss 2021), the high degree of specificity observed here in picoplankton over differing benthic cover or reef organisms exceeds distance thresholds needed to avoid these risks. Furthermore, where individual ASVs were identified as associated with specific benthic cover classes or organisms, they often originated from taxonomically distinct groups unlikely to be derived from artificial genome splitting alone (Fig. 3). Patterns of picoplankton specificity were observed for all benthic organisms detected across reef sites, with an average of 63.6% site specificity for samples taken over live coral, 59.9% for sponges, and 61.9% for macroalgae. These results suggest that organismal ecospheres are a major source of variability in picoplankton communities near the benthos, and this variability is likely to be constrained by processes occurring at the scale of individual reef sites.

4.3. Residual variation in reef-associated seawater microbiomes

This study identifies both reef site and organismal effects as having significant influence on the composition of reef picoplankton communities, but more than half of the variation in this system (57.9%) was not explained by these factors. This residual unexplained variation represents a considerable opportu-

nity for the future study of abiotic and biotic drivers of picoplankton structure on coral reefs. Prior research has shown that light environments vary at small scales on and within (Wangpraseurt et al. 2014) coral reefs. Temperature and nutrient availability on coral reefs can vary substantially at submeter scales (Reid et al. 2019), a pattern only recently discovered with the use of new distributed temperature sensing systems (Sinnett et al. 2020). Submarine groundwater discharge, often laden with nutrients and occurring as point-source impacts, directly alters the productivity of reef organisms including calcifiers (Silbiger et al. 2020). Hydrodynamics also play a major role in benthic–pelagic coupling on coral reefs, though this was not studied here. Increased flushing across coral reefs, or within reef cavities, directly increases removal of bacterioplankton by heterotrophy (van Duyl et al. 2006) and may shift community structure via selective feeding behavior of corals and other benthic invertebrates (McNally et al. 2017) whose feeding is facilitated by increased turbulent flow across the rugose reef canopy (Ribes & Atkinson 2007). More broadly, reef-scale circulation patterns driven by tidal forcing, as well as sporadic events (e.g. wind or wave events), can drive shifts in microbial communities over coral reefs by altering the availability of resources over short timescales (Rodier et al. 2024). These abiotic factors can directly shape the physiology and function of organisms living on coral reefs (Luehrmann et al. 2020), including picoplankton communities (Quinlan et al. 2019). As these diverse abiotic factors were not characterized in our study, we recommend that the patterns presented here should be interpreted as a snapshot in time, demonstrating (1) high spatial variability of reef picoplankton within individual reefs, (2) strong site specificity of picoplankton communities, and (3) reliable detection of organismal effects (e.g. ecospheres) wherein discrete reef organisms modulate nearby picoplankton community structure. Future studies capable of simultaneously analyzing reef picoplankton, benthic cover, and abiotic factors such as flow and temperature over short timescales will dramatically improve our understanding of benthic–pelagic coupling on coral reefs, and these studies are becoming increasingly feasible with the development of new reef-monitoring technologies (Sinnett et al. 2020, Apprill et al. 2023).

To our knowledge, this study constitutes the first effort to match fine-scale picoplankton variability (samples every 2 m) to equal-or-finer scale benthic variability, characterized using a grid of points every 10 cm around each sample. While we did not conduct a power analysis prior to conducting the study, we believe that such analysis could be useful in guiding future studies. As for resolution, finer-scale benthic annotation, for example every 1 cm, or the addition of more benthic classes was not logistically feasible in this study. However, methods to automate benthic classification are rapidly coming available (Miller et al. 2023), and by expanding both the scope and resolution of benthic mapping, these techniques will likely reveal additional associations between benthic and picoplankton communities. Using 9600 manual benthic annotations to establish benthic cover and 6600 annotations associated with picoplankton variability from seawater samples, this study revealed that organismal effects are a major driver of picoplankton structure, possibly even outweighing reef site effects. Furthermore, the residual variability unexplained here is an opportunity for future in-depth study of abiotic and biotic conditions at fine spatial scales. Our work puts into context the scale at which reef microbial communities vary and is critical if these organisms are to be used as indicators of reef health in future work.

5. CONCLUSION

Coral reefs are experiencing global decline, and their ecosystem function is changing (Williams & Graham 2019). Microbial organisms are central to the health of corals as foundational species (Bourne et al. 2016) and are increasingly monitored in the water column as indicators of disturbance to coral reefs (Kelly et al. 2014, Glasl et al. 2017). Critically, the goal of this study was not to ascribe picoplankton characteristics to reef health but rather to describe their variability in relation to benthic characteristics. Spatial variation of seawater microbial communities is poorly resolved due to the layered effects of broad geographic patterns (Ma et al. 2022), differences between reef sites, and the influence of macroorganisms (Walsh et al. 2017). We addressed this gap through fine-scale paired analysis of reef-associated seawater and benthic communities at 3 Caribbean reef sites. We determined that reef seawater microbial communities significantly vary at the scale of distinct reef sites, explaining as much as 27.9% of microbial variability, and these differences can be detected with as few as three 60 ml samples, a helpful lower bound when conducting similar studies. Within reefs, benthic cover surrounding each sample had no significant effect on overall microbial content but did produce taxon-specific enrichment. Instead, the organism or benthic cover 2 cm directly under each sample significantly explained as much as 12.1% of microbial variation,

supporting the hypothesis that reef macroorganisms alter picoplankton in their vicinity (Weber et al. 2019). Associations such as these provide valuable baseline data for benthic–pelagic coupling in reef ecosystems and, perhaps, future inference of picoplankton demographics based on visually detectable characteristics of the reef environment by autonomous vehicles (Apprill et al. 2023). By comparing inter- and intrareef drivers of microbial differentiation, we identify that as much as 57.9% of microbial variation is unexplained by reef site, benthic cover, or macroorganism influence. Our results highlight the remarkable spatial variability of coral reef picoplankton communities and the need for additional study of the biotic and abiotic factors that structure them.

Data archive. All supplementary materials (Data S1–S8), including analysis code and tables, are publicly available via a permanent repository on the Open Science Framework at https://osf.io/w495h/. S1: A compressed folder containing code files and metadata used in analysis. S2: A full-resolution orthomosaic of the Booby Rock reef site used in benthic data analysis. S3: A full-resolution orthomosaic of the Salt Pond reef site used in benthic data analysis. S4: A full-resolution orthomosaic of the Tektite reef site used in benthic data analysis. S5: A list of representative sequences for ASVs found to significantly vary by putative contaminant. S6: A list of representative sequences for ASVs found to significantly vary by reef site. S7: A list of representative sequences for ASVs found to significantly vary by local benthic cover around water samples collected. S8: A list of representative sequences for ASVs found to significantly vary by the benthic cover directly under water samples collected.

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LITERATURE CITED

Acock AC, Stavig GR (1979) A measure of association for nonparametric statistics. Soc Forces 57:1381–1386

Apprill A, McNally S, Parsons R, Weber L (2015) Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol 75:129–137

Apprill A, Girdhar Y, Mooney TA, Hansel CM and others (2023) Toward a new era of coral reef monitoring. Environ Sci Technol 57:5117–5124

Barnett DJM, Arts ICW, Penders J (2021) microViz: an R package for microbiome data visualization and statistics. J Open Source Software 6:3201

Becker CC, Weber L, Suca JJ, Llopiz JL, Mooney TA, Apprill A (2020) Microbial and nutrient dynamics in mangrove, reef, and seagrass waters over tidal and diurnal time scales. Aquat Microb Ecol 85:101–119

Becker CC, Brandt M, Miller CA, Apprill A (2022) Microbial bioindicators of stony coral tissue loss disease identified in corals and overlying waters using a rapid field-based sequencing approach. Environ Microbiol 24: 1166–1182

Becker CC, Weber L, Llopiz JK, Mooney TA, Apprill A (2024) Microorganisms uniquely capture and predict stony coral tissue loss disease and hurricane disturbance impacts on US Virgin Island reefs. Environ Microbiol 26:e16610

Bourne DG, Morrow KM, Webster NS (2016) Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. Annu Rev Microbiol 70:317–340

Briggs AA, Brown AL, Osenberg CW (2021) Local versus site-level effects of algae on coral microbial communities. R Soc Open Sci 8:210035

Burke S, Pottier P, Lagisz M, Macartney EL, Ainsworth T, Drobniak SM, Nakagawa S (2023) The impact of rising temperatures on the prevalence of coral diseases and its predictability: a global meta-analysis. Ecol Lett 26: 1466–1481

Callahan BJ, McMurdie PJ, Holmes SP (2017) Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J 11:2639–2643

Chen B, Liu H, Huang B, Wang J (2014) Temperature effects on the growth rate of marine picoplankton. Mar Ecol Prog Ser 505:37–47

Chen Q, Beijbom O, Chan S, Bouwmeester J, Kriegman D (2021) A new deep learning engine for CoralNet. Proc 2021 IEEE/CVF Int Conf Comput Vis Workshops (ICCVW), 11–17 Oct 2021, Montreal. IEEE, Piscataway, NJ, p3686–3695

Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ (2018) Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 6:226

Edmunds PJ, Bruno JF (1996) The importance of sampling scale in ecology: kilometer-wide variation in coral reef communities. Mar Ecol Prog Ser 143:165–171

Ferrier-Pagès C, Leclercq N, Jaubert J, Pelegrí SP (2000) Enhancement of pico- and nanoplankton growth by coral exudates. Aquat Microb Ecol 21:203–209

Fordyce AJ, Ainsworth TD, Heron SF, Leggat W (2019) Marine heatwave hotspots in coral reef environments: physical drivers, ecophysiological outcomes and impact upon structural complexity. Front Mar Sci 6:1–17

Fox J, Weisberg S (2019) An R companion to applied regression, 3rd edn. Sage, Thousand Oaks CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Fung T, Seymour RM, Johnson CR (2011) Alternative stable states and phase shifts in coral reefs under anthropogenic stress. Ecology 92:967–982

Galand PE, Ruscheweyh HJ, Salazar G, Hochart C and others (2023) Diversity of the Pacific Ocean coral reef microbiome. Nat Commun 14:3039

Glasl B, Webster NS, Bourne DG (2017) Microbial indicators as a diagnostic tool for assessing water quality and climate stress in coral reef ecosystems. Mar Biol 164:91

Glasl B, Bourne DG, Frade PR, Thomas T, Schaffelke B, Webster NS (2019) Microbial indicators of environmental perturbations in coral reef ecosystems. Microbiome 7:94

Griffiths JR, Kadin M, Nascimento FJA, Tamelander T and others (2017) The importance of benthic–pelagic coupling for marine ecosystem functioning in a changing world. Glob Change Biol 23:2179–2196

Haas AF, Nelson CE, Rohwer F, Wegley-Kelly L and others

(2013) Influence of coral and algal exudates on microbially mediated reef metabolism. PeerJ 1:e108
Haas AF, Fairoz MFM, Kelly LW, Nelson CE and others (2016) Global microbialization of coral reefs. Nat Microbiol 1:16042
Kelly LW, Williams GJ, Barott KL, Carlson CA and others (2014) Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors. Proc Natl Acad Sci USA 111:10227–10232
Kelly LW, Nelson CE, Haas AF, Naliboff DS and others (2019) Diel population and functional synchrony of microbial communities on coral reefs. Nat Commun 10:1691
Laurence M, Hatzis C, Brash DE (2014) Common contaminants in next-generation sequencing that hinder discovery of low-abundance microbes. PLOS ONE 9:e97876
Lesser MP (2006) Benthic–pelagic coupling on coral reefs: feeding and growth of Caribbean sponges. J Exp Mar Biol Ecol 328:277–288
Lesser MP, Slattery M (2020) Will coral reef sponges be winners in the Anthropocene? Glob Change Biol 26: 3202–3211
Ludwig W, Strunk O, Westram R, Richter L and others (2004) ARB: a software environment for sequence data. Nucleic Acids Res 32:1363–1371
Luehrmann M, Cortesi F, Cheney KL, de Busserolles F, Marshall NJ (2020) Microhabitat partitioning correlates with opsin gene expression in coral reef cardinalfishes (Apogonidae). Funct Ecol 34:1041–1052
Ma L, Becker C, Weber L, Sullivan C and others (2022) Biogeography of reef water microbes from within-reef to global scales. Aquat Microb Ecol 88:81–94
Martin BD, Witten D, Willis AD (2020) Modeling microbial abundances and dysbiosis with beta-binomial regression. Ann Appl Stat 14:94–115
McMurdie PJ, Holmes S (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLOS ONE 8:e61217
McNally SP, Parsons RJ, Santoro AE, Apprill A (2017) Multifaceted impacts of the stony coral *Porites astreoides* on picoplankton abundance and community composition. Limnol Oceanogr 62:217–234
Meron D, Rodolfo-Metalpa R, Cunning R, Baker AC, Fine M, Banin E (2012) Changes in coral microbial communities in response to a natural pH gradient. ISME J 6:1775–1785
Meunier V, Bonnet S, Pernice M, Benavides M and others (2019) Bleaching forces coral's heterotrophy on diazotrophs and *Synechococcus*. ISME J 13:2882–2886
Miller SD, Dubel AK, Adam TC, Cook DT, Holbrook SJ, Schmitt RJ, Rassweiler A (2023) Using machine learning to achieve simultaneous, georeferenced surveys of fish and benthic communities on shallow coral reefs. Limnol Oceanogr Methods 21:451–466
Morrow KM, Liles MR, Paul VJ, Moss AG, Chadwick NE (2013) Bacterial shifts associated with coral–macroalgal competition in the Caribbean Sea. Mar Ecol Prog Ser 488: 103–117
Nelson CE, Alldredge AL, McCliment EA, Amaral-Zettler LA, Carlson CA (2011) Depleted dissolved organic carbon and distinct bacterial communities in the water column of a rapid-flushing coral reef ecosystem. ISME J 5:1374–1387
Nelson CE, Goldberg SJ, Wegley Kelly L, Haas AF, Smith JE, Rohwer F, Carlson CA (2013) Coral and macroalgal exudates vary in neutral sugar composition and differentially enrich reef bacterioplankton lineages. ISME J 7: 962–979
Nelson CE, Kelly LW, Haas AF (2023) Microbial interactions with dissolved organic matter are central to coral reef ecosystem function and resilience. Annu Rev Mar Sci 15: 431–460
Nuryadi H, Nguyen TTM, Ito M, Okada N and others (2018) A metabarcoding survey for seasonal picophytoplankton composition in two coral reefs around Sesoko Island, Okinawa, Japan. J Appl Phycol 30:3179–3186
Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol 18:1403–1414
Patten NL, Wyatt ASJ, Lowe RJ, Waite AM (2011) Uptake of picophytoplankton, bacterioplankton and virioplankton by a fringing coral reef community (Ningaloo Reef, Australia). Coral Reefs 30:555–567
Polónia ARM, Cleary DFR, Freitas R, Coelho FJRDC, De Voogd NJ, Gomes NCM (2016) Comparison of archaeal and bacterial communities in two sponge species and seawater from an Indonesian coral reef environment. Mar Genomics 29:69–80
Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glöckner FO (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 35:7188–7196
Quast C, Pruesse E, Yilmaz P, Gerken J and others (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596
Quinlan ZA, Ritson-Williams R, Carroll BJ, Carlson CA, Nelson CE (2019) Species-specific differences in the microbiomes and organic exudates of crustose coralline algae influence bacterioplankton communities. Front Microbiol 10:2397
Reid EC, DeCarlo TM, Cohen AL, Wong GTF and others (2019) Internal waves influence the thermal and nutrient environment on a shallow coral reef. Limnol Oceanogr 64:1949–1965
Remple KL, Silbiger NJ, Quinlan ZA, Fox MD, Kelly LW, Donahue MJ, Nelson CE (2021) Coral reef biofilm bacterial diversity and successional trajectories are structured by reef benthic organisms and shift under chronic nutrient enrichment. NPJ Biofilms Microbiomes 7:84
Rhew K, Baca RM, Ochs CA, Threlkeld ST (1999) Interaction effects of fish, nutrients, mixing and sediments on autotrophic picoplankton and algal composition. Freshw Biol 42:99–109
Ribes M, Atkinson MJ (2007) Effects of water velocity on picoplankton uptake by coral reef communities. Coral Reefs 26:413–421
Ribes M, Coma R, Atkinson MJ, Kinzie RA III (2003) Particle removal by coral reef communities: picoplankton is a major source of nitrogen. Mar Ecol Prog Ser 257:13–23
Rix L, de Goeij JM, van Oevelen D, Struck U, Al-Horani FA, Wild C, Naumann MS (2018) Reef sponges facilitate the transfer of coral-derived organic matter to their associated fauna via the sponge loop. Mar Ecol Prog Ser 589:85–96
Roach TNF, Yadav S, Caruso C, Dilworth J and others (2021) A field primer for monitoring benthic ecosystems using structure-from-motion photogrammetry. J Vis Exp 2021: 1–14
Rodier M, Pagano M, Lhomond L, Péricaud JB, Guilloux L, Devenon JL, Chevalier C (2024) Effects of physical forcing on short-term plankton dynamics in a narrow coral

reef lagoon (Ouano, New Caledonia): a two-week high-frequency study. J Plankton Res 46:202–218
Rosenberg Y, Simon-Blecher N, Lalzar M, Yam R and others (2022) Urbanization comprehensively impairs biological rhythms in coral holobionts. Glob Change Biol 28: 3349–3364
Schloss PD (2021) Amplicon sequence variants artificially split bacterial genomes into separate clusters. MSphere 6
Silbiger NJ, Donahue MJ, Lubarsky K (2020) Submarine groundwater discharge alters coral reef ecosystem metabolism. Proc Biol Sci 287:20202743
Sinnett G, Davis KA, Lucas AJ, Giddings SN, Reid E, Harvey ME, Stokes I (2020) Distributed temperature sensing for oceanographic applications. J Atmos Ocean Technol 37: 1987–1997
Sparagon WJ, Arts MGI, Quinlan ZA, Wegley Kelly L and others (2024) Coral thermal stress and bleaching enrich and restructure reef microbial communities via altered organic matter exudation. Commun Biol 7:160
Sun H, Zheng H, Jiang Y, Liang J and others (2022) Elevated temperature alters bacterial community composition and metabolism in seawaters of coral reef ecosystem: an evidence of laboratory experiment with *Acropora digitifera* bleaching. Ecol Indic 139:108886
Sutherland KP, Griffin A, Park A, Porter JW and others (2023) Twenty-year record of white pox disease in the Florida Keys: importance of environmental risk factors as drivers of coral health. Dis Aquat Org 154:15–31
Terzin M, Laffy PW, Robbins S, Yeoh YK and others (2024) The road forward to incorporate seawater microbes in predictive reef monitoring. Environ Microbiome 19:5
Thomas Y, Garen P, Courties C, Charpy L (2010) Spatial and temporal variability of the pico- and nanophytoplankton and bacterioplankton in a deep Polynesian atoll lagoon. Aquat Microb Ecol 59:89–101
Tsai AY, Gong GC, Sanders RW, Chao CF, Chiang KP (2010) Microbial dynamics in an oligotrophic bay of the western subtropical Pacific: impact of short-term heavy freshwater runoff and upwelling. J Oceanogr 66:873–883
van Duyl FC, Scheffers SR, Thomas FIM, Driscoll M (2006) The effect of water exchange on bacterioplankton depletion and inorganic nutrient dynamics in coral reef cavities. Coral Reefs 25:23–36
Walsh K, Haggerty JM, Doane MP, Hansen JJ and others (2017) Aura-biomes are present in the water layer above coral reef benthic macro-organisms. PeerJ 5:e3666
Wangpraseurt D, Polerecky L, Larkum AWD, Ralph PJ, Nielsen DA, Pernice M, Kühl M (2014) The *in situ* light microenvironment of corals. Limnol Oceanogr 59: 917–926
Weber L, Gonzalez-Díaz P, Armenteros M, Apprill A (2019) The coral ecosphere: a unique coral reef habitat that fosters coral–microbial interactions. Limnol Oceanogr 64:2373–2388
Weber L, Soule MK, Longnecker K, Becker CC, Huntley N, Kujawinski EB, Apprill A (2022) Benthic exometabolites and their ecological significance on threatened Caribbean coral reefs. ISME Commun 2:101
Wei Y, Gu T, Zhang G, Qu K, Cui Z, Sun J (2022) Exploring the dynamics of marine picophytoplankton among the Yellow Sea, Indian Ocean and Pacific Ocean: the importance of temperature and nitrogen. Environ Res 214: 113870
Wickham H (2016) Ggplot2: elegant graphics for data analysis. Springer-Verlag, New York, NY
Williams GJ, Graham NAJ (2019) Rethinking coral reef functional futures. Funct Ecol 33:942–947
Williams SD, Walter CS, Muller EM (2021) Fine scale temporal and spatial dynamics of the stony coral tissue loss disease outbreak within the lower Florida Keys. Front Mar Sci 8:631776
Xu S, Zhan L, Tang W, Wang Q and others (2023) MicrobiotaProcess: a comprehensive R package for deep mining microbiome. Innovation 4:100388
Zaneveld JR, Burkepile DE, Shantz AA, Pritchard CE and others (2016) Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. Nat Commun 7:11833
Zhang Z, Falter J, Lowe R, Ivey G (2012) The combined influence of hydrodynamic forcing and calcification on the spatial distribution of alkalinity in a coral reef system. J Geophys Res Oceans 117:C04034

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