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Rubble fields shape planktonic protist communities in Indonesia at a local scale

Erin M. Borbee[1] | **Inna Puspa Ayu**[2] | **Paul Carvalho**[3] | **Ester Restiana**[2,4] | **Fahkrizal Setiawan**[2] | **Beginer Subhan**[2] | **Austin T. Humphries**[3] | **Hawis Madduppa**[2] | **Christopher E. Lane**[1]

[1]Department of Biological Sciences, University of Rhode Island, Kingston, Rhode Island, USA

[2]Department of Marine Science and Technology, Institut Pertainian Bogor, Bogor, Indonesia

[3]Department of Fisheries, Animal, and Veterinary Sciences, University of Rhode Island, Kingston, Rhode Island, USA

[4]Department of Fisheries, University of Jambi, Jambi, Indonesia

Correspondence
Christopher E. Lane, Department of Biological Sciences, University of Rhode Island, 120 Flagg Road, Kingston, RI 02881, USA.
Email: clane@uri.edu

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Abstract
The Coral Triangle encompasses nearly 30% of the world's coral reefs and is widely considered the epicenter of marine biodiversity. Destructive fishing practices and natural disturbances common to this region damage reefs leaving behind fields of coral rubble. While the impacts of disturbances in these ecosystems are well documented on metazoans, we have a poor understanding of their impact on microbial communities at the base of the food web. We use metabarcoding to characterize protist community composition in sites of varying fisheries management schemes and benthic profiles across the island of Lombok, Indonesia. Our study shows that rubble coverage and net primary productivity are the strongest explainers of variation in protist communities across Lombok. More specifically, rubble fields are characterized by increases in small heterotrophic protists, including ciliates and cercozoans. In addition to shifts in heterotrophic protist communities, we also observed increases in diatom relative abundance in rubble fields, which corresponded to sites with higher net primary productivity. These results are the first to characterize protist communities in tropical marine rubble fields and provide insight on environmental factors potentially driving these shifts on a local scale.

KEYWORDS
biodiversity, biogeography, cercozoans, ciliates, diatoms, foodwebs, metabarcoding

THE Coral Triangle encompasses nearly one-third of the world's coral reefs and is widely considered the epicenter of marine biodiversity (Allen, 2008; Bowen et al., 2013; Burke et al., 2012; Hoeksema, 2007; Veron et al., 2009). The coral reef ecosystems in this region are vital food and economic resources for countries across the Indo-Pacific (Cruz-Trinidad et al., 2014; Foale et al., 2013). Indonesia, the largest country in this region, is home to 1.7 million coral reef fishers and nearly 60% of its total population lives in coastal areas (Teh et al., 2013; Siry, 2007). While fisheries management strategies aim to protect these ecosystems and promote sustainable fishing practices (Gaines et al., 2010), overfishing and destructive fishing practices like cyanide and blast fishing across the country pose threats to coral reefs (Dulvy et al., 2004; Halim, 2002; Langfelder & Horvath, 2008; Pet-Soede & Erdmann, 1998). These threats to reefs are of particular concern in southwestern Indonesia where the human population and fishing pressure are highest.

Natural disturbances like earthquakes, storms, and tsunamis can also cause damage reefs in this region. While the damage caused by natural disturbances is often patchy across impacted reefs (Foster et al., 2006; Hagan et al., 2007), these disturbances also contribute to the formation of rubble fields and can exacerbate existing damage from destructive fishing practices (Campbell et al., 2007). The formation of rubble fields by various disturbances leads to a sudden drop in animal diversity and dramatic shifts in food web dynamics on the impacted reefs (Edinger et al., 1998; McManus et al., 1997). The coral fragments that do manage to survive these disturbances often die within a month (Fox et al., 2003).

Studies on the recovery and response of organisms to these disturbances in the Indo-Pacific largely center around macroscopic organisms including hard coral, soft coral, and macroalgae (Connell et al., 1997; Fox et al., 2003, 2005; Sawall et al., 2013; Williams et al., 2019). These studies have documented how unstable substrate left behind in rubble fields makes settlement of hard coral larvae difficult by essentially creating a "killing field" for coral recruits (Fox et al., 2003; Sawall et al., 2013). However, both models and experimental data suggest the extent of the damage on the reef is also an important factor in evaluating the potential for recovery of these reefs (Fox & Caldwell, 2006; Saila et al., 1993).

Despite our understanding of the responses of macroorganisms in rubble fields, we lack data on microbial communities and their responses to such disturbances. Microorganisms play important roles in biogeochemical cycling across marine environments and also play important roles as symbionts to organisms in coral reef ecosystems (Ainsworth et al., 2010; Falkowski et al., 1998; Glasl et al., 2019). Protists (microbial eukaryotes), in particular, play important roles as both primary producers and consumers in the microbial food web and engage in a wide spectrum of symbioses (Clerissi et al., 2018; Decelle et al., 2015; Field et al., 1998). Understanding the impacts of disturbances on protist communities, and how protist communities respond to such dramatic environmental change, is therefore important for understanding connectivity across different levels of the food web and for understanding how these disturbances might potentially impact nutrient cycling and other microbial processes in coral reef ecosystems.

Our study focuses on protist communities across the island of Lombok, Indonesia. These communities encompass phytoplankton, grazers, and symbionts, many of which play essential roles in coral reef ecosystems. We use metabarcoding data to evaluate protist community composition across sites of differing fisheries management schemes and benthic profiles and explore how rubble fields may impact protist community structure across the island. Our data show that rubble cover and net primary productivity (NPP) were the strongest explainers of protist community structure across Lombok. Rubble fields were characterized by an increased relative abundance of small grazers including ciliates and cercozoans. Additionally, we show that sites with high rubble cover also have increased net primary productivity (NPP) and relative abundance of diatoms. These results could potentially reflect shifting nutrient levels across the island, allowing diatoms to be more successful at those sites.

MATERIALS AND METHODS

Sample collection and preservation

Samples were collected from 18 sites around the island of Lombok, Indonesia, in July 2018 (Table S1, Figure 1A). The sites were grouped into three regions around the island (northeast, northwest, and southwest) and were chosen to span various fisheries management zones, including open access zones with no restrictions on fishing, gear-restricted zones where some fishing gear is restricted and no-take zones. At each site, water was collected at 5 m depth and surface sediment with water was collected at 10 m in 4-L containers by SCUBA. After collection, water and sediment samples were each filtered over 12-μm followed by 0.4-μm polycarbonate filters (Sterlitech) using a peristaltic pump until filters clogged. The entire 4 L water sample was filtered, but the sediment samples often clogged after 1–2 L was filtered. Following filtering, filters were cut in half and placed in 2 ml cryovials with 1 ml of DNA/RNA Shield (Zymo Research). The samples were then stored at room temperature until transported back to the lab where they were stored at 4°C.

In addition to water and sediment at each site, underwater visual census (UVC) data was collected on fish communities and benthic coverage. At each site, a total of six 50-m transects were surveyed by two observers (three transects each). For fish counts, the observers counted fish and estimated their size within 2.5 m on either side of the transect for each of the transects. The fish were classified as close to the species level as possible and were later organized into trophic and functional groups for data analysis. Benthic coverage data was estimated along each transect using the point-intercept method every 0.5 m. The benthic categories were organized into, hard coral (classified to the genus level), soft coral, macroalgae, rubble, and other. To further supplement the UVC data, we downloaded mean net primary productivity (NPP) and socio-environmental data from the Marine Socio-Environmental Covariates dataset for each of our sites (Yeager et al., 2017) (Table S1).

DNA extraction, PCR, and sequencing

We extracted DNA from each filter using the Zymo Biomics DNA Miniprep Kit following the manufacturer's protocol. Two sets of eukaryotic primers were used to amplify different regions of DNA using polymerase chain reaction (PCR) (Table 1). The primers used for the V9 hypervariable region of the small-subunit ribosomal RNA gene (18 S) were used primarily to target protists and other microbial eukaryotic groups, while the cytochrome oxidase I (*cox*1) primers were used to target metazoan groups. All PCRs were set up using Bioline MyTaq Red Mix following the volume and

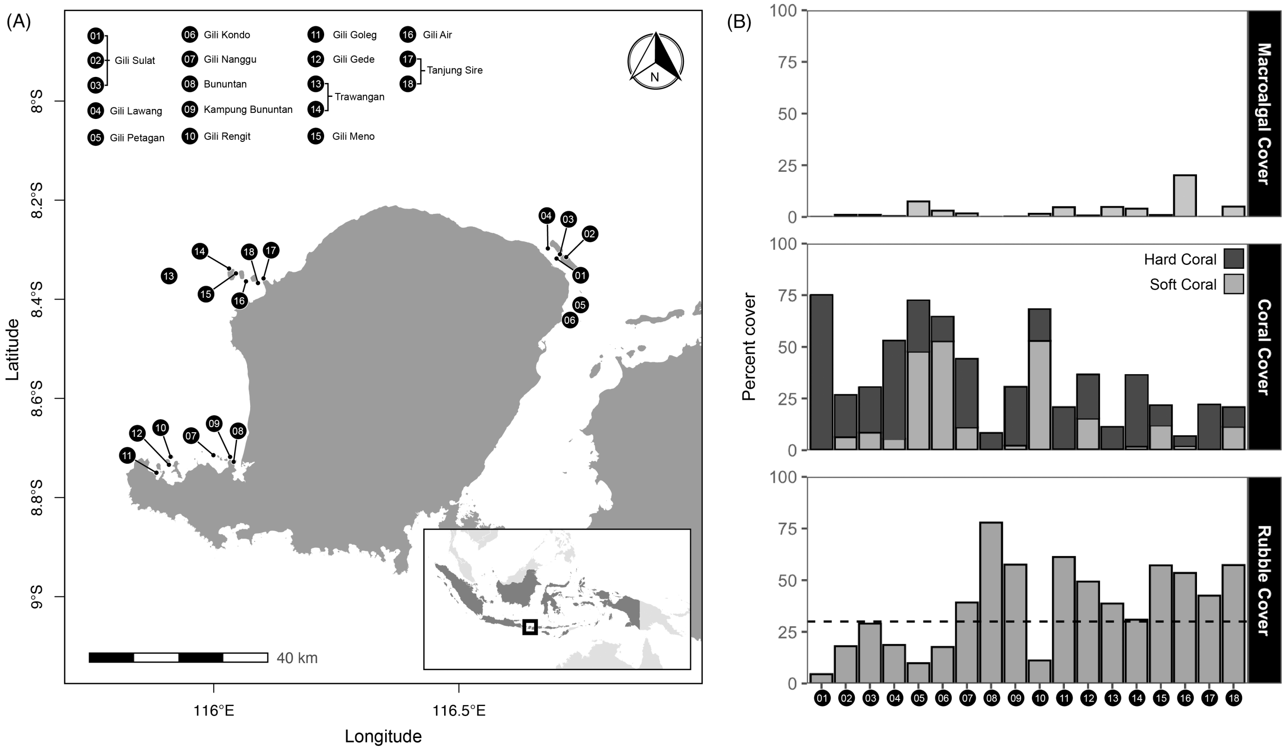

FIGURE 1 Site characteristics including a (A) map of collection sites on the island of Lombok, Indonesia and (B) corresponding benthic coverage data for each collection site as evaluated by UVC. The horizontal line across rubble coverage indicates a 30% threshold used by Sawall et al., 2013 to denote reefs impacted by blast fishing.

TABLE 1 Primer sequences and PCR cycles were used to amplify the V9 hypervariable region of 18 S rDNA (121 bp) and the portion of COI (313 bp)

Target region	Primer name/ direction	Primer sequence (5′-3′)	References	PCR cycles
18 S (V9)	1389F (Forward) 1510R (Reverse)	TTGTACACACCGCCC CCTTCYGCAGGTTCACCTAC	Amaral-Zettler et al. (2009)	Start: 3 min denaturation at 94°C Cycles (x35): *Denaturation* at 94°C for 45 s, *Annealing* at 48°C for 30 s, *Elongation* at 72°C for 30 s End: 5 min elongation at 72°C
*cox*1	mICOIintF (Forward) dgHCO2198 (Reverse)	GGWACWGGWTGAACWGTWTAYCCYCC TAAACTTCAGGGTGACCAAARAAYCA	Leray et al. (2013)	Start: 5 min denaturation at 95°C Cycles (x35): *Denaturation* at 95°C for 1 min, *Annealing* at 48°C for 45 s, *Elongation* at 72°C for 30 s End: 10 min elongation at 72°C

concentration recommendations from the manufacturer for both primers and DNA templates. Successful amplification and amplicon size were confirmed using gel electrophoresis. Library prep was done by the University of Rhode Island Genomics and Sequencing Center and the amplicons were then sequenced on the Illumina MiSeq using paired-end sequencing. The 18 S V9 samples were sequenced on a 2 × 150 bp run and the cox1 samples were sequenced on a 2 × 250 bp run using v3 chemistry.

In addition to samples from the field, a mock community was prepared using DNA samples from cultures available in the lab as a control on the sequencing run. The cultures used spanned different Stramenopile lineages, including *Fragilariopsis cylindrus* (Bacillariophyta), *Apedinella radians* (Dictyochophyceae), *Phaeothamnion confervicola* (Phaeothamniophyceae), *Chrysosaccus* sp. (Chrysophyceae), and *Tribonema minus* (Xanthophyceae). The DNA from each culture was added in equal concentrations to a single tube, and the resulting DNA sample was amplified using the same primers and PCR methods described above (Figure S1). The amplicons were then sequenced on the Illumina MiSeq on the same run as the environmental samples.

Bioinformatics

Forward and reverse reads were initially quality assessed in FastQC (Andrews, 2010). Primer sequences were trimmed from the paired reads using Cutadapt v1.9.1 (Martin, 2011). After trimming, the reads were imported into QIIME2 v2020.6 and were filtered, denoised, merged, and chimera checked using the DADA2 plugin in QIIME2 (Bolyen et al., 2019; Callahan et al., 2013). Truncation length for denoising was chosen to minimize the number of low-quality bases at the end of the reads while maximizing the amount of overlap between the forward and reverse reads to optimize merging.

The amplicon sequence variants (ASVs) resulting from denoising were then clustered into OTUs at 97% similarity using the vsearch plugin in QIIME2 (Rognes et al., 2016) and taxonomically assigned using a Naïve-Bayes classifier in QIIME2. The V9 reads were classified using the Protist Ribosomal Reference Database (PR^2) v4.12.0 (Guillou et al., 2013) and the *cox*1 reads were classified using the MIDORI database (Leray et al., 2018). Once classified, any ASV that had a classification of lower than 95% confidence at any level was filtered out to remove low-quality and low-abundance ASVs from the dataset to help reduce noise in downstream analyses. The 95% threshold was chosen after comparing the outputs of filtering at multiple thresholds (i.e. unfiltered, 85%, 90%, 95%, 97%, and 99%). Filtering at 95% confidence in assignment allowed us to reduce noise in the dataset without losing a high proportion of our ASVs. Finally, to obtain the protist community data for further analysis, the resulting ASV table was filtered to only include the sequences classifying to Stramenopiles, Alveolates, and Rhizaria. This filtering step allows us to eliminate undefined eukaryotic reads and reads classifying to metazoans, but also allows us to filter out other low abundance eukaryotic groups to reduce noise in downstream analyses. Furthermore, the Stramenopiles, Alveolates, and Rhizaria, encompass some of the most diverse and abundant protist lineages and have been shown to be the dominant protists in ocean sampling across the globe (de Vargas et al., 2015).

Data analysis and statistics

Alpha and Beta diversity statistics were calculated in R using the *vegan* package (Oksanen et al., 2020). Richness was used as the alpha diversity metric to avoid potential 18 S copy number bias in the V9 dataset across taxonomic groups. Kruskal-Wallis and Wilcoxon-pairwise tests were used to test differences across the three geographic groupings of sites (NE, NW, and SW).

Canonical Analysis of Principle coordinates (CAP) was used to evaluate potential drivers of protist community composition across Lombok (Anderson & Willis, 2003). The analysis and ordinations were done using the *phyloseq* package in R (McMurdie & Holmes, 2013). The environmental variables used in the analyses were a combination of UVC data from each site, including benthic coverage percentages and fish abundances across different trophic groups, as well as socio-environmental data from the MSEC dataset. Sequence data were also used from the *cox*1 and 18 S V9 datasets we generated to fill in gaps in the food web and better understand potential biotic drivers at play. To do this, copepod data was filtered from the V9 dataset and invertebrate data, including bivalves, anthozoans, and sponges, were filtered from the *cox*1 datasets for each site.

Co-occurrence networks were constructed using the WGCNA package in R (Langfelder & Horvath, 2008). A network was constructed encompassing all sites and then separate subsequent networks were constructed for the NE, NW, and SW sites in order to observe how co-occurrence patterns shifted across the island (Figure 5). The edges for each network were used to construct circos plots showing which taxonomic groups most closely co-occurred with one another (Figure 6).

RESULTS

Sequencing and filtering results

The V9 sequencing for these sites consisted of 68 samples and greater than 4.5 million total reads with an average of 67,160 reads per sample. After quality filtering, denoising, and merging we retained on average 85.4% of reads. After taxonomic assignment, reads were filtered again to remove ASVs that were classified at less than 95% confidence. This round of filtering eliminated an additional 30% of our reads, most of which were undefined eukaryotes and low-abundance ASVs. Nearly half of the ASVs eliminated in this filtering step belonged to Metazoa, Fungi, and Archaeplastida, which were not groups of interest in this study. The remaining sequences consisted of 18,430 ASVs, which were classified as 1595 taxa. The final filtering step isolated protist sequences, retaining only those classified as Stramenopiles, Alveolates, and Rhizaria. This step eliminated about 70% of the remaining data, resulted in 4550 ASVs that were classified as 811 taxa (Figure S2). The 70% of reads that were filtered out were primarily undefined eukaryotes (28%) and metazoans (24%), and the remaining eukaryotic groups including Fungi, Archaeplastida, Hacrobia, Excavata, Amoebozoa, and Apusozoa each made up 0%–5% of the total reads.

The *cox*1 sequencing data encompassed 28 samples with an average of 44,249 sequences per sample. After quality filtering, denoising, and merging, we retained 71% of our total reads, maintaining an average of 26,360 reads per sample. The resulting dataset consisted of

9371 ASVs that were classified as 317 taxa (Figure S3). Sequences classified as filter-feeders were filtered out to be used as metadata variables in later analysis. These sequences only made up on average 5% of reads per sample.

UVC site data

The three collection site regions showed distinct benthic profiles from one another (Figure 1B). While coral cover did not statistically differ by these regions (Kruskal–Wallis, $p > 0.05$), rubble percent and available substrate did. Rubble was significantly higher in the NW and SW sites than it was in the NE sites (Wilcoxon pairwise, $p < 0.05$). The coral cover showed the opposite trend, where it was significantly higher in the NE compared to the NW (Wilcoxon, $p < 0.05$) (Table S2). The SW was not significantly different from either the NE or NW coral cover, but that was likely due to a single site (10), which had much higher coral cover than the other sites in that region. When site 10 was excluded from the analysis, NW and SW sites have significantly higher rubble percent than NE sites. Benthic profiles correlated with changes in fish abundance per site, with rubble percent, in particular, showing a significant negative correlation with fish abundance per site ($R = -0.58$, $p = 0.016$).

Protist community composition

Protist community composition varied by geography across Lombok with significant differences in community composition between North and South Lombok across all samples and further separation between northwest and northeast sites in the 0.4 μm water samples and 12 μm sediment samples (ANOSIM, $p < 0.05$) (Table S3). In particular, the diatoms (Bacillariophyta), ciliates (Ciliophora), and cercozoans (Cercozoa), increased in relative abundance in the southwest and certain northwest sites (Figure 2). Despite these shifts in relative abundance and Beta diversity, Alpha diversity remained steady in all protist groups across Lombok (Wilcoxon pairwise, $p > 0.05$) (Table S4, Figure 3).

Canonical analysis of principal coordinates (CAP)

CAP ordinations constructed using Bray–Curtis dissimilarity provided insight into potential drivers of these community shifts (Figure 4). Across all of the CAP ordinations, the two variables with the strongest correlation to the first CAP axis, and, therefore, explaining the most variation, were mean NPP and rubble percent (Figure 4). Other variables that explained significant correlation

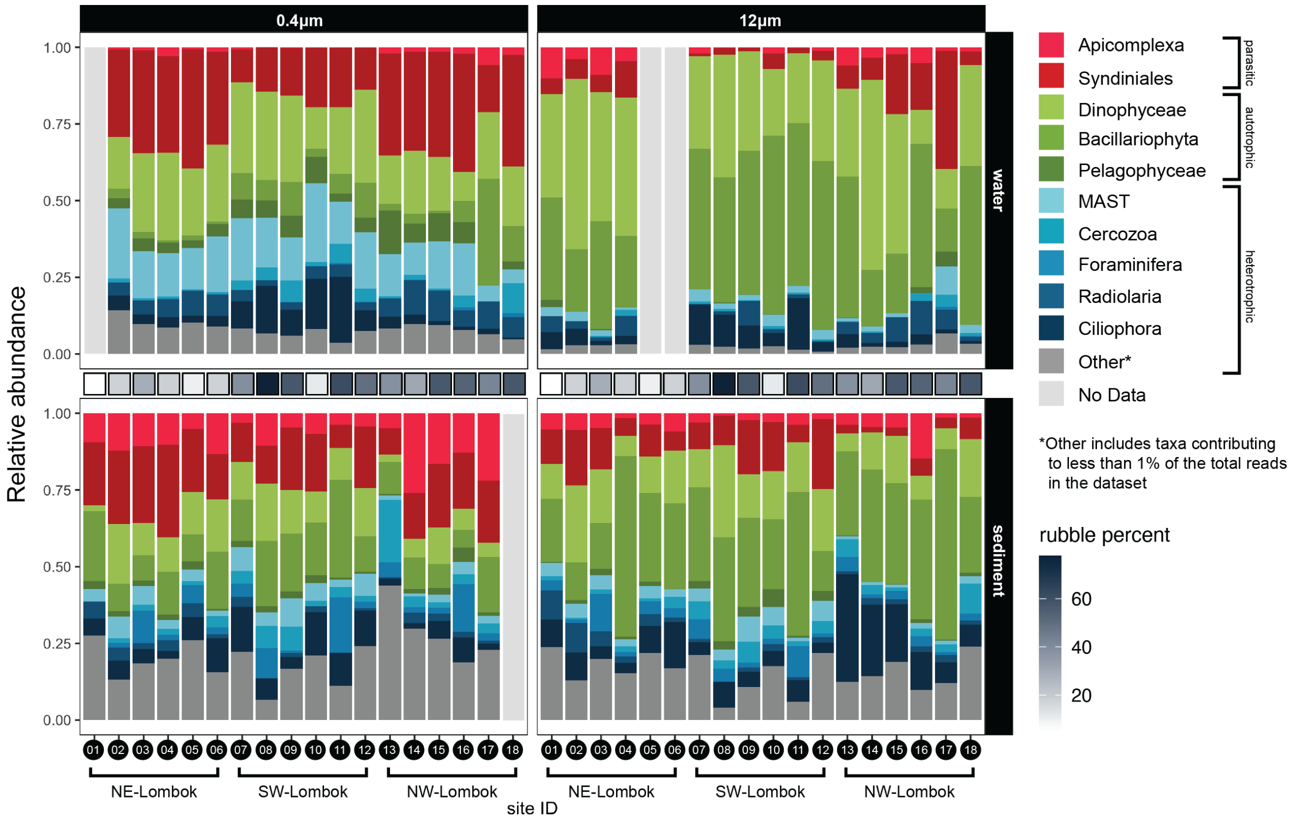

FIGURE 2 Protist community composition by collection site for water and sediment sample of both size fractions. Squares between water and sediment plots represent, rubble percent per site.

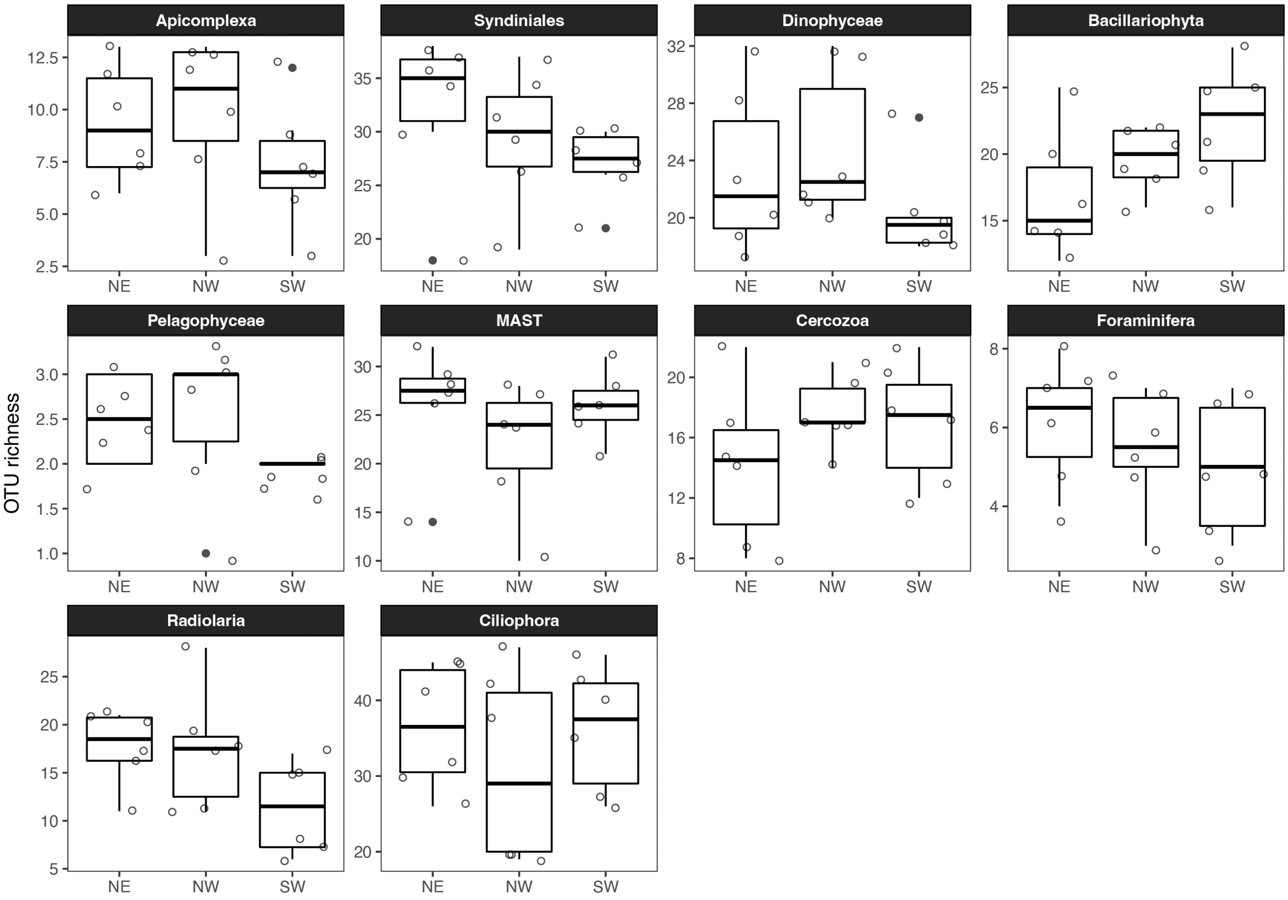

FIGURE 3 OTU richness by geographic groupings of collection sites (NE = sites 01–06, SW = sites 07–12, NW = sites 13–18). Richness did not significantly differ by groups of the site in any protist groups (Wilcoxon pairwise, $p < 0.05$).

along the first CAP axis include copepod relative abundance (V9 data), herbivore and corallivore abundance (UVC data), as well as sponge and anthozoan relative abundance (*cox*1 data).

The percent variation explained on the axes of each ordination revealed that variables used including NPP, rubble percent, and abundances of upper trophic groups, explained a higher amount of variation in the water samples than in the sediment samples. Furthermore, the percent explained by the CAP axes in the 0.4-μm water samples (47.8%) was 10.4% higher than the percent explained on the CAP axes for the 12-μm water samples (37.4%), suggesting that smaller planktonic protist communities were more strongly impacted by the variables used in these models. The sediment ordinations on the other hand explained 23.9% and 27.2% of the variation in the 0.4- and 12-μm samples, respectively. This drop in percent explanation between water and sediment samples suggests that benthic communities were not as strongly impacted by the variables used in these models as planktonic communities. The difference could also be reflective of the community differences between water and sediment samples. The sediment samples had a much larger proportion of low-abundance taxa that individually contributed to less than 1% of each sample but collectively contributed to on average 10%–20% more of the reads in sediment samples than they did in water samples at the same sites.

Co-occurrence networks

Given the significance of copepod relative abundance in explaining variation among certain samples in the CAP ordinations, the V9 dataset was re-filtered to include both protist and copepod ASVs, in order to evaluate co-occurrence among copepods and various protist groups. The network constructed using all of the collection sites across Lombok had a total of 88 edges, which was considerably less than the networks constructed for each subset of sites. The networks for the NE, NW, and SW sites had 311, 461, and 280 edges respectively, suggesting high spatial structuring of communities across the island. The networks for each individual grouping of sites had distinct patterns of co-occurrence among taxonomic groups further supporting the high spatial structuring of these communities. More specifically, there are a higher proportion of edges involving heterotrophic groups in the SW and the NW where rubble percent was higher (Figure 5). This shift was most notable in the

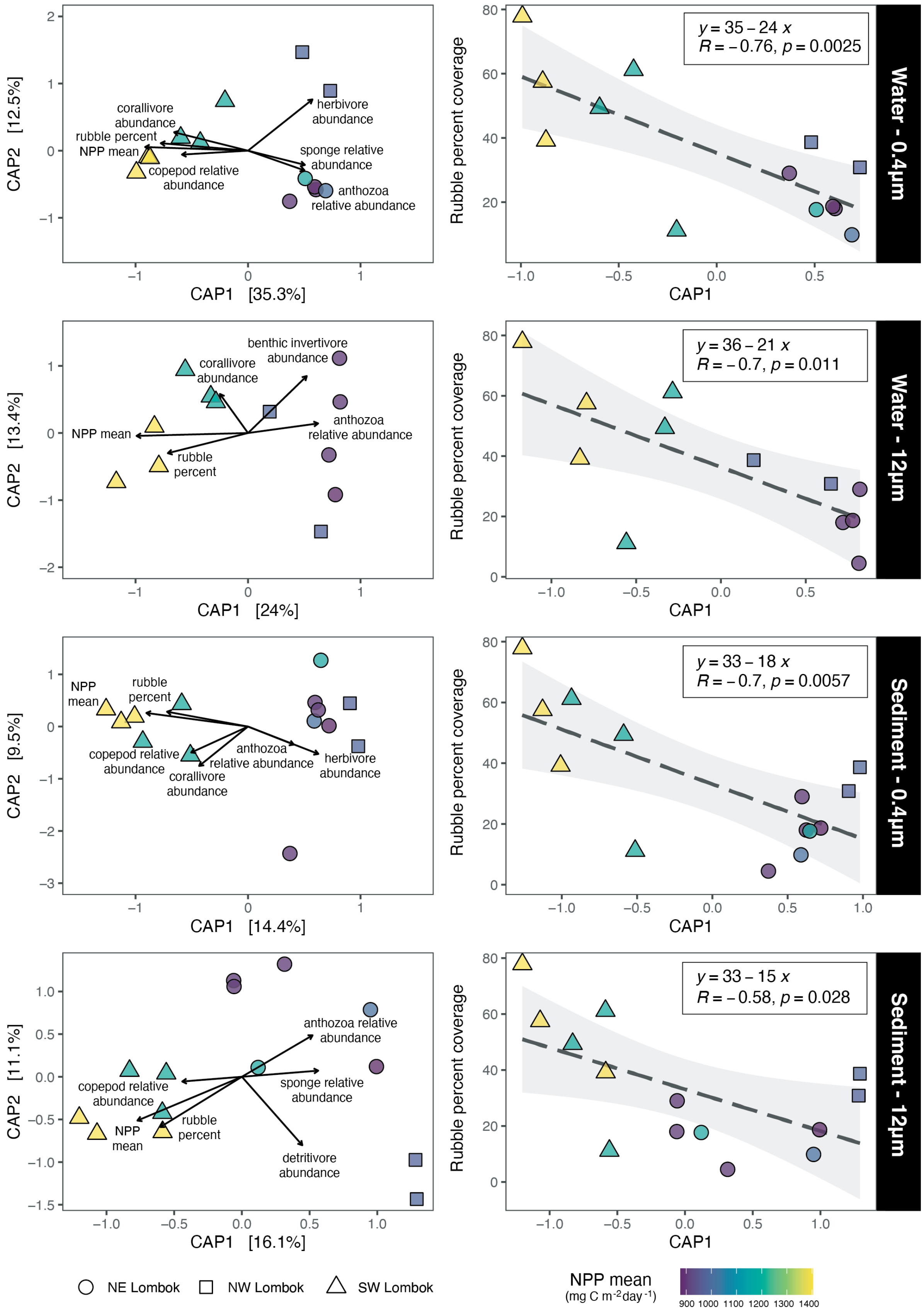

FIGURE 4 CAP ordinations of V9 samples constructed using Bray–Curtis dissimilarity, and regressions showing a significant correlation between CAP axes and rubble percent at each given site. Fish trophic level abundance data and rubble percent came from UVC data, mean NPP came from MSEC, copepod relative abundance data came from V9 sequence data, and sponge and anthozoan relative abundance came from cox1 sequence data.

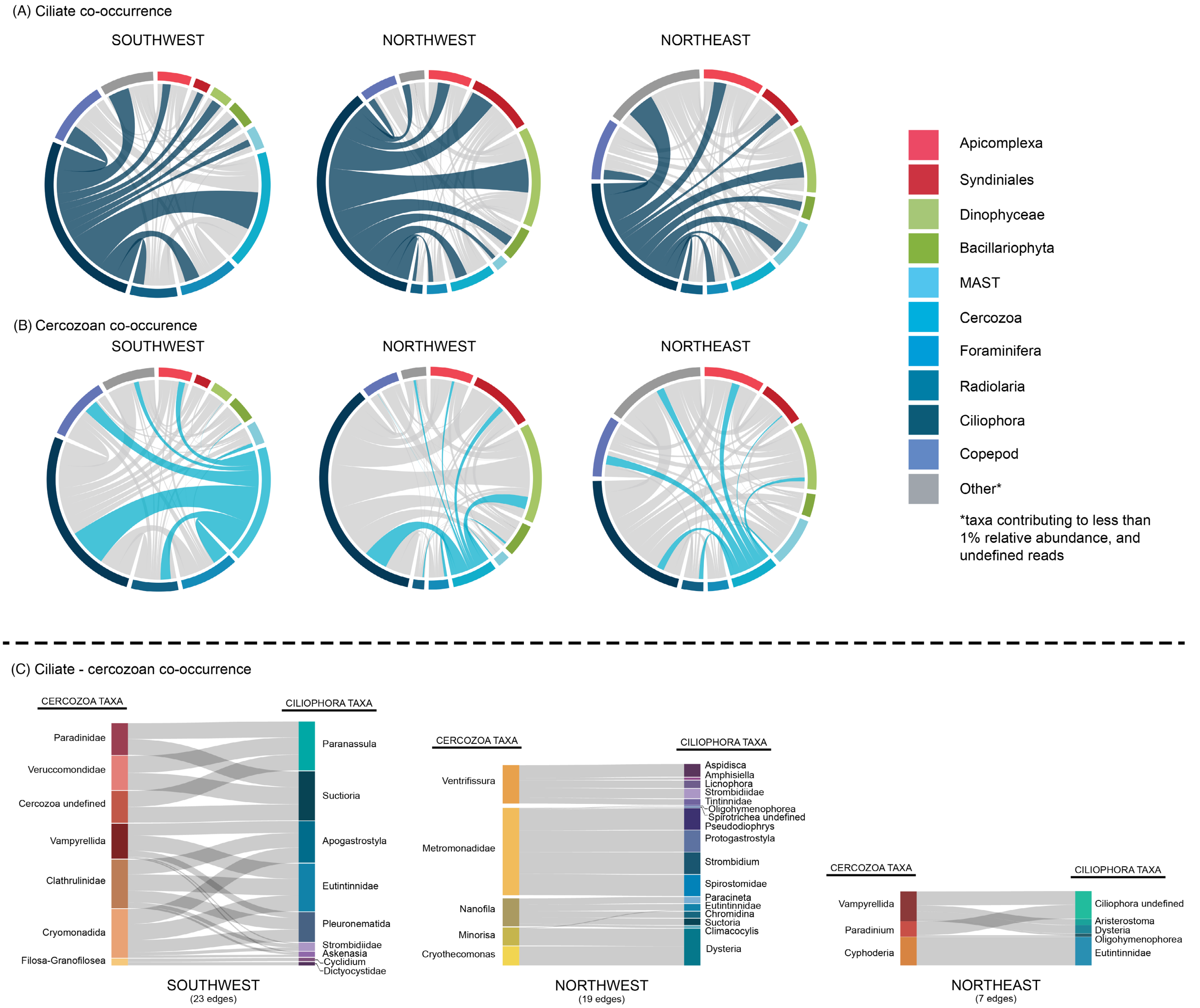

FIGURE 5 Co-occurrence networks constructed using WGCNA with (A) ciliate edges highlighted and (B) cercozoan edges highlighted, and (C) the co-occurrence between ciliates and cercozoans at lower taxonomic level, showing the stronger co-occurrence between these groups in the northwest and southwest regions. Edge thickness corresponds to the number of edges between two groups (i.e. the thicker the edge the more co-occurrence between the two groups). Color groupings in (A) and (B) were used to indicate ecological roles: Reds for parasitic groups, greens for autotrophic groups, and blues for heterotrophic groups.

ciliates, which had low co-occurrence in NE sites (111 edges, 36% of network edges), higher co-occurrence in SW sites (129 edges, 46% of network edges), and highest co-occurrence in NW sites (223 edges, 48% of network edges) (Figure 5A). Cercozoans also showed a higher number of edges involving other heterotrophic groups in the NW and SW sites compared to NE sites (Figure 5B).

Co-occurrence between the ciliates and cercozoans alone also showed a similar pattern, with the greatest number of edges between these groups occurring in the SW sites (23 edges), followed closely by the NW sites (19 edges), and the least number of edges in the NE sites (7 edges). The co-occurring ciliate and cercozoan taxa in each site grouping also varied. In the NW and SW, most edges between ciliates and cercozoans occurred between bacterivorous species and species that feed on small phytoplankton. However, the edges connecting ciliates in cercozoans in the network generated using the NE sites occurred between various functional groups including parasites, bacterivores, and other heterotrophic groups.

DISCUSSION

Variation in protist community composition across Lombok was best explained by percent rubble and mean net primary productivity (NPP) per site (Figure 4). While other variables, like anthozoa and sponge relative abundance, contributed to the percent variation explained on the canonical analysis of principal coordinates (CAP) axes, rubble cover and mean NPP were the only variables that explained significant variation in 0.4 and 12 μm size fractions in both water and sediment samples. The sites with higher rubble cover and mean NPP were

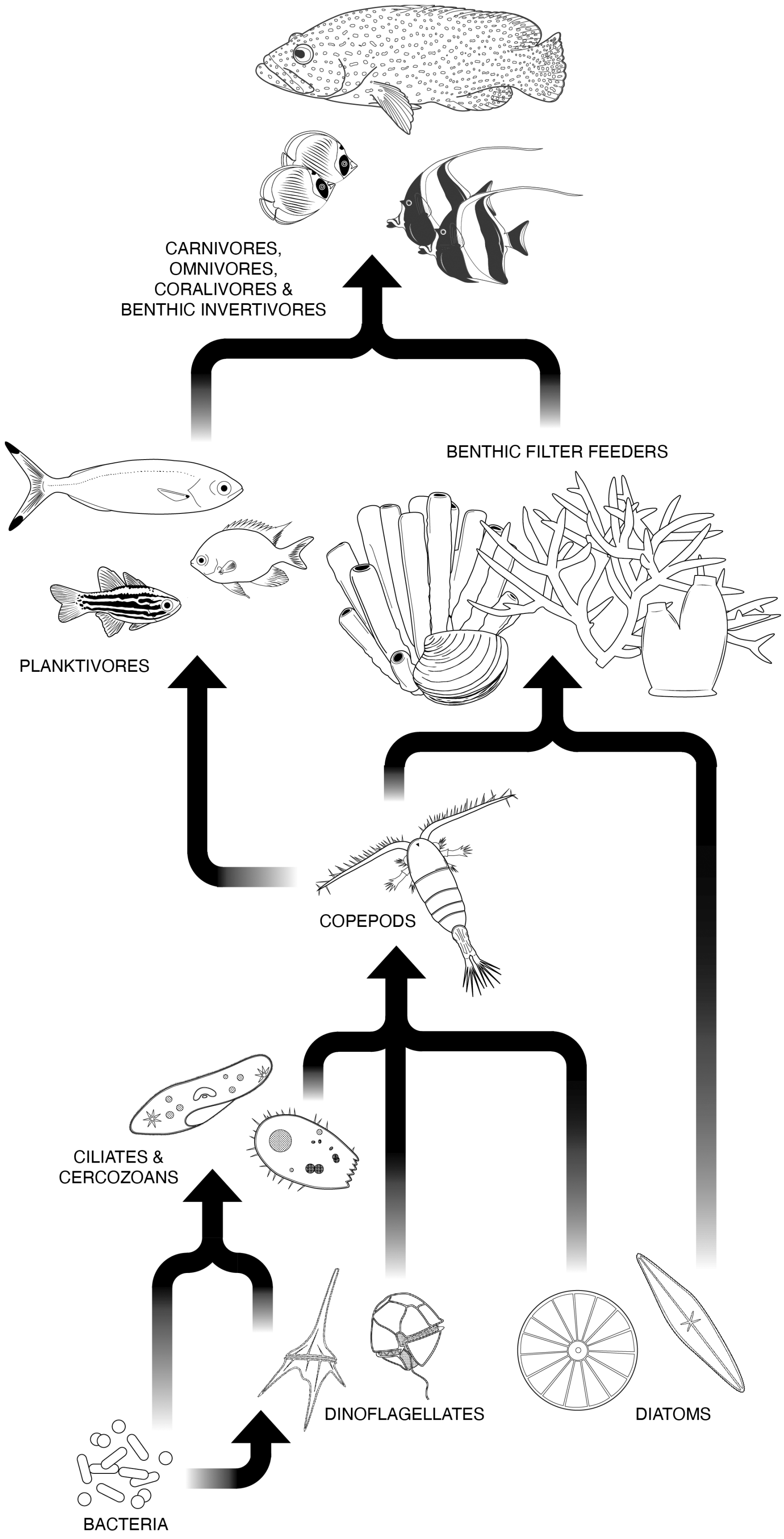

FIGURE 6 Diagram showing energy flow through the food web.

characterized by increased relative abundance in ciliates (Ciliophora), cercozoans (Cercozoa), and diatoms (Bacillariophyta) (Figure 2). Despite these shifts in relative abundance, the alpha diversity in all protist groups showed no significant change across all site groupings (Figure 3). The lack of change in alpha diversity suggests that the observed shifts in relative abundance were likely driven by certain species taking advantage of environmental differences across sites, rather than new species coming into certain sites and establishing themselves. The correlation between these shifts in community composition and rubble coverage suggests that disturbances resulting in rubble fields, and the subsequent changes in food web dynamics, play a significant role in structuring protist communities across Lombok. The increase in diatom relative abundance at sites with high rubble cover also corresponds with sites with high NPP. This correlation suggests that in addition to shifting food web dynamics as a result of disturbances, nutrient levels may also play a role in structuring these communities.

Water vs. sediment communities

Given the wide range of ecological roles protists serve in marine environments, water, and surface sediment protist communities have distinct compositions, despite their proximity to one another. While community composition in our water samples remains fairly consistent within groupings of sampling sites, the sediment samples show much more variation across all sites. Our sediment samples show a higher relative abundance of parasitic groups, like apicomplexans, compared to water samples, which is consistent with sediment communities sampled across other parts of the globe, including Antarctic marine sediments (Cleary & Durbin, 2016) and neotropical soils (Mahé et al., 2017). However, while apicomplexans are the dominant parasitic group in Antarctic marine sediments and neotropical soils, the dominant parasitic group in our dataset is the Syndiniales dinoflagellates. Our data also show on average 10–20% more undefined reads (encompassed in "Other" in Figure 2) in sediment samples compared to water samples. This high level of unknown diversity in marine sediments is consistent with other metabarcoding surveys across the globe (Forster et al., 2016; Massana et al., 2015; Santoferrara et al., 2020).

While sediment samples appeared to have more variation in community composition from site to site than water samples, the variables used to construct the CAP ordinations only explained a minimal amount of the variation across sediment samples. More specifically, the CAP ordinations for sediment explained half as much variation in the 0.4-μm samples, and a tenth of the variation in the 12-μm samples, compared to water samples in those same size fractions (Figure 4). The lack of variation explained in the sediment ordinations suggests that disturbances resulting in rubble fields, and the subsequent effects on the pelagic food web, have a minimal impact on sediment protist communities. These ordinations also suggest that we are missing other environmental variables that play more important roles in structuring benthic protist communities. The variability in benthic communities and low percent explanation of the CAP axes could also be reflective of variations in life history across protists. Benthic environments often act as a "seed bank" for planktonic diversity as planktonic protists transition between benthic and pelagic life stages (Massana et al., 2015). Populations of planktonic species that have benthic cyst stages are also hard to predict, with some lasting days, years, or occurring on seasonal cycles (Satta et al., 2010). This variability in benthic stages makes these organisms difficult to study and further complicates the understanding of variables driving benthic protist community structure.

Response of heterotrophic protists in rubble fields

Despite the importance of both free-living and symbiotic microbial communities on coral reefs, our understanding of how these communities respond to disturbances largely comes from studies on bacterial communities and their roles and responses to coral disease and bleaching events (Bourne et al., 2008, 2009; Mao-Jones et al., 2010). However, protists play important roles as both producers and consumers in cycling nutrients in these ecosystems and play a role in controlling bacterial growth on reefs (Silveira et al., 2017). Additionally, given the ability of microbial organisms to respond rapidly to changing environmental conditions (Glasl et al., 2019), protists allow us to readily identify the impacts of these disturbances at a microbial level which in turn will have consequences for ecosystem recovery throughout the food web.

Ciliates and cercozoans both play important roles as top-down controls on microbial communities across marine environments by consuming bacteria and small protists (Flues et al., 2017; Glücksman et al., 2010; Gonzalez et al., 1990; Hall et al., 1993). While there have been no studies on these groups and their response in rubble fields, there have been studies on the associations between ciliates and some of the most widespread coral diseases (Katz et al., 2014; Sweet et al., 2014; Sweet & Bythell, 2012). Whereas some of the ciliates associated with these diseases have been identified as bacterivores, others have been identified as ciliatovores and are likely feeding on the ciliates drawn to the skeletons by bacteria (Sweet & Séré, 2016). A similar dynamic could be happening in rubble fields. Ciliates are initially drawn to bacteria breaking down organic material in the rubble field, which triggers a chain reaction attracting more ciliates, and other small grazers, to feed on them. While the increase in co-occurrence among small heterotrophic protists in sites with high rubble cover does not necessarily indicate ecological interactions it does suggest that the spatial structure in these groups is likely driven by similar environmental variables (Figure 5). In the case of the NW and SW sites, those variables appear to be rubble percent and mean NPP, as indicated in the CAP ordinations. However, it is important to note, the high co-occurrence among these heterotrophic groups at those sites does not necessarily indicate that they are directly interacting with one another.

In addition to highlighting potential environmental drivers of community composition shifts, the CAP ordinations also revealed the differential impact of those variables across size fractions. In water samples, CAP axes explained 47.8% of the variation in 0.4-μm samples and 37.4% of variation in the 12-μm samples (Figure 4). The difference in percent explanation between size fractions suggests that rubble per cent and the other variables used in the analysis had stronger effects on smaller protists like ciliates and cercozoans than they did on larger protists like diatoms. This difference further suggests that other variables, not accounted for in our analysis, better explain community variation in larger cell-size communities. In particular, organisms with smaller body sizes have larger dispersal ranges than organisms with larger body sizes, due to environmental factors like surface currents, cell sinking rates, and grazing (Villarino et al., 2018). While rubble per cent and food web dynamics account for 37.4% of the variation in 12-μm samples, it is likely that variables such as grazing and sinking, which we were unable to directly account for, make up the difference in percent explanation between the 12- and 0.4-μm samples.

Phytoplankton dynamics in rubble fields

The two dominant phytoplankton groups in our samples were the dinoflagellates (Dinophyceae) and the diatoms (Bacillariophyceae), both of which have been studied extensively across the globe. Global surveys of phytoplankton communities have elucidated biogeographic structure in these communities and revealed environmental factors responsible for such community structure. In particular, these studies have highlighted the importance of abiotic factors like temperature and nutrients in structuring phytoplankton communities on a broad-geographic scale (Chust et al., 2013; Sunagawa et al., 2015). While diatoms have more success in nutrient-rich regions, dinoflagellates are typically more successful in oligotrophic regions (Edwards et al., 2015; Litchman et al., 2007). In addition to nutrient dynamics, ocean circulation also appears to play an important role in shaping phytoplankton communities. More specifically, choke points in ocean circulation at transitions between ocean basins have corresponded directly with choke points in diatom diversity (Malviya et al., 2016). Together, these results suggest that ocean circulation determines the potential geographic range of phytoplankton, but abiotic environmental factors such as nutrients and temperature determine the realized geographic range of these communities.

In our dataset, we see a higher relative abundance of diatoms in the 12-μm water samples, on the western side of Lombok. The two primary nutrient inputs to our sites from across the island would include runoff and upwelling. Runoff in this region is typically highest during the wet season (Oct–Mar) and results in high levels of regenerated nutrients entering the water. Upwelling, on the other hand, is strongest during the dry season (Jun–Sep), and results in a high supply of new nutrients to coastal waters in this region (Hendiarti et al., 2004; Ningsih et al., 2013; Susanto et al., 2001). Just as diatoms and dinoflagellates have a preference for nutrient-rich and oligotrophic regions, respectively, they also have a preference for new and regenerated nutrients, respectively. Dinoflagellates are typically better able to utilize regenerated nutrients (ammonia), which are forms of nitrogen resulting from nitrogenous waste products of heterotrophic organisms, whereas diatoms are typically more successful in the presence of new nutrients (e.g. nitrate) (Kopczynska et al., 2001; Olofsson et al., 2019). If upwelling or runoff were driving the shifts we see in phytoplankton communities across Lombok, we would expect to see consistency in phytoplankton community structure at all of our sites, due to their locations along the coasts and consistent sampling time frames. However, instead, we see a distinct shift in communities between eastern and western sites on Lombok.

While most studies on phytoplankton communities focus on broad-scale biogeographic trends and abiotic factors driving these trends, recent data suggest that biotic interactions may play a more important role in shaping these communities on a local geographic scale (Sommeria-Klein et al., 2020). In particular, these studies have focused on the impact that grazing can have on shaping phytoplankton communities from the top down. Given the size difference between most diatoms and dinoflagellates (diatoms dominate the 12 μm samples, dinoflagellates dominate the 0.4 μm samples), these groups face different pressures when it comes to grazing. Diatoms, for example, are the primary food source for copepods (Jagadeesan et al., 2017; Liu et al., 2016), while dinoflagellates face grazing pressure from smaller plankton like ciliates (Pierce & Turner, 1992).

The increase in small grazers, including ciliates and cercozoans, at sites with high rubble coverage could suggest additional top-down pressure on dinoflagellate communities. Likewise, the presence of these grazers creates additional competition for food with dinoflagellates given that many dinoflagellates are bacterivores, whereas diatoms are not. In addition to the increased grazing pressure on smaller phytoplankton at these sites, there is also a lower abundance of larger grazers like planktivorous fish. This may, in turn, remove some grazing pressure on larger phytoplankton-like diatoms, allowing them to be more successful at those sites (Figure 6). The increased relative abundance in diatoms could also help explain why copepod relative abundance also appears connected to rubble percent and mean NPP in the CAP ordinations (Figure 4). Both diatoms and ciliates are important components of copepod diets (Calbet & Saiz, 2005; Jagadeesan et al., 2017), and the increased relative abundance of both of these groups, combined with a decrease in abundance of higher trophic level predators at sites with high rubble percent, could allow for copepods to be more successful there.

Characterizing protist community response to varying environmental conditions and disturbances on a small geographic scale is important for understanding how those disturbances potentially impact the microbial food web, and in turn, biogeochemical cycling in the ecosystem. Our study shows that sites with high rubble coverage are characterized by an increased relative abundance of small grazers, including ciliates and cercozoans. In addition to small grazers, these sites were also characterized by an increased relative abundance of diatoms, which is likely reflective of shifting food web dynamics in rubble fields as opposed to abiotic factors like nutrients resulting from upwelling or runoff. These results provide insight on how protist communities on coral reefs respond to sudden and dramatic ecosystem disturbances and also provide insight on what environmental factors appear most important in shaping these communities on a local level.

AUTHOR CONTRIBUTIONS

EB processed the samples in the lab, ran the data analysis, and wrote the manuscript. PC, FS, and BS collected and cleaned the UVC data. ER and IPA helped EB process samples in the lab. HM, AH, and CL, helped collect samples in the field and secured funding for the project. All authors have contributed to editing the manuscript.

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DATA AVAILABILITY STATEMENT

Raw sequence data is available on NCBI in the sequence read archive (SRA) under BioProject number PRJNA846789. All metadata and scripts used in data analysis are publicly available on GitHub (https://github.com/ErinBorbee/Lombok_protistDynamics).

ORCID

Erin M. Borbee https://orcid.org/0000-0002-2659-1828
Christopher E. Lane https://orcid.org/0000-0003-2558-2767

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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