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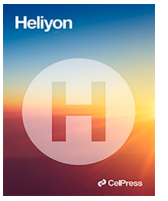

Research article

Water depth outweighs reef condition in shaping non-geniculate coralline algae-associated microbial communities in coral reefs: A case study from Thailand

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ABSTRACT

Red calcified non-geniculate coralline algae (NGCA) provide habitat structures, stabilize reef structures, and foster coral larval settlement and metamorphosis. Moreover, the microbes associated with NGCA are dependent on the NGCA host species and are affected by environmental factors; however, little is known about the influence of reef conditions and depth gradients on the associated microbial communities and NGCA. In this study, we collected NGCA under different reef conditions and depth gradients and characterized the microbial communities using the V3–V4 hypervariable regions of the 16S rRNA gene. Metagenomic analysis revealed 2 domains, 51 phyla, 123 classes, and 210 genera. The NGCA-associated bacterial communities were dominated by Proteobacteria, Bacteroidetes, Chloroflexi, Actinobacteria, and Acidobacteriota. Gammaproteobacteria and Alphaproteobacteria were the most abundant bacterial classes. Differences in microbial diversity and richness were not apparent between reef conditions and depth gradients. However, there was a significant difference in bacterial evenness among the depth gradients. The bacterial abundance associated with NGCA was greater in deep zones than in shallow zones. The shallow zone exhibited a greater relative abundance of all gene functions than the deep zone, indicating differences in the distribution of gene functions. This study showed that the microbial communities associated with red calcified NGCA are diverse, and that the depth gradient affects their abundance and evenness, highlighting the need for further research to understand the functional roles of these microbial communities in coral reef conservation.

1. Introduction

Non-geniculate coralline red algae (NGCA) are important ecosystem engineers, reef builders, and carbon sequestrators [1,2]. Non-geniculate coralline red algae can create and provide habitats and settlement substrata for diverse marine organisms and host a variety of microbial assemblages [3]. They can foster the recruitment, settlement, and metamorphosis of the larval stages of numerous invertebrates, such as corals, economically important molluscs, abalone, echinoderms, and sponges, through chemical cues and bacterial biofilms that accumulate on the surface of NGCA [3–5].

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Currently, NGCA abundance and community structure are influenced by environmental changes and stress, such as climate change, ocean acidification, bleaching, sedimentation, light, and nutrient enrichment [6–9]. These environmental variables can decrease algal growth, calcification, and cover [10]. Consequently, the loss of NGCA habitats can affect the settlement and metamorphosis of many larval stages of flora and fauna, as well as trigger shifts in the microbial composition and abundance [7]. Although NGCA has been reported to be an important habitat for corals, invertebrates, and bacteria, little is known about the abundance, community structure, and diversity of the microbial communities associated with NGCA.

More recently, a few studies have investigated the relationship between crustose coralline algae and -microbial communities in terms of biomineralization and metamorphosis [11]. Alga-microbial communities are species-specific and dynamic in response to environmental changes and stress [2,3,7]. The relative abundances of microbial communities are affected by salinity, light intensity, water temperature, nutrient concentration, depth, habitat type, and algal health statuses [2,7,12–18].

Evaluating different habitats and depth gradients, depth is reported as an important factor driving changes in the bacterial composition [19] related to nutrient availability and sedimentation rates [20]. Coral reef degradation, coral bleaching, and coralline algal bleaching commonly occur under the influence of climate change and anthropogenic stresses, which can affect microbial communities [21,22]. Yang et al. [2] found that *Alphaproteobacteria*, *Gammaproteobacteria*, and *Bacteroidetes* were the most dominant phyla, and that the bacterial composition was similar between healthy and bleached crustose coralline alga, *Porolithon onkodes*. However, they found differences in the relative abundances among algal health conditions. Similarly, the bacterial compositions in healthy and bleached corals were not significantly different [23]. Nevertheless, few studies have been conducted on how depth and reef conditions (degraded *vs*. fair reefs) influence the microbial communities associated with NGCA and how these communities shift in response to environmental changes.

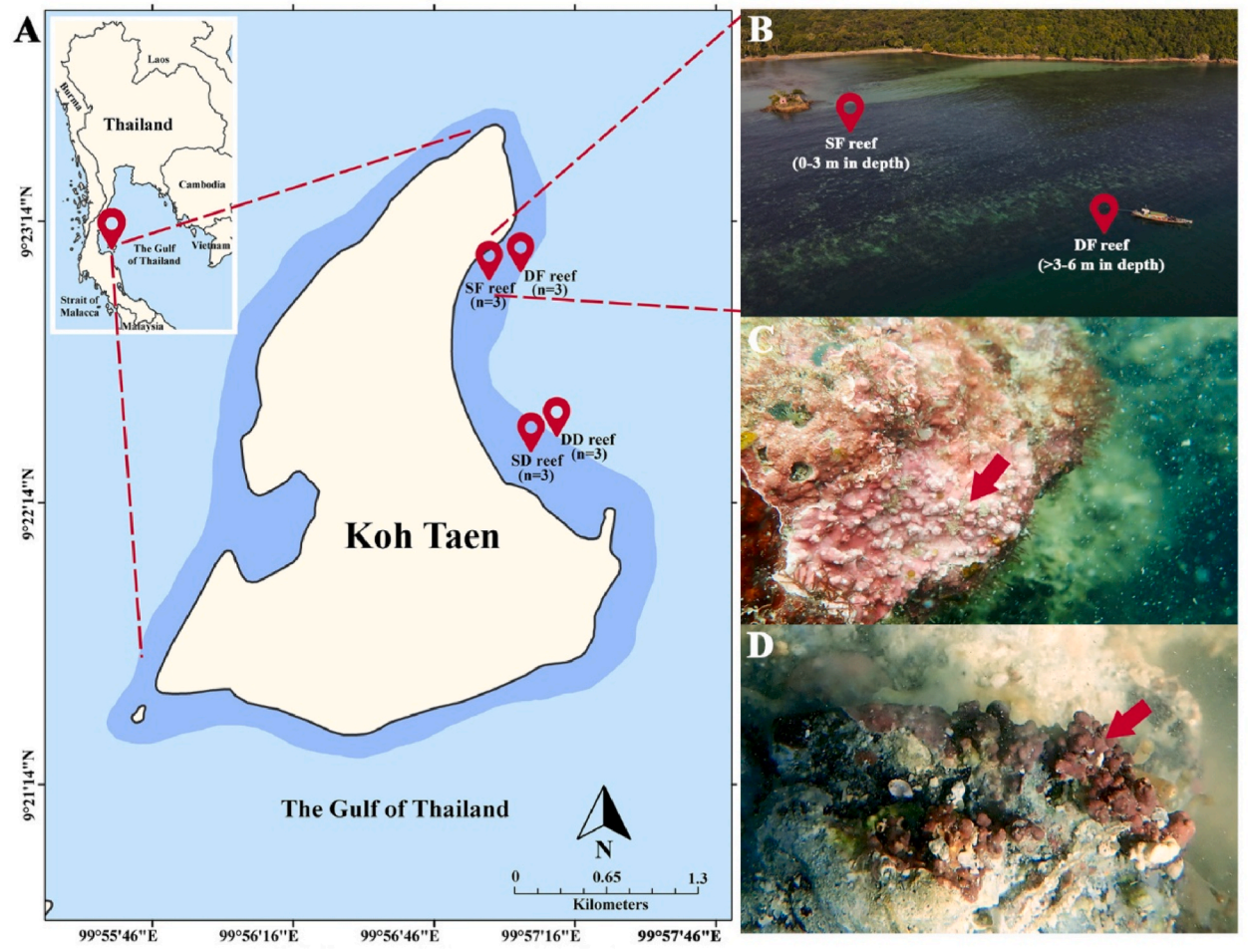

Fig. 1. Study site having a gently sloping reef on the east side of Koh Taen, Surat Thani Province in the lower Gulf of Thailand. Twelve samples of non-geniculate coralline algae were collected from the coral reefs at four different sites. (A) The reefs presented different conditions at each site: shallow zone of a fair reef (SF), deep zone of a fair reef (DF), shallow zone of a degraded reef (SD), and deep zone of a degraded reef (DD). (B) View of the subtidal fair reef (SF and DF) taken using a drone (DJI Mavic Air 2; SZ DJI Technology Co., Ltd., China). (C) *Parvicellularium* and (D) *Sporolithon*, indicated by the red arrow, were collected and placed immediately in sterile microcentrifuge tubes or plastic bags and stored at −20 °C for DNA extraction and sequencing.

The aim of the present study was to examine the diversity of microbial communities associated with NGCA under different coral reef conditions and depth gradients using next-generation sequencing of the V3–V4 hypervariable regions of the 16S rRNA gene. The results of this study are expected to contribute to our understanding of shifts in microbial communities between different coral reef conditions and depth gradients. Additionally, providing information on beneficial bacterial NGCA that can aid in coral settlement may help bolster the resilience of coral reefs.

2. Materials and methods

2.1. Sample collection

The collection site was located at Koh Taen, Mu Ko Thale Tai National Park on the southern coast of the Gulf of Thailand (GPS location; 9°23' N and 99°57' E, Fig. 1A and B). This region has two distinct seasons: at dry season, which lasts from February to September, and at rainy season, which lasts from October to January. *Porites lutea* is a massive coral that dominates the shallow zone (around 3 m), whereas branching corals such as *Acropora* spp. and *Pavona decussata* dominate the deep zone and grow alongside massive corals such as *P. lutea*, *Diploastrea heliopore*, *Platygyra daedalea*, and *Symphyllia* sp. Marine algae are abundant, with approximately 60 identified species [24]. Primary macroalgal genera (or groups) observed are *Padina*, *Sargassum*, *Lobophora*, *Rhipidosiphon*, *Parvocaulis*, red turf algae, and coralline algae (based on personal observation). According to our DNA barcode analyses, two genera of NGCA, *Parvicellularium* Caragnano, Foetisch, Maneveldt & Payri, 2018 (Fig. 1C), and *Sporolithon* Heydrich, 1897 (Fig. 1D), are present in the study site, with NGCA showing a higher abundance in the deep zone.

The study site was divided into two reef conditions, a fair reef (9°22'28" N, 99°57'21" E, Fig. 1B) and a degraded reef (9°23'04" N, 99°57'06" E). The reefs were located approximately 1.3 km apart. Coral reef conditions were categorized based on the Department of Marine and Coastal Resources (DMCR), Thailand classification of the ratio of live: dead coral cover of 1:1 as a fair reef and 1:>1 as a degraded reef. Koh Taen is a one destination for snorkeling and SCUBA diving of shallow subtidal coral reefs. Mooring buoys on tourist boats, canal digging, touching, and stepping on living corals are significant factors in degradation, especially in degraded reef areas. For each reef condition, NGCA samples were collected from two depth gradients: a shallow zone (0–3 m) and a deep zone (3–6 m**)**. The distance between these two depths was approximately 600–650 m. Therefore, there were four reef zones in this study: 1) the shallow zone of the fair reef (SF), 2) the deep zone of the fair reef (DF), 3) the shallow zone of the degraded reef (SD), and 4) the deep zone of the degraded reef (DD). Regarding environmental factors, the light intensities were 286.54 ± 177.45, 260.22 ± 178.01, 168.07 ± 50.83, and 165.37 ± 148.46 μmole photon m^{-1}. s^{-1} in the SF, DF, SD, and DD sites, respectively. The water temperatures in the SF, DF, SD, and DD sites were 31.06 ± 2.06, 30.43 ± 1.62, 30.29 ± 1.29, and 30.48 ± 1.42 °C, respectively. The suspended sediment concentrations in the SF, DF, SD, and DD sites were 0.023, 0.017, 0.022, and 0.022 g/L, respectively.

In February 2021, the whole NGCA specimens (50–100 g) from three patches of dead coral (10 × 10 cm^2) were randomly collected from each reef zone. All 12 specimens were placed in sterile microcentrifuge tubes or plastic bags filled with 50 mL 0.2 μm-filtered seawater and stored at −20 °C until DNA extraction. The three environmental factors were measured at each site. Light intensity and water temperature were measured using an Onset Hobo data logger (Model UA-002-64; Onset Computer Corporation, Contoocook, NH, USA). The suspended sediment concentration was calculated as the dry weight of the suspended sediment per 1 L of seawater.

2.2. DNA extraction and 16S rRNA gene sequencing

Total bacterial genomic DNA was extracted from the whole NGCA specimen using the DNeasy® PowerSoil® Pro Kit (QIAGEN Co., Ltd., Hilden, Germany) according to the manufacturer's instructions. Genomic DNA integrity was verified by agarose gel electrophoresis. The concentration of extracted DNA was quantified using at DS-11 Series spectrophotometer (DeNovix Inc., Wilmington, USA). DNA extracts were placed on dry ice and sent to GENEWIZ Biological Technology Co., Ltd. (Suzhou, China) for sequencing. The purified genomic DNA was used as the template for amplification of the partial 16S rRNA gene using the universal bacterial primers PCR 319F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GACTACHVGGGTATCTAATCC-3′) to capture the V3–V4 hypervariable regions. The total volume of each polymerase chain reaction was 25 μL, containing 2.5 μL of TransStart Buffer (TransGen, Beijing, China), 2 μL of dNTPs, 1 μL of each primer, and 20–30 ng of template DNA [25]. With the Index PCR product, the final libraries were purified using AMPure XP beads (Beckman Coulter, Indianapolis, USA) before quantification. Sequencing was performed on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) to generate a 2 × 300 bp paired-end sequence.

2.3. Bioinformatics and statistical analysis

The raw sequence datasets were analyzed using the QIIME2 pipeline v.2022.2 [26]. Quality control (Q30) and denoising were filtered before the feature table was constructed using the DADA2 pipeline [27]. Representative sequences retrieved from the DADA2 results were used to perform multiple sequence alignments using the MAFFT algorithm [28] and to construct the phylogenetic tree using FastTree [29]. The taxonomic composition of the samples was classified using a naïve Bayes classifier trained on the Silva database v.138.1 [30] at 95 % similarity.

Microbial data are reported as relative abundance. The species diversity was calculated using the Shannon diversity index. Species richness and evenness were calculated using the observed features and Pielou's evenness index. Species richness and evenness among conditions were verified using the pairwise Kruskal–Wallis test. Principal coordinate analysis (PCoA) was based on an unweighted UniFrac distance matrix, and the differences in microbial communities between reef conditions and depth gradients were quantified

and tested with permutational multivariate analysis of variance (PERMANOVA) using beta and beta-phylogenetic methods of the QIME2 software [31]. Linear discriminant analysis for effect size (LEfSe) [32] was used to determine the significant differences between each group of specific prokaryotic microbial taxa. The LEfSe was performed on the website http://huttenhower.sph.harvard.edu/galaxy/with an LDA threshold score of 2.0. Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) [33] was used to predict the microbial community function using 16S rRNA profiles.

3. Results

The diversity and abundance of bacteria associated with NGCA were investigated using next-generation sequencing. Taxonomic classification using QIIME identified 2 domains 51 bacterial phyla, 123 classes, and 210 genera associated with NGCA. According to the dataset, the phyla in the Archaea domain were found to have a low relative abundance of <2 % at all sites, whereas the phyla in the Bacteria domain were dominant, with relative abundances ranging from 98.74 % to 99.93 % (minimum–maximum). Altogether, 51 bacterial phyla were found: Acetothermia, Acidobacteriota, Actinobacteriota, AncK6, Armatimonadota, Bacteroidetes, Bdellovibrionota, Calditrichota, Campilobacterota, Chloroflexi, Cloacimonadota, Cyanobacteria, Dadabacteria, Deferrisomatota, Deinococcota, Dependentiae, Desul-fobacterota, Elusimicrobiota, Entotheonellaeota, FCPU426, Fibrobacterota, Firmicutes, Fusobacteriota, Gemmatimonadota, Halanaerobiaeota, Hydrogenedentes, Latescibacterota, LCP-89, Margulisbacteria, Marinimicrobia, MBNT15, Modulibacteria, Myxococcota, NB1-j, Nitrospinota, Nitrospirota, Patescibacteria, PAUC34f, Planctomycetota, Poribacteria, Proteobacteria, SAR324, Schekmanbacteria, Spirochaetota, Sumerlaeota, Sva0485, Synergistota, Verrucomicrobiota, WPS-2, WS2, and Zixibacteria. The phylum Proteobacteria was the most abundant (27.80–57.41 %), followed by Bacteroidetes (2.96–16.42 %), Chloroflexi (0.94–32.49 %), Actinobacteriota (2.68–8.36 %), and Acidobacteriota (1.63–8.00 %), respectively (Fig. 2).

Under the four different reef conditions, the most abundant bacterial phylum was Proteobacteria presenting a relative abundance of 43.22 ± 8.08 % (mean ± SE) at the DD site, 49.11 ± 4.21 % at the DF site, 51.91 ± 3.47 % at the SD site, and 50.12 ± 1.92 % at the SF site. Within the class level, most microbial communities belonged to Gammaproteobacteria (18.91 ± 41.35 %) and Alphaproteobacteria (8.87 ± 27.55 %). The highest relative abundance of Gammaproteobacteria (36.70 ± 4.14 %) was found at SD, and whereas the lowest abundance (26.53 ± 4.14 %) was found at DD (Fig. 3). The Gammaproteobacterium *Woeseia* (family Woeseiaceae) was found at all study sites, followed by *Endozoicomonas* and *KI89A* (Table S1, Supplementary Information). Additionally, Alphaproteobacteria showed the highest relative abundance (19.86 ± 2.30 %) at SF, and the lowest (14.74 ± 2.30 %) at DF (Fig. 3). The

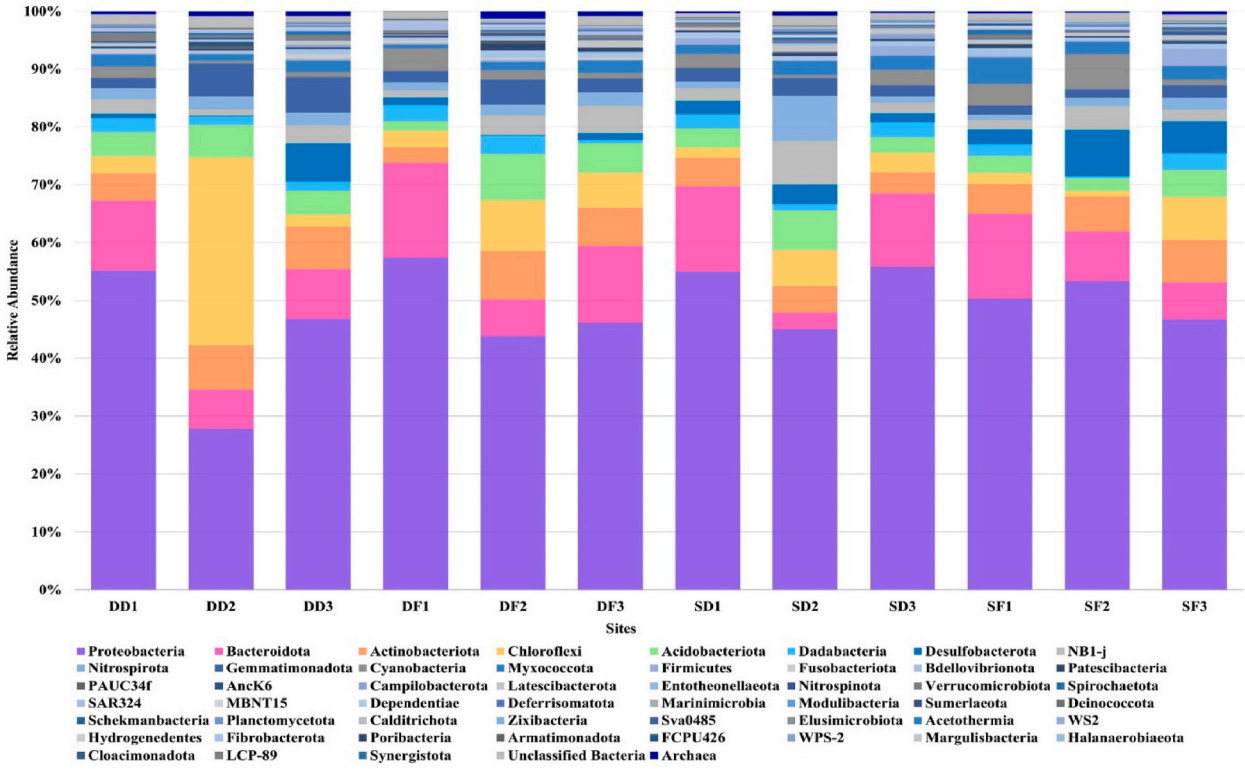

Fig. 2. Relative abundance (% sequences) of microbial assemblages associated with non-geniculate coralline algae (NGCA) at the phylum taxonomic level from the deep zone of a degraded reef (DD), shallow zone of a degraded reef (SD), deep zone of a fair reef (DF), and shallow zone of a fair reef (SF).

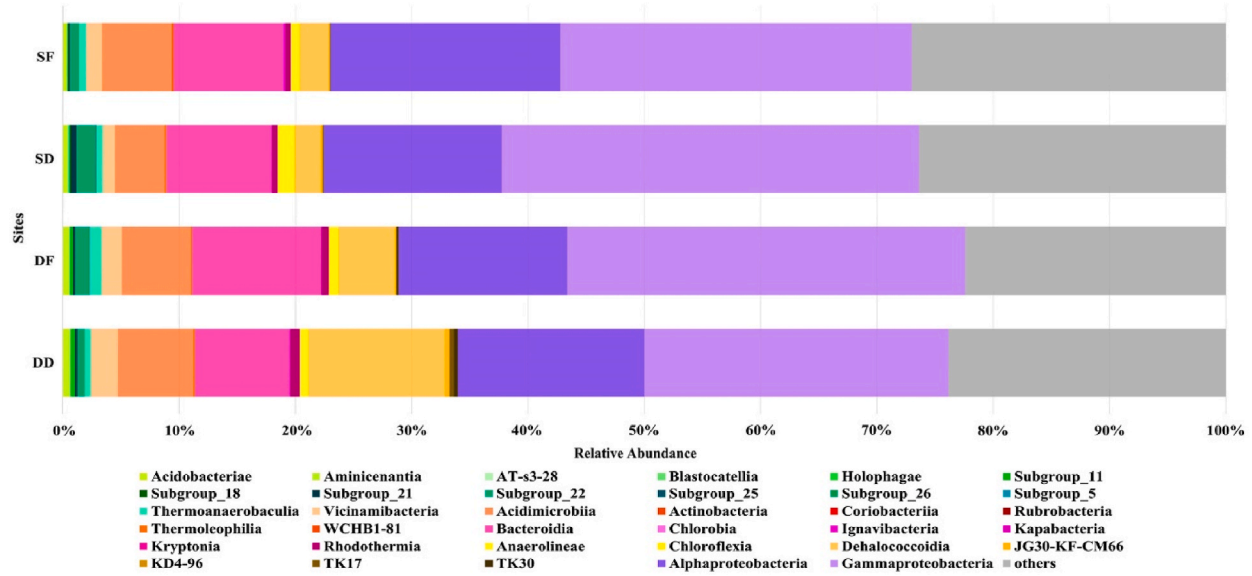

Fig. 3. Relative abundance of non-geniculate coralline algae (NGCA)-associated bacteria among the combination of water depth and reef conditions. The most abundant ones: Acidobacteriota (green), Actinobacteria (orange), Bacteroidetes (pink), and Proteobacteria (purple). DD, deep zone of a degraded reef; DF, deep zone of a fair reef; SD, shallow zone of a degraded reef; and SF, shallow zone of a fair reef.

Alphaproteobacterium *Ruegeria* (family Rhodobacteraceae) was isolated from the collection sites (Table S1).

Bacteroidetes showed relative percentages of 9.20 ± 1.58 %, 12.04 ± 2.96 %, 10.14 ± 3.64 %, and 9.91 ± 2.47 % at the DD, DF, SD, and SF sites, respectively. The Bacteroidetes bacterium *Muricauda* (family Flavobacteriaceae) was found throughout the subtidal reef (Table S1). The relative abundance of Chloroflexi was 12.51 ± 9.99 %, 5.98 ± 1.72 %, 3.80 ± 1.32 %, and 3.40 ± 2.01 % at the DD, DF, SD, and SF sites, respectively. A Chloroflexi bacterium *SAR202* (class Dehalococcoidia) and an Actinobacterium *Sva0996 marine group* were highly abundant at the DD site (9.93 ± 8.69 % and 4.44 ± 1.16 %, respectively). The relative abundance of Actinobacteria was 6.58 ± 0.95 %, 5.89 ± 1.68 %, 4.39 ± 0.37 %, and 6.18 ± 0.62 % at the DD, DF, SD, and SF sites, respectively. Other bacterial phyla, such as Gemmatimonadota (e.g., *BD2-11 terrestrial group*), NB1-j, and Nitrospirota (e.g., *Nitrospira*) were also commonly found in the NGCA tissue and surface (Table S1).The highest Shannon diversity index was found in the deep zones with values of 5.18 and 5.16 for the degraded and fair reefs, respectively. In the shallow zone, the index values were 5.09 and 4.99 for the fair and degraded reefs, respectively. However, there were no significant differences in bacterial richness among the four locations according to Faith's phylogenetic diversity metric (Kruskal-Wallis test, $p > 0.05$, Fig. 4A). In contrast, bacterial evenness was significantly different in the DF compared with that in the SD and SF sites (Kruskal–Wallis test, $p = 0.05$, Fig. 4B). Overall bacterial abundance was higher in the deep zones than in the shallow zones (Two-way ANOVA, $p < 0.05$, Table 1). Regarding microbial community composition, the unweighted UniFrac distance showed no significant differences in homogenous bacterial communities among the four sampling sites (Pairwise PERMANOVA test, $p > 0.05$, Fig. 5A), and the PCoA plot using unweighted UniFrac distance also suggested that dissimilarity was low among the microbiome structures (Fig. 5B).

According to the LEfSe analysis, 40 clades of prokaryotes were screened with an LDA threshold score of 2.0. The abundance of the family Phormidesmiaceae, order Gastranaerophilales, genus *Blastocatella* (a member of the family Blastocatellaceae), and 11 genera of uncultured bacteria, including JG30-KF-CM45, was high at the SF site. Class Nitrospiria, order Nitrospirales, family Nitrospiraceae, Margulisbacteria, Ardenticatenales, and Latesscibacteriaceae were highly abundant at the SD site, whereas *Roseimarinus* and uncultured Bacteroidetes were the dominant genera at the DF site. At the DD site, the uncultured bacteria JG30-KF-CM66 and TK17 of Phylum Chloroflexi were dominant (Fig. 6A and B).

Regarding the functions of bacterial communities in the deep and shallow zones, a summary of the distribution patterns of some ecologically potential bacteria associated with NGCA compared between depth gradients is provided in Table S1. The ecological importance of most bacteria is related to their marine biogeochemical cycles, including their ability to utilize carbon, nitrogen, phosphorus, and sulfur. For example, the nitrate reducer *Nitrospira calida*, sulfate oxidizer *Fusibacter* sp., and organic matter decomposer genera *BD2-11, Dadabacteria, KI89A*, and *SAR202* were present at all sites. Moreover, the coral and fish pathogens *Vibrio ponticus* and *Tenacibaculum maritimum* were found in the reef patches. Two pathogenic genera, *Francisella* and *Malaciobacter* of various marine hosts were found only in the deep zone, whereas the pathogen *Halarcobacter* was found in the shallow zone. Notably, three coral probiotics, *Acinetobacter, Endozoicomonas atrinae*, and *Ruegeria* spp., were collected from all the study sites. Although bacterial diversity was not significantly different among the sites, bacterial abundance differed along the depth gradients (Table 1).

A total of 103 bacterial genera were found in the shallow zone. Many organic compound decomposers and fermenters such as *Bacillus*, *Clostridium sensu stricto 1*, *Clostridium sensu stricto 13*, *Hydrogenispora*, *Lactobacillus*, *Lewinella*, *Lutibacter*, *Portibacter*, *Rubidimonas*, *Sunxiuqinia*, *V2072-189E03*, and *WCHB1-81* were only recorded in the shallow zone. A high abundance of Desulfobacterota,

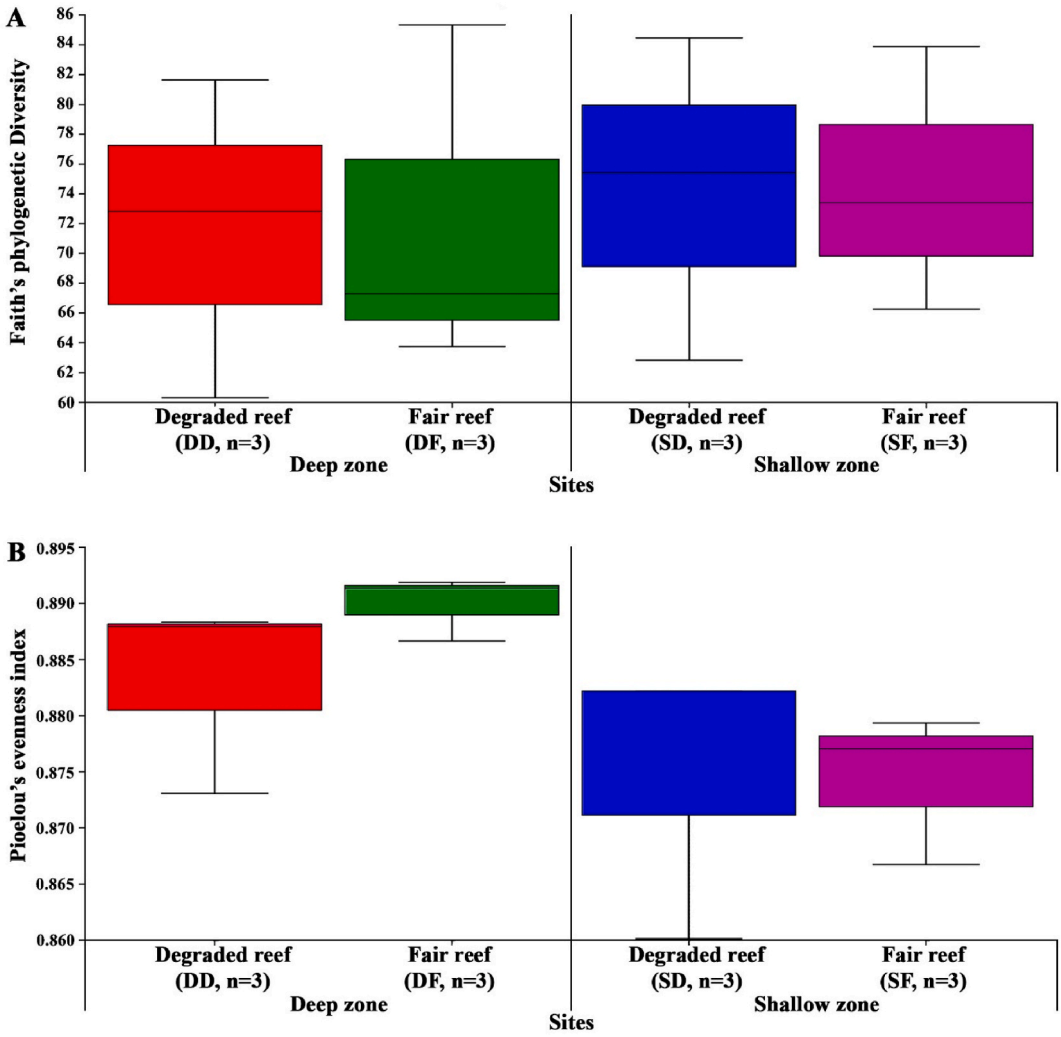

Fig. 4. Boxplots of bacterial richness and evenness analyzed by (A) Faith's phylogenetic diversity (PD Faith), and (B) Pielou's evenness using the Kruskal–Wallis test within all samples of each site. DD, deep zone of a degraded reef; DF, deep zone of a fair reef; SD, shallow zone of a degraded reef; and SF, shallow zone of a fair reef.

Table 1
Bacterial richness and evenness were analyzed using two-way ANOVA between reef conditions (Fair vs. Degraded) and depth gradients (Deep vs. Shallow).

		df	Mean Square	F	Sig.
Richness	depth gradients	1	0.333	0.000	0.991
	reef statuses	1	8.333	0.003	0.957
	depth gradients × reef statuses	1	645.333	0.241	0.637
	Error	8	2677.583		
	Total	12			
Evenness	depth gradients	1	76366165	6.769	0.032
	reef statuses	1	318828	0.028	0.871
	depth gradients × reef statuses	1	7990272	0.708	0.424
	Error	8	11281243		
	Total	12			

including *Desulfatiglans*, *Desulfobulbus*, *Desulfofaba*, *Desulfosarcina*, and *Desulfurivibrio*, which play important roles in the carbon and sulfur cycles, was determined. Regarding the deep zone, a total of 92 bacterial genera appeared specifically in the deep zone, among which the coral larval metamorphosis, inducing *Pseudoalteromonas luteoviolacea* and a biocontrol bacterium that inhibits the coral pathogen *Aureispira* sp. were found in the deep zone. Some bacterial genera found in our samples (e.g., *Albidovulum*, *Labrenzia*, and

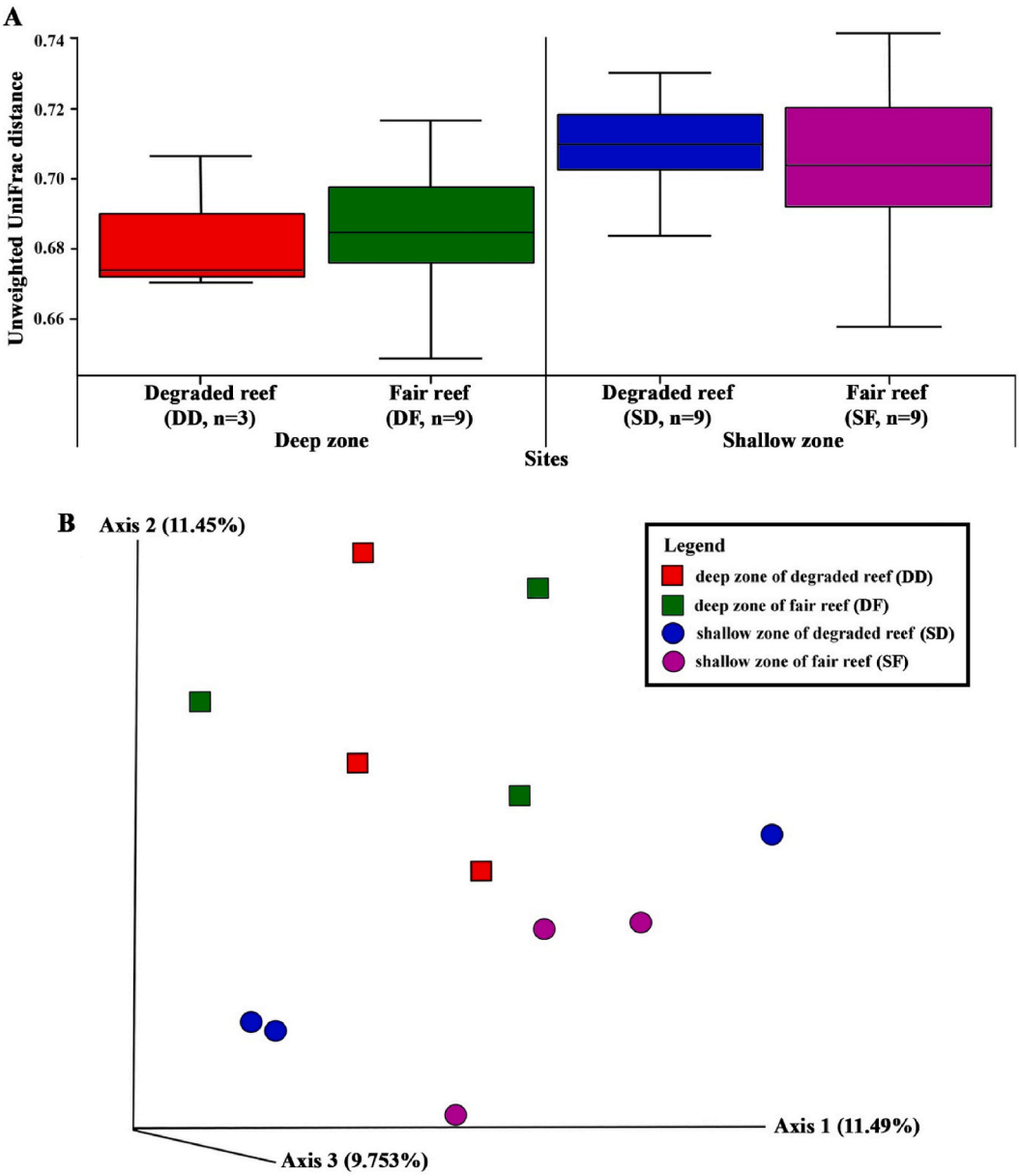

Fig. 5. Beta diversity boxplots (A) comparing the bacterial communities in the deep zone of the degraded reef (DD) with those of the other groups. The unweighted UniFrac distance method was used. DF, deep zone of a fair reef; SD, shallow zone of a degraded reef; and SF, shallow zone of a fair reef. (B) The PCoA plot was constructed using the unweighted UniFrac distances of all study sites.

Photobacterium) could cleave dimethylsulfoniopropionate (DMSP).

According to the PICRUSt functional prediction, all sites showed 38 functional subgroups (level 2) that belonged to the main groups (level 1) of cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organismal systems. The shallow zone exhibited a higher relative abundance of gene functions than the deep zone for all gene functions (Fig. 7A). In addition, 328 KEGG pathways were identified across all samples, of which the top 40 most frequently occurring abundances among the conditions are presented in a heatmap. The dominant pathways under all conditions were the transporter, ABC transporter, general function, DNA repair, and recombination protein pathways (Fig. 7B).

4. Discussion

The composition of the microbial community associated with NGCA in Thai waters was assessed. Our study shows that, as markers for species-level microbial classification, the V3–V4 hypervariable regions of the 16S rRNA gene have potential, similar to that

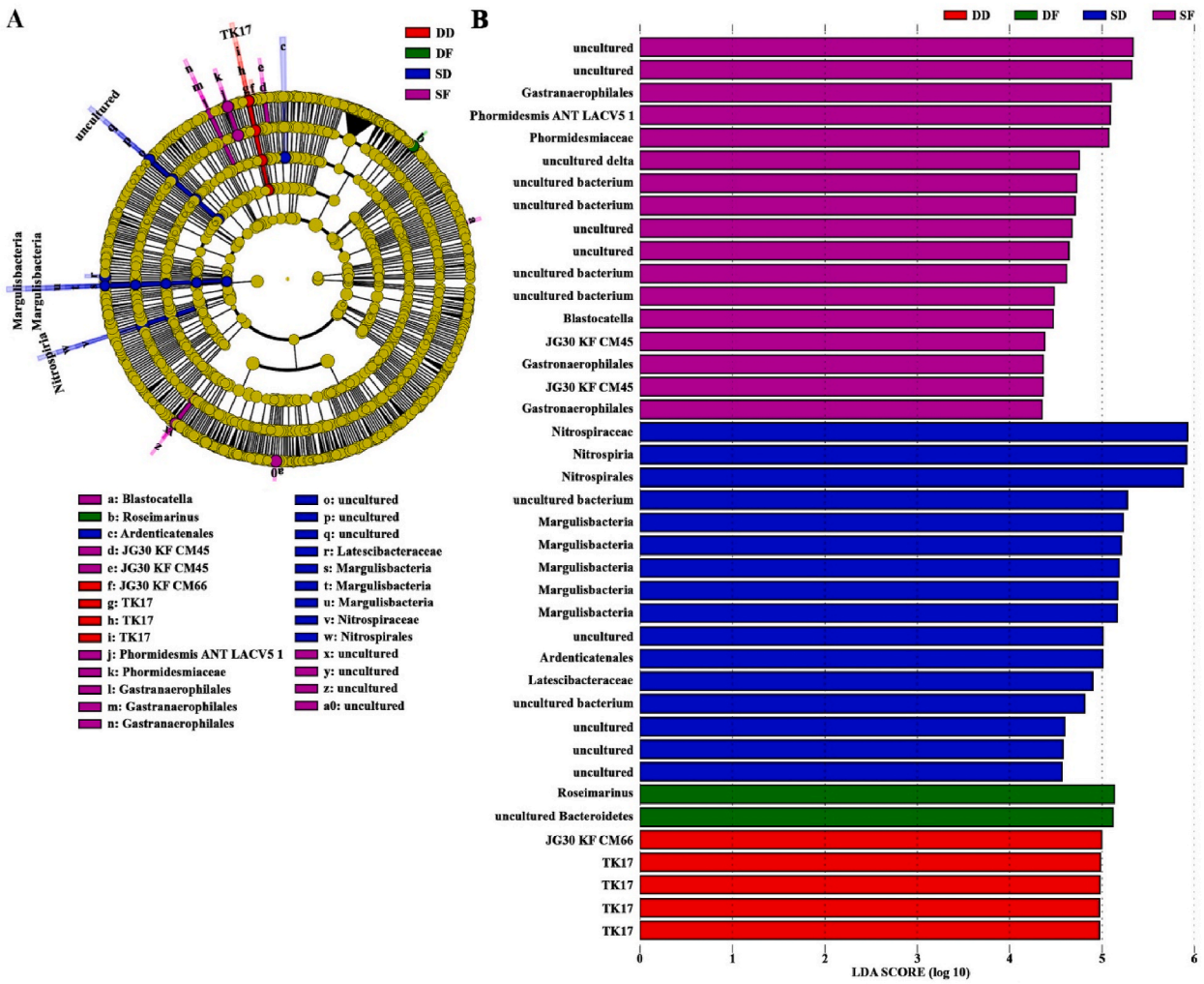

Fig. 6. (A) Microbial abundances from the four study sites are presented using the linear discriminant analysis effect size (LEfSe) plot. (B) The LEfSe cladogram and LDA threshold score (2.0) results show taxa with significant differences between reef conditions and depth gradients. DD, deep zone of a degraded reef; DF, deep zone of a fair reef; SD, shallow zone of a degraded reef; and SF, shallow zone of a fair reef.

reported in previous studies [2,34,35]. In the present study, the Archaea domain was found to have a relative abundance of less than 2 % in the subtidal reef. Low abundance of the phylum Archaea is found in coral reefs, but this is usually the case in polluted marine environments [36]. A total of 51 bacterial phyla were recorded, of which Proteobacteria was the core member, as previously reported by Nimnoi and Pongsilp [37] and Saipan et al. [38] who studied bacterial communities in seawater in the upper Gulf of Thailand and Koh Tachai in the Andaman Sea. Proteobacteria is commonly suggested to be a dominant bacterial phylum associated with the coral *Porites lutea* Quoy & Gaimard, 1833 [39] and NGCA *Neogoniolithon* sp [40].

Gammaproteobacteria and Alphaproteobacteria were highly abundant in the reefs. Members of Gammaproteobacteria and Alphaproteobacteria are widely distributed in marine ecosystems and can metabolize organic compounds and nutrients [41]. These results are consistent with those of previous studies [19,37–39,42]. For example, the globally prominent Gammaproteobacteria genera *Woeseia* and *KI89A* play important ecological roles in biogeochemical cycling in marine sediment environments [43]. The three coral probiotic genera, *Acinetobacter*, *Endozoicomonas* (phylum Gammaproteobacteria), and *Ruegeria* (phylum Alphaproteobacteria), contribute to carbon fixation, sulfate reduction, and nutrient translocation within their host. Photosymbionts can produce DMSP and various breakdown products (e.g., DMS, acrylate, and methanesulfonic acid) performing a protective function against heat or oxidative stress on the host surface [44]. Therefore, these bioactive compounds may prevent bleaching and pathogenic invasion of corals [45]. The present study suggests that three beneficial bacteria, *Acinetobacter*, *Endozoicomonas*, and *Ruegeria*, are associated with NGCA, possibly supporting host metabolism.

Gammaproteobacteria, Alphaproteobacteria, and Actinobacteria are the most dominant classes of the four crustose coralline algal species in the Caribbean Sea [3]. In the sea southwest of the United Kingdom, Brodie et al. [13] found the highest abundances of Gammaproteobacteria, Alphaproteobacteria, Flavobacteria, and Bacteroidetes on the surface of the geniculated coralline algae,

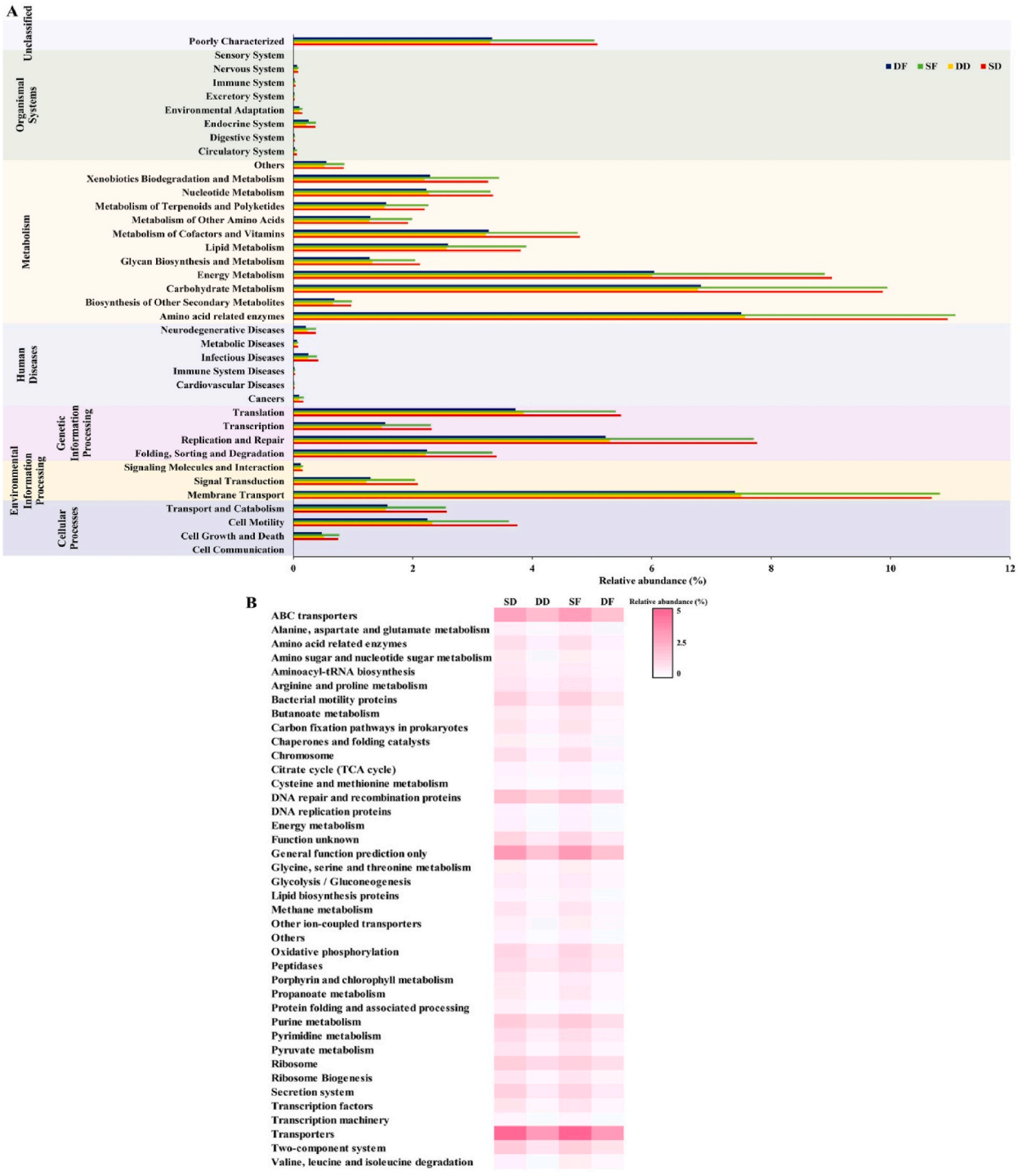

Fig. 7. (A) PICRUSt analysis showing the predicted functional main groups (level 1) and subgroups (level 2) from all site conditions. (B) Heatmap showing the top 40 most dominant pathways among different conditions. DD, deep zone of a degraded reef; DF, deep zone of a fair reef; SD, shallow zone of a degraded reef; and SF, shallow zone of a fair reef.

Corallina officinalis Linnaeus, 1758. However, they found differences in bacterial communities at different sites and depths.

In the present study, we found that diversity relative abundance of dominant NGCA-associated bacteria was not significantly different between fair and degraded coral reefs. Among these dominant bacteria, Alphaproteobacteria, Gammaproteobacteria and Bacteroidetes were the most abundant bacteria at all sites, indicating the core microbiome in our NGCA samples regardless of reef

conditions. Yang et al. [2] found that Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes were the dominant phyla and their relative abundances differ among bleached, semi-bleached, and healthy coralline algae (*P. onkodes*). Thus, we hypothesized that the health status of NGCA plays more important role than surrounding reef conditions on the NGCA-associated bacterial relative abundance. Alternatively, other unexamined abiotic factors play more important role in shaping their relative abundance than reef conditions. We envisage that further expanding experiments would shed light into this aspect.

In our study, we found the coral pathogenic bacteria *Francisella*, *Malaciobacter*, *Halarcobacter*, and *Vibrio* in the surveyed reefs. These genera are typically found in degraded coral reefs [46,47]. At our study site, the deterioration in reef areas is caused by careless tourism (use of mooring buoys by tourist boats and stepping on living corals), canal excavation, and local fisheries. These factors can result in coral mortality, bleaching, disease, decreased NGCA, and proliferation of fleshy macroalgae, leading to changes in bacterial communities and abundance [48–50]. The dead or diseased coral colony and other benthic macroalgae could then serve and host different compositions of the microbial community [51–53], which might explain why major marine pathogens occasionally appear in degraded reefs.

In the present study, there was a significant difference in the microbial-NGCA communities between the depth gradients. Bacterial abundance was higher in deep zones than in shallow zones. This finding was consistent with the higher percentage cover of NGCA in the deep zones. A greater coralline algal surface area and structural complexity provide more opportunities for microbial colonization [54]. In the present study, we found a high abundance of different marine pathogens such as *Francisella* sp., *Tenacibaculum maritimum*, *Malaciobacter* sp., and *Vibrio ponticus* in the deep zone. In addition, the filamentous bacterium *Aureispira* sp. was abundant in the samples. This bacterium suppresses the growth of *Vibrio* by inhibiting calcium ion uptake [55,56]. A beneficial *Aureispira* species may support the recruitment of young corals that grow over NGCA colonies. In addition, the coral metamorphosis-inducing bacterium *Pseudoalteromonas luteoviolacea* possesses antimicrobial enzyme activity that destroys other coral pathogens, especially *Vibrio* spp [57]. The probiotics *Aureispira* and *Pseudoalteromonas* were only present in the deep zone of the fair reef, and one year later (February 2022), we found that young coral settlement occurred above the NGCA in the deep zone of this study site. Therefore, these two bacteria may be suggested as biological indicators for assessing the suitable coral-NGCA health for future coral restoration. Moreover, the importance of DMSP-degrading bacteria could positively affect the high abundance of other microorganisms associated with NGCA in the deep zone. Dimethylsulfoniopropionate-demethylating bacteria (family Rhodobacteraceae) such as *Albidovulum, Labrenzia*, and *Photobacterium* vary with depth. The breakdown of DMSP supports coral-NGCA growth by increasing the availability of carbon and sulfur. The antibacterial compound of the DMSP production pathway inhibits coral pathogens and contributes to the control of microbial communities associated with corals [44] and NGCA.

Previous studies have reported that microbial abundance is affected by environmental factors such as seawater temperature, light intensity, turbidity, nutrient levels, and habitat [13,16,58–61]. Depth gradients are related to other local factors such as light intensity, organic matter concentration, and nutrient availability [20]. In the present study, the suspended matter content was high in the shallow zone, which could be an indirect factor driving the bacteria-NGCA community. We found that the bacterial decomposers in the phylum Bacteroidetes, such as *WCHB1-81, Lewinella, Portibacter*, and *Rubidimonas*, generally occurred when total organic matter was high in the shallow zone. The several organic matter–degrading bacteria such as *SAR202, Geothermobacter, Weissella, Paenibacillus, BD2-11 terrestrial group*, and *Phycisphaera* showed considerably high abundances in the deep zone under conditions of low sedimentation. Different organic matter concentrations can provide particular growth in various bacterial communities [62]. In a study conducted on the Great Barrier Reef in Australia, Hernandez-Agreda et al. [14] suggested that the bacteria associated with the coral community increased in diversity with depth, probably because increased exposure to water currents in deep reefs allowed for greater acquisition of nutrients by the bacterial community. Román et al. [17] also reported that the proportion and occurrence of microbial communities on the deep-sea floor were high because of the nutrient enrichment that occurs from coastal depths to deep waters and the pelagic productivity that modulates food availability in the deep microbial biosphere.

According to the metagenomic functional content prediction from the 16S rRNA gene, highly abundant functions in the shallow-water environment, especially in cellular processes, environmental information processing, genetic information processing, and metabolism, are probably related to diverse autotrophic and heterotrophic microorganisms. The majority of transporter pathways enabled strong biological productivity and biogeochemical heterogeneity, consistent with the observations reported by Pop Ristova et al. [63]. Upward microbial process activity in shallow areas results in high environmental and genetic expression patterns. However, to predict the functional potential of a reef ecosystem, a long-term investigation into the impact of seasonal variability on bacterial communities and description of the different associations between the functional groups of bacteria and NGCA species is necessary.

Thus, our study showed that the increased bacterial composition in the deep zone of the subtidal reef was correlated with NGCA cover, which increased when the algal cover and surface area increased and was related to changing environmental factors across the depth gradient. However, the effects of major environmental factors on the NGCA-bacterial community association require long-term monitoring and more replication in other areas with the same experimental design. Future work should investigate responses to extreme environmental stresses in the process of bacterial-NGCA association. Additionally, the functional roles of NGCA-microbial interactions should be investigated. The current investigation contributes to a better understanding of the interactive effects of environmental factors on NGCA-microbial communities, reef acclimatization, and resilience.

5. Conclusion

This study provided information on the diversity and abundance of bacteria associated with NGCA in Thai waters under different coral reef conditions and depth gradients. The V3–V4 hypervariable regions of the 16S rRNA gene were used as a marker for bacterial identification and classification. The core microbiomes Gammaproteobacteria and Alphaproteobacteria, and Bacteroidetes were the

most abundant at all sites. Bacterial diversity and richness were not significantly different among the reef conditions and depth gradients; however, there was a significant difference in bacterial evenness between the depth gradients. Given that the shallower reef area often encountered higher human and environmental disturbance, we found that bacterial relative abundance associated with NGCA was greater in the deep zone than in the shallow zone, and relative abundance in the degraded reef was slightly higher than in the fair reef. Important coral and fish pathogens *Francisella*, *Halarcobacter*, *Malaciobacter*, *Tenacibaculum maritimum*, and *Vibrio ponticus*, were identified in this study. Based on our observations, it appears that the sensitivity of NGCA-associated bacterial diversity to the environment makes them a promising ecological indicator for monitoring reef condition.

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Ethics declaration

Review and/or approval by an ethics committee was not required because the study did not meet the requirements based on the research involved.

Data availability statement

Genomic DNA sequences are deposited in the NCBI Sequence Read Archive under Bioproject No. PRJNA918332 for microbial diversity and NCBI Accession No. OQ064627– OQ064628 for *Parvicellularium* and *Sporolithon* sequences, respectively.

CRediT authorship contribution statement

Kattika Pattarach: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition. **Komwit Surachat:** Writing – review & editing, Software, Project administration, Methodology, Formal analysis, Conceptualization. **Shao-Lun Liu:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Jaruwan Mayakun:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25486.

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