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Microbial Community Composition and Putative Biogeochemical Functions in the Sediment and Water of Tropical Granite Quarry Lakes

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Abstract

Re-naturalized quarry lakes are important ecosystems, which support complex communities of flora and fauna. Microorganisms associated with sediment and water form the lowest trophic level in these ecosystems and drive biogeochemical cycles. A direct comparison of microbial taxa in water and sediment microbial communities is lacking, which limits our understanding of the dominant functions that are carried out by the water and sediment microbial communities in quarry lakes. In this study, using the 16S rDNA amplicon sequencing approach, we compared microbial communities in the water and sediment in two re-naturalized quarry lakes in Singapore and elucidated putative functions of the sediment and water microbial communities in driving major biogeochemical processes. The richness and diversity of microbial communities in sediments of the quarry lakes were higher than those in the water. The composition of the microbial communities in the sediments from the two quarries was highly similar to one another, while those in the water differed greatly. Although the microbial communities of the sediment and water samples shared some common members, a large number of microbial taxa (at the phylum and genus levels) were prevalent either in sediment or water alone. Our results provide valuable insights into the prevalent biogeochemical processes carried out by water and sediment microbial communities in tropical granite quarry lakes, highlighting distinct microbial processes in water and sediment that contribute to the natural purification of the resident water.

Keywords Quarry lake · Sediment · 16S rDNA amplicon sequencing · Microbial community

Introduction

Quarry lakes form when quarrying activity ceases and the disused sites are naturally filled with rainwater, surface runoff, and/or groundwater. Re-naturalized quarry lakes are important ecosystems which support complex communities of flora and fauna. Microorganisms, the primary colonizers of the quarry lake ecosystem, are believed to play important roles in various environmental processes critical to the re-naturalization of quarry lake ecosystems [15]. In a quarry rich in acidic sulfate soil, microorganisms were found to promote the growth of grasses and shrubs [41]. Microorganisms are also involved in the degradation of anthropogenic chemicals in quarry lakes. For example, microorganisms in the water and sediments were reported to drive the degradation of anthropogenic contaminants such as acrylamide in a quarry lake [27]. In addition, through interacting with solid minerals, microorganisms in quarry lakes also contribute significantly to the biogeochemical cycles of elements (e.g., iron and manganese cycles), weathering, and biodeterioration of solid minerals [9, 25, 46, 63].

Microorganisms in quarry lakes are primarily associated with the sediments and the water. Sediment microbial communities in lakes and marine ecosystems have been frequently linked to methane production, phosphorus cycling, and metal transformation [22–24, 54]. Microorganisms in the water of lakes and other natural water bodies, for example,

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Actinobacteria and *Cyanobacteria*, are often heavily involved in the decomposition of organic matter, carbon fixation, and production of nutrients for heterotrophs [4, 55]. Although microbial communities in both the sediment and water have been reported to contribute greatly to the health of the lakes and other natural water bodies, they may have distinct community structures and functions. In particular, for the quarry lake ecosystem, a direct comparison of microbial taxa in water and sediment microbial communities is lacking, which limits our understanding of the dominant functions that are carried out by the water and sediment microbial communities, respectively, in the recycling of nutrients and purification of resident water in quarry lakes.

The objective of this study was to compare the microbial communities in the water and sediment in re-naturalized quarry lakes. Specifically, we conducted 16S rDNA amplicon sequencing using the water and sediment samples collected from two quarry lakes in Singapore and elucidated dominant potential functions of the sediment and water microbial communities in driving major biogeochemical processes in these ecosystems.

Materials and Methods

Sampling Sites

Water and sediment samples were collected from the lakes formed from the disused Singapore quarry (1° 21′ 23.7″ N, 103° 46′ 20.6″ E) and Ubin quarry (1° 24′ 29.2″ N, 103° 57′ 27.3″ E) in Singapore (Fig. 1a). Both quarry lakes were formed after the antecedent granite quarries ceased operating in the 1990s. The Singapore quarry lake is located near the central catchment of the main island of Singapore in a small (1.64 km^2) nature reserve and is surrounded by a dense rainforest housing over 900 species of trees and ferns and nearly 500 species of fauna [14, 29]. The Ubin quarry lake is located on Pulau Ubin, an offshore island northeast to the main island of Singapore. Pulau Ubin is one of the last rural areas in Singapore and supports an abundance of plant and animal species. Unlike the Singapore quarry lake in which public access is restricted, the Ubin quarry lake is used for recreation frequently.

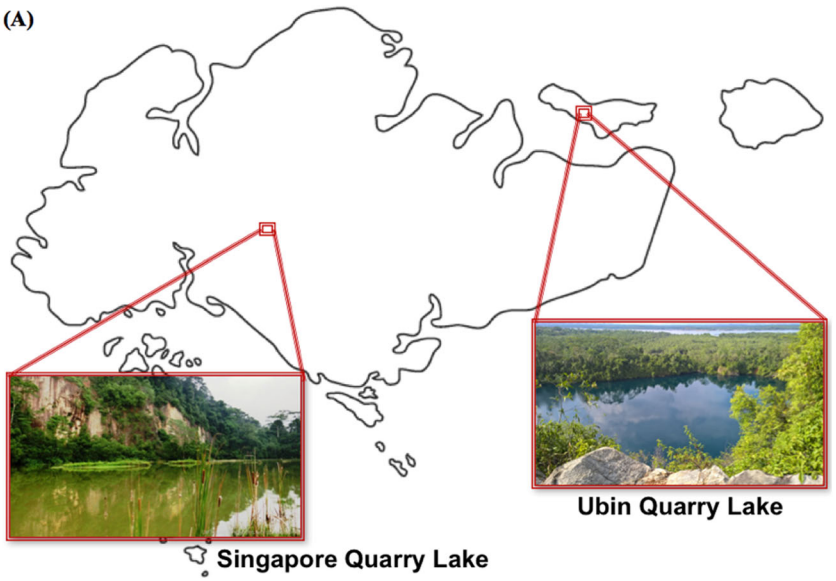

Fig. 1 Geographic locations of the quarry lakes and details of the sediment and water samples. **a** The Singapore quarry lake is located in the mainland Singapore (1° 21′ 23.7″ N, 103° 46′ 20.6″ E) while the Ubin quarry lake (1° 24′ 29.2″ N, 103° 57′ 27.3″ E) is located on an offshore island, Pulau Ubin. **b** Sample, location, depth, and code of sediment and water samples collected. Sediment and water samples were collected from the surface of the Singapore quarry lake. Sediment and water samples were collected from the surface as well as at a depth of 25 m from the Ubin quarry lake

(B)

Sample	Location	Depth	Sample Code
Water	Singapore quarry lake	Surface	W.00.SQ
Sediment	Singapore quarry lake	Surface	S.00.SQ
Water	Ubin quarry lake	Surface	W.00.UQ
Water	Ubin quarry lake	25 m	W.25.UQ
Sediment	Ubin quarry lake	Surface	S.00.UQ
Sediment	Ubin quarry lake	25 m	S.25.UQ

Sample Collection and Water Quality Measurement

The sediment samples from the two quarry lakes were collected using a Ponar sediment grab (Wildco Instruments, Wildlife Supply Company, USA), while the water samples were collected using a Van Dorn water sampler (Wildco Instruments, Wildlife Supply Company, USA). Sediment and water samples were collected from the surface (< 1 m below water level) for the Singapore quarry lake (S.00.SQ and W.00.SQ) and from the surface (< 1 m below water level) (S.00.UQ and W.00UQ) as well as 25 m below the surface for the Ubin quarry lake (S.25.UQ and W.25.UQ) (Fig. 1b). Samples were collected in triplicate. Samples from deeper locations in the Singapore quarry lake could not be collected as the access to those areas of the quarry lake is restricted. The collected water and sediment samples were immediately transferred to ice-packed clean autoclaved carboys and sterile plastic bags, respectively, and transported to the laboratory and stored at −80 °C for further use.

Water quality parameters, including turbidity, pH, oxidation-reduction potential (ORP), nitrate, ammonium, and ammonia, were measured in situ using an EXO2 multiparameter Sonde fitted with water quality sensors (Xylem Analytics, Hemmant, Australia).

Sample Processing and Sequencing

DNA from the sediment samples was extracted using the FastDNA® SPIN Kit for soil (MP Biomedicals, Santa Ana, USA). To extract DNA from water samples, 5 L water was filtered through nitrocellulose filters (diameter 47 mm, pore size 0.2 μm) and the genomic DNA was isolated from the filters using the PowerWater® DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, USA) according to the manufacturer's instructions. Degenerate primers 16SF (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGCMGCCGCGGTAA) (M = A/C) and 16SR (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACNVGGGTATCTAATCC) (N = A/T/C/G, V = G/C/A) were used to amplify an approximately 300 bp amplicon of the hypervariable region four (HV4) of bacterial and archaeal 16S rDNA [36]. The primers were designed to include the Illumina-specific overhang adapter sequences for compatibility with the Illumina index and sequencing adapters. 16S rDNA amplicons were amplified using the 2X Kapa HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington, MA). PCR cycling was carried out by using an initial denaturation step of 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. The final extension step was performed at 72 °C for 5 min. The amplicons were purified by cleaning with Agencourt AMPure XP beads (Beckman Coulter, Brea, CA). Amplified DNA was quantified by Invitrogen Qubit fluorometric quantitation (Thermo Fisher Scientific, Waltham, MA). Amplicons were sequenced using the PCR-free paired-end sequencing approach on an Illumina MiSeq sequencing by synthesis (SBS) platform.

Data Processing

The sequences were uploaded onto the MG-RAST server (version 3.0) [26, 65] under project ID "Quarry Amplicons" (MG-RAST IDs: mgm4706502.3–mgm4706519.3). Reads were preprocessed by using "SolexaQA" to trim low-quality regions. Artificial replication reads were analyzed and removed using "duplicate read inferred sequencing error estimation" (DRISEE). The sequences were screened for contamination using "Bowtie" against *Homo sapiens* NCBI v36 as a reference database [69]. An initial search using "vsearch" against a reduced RNA database, which is a 90% identity-clustered version of SILVA, Greengenes, and RDP databases, was used for rDNA detection. The reads were then clustered at 97% identity using cd-hit, and the longest sequence was picked as the cluster representative. A BLAT (https://genome.ucsc.edu/FAQ/FAQblat) similarity search for the longest cluster representative sequences was performed against SSU, M5RNA, Greengenes, and RDP databases with an E-value cut-off of 1E−10, minimum identity of 97%, and a minimum alignment of 50 bp. The RDP tool on the MG-RAST server produced the highest number of hits in comparison to the other three databases, and hence, it was used for further analysis. The operational taxonomic unit (OTU) table was generated and downloaded in a comma-separated value format for further downstream analysis. The OTU table was filtered to exclude eukaryotic and chloroplast sequences as well as sequences from unidentified domains, and only prokaryotic sequences were kept.

Data Analyses

Subsequent analyses were performed in R using "vegan" and "phyloseq" packages for the analysis of ecological data [42, 49]. All samples were rarefied to a sequencing depth of least-abundant sample for the estimation of diversity and clustering and ordination analyses. Rarefaction was performed using the "phyloseq" package, and the diversity indices (Shannon, Simpson, Chao, and Ace) were estimated from the rarefied data using the "vegan" package. Nonparametric statistical analyses on the rarefied data were performed using "adonis," "anosim," and "betadisper" functions in R [2, 12, 68]. Hierarchical cluster analysis was performed using "hclust" function by Ward's minimum variance method [44, 64]. Nonmetric dimensional scaling (NMDS) ordination was performed using the weighted "UniFrac distance metric" in the "phyloseq" package. NMDS ordination was also performed

using the "Bray-Curtis dissimilarity index" in the vegan package.

For the comparison of OTU abundances across the samples, normalized OTU tables were used instead of rarefied data. All the samples were normalized against the total OTU abundance of individual samples. To compare the OTU abundances among the groups of samples, Welch's test statistics was used. Functional annotation of taxa was performed using the program "functional annotation of prokaryotic taxa" (FAPROTAX) on the normalized OTU table [40]. FAPROTAX is a manually constructed database that maps prokaryotic taxa (e.g., genera or species) to putative functions based on the literature on cultured representatives. Functions represented in FAPROTAX focus on marine and lake biogeochemistry. It comes with a Python script for converting OTU tables into putative functional tables based on the taxa identified in a sample and their functional annotations in the FAPROTAX database. The main limitation of applying this approach to our data is the implicit assumption of FAPROTAX that if all cultured members of a taxon can perform a particular function, then all members of the taxon (cultured and noncultured) can perform that function. Even considering this caveat, we believe that predicting putative functional groups using this approach is superior to genomic prediction approaches based on sequence homology. The predicted abundances of functions among the groups of samples were also compared using Welch's test statistics. Significant differences in the mean values were calculated at the 95% confidence interval ($p < 0.05$). Bonferroni correction was applied, where appropriate, to control for the effect of testing multiple hypotheses simultaneously.

Results and Discussion

Microbial Communities Are Richer and More Diverse in Sediment Than in Water

After removing low-quality sequences and mismatches, a total of 15,886,002 16S rDNA reads were obtained from the 18 samples, which were binned into 3346 OTUs based on 97% or higher sequence similarity. Rarefaction curves of the individual samples were asymptotic, indicating that reasonable sequencing depth was attained (Fig. A.1).

The evenness indices were significantly higher for the microbial communities in the sediments (S.00.SQ, S.00.UQ, and S.25.UQ) than in the corresponding water (W.00.SQ, W.00.UQ, and W.25.UQ) samples ($p < 0.05$) for both the Singapore quarry lake and the Ubin quarry lake (Table 1). The evenness indices ranged from 0.50 ± 0.01 for the deep-sediment sample from the Ubin quarry lake to 0.55 ± 0.02 for the surface sediment collected from the Singapore quarry lake, while those for the water samples were estimated to be in the range of 0.45 ± 0.04 to 0.46 ± 0.01. The Ace and Chao indices, which extrapolate the data to estimate what the actual number of species would have been based on the occurrence of rare species in the samples [13], estimated significantly higher ($p < 0.05$) OTU richness for the deep sediment from the Ubin quarry lake as well as from the surface sediment of the Singapore quarry lake in comparison to the corresponding water samples. The richness estimates for the surface sediment and water from the Ubin quarry lake were comparable.

The γ-diversity, calculated based on Shannon's index, of the microbial communities in the sediments appeared to be considerably higher than that in the water samples (Table 1). The γ-diversity ranged from 3.51 for the deep sediment from the Ubin quarry lake to 3.98 for the surface sediment from the

Table 1 Richness, evenness, and diversity of the groups of samples from the same niche

Sample identity	Observed richness[a]	Richness estimators		Evenness[d]	α-Diversity		γ-Diversity (Shannon)
		Ace[b]	Chao[c]		Simpson[e]	Shannon[f]	
S.00.SQ	1104 ± 46	1255 ± 63	1251 ± 70	0.55 ± 0.02	0.89 ± 0.04	3.85 ± 0.07	3.98
S.00.UQ	1162 ± 17	1347 ± 41	1352 ± 21	0.51 ± 0.02	0.84 ± 0.14	3.61 ± 0.14	3.72
S.25.UQ	1004 ± 7	1143 ± 21	1137 ± 29	0.50 ± 0.01	0.84 ± 0.08	3.42 ± 0.06	3.51
W.00.SQ	735 ± 44	886 ± 43	892 ± 19	0.46 ± 0.01	0.89 ± 0.05	3.04 ± 0.02	3.20
W.00.UQ	1142 ± 118	1320 ± 141	1327 ± 130	0.45 ± 0.04	0.84 ± 0.05	3.15 ± 0.33	3.34
W.25.UQ	814 ± 47	916 ± 46	912 ± 47	0.45 ± 0.02	0.87 ± 0.05	3.04 ± 0.07	3.20

[a] Mean of OTUs observed at a cut-off value of 0.03 (97% sequence identity)

[b] Ace is used to evaluate the actual community richness based on the OTUs containing 1 and 2 reads

[c] Chao is used to evaluate the actual community richness based on the OTUs containing 1–10 reads

[d] Evenness is a measure of relative abundance of different taxa in the community

[e] Simpson's index focuses on major taxa and assesses community diversity as a function of dominance

[f] Shannon's index is used to assess community diversity taking the contributions of rare taxa into account

Singapore quarry lake, while for the water samples, it ranged from 3.20 for the water from the Singapore quarry lake to 3.34 for the water from the Ubin quarry lake. Similarly, the means of α-diversity indices (based on Shannon's index) were significantly higher ($p < 0.05$) for the sediment samples than for the water samples. The Simpson's diversity indices (ranging from 0.84 ± 0.14 for the surface sediment in the Ubin quarry lake to 0.89 ± 0.05 for the water in the Singapore quarry lake) did not differ significantly across the samples.

Differences in microbial diversity between the sediment and water samples from lake ecosystems have been reported previously in a few studies [20, 21, 43, 52]. For example, Qu et al. [52] showed that, in a lake ecosystem in China, Shannon's diversity index was higher for the sediment samples in comparison with the water samples. In the same study, the Ace and Chao estimates for the richness were also found to be higher for the sediment samples. Our results were consistent with those previously reported in literature that Shannon's diversity of sediments is usually higher than that of the associated water [20, 52]. On the other hand, we observed no significant variation in Simpson's diversity across all the six samples. Since Simpson's diversity index focuses on major taxa, our results suggest that the differences in microbial diversity across the samples arose mainly from the differences in rare taxa.

Community Compositions of Sediments Are Similar while those of Water Differ Greatly

A nonparametric statistical test using "anosim" and "adonis2" showed that (i) the difference between microbial communities across the sediment and water samples among the six groups was significantly greater than the difference among replicates within the same group ($p < 0.01$) and (ii) there was a significant difference between the microbial communities in the Ubin and Singapore quarry lakes ($p < 0.001$), as well as between the microbial communities in water and sediment samples ($p < 0.001$). Analysis of variance using "betadisper" further indicated that the differences in the community composition of the samples were not merely due to the differences in their variance ($p > 0.14$). These tests showed that the maximum difference in the microbial community composition was between the samples from the sediment and the water group (Table A.2). While differences were also expected in the microbial community composition between the two quarry lakes, these differences were not as prominent as those between the sediment and the water.

The dendrogram generated using unsupervised hierarchical clustering of the 18 samples showed two very distinct clusters, one consisting of all the sediment samples and the other consisting of all the water samples (Fig. A.2). In addition, the distances between the samples in the sediment groups were lower compared to those between the samples in the water groups, suggesting a higher similarity among the sediment samples.

NMDS ordination plot produced based on the Bray-Curtis dissimilarity also showed that the sediment samples were clustered away from the water samples and there was more variability among the samples in the water group in comparison to the sediment group (Fig. 2a). The water samples from the Singapore quarry lake were clustered relatively far from the water samples from the Ubin quarry lake, suggesting a high dissimilarity in the community composition between the water in the two quarries. In contrast, the sediment samples from different groups were clustered close to one another, indicating similar community composition in the sediment samples from the two quarries as well as from the surface and the deep sediment in the Ubin quarry lake. In addition, the water and sediment samples from the two quarries were clustered on separate corners of the ordination plot. Hence, some differences in the overall community composition between the two quarries were expected as well. In addition, the water samples from different groups were clustered far away not only from the sediment samples but also from each other, suggesting that the water samples were highly dissimilar from the sediments as well as from each other. Similar observations were made with the NMDS ordination plot produced based on the UniFrac distance metric (Fig. 2b). Although the exact placements of the different samples on the ordination plot varied a little, there were no major discrepancies in the conclusions arising from the two ordination plots.

Major Microbial Taxa in the Quarry Lake Samples

Less than 2% (60 OTUs) of the total 3346 OTUs were classified as the domain Archaea and the rest as bacteria. It should also be noted that less than 1% of the total reads were assigned to the domain Archaea. OTUs were further assigned to lower taxonomic ranks, and abundance for each rank was estimated across different groups. A total of 31 microbial phyla were identified which consisted of 61 classes, 130 orders, 297 families, 1004 genera, and 3346 species. Of these, 5 phyla consisting of 12 classes, 18 orders, 24 families, 39 genera, and 60 species belonged to the domain Archaea. A large fraction of the reads (~36%) could not be classified into any known phyla (unclassified sequences). Together with the unclassified sequences, *Proteobacteria* (17.2%), *Actinobacteria* (12.3%), *Cyanobacteria* (9.5%), *Firmicutes* (7.4%), and *Bacteroidetes* (6.8%) accounted for approximately 90% of the total reads. The other major phyla were *Verrucomicrobia* (2.3%), *Planctomycetes* (1.9%), *Nitrospirae* (1.6%), *Chloroflexi* (1.5%), *Spirochaetes* (0.6%), *Acidobacteria* (0.5%), *Gemmatimonadetes* (0.5%), and *Fusobacteria* (0.5%) (Fig. A.3). The major phyla belonging to the domain Archaea were *Thaumarchaeota* (0.4%), *Crenarchaeota* (0.1%), and *Euryarchaeota* (0.1%). Approximately 0.002%

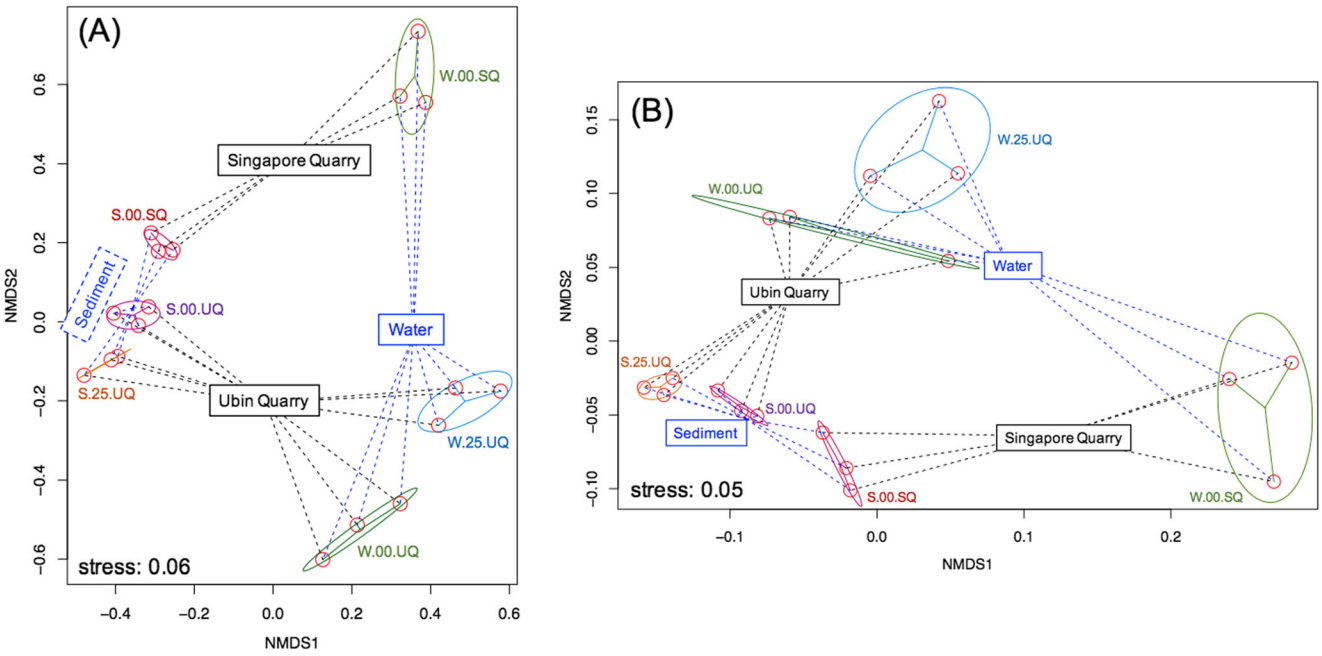

Fig. 2 Ordination plots produced based on **a** Bray-Curtis dissimilarity and **b** UniFrac distance metric. The sediment samples were clustered together while the water samples were clustered far apart from each other, indicating that the community composition in the sediment samples was more similar than that in the water samples

of the total reads were assigned to unclassified sequences derived from archaea.

Actinobacteria, *Bacteroidetes*, *Cyanobacteria*, *Chloroflexi*, and *Verrucomicrobia* Dominate in Water

Actinobacteria, *Bacteroidetes*, *Cyanobacteria*, *Chloroflexi*, and *Verrucomicrobia* were mainly associated with the water samples in the two quarry lakes (Fig. A.4). *Actinobacteria* and *Verrucomicrobia* were in significantly higher proportion ($p < 0.01$) in the water from the Singapore quarry lake in comparison to the other sample groups (Fig. 3). This is consistent with previous studies where bacteria from these two phyla have been reported to be dominating in the water of a freshwater lake [45]. The dominant genera in these two phyla were represented in significantly higher proportion in the water samples in comparison to the sediment samples ($p < 0.01$). For example, the top five dominant genera identified in the phylum *Actinobacteria*, including *Tetrasphaera* and *Streptomyces*, were relatively more abundant in the water samples as compared to the sediment samples (Fig. A.5). Similarly, the dominant genera identified in the phylum *Verrucomicrobia* (*Chthoniobacter*, *Rubritalea*, and *Prosthecobacter*) were more abundant in the water samples as compared to the sediment samples.

In addition, the phylum *Cyanobacteria* was in higher proportion in the water samples as compared to the sediment samples. Nonetheless, some genera in this phylum were also prevalent in sediments (Fig. A.6). The most dominant genus in this phylum, *Synechococcus*, was in much higher proportion in the water samples as compared to the sediment samples, while *Microcystis* was more abundant in the deep-water samples compared to the surface water. Many taxa in the phylum *Cyanobacteria*, such as *Synechococcus*, have an affinity for the bright sunlight [1], while other genera, such as *Microcystis*, are known to remain in the sediments for a prolonged period of time for reasons not clearly understood [37]. *Oscillatoria* and *Anabaena*, along with other benthic *Cyanobacteria* genera such as *Pleurocapsa*, *Arthrospira*, *Symploca*, and *Lyngbya*, were represented in higher proportion in the sediments in comparison to water. These observations are consistent with previous reports where these cyanobacteria were found to form mats associated with aquatic sediments instead of freely floating in water [16, 18, 60].

Other phyla such as *Bacteroidetes* and *Chloroflexi* were found to be well represented in water as well as in sediments. In the phylum *Chloroflexi*, the dominant genus *Chloroflexus* was more abundant in the water samples while the other genus *Herpetosiphon* was more abundant in the sediment samples. In the phylum *Bacteroidetes*, the dominant genera *Saprospira* and *Alistipes* were more abundant in the water samples, while other genera such as *Prolixibacter*, *Cytophaga*, and *Flexibacter* were more abundant in the sediment samples (Fig. A.7). *Prolixibacter* was reported to be prevalent in marine sediments in a previous study [32]. Here, we found that these microorganisms were relatively more represented in the water of the quarry lakes rather than in the sediments. The reasons why these microorganisms appeared in higher

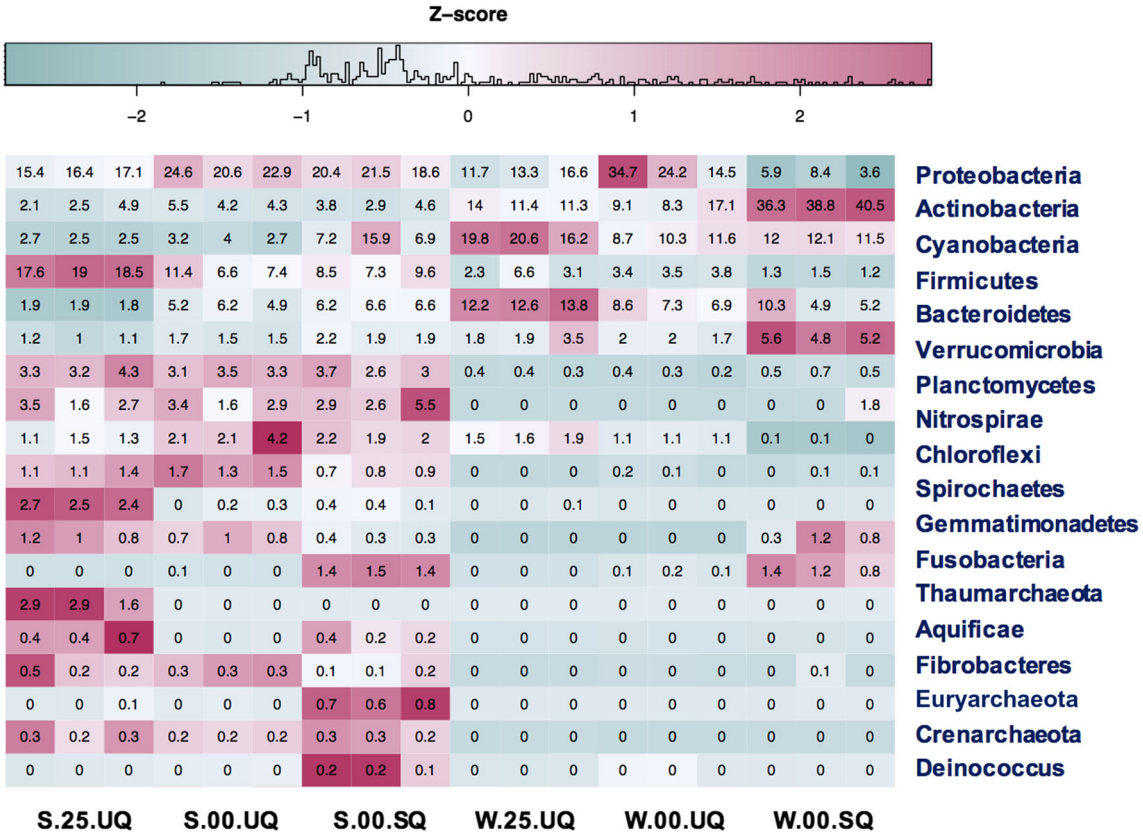

Fig. 3 Heatmap representing major differences in prokaryotic phyla among different samples. *Actinobacteria*, *Firmicutes*, *Verrucomicrobia*, *Cyanobacteria*, *Chloroflexi*, and *Bacteroidetes* were more abundant in water whereas *Nitrospirae*, *Aquificae*, and the phyla of *Archaea* were dominant in sediments. *Proteobacteria* were well represented in water as well as in sediments. The color code represents the row *z*-score; the number of standard deviations of a value differs from the mean. The numeric values represent relative abundance in a column in arbitrary units. The line profile in the color key is the histogram of all the values

proportion in the water samples rather than in the sediment samples remain elusive. Nonetheless, it has been reported that the turbulent mixing in the ecosystem may cause the upwelling of these microorganisms from sediment into the water [62], which could possibly be a reason for the relatively higher proportion of these microorganisms in the water samples rather than in the sediment samples.

Proteobacteria Are Well Represented in Water as well as in Sediments

Proteobacteria, the phylum with maximum number of reads, was well represented in both the water and the sediment samples from the two quarry lakes (Fig. 3). Most *Proteobacteria* identified in this study are commonly found in water and sediments of aquatic environments and are the dominant bacteria in a variety of freshwater biofilms such as river and streams and drinking water biofilms [3, 5, 19, 30, 71]. The *Alphaproteobacteria* class was in higher proportion in the water samples, while *Gammaproteobacteria* and *Deltaproteobacteria* were in higher proportion in the sediment samples (Fig. A.8). The abundances of other genera from this phylum also differed between the water and sediment samples. For example, *Hyphomicrobium*, *Methylocystis*, and *Vibrio* were in higher proportion in water, while *Rhodovulum*, *Geobacter*, *Thioalkalivibrio*, *Gluconacetobacter*, and *Desulfococcus* were more abundant in sediments.

Nitrospirae, *Firmicutes*, *Aquificae*, and the Phyla of *Archaea* Dominate in Sediments

Planctomycetes, *Firmicutes*, *Nitrospirae*, *Chloroflexi*, *Spirochaetes*, *Acidobacteria*, *Aquificae*, and *Fibrobacteres* were dominant in the sediment samples but scarce in the water samples for both quarry lakes. In addition, all phyla in the domain Archaea were in much higher proportion in the sediment samples than in the water.

Overall, the phylum *Firmicutes* was abundant in sediments (Fig. 3). The major genera in this phylum, e.g., *Desulfotomaculum*, *Paenibacillus*, and *Sporanaerobacter*, were dominant in sediments and scarce in water, although a few other genera in this phylum such as, *Seinonella* and *Fusibacter*, were more abundant in water (Fig. A.9).

All the three genera in the phylum *Nitrospirae*, i.e., *Leptospirillum*, *Nitrospira*, and *Thermodesulfovibrio*, were in significantly higher proportion in the sediment samples as compared to the water samples. Similarly, in the phylum *Aquificae*, the four dominant genera *Sulfurihydrogenibium*, *Persephonella*, *Hydrogenobacter*, and *Hydrogenobaculum* were more abundant in the sediment samples (Fig. A.10).

In the phylum *Thaumarchaeota*, all the three genera *Nitrososphaera*, *Cenarcheum*, and *Nitrosopumilus* were in higher proportion in the Ubin quarry lake sediments as compared to the Singapore quarry lake sediments (Fig. A.11). *Nitrosocladus*, the dominant genera in the phylum *Crenarchaeota*, was equally represented in the sediments of both quarry lakes. In the phylum *Euryarchaeota*, the dominant genera (*Methanobacterium*, *Methanococcus*, *Methanosaeta*, *Methanolinea*, and *Methanoregula*) were more abundant in the sediment samples from the Singapore quarry lake, while the subordinate genera (*Methanothermus*, *Methanosarcina*, and *Methanomethylovorans*) were more dominant in the sediment samples from the Ubin quarry lake.

Bacteria genera such as *Geobacter*, *Desulfotomaculum*, and *Sporanaerobacter* which were in higher proportion in the sediments in the current study have also been reported to be found mainly in sludge, soils, and sediments in previous studies [53, 58]. Similarly, gliding bacteria like *Saprospira* and *Cytophaga*, which were dominant in the sediment samples in this study, are abundant in epilithic biofilms in freshwater aquatic habitats like rivers and lakes [48]. A few other examples of bacteria that were abundant in sediments included *Desulfotomaculum* and *Sporanerobacter* which are anaerobic spore-forming bacteria commonly found in sediments and sludge [17, 31, 50, 51].

Potential Roles of Key Microbial Players in the Sediment and Water Samples

A number of microorganisms identified are known to be involved in crucial biogeochemical processes and interspecies interactions which occur primarily in sediments. For example, *Geobacter*, a metal reducer in soils and sediments, may play an important role in direct electron transfer to iron oxide as well as in interspecies electron transfer to methane-producing archaea [53, 58]. Similarly, major taxa in the domain Archaea, *Thaumarchaeota* and *Crenarchaeota*, may be involved in the oxidation of ammonia to nitrite, while *Euryarchaeota* is involved in methanogenesis [11, 34]. Bacteria in the phylum *Spirochaetes* (*Leptospira*, *Treponema*, and *Aminobacterium*) may contribute to the degradation of organic matter in sludge and sediments [6, 28]. To identify the potential biogeochemical processes in the water and sediment of these quarry lakes, a comprehensive assignment of microbial taxa to function was performed (Fig. 4).

Among the putative functions, aerobic chemoheterotrophy and phototrophy were more abundant in water as compared to sediments. On the other hand, degradation of biomass, aromatic compounds, and hydrocarbons, as well as transformation of metals and sulfur, was more abundant in sediments as compared to water. Aerobic chemoheterotrophy in the water of the Ubin and Singapore quarry lakes was mainly attributed to the abundance of bacteria such as *Tetrasphaera*, *Saprospira*, *Microbacterium*, and *Streptomyces*, to name a few (Fig. A.12). *Synechococcus* was the most abundant genus in the phylum *Cyanobacteria* and was associated with phototrophy (Fig. A.13). *Bacillus*, *Cytophaga*, *Fibrobacter*, *Chitinophaga*, and *Rhodothermus* were the predominant genera associated with extracellular hydrolysis in the sediments (Fig. A.14). *Bacillus* and *Cytophaga* are soil bacteria which are known to degrade cellulosic materials [38, 66, 67]. Degradation of aromatic compounds in the sediments was attributed mainly to *Clostridia* (Fig. A.15). *Clostridium* has been previously found in sludge and predicted to metabolize aromatic compounds to methane in anaerobic conditions [70]. Other major genera, such as *Rhodococcus*, capable of degrading both aromatic and nonaromatic hydrocarbons [7, 35], were found primarily in the sediments of the two quarries. Metal transformation was attributed to *Geobacter*, *Desulfobacterium*, *Desulfomonas*, *Shewanella*, and *Leptospirillum* in the sediments. Transformation of sulfur compounds could be attributed mainly to *Desulfotomaculum*, *Desulfobacterium*, *Desulfococcus*, *Sporanaerobacter*, and *Thioalkalivibrio* (Fig. A.16). Most of the nitrifiers were found primarily in sediments, and hence, nitrification was predicted to occur primarily in the sediments (Fig. A.17). This is counterintuitive as nitrification is an aerobic process and the oxygen concentration in the sediments is expected to be lower than that in the water. However, it should be noted that many of these microorganisms have often been found in sludge, sediments, or the transition zone at the oxic-anoxic interface [8, 33, 39, 56, 61]. Previous studies have shown that nitrification is inhibited by sunlight [10], which could be a possible reason for the localization of these nitrifying bacteria in the sediments during mid-day, when the samples were collected from the quarry lakes. In oxygenated waters, nitrifiers are believed to be prevalent at the sediment-water interface, and nitrification takes place primarily in the upper layers of sediments [54, 57, 59].

It should be noted that the functional assignment of the quarry lake ecosystems based on microbial taxonomy is putative. Nevertheless, these predictions provide insights into the potential functions of water and sediment microbial communities in quarry lakes. Additionally, the temporal microbial dynamics of the quarry lake ecosystems are not explored in this study as the quarry lakes in Singapore are protected areas with restricted access. Nonetheless, it is likely that there are no significant temporal variations in these quarry lakes as there is

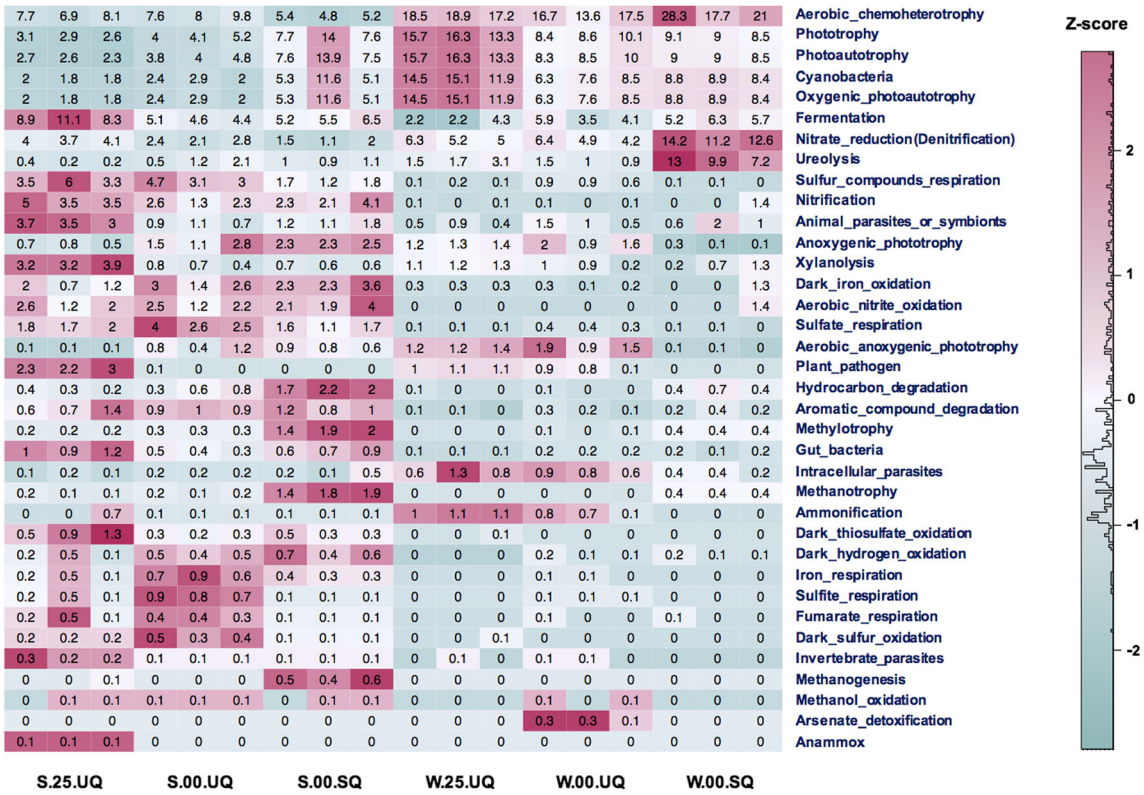

Fig. 4 Heatmap representing major differences in predicted functions among different sample groups. Methanogenesis, methylotrophy, degradation of aromatic compounds and hydrocarbons, and metal and sulfur transformation as well as nitrification were associated with sediments. Phototrophy, aerobic chemoheterotrophy, and denitrification were abundant in water. The color code represents the row *z*-score; the number of standard deviations of a value differs from the mean. The numeric values represent relative abundance in a column in arbitrary units. The line profile in the color key is the histogram of all the values

little seasonal variation in tropical regions. For example, a recent study on bacterial communities in reservoir water in Singapore showed a weak temporal variation in the overall bacterial community composition [47].

Conclusions

The richness and diversity of microbial communities in sediments of the quarry lakes were higher than those in the water. In addition, the compositions of the microbial communities in the sediments from the two quarries were highly similar to one another, while those in the water differed greatly. Although the microbial communities of the sediment and water samples shared some common members, a large number of microbial taxa (at the phylum and genus levels) were predominantly found either in sediment or in water. Bacteria in the phyla *Actinobacteria*, *Verrucomicrobia*, *Cyanobacteria*, *Chloroflexi*, and *Bacteroidetes* were more abundant in water whereas *Nitrospirae*, *Aquificae*, and *Firmicutes* along with Archaea were abundant in sediments. *Proteobacteria* was well represented in water as well as in sediments. Phototrophy and aerobic chemoheterotrophy were expected to be the most prevalent microbial processes in water, while degradation of organic matter and transformation of metals and sulfur compounds were expected to occur mainly in sediments. Intriguingly, nitrification, which is traditionally expected to occur in water, was implied to occur primarily in the sediments. Our results provide valuable insights into the putative microbial processes in water and sediment that potentially contribute to the biogeochemical processes carried out by water and sediment microbial communities in tropical granite quarry lakes.

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Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

Disclaimer Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the L2 NIC.

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