Pollution and environmental stressors modulate the microbiome in estuarine mangroves: a metagenome analysis

Mandar S. Paingankar[1,2] and Deepti D. Deobagkar[1,*]

[1]Molecular Biology Research Laboratory, Department of Zoology, Savitribai Phule Pune University, Pune 411 007, India
[2]ISRO Science and Technology Cell & Department of Zoology, Centre for Advanced Studies, Savitribai Phule Pune University, Pune 411 007, India

The microbial communities of mangroves which form important links in elemental cycling and bioremediation have not been elucidated in most parts of the world. Due to urbanization and deforestation mangroves are also under threat. In the present study, high throughput next generation sequencing technology, based on 16S rRNA amplicon analysis using Illumina platform, was employed to unravel the microbial diversity present in different mangrove areas in the west coast of India. It could be seen that in mangroves, Proteobacteria and Bacteroidetes were most common, followed by taxon such as Firmicutes, Spirochaetes, Chloroflexi and Verrucomicrobia. In proteobacteria group, Gammaproteobacteria, Alphaproteobacteria and Deltaproteobacteria were most abundant. Interestingly, bacteria having the capacity to utilize sulphate were present along with methanogens in all samples, suggesting that anaerobic and sulphur-based metabolic pathways play an important role in these mangrove ecosystems. The differences in bacterial diversity can be partly attributed to biotic and abiotic factors such as physico-chemical characteristics of the samples, geographical location and natural and human-induced changes in the locality. The metagenomics analysis of mangrove sediment samples has helped in elucidating the baseline data on bacterial diversity along mangroves in Maharashtra along the west coast of India and can provide pointers for effective measures of conservation.

Keywords: Anthropogenic stressors, bacterial communities, metagenomics, microbial ecology, pollution.

MANGROVES are salt-tolerant, arboreal, flowering plant forests that are primarily situated at the confluence of land and sea in tropical and sub-tropical regions[1]. Morphological, ecophysiological and adaptive characteristics of mangroves make them structurally and functionally unique ecosystems. Furthermore, mangroves have important ecological and economic implications in the protection of coasts from soil erosion and from organic and inorganic matter, pesticides, and fertilizers and are known to provide nursery habitat for aquatic animals[2]. The mangrove ecosystem functions are maintained by taxonomically diverse group of plants and microbial communities which are interdependent and play a fundamental role in the productivity, functioning and maintenance of the mangrove ecosystem[3]. The abundance, activity and composition of microbial communities in mangroves play an integral role in providing nutrients to the ecosystem and thus determine the sustainable productivity[4]. The mangrove microbial community is strongly influenced by nutrient cycle, organic and inorganic compounds in the sediment, biogeographical and ecological parameters and anthropogenic stressors[5]. Furthermore, salinity and the frequent anaerobic condition caused by tidal variation make mangrove forests hotspots for microbial diversity. The floral, faunal and some microbial diversity studies have been carried out to understand the role of these communities in the complex functioning of mangrove ecosystems[6]. In recent years, this ecosystem is rapidly getting destroyed and is threatened due to anthropological activities and modernization. It is therefore important to document and account for the existing rich diversity of this ecosystem.

The complexity of microbial communities and the technical constraints to identify and measure the diversity have hampered our understanding of the relationships between mangrove functioning and microbial diversity. Several microbes are unculturable and therefore their abundance and diversity cannot be assessed by conventional culture-based method. However, advances in next generation sequencing technology can be employed to study the diversity and functional genomics of microbial communities at higher resolution and in greater depth and coverage. Metagenomic analyses have been extensively employed in diverse ecosystems including marine waters[7–10], sediments[6,10,11], agricultural soils[12,13], forest soil[14,15] and in some other extremely challenging environments[16–19]. However, very few studies have been targeted towards understanding the diversity of microbial communities in mangrove forests[6,20–27].

Mangroves in India are spread over an area of 4921 sq. km and are present on western coastline, eastern coastline and in the Andaman and Nicobar Islands of

*For correspondence. (e-mail: deepti.deobagkar@gmail.com)

Table 1. Sampling sites

Place	District	Sample code	Latitude	Longitude	River	Threats
Arnala	Palghar	ANMN1	19.473	72.760	Vaitarana	Less polluted
Arnala	Palghar	ANMN2	19.474	72.760	Vaitarana	Less polluted
Dighi	Raigad	DGMG1	18.258	72.971		Industrial pollution
Murbe	Palghar	MBMN2	19.735	72.697	Banganga	Industrial pollution
Murud	Raigad	MRMG1	18.373	72.931		Less polluted
Murud	Raigad	MRMG2	18.374	72.933		Less polluted
Panvel	Raigad	PNMN1	19.029	73.079	Taloje	Urban pollution
Panvel	Raigad	PNMN2	19.031	73.082	Taloje	Urban pollution
Rajapuri	Raigad	RPMG1	18.316	72.970		Less polluted
Rajapuri	Raigad	RPMG2	18.318	72.971		Less polluted
Vasai Creek	Palghar	VSMN1	19.287	72.905	Ulhas	Urban pollution
Vasai Creek	Palghar	VSMN2	19.291	72.906	Ulhas	Urban pollution

India. Mangroves of western coastline of India are mainly present in Gujarat and Maharashtra states and are under threat of conversion into agricultural land and urban development[28]. In Maharashtra, mangroves are mainly concentrated in Mumbai, Thane, Raigad and Ratnagiri areas, and are under tremendous pressure of urban development and agricultural practices. The plants and animals of mangrove community are well studied in India. Although a few studies are available, a comprehensive description of the microbial life in the mangrove ecosystem is lacking. Comparisons among distinct mangroves based on metagenomics will significantly contribute to a better overview of the microbial community structure in the mangroves of India and a greater understanding of the dynamics of this ecosystem.

In the current study, we have employed Illumina MiSeq sequencing platform to understand and document the microbial community structure present in different mangrove areas along the west coast in Maharashtra, India.

Material and methods

Ethics statement

The collection sites used in the current study are not part of any reserve forests, national parks or privately owned areas and therefore no specific permits were needed for the field studies. Endangered or protected species were not collected or included in the study.

Sample collection

Information on mangroves of India and shape files for GIS mapping was retrieved from earlier reports[1,29]. Sediment samples were collected from mangrove forests and these are dominated mainly by *Sonneratia caseolaris*, *Sueda fruticosa*, *Urochondra setulosa*, etc. The samples were collected superficially (0–5 cm depth) during the period of low tide at the GPS coordinates Arnala (19°28′26.4″N, 72°45′36.0″E), Dighi (18°15′28.8″N, 72°58′15.6″E), Murbe (19°44′06.0″N, 72°41′49.2″E), Murud (18°22′26.4″N, 72°55′58.8″E), Panvel (19°01′51.6″N, 73°04′56.1″E), Rajapuri (18°18′57.6″N, 72°58′12.0″E) and Vasai (19°17′27.6″N, 72°54′21.6″E). These mangrove forests were located in estuarine regions of the rivers Banganga, Taloje, Ulhas and Vaitarana of Maharashtra, India. These mangroves can be categorized into areas facing urbanization threats (UT), areas facing industrialization threats (IT) and areas relatively less polluted (LP) (Table 1).

Environmental parameters

The average temperature, average rainfall, pH, SO_4, NO_3, PO_4, Mg, Cl_2 and salinity of samples were measured and are depicted in Table 2.

DNA extraction

Soil collected from respective mangrove samples was processed for DNA isolation using Powersoil DNA isolation kit (MoBio Laboratories Inc. Carlsbad, CA) according to manufacturer's instructions. DNA concentration was measured using the Quantus Fluorimeter (Promega, USA).

Amplification primers and sequence analysis

Regions corresponding to V3 and V4 regions of 16S rRNA gene were amplified with appropriate sample barcoding index sequences and Illumina adapter sequences. PCR conditions were optimized for each primer set that are different only by the barcoding indices. The amplified DNA was purified by agarose gel electrophoresis, quantified and normalized; equimolar pool of all the samples was made and this multiplexed library was further subjected to QC using an Agilent Bioanalyser DNA chip. The sequencing library generated from V3 and V4 amplicons from all the samples was sequenced using an Illumina

Table 2. Soil chemical properties at different sampling sites

Sample code	Average temperature	Average rainfall	pH	SO_4	NO_3	PO_4	Mg	Cl_2	Salinity
ANMN1	26.7	2208	7.3	2191.7	97.9	1431.4	10200	8860.7	16.008
ANMN2	26.7	2208	7.2	2200	102	1435	10230	8896	16.071
DGMG1	26	3329	7.3	4927	30.9	1568	3057	17732	32.034
MBMN2	26.5	1955	7.3	1641.2	30	1641.2	7900	18211.6	32.901
MRMG1	26.6	2827	7.9	2604	0.1	2284	5047	10426	18.835
MRMG2	26.6	2827	7.9	2609	0.2	2298	5050	10432	18.846
PNMN1	27	3267	5.7	1467	109.4	1456.5	7100	6126.1	11.067
PNMN2	27	3267	5.6	1475	108	1457	7150	6122.3	11.061
RPMG1	26.6	2861	8.1	2382	0.1	4131	8714	8100	14.633
RPMG2	26.6	2861	8.1	2386	0.2	4135	8724	8112	14.655
VSMN1	26.7	2377	7.6	1873.7	36	1895	10400	9782.3	17.672
VSMN2	26.7	2377	7.5	1882	39	1904	10455	9756	17.625

Table 3. Metagenomics reads information and taxonomic affiliations of bacteria present in soil samples

Sample code	Reads passed quality filtering (No. of reads)	Unclassified at bacteria (No. of reads)	Kingdom level (No. of reads)	Archaea (No. of reads)	Bacteria		
					Class	Order	Family
ANMN1	509,408	485,991	23,337	80	54	111	239
ANMN2	446,190	420,787	25,379	24	54	109	236
DGMG1	463,241	396,887	66,013	341	53	106	241
MBMN2	513,475	447,988	65,455	32	54	111	239
MRMG1	429,852	401,826	28,025	1	57	117	254
MRMG2	400,623	388,780	11,841	2	58	115	247
PNMN1	433,235	415,973	17262	0	50	100	224
PNMN2	429,505	394,295	35210	0	53	111	245
RPMG1	531,638	504,074	27,557	7	58	114	253
RPMG2	492,163	445,138	46,987	38	57	113	249
VSMN1	459,714	428,565	31,149	0	54	114	250
VSMN2	428,232	384,388	43844	0	53	114	255

MiSeq sequencing platform employing paired-end overlapping sequencing. Sequence reads were binned according to index sequences and QC of the raw sequence data was performed by custom scripts; low quality reads were filtered out and trimmed based on observed quality pattern in the dataset. Read pairs with individually high sequence quality that match each other at the overlapping region were fused together to obtain a single read traversing full length of V3 and V4 regions. Fused sequences from the samples were used directly for further analysis for detecting microbes present in soil flora. Diversity and abundance were analysed using available standard bioinformatic pipelines with necessary custom modifications. The taxonomic assignment of unassembled metagenomic sequences was performed using BLASTX against the SEED and Pfam databases on the MG-RAST server v2.0 (http://metagenomics.nmpdr.org) using a cut-off E-value of 1e-10. BLASTX was also used to conduct a similarity search against the NCBI-NR database, and MetaGenome Analyzer software (MEGAN v5.0) with the LCA algorithm (maximum number of matches per read: 5, min support: 5, min score: 35, top per cent: 10) was used to visualize results. Metagenomics reads information samples are depicted in Table 3.

Statistical analysis

Several indices of clonal diversity were estimated namely, Taxa_S, Individuals, Dominance_D, Simpson_1-D, Shannon_H, Evenness_e^H/S, Brillouin, Menhinick, Margalef, Equitability_J, Fisher_alpha, Berger-Parker and Chao-1 using the PAST3 program available from the University of Oslo website link (http://folk.uio.no/ohammer/past). Similarly, beta diversity indices were calculated using PAST3 program. A similarity matrix was generated using a probabilistic distance metric. This metric was designed to account for unseen shared species in the sampled data that have many rare species and unequal sample sizes, typical of microbial pyrosequencing data. The statistical significance of the relationship between differences in chemical composition and (i) species diversity, (ii) species alpha diversity indices and (iii) species beta diversity indices was tested by Mantel tests.

P values were calculated using 9999 permutations on rows and columns of dissimilarity matrices.

Results

Description of the community

The present study is based on NGS metagenomic analysis of soil sediments from mangroves localized in Maharashtra from Arnala (ANMN1, ANMN2), Murbe (MBMN2), Vasai Creek (VSMN1, VSMN2) in Palghar district and Dighi (DGMG1), Murud (MRMG1, MRMG2), Panvel (PMPG1, PMPG2), Rajapuri (RPMG1, RPMG2) in Raigad district (Table 1 and Figure 1) as described earlier. A total of 5,537,276 (461,440 ± 41,268.2 per sample) bacterial 16SV3–V4 high-quality sequences with an average read length of 258 ± 14, were obtained. Sequence clustering resulted in the identification of 2346 (962 ± 84.03 per sample) different bacterial species (Supplementary Information). In general, Proteobacteria and Bacteroidetes were more abundant and other taxon such as Firmicutes, Spirochaetes, Chloroflexi and Verrucomicrobia were present in low abundance. The Spirochaetes were more abundant in Murbe sample whereas Tenericutes and Thermi showed moderate abundance in the Panvel sample. The numbers of sequences affiliated with each taxon are depicted in Figure 2 *a*, with a major abundance of Gammaproteobacteria (14.27–28.46%), Flavobacteria (3.22–18.76%), Sphingobacteria (4.46–12.81%), Alphaproteobacteria (5.02–18.63%), Deltaproteobacteria (1.3–19.94%), Bacilli (16.55–33.15%) and Actinobacteria (2.56–7.29%) followed by other minor classes represented by Clostridia (1.23–4.91%), Nostocophycideae (3.18–4.81%). Among the distinct mangrove sets, the following differences were observed: higher abundance of Bacilli in DGMG1 (33.15%) and PNMN1 (16.55%), a lower number of sequences of Anaerolineae (2.57%) in PNMN2, and lower occurrence of Nostocophycideae in PNMN1 (3.21%), RPMG2 (4.48%), VSMN1 (4.81%) and VSMN2 (3.18%). A more detailed analysis of data based on the species level revealed that the top 100 species could account for 43.12% of all bacterial sequences (Figure 2 *b*). The microbial community consisted of bacteria having the capacity to utilize the sulphate. Species such as *Desulfobacca acetoxidans*, *Desulfotalea psychrophila*, *Desulfobulbus propionicus* were present in higher numbers in all samples. *Marinobacterium sediminicola*, *Desulfomonile tiedjei*, *Methylophaga* sp., *Desulfovibrio oryzae*, *Desulfosarcina* sp. were present in moderate numbers in all collected samples. These sulphate reducers were accompanied by methanogens at all sites. *Thiorhodococcus pfennigii*, *Paracoccus sulfuroxidans*, *Psychroflexus gondwanensis*, *Balneola vulgaris*, *Desulfovibrio butyratiphilus* showed high abundance in all samples except the DGMG1 sample which was located in the area threatened by paint, chemical and refinery industry, suggesting that industrial pollutants such as hydrocarbons and organic solvents are significantly affecting the sulphur cycle in mangroves.

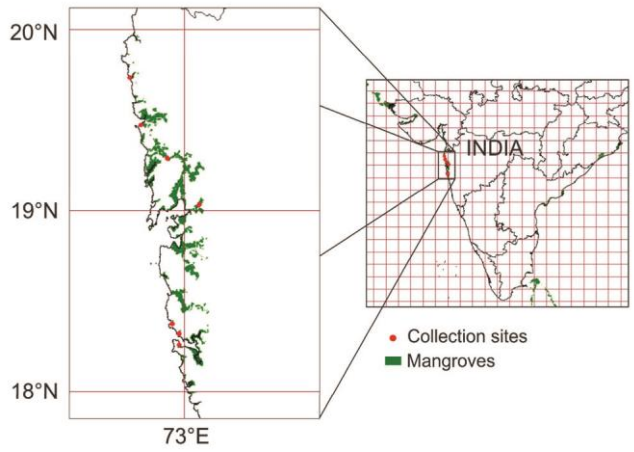

Figure 1. Map of the mangrove sample collection sites.

Alpha and beta diversity of bacterial communities in different mangrove soil samples

Alpha and beta diversity analysis revealed rich taxonomic diversity and dominance of few species in mangroves samples (Table 4). Interestingly, soil samples from MBMN2 ($D = 0.02988$), PNMN1 ($D = 0.02869$) and VSMN2 ($D = 0.02226$) showed higher dominance of fewer bacterial groups. Chao-1 analysis identified 1500–1900 (1562–1975) species in each sample. At species level, high beta diversity was observed in all mangrove samples (Table 5) wherein 638 species were common in all 12 samples. DGMG1 sample showed maximum number of unique species.

The samples were grouped in three categories: mangroves having urbanization threats (UT), industrialization threats (IT) and less pollution (LP). 1328 species were found common in all groups. It could be seen that 91 species were unique to samples having IT, 250 unique to samples having UT and the LP area samples showed 272 unique species (Figure 3 *a*). Mangroves suffering from exposure to industry waste (IT) showed a higher Shanon's and Evenness index compared to LP and UT (Figure 3 *b* and *c*). Rarefaction curves revealed distinction between the bacterial communities of different mangrove samples (Figure 4). These observations suggest that exposure to pollution plays an important role in affecting the natural microbiota of mangroves, thereby threatening the community structure and eliminating the rich diversity.

Habitat type differences in communities

In order to examine the community structure and its specific features, non-metric multidimensional scaling

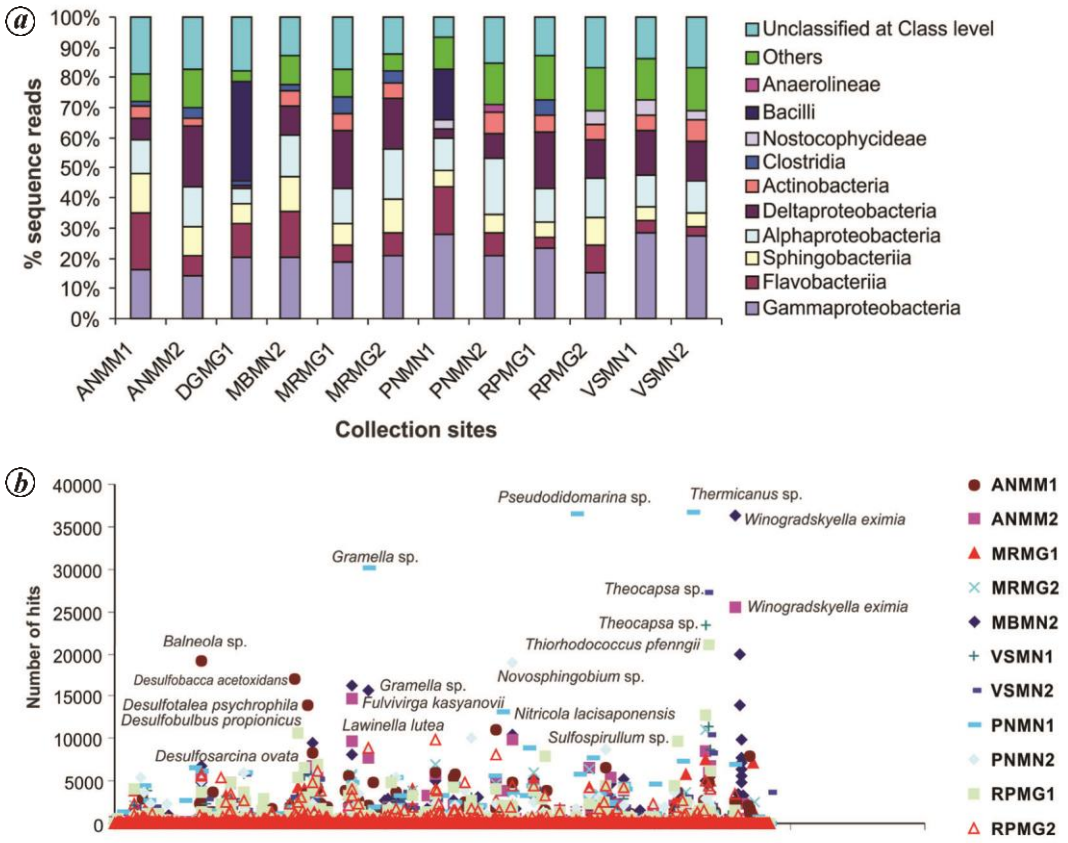

Figure 2. Distribution of predominant bacterial class in samples based on 16S rRNA gene sequencing. ***a***, Phyla distribution. Observations are displayed as stacked bar charts for individual mangrove sample (*x*-axis) against the taxa abundance (*y*-axis). ***b***, Abundance of species. Observations are displayed as scatter plot for individual mangrove sample (*x*-axis) against the species abundance (*y*-axis).

Table 4. Alpha diversity of bacteria in mangroves samples

Collection site	OTU	Dominance _D	Simpson _1-D	Shannon _H	Evenness _e^H/S	Margalef	Equitability _J	Fisher _alpha	Berger-Parker	Chao-1
ANMM1	1354	0.01798	0.982	5.005	0.1101	103	0.6941	169	0.08695	1673
ANMM2	1293	0.01669	0.9833	4.993	0.114	99.32	0.6969	163.4	0.05716	1597
DGMG1	1419	0.06666	0.9333	4.023	0.03936	108.7	0.5543	180.8	0.1956	1678
MBMN2	1372	0.02988	0.9701	4.718	0.08162	104.3	0.6532	171.4	0.1275	1632
MRMG1	1461	0.0145	0.9855	5.227	0.1274	112.6	0.7173	189	0.0652	1723
MRMG2	1388	0.01135	0.9886	5.282	0.1418	107.5	0.73	180.1	0.05567	1682
PNMN1	1297	0.02869	0.9713	4.468	0.06724	99.85	0.6234	164.7	0.08481	1562
PNMN2	1486	0.01737	0.9826	5.071	0.1072	114.5	0.6943	192.8	0.08198	1780
RPMG1	1564	0.0132	0.9868	5.232	0.1197	118.6	0.7114	198.1	0.05311	1856
RPMG2	1561	0.01838	0.9816	5.132	0.1085	119	0.6979	199.9	0.09547	1897
VSMN1	1533	0.01698	0.983	5.118	0.109	117.5	0.6978	197.8	0.06776	1797
VSMN2	1590	0.02226	0.9777	5.032	0.09634	122.5	0.6826	208.4	0.1024	1975

(MDS) using PCA analysis was carried out. Most samples clustered closely together, indicating that the microbial communities residing in these mangroves are similar and represent characteristic and typical community structures. Interestingly, communities in mangroves facing industrialization threats, DGMG1 and MBMN2 are distinct from all other, with more sequences being assigned to the firmicutes, i.e. Bacilli (Figure 5 *a*). Similarly, nMDS based only on chemical parameters revealed the distinctness of MBMN2 and DGMG1 samples in chemical properties (Figure 5 *b*). To explore the inter-relations between the different mangrove samples and chemical composition of mangrove soil samples, a correlation matrix was generated by calculating the Pearson's correlation

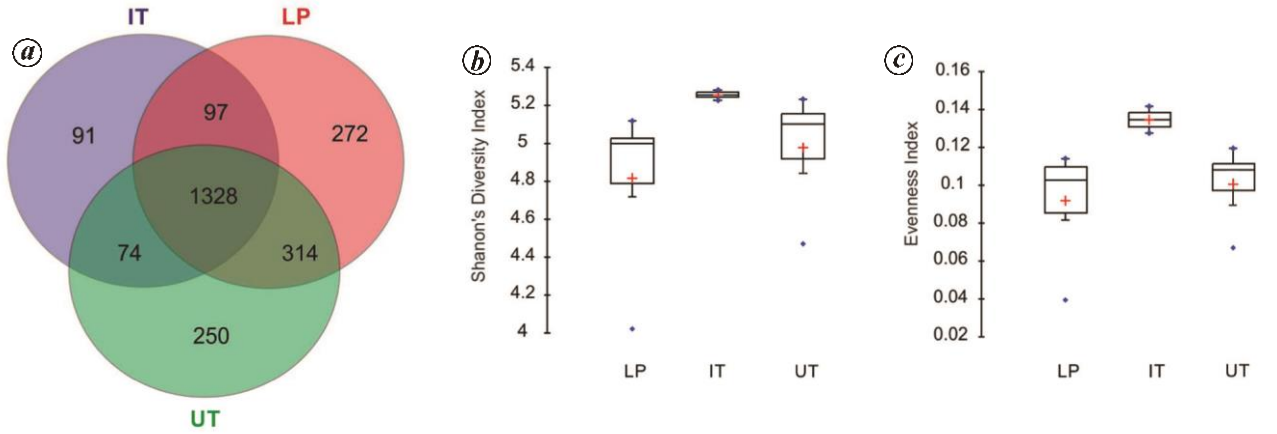

Figure 3. Species diversity and variation in alpha diversity of mangroves samples. Based on threats, mangroves samples were categorized into three groups, less polluted (LP), industrialization threats (IT) and urbanization threats (UT). ***a***, Venn diagram shows the number of unique and shared operational taxonomic units (OTUs) between the three groups. Comparison of number of OTUs observed in mangroves samples. ***b***, Shannon diversity indices; ***c***, Evenness indices.

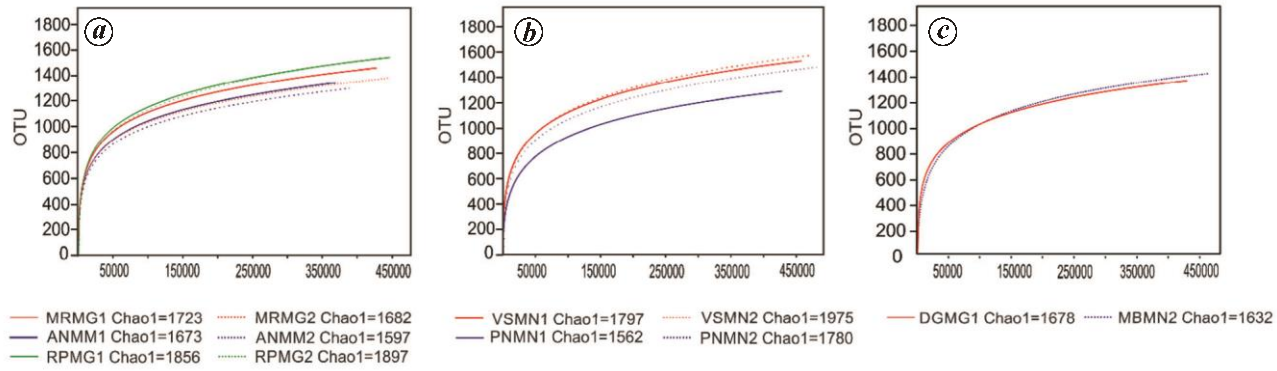

Figure 4. Rarefaction curves for mangroves samples. ***a***, Samples experiencing less pollution; ***b***, Samples having urbanization threats; ***c***, Samples facing industrialization threats. Each curve compares the number of sequence reads with the number of detected species.

Table 5. Global beta diversity indices of mangrove soil samples

Beta diversity index	
Whittaker	0.83811
Harrison	0.07619
Cody	3108
Routledge	0.14936
Wilson-Shmida	2.3548
Mourelle	0.21408
Harrison 2	0.06005
Williams	0.39777

coefficient (Figure 6 *a*). Mantel test was used to determine the factors (diversity, chemical parameters and Whitaker beta diversity) which best predicted the community diversity across the different mangrove area samples (Figure 6 *b*). Differences in chemical parameters correlated significantly with diversity across different collection sites ($r = 1$; $P = 0.0001$). Similarly, beta diversity changed significantly with chemical parameters of soil ($r = 0.345$; $P = 0.0001$). However, no significant correlation was observed in alpha diversity and chemical properties of soil ($r = –0.149$; $P = 0.225$). The correlations between environmental factors and alpha diversity indices were assessed by canonical correlation analysis (CCorA). A strong positive correlation could be seen for SO_4 concentration and relative dominance of a few species. Presence of PO_4 enhanced certain species whereas a positive correlation was observed in Mg concentration with evenness in samples (Figure 6 *c*). These results indicated that soil chemical composition and presence of pollutants play an important role and influence the bacterial diversity.

Discussion

Mangrove sediments entrap the minerals and the sedimentary catchments of mangroves respond quickly to external drivers making them highly sensitive to natural

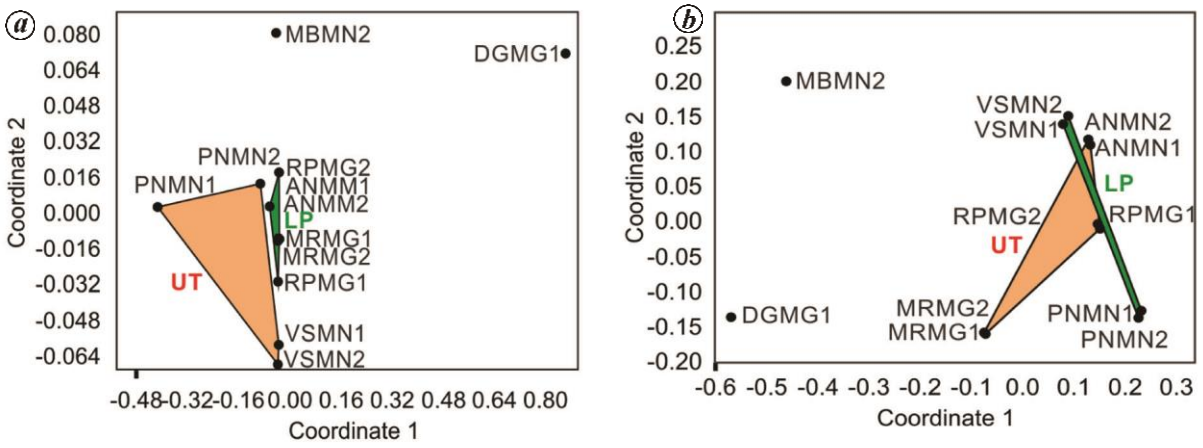

Figure 5. Non-metric multidimensional scaling (MDS) representation of the similarity matrix generated by cluster analysis. Each of the mangrove samples is represented by a different shape, and the distance between dots represents relatedness obtained from the similarity matrix. ***a***, MDS based on microbiota present in soil sample; ***b***, MDS based on soil chemical properties.

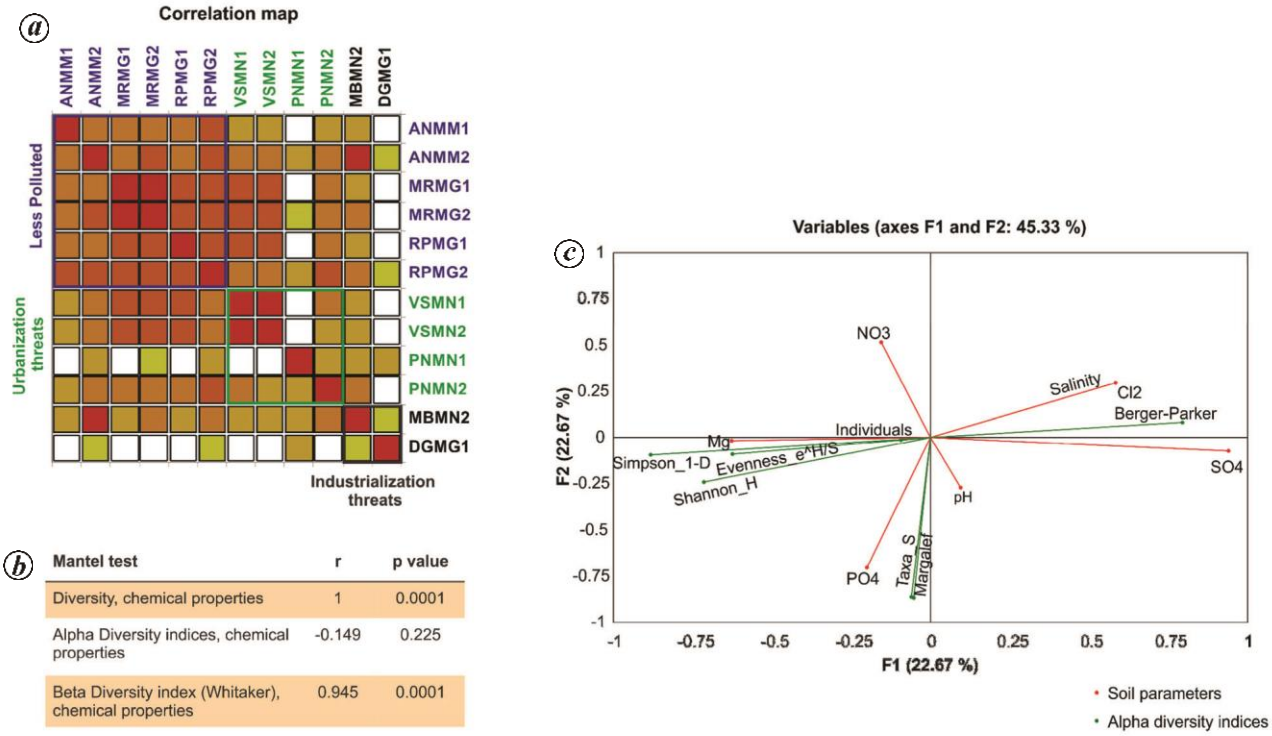

Figure 6. ***a***, Correlation map; ***b***, Mantel test; ***c***, Canonical correlation analysis.

and anthropogenic processes[30,31]. The mangroves of India face various anthropogenic stressors and are declining[30]. North Maharashtra along the west coast of India is one of the rapidly industrializing and urbanizing regions and faces pollution[30,32,33].

Mangroves from different ecologically sensitive areas like LP areas, urban areas (UT) and industrial areas (IT) from Maharashtra were analysed in this study. Arnala, Murud and Rajapuri areas facing organic and inorganic pollution, Panvel and Vasai creek areas with pollution from Panvel and Vasai cities, Murbe with proximity to Tarapur industrial estate and Dighi facing industrial and port pollution were examined. These areas are different from each other in terms of catchment area, freshwater input as well as addition of industrial and domestic wastes. The objective of the present study is to provide baseline data on bacterial diversity in the mangroves along northern coast of Maharashtra.

Metagenomic analysis helps to document the rich microbial community structure and dynamics. Although a few studies have been carried out in Sundarbans of India, northeast and Andaman and Nicobar[27,34,35], the metagenomic profiles of mangroves of western coast of India have not been examined. The taxonomic analysis of Sundarban mangrove samples revealed the abundance of Bacteroidetes, Acidobacteria, Firmicutes, Actinobacteria, Nitrospirae, Cyanobacteria, Planctomycetes and Fusobacteria[25,27]. Proteobacteria were abundant in this study and also in the Sundarbans and Bhitarkanika mangrove dataset[23,25,27]. Actinobacteria, Acidobacteria, Nitrospirae, and Verrucomicrobia were detected in inner mangrove sediments whereas Proteobacteria and Deferribacteria were present in high numbers in outer mangrove sediments of Mai Po Ramsar Wetland in Hong Kong, SAR, China[24]. The Brazilian mangrove sediment microbiomes[36] and Indian mangrove sediment microbiomes showed presence of higher abundance of bacteria. Red sea mangrove samples have been reported to possess relatively higher number of Achaea compared to the microbiomes from Brazilian and Indian mangrove sediments. Andreote *et al.*[36] have reported high abundance of Acidobacteria and absence of Thaumarchaeota (Nitrosopumilales) in red sea samples whereas Nitrosopumilales were present in moderate numbers. Samples from Indian mangroves showed the presence of high numbers of sulphate reducing bacteria. Importantly, Firmicutes were abundant in some samples (DGMG1 and MBMN2). The differences in bacterial diversity can be attributed to the different geographical locations, plant species, and/or physico-chemical characteristics of the samples, which would lead to differences in the community compositions. Additional divergence could also be attributed to anthropogenic stressors acting on mangrove areas, chemical composition of the area and pollution. Proteobacteria, well known for their role in nitrogen fixation in marine ecosystems, are the most abundant group in mangrove samples[24,27,36–38]. Among the Proteobacteria detected, Gammaproteobacteria were most abundant followed by Deltaproteobacteria and Alphaproteobacteria. The *nifH* gene, the key player in nitrogen fixation, has been detected predominantly in Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria[39–41]. Interestingly, these bacteria are also known to be hydrocarbon degraders. Similarly, Bacteroidetes are frequent in tidal mudflats, near-shore sediments, as well as in hydrocarbon-contaminated environments, a few of which are hydrocarbon degraders[42]. Despite frequent detection in soils, little is known about the possible role of Chloroflexi and Verrucomicrobia. Substantial abundance of Bacteroidetes in the rhizospheres of mangroves has been previously noted[22]. The mixed marine and terrestrial conditions in mangroves are suitable for nitrogen fixer Proteobacteria and Bacteroidetes. Mangroves can thus serve as reservoirs for these genetically and ecologically novel microbes.

Sulphate reducing bacteria, such as *Desulfobacca acetoxidans*, *Desulfotalea psychrophila*, *Desulfobulbus propionicus* were present in high numbers in all mangrove sediment samples analysed in this study from the west coast of India. The high concentration of sulphate in sea water present in mangroves, the anoxic conditions and very low redox potential provide an ideal environment in mangroves for sulphate reducers and methanogens[20]. It can be seen that sulphate reducers and methanogens show co-occurrence in mangroves which can be linked to the use of different metabolic pathways and utilization of non-competitive substrates[43,44]. In the mangrove ecosystem, sulphate reducers play an important role in the oxidation of organic matter, degradation of long-chain and aromatic compounds[45], production of H_2S which reacts with insoluble iron phosphates[46], releasing phosphate and other ions which are essential for plant growth[47]. The higher abundance of sulphate reducers in Indian mangrove soil sediments clearly suggests that these organisms are important players in mangrove ecology. In mangroves, the frequent anaerobic conditions (anoxia) could drive selection of specific microbial groups such as sulphate reducing bacteria[20]. Sulphur can play a role in supporting microbial metabolism (i.e. sulphate reduction) or exerting a toxic effect in case of specific microbial groups (in the form of H_2S). Sulphur cycle performed by these sulphate reducing bacteria fuels nitrate reduction, thereby supplying additional substrates (nitrite and ammonia) for anammox bacteria.

The alpha and beta diversity indices for microbial diversity of these mangrove samples from the west coast of India emphasize the species richness and high diversity in these samples. Chao-1 analysis suggested that this metagenomic analysis identified around 80% species inhabited in these mangrove sediments. One important outcome of the present study is that it clearly documents the threat to mangrove microbial community due to industrial pollution. Only 91 species were found unique to samples which are facing industrial pollution, whereas 272 and 250 species were found unique and specific to mangroves which are located near less polluted areas and urban areas respectively. Although Shanon's diversity index and evenness index remained similar in these three groups, microbial community structure and dynamics are substantially influenced by anthropogenic stressors. Notable example was the high abundance of bacilli in Murbe and Dighi samples. nMDS analysis indicated that mangroves of the west coast of India harboured similar microflora typical to the mangroves specific niche. However, the Dighi and Murbe samples differed from all other studied samples with respect to the chemical parameters. It could be seen that chemical properties of these samples significantly influenced microbial diversity. Correlation maps also suggested that bacterial diversity in Dighi and Murbe samples is significantly different from other mangroves samples analysed in this study. The Mantel test clearly

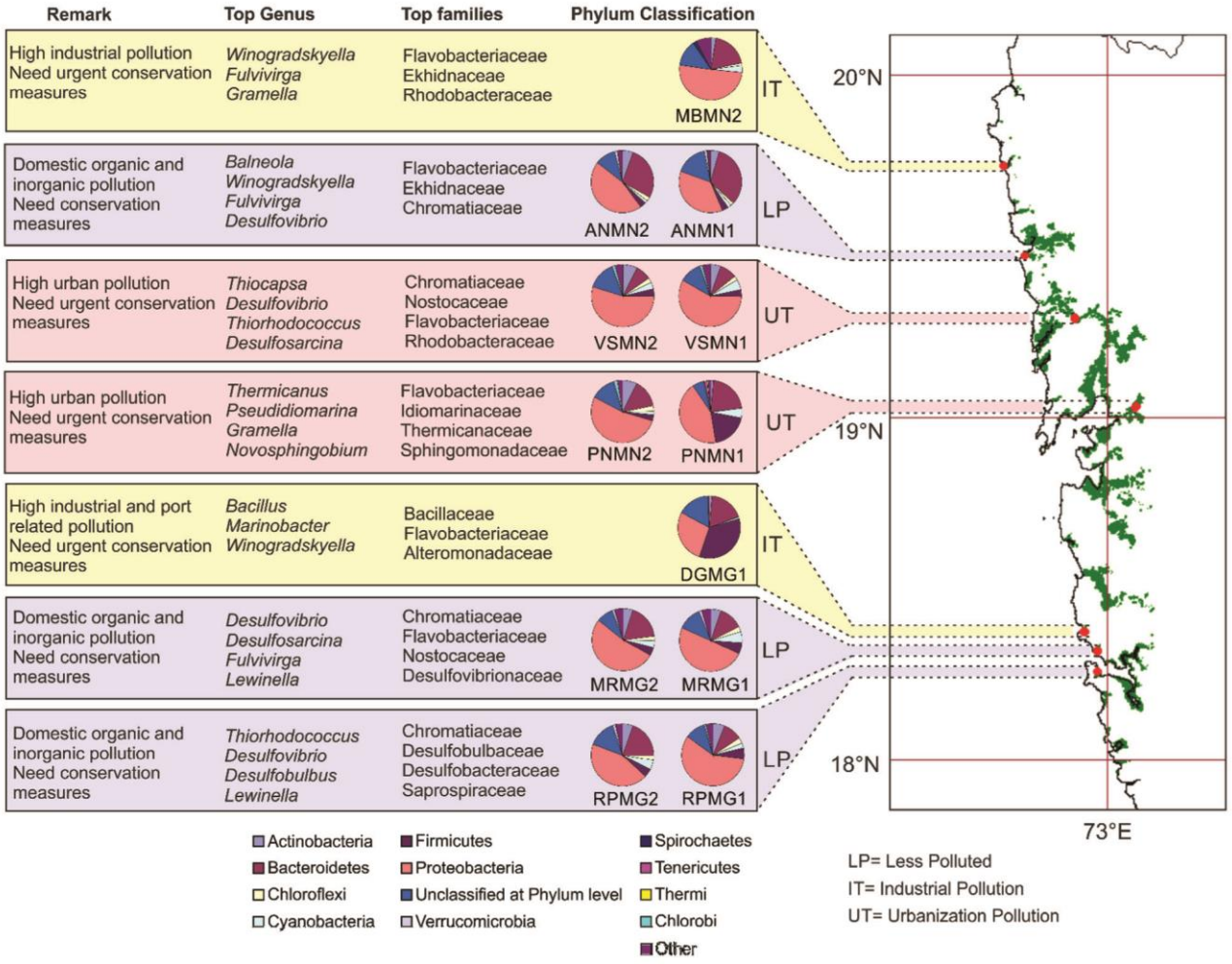

Figure 7. Microbial diversity at various mangrove sites.

indicated that chemical properties of soil sediments influenced bacterial diversity in these mangrove areas. Canonical correlation analysis highlighted that SO_4 concentration led to the dominance of fewer species. It is likely that H_2S production by sulphate reducers may exert toxic effect on some species, thereby reducing the diversity. PO_4 concentration enhanced the number of species whereas Mg concentration played an important role in determining the evenness in the samples. The innate resilience of mangroves helps the mangrove ecosystem to cope with environmental changes. Along the west coast of India, the mangroves ecosystem, although rich in microbial communities, is under tremendous pressure of anthropogenic stressors. The microbial communities of mangroves of Murbe and Dighi are now replaced with non-native mangrove bacteria such as Bacilli. Microflora of Panvel and Vasai is largely affected by domestic waste and urbanization. The mangroves of Murud, Rajapuri and Arnala which are relatively undisturbed have good abundance of native mangrove flora such as sulphur reducing bacteria and methanogens. The present study highlights that industrial waste and chemicals are major factors in altering the mangrove ecosystems. The synergistic effects of human-induced and natural changes need to be taken into account while planning and implementing conservation strategies. There is an urgent need to develop a conservation strategy which should consider the upstream development, microbial communities of sediment, human-induced changes and climate change-oriented transformation in mangrove ecosystems.

In conclusion, the present study indicates the presence of novel groups of bacteria in the mangrove sediment of west coast of India and suggests their association with biochemical cycles of sulphur, carbon and other elements. The phylogenetic affiliation of the sequences obtained in this study has allowed a robust comparison of the taxonomically dominant groups in distinct mangroves (Figure 7). We have generated the baseline data of bacterial diversity of sediments of mangroves of west coast of India. The current observations clearly indicate that soil chemical composition and presence of pollutants play an important role in determining the bacterial diversity. The

diversity of microbial communities highlights the importance of understanding the dynamics and complexity of mangrove sediments which can provide pointers for inferring a critical ecological role for these microorganisms in the mangrove ecosystems. Future research considering deeper soil and involving more sampling along with functional metagenomics will lead to in-depth exploration of the biodiversity of these communities and the community structure. Mangroves of the west coast of India are under tremendous pressure of pollution and deforestation. These anthropogenic activities near mangrove areas are playing an important role in declining of the ecological niche and in turn the carbon and nitrogen cycle in mangroves which thus affects the mangrove ecosystems. Moreover, these human-induced changes near mangrove forests are hampering the diversity of these genetically and ecologically novel microbes. Therefore, there is an urgent need to apply resilience principles such as maintaining diversity and redundancy, fostering complex adaptive systems thinking and broadening the participation of stakeholders in all development and conservation programmes and planning strategies to conserve ecologically sensitive and important sites.

1. Alongi, D. M., Present state and future of the world's mangrove forests. *Environ. Conserv.*, 2002, **29**(3), 331–349.
2. Beck, M. W. *et al*., The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates: a better understanding of the habitats that serve as nurseries for marine species and the factors that create site-specific variability in nursery quality will improve conservation and management of these areas. *Bioscience*, 2001, **51**, 633–641.
3. McLusky, D., *The Estuarine Ecosystem*, Springer Science and Business Media, 2013.
4. Field, C. *et al*., Mangrove biodiversity and ecosystem function. *Global Ecol. Biogeogr. Lett*., 1998, **7**, 3–14.
5. Duke, N. C. *et al*., A world without mangroves. *Science*, 2007, **317**, 41.
6. Gomes, N. C. M., Borges, L. R., Paranhos, R., Pinto, F. N., Mendonça-Hagler, L. C. and Smalla, K., Exploring the diversity of bacterial communities in sediments of urban mangrove forests. *FEMS Microbiol. Ecol*., 2008, **66**(1), 96–109.
7. DeLong, E. F. *et al*., Community genomics among stratified microbial assemblages in the ocean's interior. *Science*, 2006, **311**, 496–503.
8. Konstantinidis, K. T., Braff, J., Karl, D. M. and DeLong, E. F., Comparative metagenomic analysis of a microbial community residing at a depth of 4000 meters at station ALOHA in the North Pacific subtropical gyre. *Appl. Environ. Microbiol*., 2009, **75**(16), 5345–5355.
9. Shi, Y., Tyson, G. W., Eppley, J. M. and DeLong, E. F., Integrated metatranscriptomic and metagenomic analyses of stratified microbial assemblages in the open ocean. *ISME J*., 2010, **5**(6), 999–1013.
10. Quaiser, A., Zivanovic, Y., Moreira, D. and Lopez-Garcia, P., Comparative metagenomics of bathypelagic plankton and bottom sediment from the Sea of Marmara. *ISME J*., 2011, **5**(2), 285–304.
11. Kimes, N. E. *et al*., Metagenomic analysis and metabolite profiling of deep-sea sediments from the Gulf of Mexico following the Deepwater Horizon oil spill. *Front. Microbiol*., 2013, **4**, 50; doi:10.3389/fmicb.2013.00050.
12. Tringe, S. G. *et al.*, Comparative metagenomics of microbial communities. *Science*, 2005, **308**, 554–557.
13. De Angelis, K. M. *et al*., Strategies for enhancing the effectiveness of metagenomic based enzyme discovery in lignocellulolytic microbial communities. *Bioenergy Res.*, 2010, **3**(2), 146–158.
14. Baldrian, P. *et al*., Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J*., 2012, **6**, 248–258.
15. Fierer, N. *et al.*, Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proc. Natl. Acad. Sci. USA*, 2012, **109**, 21390–21395.
16. Simon, C., Wiezer, A., Strittmatter, A. W. and Daniel, R., Phylogenetic diversity and metabolic potential revealed in a glacier ice metagenome. *Appl. Environ. Microbiol.*, 2009, **75**(23), 7519–7526.
17. Bodaker, I. *et al*., Comparative community genomics in the Dead Sea, an increasingly extreme environment. *ISME J*., 2010, **4**, 399–407.
18. Inskeep, W. P. *et al*., Metagenomes from high-temperature chemotrophic systems reveal geochemical controls on microbial community structure and function. *PLoS ONE*, 2010, **5**(3), e9773.
19. Amaral-Zettler, L. A. *et al*., Microbial community structure across the tree of life in the extreme Río Tinto. *ISME J*., 2011, **5**(1), 42–50.
20. Taketani, R. G., Yoshiura, C. A., Dias, A. C. F., Andreote, F. D. and Tsai, S. M., Diversity and identification of methanogenic archaea and sulphate-reducing bacteria in sediments from a pristine tropical mangrove. *A van Leeuw J. Microb*., 2010, **97**(4), 401–411.
21. Ghosh, A., Dey, N. and Bera, A., Culture independent molecular analysis of bacterial communities in the mangrove sediment of Sundarbans, India. *Saline Syst.*, 2010, **6**(1), 1; http://dx.doi.org/10.1186/1746-1448-6-1.
22. Gomes, N. C. M. *et al*., Taking root, enduring effect of rhizosphere bacterial colonization in mangroves. *PLoS ONE*, 2010, **5**(11), e14065.
23. Mishra, R. R., Swain, M. R., Dangar, T. K. and Thatoi, H., Diversity and seasonal fluctuation of predominant microbial communities in Bhitarkanika, a tropical mangrove ecosystem in India. *Rev. Biol. Trop.*, 2012, **60**(2), 909–924.
24. Jiang, X. T., Peng, X., Deng, G. H., Sheng, H. F., Wang, Y. and Zhou, H. W., Illumina sequencing of 16S rRNA tag revealed spatial variations of bacterial communities in a mangrove wetland. *Microb. Ecol.*, 2013, **66**, 96–104.
25. Basak, P. *et al*., Spatiotemporal analysis of bacterial diversity in sediments of Sundarbans using parallel 16S rRNA gene tag sequencing. *Microb. Ecol.*, 2014, **69**, 500–511.
26. Prakash, S. *et al*., Influence of physicochemical and nutritional factors on bacterial diversity in mangrove sediments along the southwest coast of Tamil Nadu, India. *Environ. Monit. Assess.*, 2015, **187**, 562.
27. Basak, P. *et al.*, Bacterial diversity assessment of pristine mangrove microbial community from Dhulibhashani, Sundarbans using 16S rRNA gene tag sequencing. *Genom. Data*, 2016, **7**, 76–78.
28. Upadhyay, V. P., Ranjan, R. and Singh, J. S., Human-mangrove conflicts: the way out. *Curr. Sci.*, 2002, **83**, 1328–1336.
29. Giri, C. *et al*., Status and distribution of mangrove forests of the world using earth observation satellite data. *Global Ecol. Biogeogr*., 2011, **20**, 154–159.
30. Sahu, S. C., Suresh, H. S., Murthy, I. K. and Ravindranath, N. H., Mangrove area assessment in India, implications of loss of mangroves. *J. Earth Sci. Clim. Change*, 2015, **6**, 280; doi:10.4172/2157-7617.1000280.
31. Kulkarni, R., Deobagkar, D. and Zinjarde, S., Metals in mangrove ecosystems and associated biota: a global perspective. *Ecotoxicol. Environ. Saf.*, 2018, **153**, 215–228.

32. Jha, S. K., Chavan, S. B., Pandit, G. G., Negi, B. S. and Sadasivan, S., Behaviour and fluxes of trace and toxic elements in creek sediment near Mumbai, India. *Environ. Monit. Assess.*, 2002, **76**, 249–262.
33. Fernandes, L., Nayak, G. N., Ilangovan, D. and Borole, D. V., Accumulation of sediment, organic matter and trace metals with space and time, in a creek along Mumbai coast, India. *Estuar. Coast Shelf Sci*., 2011, **91**, 388–399.
34. Kandasamy, K. A., Review of studies on Pichavaram mangrove, Southeast India. *Hydrobiologia*, 2000, **430**(1), 185–205.
35. Ragavan, P., Mohan, P. M., Saxena, A., Jayaraj, R. S. C., Ravichandran, K. and Saxena, M., Mangrove floristics of the Andaman and Nicobar Islands: critical review and current scenario *Mar. Biodivers.*, 2016, **48**, 1291–1311; https://doi.org/10.1007/s12526-016-0581-3.
36. Andreote, F. D. *et al*., The microbiome of Brazilian mangrove sediments as revealed by metagenomics. *PLoS ONE*, 2012, **7**(6), e38600.
37. Alzubaidy, H. *et al*., Rhizosphere microbiome metagenomics of gray mangroves (*Avicennia marina*) in the Red Sea. *Gene*, 2016, **576**, 626–636.
38. Salter, S. J. *et al*., Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biol*., 2014, **12**(1), 87.
39. Bagwell, C. E., La Rocque, J. R., Smith, G. W., Polson, S. W., Friez. M. J., Longshore, J. W. and Lovell, C. R., Molecular diversity of diazotrophs in oligotrophic tropical seagrass bed communities. *FEMS Microbiol. Ecol*., 2002, **39**, 113–119.
40. Bird, C., Martinez, J. M., O'Donnell, A. G. and Wyman, M., Spatial distribution and transcriptional activity of an uncultured clade of planktonic diazotrophic γ-proteobacteria in the Arabian Sea. *Appl. Environ. Microbiol*., 2005, **71**, 2079–2085.
41. Church, M. J., Short, C. M., Jenkins, B. D., Karl, D. M. and Zehr, J. P., Temporal patterns of nitrogenase gene (*nifH*) expression in the oligotrophic North Pacific Ocean. *Appl. Environ. Microbiol*., 2005, **71**, 5362–5370.
42. Kim, S. J. and Kwon, K. K., *Bacteroidetes*, Handbook of Hydrocarbon and Lipid Microbiology, 2010, pp. 1813–1817.
43. Lyimo, T. J., Pol, A. and Op den Camp, H. J. M., Methane emission, sulphide concentration and redox potential profiles in Mtoni mangrove sediment, Tanzania. *Western Indian Ocean J. Mar. Sci*., 2002, **1**, 71–80.
44. Lyimo, T. J., Pol, A. and Op den Camp, H. J. M., Sulfate reduction and methanogenesis in sediments of Mtoni mangrove forest, Tanzania. *Ambio*, 2002, **31**, 614–616.
45. Muyzer, G. and Stams, A. J., The ecology and biotechnology of sulphate-reducing bacteria. *Nat. Rev. Microbiol*., 2008, **6**(6), 441–454.
46. Alongi, D. M., Boto, K. G. and Robertson, A. I., *Nitrogen and Phosphorus Cycles in Tropical Mangrove Ecosystems*, Am Geophys Univ, Washington DC, 1992, vol. 41, pp. 251–292.
47. Rodríguez, H. and Fraga, R., Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.*, 1999, **17**(4–5), 319–339.

ACKNOWLEDGEMENTS. We thank Prof. Dileep N. Deobagkar for his valuable suggestions. The authors would like to acknowledge financial support provided by the Ministry of Earth Sciences (MoES), New Delhi and UGC-UPE-II.

Received 17 July 2017; revised accepted 25 July 2018

doi: 10.18520/cs/v115/i8/1525-1535