

## Seasonal Variability In Environmental Parameters Influence Bacterial Communities In Mangrove Sediments Along An Estuarine Gradient.

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### Abstract

Mangrove ecosystems are repositories of divergent microbial communities which respond differently to environmental characteristics. Hence, it is important to understand the community structure with respect to pertinent controlling environmental factors. Seasonal down-core variations in bacterial communities were investigated at 3 mangrove sites at upstream, mid-stream and mouth region along the economically-important Mandovi estuary, west coast of India. Genomic DNA extracted from 45 sediment samples were analyzed by 454 pyrosequencing. A total of 15008 operational taxonomic units (OTUs) were assigned to 54 phyla and 127 classes re-affirming mangroves to be biodiversity hotspots. Members of class Alphaproteobacteria constituted up to 35% of the tag sequences and showed marked seasonal variability at all locations (Kruskal-Wallis test,  $p < 0.05$ ). Occurrence of other abundant phyla like Bacteroidetes, Firmicutes, Actinobacteria, Chloroflexi and Acidobacteria at the study sites indicated their participation in biogeochemical cycling. Seasonal fluctuations in the environmental variables influenced the bacterial communities. Statistical analysis revealed that during the monsoon, bacterial communities in mouth region were largely influenced by pore water  $\text{NH}_4^+$  concentrations due to its accumulation in the sub-surface sediments. At the upstream site, elevated organic carbon and  $\text{NO}_2^-$  content during the post-monsoon caused variations in the bacterial community.

**Keywords:** Organic matter, environment, phylum, functions, pyrosequencing, microbes.

## 1.1 Introduction

Mangrove sediments are reservoirs of organic matter (Koster and Meyer-Reil, 2001), absorbed nutrients (Hou *et al.*, 2013), pesticides (Enrich-Prast, 2006) and heavy metals (Li *et al.*, 2016). They are complex systems affected by the interaction between geological, hydrological, physico-chemical and biological factors (Zheng *et al.*, 2014). Bacteria residing in these mangrove sediments have fundamental roles in mediating biogeochemical cycles of elements, thus leading to the uptake and release of chemicals (Brune *et al.*, 2000; Spring *et al.*, 2000; Wetzel, 2000; Borsodi *et al.*, 2003; Micsinai *et al.*, 2003). Besides, bacterial communities mediate the oxidation of complex organic matter leading to the generation of inorganic nutrients which are recycled to the overlying water column to sustain primary production (Carpenter and Capone, 1983; Sloth *et al.*, 1995; Herbert, 1999). Hence, the presence of metabolically active and dominant groups within the bacterial communities indicate different degrees of biochemical activities. This could be of peculiar importance in ecological studies (Azam and Worden, 2004; Martiny *et al.*, 2006; Cottrell and Kirchman, 2003). Therefore, an in-depth analysis of microbial communities along with environmental parameters is crucial in understanding their contribution to ecosystem functioning.

Culturable bacteria accounts for 0.001 to 3 % of the total microbial cells (Amann *et al.*, 1995). Hence, culture-independent techniques for detecting microbial genes in environmental samples are considered as a powerful tool to study the structure and function of bacterial communities in varied habitats (Hugenholtz *et al.*, 1998; Roesch *et al.*, 2007; Qian *et al.*, 2011; Kim *et al.*, 2011; Zhu *et al.*, 2013; Zheng *et al.*, 2014). Few culture-independent studies have been carried out to assess the taxonomic diversity of bacteria in mangrove sediments (Sjöling *et al.*, 2005; Liang *et al.*, 2007; Gomes *et al.*, 2013). Recent studies reported the adaptability of microbial diversity with changing environmental conditions (Tavares *et al.*, 2021; Zhang *et al.*, 2019; Chen *et al.*, 2021; Pramanik *et al.*, 2019). Also, the influence of anthropogenic activity on the bacterial community was studied in the Mandovi estuarine mangrove ecosystems of Goa, India (Fernandes *et al.*, 2014; Khandeparker *et al.*, 2017). However, the aforementioned studies were limited only to surface sediment samples. This study highlights the influence of environmental factors on spatial, temporal and depth-wise changes in the microbial community structure.

In an estuary, the presence of salinity gradient caused due to mixing of fresh water and seawater can lead to strong chemical and biological gradients. This makes estuaries an ideal environment to examine shifts in bacterioplankton communities along a spatial gradient. Changes in benthic microbial communities can be useful as an indicator for assessing the pollution states of coastal marine environments like the mangrove ecosystems that are prone to anthropogenic influence

(Zheng *et al.*, 2014; Aguirre *et al.*, 2017; Kawalekar, 2015; Araujo *et al.*, 2018). Changing environmental factors can directly impact the biological functioning of these systems. Given the importance of bacterially-mediated processes to ecosystem functioning, it is critical to study bacterial diversity in the context of shifting environmental drivers in adjoining mangrove ecosystems. In this study, a pyrosequencing approach was used to investigate the following: (i) composition of bacterial communities with reference to the spatio-temporal and depth-wise variation in environmental parameters in mangrove sediments along an economically-important estuary (ii) structure of the bacterial community that is influenced by the environmental variables. It is hypothesized that variations in physico-chemical parameters mainly the disparity in the extent of organic loading at sampling sites along the estuary would affect bacterial communities prevailing in their respective environments.

## **1.2 Materials and methods**

### *1.2.1 Study area and sampling*

The Mandovi estuary (length ~52 km, area ~29 km<sup>2</sup>, average depth ~4 m) on the west coast of India is described as the lifeline of the state of Goa as it has been used by the mining industry as an efficient waterway for transporting Fe-Mn ore (Kessarkar *et al.*, 2015; Prajith *et al.*, 2016; Singh *et al.*, 2014) from mines located upstream. The average annual river discharge is 6004 Mm<sup>3</sup> (Manoj *et al.*, 2012). Runoff into the estuary varies from ~1000 m<sup>3</sup> s<sup>-1</sup> in the wet season to ~1 m<sup>3</sup> s<sup>-1</sup> at end of the dry season (Vijith *et al.*, 2016). During dry season, tidal circulation dominates and saline waters penetrate ~ 45 km upstream from the river mouth (Shynu *et al.*, 2015). It also receives a considerable supply of inorganic nitrogenous nutrients from various sources such as mining wastes (Divya *et al.*, 2009), land runoff (Sardessai and Sundar, 2007) and sewage effluents (Maya *et al.*, 2011; Araujo *et al.*, 2018). There have been reports of sewage effluents being discharged from the capital city of Panaji into the river Mandovi at Campal located ~ 3 km from the mouth at a rate of about 7×10<sup>3</sup> m<sup>3</sup> d<sup>-1</sup> (Ansari *et al.*, 1986; Nanajkar and Ingole, 2010).

The Mandovi estuary is fringed with a luxuriant mangrove cover. The diverse community of mangroves here are a rich repository of other associated faunal species. In addition, these mangrove systems are known to help in depuration of excess nutrients from the aquatic system (Fernandes and LokaBharathi, 2011). Three mangrove regions along the Mandovi estuary (Fig. 1) were selected for detailed studies i.e. at upstream which is 21 km away from the mouth; 15°29'41" N and 74°00'38" E, mid-stream which is 5.8 km from the mouth; 15°30'45" N and 73°52'14" E and proximal to the mouth; 15°30'19" N and 73°49'31" E. At the upstream site, salinity values are ~ 10 during the dry

season while in the monsoon season fresh water pre-dominates (Manoj, 2008). Along the upstream site of the Mandovi estuary mangroves like *Sonneretia caseolaris*, *Acanthus illicifolius* and *Aegiceras corniculatum* predominate (Department of Forests, Government of Goa). The mid-stream mangrove ecosystem is situated at the confluence of the Mandovi and Mapusa river. About 2.5 km<sup>2</sup> of mangrove forest at the mid-stream site was declared as a bird sanctuary in 1987 by the Government of Goa for the purpose of conservation and has been named as the Dr. Salim Ali Bird Sanctuary. This site houses 15 species of mangroves most of which are found in Goa (Dhargalkar *et al.*, 2014). Some of the dominant mangrove species at this site are *Rhizophora mucronata*, *Avicennia alba*, *A. officinalis*, *Sonneratia alba* and *S. caseolaris* (Dhargalkar *et al.*, 2014). A variety of algae, fishes, migratory birds, reptiles and mammals also contribute to the biodiversity. The site adjacent to the mouth of the estuary is characterized by anthropogenic activities such as the presence of trawlers, offshore casinos, plying of ferry service and land-derived sewage/rainfall discharge. *Sonneratia alba*, *R. mucronata* and *A. alba* have been reported at the mouth region of the estuary (Department of Forests, Government of Goa).

Sampling was carried out at low tide during May (pre-monsoon), July (monsoon) and November (post-monsoon), 2014. A total of 12 cores were collected at each sampling and were used as follows: (i) Three cores were collected using PVC push cores (7.5 cm inner diameter, 20 cm length) and capped at both ends. The variation in most of the physico-chemical parameters was measured on field. (ii) Six cores of dimensions described above were collected for chemical and microbiological analyses. These were transported to the laboratory in an ice box and then were stored at 4 °C until analyses which were completed within 24 h of sample collection. (iii) Three sediment cores were collected for molecular biology analysis. On arrival to the laboratory, the sediment samples were sectioned aseptically at respective depths. A cut-off 10 mL syringe was used to draw the sediment sample and transfer it in 50 mL centrifuge tubes (~3 g). The samples were lyophilised (model no. LYOLAB 1500), sealed with parafilm and stored at -80 °C until further processing.

### 1.2.2 Physico-chemical and biochemical analysis

Sediment temperature, hydrogen ion concentration (pH) and oxidation-reduction potential at each section was measured using an IQ Scientific Waterproof ISFET pH/Ion Meter Kit (Cole-Parmer-99361-12). Salinity of the pore water was measured using an ATAGO hand held refractometer (model: MASTER-S28 $\alpha$ ). About 10 g of sediment was taken in 50 mL centrifuge tubes, centrifuged at 5000 rpm for 10 min at room temperature. Pore water salinity was measured as explained by Das *et al.* (2011). For nutrient analyses, 5 g of sediment was transferred to centrifuge tubes containing 30 mL of seawater whose blank was measured and corrected for respective

nutrients. The contents were gently homogenized using a glass rod. The resultant slurry was centrifuged at 4 °C for 10 min at 5000 rpm. The supernatant was filtered through a 0.2 µm filter. Ammonia (Koroleff, 1969), nitrite (Bendschneider and Robinson, 1952), nitrate (Wood *et al.*, 1967) and phosphate (APHA, 1992) were measured colorimetrically.

For analysis of total organic carbon (TOC), sediment samples were dried in a hot air oven at 60 °C for 48 h. Organic carbon was determined by the wet oxidation method with a precision of 0.01% (El Wakeel & Riley, 1957).

Total proteins in sediments were estimated by Lowry's Folin Ciocalteu method using bovine serum albumin as standard (Lowry *et al.*, 1951). Total carbohydrates in sediments were estimated by phenol-sulfuric acid method using glucose as standard (Kochert, 1978). Total lipids in sediments were estimated according to Bligh & Dyer (1959) using stearic acid as standard. The sum of total proteins, carbohydrates and lipids was expressed as labile organic matter (LOM) (Danovaro *et al.*, 1993).

### *1.2.3 Enumeration of total prokaryotic cells*

The cores were sectioned aseptically as described above. Sub-samples of ~1 g wet weight sediment were diluted with 9 mL of sterile estuarine water. The sediments were vortexed thoroughly and allowed to settle. One mL of sample was transferred to sterile eppendorf tubes and fixed with buffered formalin at a final concentration of 2%. The samples were stored in 4 °C until further processing. During the enumeration of total bacterial cells, the stored samples were sonicated at 15 hertz for 15 s. The samples were stained with 50 µL of 0.01% acridine orange for 10 min in the dark and filtered on 0.22 µm black polycarbonate filter paper (Millipore, USA), using a Millipore filtration unit. The cells were enumerated using the epifluorescence microscope (Nikon Eclipse E50i microscope) by the acridine orange direct count (AODC) method as described by Hobbie *et al.* (1977). Cells were counted from 10-100 microscopic fields. The sediment samples were filtered using a pre-weighed Whatman filter paper. Sediments were oven dried and the weight obtained was used to calculate the total prokaryotic abundance in one gram of sediment.

### *1.2.4 DNA extraction*

Genomic DNA was extracted from 0.25 g of freeze dried sediment from each section of the cores. The Power Soil DNA isolation kit from MO BIO Laboratories, (CA, USA) was used to extract the DNA according to the manufacturer's protocol. Thereafter, DNA extracts from each section of the triplicate cores were pooled, stored at -20 °C for use as templates for PCR amplifications and for pyrosequencing analysis.

### 1.2.5 Bacterial 16S rRNA gene amplification and pyrosequencing

The V1-V3 hyper variable regions of bacterial 16S rRNA gene were amplified from the extracted DNA templates by polymerase chain reaction (PCR). The 27F forward primer with multiplex identifier

MID:5'CCATTCATCCCTGCGTGTCTCCGACT

CAGXXXXXXXXXXAGAGTTTGATCMTGGCTCAG-3', where X's represents the sample-specific multiplex identifier-MID (Kim *et al.*, 2011) was used as the forward primer while

519R with adaptor:

5'CCTATCCCCTGTGTGCCTTGGCAGTCTCAG

(GWATTACCGCGGCKGCTG 3' was used as the reverse primer (Kim *et al.*, 2011). The

PCR reactions were carried out in 20  $\mu$ L volumes and in triplicates. The PCR mixture consisted of 2  $\mu$ L of DNA template, 13.1  $\mu$ L molecular biological grade double distilled water, 0.6  $\mu$ L (5  $\mu$ M) of each primer, 2  $\mu$ L 10X Ex Taq Buffer (TaKaRa Bio Inc., Shiga, Japan), 1.6  $\mu$ L dNTP mixture (TaKaRa Bio Inc.), and 0.1  $\mu$ L Ex Taq HS Polymerase (TaKaRa Bio Inc.). Thermal cycling was carried out for a total of 25 cycles as per the following conditions: initial denaturation at 94 °C for 4 min, denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, elongation at 72 °C for 1 min and final elongation at 72 °C for 10 min. After amplification, the desired length of the 16S rRNA gene was confirmed by agarose gel electrophoresis. The amplified DNA products were pooled and purified using Agencourt AMPure XP (Beckman Coulter Inc., Indianapolis, USA). Thereafter, purified products were visualized using agarose gel electrophoresis. The target bands were excised and purified using a gel extraction kit (Qiagen, Hilden, Germany). The purified DNA amplicon was then quantified using Quant-iT Picogreen ds DNA Kit (Invitrogen, Carlsbad, USA). The bacterial 16S rRNA gene amplicons were then sequenced using the GS Junior sequencer (Roche Applied Science, Indianapolis, USA) according to the manufacturer's protocol for 454 GS Junior Titanium Series.

### 1.2.6 Pyrosequencing data set analyses

The open-source MOTHUR program (Schloss *et al.*, 2009) was used for sequence analysis, quality checking and arrangement of the obtained sequences following the guidelines available to the operation manual for 454 ([http://www.mothur.org/wiki/454\\_SOP](http://www.mothur.org/wiki/454_SOP)). Initially, unique sequences were selected and then the similar sequences were clustered and aligned against the SILVA bacterial databases. Thereafter, the pre-cluster method (Huse *et al.*, 2010) was applied to reduce the sequencing errors by screening, filtering and de-noising. The chimera.uchime command was used for checking and removing the chimeras. The sequences were subsequently classified against the SILVA v123 database and inactive components such as chloroplast, mitochondria, etc. were removed from the dataset to improve data quality. High-quality sequences were then used to generate distance

matrix and clustered assigning to operational taxonomic units (OTUs) at 97% identity level (Schloss and Westcott, 2011). To standardize the number of sequences between samples, they were randomly re-sampled to the sample with the lowest reads (1500 reads) using the ‘phyloseq’ package in R version 3.5.0 (R Core Team, 2018). A total of 38 out of 45 samples (upstream: n = 12; mid-stream: n = 11; mouth: n = 15) were retained after subsampling and used for downstream analysis. Species richness was calculated using the ace Chao1 index (Chao *et al.*, 2005), diversity was estimated using the Shannon diversity index and inverse Simpson (Invsimpson) index (Simpson, 1949) using the ‘phyloseq’ package.

### *1.2.7 Nucleotide sequence accession numbers*

The 16S rRNA gene sequences reported in this study has been submitted to the DDBJ database and the DRA accession number is DRA011696.

### *1.2.8 Statistical analyses*

Multivariate analysis was performed in R 3.5.0 implemented in the ‘vegan’ package (Oksanen, 2016). The association between bacterial assemblages and environmental parameters were examined using canonical correspondence analysis (CCA).  $\log_2(x+1)$  transformation was applied to bacterial abundance data in order to correct for possible statistical errors associated with the relative abundance difference between major and rare bacterial classes. Environmental parameters with variance inflation factor (VIF) of less than 10 was used as a criterion for analysis (Oksanen, 2016). Permutation tests by marginal effects with 1,000 permutations were applied to estimate the significance of the VIF-based model. The correlation between the environmental variables and bacterial classes at each sampling site were also examined using the Spearman’s rank correlation coefficient and visualized using the ‘ggplot2’ package in R. Kruskal-Wallis test was used to estimate the significant seasonal variability in the bacterial communities.

## **1.3 Results**

### *1.3.1 Physico-chemical and biochemical parameters*

Data for physico-chemical characteristics of the samples studied have been tabulated in Table 1. During the pre-monsoon and monsoon, temperature varied marginally with depth, at all sampling locations. However, during post-monsoon, significantly higher sediment temperatures were recorded at the upstream site (ANOVA,  $p < 8.87 \times 10^{-9}$ ,  $df = 2$ ) and proximal to the mouth ( $p < 2.99 \times 10^{-15}$ ,  $df = 2$ ). A sub-surface increase in temperature of up to a maximum of 2.44 °C was recorded at 3-4 cm at the upstream site followed by a steady decrease thereafter (Table 1).

The sediments at the upstream site were weakly acidic with pH values ranging from  $6.12 \pm 0.02$  to  $6.52 \pm 0.02$  and followed a similar down-core pattern of variation during the monsoon and post-monsoon (Table 1). At pre-monsoon, pH at the surface was  $7.71 \pm 0.02$  which decreased with depth. Along the mid-stream site, the sediments were weakly acidic during the pre-monsoon and monsoon while neutral pH values were recorded during the post-monsoon (Table 1). At the mouth, pH values varied marginally from  $7.03 \pm 0.15$  to  $7.61 \pm 0.02$  during the pre-monsoon and monsoon (Table 1). However, at post-monsoon, pH in these sediments varied from  $5.83 \pm 0.02$  at 3-4 cm to  $6.34 \pm 0.02$  at 14-15 cm.

Surface sediments at all sampling locations were relatively more reduced in nature at all the seasons with Eh values of up to  $-38.90 (\pm 4.0)$  mV along the upstream site (Table 1). In the monsoon season, intense reducing conditions prevailed between 6-8 cm at the upstream and mouth (Tables 1). At post-monsoon, reducing conditions at the sampling locations intensified much further into the sediments i.e. at 14-15 cm.

Mangrove sediments were mostly saline during the pre-monsoon (25-39; Table 1). During this season, salinity of pore water was relatively higher at  $> 10$  cm depth compared to other depths examined. The onset of south-west monsoon led to low saline conditions in the estuary which was evident from salinity values varying from 1-11. Interestingly, lowest salinity values of 1-2 were recorded at the mid-stream station (Table 1). In the post-monsoon season, salinity at the mid-stream and mouth were in similar range to those recorded during the pre-monsoon season. However, along the upstream, salinity was 15 (Table 1).

At the upstream site, maximum pore water  $\text{NH}_4^+$  content of  $25.51 \pm 0.88 \mu\text{mol L}^{-1}$  was recorded in the surface sediments (Table 2). On a seasonal scale, significant variations in  $\text{NH}_4^+$  concentrations were also observed at this site ( $p < 1.83 \times 10^{-6}$ ,  $df = 2$ ) and at the mouth ( $p < 2.86 \times 10^{-5}$ ,  $df = 2$ ). Ammonia accumulation of  $37.05 \pm 0.31 \mu\text{mol L}^{-1}$  was observed at 6-8 cm in the sediments at the mouth during the monsoon (Table 2). Contrastingly, the mid-stream sediments exhibited low concentrations of the nutrient at this depth range and during all seasons (Table 2).

Nitrite concentrations at the sampling locations varied from non-detectable levels to  $15.45 \pm 9.86 \mu\text{mol L}^{-1}$  (Tables 2). Significant seasonal variations in  $\text{NO}_2^-$  concentrations were at the upstream ( $p < 0.00097$ ,  $df = 2$ ) and mid-stream sites ( $p < 0.05$ ,  $df = 2$ ) mainly due to accumulation at 3-4 cm during the wet season.

Overall,  $\text{NO}_3^-$  concentrations in mangrove sediments varied from  $1.43 \pm 0.29$  to  $23.33 \pm 4.01 \mu\text{mol L}^{-1}$  (Table 2). In the upstream sediments, there was a sub-surface (3-11 cm) increase in



NO<sub>3</sub><sup>-</sup> concentration during the pre-monsoon (Table 2). However, there was a reverse trend in the monsoon and post-monsoon seasons. Down-core variations in NO<sub>3</sub><sup>-</sup> concentrations were more pronounced on a seasonal scale at mid-stream ( $p < 0.04$ ,  $df = 2$ ) and at the mouth ( $p < 0.04$ ,  $df = 2$ ).

Though PO<sub>4</sub><sup>3-</sup> concentrations were  $< 7.53 \pm 0.61 \mu\text{mol L}^{-1}$ , significant seasonal variability was observed at all sampling locations ( $p < 0.001$ ,  $df = 2$ ). Elevated levels of the nutrient were recorded between 3-8 cm in the upstream sediments during the post-monsoon and at mid-stream and proximal to the mouth during the monsoon (Table 2).

Biochemical components in mangrove sediments generally followed the order: carbohydrates > proteins > lipids (Tables 3). Maximum carbohydrate ( $6.24 \pm 0.01 \text{ mg g}^{-1}$  at 0-2 cm) and lipid concentration ( $1.38 \pm 0.00 \text{ mg g}^{-1}$  at 6-8 cm) were recorded in the upstream sediments in the post-monsoon season (Table 3). Protein content in the sediments ranged from  $0.25 \pm 0.01 \text{ mg g}^{-1}$  to  $3.59 \pm 0.00 \text{ mg g}^{-1}$ . Strong variations in the down-core distribution of LOM seasonally were observed at all sampling locations ( $p < 0.001$ ,  $df = 2$ ).

Organic carbon content in mangrove sediments varied significantly on a seasonal scale ( $p < 0.001$ ,  $df = 2$ ). At pre-monsoon season, maximum organic carbon content of  $5.08 \pm 0.20 \%$  was at the surface sediments of the mid-stream site (Table S1). During the monsoon season, TOC content was maximum in the mid-stream sediments as compared to other sampling locations and increased to  $8.83 \pm 0.12 \%$  at 6-8 cm. At post-monsoon season, the upstream sediments had maximum organic carbon loading which varied from  $7.05 \pm 0.06$  to  $8.91 \pm 0.04 \%$  (Table S1).

### *1.3.2 Total prokaryotic cells*

The microbial load in mangrove sediments varied from  $10^{8-9}$  cells  $\text{g}^{-1}$  (Tables S1). No significant variation in their abundance was observed with depth except near the mouth where it peaked at 10-12 cm during the monsoon season ( $p < 3.86 \times 10^{-5}$ ,  $df = 2$ ). In the mid-stream sediments, higher microbial cells during the monsoon season was responsible for strong seasonal differences ( $p < 7.09 \times 10^{-9}$ ,  $df = 2$ ).

### *1.3.3 Composition and diversity of bacterial assemblage*

After quality checking and data filtering, a total of 57,000 tag sequences were obtained from 38 sediment samples, with an average read length of 264 bp per sample. Clustering of these tag sequences at 97% similarity threshold resulted in 15,008 OTUs. A total of 54 phyla were detected in the entire data set (Fig. 2 a-c). Phylum Proteobacteria was the most dominant at all sampling locations accounting for 35-77% of the total sequences. On a seasonal scale, Proteobacterial abundance varied significantly at the upstream site (Kruskal-Wallis test,  $H = 9.36$ , (2,  $N = 15$ )  $p <$

0.05) and mid-estuary ( $H = 6.045$ , ( $2, N = 15$ )  $p < 0.05$ ). The other most frequently recorded phyla in mangrove sediments were Bacteroidetes ( $\leq 24$  %), Firmicutes ( $\leq 20$  %), Actinobacteria ( $\leq 17$  %), Chloroflexi ( $\leq 14$  %) and Acidobacteria ( $\leq 14$  %).

The bacterial community in mangrove sediments could be classified into 127 different classes. Alphaproteobacteria constituted up to 35% of the tag sequences. Their abundance was more in upstream and mid-stream whereas Gammaproteobacteria predominated at the mid-stream and proximate to the mouth (Fig. 3 a-c). The abundance of Alphaproteobacteria varied significantly on a seasonal scale at all locations (Kruskal-Wallis test, ( $2, N = 15$ ),  $p < 0.05$ ). A significant seasonal variation in the relative abundance of Betaproteobacteria was observed at upstream (Kruskal-Wallis test,  $H = 9.98$ , ( $2, N = 15$ )  $p < 0.05$ ) and near the mouth ( $H = 12.25$ , ( $2, N = 15$ )  $p < 0.05$ ) stations. Marked seasonal variability in abundance of Betaproteobacteria (Kruskal-Wallis test,  $H = 9.38$ , ( $2, N = 15$ )  $p < 0.05$ ) was observed only proximate to the mouth of the estuary. Flavobacteriia, Acidobacteria, Phycisphaerae, Cytophagia, Actinobacteria and Gemmatimonadetes accounted for up to 19, 14, 13, 10, 7 and 5 % of the total sequences respectively (Fig. 3 a-c).

Most of the minor orders (relative abundance  $< 1.5$  %) contributed to a majority (19.61-85.26%) of the tag sequences (data not shown). The Betaproteobacteria were largely represented by members of the order Nitrosomonadales accounting for up to 26% of the tag sequences. The Flavobacteriia accounted for the second largest fraction at order level ( $\leq 18$ %). Sequences of class Alphaproteobacteria were predominantly affiliated with members of the order Rhodospirillales (1-15%), Rhizobiales (0-13%) and Rhodobacterales (1-13%). In general, notable spatio-temporal shifts in bacterial composition along the Mandovi estuary were clearly evidenced (Fig. 2 a-c, Fig. 3 a-c).

Richness and diversity estimates of the bacterial community have been presented in Table S2. According to the number of OTUs, Chao1 estimator revealed highest bacterial richness in the surface sediments at upstream during post-monsoon, followed by surface sediments proximal to the mouth and mid-stream at pre-monsoon season respectively (Table S2). Though samples with highest richness differed using the ACE estimator, from both Chao1 and ACE estimators, the lowest richness was at 10-12 cm sample from the mid-stream (pre-monsoon). The Shannon index ( $H'$ ) varied from 5.44-6.68 with highest value in the surface sediment samples at the upstream site during the post-monsoon (Table S2). The lowest values of  $H'$  were recorded at the mid-stream site during the pre-monsoon. A significant seasonal variation in  $H'$  was observed at the station proximal to the mouth (Kruskal-Wallis test,  $H = 8.0$ , ( $2, N = 15$ )  $p < 0.05$ ).

### 1.3.4 Influence of environmental variables on bacterial diversity

Spearman correlation analysis revealed the influence of environmental factors on the relative abundance of bacterial tag sequences (class level data; Fig. S1 a-c). Some important variables in determining changes in bacterial community structure (OTU level) were evaluated by CCA to elucidate the response of phylogenetically divergent bacterial lineages co-existing in mangrove sediments.

About 25.7 % of the total variability could be explained based on CCA (Fig. 4). About 14.3 % of the constrained variability could be explained by the first axis and 12.7 % by the second axis. Nevertheless, CCA revealed that pore water salinity,  $\text{NH}_4^+$  and pH had the largest influence on bacterial community in sub-surface sediments proximal to the mouth mainly during the monsoon period and to a certain extent in mid-stream samples. Bacterial community at the mid-stream were mostly affected by phosphate, nitrate and total organic carbon content in the sediments. The influence of temperature, nitrite and reducing potential of the sediment was also evident on the bacterial community mostly at the head and mouth region during the post-monsoon (Fig. 4).

## 1.4 Discussion

Bacterial communities in estuarine systems are complex and, a sizeable portion is composed of uncultured lineages (Fernandes *et al.*, 2014; Shu and Huang, 2021). In biodiversity hotspots like mangroves, bacteria mediate transformation of C, N, S, metals, etc. Hence, study on the distribution of bacteria in mangrove sediments can provide valuable insights into various bacterially-mediated biogeochemical transformations. To extend our knowledge on seasonal changes of bacterial community structure in near shore systems, the down-core and spatial variation in their taxonomic diversity along different sections of an anthropogenically-influenced estuary through three seasons was documented. The present study elucidated that a large portion of tag sequences (up to 77%) were affiliated to phylum Proteobacteria (Fig. 2a-c). This corroborates with previously reported works (Feng *et al.*, 2009; Fernandes *et al.*, 2014; Wang *et al.*, 2015).

On a seasonal scale, there were significant differences in the distribution of Proteobacteria mainly at the upstream and mid-stream stations. This could be mainly due to a decrease in their abundance during the post-monsoon. Kunihiro *et al.* (2011) has reported an increase of Alphaproteobacteria in association with deposit-feeding polychaetes in organically-rich marine sediments. The authors suggested that these bacteria decompose organic matter with the assistance of bioturbators like polychaetes and in turn serve as food for macrofauna. Studies conducted in a mangrove system on the east coast of India have shown highest macrofaunal abundance and diversity

during the post-monsoon (Kumar and Khan, 2013). It is reasonable to envisage that the benthic bacterial community in mangrove sediments along the Mandovi is possibly grazed upon by macrofauna (Pascal *et al.*, 2014) during the post-monsoon leading to a decline in their abundance. Though sequences belonging to phylum Proteobacteria were encountered at all depths (Fig. 2a-c), their relative abundance showed marked variability with depth at the mouth station (Fig. 2c). However, statistically significant differences in depth-wise distribution of various Proteobacterial classes were not evident at this location.

Within phylum Proteobacteria, class Alphaproteobacteria dominated at the upstream site whereas Gammaproteobacteria predominated in sediments with higher salinity (Fig. 3 a-c). Bacteria belonging to class Alphaproteobacteria isolated from mangrove sediments grow photoheterotrophically with a number of organic compounds as carbon sources and electron donors (Nupur *et al.*, 2014). In mangrove sediments, cellulosic glucose and xylose are the two most labile neutral sugars (Marchand *et al.*, 2005). Alphaproteobacteria may contribute to the assimilation of glucose in these environments as they have been reported to assimilate ~ 60 % glucose in saline water (Elifantz *et al.*, 2005). This gains significance as diagenesis patterns have shown uptake of glucose to surpass other sugars like galactose, rhamnose, fucose, xylose and mannose indicating the importance of carbohydrates in supporting heterotrophic production in marine sediments (Oakes *et al.*, 2010). Mangrove sediments from the Mandovi estuary are rich in organic matter. The LOM fractions comprises 0.02-0.62% of the TOC in these sediments indicating that a considerable fraction of TOC is in the labile state (Tables S1). Since Alphaproteobacteria are capable of exploiting several organic resources available in mangrove sediments, they could play a vital role in carbon cycling and regulating DOM turnover. Moreover, Alphaproteobacteria could mediate metabolisation of sulfur, production of antibacterial compounds and could have symbiotic relationships with diverse marine eukaryotes (Moran *et al.*, 2003; Brinkhoff *et al.*, 2008).

In coastal sediments, Gammaproteobacteria are metabolically flexible mediating important parts of marine carbon, nitrogen and sulfur cycles (Fernandes *et al.*, 2014; Dykema *et al.*, 2016). In the present study their abundance was relatively higher at the mid-stream and mouth stations compared to the upstream site. Molecular investigations of bacterial community in freshwater to marine conditions in the Baltic Sea have also revealed strong correlation of Gammaproteobacteria with salinity (Klier *et al.*, 2018) indicating their preference to thrive in higher salinity conditions. Some studies have linked Gammaproteobacterial abundance to low pH while Alphaproteobacteria predominate at intermediate pH (Wei *et al.*, 2017). Many Betaproteobacteria like *Bacillus* and *Clostridium* species (Roadcap *et al.*, 2006) and magnetotactic

bacteria (MTB) in the class Deltaproteobacteria (Lefèvre *et al.*, 2011) are abundant in highly alkaline environments (Griffiths *et al.*, 2011; Wei *et al.*, 2017). In the present study, significant relationship of Alpha-, Beta- and Gamma- proteobacteria with sediment pH was absent (Fig. S1 a-c). Though not statistically significant, a positive relationship of Deltaproteobacteria with sediment pH was observed at upstream (Fig. S1a) and mid-stream sites (Fig. S1b) during the post-monsoon corroborating the findings of Griffiths *et al.* (2011). Sulfate reducing activity (SRA) is mainly mediated by members affiliated to class Deltaproteobacteria (Foti *et al.*, 2007). In mangrove sediments, SRA tends to increase pH of sediments re-affirming the positive relationship of Deltaproteobacteria with sediment pH (Attri *et al.*, 2011).

Other common bacterial phyla viz., Bacteroidetes, Firmicutes, Actinobacteria, Chloroflexi and Acidobacteria were identified in this study (Fig. 2 a-c) as in marine sediments elsewhere (Piza *et al.*, 2004; Zhang *et al.*, 2009; Zheng *et al.*, 2014). These phyla are important drivers of C and N cycles (Hunter *et al.*, 2006) and are likely to participate in many nutrient transformations (Li *et al.*, 2017). Proteins account for a large proportion of bioavailable C and N for sediment communities (Wakeham *et al.*, 1997). Bacteroidetes members have been known to be involved in protein degradation (Baker *et al.* 2015). An inverse relationship between protein content and Bacteroidia during the monsoon at the upstream (Fig. S1a) and during the pre-monsoon and post-monsoon at mid-stream stations (Fig. S1b) could be linked to enhanced protein utilisation by members of this class. Bacteroidetes and Chloroflexi could be possibly involved in the breakdown of carbohydrates in mangrove sediments as they have genes that encode for carbohydrate degradation (Baker *et al.*, 2015). Bacteria affiliated to class Actinobacteria represented up to 17% of the total sequences in the entire data set. Their presence in the mangrove benthic system could be useful in the degradation of leaf litter (Schneider *et al.*, 2012), hydrocarbons (Harwati *et al.*, 2007), metal oxidation (Bryan and Johnson, 2008; Johnson *et al.*, 2009), and  $\text{NO}_3^-$  reduction (Van *et al.*, 2005). Many Actinobacteria are of economic importance (Ward and Bora, 2006) as they are a source of antibiotics (Adinarayana *et al.*, 2006; Ellaiah and Zeeck, 2006; Manivasagan *et al.*, 2009). They are capable of using  $\text{NO}_2^-$  as N source, respond to soil macro-, micro nutrients and soil acidity, express multiple active transporters degrade gellan gum and produce exopolysaccharide (Kielak *et al.*, 2016). They are as metabolically diverse as Proteobacteria and are versatile heterotrophs. Their ability to use C sources like hemicellulose, cellulose and chitin (Ward *et al.*, 2009) which are the components of leaf litter (Yadav and Malanson, 2007) can help to degrade plant material like leaves and twigs which are major contributors of organic matter in mangrove systems. Considerable litterfall has been reported to occur in mangroves along the Mandovi estuary (Wafar *et al.*, 1997; Fernandes *et al.*, 2016). The

PN/POC and  $\delta^{13}\text{C}_{\text{org}}$  values have indicated dominance of terrestrial plant-derived organic matter (OM) in Mandovi during the monsoon (Shynu *et al.*, 2015). At the mid-stream site, sediment OM was elevated during the pre-monsoon and monsoon and at post-monsoon season in the upstream and adjacent to the mouth station (Table S1). In the present study, almost 14% of the sequences in the data were represented by members of class Acidobacteria. A significant negative relationship of Acidobacteria with TOC in the mid-stream sediments during the post-monsoon ( $P < 0.05$ ; Fig. S1b) is suggestive of degradation of accumulated OM by bacteria belonging to this phylum. Their ubiquity and abundance in mangrove sediments is also suggestive of their role in the degradation of plant-derived organic matter. Thus, it is evident that presence of dominant phyla may be linked to changes in sediment properties among different sites.

In the present study, some of the predominant orders known to have metabolic capabilities for transforming C as well as N compounds (Dubbs and Tabita, 2004; Carvalho *et al.*, 2010; Gomes *et al.*, 2013; Baldani *et al.*, 2014; Kutvonen *et al.*, 2015) were Flavobacteriales, Nitrosomonadales (class Betaproteobacteria), Rhodospirillales, Rhizobiales and Rhodobacterales (class Alphaproteobacteria) (Fig. 3 a-c). These findings highlight the important ecological role of major bacterial lineages in mangrove sediments. These bacteria also play a role towards modulating the levels of nutrients and organic carbon content which was corroborated in studies by Fernandes and LokaBharathi (2011).

Shannon diversity indices indicated the presence of high bacterial richness and diversity as compared to mangrove sediments elsewhere (dos Santos *et al.*, 2011; Mendes and Tsai, 2014). According to the Chao1 estimator and Shannon index, highest bacterial richness and diversity was in the upstream surface sediments during the post-monsoon season. During this season, sediments at the upstream station were relatively more oxic compared to the other seasons. Besides, there was an increase of LOM,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and prokaryotic cells in the surface sediments (Tables 2 & Tables S1). The upstream locations along the Mandovi are mainly colonized by *A. illicifolius* which is known to shed large amount of leaves (Ball, 1998) as compared to other mangroves like *R. mucronata* or *A. marina* (Fernandes *et al.*, 2016) thus contributing to the benthic organic C pool. Such organic matter addition can lead to the elevation of microbial respiration in the benthic system, activity of C-acquiring extracellular enzymes and can also alter bacterial community composition in these estuarine ecosystems as was reported in low salinity soils (Van Horn *et al.*, 2014). At the upstream station during the post-monsoon season, relative abundance of sequences belonging to phyla Chloroflexi, Gemmatimonadetes, Planctomycetes and Latescibacteria increased (Fig. 2a). In the same season the presence of members of phylum Chloroflexi can lead to a comparatively more

active sediment carbon cycling and respiration of sugars, fermentation, CO<sub>2</sub> fixation, acetogenesis with ATP formation by substrate-level phosphorylation (Hug *et al.*, 2013) in these sediments. The phylum Gemmatimonadetes usually reported from systems with high nutrient input (Li *et al.*, 2006) was found in upstream sediments, which may be due to the large organic input during this season, in addition to the high litterfall during the post-monsoon season (Wafar *et al.*, 1997). Bacteria of phylum Latescibacteria is known to enable hydrolysis of the complex organic compounds in mangrove litter (Baker *et al.*, 2015). Besides, the occurrence of Planctomycetes in these mangrove sediments could aid in carbohydrate fermentation and sulfur reduction (Elshahed *et al.*, 2007). Thus, presence of the different bacterial phyla could aid in remineralisation and cycling of organic matter. In addition, the various organic and inorganic nutrients along with prevailing environmental conditions play a role in proliferation of bacterial communities involved in C and N cycling along the upstream sediments during the post-monsoon season.

Considering the geographical distance between sampling sites, CCA analysis revealed the influence of salinity on the bacterial community which was most prominent at the mouth and mid-stream stations during the monsoon season (Fig. 4). The Mandovi is a flood dominant estuary as the amplitude and phase of major diurnal and semi-diurnal constituent increases toward the upstream regions (Manoj *et al.*, 2009). The mouth and mid-stream stations are prone to experience wider variations in salinity due to tidal forcing. Moderate changes in salinity affect bacterial community composition leading to altered growth characteristics. This is because salinity optimum is required for the growth of specific bacterial strains as well as broader phylogenetic groups (Langenheder *et al.*, 2003). A study carried out along a brackish to marine salinity gradient in the Baltic Sea has revealed phylogenetically clustered ecological preferences arising due to differences in salinity (Herlemann *et al.*, 2016). Indices of bacterial richness and diversity of the bacterial community in the present study varied along the estuary reflecting active mixing between the freshwater and marine ends of the estuary, as was also reported by Campbell and Kirchman (2013). The CCA plot also clearly indicated the influence of NH<sub>4</sub><sup>+</sup> concentration on composition of the bacterial community towards down-stream locations mainly during the monsoon. In addition to extraneous input, organic matter brought into the estuary at monsoon or via litterfall is gradually re-mineralized over time resulting in generation and accrual of NH<sub>4</sub><sup>+</sup> in the sediments as observed at the mid-stream and near mouth stations (Table 2). Earlier studies by Krishnan *et al.* (2009) have shown highest TOC and NH<sub>4</sub><sup>+</sup> content in mangrove sediments along the mid-region of Mandovi during the post-monsoon. The post-monsoon season represents a transition period wherein the organic carbon (OC): dissolved inorganic nitrogen (DIN) ratio is gradually increased. Organic carbon is either unlimited or

dominated by the recalcitrant fraction prompting anaerobic autotrophic nitrification to occur at the expense of metals like Fe (Krishnan *et al.*, 2009). An accumulation of nitrate in the sediments during the post-monsoon (Table 2) is suggestive of the  $\text{NH}_4^+$  transformation to  $\text{NO}_3^-$  via the aforementioned pathway.

## **Conclusions**

This is the first comprehensive investigation of spatio-temporal variability in benthic bacterial communities from mangroves fringing the economically important Mandovi estuary. The Central Pollution Control Board (CPCB) of India has identified 29 km stretch of this estuary as polluted mainly due to influx of domestic sewage. Our observations revealed a rich and diverse bacterial community dominated by Proteobacteria (mainly Alpha- and Gamma- proteobacteria) even in the deeper layers in mangrove sediments along the Mandovi. Other dominant phyla included Bacteroidetes, Firmicutes, Actinobacteria, Chloroflexi and Acidobacteria. Along with Proteobacteria, they could be playing an important role in modulating excess C and N content in the benthic system. Down-core distribution of major phyla/classes showed no significant variations. Seasonal changes in local environmental parameters were crucial in influencing composition and diversity of autochthonous benthic bacterial communities. In higher salinity regions such as the midstream and mouth regions of the estuary, variations in  $\text{NH}_4^+$  content influenced bacterial community composition. At the upstream location, elevated bacterial richness and diversity in surface sediments could be linked to an increase in sediment organic carbon content mainly during the post-monsoon. As hypothesized, sustenance of bacterial communities could be dependent on the level of organic loading along the estuary. Future studies would involve probing the functional diversity of microbial communities in mangrove sediments in order to improve our understanding on their role in biogeochemical cycling.

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### Legends to figures

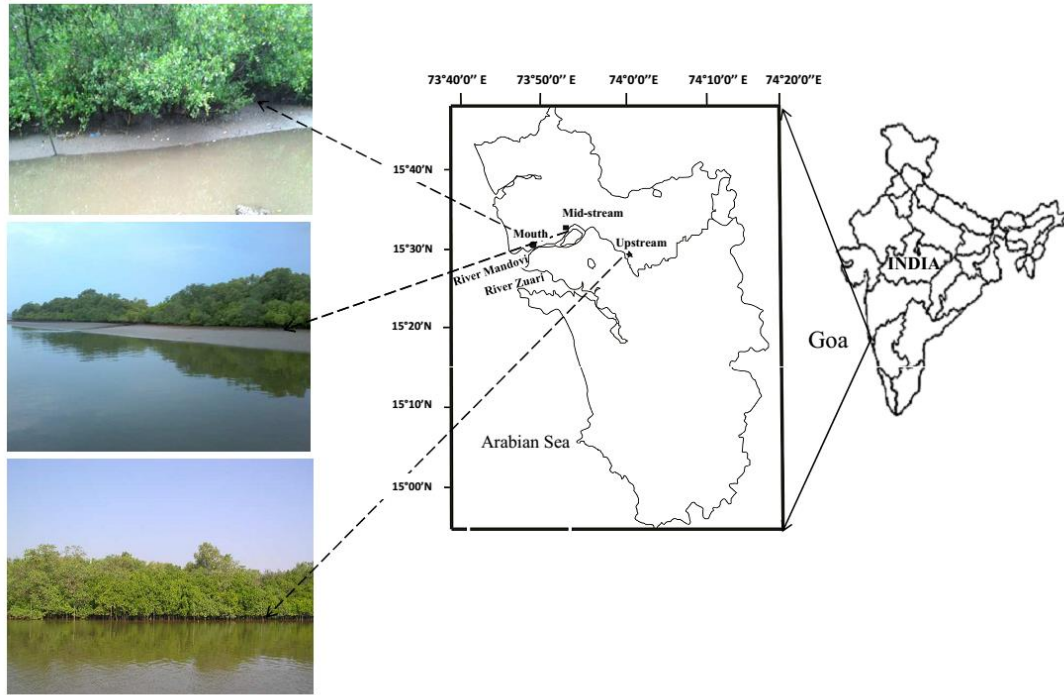
- Figure 1.** Location of sampling stations at upstream, mid-stream and proximal to the mouth of the Mandovi estuary
- Figure 2.** Bacterial community compositions at phylum level as revealed by pyrosequencing from sediment samples at stations (a) upstream (b) mid-stream and (c) proximal to the mouth of the Mandovi estuary. The relative abundance was defined as the percentage of tag sequences of a given phylum from the total sequences in the data set using the SILVA database. Phyla making up less than 0.1% of total composition in the dataset were classified as 'other'. (Results obtained from a total of 38 out of 45 samples (upstream: n = 12; mid-stream: n = 11; mouth: n = 15) that were retained after subsampling to 1500 reads have been depicted in this plot)
- Figure 3.** Bacterial community compositions at class level as revealed by pyrosequencing from sediment samples at stations (a) upstream (b) mid-stream and (c) proximal to the mouth of the Mandovi estuary. Classes making up less than 0.5% of total composition in the dataset were classified as 'other'. Results obtained from a total of 38 out of 45 samples (upstream: n = 12; mid-stream: n = 11; mouth: n = 15) that were retained after subsampling to 1500 reads have been depicted in this plot)
- Figure 4.** Canonical correspondence analysis (CCA) of sediment bacterial community structure based on the relative abundance of detected sequences (at class level) in relation to pertinent environmental variables. Sediment characteristics are represented by vectors. The vectors with longer arrows represent the larger effects, while the vectors forming smaller angles with an ordination axis represent a strong correlation with that axis. Unit for depth = cm.

### Legends to tables

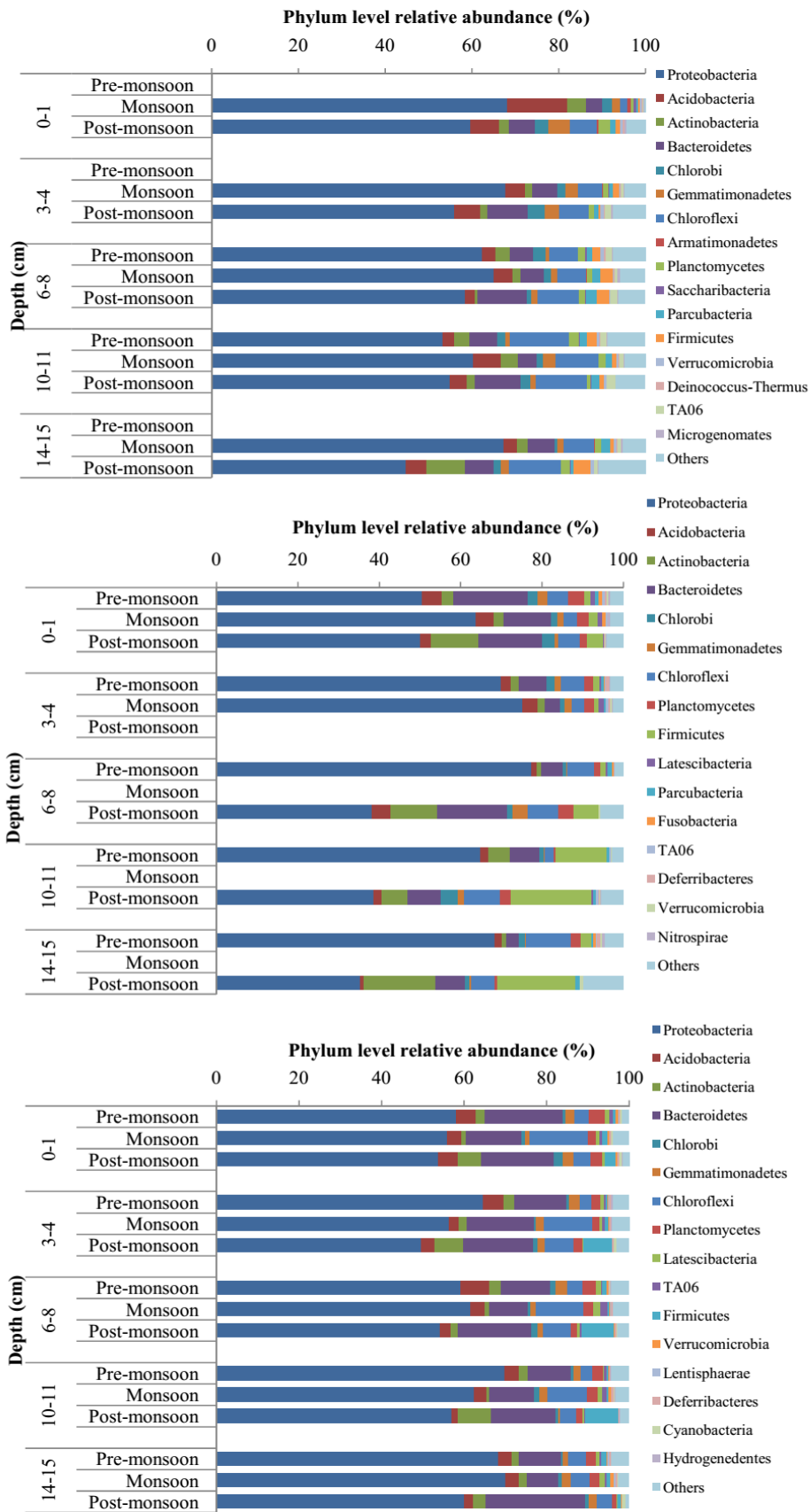
- Table 1** Average and standard deviation ( $\pm$ ) values for down-core variation of physico-chemical variables of sediment samples at stations upstream, mid-stream and proximal to the mouth of the Mandovi estuary.
- Table 2** Average and standard ( $\pm$ ) values for down-core variation of nutrient concentrations of sediment samples at stations upstream, mid-stream and proximal mouth of the Mandovi estuary.
- Table 3** Average and standard deviation ( $\pm$ ) values for down-core variation of biochemical parameters of sediment samples at stations upstream, mid-stream and proximal to the mouth of the Mandovi estuary.

### Legends to supplementary data

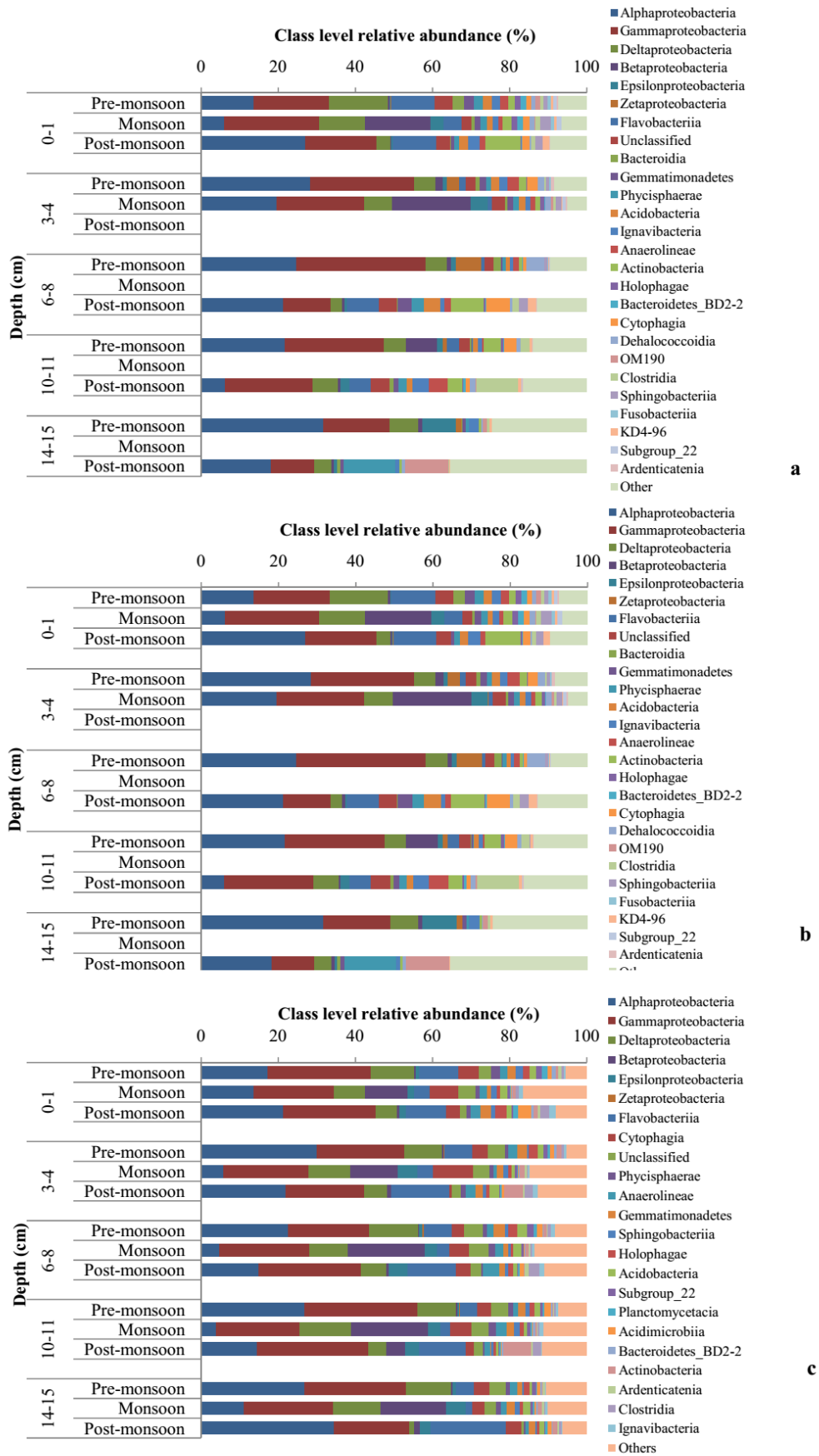
- Figure S1** Spearman correlation heat map of environmental variables and the top 32 bacterial classes at the (a) upstream (b) mid-stream and (c) proximal to the mouth with correlation coefficient and significance levels. Asterisks mark on the plots denotes the level of significance (p-value): \*\*\* =  $p < 0.001$ ; \*\* =  $p < 0.01$ , \* =  $p < 0.05$ . On the primary X-axis, V = upstream, C = mid-stream and B = mouth while a = pre-monsoon, b = monsoon and c = post-monsoon season. Environmental variables have been depicted on the secondary X-axis where TPC = total prokaryotic cells; TOC = total organic carbon; LOM = labile organic matter; Temp = sediment temperature. There were insufficient sample number for Spearman rank test for Volvo and Chora after subsampling at 1500 reads.
- Table S1** Average and standard deviation ( $\pm$ ) values for down-core variation of geo-microbiological variables of sediment samples at stations upstream, mid-stream and proximal to the mouth of the Mandovi estuary.
- Table S2** Diversity estimates for bacterial communities in the sediment samples from stations upstream, mid-stream and proximal to the mouth of the Mandovi estuary.



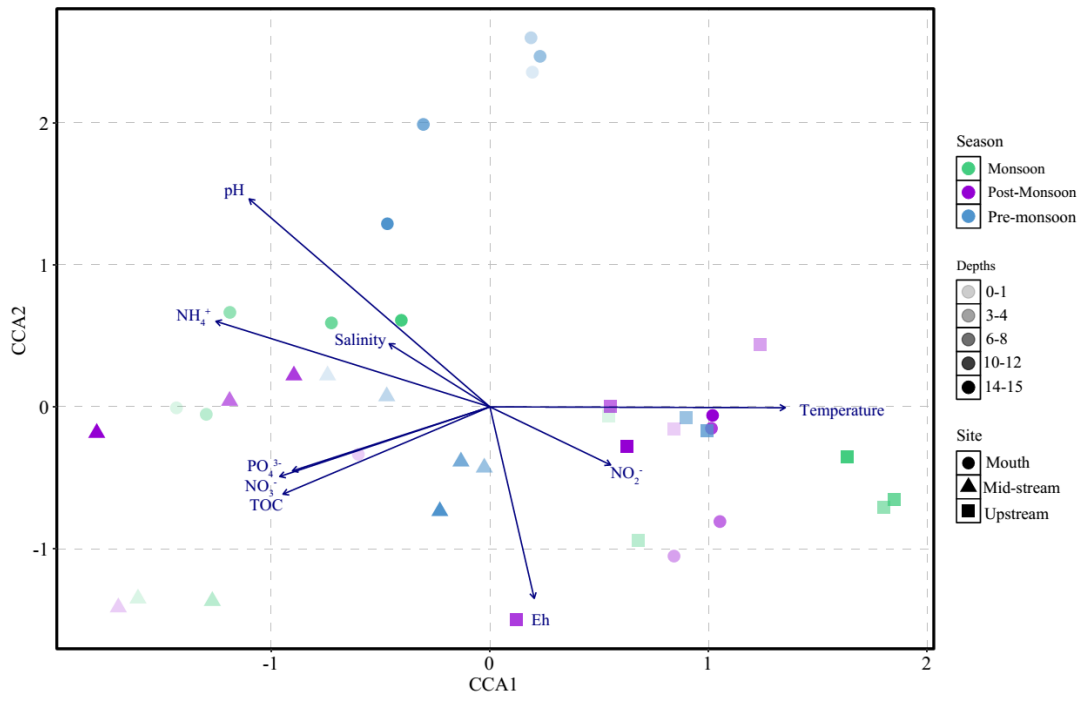
**Fig. 1**



**Fig. 2**



**Fig. 3**



**Fig. 4**

**Table 1** Average and standard deviation ( $\pm$ ) values for down-core variation of physico-chemical variables of sediment samples at stations upstream, mid-stream and proximal to the mouth of the Mandovi estuary.

Parameters	Depth (cm)	Temp ( $^{\circ}$ C)			pH			Eh (mV)			Salinity		
Seasons	Stations	Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth
Pre-monsoon	0-1	26.90 ( $\pm 0.00$ )	27.60 ( $\pm 0.00$ )	28.90 ( $\pm 0.01$ )	7.71 ( $\pm 0.02$ )	6.92 ( $\pm 0.02$ )	7.61 ( $\pm 0.02$ )	-38.90 ( $\pm 4.00$ )	5.40 ( $\pm 0.75$ )	-23.75 ( $\pm 6.65$ )	17.00 ( $\pm 0.00$ )	39.00 ( $\pm 0.00$ )	25.00 ( $\pm 0.00$ )
	3-4	26.90 ( $\pm 0.00$ )	27.90 ( $\pm 0.01$ )	27.7 ( $\pm 0.01$ )	6.96 ( $\pm 0.13$ )	7.12 ( $\pm 0.02$ )	7.57 ( $\pm 0.02$ )	11.43 ( $\pm 1.75$ )	12.20 ( $\pm 1.01$ )	-31.86 ( $\pm 6.86$ )	17.00 ( $\pm 0.00$ )	37.00 ( $\pm 0.00$ )	26.00 ( $\pm 0.00$ )
	6-8	27.10 ( $\pm 0.00$ )	27.90 ( $\pm 0.02$ )	27.23 ( $\pm 0.06$ )	6.86 ( $\pm 0.02$ )	6.68 ( $\pm 0.01$ )	7.53 ( $\pm 0.02$ )	10.87 ( $\pm 1.10$ )	20.50 ( $\pm 1.35$ )	-27.69 ( $\pm 7.02$ )	17.00 ( $\pm 0.00$ )	36.00 ( $\pm 0.00$ )	25.00 ( $\pm 0.00$ )
	10-12	26.90 ( $\pm 0.01$ )	28.10 ( $\pm 0.01$ )	27.07 ( $\pm 0.06$ )	6.71 ( $\pm 0.02$ )	6.47 ( $\pm 0.01$ )	7.48 ( $\pm 0.02$ )	20.87 ( $\pm 2.00$ )	35.91 ( $\pm 2.91$ )	-27.35 ( $\pm 5.37$ )	17.00 ( $\pm 0.00$ )	39.00 ( $\pm 0.00$ )	29 ( $\pm 0.00$ )
	14-15	27.10 ( $\pm 0.00$ )	28.30 ( $\pm 0.00$ )	26.80 ( $\pm 0.00$ )	6.73 ( $\pm 0.02$ )	6.41 ( $\pm 0.02$ )	7.36 ( $\pm 0.02$ )	23.05 ( $\pm 1.93$ )	37.90 ( $\pm 6.06$ )	-20.52 ( $\pm 21.98$ )	17.00 ( $\pm 0.00$ )	37.00 ( $\pm 0.00$ )	35.00 ( $\pm 0.00$ )
Monsoon	0-1	28.00 ( $\pm 0.00$ )	26.40 ( $\pm 0.00$ )	26.20 ( $\pm 0.00$ )	6.52 ( $\pm 0.02$ )	6.52 ( $\pm 0.02$ )	7.23 ( $\pm 0.02$ )	22.07 ( $\pm 2.40$ )	33.01 ( $\pm 17.89$ )	23.00 ( $\pm 7.00$ )	2.00 ( $\pm 0.00$ )	2.00 ( $\pm 0.00$ )	11.00 ( $\pm 0.00$ )
	3-4	28.07 ( $\pm 0.06$ )	26.60 ( $\pm 0.01$ )	26.17 ( $\pm 0.06$ )	6.40 ( $\pm 0.02$ )	6.32 ( $\pm 0.02$ )	7.22 ( $\pm 0.02$ )	17.17 ( $\pm 9.25$ )	29.00 ( $\pm 6.90$ )	13.17 ( $\pm 4.25$ )	2.00 ( $\pm 0.00$ )	1.00 ( $\pm 0.00$ )	7.00 ( $\pm 0.00$ )
	6-8	27.90 ( $\pm 0.00$ )	26.70 ( $\pm 0.01$ )	26.00 ( $\pm 0.00$ )	6.12 ( $\pm 0.02$ )	6.30 ( $\pm 0.02$ )	7.11 ( $\pm 0.02$ )	-18.97 ( $\pm 6.72$ )	15.04 ( $\pm 5.04$ )	-21.20 ( $\pm 21.70$ )	3.00 ( $\pm 0.00$ )	1.00 ( $\pm 0.00$ )	7.00 ( $\pm 0.00$ )
	10-12	28.00 ( $\pm 0.00$ )	26.20 ( $\pm 0.00$ )	26.07 ( $\pm 0.06$ )	6.20 ( $\pm 0.02$ )	6.20 ( $\pm 0.02$ )	7.23 ( $\pm 0.02$ )	3.04 ( $\pm 2.94$ )	-20.01 ( $\pm 27.98$ )	-11.00 ( $\pm 4.00$ )	3.00 ( $\pm 0.00$ )	2.00 ( $\pm 0.00$ )	6.00 ( $\pm 0.00$ )
	14-15	28.00 ( $\pm 0.00$ )	26.50 ( $\pm 0.01$ )	26.20 ( $\pm 0.01$ )	6.39 ( $\pm 0.02$ )	6.22 ( $\pm 0.02$ )	7.03 ( $\pm 0.15$ )	10.00 ( $\pm 4.00$ )	13.01 ( $\pm 10.44$ )	-2.01 ( $\pm 2.49$ )	4.00 ( $\pm 0.00$ )	1.00 ( $\pm 0.00$ )	6.00 ( $\pm 0.00$ )
Post-monsoon	0-1	28.83 ( $\pm 0.06$ )	26.00 ( $\pm 0.01$ )	28.90 ( $\pm 0.00$ )	6.61 ( $\pm 0.02$ )	7.21 ( $\pm 0.02$ )	6.24 ( $\pm 0.02$ )	30.90 ( $\pm 8.00$ )	16.40 ( $\pm 12.20$ )	34.77 ( $\pm 9.10$ )	15.00 ( $\pm 0.00$ )	31.80 ( $\pm 0.00$ )	30.20 ( $\pm 0.00$ )
	3-4	31.27 ( $\pm 0.06$ )	25.90 ( $\pm 0.01$ )	28.70 ( $\pm 0.00$ )	6.44 ( $\pm 0.02$ )	7.14 ( $\pm 0.02$ )	5.83 ( $\pm 0.02$ )	40.73 ( $\pm 8.05$ )	-10.20 ( $\pm 1.91$ )	57.73 ( $\pm 5.10$ )	15.00 ( $\pm 0.00$ )	31.80 ( $\pm 0.00$ )	30.20 ( $\pm 0.00$ )
	6-8	30.50 ( $\pm 0.00$ )	26.00 ( $\pm 0.00$ )	28.30 ( $\pm 0.00$ )	6.30 ( $\pm 0.02$ )	7.12 ( $\pm 0.02$ )	5.89 ( $\pm 0.02$ )	49.87 ( $\pm 9.95$ )	-9.00 ( $\pm 7.00$ )	14.62 ( $\pm 5.93$ )	18.00 ( $\pm 0.00$ )	31.80 ( $\pm 0.00$ )	30.20 ( $\pm 0.00$ )
	10-12	27.87 ( $\pm 0.06$ )	26.20 ( $\pm 0.01$ )	28.87 ( $\pm 0.00$ )	6.14 ( $\pm 0.02$ )	7.22 ( $\pm 0.02$ )	6.13 ( $\pm 0.15$ )	57.50 ( $\pm 8.10$ )	-15.90 ( $\pm 18.39$ )	-14.67 ( $\pm 3.51$ )	15.00 ( $\pm 0.00$ )	31.80 ( $\pm 0.00$ )	30.20 ( $\pm 0.00$ )
	14-15	28.57 ( $\pm 0.06$ )	26.30 ( $\pm 0.00$ )	28.30 ( $\pm 0.00$ )	6.64 ( $\pm 0.02$ )	7.18 ( $\pm 0.02$ )	6.34 ( $\pm 0.02$ )	27.87 ( $\pm 7.50$ )	-14.03 ( $\pm 16.05$ )	-15.84 ( $\pm 6.64$ )	15.00 ( $\pm 0.00$ )	31.80 ( $\pm 0.00$ )	30.20 ( $\pm 0.00$ )



**Table 2** Average and standard ( $\pm$ ) values for down-core variation of nutrient concentrations of sediment samples at stations upstream, mid-stream and proximal to the mouth of the Mandovi estuary.

Parameters	Depth (cm)	NH <sub>4</sub> <sup>+</sup> ( $\mu\text{M L}^{-1}$ )			NO <sub>2</sub> <sup>-</sup> ( $\mu\text{M L}^{-1}$ )			NO <sub>3</sub> <sup>-</sup> ( $\mu\text{M L}^{-1}$ )			PO <sub>4</sub> <sup>3-</sup> ( $\mu\text{M L}^{-1}$ )		
		Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth
		Stations → Seasons ↓											
Pre-monsoon	0-1	25.51 ( $\pm 0.88$ )	36.16 ( $\pm 0.32$ )	33.39 ( $\pm 0.13$ )	9.10 ( $\pm 4.62$ )	2.26 ( $\pm 2.61$ )	9.65 ( $\pm 0.82$ )	2.97 ( $\pm 1.89$ )	3.25 ( $\pm 1.18$ )	5.96 ( $\pm 2.87$ )	0.99 ( $\pm 0.15$ )	1.40 ( $\pm 0.36$ )	0.77 ( $\pm 0.00$ )
	3-4	19.08 ( $\pm 0.37$ )	30.95 ( $\pm 0.54$ )	35.67 ( $\pm 0.17$ )	1.94 ( $\pm 1.527$ )	4.99 ( $\pm 0.39$ )	1.33 ( $\pm 0.52$ )	4.06 ( $\pm 2.08$ )	7.59 ( $\pm 7.67$ )	3.32 ( $\pm 2.17$ )	0.77 ( $\pm 0.00$ )	0.64 ( $\pm 0.06$ )	0.77 ( $\pm 0.17$ )
	6-8	25.00 ( $\pm 0.11$ )	26.49 ( $\pm 0.10$ )	34.07 ( $\pm 0.11$ )	1.27 ( $\pm 0.14$ )	2.26 ( $\pm 0.20$ )	ND	3.64 ( $\pm 3.02$ )	5.18 ( $\pm 3.66$ )	2.67 ( $\pm 1.87$ )	0.67 ( $\pm 0.10$ )	0.64 ( $\pm 0.06$ )	0.64 ( $\pm 0.11$ )
	10-12	23.54 ( $\pm 0.25$ )	35.85 ( $\pm 0.20$ )	36.80 ( $\pm 0.41$ )	1.46 ( $\pm 0.71$ )	1.48 ( $\pm 0.56$ )	ND	4.66 ( $\pm 3.12$ )	3.17 ( $\pm 0.50$ )	3.65 ( $\pm 1.54$ )	0.67 ( $\pm 0.10$ )	0.80 ( $\pm 0.15$ )	0.64 ( $\pm 0.06$ )
	14-15	18.86 ( $\pm 0.08$ )	35.36 ( $\pm 0.41$ )	31.40 ( $\pm 0.16$ )	0.82 ( $\pm 0.44$ )	4.25 ( $\pm 0.47$ )	0.87 ( $\pm 1.03$ )	1.69 ( $\pm 0.64$ )	6.13 ( $\pm 6.92$ )	2.13 ( $\pm 0.25$ )	0.67 ( $\pm 0.00$ )	0.80 ( $\pm 0.11$ )	0.83 ( $\pm 0.20$ )
Monsoon	0-1	24.37 ( $\pm 0.26$ )	31.15 ( $\pm 0.15$ )	34.96 ( $\pm 0.37$ )	5.14 ( $\pm 1.13$ )	4.23 ( $\pm 4.43$ )	0.78 ( $\pm 0.27$ )	2.38 ( $\pm 0.50$ )	6.55 ( $\pm 0.36$ )	8.16 ( $\pm 6.43$ )	0.73 ( $\pm 0.06$ )	5.59 ( $\pm 0.62$ )	2.23 ( $\pm 0.64$ )
	3-4	16.32 ( $\pm 0.05$ )	34.71 ( $\pm 0.12$ )	36.00 ( $\pm 0.35$ )	15.45 ( $\pm 9.86$ )	5.83 ( $\pm 0.16$ )	1.59 ( $\pm 2.02$ )	1.78 ( $\pm 0.52$ )	5.26 ( $\pm 5.45$ )	9.83 ( $\pm 5.41$ )	1.05 ( $\pm 0.00$ )	2.68 ( $\pm 0.00$ )	4.82 ( $\pm 0.15$ )
	6-8	16.89 ( $\pm 0.18$ )	31.65 ( $\pm 0.13$ )	37.05 ( $\pm 0.31$ )	9.99 ( $\pm 1.12$ )	0.36 ( $\pm 0.24$ )	0.09 ( $\pm 0.12$ )	1.50 ( $\pm 0.18$ )	6.48 ( $\pm 1.54$ )	5.18 ( $\pm 3.08$ )	1.12 ( $\pm 0.15$ )	7.53 ( $\pm 0.61$ )	5.59 ( $\pm 0.62$ )
	10-12	11.36 ( $\pm 0.29$ )	36.60 ( $\pm 0.57$ )	36.95 ( $\pm 0.62$ )	7.63 ( $\pm 8.72$ )	0.43 ( $\pm 0.34$ )	ND	2.16 ( $\pm 0.79$ )	2.79 ( $\pm 0.28$ )	6.63 ( $\pm 1.73$ )	0.48 ( $\pm 0.25$ )	2.59 ( $\pm 0.17$ )	0.96 ( $\pm 0.35$ )
	14-15	12.32 ( $\pm 0.29$ )	36.67 ( $\pm 0.71$ )	36.45 ( $\pm 0.16$ )	2.75 ( $\pm 0.35$ )	ND	ND	3.00 ( $\pm 1.42$ )	2.18 ( $\pm 0.05$ )	2.35 ( $\pm 0.33$ )	1.21 ( $\pm 0.15$ )	0.67 ( $\pm 0.10$ )	0.77 ( $\pm 0.10$ )
Post-	0-1	23.44	31.96	36.59	1.44	0.98	1.07	4.03	23.33	3.55	1.15	1.15	0.67

monsoon		(±0.09)	(±0.11)	(±0.41)	(±0.63)	(±0.29)	(±0.15)	(±1.57)	(±4.01)	(±2.33)	(±0.17)	(±0.35)	(±0.00)
	3-4	21.24 (±0.12)	36.73 (±0.03)	36.19 (±0.21)	2.14 (±3.70)	0.74 (±0.46)	0.83 (±0.18)	2.07 (±0.75)	2.29 (±0.61)	4.27 (±0.75)	0.99 (±0.15)	1.05 (±0.29)	1.56 (±0.97)
	6-8	19.75 (±0.14)	35.51 (±0.51)	37.04 (±0.49)	ND	ND	1.23 (±0.37)	1.43 (±0.29)	4.07 (±1.15)	7.77 (±3.81)	2.17 (±1.56)	1.18 (±0.72)	1.34 (±0.42)
	10-12	17.98 (±0.10)	35.90 (±0.26)	36.44 (±0.71)	1.50 (±2.49)	ND	3.31 (±3.04)	3.06 (±1.45)	6.08 (±0.25)	5.23 (±2.71)	1.40 (±0.15)	0.77 (±0.17)	0.93 (±0.06)
	14-15	11.36 (±0.29)	34.43 (±0.41)	35.24 (±0.05)	0.22 (±0.39)	3.78 (±0.10)	1.75 (±0.22)	2.09 (±0.36)	3.04 (±1.21)	4.00 (±2.28)	1.09 (±0.11)	1.18 (±0.39)	1.15 (±0.10)

**\*Note:** ND = Not detectable

**Table 3** Average and standard deviation ( $\pm$ ) values for down-core variation of biochemical parameters of sediment samples at stations upstream, mid-stream and proximal to the mouth of the Mandovi estuary.

Parameters	Depth (cm)	Proteins (mg g <sup>-1</sup> )			Carbohydrates (mg g <sup>-1</sup> )			Lipids (mg g <sup>-1</sup> )		
Season ↓ Stations →		Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth
Pre-monsoon	0-1	0.27 (±0.00)	1.52 (±0.01)	0.66 (±0.01)	1.20 (± 0.00)	1.29 (±0.01)	1.39 (± 0.01)	0.03 (± 0.00)	0.03 (± 0.01)	0.02 (± 0.00)
	3-4	0.42 (±0.00)	1.57 (±0.01)	0.49 (±0.01)	1.92 (± 0.00)	1.36 (±0.01)	1.25 (± 0.01)	0.04 (± 0.00)	0.02 (± 0.00)	0.01 (± 0.00)
	6-8	1.30 (±0.01)	1.31 (±0.01)	1.14 (±0.01)	1.81 (± 0.00)	1.16 (±0.01)	1.37 (± 0.01)	0.05 (± 0.00)	0.04 (± 0.00)	0.03 (± 0.00)
	10-12	1.36 (±0.00)	1.68 (±0.01)	1.61 (±0.01)	0.54 (± 0.01)	1.33 (±0.01)	1.45 (± 0.02)	0.04 (± 0.00)	0.04 (± 0.01)	0.02 (± 0.00)
	14-15	1.31 (±0.01)	1.98 (±0.01)	1.50 (±0.01)	1.66 (± 0.01)	1.30 (±0.02)	1.44 (± 0.01)	0.05 (± 0.00)	0.03 (± 0.01)	0.02 (± 0.00)
Monsoon	0-1	2.27 (±0.01)	2.19 (±0.01)	1.38 (±0.01)	3.39 (± 0.01)	3.53 (±0.02)	3.96 (± 0.01)	0.08 (± 0.00)	0.06 (± 0.01)	0.03 (± 0.00)
	3-4	1.31 (±0.02)	2.50 (±0.01)	1.35 (±0.01)	3.37 (± 0.01)	3.69 (±0.01)	3.65 (± 0.01)	0.04 (± 0.00)	0.06 (± 0.01)	0.04 (± 0.00)

	6-8	0.57 (±0.01)	3.06 (±0.01)	2.03 (±0.01)	4.13 (± 0.01)	1.78 (±0.01 )	1.75 (± 0.01)	0.01 (± 0.00)	0.06 (± 0.00)	0.04 (± 0.00)
	10-12	0.40 (±0.01)	2.39 (±0.01)	1.76 (±0.01)	4.43 (± 0.01)	4.00 (±0.02 )	3.40 (± 0.01)	0.01 (± 0.00)	0.05 (± 0.01)	0.03 (± 0.00)
	14-15	0.25 (±0.01)	2.16 (±0.01)	1.32 (±0.01)	3.95 (± 0.01)	4.76 (±0.01 )	4.02 (± 0.01)	0.01 (± 0.00)	0.05 (± 0.00)	0.02 (± 0.00)
Post-monsoon	0-1	1.64 (±0.01)	3.59 (±0.01)	1.32 (±0.01)	6.24 (± 0.01)	1.29 (±0.02 )	1.39 (± 0.01)	0.47 (± 0.01)	0.14 (± 0.01)	0.06 (± 0.01)
	3-4	2.90 (±0.01)	2.23 (±0.01)	1.42 (±0.01)	1.65 (± 0.01)	1.36 (±0.01 )	1.25 (± 0.01)	0.44 (± 0.00)	0.11 (± 0.01)	0.06 (± 0.01)
	6-8	2.17 (±0.01)	1.57 (±0.01)	1.30 (±0.01)	1.14 (± 0.01)	1.16 (±0.02 )	1.37 (± 0.01)	1.38 (± 0.00)	0.28 (± 0.01)	0.03 (± 0.00)
	10-12	1.08 (±0.01)	1.96 (±0.01)	1.37 (±0.01)	3.82 (± 0.01)	1.33 (±0.01 )	1.45 (± 0.01)	0.31 (± 0.01)	0.10 (± 0.01)	0.11 (± 0.01)
	14-15	0.89 (±0.01)	2.17 (±0.01)	0.65 (±0.01)	3.39 (±0.01)	1.30 (±0.02 )	1.44 (± 0.01)	1.12 (±0.01)	0.06 (± 0.01)	0.10 (± 0.00)

## Supplementary data

**Table S1** Average and standard deviation ( $\pm$ ) values for down-core variation of geo-microbiological variables of sediment samples at stations upstream, mid-stream and proximal to the mouth of the Mandovi estuary.

Parameters	Depth (cm)	TOC (%)			LOM (mg g <sup>-1</sup> )			TPC (10 <sup>9</sup> cells g <sup>-1</sup> )		
		Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth
Stations → Seasons ↓										
Pre-monsoon	0-1	1.94 (± 0.19)	5.08 (± 0.20)	1.51 (± 0.41)	1.15 (± 1.13)	1.57 (± 1.09)	1.15 (± .80)	1.49 (± 0.01)	1.65 (± 0.01)	1.15 (± 0.01)
	3-4	1.21 (± 0.06)	4.20 (± 0.72)	1.90 (± 0.21)	1.35 (± 0.97)	1.64 (± .13)	0.98 (± .68)	1.26 (± 0.01)	1.50 (± 0.01)	1.37 (± 0.01)
	6-8	0.93 (± 0.17)	4.15 (± 0.07)	1.47 (± 0.02)	1.50 (± 0.78)	1.39 (± 0.97)	1.41 (± .98)	1.32 (± 0.01)	1.33 (± 0.02)	1.21 (± 0.01)
	10-12	1.21 (± 0.04)	3.46 (± 0.06)	4.45 (± 0.05)	1.18 (± 0.92)	1.69 (± 1.17)	1.71 (± .18)	1.16 (± 0.01)	6.71 (± 0.01)	1.11 (± 0.01)
	14-15	0.23 (± 0.05)	4.41 (± 0.30)	4.62 (± 0.03)	1.43 (± 0.73)	1.84 (± 1.27)	1.64 (± 1.14)	1.24 (± 0.01)	7.51 (± 0.01)	1.60 (± 0.01)
Monsoon	0-1	5.08 (± 0.22)	6.10 (± 0.15)	6.16 (± 0.06)	3.19 (± 2.21)	3.21 (± 2.22)	2.98 (± 2.06)	1.57 (± 0.01)	7.68 (± 0.01)	2.09 (± 0.01)

							)		)	
	3-4	5.46 (± 0.04)	7.22 (± 0.08)	5.83 (± 0.04)	2.62 (± 1.82)	3.47 (± .40)	2.80 (± .94)	1.50 (±0.01)	8.50 (±0.01)	1.60 (±0.01)
	6-8	3.03 (± 0.09)	8.83 (± 0.12)	5.39 (± 0.10)	2.62 (± 1.81)	2.72 (± .88)	2.12 (± .47)	1.33 (±0.01)	6.13 (±0.01)	1.40 (±0.01)
	10-12	1.72 (± 0.03)	8.02 (± 0.10)	6.37 (± 0.05)	2.69 (± 1.86)	3.58 (± .48)	2.89 (±2.00)	1.32 (±0.01)	6.92 (±0.01)	1.32 (±0.01)
	14-15	2.18 (± 0.03)	8.58 (± 0.11)	5.71 (± 0.05)	2.34 (± 1.62)	3.87 (± .68)	2.98 (±2.06)	1.11 (±0.01)	6.72 (±0.01)	1.09 (±0.01)
Post- monsoon	0-1	7.10 (± 0.10)	6.59 (± 0.13)	8.35 (± 0.07)	4.64 (± 3.22)	2.79 (± .93)	1.53 (±1.06)	1.67 (±0.01)	1.63 (±0.01)	2.09 (±0.01)
	3-4	8.29 (± 0.04)	5.98 (± 0.19)	2.83 (± 0.04)	2.78 (± 1.92)	2.05 (± .42)	1.52 (±1.05)	1.14 (±0.01)	1.26 (±0.01)	1.65 (±0.01)
	6-8	8.91 (± 0.04)	4.44 (± 0.11)	3.09 (± 0.09)	2.61 (± 1.81)	1.67 (± .16)	1.50 (±1.04)	1.33 (±0.01)	1.16 (±0.01)	1.33 (±0.01)
	10-12	7.45 (± 0.03)	7.54 (± 0.05)	5.23 (± 0.03)	2.89 (± 2.00)	1.89 (±1.31)	1.63 (±1.13)	1.46 (±0.01)	0.67 (±0.01)	1.35 (±0.01)
	14-15	7.05 (±0.06)	9.01 (± 0.10)	4.42 (± 0.03)	3.00 (±2.08)	1.96 (±1.36)	1.22 (±0.84)	1.38 (±0.01)	0.75 (±0.01)	1.00 (±0.01)

**\*Note:** ND: Not detectable, TOC: total organic carbon, LOM: labile organic matter, TPC: total prokaryotic cells.

**Table S2** Diversity estimates for bacterial communities in the sediment samples from stations upstream, mid-stream and proximal to the mouth of the Mandovi estuary.

Parameters	Depth (cm)	Chao			ACE			Shannon			Invsimpson		
Seasons	Stations → ↓	Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth	Upstream	Mid-stream	Mouth
		Pre-monsoon	0-1		1512.52	1675.58		1638.02	1844.80		6.46	6.52	
	3-4		867.08	1347.02		969.22	1513.83		5.95	6.29		176.42	316.72
	6-8	1010.70	679.09	1631.58	1111.70	711.75	1861.63	6.26	5.44	6.54	326.47	85.98	481.80
	10-12	1165.01	617.25	1374.17	1270.17	634.59	1543.21	6.36	5.76	6.22	368.37	166.35	196.71
	14-15		1081.97	1435.79		1225.15	1587.28		6.14	6.38		254.01	369.70
Monsoon	0-1	912.31	1017.11	1464.34	905.06	1223.75	1633.51	5.77	6.14	6.27	185.12	279.71	256.73
	3-4	1502.50	1335.81	1124.08	1711.83	1625.78	1224.92	6.07	6.10	6.07	133.15	186.01	186.94
	6-8	1175.67		1041.52	1301.47		1179.79	5.93		6.02	107.05		177.70
	10-12	1492.08		1081.96	1702.01		1246.23	6.42		6.06	391.17		184.49
	14-15	943.69		1502.23	1051.14		1699.79	6.17		6.47	307.46		457.69
Post-monsoon	0-1	1974.47	795.24	1265.57	2164.56	863.57	1366.05	6.68	5.84	6.25	605.16	186.04	297.07
	3-4	1466.35		824.00	1545.36		882.77	6.38		5.73	290.32		130.54
	6-8	1268.16	878.50	644.39	1410.88	920.28	706.31	6.08	5.75	5.50	179.40	126.39	102.38
	10-12	1409.80	810.45	909.35	1541.59	928.54	997.63	6.30	5.71	6.04	275.33	121.06	253.84
	14-15	1827.23	1395.58	873.59	2142.14	1470.50	920.32	6.45	5.69	5.77	324.77	67.32	176.91

**\*Note:** Chao: OTU richness estimator, ACE: abundance-based coverage estimator, Shannon: Shannon index of diversity, higher numbers represent higher levels of diversity, Invsimpson: the inverse Simpson index of diversity.

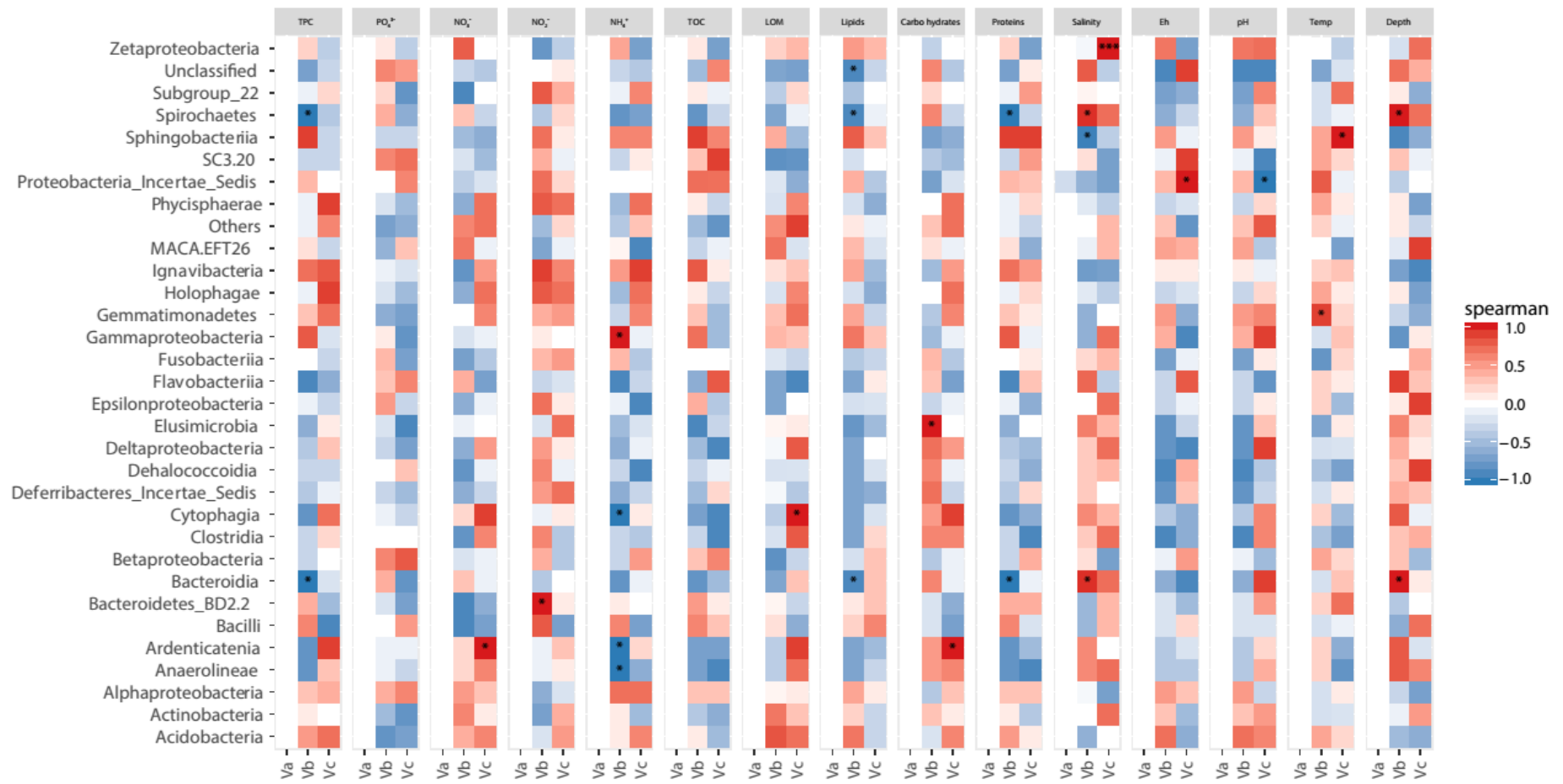


Fig S1 a



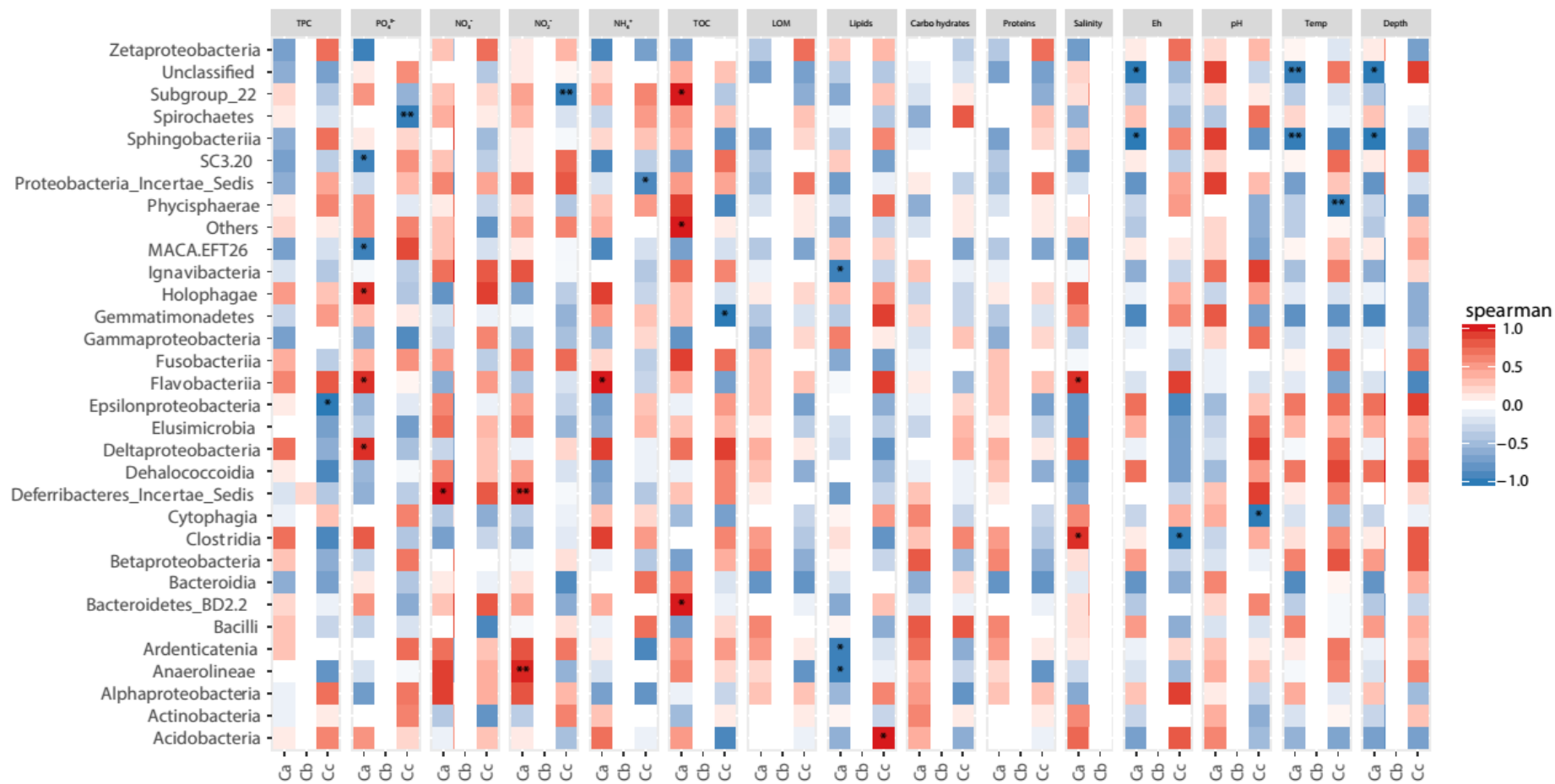


Fig S1 b

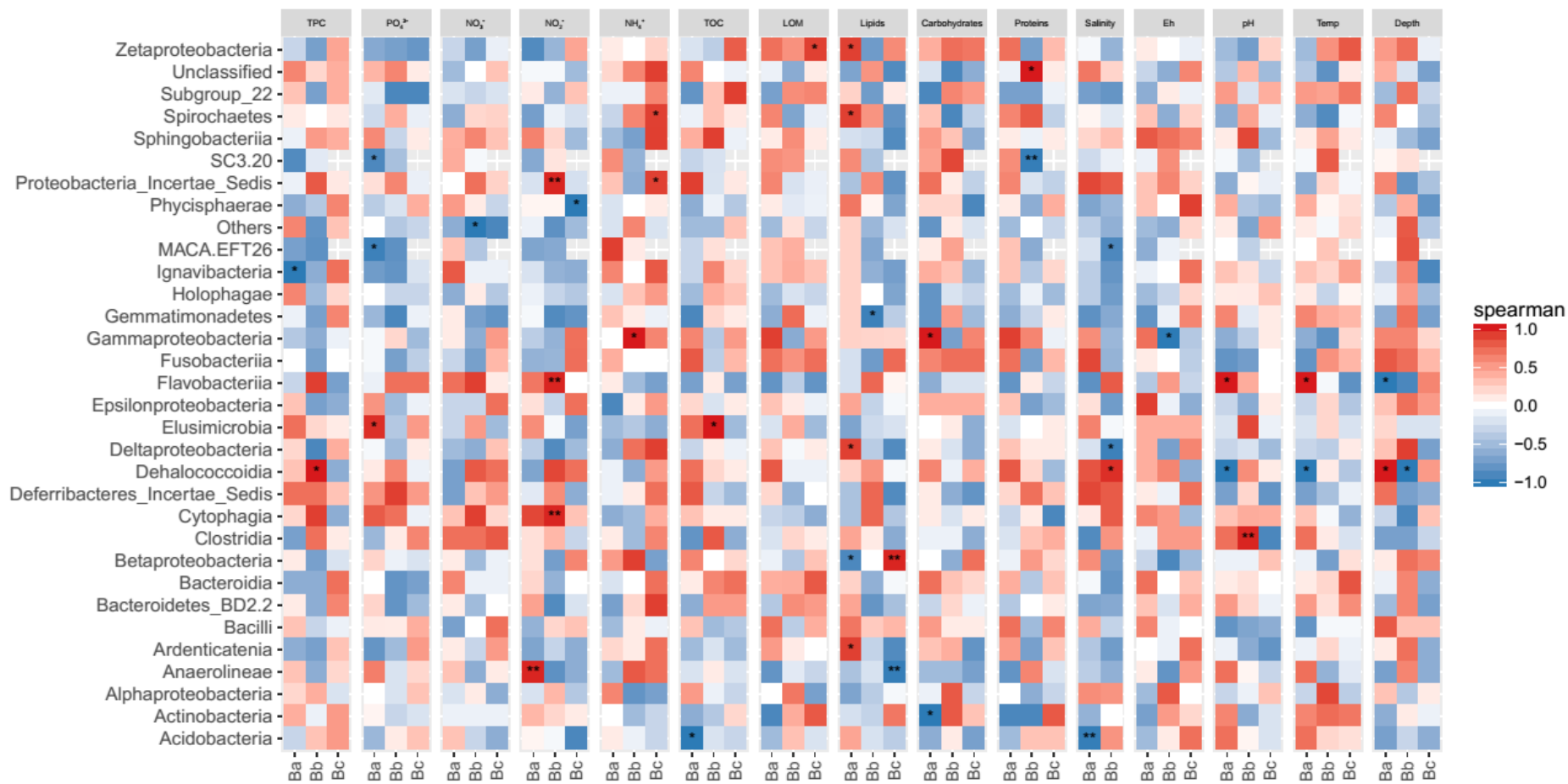


Fig S1 c