# Insights on aquatic microbiome of the Indian Sundarbans mangrove areas

**Paltu Kumar Dhal[1]*, Gérmán A. Kopprio[2], Astrid Gärdes[2]**

[1]Department of Life Science and Biotechnology, Jadavpur University, [2]Tropical Marine Microbiology, Department of Biogeochemistry and Geology, Leibniz Center for Tropical Marine Research, Bremen, Germany

***Corresponding address:**

Dr. Paltu Kumar Dhal
Department of Life Science and Biotechnology,
Jadavpur University
188, Raja Subodh Chandra Mallick Road, West Bengal,
Kolkata 700032

# Insights on aquatic microbiome of the Indian Sundarbans mangrove areas

**Paltu Kumar Dhal[1]*, Gérmán A. Kopprio[2], Astrid Gärdes[2]**

[1]Department of Life Science and Biotechnology, Jadavpur University, [2]Tropical Marine Microbiology, Department of Biogeochemistry and Geology, Leibniz Center for Tropical Marine Research, Bremen, Germany

## ABSTRACT:

**Background:** Anthropogenic perturbations have strong impact on water quality and ecological health of mangrove areas of Indian Sundarbans. Diversity in microbial community composition is important causes for maintaining the healthy of the mangrove ecosystem. However, microbial communities of estuarine water in Indian Sundarbans mangrove areas and environmental determinants that contribute to those communities were seldom studied.

**Methods:** Nevertheless, this study attempted first to report bacterial and archaeal communities simultaneously in the water from Matla River and Thakuran River of Maipith coastal areas more accurately using 16S rRNA gene-based amplicon approaches. Attempt also been made to assess the capability of the environmental parameters for explaining the variation in microbial community composition.

**Results:** Our investigation indicates the dominancy of halophilic marine bacteria from families *Flavobacteriaceae* and OM1 clade in the water with lower nutrient load collected from costal regions of a small Island of Sundarban Mangroves (ISM). At higher eutrophic conditions, changes in bacterial communities in Open Marine Water (OMW) were detected, where some of

the marine hydrocarbons degrading bacteria under families *Oceanospirillaceae* and *Spongiibacteraceae* were dominated. While most abundant bacterial family *Rhodobacteracea* almost equally (18% of the total community) dominated in both sites. Minor variation in the composition of archaeal community was also observed between OMW and ISM. Redundancy analysis indicates a combination of total nitrogen and dissolved inorganic nitrogen for OMW and for ISM, salinity and total nitrogen was responsible for explaining the changes in their respective microbial community composition.

**Conclusions:** Our study can serve as baseline approaches, which should focused on how do multiple environmental/anthropogenic stressors (salinity, pollution, eutrophication, land-use) affect the estuary water and consequently the microbial communities in concert. However, systematic approaches with more samples for evaluating the effect of environmental pollutions on mangrove microbial communities are recommended.

**Keywords:** Indian Sundarbans, mangrove water, Microbial community structure, 16S Illumina sequencing.

## 1. INTRODUCTION:

Sundarbans, the largest mangrove forest of the world, is situated in the joint delta of Ganges, Brahmaputra and Meghna rivers at Bay of Bengal (Ghosh and Bhadury 2017). This UNESCO World Heritage site comprises the Indian state of West Bengal and southwest Bangladesh (Bhattacharyya *et al.* 2015). Livelihood and well being of millions of people live in and around of Sundarbans, depends on its status and ecological services. Despite its high ecological and economical values, Sundarbans is seriously threatened by different anthropogenic activities. Since the early 19$^{th}$ century, landscapes of Sundarbans have also been changing due to saline and

freshwater imbalances. Water quality of this ecosystem is largely affected by sewage pollutant originated from industries located upstream and urban areas of West Bengal. Sewage entering into coastal water contains diverse chemical and microbiological pollutants and a wide variety of organic and inorganic wastes (Mitra *et al.* 2009, Banerjee *et al.* 2017), driving changes on its ecological and physiological health.

Microbial communities of mangroves are responsible for nutrient cycling and play a vital role in productivity, conservation and rehabilitation of mangrove ecosystems (Holguin *et al.* 2006). Therefore, understanding their responses to environmental changes is essential to predict changes in service-provisioning (Mishra *et al.* 2012). Several recent studies described the microbial community compositions of surface sediments and water of Indian Sundarban mangrove areas. Surface sediments this area dominanted with *Deltaproteobacteria* followed by *Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, and Epsilonproteobacteria* under phylum *Proteobacteria.* Abundant bacterial orders are *Desulfobacterales, Desulfuromonadales, Myxococcales, and Bdellovibrionales*. (Basak *et al.* 2015 ; Chakraborty *et al.* 2015; Basak *et al.* 2016). While bacterioplankton communities in the water of this region were found to be abundant with *Gammaproteobacteria* and *Alphaproteobacteria.* At the family level dominancy of *Hyphomicrobiaceae, Rhodobacteraceae, Pseudomonadaceae, Erythrobacteraceae, Kordiimonadaceae, Hyphomonadaceae,* and *Ruminococcaceae* were observed (Ghosh and Bhadury 2017; Ghosh and Bhadury 2018; Ghosh and Bhadury 2019). However, sampling locations of those studies on microbial communities in the Indian Sundarban mangrove water mainly restricted near to an island (Sagar Island), therefore the major conclusions of these studies were made based on a single site specific with limited number of samples. Moreover, not much effort has been made to investigate the archaeal community of this

region except single report by Bhattacharyya *et al*. (2015) on surface and subsurface sediments of Indian Sundarban mangrove forest. Moreover, the above studies have rarely analyzed the bacterial and archaeal community structures of the same samples at the same time. Therefore, our knowledge on those communities as well as information on how they are controlled by environmental parameters is limited. In order to assess the microbial communities of marine ecosystem via high-throughput sequencing of amplified 16S rRNA genes with high resolution and fidelity, it is extremely important to select the proper primer set that can't underestimated or overestimated any common marine taxa (Parada *et al*. 2016). However, this will be the first attempt to visualize the accurate and well-resolved picture of bacterial and archaeal communities simultaneously of marine water in Sundarbans mangrove using next-generation amplicon sequencing of the 16S rRNA gene using recently developed 515F-Y/926R primers. We also tried to explore the environmental determinants that contribute to the variation of their microbial communities. This study will provide baseline knowledge on microbial ecology of the World Heritage site and serve as a baseline for monitoring programs and predicting changes at impacted sites.

## 2. MATERIAL AND METHODS

### 2.1 Study sites and Sample collection:

In the present study sampling was conducted in the Sundarbans mangrove ecosystem that shared between India and Bangladesh and lies in the Ganga-Brahmaputra-Meghna (GBM) delta. This mangrove ecosystem contains over 102 islands with a network complex of many rivers, rivulets, and creeks (Das 2017). Sampling was carried out at two different locations on Thakuran River - Matla River estuarine complex of Maipith coastal areas in the Indian Sundarbans mangroves

during March 2017 (Fig 1). They are designated as Island of Sundarban Mangroves (ISM) and Open Marine Water (OMW). ISM is an uninhabited small island with lesser anthropogenic disturbance situated in Thakuran and Matla river complex in low-lying costal plain. This river has no reports for perennial fresh water source (Das 2017). Water from three different costal regions of this island is selected for sampling. OMW is an open marine site around same regions, which is supposedly continually influenced by the wastewaters from upstream regions of Matla River. Three independent replicated water samples (1 L) from each of three different sampling sites of both ISM and OMW were collected collected in sterile containers and immediately stored at a chilled box until further laboratory analysis.

**2.2 Environmental Parameters and nutrients analysis:**

Physiochemical parameters (salinity and pH) of all collected samples were measured using Eureka 2 Manta multiprobe (Eureka Environmental Engineering, Texas, USA). Total 50 mL of each sample was filtered through a 0.7 µm syringe filter and poisoned with 200 µL of 3.5 g/100 mL $HgCl_2$ solution for nutrient analysis. Dissolved organic carbon (DOC) and total nitrogen (TN) as well as dissolved inorganic nutrients (DIN), that including nitrate and nitrite (NOx), phosphate ($PO_4^{3-}$), and silicate (Si) were analyzed using a continuous flow analyzer (Flowsys by Unity Scientific, Brookfield, USA) (https://doi.pangaea.de/10.1594/PANGAEA.889699). Each of the samples was filtered through a 0.7 µm pore size GF/F filter (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) before DOC and nutrients measurements. The inorganic nutrients (nitrite, nitrate, phosphate, and silicate) were measured using spectro-photometrical analysis with a Flowsys continuous flow analyzer (Systea, Anagni, Italy). For measurements of DOC, the filtered samples were acidified with concentrated HCl (pH <2) and analyzed by high-temperature oxic combustion method using a TOC-$V_{CPN}$ analyzer (Shimadzu, Mandel, Canada). Seawater

137 standards (Hansell laboratory, RSMAS University Miami, USA) were used for calibration and
138 quality control, and ultrapure water as a blank.

139 **2.3 DNA Extraction, PCR Amplification, and Illumina MiSeq osequencing**

140 From each site, water (1 L) was filtered (0.2 μm) and DNA was extracted using the
141 PowerWater® DNA Isolation Kit according to the manufacturer's instructions (MoBio
142 Laboratories Inc., Carlsbad, CA, USA). DNA concentrations and purity were measured
143 spectrophotometrically. Presence of bacterial and archebacterial 16S rRNA gene was in the
144 extracted metagenome was verified following previous method (Dhal and Sar, 2014). In order to
145 classify taxonomically both bacterial and archaeal community structure simultaneously,
146 sequencing of V4–V5 hypervariable regions of 16S rRNA gene were generated using primers
147 515F-Y (5′-GTGYCAGCMGCCGCGGTAA-3′) and 926R (5′-
148 CCGYCAATTYMTTTRAGTTT-3′) (Parada *et al.*, 2016) on the Illumina MiSeq platform
149 (CeBiTec Bielefeld, Germany), in a 2 × 300 bp paired-end run.

150 **2.4 High Throughput sequencing data processing：**

151 Primer sequences were removed using *cutadapt* from the raw paired-end reads (Martin, 2011).
152 The primer-trimmed sequences are available on Sequence Read Archive (SRA) (accession no.
153 SRP144285). Sequences were quality trimmed with *trimmomatic* v0.32 (Bolger *et al*. 2014) using
154 a sliding window of 4 bases and a minimum average quality of 15, and merged with PEAR
155 v0.9.5 (Zhang *et al.* 2014). Quality-filtered sequences were clustered into OTUs with *swarm*
156 algorithm using default parameters (Mahe *et al.* 2014). One single representative sequence per
157 OTU was taxonomically classified with SINA (SILVA Incremental Aligner; v1.2.11; Silva
158 reference database release 132) at a minimum alignment similarity of 0.9, and a last common
159 ancestor consensus of 0.7 (Pruesse *et al*. 2012). OTUs that were unclassified on the domain level

and those matching to chloroplast and mitochondrial sequences were excluded from the analysis using well-standardized r script (Kunda *et al.* 2018). The final OTU tables are accessible at (https://doi.pangaea.de/10.1594/PANGAEA.890757).

**2.5 Statistical analysis:**

Principal component analysis (PCA) was performed to cluster the sampling sites based on their environmental parameters. Differences in environmental parameters among ISM and OMW were assessed using general linear mixed models (GLMM) with sampling station as a random factor (Kuznetsova *et al*. 2017).

Alpha-Diversity indices were calculated to assess richness and evenness of the microbial communities (Hill 1973) in the studied samples, based on repeated random subsampling of the amplicon data sets after randomly rarefying the data set to the minimum library size (50517 sequences). Significant differences in alpha-diversity indices between the studied stations were determined by using the non-parametric Kruskal test followed by p-value adjusted Wilcoxon tests (Hassenruck et al 2016).

To assess the differences in community structure between two sampling sites (beta-diversity), Bray–Curtis dissimilarities were calculated using the relative OTU abundances and also non-metric multidimensional scaling (NMDS) plot was produced. Analysis of similarity (ANOSIM) was calculated to assess the separation of bacterial communities between the two sites. P-values of all multiple pairwise comparisons were adjusted using the false discovery rate (fdr) correction method by Benjamini and Hochberg (1995). In order to evaluate the environmental parameters as drivers of the variations in community compositions, redundancy analysis (RDA) was used with centered log ratio (clr)-transformed sequence counts using the R function aldex.clr of the ALDEx2 package via median values of 128 Monte-Carlo instances (Fernandes *et al.* 2014). To

compare the explanatory power of all measured environmental parameters, additional RDA models were constructed with environmental parameters as predictors. Forward model selection was used after checking for variance inflation to determine which of parameters would be included in the RDA models. When more than one parameter was included, pure effects were also tested accounting for the variation explained by the other factors in the model. Collinearity among predictors was determined via Variance inflation factors (VIFs) of the individual parameters. All of the parameters in any of the RDA models displayed VIFs less than 10. The adjusted $R^2$ is provided as goodness-fit-stat. All statistical analyses were conducted in R using the core distribution, version 3.3.2 and R-Studio, version 1.0.153,with following packages: vegan (Oksanen *et al.* 2016), lmerTest for the GLMM (Kuznetsova *et al.* 2017), ALDEx2 (Oksanen *et al.* 2016) and multcomp (Fernandes *et al.* 2014).

## 3. Result:

### 3.1 Environmental characterization

Environmental parameters (pH, salinity) and nutrients (including DOC, TN, NOx, nitrate, DIN, phosphate, and silicate) concentrations for all samples were measured (**Table 1)**. Samples were slightly alkaline (pH 8.0 to 8.7) in nature. The GLMMs analysis indicated that the measured water nutrients that differed significantly among the two sampling station were mainly TN, DIN and $PO_4^{3-}$ (Table 1; **Table S1**). The PCA ordination (**Fig 2)** showed that first two principal components (PC1 and PC2) represented 74.4% of data variation among sites. PC1 alone represents 60.1% of total variation and influenced by most of the measured parameters, while pH showed a strong correlation with PC2 (**Fig S1)**. Noteworthy to mention, the samples were separated into two clusters by PC1. One cluster is mainly composed with the samples from ISM

(except TH2.3 of OMW) and other cluster accommodating samples collected from OMW. This ordination probably indicates elevated eutrophication in samples from the OMW compared to ISM.

### 3.2. Microbial communities

Total numbers of reads generated per sample ranged between 50517 to 90468 (after merged) corresponding to 3,644 to 6,470 swarmed, non-singleton 16S OTUs **(Fig S2)**. After rarefaction, numbers of bacterial and archaeal OTUs ranged between 3390 to 5415 and 37 to 91, respectively (**Fig S2**). None of the measured diversity indices (Average Shannon diversity and inverse Simpson indices) were found significance differences in between OMW and ISM (**Fig 3**; **Table S2**) indicated by Kruskal - Wilcoxon test ($p > 0.5$), although values varied considerably.

The microbial community of marine estuary water from Sundarbans was dominated with bacteria occupying more than 96 % of total community and archaea represented only 4 %. Bacterial assemblage of two different sites this area showed to have a distinct community. In class level, among the dominant bacterial groups, *Flavobacteria* (ISM: 15.9 % vs OMW: 8.6%), *Alphaproteobacteria* (ISM: 29.5% vs OMW: 28%), and *Acidimicrobiia* (ISM: 6.6% vs OMW: 5.0%) were dominant in ISM while OMW was dominated with mainly with *Gammaproteobacteria* (ISM: 22.6% vs OMW: 35.3%) **(Fig S3)**. At higher taxonomic resolution levels **(Fig 4)**, bacterial communities were composed with a total of 474 and 915 different bacterial family and genus, respectively. The most dominant bacterial family was *Rhodobacteraceae* (18.6%), almost equally distributed between studied two sites. Other dominant bacterial families of ISM were *Flavobacteriaceae* (14.8%) and OM1 clade (5.2%) whereas in OMW, *Oceanospirillaceae* (16%) and *Spongiibacteraceae* (4 %) were the most abundant.

We observed dominancy of Marine Group (MG I) (currently known as *Thaumarchaeota*) and *Euryarchaea* MG II in archaeal community assemblages with 78.5% and 16.9% of relative abundance, respectively while the presence of *Woesearchaeota* (2.1%) was also evident **(Fig 5)**. MG I was found in relatively higher abundant at ISM constituting on average 82.1% of sequences as opposed to 75% at OMW. *Euryarchaeota* MG II comprised about 20.3% at OMW compared to 13.7% at ISM. Among total twenty-nine (29) archaeal genera, *Candidatus Nitrosopumilus* and *Candidatus Nitrosopelagicus* accounted for the 40.7% and 21.4% of total relative abundance, respectively (**Fig S4**).

### 3.3 Environmental drivers of bacterial communities

At OTU resolution level also, distinct microbial communities were observed between OMW and ISM based on changes in community structure (beta diversity) which is quantified by non-metric multidimensional scaling (NMDS) plot by calculating Bray–Curtis dissimilarly (**Fig 6**). This pattern is confirmed by the ANOSIM test that indicated a significant difference in microbial community structure between ISM and OMW (ANOSIM, $R = 0.24$, $p < 0.001$). Redundancy analyses attempted to identify the water quality parameters that had strong explanatory power for microbial communities. We observed that total nitrogen (TN) and dissolved inorganic nitrogen (DIN) accounted for almost 10% of the variability in microbial community of OMW where TN alone explain 6% variation of microbial community (RDA, $R^2 = 0.06$, $F_{(1,7)} = 1.34$, $p < 0.05$). In contrast, salinity and TN explained approximately 9% of the variability in community composition of ISM (RDA, $R^2 = 0.06$, $F_{(1,7)} = 1.34$, $p < 0.05$) and alone salinity responsible for explaining 7% microbial variation of this site (**Table 2**).

## 4. Discussion:

The pH values (8.0- 8.7) indicates the water of ISM and OMW slightly alkaline in nature which supports the previous findings in similar samples from Sundarbans Mangrove forest areas ( Sarkar and Bhattacharya, 2010). Such ranges of pH may be attributed by the buffering capacity of water that support high biological activity (Balasubramanian and Kannan 2005; Sarkar and Bhattacharya 2010). The water of Sundarbans is characterized by elevated salinity values in line with previous reports (Balasubramanian and Kannan 2005). The long-term changes in water properties in the eastern part of Sundarbans, sampling regions of our study, indicating increased trends on salinity and pH (Bhattacharyya *et al.* 2015). Our result shows differences in measured environmental parameters between two sites represented by three sampling stations and nine samples each leading to their segregation into two clusters (in PCA analysis) along with their sampling sites. This ordination as a result of different nutrient loads and this is reflected by potential eutrophication in water from OWM. Influences from the Thakuran and Matla rivers reprted to have a strong impact on the estuary water quality represented by OWM (Mitra *et al.* 2009, Banerjee *et al.* 2017), that also reflected in our study. The perennial discharge in Thakuran and Matla Estuary from upstream regions brings in a high suspended matter load throughout the year (Sarkar and Bhattacharya 2010). Those estuaries severely contaminated with huge organic load and sediment flux originated from upstream domestic sewage, aquaculture, intensive trawling activities, agricultural runoff as well as soil erosion (Sarkar *et al.* 2004; Mukherjee *et al.* 2009).

Because of the relevance of microbial community of Indian Sundarbans, several investigators attempted on surface sediments samples (Ghosh *et al.* 2010; Basak *et al.* 2015; Bhattacharyya *et al.* 2015; Eloe-Fadrosh *et al.* 2015; Basak *et al.* 2016; Mallick *et al.* 2018) as well as recently on water column of this regions (Ghosh and Bhadury 2018; Ghosh and Bhadury 2019) using 16S

rRNA gene metagenomic approaches. Unlike previous studies, this investigation attempted to asses both the bacterial and archaeal community at a same time of water from relative less anthropogenic disturbance sites using an efficient primer set to target V4-V5 variable region of 16S rRNA gene in order to avoid the problems of underestimated or overestimated common marine taxa (Parada et al. 2016), therefore our investigation gives more accurate and well-resolved picture of microbial communities of these sites.

Although insignificant differences, elevated trends of α-diversity of the marine estuary water samples (OWM) might be an indications of relatively rich bacterial community compared to ISM of Sundarbans might be attributed toward their elevated eutrophication. This observation was supported by previous reports that indicate a higher diversity and equitability in the human impacted estuary because of proliferation of several different microorganisms (Nogales et al., 2011; Borin et al., 2009).

At phyla level, bacterial assemblages of the studied samples (specially OMW) showed similarity with the previously reported bacterial community of marine sediments and water samples of Sundarban Mangrove areas (Basak *et al.* 2015; Basak *et al.* 2016; Ghosh and Bhadury 2017; Ghosh and Bhadury 2018). The most dominant bacterial family presents both the station with almost equal proportion is *Rhodobacteraceae*. Dominancy of members of this family in marine water microbial community previously reported and known to be associated with marine phytoplankton blooms where it plays important role in transforming phytoplankton-derived organic matter (Ghosh *et al.* 2010; Buchan *et al*. 2010; Simon *et al.* 2017). The abundant OTUs of *Rhodobacteraceae* are classified as anoxygenic phototrophs *Nautella,* reported to serve an indicator of marine eutrophication, predominantly found in higher eutrophic OMW samples. The other dominant one under the same family is marine heterotrophs *Ruegeria*s (almost equally

297 distributed among both ISM and OMW) serve as the model of marine sulfur and carbon cycle
298 (Buchan *et al.*, 2005, Dang *et al.* 2008; Doberva *et al.* 2017).

299 Interestingly enrichment of several OTUs from the *Flavobacteria* is observed in the oligotrophic
300 ISM compared to the eutrophic OMW. They are specialized in utilization of biopolymers and
301 organic substances in oligotrophic environment i.e., when organic substances present even at
302 very low concentrations. Higher abundance of these polymers degrading bacteria biopolymers
303 promotes the growth of heterotrophic bacteria at oligotrophic environments (Lauro et al 2009;
304 Jessen *et al.* 2013; Kegler *et al.* 2018), might plays central role in microbial ecology of ISM by
305 creating the supporing environment for heterotrophs/copiotrophic organisms in relatively
306 oligotrophic conditions. The dominant OTUs under family *Flavobacteriaceae* were mainly
307 classified as *Aureimarina* and NS5 marine group genus. Roles of *Aureimarina* in marine
308 biogeochemistry has not been investigated much although few studies reported on their
309 presences in coastal seawater and saline estuarine (Teeling *et al.* 2012; Campbell *et al.* 2015).
310 However, this is the first report of their abundance in marine water of Sundarbans. The NS5
311 marine group which are equally dominated in both the studied sites are reported to be ubiquitous
312 in the seawater-related samples and known for phytoplankton-derived macromolecules (Tanaka
313 *et al.* 2008; Dupont *et al.* 2012 Seo et al, 2017).

314 This investigation identified *Actinobacteria* constituted a predominant fraction both in OMW
315 and ISM but elevated amount in the later samples. Bacteria under this group are consisted of
316 both copiotrophic and oligotrophic members with abundance in oligotrophic marine
317 environments (Ho et al 2017; Nouioui et al 2018). As marine *Actinobacteria* are the richest
318 sources of secondary metabolites thus, have been well reported as potential sources of bioactive
319 compounds (Manivasagan et al 2014)**.** Therefore, their abundance in our studied sites (specially

ISM) would be potential hotspot for isolating bioactive molecules from Indian Sundarban mangrove forest. The OM1 clade (dominating in ISM), an uncultured Actinobacterial clade, frequently recovered from various marine environments with higher abundance at near coastal sites than open marine areas however supports our reports (Giovannoni and Stingl 2005; Morris *et al.* 2012; Ngugi and Stingl 2018). The dominant OTUs of this family were classified as *Candidatus Actinomarina*. Those photoheterotrophs are one of smallest free-living prokaryotes are reported to be ubiquitous in marine systems. Not many reports are described their role in the marine biogeochemical cycle and first reports on their presence in Sundarban mangrove areas.

The OTUs affiliated to families *Oceanospirillaceae* and *Spongiibacteraceae* of *Gammaproteobacteria* showed increase abundance in the impacted site OMW. Bacteria from these families are known to be present in eutrophic marine environments. They are known as polymer degraders and can utilize polyhydroxy alkanoate compounds and proteorhodopsin, for harvesting an additional energy, supports their living in eutrophic water samples (Mizuno *et al.* 2015; Hoffmann *et al.* 2017; Ribicic *et al.* 2018). The dominant OTUs of *Oceanospirillaceae* are affiliated to chemoheterotrophic genus *Marinobacterium*. Their presence in mangroves as well as surface seawater have already been described in previous studies and known to be associated with hydrocarbon biodegradation (Dos Santos *et al.* 2011; Spring *et al.* 2015). The other dominated bacterial family in the samples from OMW is *Spongiibacteraceae*. They comprise mainly marine bacteria known as Oligotrophic Marine *Gammaproteobacteria* (OMG) group (Cho and Giovannoni 2004; Yilmaz *et al*. 2016). We recorded the dominant OTUs of this family are affiliated with BD1-7 which is a cosmopolitan group of *Gammaproteobacteria* is mostly autochthonous, reported to inhabits at diverse marine habitats (Huggett and Rappé 2012; Spring *et al.* 2015; Zhou *et al.* 2018). In line with previous reports this investigation, therefore, indicates

343 proliferation of bacterial groups under *Gammaproteobacteria* with respond to increased nutrient
344 concentrations in estuary (Nogales et al 2011).

345 However, in contrast to sediments reported in previous investigations, an archaeal community of
346 marine waters in the Sundarban mangroves is dominated with *Thaumarchaeota* Marine Group
347 (MG I) and *Euryarchaea* MG II. The chemolithoautotrophic MG I which are in higher in number
348 on ISM are responsible for oxidation of ammonia and showed ability in inorganic carbon fixation
349 (Haro-Moreno et al 2017) thus important players in global Carbon (C) and Nitrogen (N)
350 biogeochemical cycles. In contrast enrichment of heterotrophic MG II, which is more dominate
351 in OMW, also are observed in the marine aquatic environment (Liu et al 2017). Their abilities in
352 organic carbon degradation and in the photic zone, they acquired energy in presences of light.
353 Dominant OTUs of MGI group are affiliated with ammonia-oxidizing archaeal, *Candidatus*
354 *Nitrosopumilus* and *Candidatus Nitrosopelagicus*, play important roles in nitrogen and carbon
355 cycling of marine ecosystem (Bhattacharyya *et al.* 2015) however, this investigation reports first
356 on their present in this areas. Therefore, the biological and geochemical processes at estuary
357 water habitats in the Indian Sundarban Mangrove areas have likely influenced the archaeal
358 community structure.

359 **5. CONCLUSION**

360 This investigation provides the first details description of bacterial and archeal communities
361 concurrently of Thakuran and Matla river complex of Maipith coastal areas in the Indian
362 Sundarbans mangroves areas. Our study indicates along with the elevated level of average pH
363 and salinity, the open marine water (OMW) showed eutrophication probably leads to an
364 observed bacterial shift toward more copiotrophic and photoheterotrophic bacterial
365 (*Oceanospirillaceae* and *Spongiibacteraceae*) and archaeal community (*Euryarchaea* MG II)

and compared to the more oligotrophic microbial community (*Aureimarina*, NS5 marine group, OM1 clade and *Thaumarchaeota* MG I) of costal water of a small Island of Sundarban Mangroves (ISM). These microbial assembles thus might represent key players in biogeochemical cycle of this mangrove and the studied areas represent a hotspot for bacterial having potential to produce the commercially important secondary metabolites. This investigation also reports that total nitrogen and dissolved inorganic nitrogen are the major environmental contributors on determining the microbial communities for OMW and for ISM it is combination of total nitrogen and salinity.

However, given the rising burden on Indian mangrove coastal ecosystems, this study suggests that sewages from urban areas lacking proper treatment can alter microbial communities that may play vital role in biogeochemical cycle of mangrove ecosystem and consequently may impact on the climate in the tropical country.

## 6. ACKNOWLEDGEMENTS

Foremost PKD would like to thank NAM S&T Centre New Delhi, India and ZMT, Bremen for fellowship selection. PKD also acknowledge to Jadavpur University for a travel grant and granting the leave to avail this fellowship. We sincerely thank the faculty and technical staff of ZMT Bremen for their generous help to carry this research work. Many thanks to Dr Halina Tegetmeye of CeBiTec Bielefeld, Germany for sequences. The assistance of conducting analyses at the laboratories at the ZMT in Bremen by Matthias Birkicht, Sonja Peters and Achim Meyer also acknowledge.

## 6. FUNDING

This work was part of the Joint NAM S&T Centre – ZMT Bremen Fellowship in Tropical Coastal Marine Research that was supported by the Leibniz Zentrumfür Marine Tropenforschung (ZMT), Bremen, Germany.

## 7. REFERENCES:

1. Balasubramanian R, Kannan L. Physico-chemical characteristics of the coral reef environs of the Gulf of Mannar Biosphere Reserve, India. Int J Ecol Environ Sci 2005;31: 273-8.
2. Banerjee K, Gatti RC, Mitra A. Climate change-induced salinity variation impacts on a stenoecious mangrove species in the Indian Sundarbans. Ambio 2017;46: 492-9.
3. Basak P, Majumder NS, Nag S et al. Spatiotemporal analysis of bacterial diversity in sediments of Sundarbans using parallel 16S rRNA gene tag sequencing. Microb Ecol 2015;69: 500-11.
4. Basak P, Pramanik A, Sengupta S et al. Bacterial diversity assessment of pristine mangrove microbial community from Dhulibhashani, Sundarbans using 16S rRNA gene tag sequencing. Genom Data 2016;7: 76-8.
5. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the royal statistical society Series B (Methodological) 1995: 289-300.
6. Bhattacharyya A, Majumder NS, Basak P et al. Diversity and Distribution of Archaea in the Mangrove Sediment of Sundarbans. Archaea 2015;2015: 968582.
7. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30: 2114-20.
8. Borin S, Brusetti L, Daffonchio D, Delaney E & Baldi F. Biodiversity of prokaryotic communities in sediments of different sub-basins of the Venice lagoon. Res Microbiol2009;160:307–314
9. Buchan A, González JM, Moran MA. Overview of the marine Roseobacter lineage. Applied and environmental microbiology 2005;71: 5665-77.
10. Campbell AM, Fleisher J, Sinigalliano C et al. Dynamics of marine bacterial community diversity of the coastal waters of the reefs, inlets, and wastewater outfalls of southeast F lorida. MicrobiologyOpen 2015;4: 390-408.
11. Chakraborty A, Bera A, Mukherjee A et al. Changing bacterial profile of Sundarbans, the world heritage mangrove:impact of anthropogenic interventions. World Journal of Microbiology and Biotechnology 2015;31: 593-610.
12. Cho J-C, Giovannoni SJ. Cultivation and growth characteristics of a diverse group of oligotrophic marine Gammaproteobacteria. Applied and Environmental Microbiology 2004;70: 432-40.
13. Dang H, Zhang X, Sun J et al. Diversity and spatial distribution of sediment ammonia-oxidizing crenarchaeota in response to estuarine and environmental gradients in the Changjiang Estuary and East China Sea. Microbiology 2008;154: 2084-95.
14. Das, Gautam. (2016). Geomorphic Environments of the Thakuran River of the Sunderbans, India. Earth Science India. 9. 974-8350. 10.31870/ESI.09.3.2016.9.
15. Dhal, P. K.; Sar, P. Microbial communities in uranium mine tailings and mine water sediment from Jaduguda U mine, India: A culture independent analysis J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.2014, 49, 694– 709DOI: 10.1080/10934529.2014.865458
16. Doberva M, Stien D, Sorres J et al. Large Diversity and Original Structures of Acyl-Homoserine Lactones in Strain MOLA 401, a Marine Rhodobacteraceae Bacterium. Frontiers in microbiology 2017;8: 1152.
17. Dos Santos HF, Cury JC, Do Carmo FL et al. Mangrove bacterial diversity and the impact of oil contamination revealed by pyrosequencing: bacterial proxies for oil pollution. PLoS One 2011;6:

e16943.
18. Dupont CL, Rusch DB, Yooseph S et al. Genomic insights to SAR86, an abundant and uncultivated marine bacterial lineage. The ISME journal 2012;6: 1186.
19. Fernandes AD, Reid JN, Macklaim JM et al. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. Microbiome 2014;2: 15.
20. Ghosh A, Bhadury P. Insights into bacterioplankton community structure from Sundarbans mangrove ecoregion using Sanger and Illumina MiSeq sequencing approaches: A comparative analysis. Genom Data 2017;11: 39-42.
21. Ghosh A, Bhadury P. Exploring biogeographic patterns of bacterioplankton communities across global estuaries. MicrobiologyOpen. 2019;8:e741.
22. Ghosh A, Bhadury P. Investigating monsoon and post-monsoon variabilities of bacterioplankton communities in a mangrove ecosystem. Environmental Science and Pollution Research 2018;25: 5722-39.
23. Ghosh A, Dey N, Bera A et al. Culture independent molecular analysis of bacterial communities in the mangrove sediment of Sundarban, India. Saline Systems 2010;6: 1.
24. Giovannoni SJ, Stingl U. Molecular diversity and ecology of microbial plankton. Nature 2005;437: 343.
25. Hassenruck C, Fink A, Lichtschlag A et al. Quantification of the effects of ocean acidification on sediment microbial communities in the environment: the importance of ecosystem approaches. FEMS Microbiol Ecol 2016;92: fiw027.
26. Haro-Moreno JM, Rodriguez-Valera F, López-García P, Moreira D, Martin-Cuadrado A-B. (2017). New insights into marine group III Euryarchaeota, from dark to light. *ISME J* **11**: 1102–1117.
27. Hill MO. Diversity and evenness: a unifying notation and its consequences. Ecology 1973;54: 427-32.
28. Ho Adrian, Paolo Di Lonardo D., Bodelier Paul L. E. Revisiting life strategy concepts in environmental microbial ecology, FEMS Microbiology Ecology, Volume 93, Issue 3, March 2017.
29. Hoffmann K, Hassenruck C, Salman-Carvalho V et al. Response of Bacterial Communities to Different Detritus Compositions in Arctic Deep-Sea Sediments. Front Microbiol 2017;8: 266.
30. Holguin G, Gonzalez-Zamorano P, de-Bashan LE et al. Mangrove health in an arid environment encroached by urban development--a case study. Sci Total Environ 2006;363: 260-74.
31. Huggett MJ, Rappé MS. Genome sequence of strain HIMB30, a novel member of the marine Gammaproteobacteria. Journal of bacteriology 2012;194: 732-3.
32. Jessen C, Lizcano JFV, Bayer T et al. In-situ effects of eutrophication and overfishing on physiology and bacterial diversity of the Red Sea coral Acropora hemprichii. PLoS One 2013;8: e62091.
33. Kegler HF, Hassenrück C, Kegler P et al. Small tropical islands with dense human population: differences in water quality of near-shore waters are associated with distinct bacterial communities. PeerJ 2018;6: e4555.
34. Kunda P, Dhal PK, Mukherjee A. Endophytic bacterial community of rice (Oryza sativa L.) from coastal saline zone of West Bengal: 16S rRNA gene based metagenomics approach. Meta Gene 2018;18: 79-86.
35. Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest package: tests in linear mixed effects models. Journal of Statistical Software 2017;82.
36. Lauro, F. M. *et al.* The genomic basis of trophic strategy in marine bacteria. *Proc. Natl Acad. Sci. USA* **106**, 15527–15533 (2009)
37. Liu H, Zhang CL, Yang C, Chen S, Cao Z, Zhang Z et al. Marine Group II dominates planktonic Archaea in water column of the Northeastern South China Sea. Front Microbiol. 2017;8:1098
38. Mahe F, Rognes T, Quince C et al. Swarm v2: highly-scalable and high-resolution amplicon clustering. PeerJ 2015;3: e1420.

39. Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal, 17(1), pp. 10-12. doi:https://doi.org/10.14806/ej.17.1.200
40. Mallick I, Bhattacharyya C, Mukherji S et al. Effective rhizoinoculation and biofilm formation by arsenic immobilizing halophilic plant growth promoting bacteria (PGPB) isolated from mangrove rhizosphere: A step towards arsenic rhizoremediation. Sci Total Environ 2018;610-611: 1239-50.
41. Manivasagan P, Kang KH, Sivakumar K, Li-Chan EC, Oh HM, Kim SK. Marine actinebacteria: an important source of bioactive natural products. Environ Toxicol Pharmacol 2014; 38(1): 172-88.
42. Mishra RR, Swain MR, Dangar TK et al. Diversity and seasonal fluctuation of predominant microbial communities in Bhitarkanika, a tropical mangrove ecosystem in India. Rev Biol Trop 2012;60: 909-24.
43. Mitra A, Gangopadhyay A, Dube A et al. Observed changes in water mass properties in the Indian Sundarbans (northwestern Bay of Bengal) during 1980–2007. Current Science 2009: 1445-52.
44. Mizuno CM, Rodriguez-Valera F, Ghai R. Genomes of planktonic acidimicrobiales: widening horizons for marine actinobacteria by metagenomics. MBio 2015;6: e02083-14.
45. Morris RM, Frazar CD, Carlson CA. Basin-scale patterns in the abundance of SAR11 subclades, marine Actinobacteria (OM1), members of the Roseobacter clade and OCS116 in the South Atlantic. Environmental microbiology 2012;14: 1133-44.
46. Mukherjee D, Mukherjee A, Kumar B. Chemical fractionation of metals in freshly deposited marine estuarine sediments of sundarban ecosystem, India. Environmental geology 2009;58: 1757-67.
47. Ngugi DK, Stingl U. High-Quality Draft Single-Cell Genome Sequence of the NS5 Marine Group from the Coastal Red Sea. Genome announcements 2018;6: e00565-18.
48. Nogales, B., Lanfranconi, M.P., Piña-Villalonga, J.M., and Bosch, R. Anthropogenic perturbations in marinemicrobial communities.FEMS Microbiol Rev2011;35:275–298.
49. Nouioui, I., Carro, L., Garcia-Lopez, M., Meier-Kolthoff, J. P., Woyke, T., Kyrpides, N. C., et al. (2018). Genome-based taxonomic classification of the phylum *Actinobacteria*. *Front. Microbiol.* 9:2007. doi: 10.3389/fmicb.2018.02007
50. Oksanen J, Blanchet FG, Kindt R et al. 2016. Vegan: Community Ecology Package.
51. Parada AE, Needham DM, Fuhrman JA. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol 2016;18: 1403-14.
52. Pruesse E, Peplies J, Glockner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 2012;28: 1823-9.
53. Ribicic D, Netzer R, Hazen TC et al. Microbial community and metagenome dynamics during biodegradation of dispersed oil reveals potential key-players in cold Norwegian seawater. Marine pollution bulletin 2018;129: 370-8.
54. Sarkar KS and Bhattacharya DB (2010) Water Quality analysis of the Coastal Region of Sunderban Mangrove Wetland, India, and Using Multivariate Statistical Techniques. Sciyo, Croatia, pp 258..
55. Sarkar SK, Frančišković-Bilinski S, Bhattacharya A et al. Levels of elements in the surficial estuarine sediments of the Hugli River, northeast India and their environmental implications. Environment international 2004;30: 1089-98.
56. Seo J-H, Kang I, Yang S-J, Cho J-C. 2017. Characterization of spatial distribution of the bacterial community in the South Sea of Korea. PLoS One 12:e0174159. doi:10.1371/journal.pone.0174159
57. Simon M, Scheuner C, Meier-Kolthoff JP et al. Phylogenomics of Rhodobacteraceae reveals evolutionary adaptation to marine and non-marine habitats. The ISME journal 2017;11: 1483.
58. Spring S, Scheuner C, Göker M et al. A taxonomic framework for emerging groups of ecologically important marine gammaproteobacteria based on the reconstruction of evolutionary relationships using genome-scale data. Frontiers in microbiology 2015;6: 281.
59. Tanaka D, Tanaka S, Yamashiro Y et al. Distribution of oil-degrading bacteria in coastal seawater, Toyama Bay, Japan. Environmental Toxicology: An International Journal 2008;23: 563-9.
60. Teeling H, Fuchs BM, Becher D et al. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science 2012;336: 608-11.

61. Yilmaz P, Yarza P, Rapp JZ et al. Expanding the world of marine bacterial and archaeal clades. Frontiers in microbiology 2016;6: 1524.
62. Zhang J, Kobert K, Flouri T et al. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 2014;30: 614-20.
63. Zhou J, Richlen ML, Sehein TR et al. Microbial community structure and associations during a marine dinoflagellate bloom. Frontiers in microbiology 2018;9.

562 Table 1: Environmental conditions in Indian Sundarban mangroves and details of the sampling sites.

| Station id | Site Id | GPS (DD COORDINATES) | Sample ID | Salinity | pH | DOC (µM ) | TN ( µM ) | NOx (µM) | $NO_3$ (µM) | DIN (µM) | $PO_4$ (µM) | Si (µM) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Open Marine Water (OMW) | TH 2 | N 21.82389 E 88.50585 | TH2.5 | 26.6 | 8.2 | 112.6 | 12.5 | 1 | 1 | 1 | 0.4 | 11.7 |
| | | | TH2.2 | 27.0 | 8.2 | 111 | 11.4 | 1.4 | 1.4 | 1.5 | 0.2 | 13.1 |
| | | | TH2.3 | 25.7 | 8.3 | 66.2 | 6.4 | 0 | 0 | 0 | 0.1 | 7.7 |
| | KL 2 | N 21.8172 E 88.53658 | KL2.3 | 26.6 | 8.3 | 129.5 | 13.6 | 2.6 | 2.5 | 2.6 | 0.2 | 14.3 |
| | | | KL2.6 | 26.9 | 8.1 | 162.4 | 10 | 1.2 | 1.2 | 1.3 | 0.1 | 26.3 |
| | | | KL2.1 | 26.3 | 8.2 | 116.3 | 12.1 | 1.5 | 1.4 | 1.5 | 0.6 | 15.9 |
| | BL 1 | N 21.78962 E 88.50534 | BL1.6 | 26.8 | 8.5 | 121.1 | 14.8 | 1 | 1 | 1.1 | 0.2 | 16.3 |
| | | | BL1.5 | 26.9 | 8.4 | 114.1 | 13.2 | 1.7 | 1.7 | 1.8 | 0.2 | 12.6 |
| | | | BL1.2 | 26.5 | 8.7 | 111.4 | 12.7 | 2.3 | 2.2 | 2.3 | 0.2 | 16.1 |
| Island of Sundarban Mangroves (ISM) | KL 1 | N 21.85189 E 88.51168 | KL1.3 | 23.6 | 8.3 | 56.1 | 4.4 | 0.2 | 0.2 | 0.3 | 0.1 | 6.2 |
| | | | KL1.6 | 22.9 | 8.0 | 58 | 6 | 0.9 | 0.8 | 0.9 | 0.1 | 6.1 |
| | | | KL1.2 | 24.3 | 8.1 | 62 | 5.1 | 0.2 | 0.2 | 0.2 | 0.1 | 6.5 |
| | KP 1 | N 21.85604 E 88.51191 | KP1.6 | 25.8 | 8.1 | 78.5 | 5.7 | 0 | 0 | 0.1 | 0.1 | 12.2 |
| | | | KP1.1 | 25.3 | 8.1 | 92.5 | 9 | 0 | 0 | 0.1 | 0.2 | 15.4 |
| | | | KP1.3 | 24.9 | 8.1 | 81.2 | 7 | 0.4 | 0.4 | 0.5 | 0.1 | 12.3 |
| | TH1 | N 21.85706 E 88.51638 | TH1.4 | 21.5 | 8.3 | 91.9 | 8.3 | 0 | 0 | 0.1 | 0.2 | 8.7 |
| | | | TH1.3 | 21.8 | 8.3 | 86.8 | 8.9 | 0.1 | 0 | 0.1 | 0.1 | 8.7 |
| | | | TH1.2 | 21.2 | 8.3 | 84.8 | 7.4 | 0.6 | 0.6 | 0.6 | 0.1 | 9.5 |

563

564

565 Table 2: Contribution of environmental parameters including nutrient content of six sampling sites to
566 explaining the variation in microbial community composition based on redundancy analysis (RDA).

567

| Sampling station | Explanatory variable | Adjusted $R^2$ | F | df | p-Value |
|---|---|---|---|---|---|
| OMW | TN + DIN | 6 % | 1.24 | 2, 6 | 0.022* |
| | TN | 4 % | 1.34 | 1, 7 | 0.029* |
| | DIN | - | 1.40 | 1, 7 | 0.125 |
| ISM | Salinity + TN | 7% | 1.31 | 2,6 | 0.043* |
| | Salinity | 9% | 1.77 | 1,7 | 0.004** |
| | TN | 0.6% | 1.05 | 1,7 | 0.326 |

568

569

570

571 p-Values defined as significant at a threshold of 0.05 are highlighted in asterisks mark,
572 Adjusted $R^2$ are provided as goodness-of-fit metrics
573 df degrees of freedom (numerator, denominator).
574

575

576 **Figure legends:**

577 Figure 1: Map of the sampling area: water samples were collected from three stations (KL 1, KP
578 1 and TH1) of a small Island are named as ISM and open marine water samples named as OWM
579 (TH 2, KL 2 and BL 1). Three biological replicates from each of the six stations; therefore, total
580 eighteen (18) samples (nine from ISM and another nine from OMW) were collected and further
581 processed for this investigation.

582 Figure 2: Principal component analysis (PCA) to ordinate the eighteen collected water samples
583 collected samples from ISM and OMW based on their environmental parameters. The arrows
584 show the direction of the environmental parameters. DIN, dissolved inorganic nitrogen; TN, total
585 nitrogen; DOC, dissolved organic carbon.

586 Figure 3: Alpha diversity of the water microbial community at two different sites (ISM and
587 OMW) of Sundarban mangrove forest areas. Values are calculated based on repeated random
588 subsampling to the lowest number of sequences per sample. The median per group presented in
589 black line.

590 Figure 4: Taxonomic composition of dominant bacterial taxa on family level across eighteen
591 samples under sites ISM and OWM (nine samples each). Ten (10) most abundant bacterial
592 families for each of the samples were reported here and rests less dominant members are label as
593 "other".

594 Figure 5: Taxonomic compositions of dominate archaeal phyla across eighteen samples
595 represents two sites ISM and OWM (nine samples each). Ten (10) most abundant phyla for each
596 of the samples were reported here and rests less dominant members are label as "other"

597 Figure 6: Non-metric multidimensional scaling (NMDS) plot of bacterial community
598 composition of the bacterial communities of each sampled at the inhabited island (ISM) and open
599 marine areas (OWM).

600 **Supporting Information:**

601 Figure S1: Heatmap of pairwise correlations between the different environmental parameters and
602 three principal components. Levels of correlations are indicated with different color bar.

603 Figure S2: The rarefaction curve of the eighteen (18) samples, indicated by the number of OTUs
604 as a function of the number of reads. The curve approaching plateau indicates that the number of
605 reads are enough to describe the OTUs representing the community.

606 Figure S3: Taxonomic composition of the ten (10) most abundant bacterial phyla in the studied
607 two sites ISM and OWM represented by eighteen samples (nine samples each).

608 Figure S4: Taxonomic composition of the ten (10) most abundant archaeal genus in the studied
609 two sites ISM and OWM represented by eighteen samples (nine samples each)

610 Table S1: Kruskal-Wallis test for Environmental parameters at the six sampling sites

611 Table S2: Kruskal-Wallis test for alpha diversity of the six sampling sites.

612

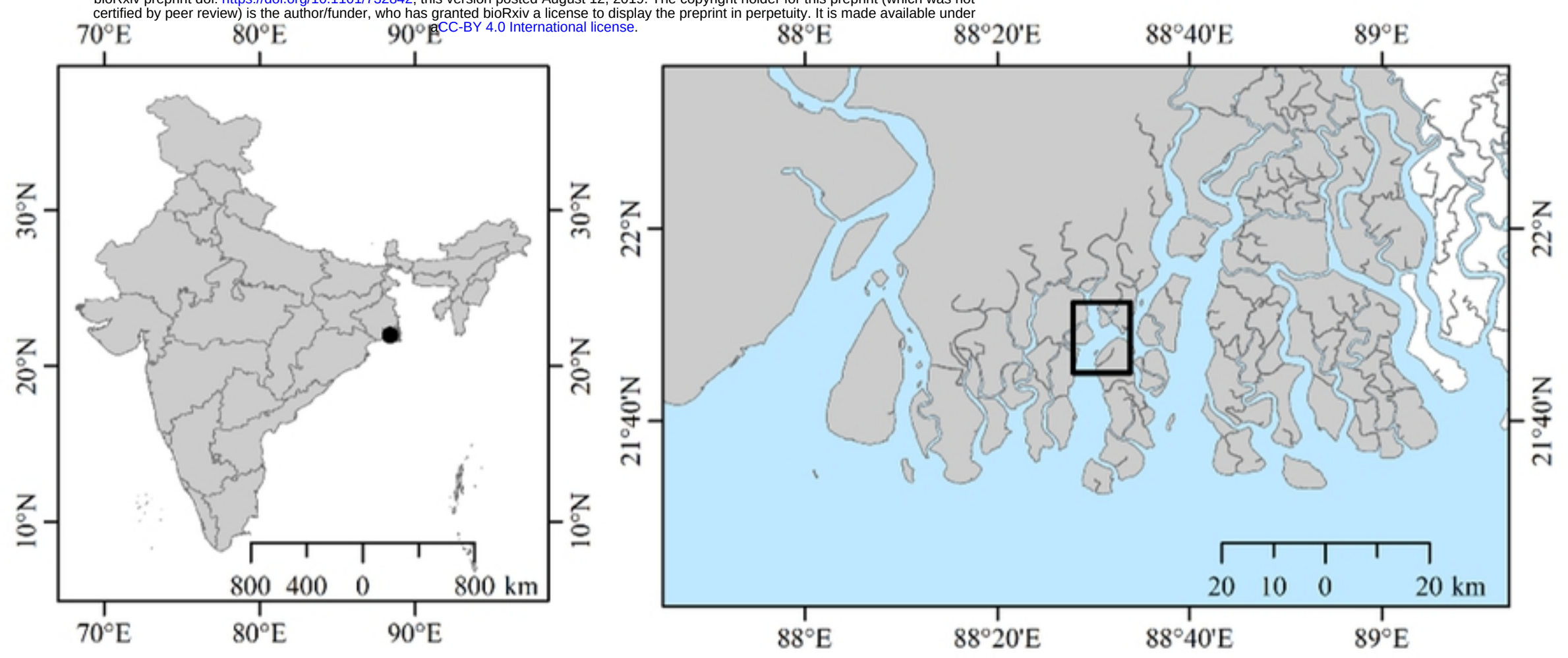



Figure 1

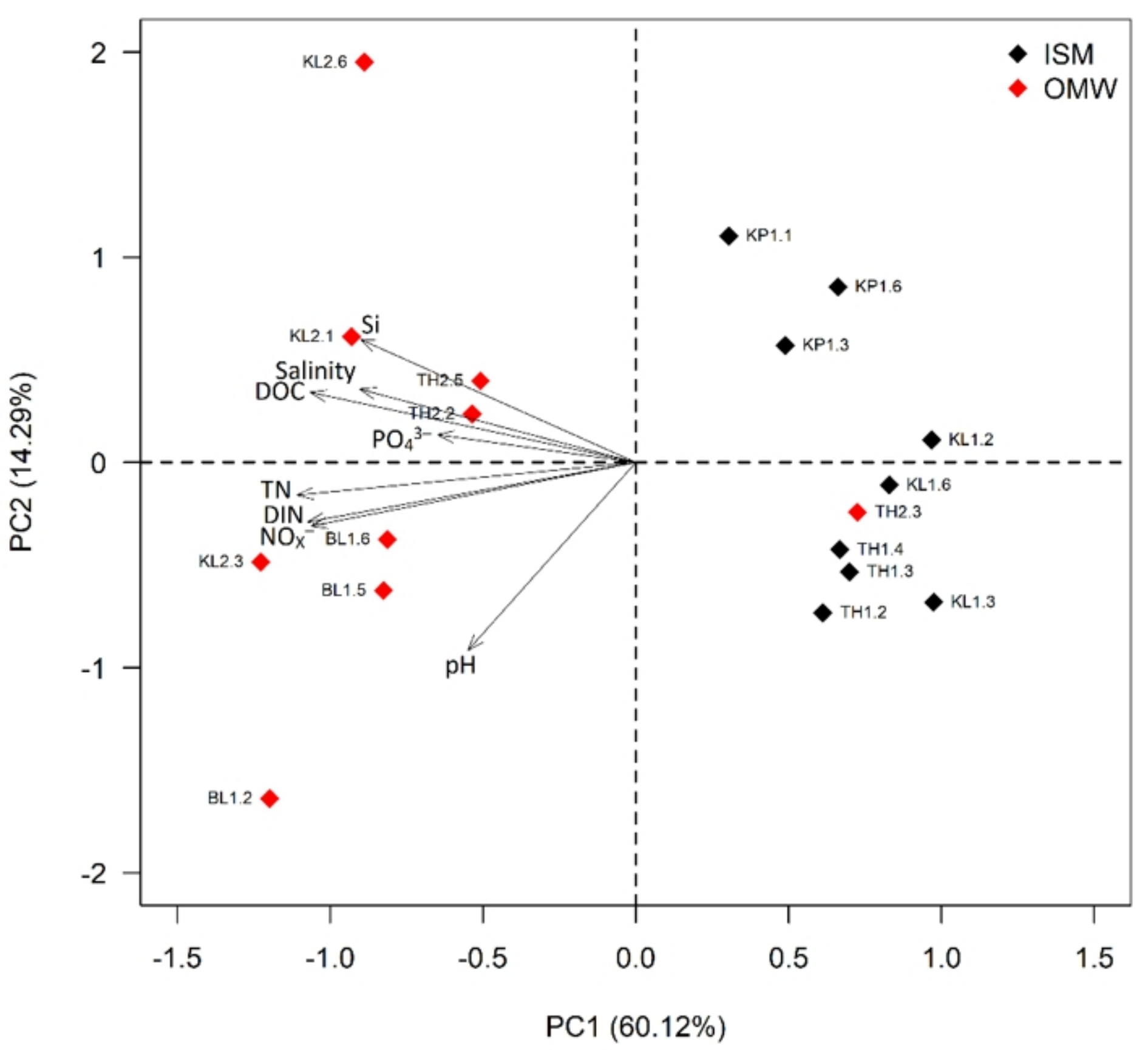
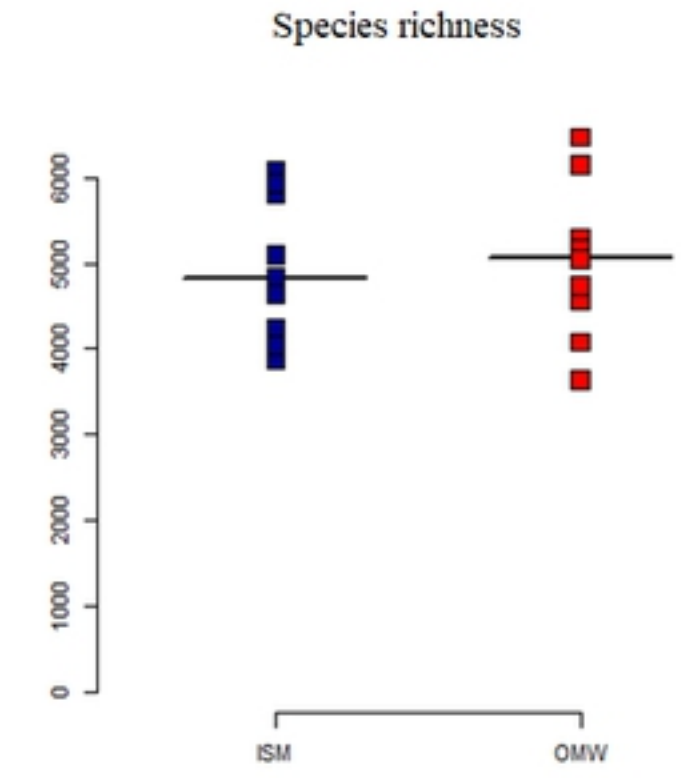
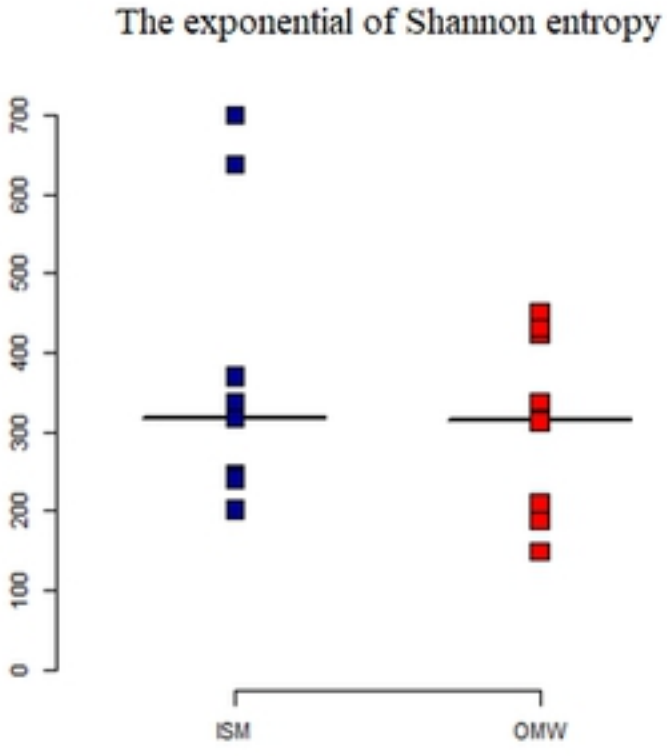

ISM
OMW
KL2.6
KP1.1
KP1.6
KP1.3
KL2.1
Si
Salinity
DOC
TH2.5
TH2.2
$PO_4^{3-}$
KL1.2
TN
DIN
$NO_X^-$
BL1.6
KL1.6
TH2.3
KL2.3
TH1.4
TH1.3
BL1.5
KL1.3
TH1.2
pH
BL1.2
PC2 (14.29%)
PC1 (60.12%)
2
1
0
-1
-2
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5

Figure 2


Species richness
0
1000
2000
3000
4000
5000
6000
ISM
OMW


The exponential of Shannon entropy
0
100
200
300
400
500
600
700
ISM
OMW

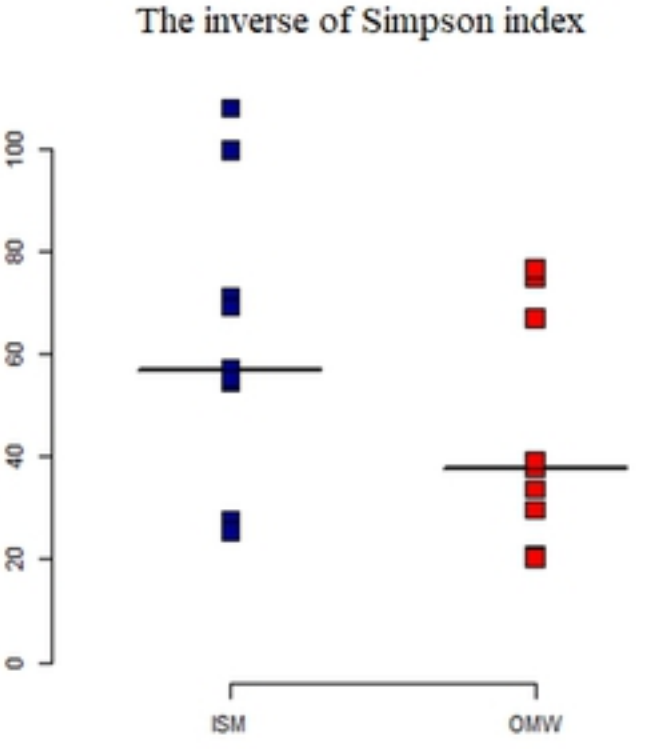

The inverse of Simpson index
0
20
40
60
80
100
ISM
OMW

Figure 3

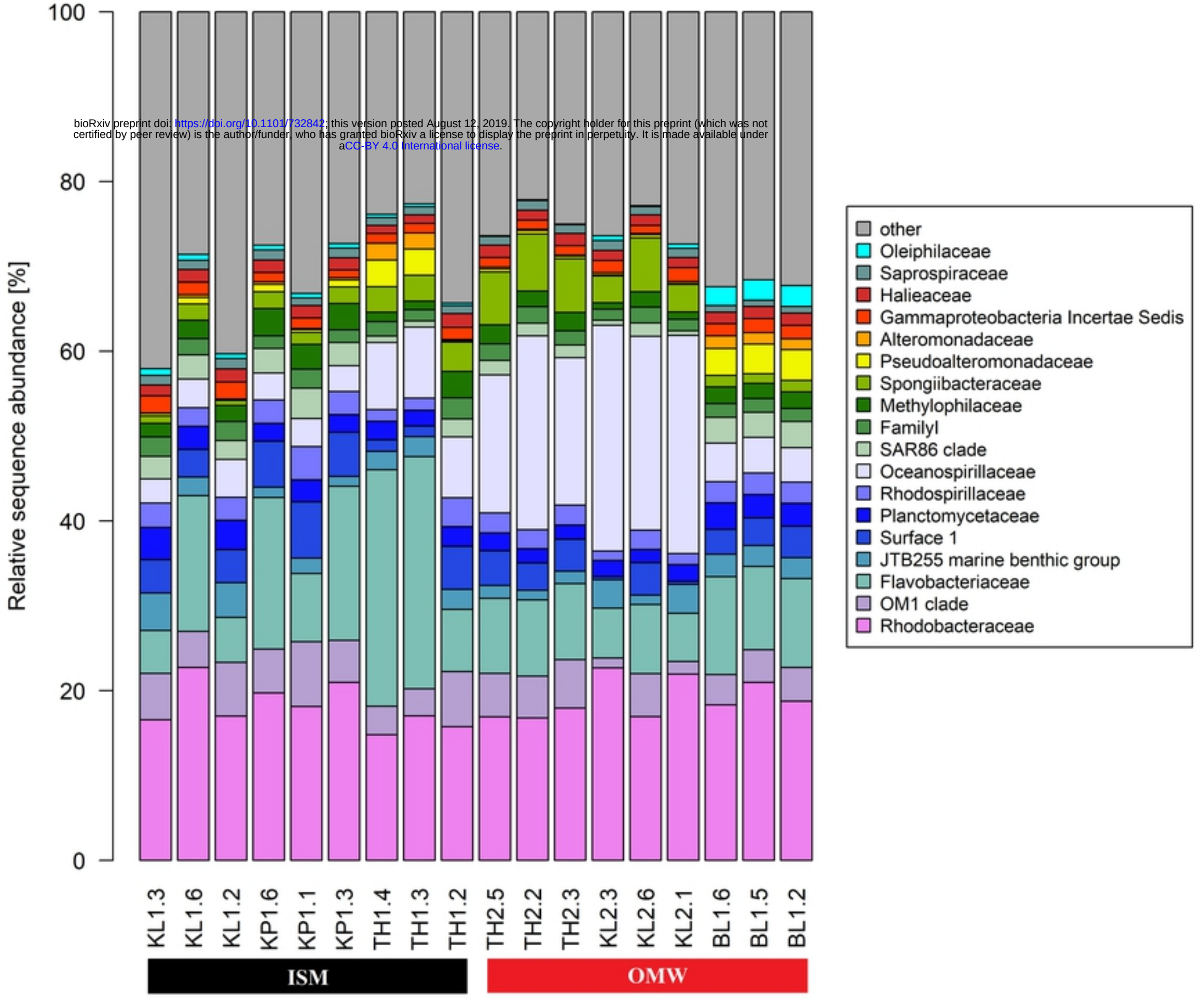



Figure 4

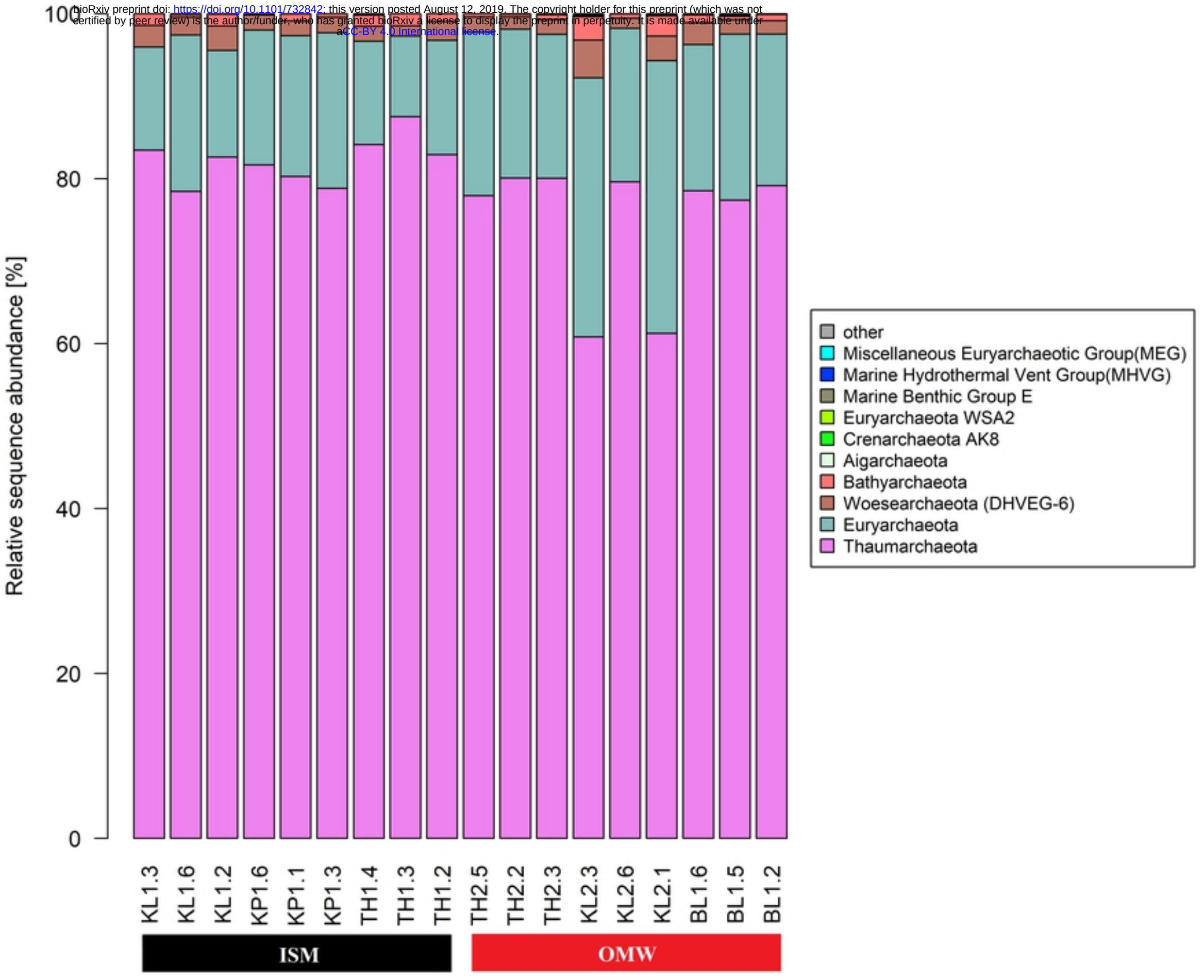



Figure 5

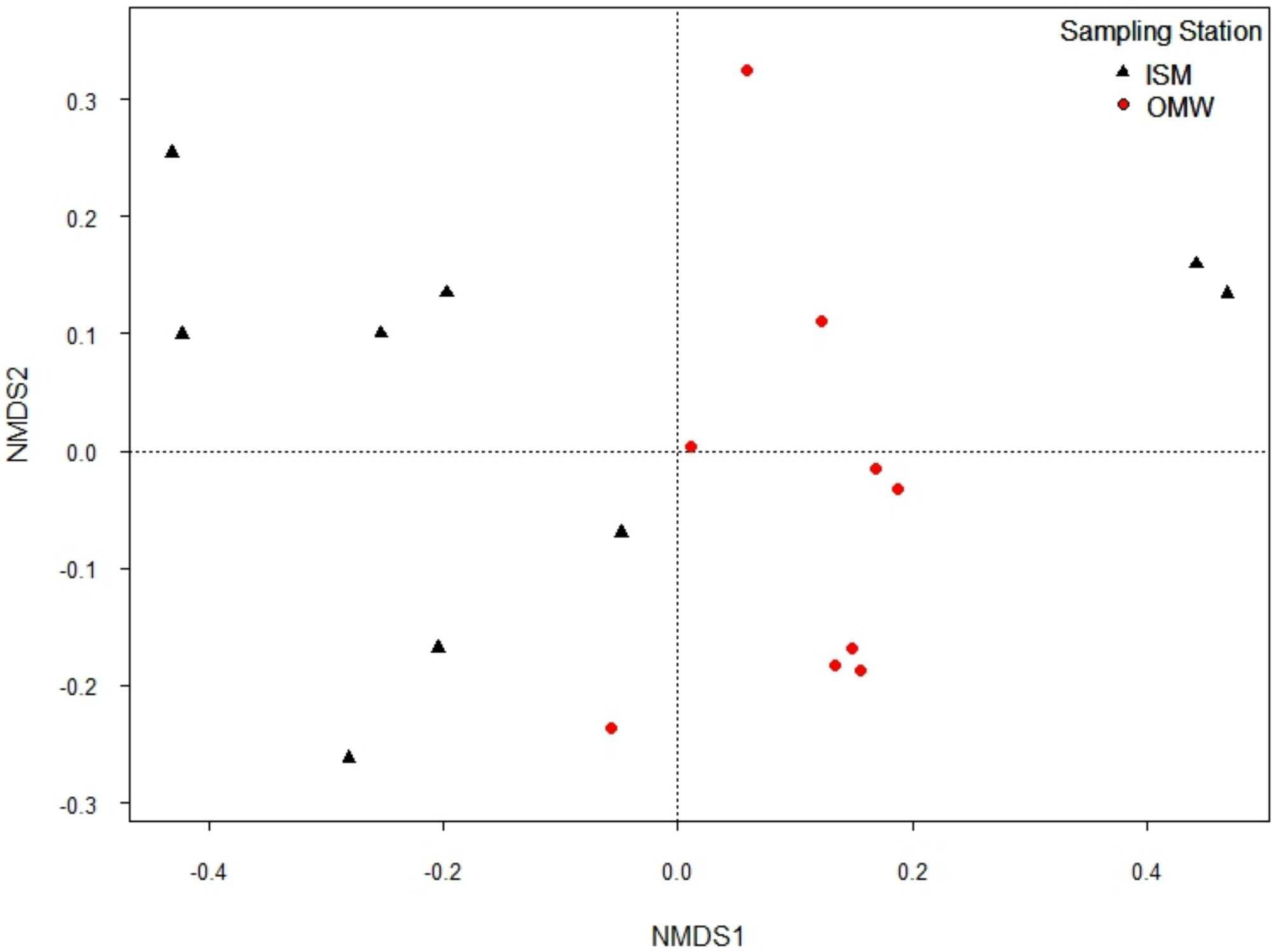

Sampling Station
ISM
OMW
NMDS2
NMDS1
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.2
0.0
0.2
0.4

Figure 6