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Legacy of a Pleistocene bacterial community: Patterns in community dynamics through changing ecosystems

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ABSTRACT

Bacterial communities are resilient to the environmental changes, yet the effect of long term ecological changes on bacterial communities remain poorly explored. To study the effect of prolonged environmental changes, a 25 m long sediment core was excavated from a paleo beach ridge located on the Cauvery delta, south east coast of India. Geological evidences suggested that the site has experienced multiple marine transgressions and regressions. The three paleosols from Vettaikaraniruppu (VKI) beach ridge, VKI-2 (2.8 m bgl; 3 kybp), VKI-5 (7.2 m bgl; 6 kybp) and VKI-14 (24.5 m bgl; 146 kybp) was chosen for bacterial community analysis based on their formation period. Bacterial community structure of paleosols was reconstructed using V3 hypervariable region of bacterial 16S rDNA targeted Illumina sequencing. The VKI-5 sediment layer which formed under marine environment contained highest bacterial diversity, and the community was a mix up of terrestrial and marine bacterial population. The final community VKI-2 exhibited an approximate structural pattern witnessed in the native bacterial community VKI-14 which formed during marine regression. Furthermore, marine transgression and regression experienced in VKI resulted in the formation of distinct biogeographic patterns.

1. Introduction

Bacteria play a major role in climate change caused by feedback responses and in turn, controlled by climate changes (Judd et al., 2006; Singh et al., 2010; Bailey et al., 2018). The climate change may enhance insignificant bacterial processes which alter ecosystem stability (Allison and Martiny, 2008). Changes in physico-chemical components of the ecosystem alters bacterial community composition and structure over the time and induces the formation of biogeographical patterns (Martiny et al., 2006; Andam et al., 2016; Hanson et al., 2016). These biogeographical patterns can be considered as an index of historical environmental changes. Even with increasing the number of microbiome research, bacterial response to future climate changes remains an enigma (Salazar and Sunagawa, 2017). To understand the effect of environmental changes on bacterial communities and to predict their responses that occur in future, it is essential to collect enough data on bacterial community responses related to the past environmental changes. Many recent studies analyzed the bacterial community responses in stimulated environments, but most of these studies considered only a single environmental parameter (eg: temperature, pH) and that too for shorter time periods (Liu et al., 2013). For accurate predictions, bacterial responses should be obtained from real scenarios happened over longer time scales. Sediment layers formed under different environmental conditions get imprinted by bacterial community structure existed at the time of burial (Inagaki et al., 2005; Vuillemin et al., 2014). Reconstruction of bacterial community structure from various sediment layers can be used to delineate the bacterial community response to the changing environments. Molecular tools and next generation sequencing with increased resolving power made reconstruction of bacterial communities from millions of year old sediments more accurately (Forschner et al., 2009; Grund et al., 2014; Epure et al., 2017). Environmental changes often result from global climate variations. One of the most prominent effects of global climate change is sea-level fluctuations, which affects the coastal ecosystem. Marine transgressions and regressions are real scenarios to identify the effect of varying environmental parameters on bacterial community. Effect of sea-level rise on coastal bacterial communities is not yet characterized.

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Further, bacterial biogeographic patterns are widely studied across the globe and it is a fact that bacterial communities differ across the geographical space. The factors responsible for varying biogeographical patterns are identified as dispersal limitation, mutation, and speciation (Martiny et al., 2006). The present study opens a window to study the effect of sea-level fluctuations on bacterial biogeographical patterns.

Cauvery delta which is located on the south east coast of India experienced marine transgressions and regressions in the past. The delta region, especially the region between Point Calimere and Nagapattinam contains many discontinuous, wide, successive beach ridges, varying in width from few meters to several tens of meters. These ridges are considered as the remnants of palaeo-strandlines formed during late Holocene sea-level high stand (Alappat et al., 2010, 2011). Paleo beach ridges are indicators of paleo sea-levels. Previous studies (Prabakaran and Anbarasu, 2010; Prabakaran, 2011) describing different beach ridges on the Cauvery delta at varying distances from the present day shore, is the evidence that the delta was once under the sea.

For the present study, samples were collected from a sediment core excavated at a paleo beach ridge which is situated 2.6 km inland. The sediment layer formed during marine transgression was identified using geological tools. The bacterial communities of sediment layer formed under the marine influence were compared with that of sediment layers formed before and after marine transgression. The comparisons were carried out with the following objectives: (i) to check the bacterial community dynamics after marine transgression and regression, (ii) to find out the effect of marine transgression on native bacterial community and (iii) to check whether final bacterial community bears any traces of niche change.

2. Materials and methods

A paleo beach ridge located ~2.6 km from the present day shore was selected as the study site. The site was primarily located using Geomorphology maps. The precise location which was situated at Vettaikaraniruppu village (VKI: 10.553467 N, 79.835450 E) on Cauvery delta, Tamil Nadu, IN (Fig. 1) has been found through satellite images and Global Positioning System. A sediment core (63 mm diameter) was collected upto 25 m depth through calyx core drilling. The cores were immediately transferred to the lab. The cores were split into half, one-half was kept for geological studies and subsamples for bacterial community analysis were collected at an equal interval from the other half of the core and stored at -80^{0}C till further studies.

To identify the sediment layers formed under marine conditions, the sediment layers were searched for Foraminifers, which is a conventional geological proxy for paleo sea-level reconstructions. The foraminifers were separated from sediments by following Cushman's (1959) method. The age of the sediment layers were detected using Optically Stimulated Luminescence (OSL) dating, following the Single Aliquot Regeneration dose protocol described by Murray and Wintle (2000). Based on the OSL and Micropaleontological result, three sediment layers: sediment layer formed before marine transgression at 24.5 m depth (VKI-14), formed during transgression at 7.2 m depth (VKI-5) and formed after transgression at 2.8 m depth (VKI-2), were selected for bacterial community analysis. In the present study, VKI-14 was considered as native population and VKI-2 was considered as the final bacterial community.

The sample name "VKI" stands for Vettaikaraniruppu from where the samples were collected and the number 2, 5 and 14 stand for the core tube number from which the subsamples were retrieved. Samples were collected and processed at the same time to minimize the errors. Paleo Environmental DNA (PalEnDNA) was isolated from 0.5gm of each sediment by CTAB method (Thomas et al., 2018). PalEnDNA was purified and quantified. V3 region of bacterial 16S rDNA was amplified from community DNA using specific primers 341 F and 518R (Hussain et al., 2016; Gaikwad et al., 2017). The library was prepared from amplified V3 regions using Illumina 16S V3 library preparation kit. The prepared library was quantified using Qubit fluorometer and was checked on Agilent D1000 Tape station for size distribution. Sequencing was carried out at on Illumina NextSeq 500 sequencer at Genotypic Technology Pvt. Ltd. Bengaluru, IN. All experiments contained negative controls to check contamination.

Low-quality reads were discarded and demultiplexed. The paired end reads were merged in QIIME 1.9.1 (Caporaso et al., 2010; Kuczynski et al., 2011) and uclust, a closed reference OTU picking method at 97% similarity (Edgar, 2010). Taxonomy assignment was carried out in QIIME pipeline using Greengenes database (May 2013 release). This method also enabled the removal of chimeric sequences and increased the probability of assigning reads to valid bacteria (DeSantis et al., 2006; McDonald et al., 2012). Few hits assigned to archaea, chloroplast, and mitochondrial genes were removed for further analysis. The OTU table was rarefied according to the sample with the lowest number of sequences. Alpha and beta diversity indices were calculated using the filtered OTU Table. Rarefaction curves, diversity indices were created in QIIME and plotted using R (R core team, 2014). Further analysis was carried out using the filtered rarefied OTU table.

To analyze the variation among the bacterial communities and to determine the biogeographical patterns formed in VKI sediment layers due to marine transgression, Beta diversity analyses: a) Bray-Curtis dissimilarity index and b) Weighted Unifrac distance were used and calculated using QIIME. The distance matrices were calculated using UPGMA based clustering and was visualized in Figtree (Rambaut, 2006). The clusters were used to interpret biogeographical patterns. A OTU network map was constructed to identify the distribution of OTUs across the samples. For visualization a new OTU table was constructed by filtering out the OTUs representing less than 100 sequences from the rarefied OTU table, and these rare OTUs were discarded from network construction. The node and edge tables were constructed by *make_otu_network.py* script in QIIME. The network map was visualized using Cytoscape Version 3.2.0 (Shannon et al., 2003; Pope et al., 2012). Functional Diversity of the samples was analyzed using Faprotax ver 1.1 (Louca et al., 2016a, b; Schiff et al., 2017). A heat map of selected functions was constructed in R. Paired end Illumina sequence data used in this study are available in NCBI Sequence Read Archive (SRA) under the accession number SRA505627.

3. Results

3.1. Geology of the samples

The Beach ridge explored in the study was located 2.6 km inland from present day shore and the elevation was 7 m from mean sea-level (MSL). The location and geomorphological features of the sampling location are illustrated in Fig. 1 and Supplementary file 1. Micropaleontological and OSL studies show that there was a 3000-year long marine transgression occurred at the study site. The sediments formed between the marine transgression of 9 kybp and 6 kybp started from 10.9 m depth and lasted till 5.6 m depth respectively. OSL dating revealed that the bottom sample VKI-14 collected at 24.5 m depth was formed 146.64 ± 36.81 kybp consisted compactly packed yellow and grey mixed clayey sand. The middle sample VKI-5 collected at 7.2 m depth was formed 6.04 ± 1.25 kybp consisted black compact clay. The top sample, VKI-2 collected from 2.6 m depth was formed 3.36 ± 0.42 kybp consisted silty sand. The presence of foraminifers' fossil (Supplementary file 2) confirmed that the VKI-5 sediments formed under marine environment. No trace of foraminifera was observed in VKI-14 and VKI-2.

3.2. Sequence yield and diversity indices

The PalEnDNA obtained for VKI-14, VKI-5 and VKI-2 was 345, 72 and 401 ng/0.5 mg of the soil samples respectively. The 16S rDNA sequences obtained from VKI-14, VKI-5, and VKI-2 were 808688, 340931

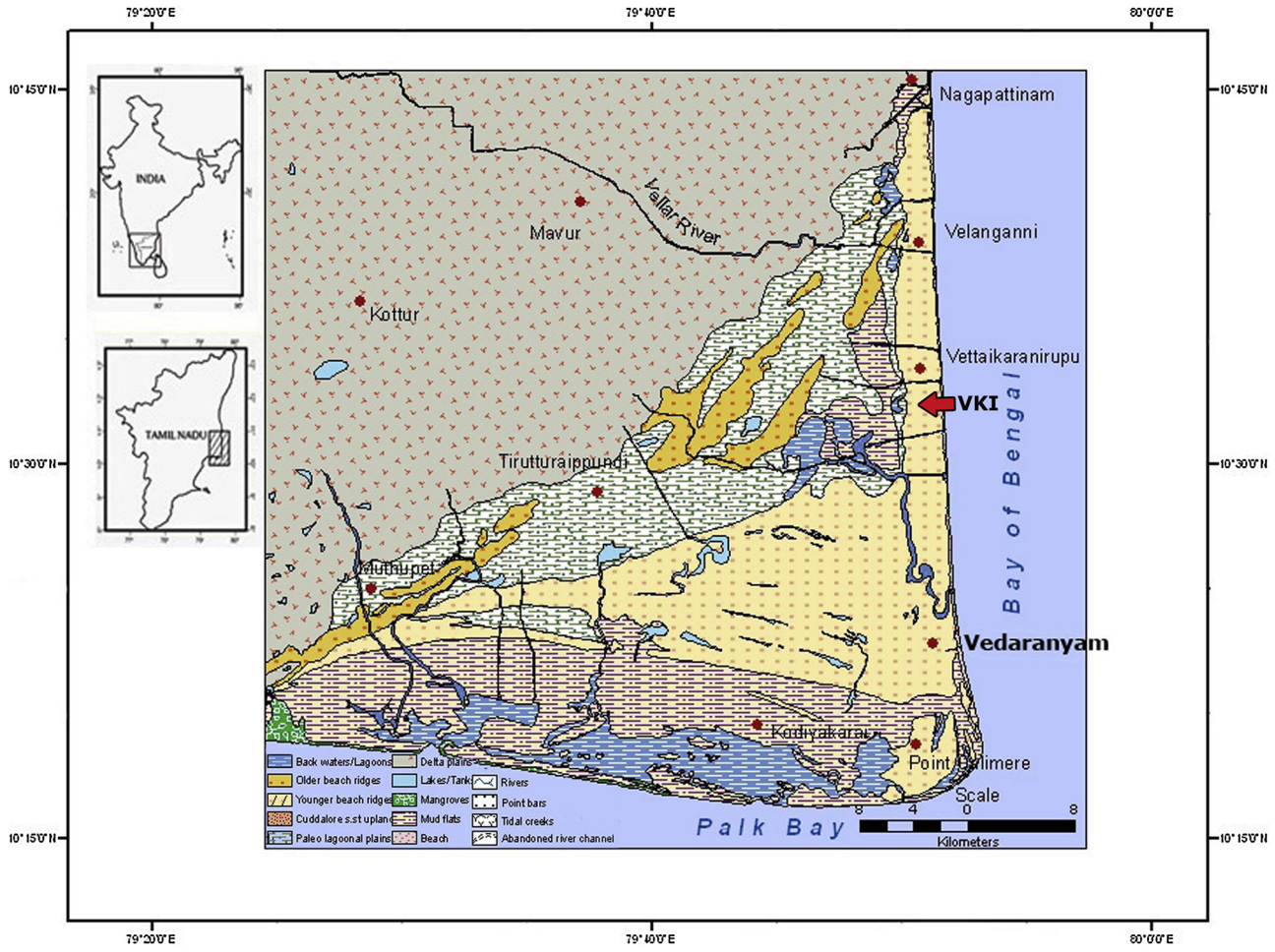

Fig. 1. Sample location (VKI).

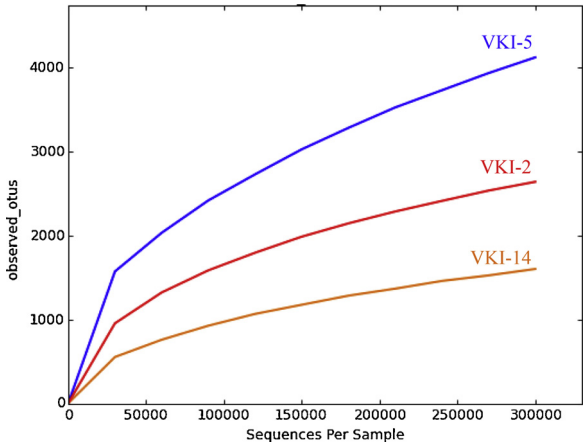

Fig. 2. Rarefaction curves of the VKI samples.

and 725902 respectively. The sequences belonged to chloroplast, mitochondria and Archaea were removed from the OTU table and rarefied according to the least number of sequences observed in the samples (ie. VKI-5: 337128; Supplementary file 3 and 4). The resulting OTU table contained 1654, 4296 and 2769 OTUs for VKI-14, VKI-5 and VKI-2 respectively (Supplementary file 5). The rarefaction curves (Fig. 2) for VKI-14 and VKI-2 reached near plateau and leveled off but the VKI-5 sample showed a steep curve towards right and did not level off as a plateau, indicating that intense sampling could have yielded more diversity.

The Good's coverage indicated that the overall diversity of the samples was well represented (Fig. 3). The Shannon and Simpson reciprocal diversity indices revealed that VKI-5 sample had more species evenness and bacterial diversity when compared to VKI-14 and VKI-2 (Fig. 3). The VKI-5 community was also phylogenetically diverse than the other two communities as showed by Faith's Phylogenetic Distance (Fig. 3).

3.3. Bacterial community structure

The bottom sample VKI-14 considered to be the native population consisted 11 bacterial phyla. *Proteobacteria* constituted 67.55% of the total bacterial community, followed by *Firmicutes* (12.88%), *Bacteroidetes* had a relative abundance (11.03%) closer to *Firmicutes* and *Actinobacteria* constituted 5.19% of total population. Approximately 96% of total population was formed by these four phyla - *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Actinobacteria* (Fig. 4 and Krona charts in Supplementary files: 6a–c). Other bacterial phyla found at VKI-14 were *Cyanobacteria, Verrucomicrobia, Chloroflexi, Nitrospira, Elusimicrobia, OD1*, and *Spirochaetes.*

The bacterial diversity at VKI-5 was much higher than VKI-14. 25 new phyla were appeared in VKI-5, increasing the total of phyla to 37. Unlike VKI-14, VKI-5 showed an even distribution of dominant phyla. *Proteobacteria* (34.22%), *Firmicutes* (29.05%) and *Bacteroidetes* (24.94%) were the top three abundant phyla in VKI-5. Out of the 37 phyla, 14 were candidate phyla in VKI-5. *Firmicutes* dominated *Bacteroidetes* at VKI-14, whereas in VKI-5 *Bacteroidetes* dominated *Firmicutes*. The abundance of *Actinobacteria* at VKI-5 decreased to 3.9%

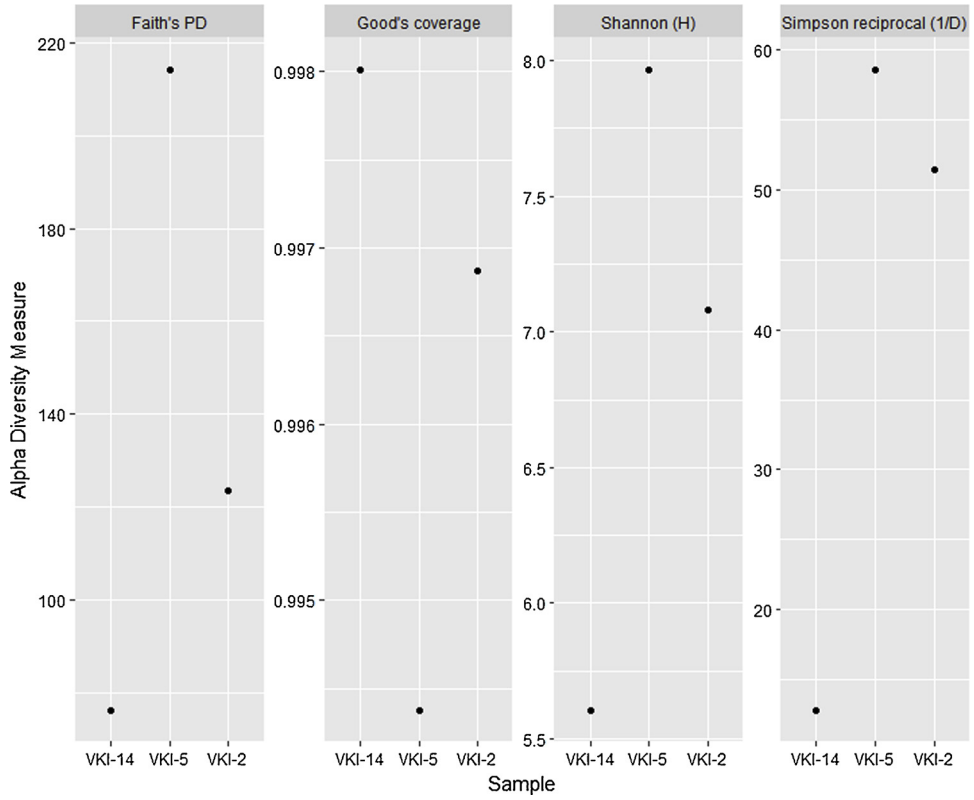

Fig. 3. Alpha diversity indices of the VKI samples.

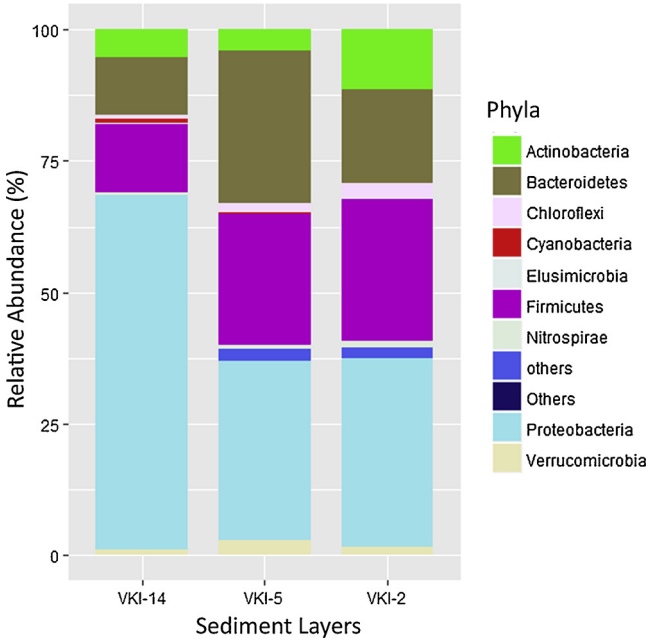

Fig. 4. Relative abundances of bacterial phyla of the VKI samples.

from 5.19%. Out of 11 phyla at VKI-14, four phyla (*Proteobacteria, Actinobacteria, Cyanobacteria and Elusimicrobia*) showed a decreasing trend at VKI-5.

VKI-2, the youngest sample, formed after marine transgression, lacked 18 phyla of VKI-5 making total number of phyla to 19. *Proteobacteria* (35.76%) and *Firmicutes* (27.21%) population increased in VKI-2 and the *Firmicutes* dominated *Bacteroidetes* (17.86%). *Actinobacteria* (11.34%) had a high rise in their population and the *Cyanobacteria* (0.01%) population was reduced. Only six candidate phyla were identified in VKI-2 and sequences of unclassified phyla were completely absent.

At order level, *Rickettsiales* dominated (25.42%) the VKI-14 sample, followed by *Rhizobiales* (10.30%), *Bacillales* (9.96%) and *Pseudomonadales* (9.68%) and these orders showed a decreased population at VKI-5 (Supplementary file 7). The dominated orders at VKI-5 were *Bacteroidales (26.31%), Clostridiales (22.44%)* and *Aeromonadales (6.28%)*. In VKI-2, the population size of *Bacteroidales* decreased to 13.73% but managed to remain as the dominating order, and *Bacillales* (11.60%), *Rhizobiales* (8.72%), and *Pseudomonadales* (8.68%) were able to attain the native population size (similar to VKI-14).

The total number of genera observed was 314, 598 and 406 for VKI-14, VKI-5, and VKI-2 respectively. *Wolbachia* was the predominant genus in VKI-14, followed by *Methylobacterium* and *Pseudomonas*. A steep decrease was observed in the relative abundance of *Wolbachia* population at VKI-5 (Supplementary file 8). The genus *Tepidiomonas* which comprised 1.5% of the total population of VKI-14 became virtually absent in VKI-5. In contrast, the genus *Prevotella*, which comprised 1.89% of VKI-14, dominated VKI-5 population with 22.42%. Out of the 25 most abundant genera of VKI-14, only *Prevotella* and *Prosethecobacter* showed an increased abundance at VKI-5. *Methylobacterium* and *Pseudomonas* which suffered a decrease in relative abundance at VKI-5, were able to restore their original structure at VKI-2. The restoration of native bacterial community structure was clearly visible at the family level (Fig. 5).

3.4. Functional diversity

The VKI-5 community showed a different functional pattern when compared to VKI-14, whereas VKI-2 community showed a functional pattern similar to VKI-5 (Fig. 6). Functional diversity analysis also revealed that some newly introduced functional traits at VKI-5 were not present in VKI-2. Chemo-heterotrophic bacteria accommodated a larger portion of the total diversity. The phototrophic bacterial diversity was

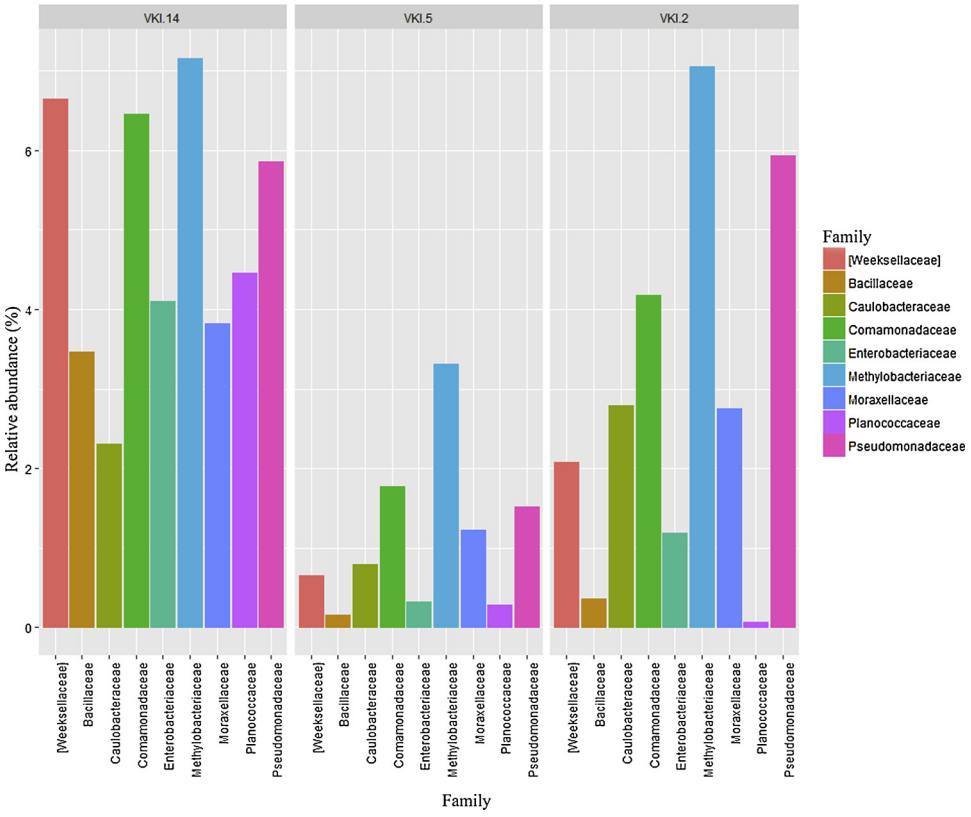

Fig. 5. Family level distribution of bacterial communities of the VKI samples.

less when compared to other trophic modes: in VKI-14, phototrophic bacteria constituted 3.9% of total diversity, but in VKI-5 their population decreased to 0.73% and their relative abundance was found to be further decreased at VKI-2. Sulfur metabolism was detected in low levels at VKI-14, but in VKI-5 it increased significantly and decreased yet again in VKI-2 (Supplementary file 9). Functions related to nitrogen cycle increased at VKI-5 and the VKI-2 population was able to maintain these functions. The VKI-5 community had a large number of symbiotic bacteria than VKI-14 and VKI-2.

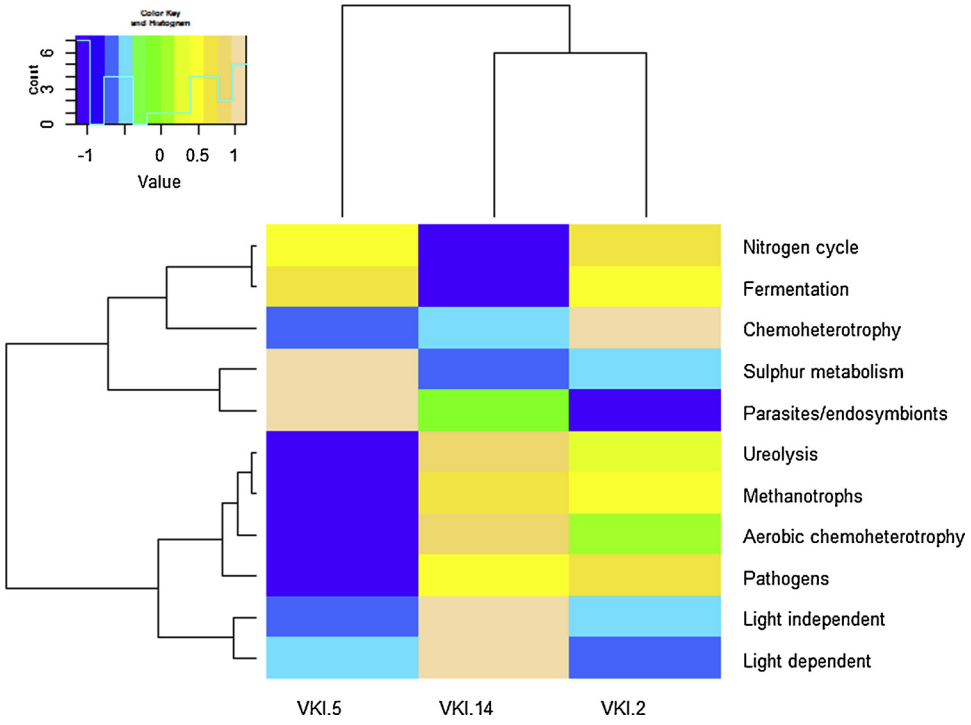

Fig. 6. Heat map depicting the distribution of major functional group of the VKI samples.

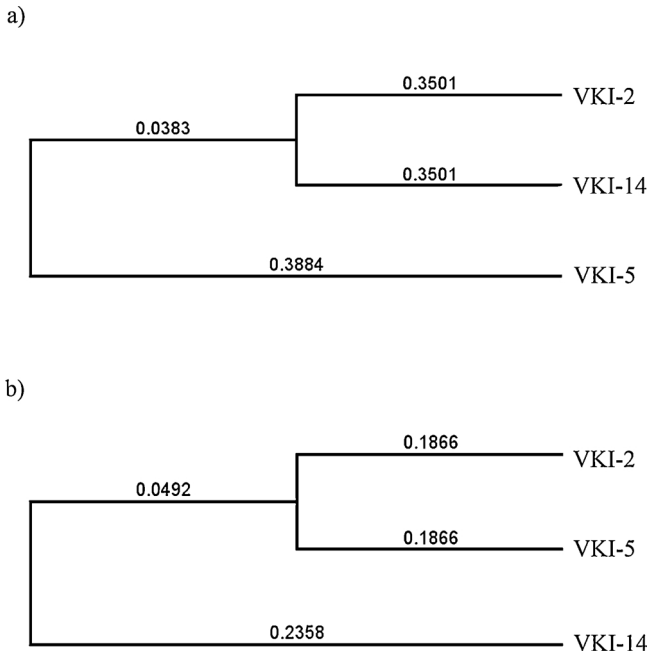

Fig. 7. Beta diversity indices of the VKI samples.
a) Clustering of VKI bacterial communities based on Bray-Curtis dissimilarity.
b) Clustering of VKI bacterial communities based on weighted Unifrac distance.

3.5. Biogeographic patterns based on Beta diversity analysis

The Bray-Curtis dissimilarity index of bacterial communities from the three VKI sediment layers showed that VKI-5 community was different from VKI-14 and VKI-2 which clustered together in hierarchical clustering analysis based on Bray-Curtis distance. The weighted Unifrac showed the clustering of VKI-5 and VKI-2 together, whereas VKI-14 diverged separately (Fig. 7). Network mapping depicted few OTUs were unique in VKI-5 and few abundant OTUs of VKI-5 were also found to be present in VKI-2 (Fig. 8). These data describes the difference between bacterial communities at the three sediment layers and thereby indicated the formation of distinct biogeographic patterns.

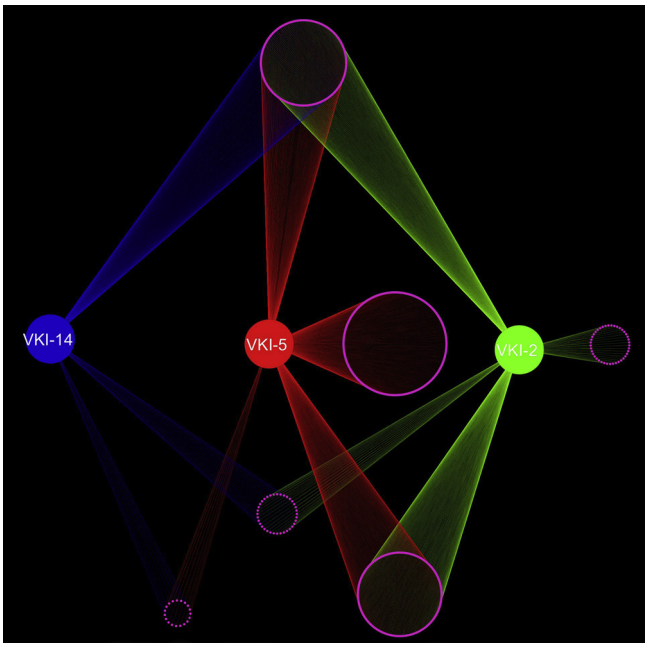

Fig. 8. Network map depicting the distribution of OTUs across VKI samples.

4. Discussion

The bacterial community response to marine transgression and regression has been illustrated in the study. Choosing paleo beach ridge as sampling location enabled the recovery of samples from both marine and non-marine environments. Rinnan et al. (2007) studied that bacterial communities are initially resistant to environmental changes or natural disturbances. Therefore, for the present study, the sediment from the marine environment was chosen at 7.2 m depth of the VKI beach ridge, as the sediment layer was formed 3000 years after marine transgression, the bacterial responses are supposed to be more prominent in this layer.

The recovery of DNA from 146,000-year-old samples confirmed the DNA preservation in silica enriched samples. The VKI-14 and VKI-2 samples contained 76–78% silica, where in VKI-5 it was 55%. The VKI-5 sample was a clayey layer and generally, clay particles have an affinity for DNA (Cai et al., 2006) which made the separation difficult. Although the affinity of clay towards DNA, caused a reduction in DNA yield in VKI-5, it prevented the DNA leaching to lower sediments, which in turn prevented the contamination of DNA pool with relatively newer DNA. These results are in consonance with the study of Lyra et al. (2013) which stated that DNA does not migrate vertically and the affinity of DNA to clay and silica increases the stratigraphic reliability.

Bacterial community studies of sediments showed that diversity decreases with depth and age (Bowman and McCuaig, 2003; Shivaji et al., 2011; Jangid et al., 2013). Yet, VKI-5 retrieved at 7.2 m showed higher diversity than VKI-2 despite the decreased sequence yield. The Shannon and Simpson indices of VKI-5 were also higher than VKI-14. The rarefaction curve showed that more diversity was yet to be discovered in VKI-5. Therefore, the actual diversity of VKI-5 ought to be higher than the observed diversity, which is also substantiated by Good's coverage, that revealed more sequences remain to be discovered in VKI-5. The bacterial diversity and abundance are also linked with the grain size of the sediments. When the sediments contain finer particles, the surface area is higher for bacterial communities (Epure et al., 2017). The higher diversity of the clayey VKI-5 sediment also positively correlated with high organic matter content in relation to the fact, the higher the organic matter, higher the diversity. From OSL dating, the age of the VKI-5 sample was calculated as ~6kybp. After the Last Glacial Maximum (LGM; 11k years ago), the global mean temperature started to rise and a sea-level rise was observed at this time period across the globe (Bird et al., 2010). The transgression occurred at a slow pace, thereof the native and introduced bacterial communities were able to adapt to the changing environment. When combining with geological observations, the rise in diversity in VKI-5 can be attributed to marine transgression which resulted in the formation of the mixed population (native and introduced species).

The three sediment layers were dominated by *Proteobacteria, Bacteroidetes, and Firmicutes*. The fall in *Proteobacteria* population at VKI-5 and VKI-2 can be attributed to the decrease in *Wolbachia* population when compared to VKI-14. The *Cyanobacteria* population showed continuous decrease along the depth gradient. *Cyanobacteria* are planktic organisms and primary producers and are abundant in ocean waters. Their decreased population in sediments is because of predatory stress at both planktic and benthic levels. At benthic levels, the remnants of *Cyanobacteria* are utilized as food by heterotrophic organisms and that is the reason why the *Cyanobacteria* population was considerably low in VKI-5 (Vuillemin et al., 2016, 2018).

Actinobacterial population at VKI-2 was higher than that of VKI-14. While rest of the bacterial phyla showed a restoring tendency at VKI-2, *Actinobacteria* showed almost twice the relative abundance of VKI-14. This rise in actinobacterial population can also be due to marine transgression, which causes the accumulation of Actinobacterial spores in mud and sediments, where it can survive but cannot grow. After marine transgression, the accumulated spores gets revived under favorable conditions (Ranjani et al., 2016) which could be the possible

reason for the rise in relative abundance of *Actinobacteria* in VKI-2 with respect to the native bacterial community VKI-14.

High abundance of *Bacteroidetes* was recorded in Cold waters, phytoplankton blooms and in oligotrophic marine surface waters (Simon et al., 1999; Abell and Bowman, 2005; Fernandez-Gomez et al., 2013). The *Bacteroidetes* especially *Prevotella* are capable of breaking down alginate, a compound produced by marine brown algae abundantly present in the coastal environment and due to this *Prevotella* was found abundant in VKI-5. The alginate utilization capacity is an adaptive advantage in coastal ecosystem as well as human gut. (Ramnani et al., 2012; Thomas et al., 2012; Yang et al., 2015). This capability of *Bacteroidetes* especially *Prevotella*, explains their abundance in VKI-5.

The predominant candidate phyla *OD1* (also referred as *Parcubacteria*) are assumed to be symbionts. They are widely spread in anoxic marine and terrestrial habitats enriched with sulphur. *OD1* is suspected to survive in partnership with *TM7* which also isolated from a wide variety of habitats. *TM7* was virtually absent in VKI-2 and relative abundance of *OD1* decreased slightly in VKI-5 and it was not possible to predict whether the decrease of *OD1* population was related to the absence of *TM7*.

The VKI-14 sample was dominated by the *Wolbachia* genus. *Wolbachia* are insect symbionts and present in sand flies. When considering the sandy lithology of VKI-14, the increased population of *Wolbachia*, might be the remnants of a sand fly population and not a part of the soil population, which correlates with the studies of Roy and Harry (2007); Azpurua et al. (2010); Tanganelli et al. (2014). The dramatic decrease of *Wolbachia* population in VKI-5 with respect to VKI-14 supports this conclusion. The other dominated genera of VKI-14 (*Methylobacterium*, *Pseudomonas*, and *Wautersiella*) also showed a decreased relative abundance in VKI-5, but in VKI-2 their relative abundance was almost restored. This shows the resilience of bacterial community to the original community structure once the native ecological conditions were restored Although, the native population was able to survive the niche change, their decreased population size in VKI-5 might be due to the competition with the introduced population. Since the environment changed to marine, the marine population introduced in VKI-5 had an advantage over the native community.

The VKI-5 population was predominated by endosymbiotic bacteria. Tara Ocean expeditions revealed that 73% of ocean microbiome is similar to human microbiome despite the physiological differences between them (Sunagawa et al., 2016). There is a possibility of human waste contamination on the site, but the common indicator of faecal contamination *E. coli*, was virtually absent in the VKI-5 sample. There are some studies indicating the possibility that 16S rDNA is less effective in differentiating *E. coli* and *Shigella* (Garcia-Mazcorro et al., 2017). But *Shigella* was present in fewer values in VKI-14 and absent in VKI-5. The *Enterobacteriaceae* family constituted 4.10% of total population at VKI-14, but reduced to 0.32% at VKI-5 which eliminated the possibility of faecal contamination in VKI-5.

Studies observed an increase in symbiotic and pathogenic bacteria in coastal waters during increased Sea Surface Temperature (SST). The sea-level at VKI-5 was higher than the present level, and indicated that the temperature at VKI-5 was higher than present temperature. The increased temperature was favorable for the growth of endosymbionts and pathogens which might be the possible reason for their increase at VKI-5.

Many studies concluded oxygen become scarce few centimeters below the ground level (Vuillemin and Ariztegui, 2013). In VKI-14 and VKI-2 the aerobic bacterial diversity was higher than that of anaerobic bacteria, but in VKI-5, anaerobic bacterial diversity was higher. Yet, in all the layers the dominating genera was anaerobic. Furthermore, in VKI-14 and VKI-2 the anaerobic bacteria dominated over the aerobic bacteria after sedimentation. This fluctuation in aerobic and anaerobic bacterial population can also be due to the marine transgression. VKI-14 and VKI-2 were formed under terrestrial environments obtained access to oxygen, but the VKI-5 layer formed under marine environment with anoxic conditions might have favored the growth of anaerobes. This can also be correlated with the reduction in phototrophy in VKI-5 sample, but phototrophy further reduced in VKI-2 unlike the revival of aerobic population. The methanotroph population almost remained the same in VKI-2 when compared toVKI-14, but decreased in VKI-5 due to increased precipitation and temperature as studied by Singh et al. (2010).

The VKI-5 bacterial community formed during marine transgression had a different taxonomical structure and functional composition when compared to native bacterial community. Sunagawa et al. (2015) noted that although taxonomical structure of bacteria varies with time, their functional composition remains the same. In the present study, VKI-5 showed a different functional profile when compared to VKI-14. Beta diversity analysis of bacterial communities based on Bray-Curtis and Unifrac depicted different clustering pattern. Bray-Curtis dissimilarity index which considers the abundance of individuals in a community showed clustering of VKI-14 and VKI-2. Whereas weighted Unifrac which considers both abundance and phylogenetic distance (Lozupone et al., 2010) among the communities showed clustering of VKI-5 and VKI-2. VKI-14 diverged separately since many of the OTUs which are phylogenetically distant from VKI-14 appeared in VKI-5, and many of these OTUs were able to reach VKI-2 making VKI-2 phylogenetically similar to VKI-5. The variation in Bray- Curtis and Weighted UniFrac can be attributed to the introduction of new species, thereby, as Bray-Curtis method considers only the abundance of OTUs (Lyra et al., 2013), validated that VKI-5 with large population clustered separately. These results described that the abundant OTUs of VKI-14 were able to survive through marine transgression and regression. Few OTUs were unique in VKI-5 whereas few newly appeared abundant OTUs of VKI-5 were also found to be present in VKI-2.

The biogeographical patterns of VKI sediments can be explained mainly as two mechanisms: dispersal limitation and niche filtering. The presence of exotic or new species at VKI-5 was due to the removal of barriers between marine population and terrestrial environment. During marine transgression the terrestrial location gets converted to marine habitat and becomes accessible to marine population. The entire population of VKI-5 was supposed to be present in VKI-2 as virtually there was no dispersal limitation, and sedimentation was rather a slow process. However a definite bacterial population which appeared in VKI-5 became virtually absent at VKI-2, due to niche filtering effects. It was also noted that few genera of VKI-14 which disappeared in VKI-5 was found to be recolonized in VKI-2. The reappearance of these OTUs might be due to the elimination of niche filtering and dispersal limitation. Moreover, the terrestrial ecosystem was restored by marine regression, the dispersal limitation and niche filtering effect won't affect the terrestrial population.

5. Conclusion

The PalEnDNA based reconstruction of bacterial community structure of the three sediment layers from the Vettaikaraniruppu paleobeach ridge, clearly indicated that sediment bacterial communities reflected the depositional environment. Reconstruction of bacterial community structure was possible even from 146,000 year old samples with latest molecular biology and sequencing techniques. The bacterial community dynamics among the sediment layers showed that marine transgression caused a mix up of environments and resulted in the increase of taxonomical diversity and altered the functional diversity of the native community. After the marine transgression, the bacterial communities had attempted to regain its original structure. These attempts erased the changes in biogeographic patterns, although the bacterial communities contained the impressions of marine transgression even after 3000 years. This study emphasized the need of geomicrobial studies in the sediment layers for the accurate interpretation of geological and geochemical proxies. Further studies are needed to accurately assess the functional dynamics of bacterial communities in the

sediments to infer the role of bacterial communities incurred in the past climatic changes.

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