

# Insights Into the Diversity of Terrestrial Soil Bacterial Communities Associated With Four Contrasting Köppen Climatic Zones.

Girish R Nair

Government Arts and Science College

Suresh S.S. Raja (✉ [sureshrajabdu@gmail.com](mailto:sureshrajabdu@gmail.com))

Government Arts and Science College <https://orcid.org/0000-0002-6090-8286>

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## Research article

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# Abstract

**Background:** The multidirectional relationship between soil, its microbiota, and climate is crucial in modulating the bacterial community diversity and its survival in the terrestrial ecosystem. Therefore, it is imperative to understand the dynamics of soil bacterial communities thriving in geographical areas of varied climatic exposure.

**Results:** The diversity of terrestrial soil bacterial communities thriving in four contrasting Köppen climatic zones of India was investigated for the first time using high-throughput sequencing. The results revealed that the bacterial species diversity, evenness and richness were highest in HSCZ (humid subtropical climatic zone). *Firmicutes* was the most abundant phylum in TWCZ (tropical wet climatic zone), ACZ (arid climatic zone), and HSCZ (humid subtropical climatic zone) while *Proteobacteria* in MCZ (Mountain climatic zone). The predominance of class *Alphaproteobacteria*, *Actinobacteria* with genera *Bradyrhizobium*, *Chthoniobacter*, and *Mycobacterium*, was observed in MCZ in contrast to class *Bacilli* with genera *Bacillus* and *Paenibacillus* in the rest of the zones. Correlation analysis showed that H' (Shannon diversity) index, S (species richness), OTU abundance were positively correlated with moisture, TOC, K, MAP (mean annual precipitation) and negatively correlated with pH, Ca, N, B, Fe, P, Mg and MAT (mean annual temperature).

**Conclusion:** This work mapped the occurrence and distribution of terrestrial soil bacterial communities in contrasting climatic zones that enabled us to assess the effect of climate in mentioned Köppen climatic zones on a taxonomic scale.

## Background

In the terrestrial ecosystem, soil plays the most crucial role in protecting the life on Earth by carrying out a large number of biological services like providing fertile grounds for crops, maintaining natural plant biodiversity, filtering and detoxifying the water before it enters the underground water table, acting as a sink for atmospheric CO<sub>2</sub> and greenhouse gases such as CH<sub>4</sub>, methyl bromide and N<sub>2</sub>O. This multi-functionality of soil is immensely attributed by the soil microbiota. Hence, understanding the diversity and composition of soil microbiota present in different soil environments holds great significance [1–5]. Although soil comprises prokaryotes (bacteria, actinobacteria, cyanobacteria) and eukaryotes (fungi, microscopic algae, protozoans), bacteria are the most abundant among all of them and considered to be the pioneer colonizers [6–8]. Bacterial community inhabiting the soil contribute to soil structure formation, decompose organic matter and recalcitrant xenobiotics, help in plant growth promotion, modulate the global biogeochemical cycle and recycle nutrients as well as essential elements such as carbon, nitrogen, phosphorous, and sulphur [9–16]. The native bacterial community of the soil can originate directly from decomposed plant matter, whereas some others can enter accidentally through agricultural runoff and the digestive tract of animals to become the part of soil microbial community [17–19]. This native bacterial community thriving in the soil microenvironment has a symbiotic-mutualistic behavior. They contribute to the proper functioning of the soil ecosystem, in turn depending

on the soil metabolites for their survival. This interplay is tightly regulated by abiotic factors like soil fertility [20–22], substrate availability [23–25], pH [26–29], climate [29, 30–33], soil temperature [34–36] and moisture [37–38], as well as shifts in seasonality [12, 21, 39] and biotic factors like plant communities [39, 40, 41] microbe food web interactions [42, 43] and farming practices [44–46]. Changes in the physicochemical characteristics of soil act as a significant factor for the bacterial community's existence. Climate is one such major abiotic factor that governs other minor factors such as pH, soil temperature, moisture, and nutrient availability. Hence, changes in the climate pattern could shape the bacterial community of a particular soil microenvironment and is very well responsible for a shift in bacterial community profiles over large geographical areas, which in turn influences the soil quality of that region.

The Köppen system of climate classification groups the world climate into several regions or zones based on the difference in temperature of a geographic location [47, 48]. India's climate is dynamic, with a variety of climates ranging from extremely hot desert regions to high altitude locations with severely cold conditions and experiences a climatic contrast [49]. According to the Köppen system, Indian climate can be divided into six major categories starting with the tropical wet climatic zone (TWCZ) experiencing tropical monsoon climate, tropical wet and dry climatic zone experiencing tropical savanna climate, arid climatic zone (ACZ) experiencing hot desert climate, semi-arid climatic zone experiencing semi-arid climate, humid subtropical climatic zone (HSCZ) experiencing humid subtropical climate and mountain climatic zone (MCZ) experiencing oceanic subpolar climate [50, 51]. The soil microenvironment in these regions is affected by the local climate, causing a variation in bacterial community profile at different climatic zones, irrespective of other factors. Such climatic hotspots are exciting avenues for exploratory studies on bacterial community diversity and understanding the intricate relationship between soil bacterial communities present in particular climate-modulated microenvironment.

Next-generation sequencing (NGS) is possibly the best effective approach for evaluating and characterizing soil microbial community profile and has undergone constant upgradation in the past years with improvements in sequence quality, depth with lesser cost and time [52–54]. Oxford Nanopore sequencer is among the newest third-generation sequencers with promising deep sequencing strategy coupled with robust characterization efficiency and has become choice of many research labs for in-depth metagenomic studies [55–57]. Recent metagenomic studies have documented the influence of climate on the diversity and activity of soil microbiota in China on a regional and spatial scale. Researchers have studied the co-occurrence network topological features of soil microbiota on a continental scale and the effect of climate, soil factors, and distance on the diversity and function of bacteria as well as fungi [58–63]. Apart from this, the culturable diversity of soil bacteria in the terrestrial ecosystem has been studied in different climatic zones of India [64–67] and some studies have also employed next-generation sequencing to determine the diversity and function of soil microbial communities in terrestrial environments [68–71]. Nevertheless, a deep sequencing strategy has not been employed in Indian soils to study and compare the diversity of native soil bacteria present in contrasting climatic zones over a large geographical area. Considering this in mind, we investigated the bacterial community profile of soils present in four different climatic zones *viz.* TWCZ, ACZ, HSCZ, and MCZ. In

addition to this, we also examined the correlation of bacterial species diversity with soil parameters and climatic factors.

## Methods

### Sample site description and soil sampling

The soil was collected in the pre-monsoon season of mid-February 2017 from four different climatic zones *viz.* TWCZ (8°26'N, 76°59'E), ACZ (26°49'N, 70°33'E), HSCZ (30°44'N, 76°43'E) and MCZ (30°53'N, 76°57'E) of India following the Köppen climate classification scheme [48]. Soil samples were taken by removing the surface soil at a depth of 15-20 cm using a sterile shovel from uncultivated areas. The soil was collected randomly from approximately 20 distant sampling spots at each climatic zone totaling 82 soil samples in sterile sampling bags (Nasco: Hi-media, India) and transported to the laboratory in ice. Later, the samples from each climatic zone were sieved through sterile 2mm mesh, pooled together to make a composite soil sample representing each climatic zone, and stored at 4°C until further processing (Additional file 1).

### Soil analysis and climatic factors

Geochemical parameters of composite soil samples were analyzed from the four climatic zones that included ten parameters like pH, moisture content, organic carbon, nitrogen, potassium, calcium, magnesium, phosphorous, iron, and boron. The total organic carbon (TOC) was calculated using the partial oxidation method [72], followed by pH using pH electrode (Shimadzu, Japan) in a saturated colloidal solution of deionized water and the moisture content using the oven-dry method [73]. Nitrogen content in the soil was determined using the micro Kjeldahl method, and total phosphorus was measured colorimetrically [74]. Determination of Iron, Boron, Calcium, Potassium, and Magnesium was carried out using a spectrophotometer [75]. Values of mean annual temperature (MAT) and mean annual precipitation (MAP) were obtained from the Indian Meteorological Department, Ministry of Earth Sciences, and taken as climate change indicators. Pearson's correlation test was performed to see the strength of correlation between soil profile and climatic factors (MAT, MAP) on the bacterial diversity determinants. Further, p values were generated using multiple regression analysis, to test the significance of correlation.

### DNA extraction and quality check

Metagenomic DNA was isolated from the air-dried soil samples using FastDNA™ Spin Kit for Soil (MP Biomedicals, USA) according to the manufacturer's protocol. The DNA concentration and purity were estimated using Nanodrop Spectrophotometer (Thermo Fisher Scientific) and Qubit Fluorometer (Thermo Fisher Scientific) after tenfold dilution in triplicate. Finally, the DNA quality was assessed using Agarose gel electrophoresis in 1.2 % Agarose [76].

### Library preparation and Nanopore sequencing

The library preparation and Nanopore sequencing was carried out at the next-generation sequencing facility of Genotypic Technology Pvt. Ltd. situated at Bengaluru, India (<http://www.genotypic.co.in>). DNA from the samples was subjected to 16S rRNA gene amplification using region-specific primers (16S rRNA barcode primer) and LongAmp Taq 2X master mix (NEB). The PCR products were purified by using 1X Ampure XP beads (Beckmann Coulter, USA). Purified PCR amplicons from each sample were pooled at equimolar concentration. These pooled barcoded samples were then subjected for sequencing adapter ligation using 16S Barcoding Kit (SQK-RAB204). Sequencing was performed on MinION Mk1b (Oxford Nanopore Technologies, Oxford, UK) using SpotON flow cell (FLO-MIN107) in a 48hr sequencing protocol on MinKNOW 1.10.11 [77-79].

## **Sequence processing and data analysis**

The reads obtained from the barcoded library using a Nanopore sequencer were demultiplexed, and basecalled 'fastqc' files were obtained by Albacore vr2 software suite of MinION Mk1b (Oxford Nanopore Technologies, Oxford, UK) [80]. Basecalled reads from each sample were subjected to microbial identification using EPI2ME desktop agent. 16S rRNA analysis was carried out using the 16S rRNA pipeline from the EPI2ME database [60, 81]. The raw reads were also processed in parallel using microbial genomics module of CLC Genomics workbench version 11.0 (CLC Bio, Qiagen, Boston, MA, USA) for OTU table generation and further downstream statistical analysis [82, 83]. The Alpha diversity parameters of the four contrasting climatic zones like Shannon-Weiner diversity index (H'), Simpsons diversity index (D), Species richness (Margalef), and Species evenness (Pielou) were calculated using the data analysis package of MS-excel software. Beta diversity of the soil samples was calculated and presented in the form of a PCA plot using PAST version 3.26 (Oyvind Hammer, Natural History Museum, University of Oslo, Oslo, Norway).

## **Data availability**

The metagenomic sequences generated in the present study have been deposited in the Sequenced Read Archive (SRA) service of the Genbank database maintained by the NCBI server under the accession numbers SRR8003384 to SRR8003387.

# **Results**

## **Geochemical properties of soil samples**

The main chemical parameters of the pooled soil samples from different climatic zones (TWCZ, ACZ, HSCZ and MCZ) were analyzed and notable differences were observed in the values of pH and moisture content. The moisture content percentage was recorded highest (13.06%) in TWCZ and lowest (0.07%) in ACZ. The pH was recorded lowest (3.94) in TWCZ compared to ACZ (8.05) and HSCZ (8.13), which were mildly alkaline. MCZ showed a mildly acidic nature (5.44) with a moderate pH value. Total organic carbon (TOC) values did not show any drastic difference and were highest for MCZ. The values of nitrogen (17.00%), calcium (6.07%), magnesium (2.43%), phosphorus (0.08%), iron (1.26%), and boron (15.12

ppm) were recorded highest in ACZ compared to other climatic zones. There was lesser difference in TOC, potassium, phosphorous, iron, and boron values among the other climatic zones. A considerable difference was observed in the values of calcium (1.8%) and magnesium (0.4), which was recorded lowest in HSCZ compared to the other climatic zones (Additional file 2).

### Bacterial diversity correlation with soil profile and climatic factors

The correlation analysis revealed that H' (Shannon diversity) index, S (species richness), OTU abundance were positively correlated with moisture, TOC, K, MAP (mean annual precipitation), with a strong positive correlation between S and K. On the other hand, a negative correlation was observed with pH, Ca, N, B, Fe, P, Mg and MAT (mean annual temperature) with a very strong negative correlation between H' and Fe (Additional file 3). The p value for Fe ( $p > 0.05$ ) showed a significant correlation with diversity parameters (Additional file 4).

### Read characteristics and bacterial diversity parameters

A total of 132130 high-quality reads were obtained from the four contrasting climatic zones that were assigned to 16556 bacterial OTUs. The maximum number of reads was obtained in TWCZ, and the minimum number of reads was obtained in ACZ. Similarly, TWCZ also had the highest number (5694) of OTUs, whereas ACZ had the lowest number (841) of OTUs among the zones. The species diversity (different species) and richness (no. of different kinds) were highest in HSCZ and lowest in ACZ. The species evenness (closeness of species) was highest in HSCZ and lowest in TWCZ (Table 1). There was not much difference in the diversity indices (H', D) among the zones except for ACZ.

**Table 1** Alpha diversity parameters of the four contrasting climatic zones. The bacterial species diversity and richness was maximum in HSCZ and minimum in ACZ.

Climatic zones	TWCZ	ACZ	HSCZ	MCZ
Reads obtained	69485	16684	57899	57547
OTUs Assigned	5694	841	5122	4899
Shannon-wiener diversity index (H')	7.371	6.678	7.449	7.254
Simpsons diversity index (D)	0.998	0.997	0.998	0.998
Species richness (Margalef)	301.041	150.476	303.234	260.940
Species evenness (Pielou)	0.916	0.921	0.927	0.920

### Taxonomic abundance and bacterial diversity

The classified sequences (known taxonomy) obtained from all the four contrasting climatic zones were affiliated with eighteen different phyla (Additional file 5), which was further narrowed down to the top eight phyla based on the relative percentage abundance and the rest of the phyla were grouped into

others that showed less than 0.2% relative abundance. Amongst these eight phyla, *Firmicutes* appeared as the most dominant phylum followed by *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, *Acidobacteria*, *Bacteroidetes*, *Verrucumicrobia*, and *Cyanobacteria*. Surprisingly, the phylum *Firmicutes*, which was dominant (>30%) in all other zones, was less abundant (<7%) in MCZ. *Proteobacteria* was more dominant in MCZ compared to *Firmicutes*. HSCZ showed a stable abundance pattern (34.82%, 14.43%, 11.16%, 4.37%, 1.43%, 0.56%, 0.43% and 0.21%) for all these eight phyla compared to other zones. Contrastingly, the abundance of *Cyanobacteria* was more in ACZ compared to the other zones. *Firmicutes* mostly dominated TWCZ with a lower percentage of unclassified bacterial phyla, which was seen as high in MCZ (Fig. 1). Other phyla that were observed in lower percentage among the top abundant were *Bacteroidetes*, *Acidobacteria*, *Verrucumicrobia*, and *Cyanobacteria*, in which abundance of *Cyanobacteria* was high in ACZ amongst other zones. A similar pattern of abundance was observed for *Verrucumicrobia*, which was prevalent only in MCZ (Fig. 1).

*Bacilli* and *Alphaproteobacteria* were the most dominant class among the four contrasting climatic zones, followed by *Actinobacteria*, *Planctomycetia*, *Clostridia* and *Thermoleophilia*. In MCZ, the class level bacterial community composition varied from the rest of the zones as *Alphaproteobacteria*, *Betaproteobacteria*, *Planctomycetia*, and *Phycisphaerae* were considerably high in MCZ, whereas, *Bacilli*, *Actinobacteria*, and *Deltaproteobacteria* were less compared to other zones (Additional file 6). Similarly, at the order level, the predominance of *Bacillales* was observed in TWCZ, ACZ, and HSCZ compared to MCZ, where *Rhizobiales* showed more predominance (Additional file 7). In family level distribution, *Bacillaceae* was predominant in all the zones except MCZ, where *Gemmataceae*, *Hyphomicrobiaceae*, *Gaiellaceae*, *Bradyrhizobiaceae* were predominant over *Bacillaceae* (Additional file 8). Further at the genus level, *Bacillus* was the most dominant genus in TWCZ (31%), ACZ (30%), and HSCZ (31%). In contrast, *Bradyrhizobium* was the most dominant genus in MCZ (8%), but a noteworthy proportion of the bacterial population remained unclassified as the genera were not assigned any nomenclature (novel genus). The pattern of the most abundant genera was different in MCZ compared to the other zones. The genus *Paenibacillus* was among the top 10 genera in all the zones except MCZ. The genera *Chthoniobacter* and *Mycobacterium* were only observed in MCZ as the top abundant ones compared to the other zones (Fig. 2). A significant share of bacterial communities present in the climatic zones was not identifiable at the species level and categorized as an unidentified bacterium. *Bacillus megaterium*, *Bacillus pumilis* and *Bacillus subtilis* were present among the top 10 abundant species in TWCZ, ACZ and HSCZ. In contrast, *Bradyrhizobium* spp. showed high prevalence in MCZ compared to the other three zones (Fig. 3). Shared bacterial community abundance was also assessed by plotting a Venn diagram, which showed a total of 18.1% bacterial community shared among all the climatic zones. Maximum shared community abundance (4.8%) was observed between HSCZ and MCZ, whereas a minimum abundance (1.2%) was observed between TWCZ and ACZ. The distribution of unique species (not shared among the climatic zones) in the different climatic zones varied, and it was found more (16.5%) in TWCZ, with a little less (11.4%) in HSCZ and almost equally distributed (4.9%) between ACZ and MCZ (Fig. 4). In conclusion, *Bacillus* emerged as a prominent genus of native soil bacterial community in all the zones except in MCZ, where *Bradyrhizobium* was the most dominant.

## Discussion

The community composition and change in the pattern of bacterial diversity in soil microenvironments located in different Köppen climates of Indian subcontinent was investigated for the first time in the present study using a metagenomic approach following which the chemical parameters of soil and climatic determinants (MAP, MAT) were taken for correlation studies with bacterial community diversity.

Upon analyzing the chemical parameters of the soil present in the four contrasting climatic zones, considerable differences were found in the values of soil pH and moisture content. It is evident from several reports that soil pH has a substantial influence on the soil diversity and bacterial community composition, suggesting that a neutral pH favours maximum bacterial diversity compared to the acidic and alkaline pH [84–87]. The pH of soil present in the four contrasting climatic zones was acidic in TWCZ, mildly acidic in MCZ, and mildly alkaline in ACZ and HSCZ. Although the soils collected from the four contrasting climatic zones had no neutral pH, species richness was observed maximum in the mildly alkaline soil (8.13) collected from HSCZ and could be considered close to neutral. Several bacterial phyla groups have adapted to different soil pH and one such phylum is *Acidobacteria* having diverse metabolic functions and is predominant in the acidic soils [88]. This fact was noticeably evident in the present study's findings. The relative OTU abundance of *Acidobacteria* was maximum in the acidic soil of TWCZ (3.94) followed by mildly acidic soil of MCZ (5.44), compared to the other two zones having a non-acidic soil. Contrastingly, the effect of pH was not seen much in the relative abundant profiles of predominant phyla *Firmicutes* and *Proteobacteria* in the four contrasting climatic zones. The amount of moisture present in the soil depends upon the water holding capacity of the soil, and it has a direct and indirect effect on the diversity of soil bacteria. Several groups of bacterial phyla have shown sensitivity towards drought and extreme rewetting. Cyanobacterial populations tend to decrease in the soil during drought conditions and have a low recovery upon rewetting. On the other hand, *Alpha-*, *Beta-* and *Gammaproteobacteria* tend to increase under drought conditions and rewetting in the soil. Some other phyla tend to resist the drought conditions in the soil by adapting themselves to the soil microenvironment [89–91]. However, there were no notable observations of the effect of soil moisture on the bacterial diversity in the four contrasting climatic zones, even though it had ACZ soil with deficient moisture level (0.07%) and TWCZ soil with the highest moisture level (13.06%). In contrast to the previous studies, which reported a decrease in Cyanobacterial abundance of soil during drought, the present finding shows a higher relative abundance of *Cyanobacteria* in ACZ than TWCZ. Having said this, the study only analyzed the soil bacterial diversity present in these zones during the pre-monsoon season. The analysis of bacterial community profile of these regions in the post-monsoon and temporal study of rainfall for more than five years should be carried out in these areas to get a better picture of the extent of influence of soil moisture on the bacterial community diversity and composition. All other studied soil parameters like TOC (total organic carbon), nitrogen, calcium, magnesium, potassium, phosphorous, iron, and boron do not seem to show considerable influence on bacterial abundance and diversity.

A correlation analysis was carried out to establish the relationship between soil parameters and bacterial diversity. A positive correlation leads to increase in the community diversity if one of the soil parameters

or climatic factor increases, whereas in negative correlation this trend is opposite and in zero correlation the parameters have no effect on the community diversity. The diversity parameters were positively correlated with TOC, K, moisture and MAP, which tend to increase the diversity of the soil bacterial community in these climatic zones. Most of the other parameters had negative correlation on the diversity profile, in which iron values showed strong negative correlation with significance ( $p > 0.05$ ) [28, 30].

A greater read length and read number would increase sequencing depth, thereby generating more information from the sample. This depends, to a great extent, on the sequencing methodology used and the MinION sequencer from Oxford Nanopore produces longer read length, which allows detailed bacterial community characterization, down to the family or even genus level at low sequencing cost [54]. However, the accuracy and sequencing output is limited compared to reads obtained using a shorter-read platform like Illumina [92, 93]. One of the reasons for low diversity in ACZ could be the sequence quality generated after sample sequencing, which is the critical parameter that affects the overall bacterial diversity. A low-quality sequence could result from different factors but primarily the quality of DNA obtained during isolation [94].

Considering the taxonomic abundance and bacterial diversity, it was observed that the two major phyla, *Firmicutes* and *Proteobacteria*, were predominating in four contrasting Köppen climatic zones. This points out that the prevailing climatic conditions and chemical nature of the soil could have only a minimal effect on these groups of the bacterial community in the terrestrial ecosystem of the Indian subcontinent. The second most dominant phyla present in the climatic zones were *Actinobacteria* and *Planctomycetes*, which showed a little variation in abundance pattern within the zone and among the zones. *Actinobacteria* constitute high G + C content bacteria that are the richest source of bioactive molecules, primarily antibiotics [95, 96]. *Planctomycetes* are basically known to exist in aquatic ecosystems; especially in freshwater during the past, but several reports now suggest their existence in the terrestrial ecosystem [97]. *Cyanobacteria* are the photosynthetic carbon fixers and are among the members of biological soil crusts present in the arid and semi-arid ecosystem, which play an important role in fixing carbon in low vegetative areas as well as drylands. *Cyanobacteria* of the biological soil crusts are much less studied in India's arid climates and are important for making the soil fertile for agriculture in desert ecosystems [98–99]. *Verrucumicrobia* are difficult to cultivate and are usually considered as less frequently available phyla in the terrestrial environment. However, studies have shown their predominance in different soil depths, biomes, and soil types [100]. The members of the genus *Bacillus* are gram-positive rods with an ability to produce spores that enables them to survive in harsh environmental conditions and stay dominant in terrestrial soils as a native bacterial community. The effect of climate in modulating the species diversity of the *Bacillus* community in India has recently been shown by our group using a culturable approach. It was seen that although the genus *Bacillus* is ubiquitously found in soil environments, its species diversity varies in different climatic zones [67]. In contrast, the members of the genus *Bradyrhizobium* are gram-negative, and most of them are symbiotic nitrogen fixers. They constitute the prominent members of the rhizospheric bacterial community, usually profound in the roots of legume plants [101]. Studies have also found the presence of *Bradyrhizobium*

spp. in deep soil and other soil bacteria inhabited by leguminous trees [102, 103]. Prevalence of this genus more in MCZ could be explained by the fact that the soil microenvironment in MCZ is in close contact with roots, originating from nearby trees providing a favourable microclimate and soil profile for its suitable existence outcompeting the genus *Bacillus*. The results mentioned above account for only a 70% bacterial community diversity in the four contrasting climatic zones represented by OTUs originating from classified sequences narrowed down to species level. At the same time, the rest remained as unclassified groups. These unclassified groups treasure novel bacteria, isolation, and identification of which demands improvised culture techniques.

## Conclusion

Analysis from the present study indicated that the bacterial community present in the four contrasting Köppen climatic zones does not show much variation in the pattern of abundance among and within the four zones, although a shift in abundance pattern was observed in MCZ. We could see only a minor change in the abundance values of certain groups of bacterial phyla and genera, which do not support the abrupt role of climate and soil chemistry in modulating the bacterial communities thriving in these soil environments of Indian subcontinent. However, these minor variations in diversity and composition of bacterial species in different climatic zones may possibly reflect from the combined effect of climate (MAT, MAP), some soil parameters and other factors. Although there are a number biotic and abiotic factors responsible for shaping the soil bacterial community in these climatic zones, the interdependency of bacterial community, soil parameters and microclimate is certain and could vary among different geographical regions. A detailed temporal study including more number of samples for more than 2 years could give us a broader picture in understanding the effect of climate and soil factors on specific groups of bacterial community present in these Köppen climatic zones.

## Declarations

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Manuscripts reporting studies involving human participants, human data or human tissue must:

- Include a statement on ethics approval and consent (even where the need for approval was waived) - Not Applicable
- Include the name of the ethics committee's reference number if appropriate- Not Applicable
- Studies involving animals must include a statement on ethics approval- Not Applicable

### CONSENT FOR PUBLICATION

Not Applicable

### AVAILABILITY OF DATA AND MATERIAL

The data supporting the findings of the present study is submitted in Sequenced Read Archive (SRA) service of the Genbank database maintained by the NCBI server under the accession numbers SRR8003384 to SRR8003387 and given in the Materials and methods section.

## COMPETING INTERESTS

Suresh S.S. Raja and Girish R Nair declare that they have no conflict of interest.

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## AUTHOR CONTRIBUTIONS

Conceptualization and Study design: Suresh SS Raja (SSR), Manuscript preparation, data analysis, review and editing: Girish R Nair (GRN).

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Not Applicable

## AVAILABILITY OF CODE

Not Applicable

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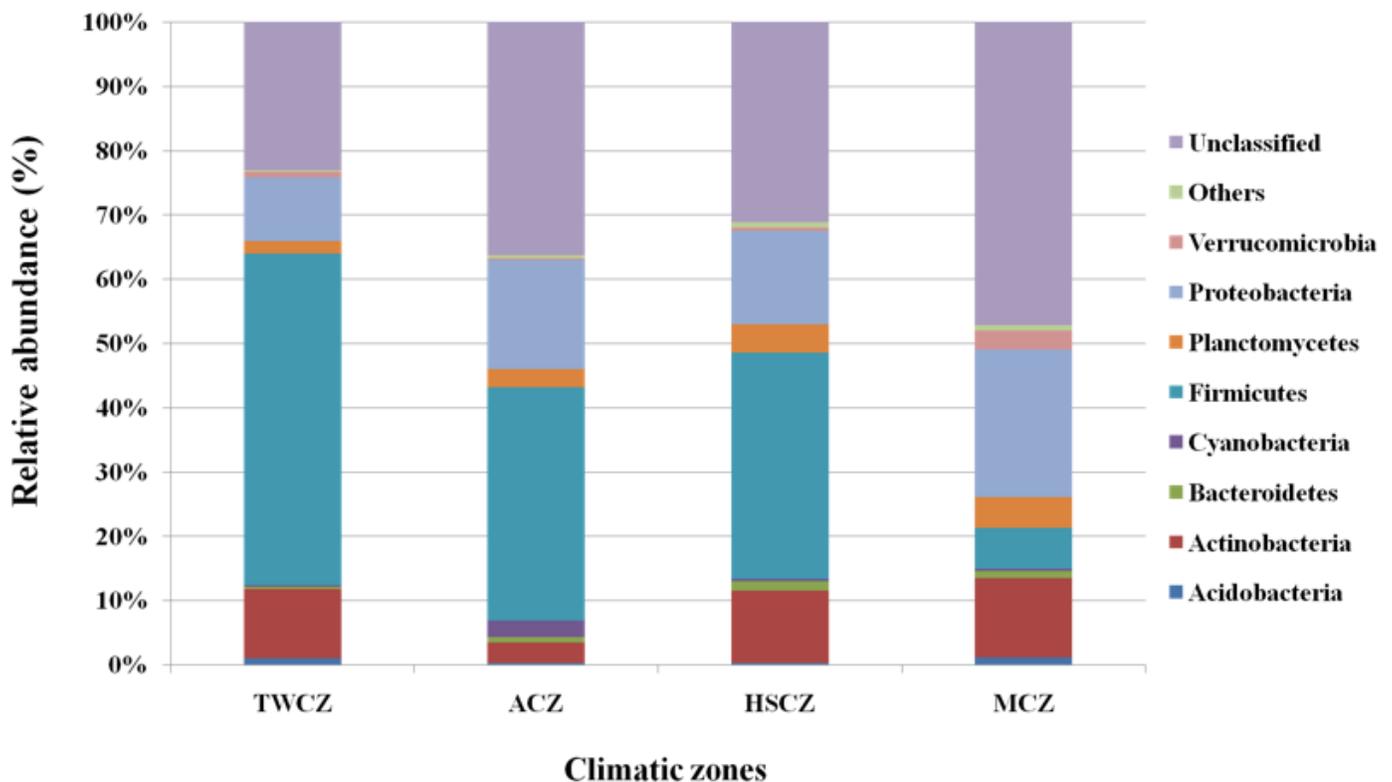
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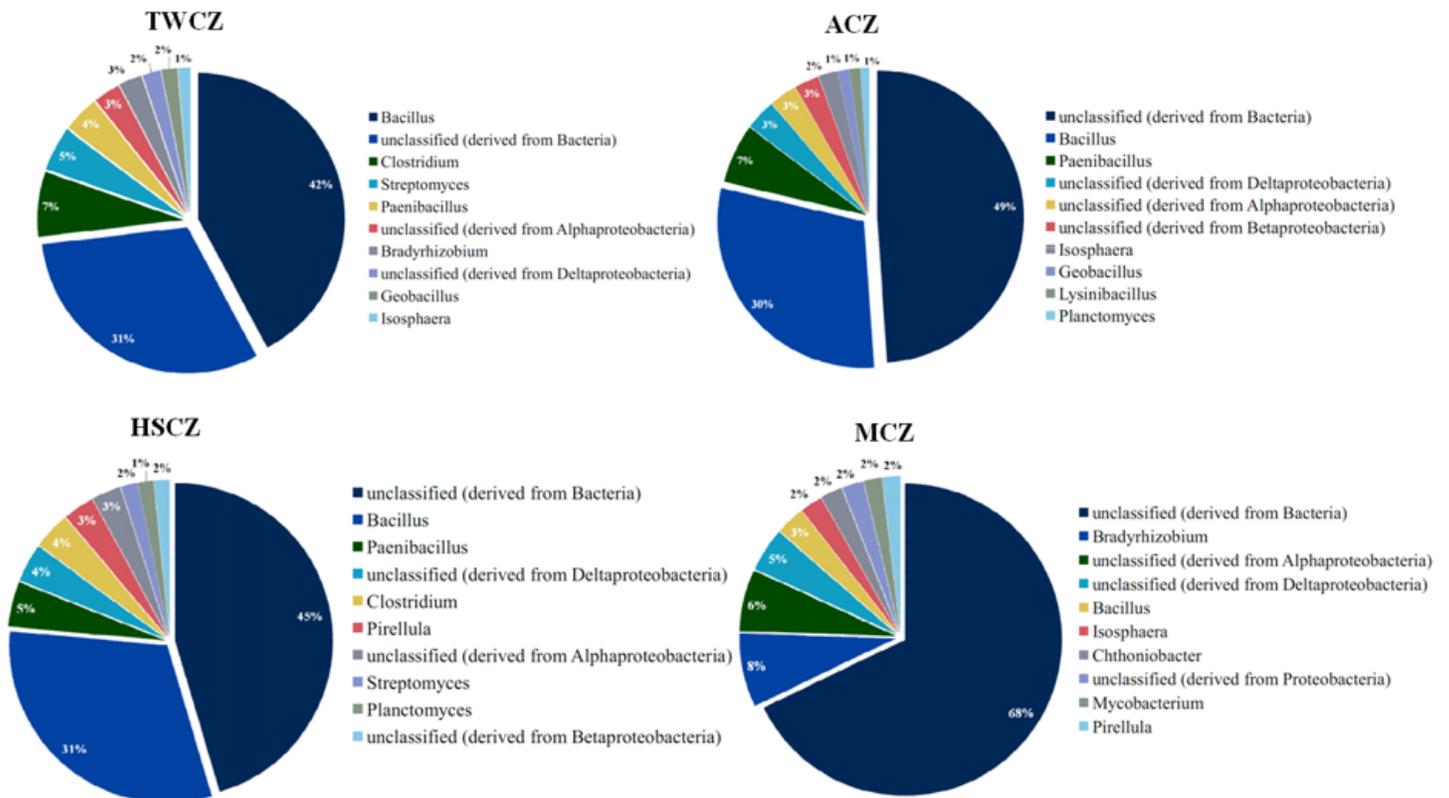
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## Figures



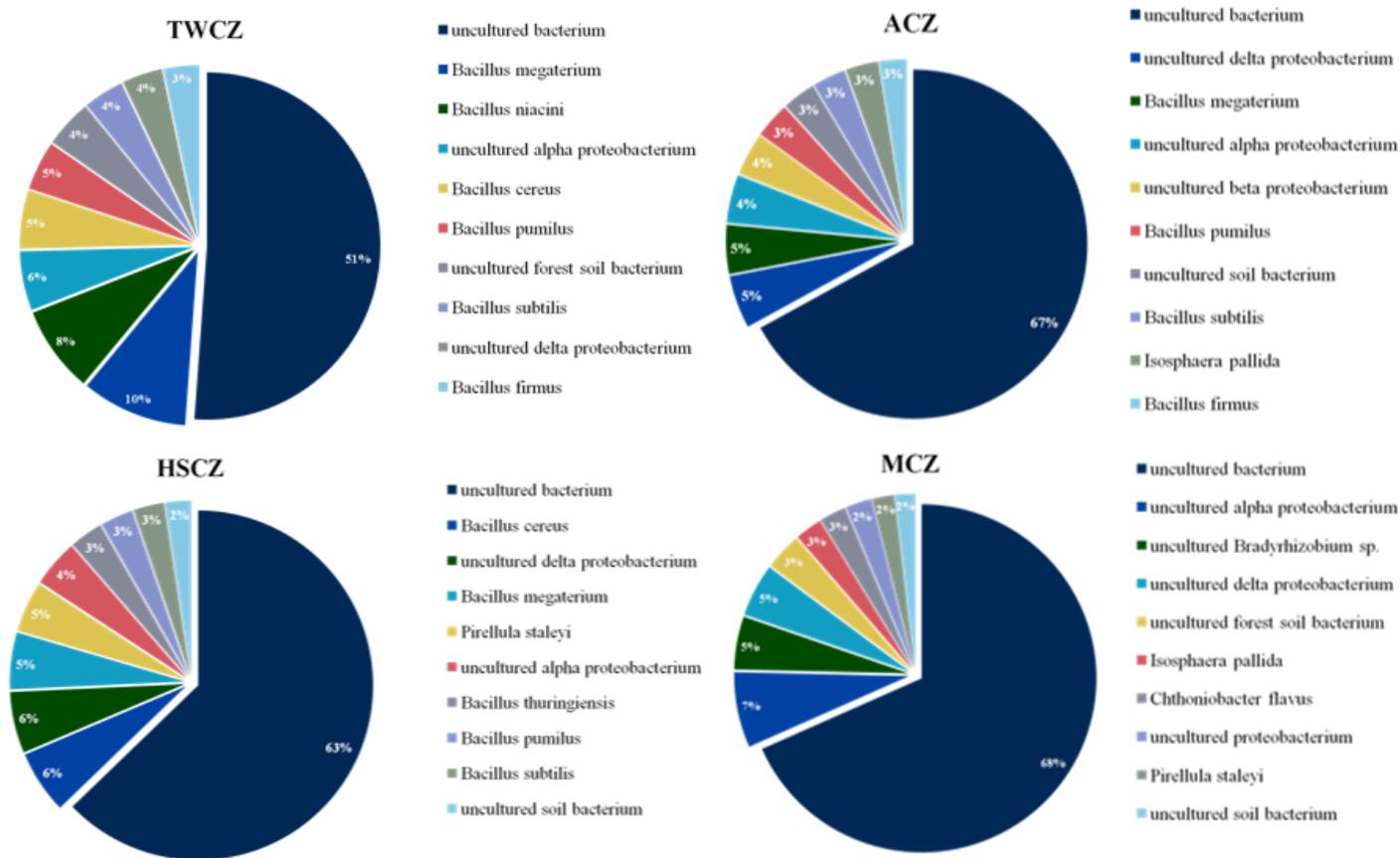
**Figure 1**

Relative abundance (%) of the major phyla detected in soils collected at four different climatic zones viz. TWCZ (tropical wet climatic zone); ACZ (Arid climatic zone); HSCZ (humid subtropical climatic zone); MCZ (Mountain climatic zone). Firmicutes and Proteobacteria were the most dominant phyla among all. "Others" contains sum of all the minor phyla.



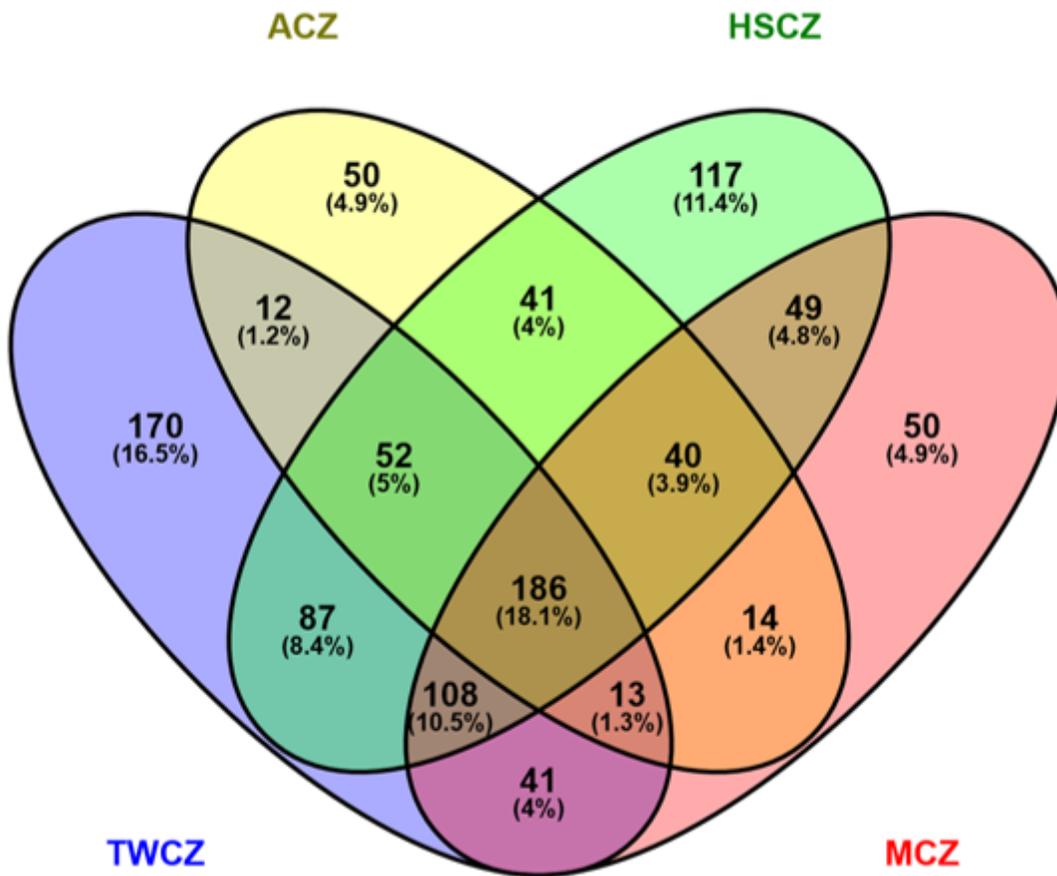
**Figure 2**

Relative abundance (%) of top 10 bacterial genera detected in the four contrasting climatic zones. viz. TWCZ (tropical wet climatic zone); ACZ (Arid climatic zone); HSCZ (humid subtropical climatic zone); MCZ (Mountain climatic zone). Bacillus was the most abundant genus in all the zones except MCZ, which was dominated by Bradyrhizobium.



**Figure 3**

Pattern of relative abundance (%) of top 10 species detected in the four contrasting climatic zones. A major proportion of the species had unassigned nomenclature and designated as uncultured bacterium.



**Figure 4**

Proportion shared bacterial community among the four contrasting climatic zones viz. TWCZ (tropical wet climatic zone); ACZ (Arid climatic zone); HSCZ (humid subtropical climatic zone); MCZ (Mountain climatic zone).

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