Malaysian Journal of Microbiology, Vol 20(2) 2024, pp. 175-187
DOI: http://dx.doi.org/10.21161/mjm.220094

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Insights into the diversity of grassland soil bacterial communities associated with four contrasting Köppen climatic zones of India

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Received 12 October 2022; Received in revised form 14 August 2023; Accepted 9 January 2024

ABSTRACT

Aims: This study investigates the community structure and diversity of terrestrial soil bacterial communities thriving in four contrasting Köppen climatic zones of India using high-throughput sequencing.
Methodology and results: Soil samples were collected for metagenomic DNA isolation and PCR amplification using primers targeting the 16S rRNA gene region. Amplicons were subjected to Oxford nanopore sequencing and data analysis. Bacterial species diversity, evenness and richness were highest in a humid sub-tropical climatic zone (HSCZ). Firmicutes were the most abundant phylum in the tropical wet climatic zone (TWCZ), arid climatic zone (ACZ) and humid sub-tropical climatic zone (HSCZ), while Proteobacteria in the mountain climatic zone (MCZ). 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. OTU abundance was positively correlated with moisture, TOC (total organic carbon), K, MAP (mean annual precipitation) and negatively correlated with pH, Ca, N, B, Fe, P, Mg and MAT (mean annual temperature). A significant correlation was only observed with Fe against Shannon diversity (H') in multiple regression analysis.
Conclusion, significance and impact of study: The multidirectional relationship between soil, its microbiota and climate is crucial in modulating bacterial community diversity and its survival in terrestrial ecosystems that significantly contribute to ecosystem function. This work mapped the occurrence and distribution of terrestrial soil bacterial communities in contrasting climatic zones for the first time, enabling us to assess the effect of climate in the mentioned Köppen climatic zones.

Keywords: Bacterial diversity, climatic zone, nanopore sequencing, OTUs, soil microbiota

INTRODUCTION

In the terrestrial ecosystem, soil plays the most crucial role in protecting life on earth by carrying out many 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_2 and greenhouse gases such as CH_4, methyl bromide and N_2O. The soil microbiota immensely attributes to this multifunctionality of soil. Hence, understanding the diversity and composition of soil microbiota present in different soil environments holds great significance (Oszust *et al.*, 2014; Gleeson *et al*., 2016; Jelen *et al*., 2016; Tan *et al*., 2020; Valliammai *et al*., 2021). Although soil comprises prokaryotes (bacteria, Actinobacteria, Cyanobacteria) and eukaryotes (fungi, microscopic algae, protozoans), bacteria are the most abundant among all of them and are considered to be the pioneer colonizers (Panikov, 1999; Classen *et al*., 2015; Vieira *et al*., 2020). Bacterial communities 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 (Balser *et al*., 2002; Schmidt *et al*., 2007; van der Heijden *et al*., 2008; Ma *et al*., 2011; Liu *et al*., 2020; Wang *et al*., 2020). 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 part of the soil microbial community (Garbeva *et al*., 2004; Dey *et al*., 2012; Barnett *et al*., 2020). This native bacterial community, such as Proteobacteria, Firmicutes and Actinobacteria, etc. thriving in the soil microenvironment,

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ISSN (print): 1823-8262, ISSN (online): 2231-7538

has a symbiotic mutualistic behavior. The native bacterial communities contribute to the proper functioning of the soil ecosystem, in turn depending on the soil metabolites for their survival. Abiotic factors like soil fertility (Bonanomi *et al*., 2020), substrate availability (Ranjard and Richaume, 2001; Girvan *et al*., 2003; Goldfarb *et al*., 2011), pH (Nicol *et al*., 2008; Lauber *et al*., 2009), climate (Fierer and Jackson, 2006; Fierer *et al*., 2007; Castro *et al*., 2010; Cao *et al*., 2016; Zhou *et al*., 2020), soil temperature (Frey *et al*., 2008; Pérez Castro *et al*., 2019) and moisture (Chen *et al*., 2020), as well as shifts in seasonality (Schmidt *et al*., 2007; Camacho-Sanchez *et al*., 2020) and biotic factors like plant communities (Stephan *et al*., 2000; O'Donnell *et al*., 2001; Camacho-Sanchez *et al*., 2020) microbe food web interactions (Sánchez-Moreno *et al*., 2011) and farming practices (Zhang *et al*., 2020) tightly regulate this interplay. Changes in the physicochemical characteristics of soil act as a significant factor for the bacterial community's existence. The climate is one such major abiotic factor that governs other 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 are very well responsible for shifts in bacterial community profiles over large geographical, which in turn influences the soil quality of that region (Zhou *et al*., 2020).

Based on the Köppen climatic classification, there are five main climate groups, and within each of these categories, there are subgroups based on the seasonality of temperature and precipitation (Köppen *et al*., 2011; Rubel and Kottek, 2011). India's climate is dynamic, with various climates ranging from extremely hot desert regions to high-altitude locations with severely cold conditions and experiencing a climatic contrast (Chang, 1967). 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 sub-polar climate (Peel *et al*., 2007; Gaughan *et al*., 2013). The local climate affects the soil microenvironment in these regions, 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 microenvironments.

Next-generation sequencing (NGS) is possibly the most effective approach for evaluating and characterizing soil microbial community profiles and has undergone constant upgradation in the past years with improvements in sequence quality and depth with lesser cost and time (Shokralla *et al*., 2012; Thomas *et al*., 2012; Nair and Raja, 2017). Oxford nanopore sequencer is among the newest third-generation sequencers with a promising deep sequencing strategy coupled with robust characterization efficiency and has become the choice of many research labs for in-depth metagenomic studies (Jain *et al*., 2016; Nicholls *et al*., 2019; Overholt *et al*., 2020). 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 (Berry and Widder, 2014; Cao *et al*., 2016; Ma *et al*., 2016; Lan *et al*., 2018; Xu *et al*., 2018; Picazo *et al*., 2019). Apart from this, the culturable diversity of soil bacteria in the terrestrial ecosystem has been studied in different climatic zones of India (Johri *et al*., 2003; Shivaji *et al*., 2011; Vasudevan *et al*., 2015; Nair and Raja, 2018) and some studies have also employed next-generation sequencing to determine the diversity and function of soil microbial communities in terrestrial environments (Patel *et al*., 2015; Bhattacharyya *et al*., 2016; Gupta *et al*., 2017). Nevertheless, a deep sequencing strategy has not been employed in Indian soils to study and compare the diversity of native soil bacteria in contrasting climatic zones over a large geographical area. Considering this, we investigated the bacterial community profile of soils present in four different climatic zones, viz. TWCZ, ACZ, HSCZ and MCZ in India. In addition to this, we also examined the correlation of bacterial species diversity with soil parameters and climatic factors.

MATERIALS AND 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 (Köppen *et al*., 2011) as described in our previous study (Nair and Raja, 2018). Soil samples were taken by removing the surface soil from uncultivated areas at a 15-20 cm depth using a sterile shovel. 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 a sterile 2mm mesh, pooled together to make a composite soil sample representing each climatic zone, and stored at 4 °C until further processing (Table 1).

Soil analysis and climatic factors

The geochemical parameters of composite soil samples from the four climatic zones were analyzed, including pH,

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 175-187
DOI: http://dx.doi.org/10.21161/mjm.220094

Table 1: Sampling description and biogeographic properties of four contrasting climatic zones.

Climatic zone	Climate	Latitude and longitude	Mean annual temperature (ºC)	Mean annual rainfall (mm)	Mean elevation (m)	Soil type	Collection depth (cm)	No. of samples
TWCZ	Tropical monsoon Climate (Am)*	8°26'N, 76°59'E	28	1871	85	Red loamy soil	20	20
ACZ	Hot desert climate (BWh)*	26°49'N, 70°33'E	33	347	135	Desert soil	20	22
HSCZ	Humid subtropical climate (Cwa)*	30°44'N, 76°43'E	23	399	321	Alluvial soil	15	20
MCZ	Humid continental climate (Cwb)*	30°53'N, 76°57'E	13	921	1800	Mountain soil	18	20

TWCZ, tropical wet climatic zone; ACZ, Arid climatic zone; HSCZ, humid subtropical climatic zone; MCZ, Mountain climatic zone.
*Denotes the code of prevailing climate in these geographical regions according to Köppen system of climate classification.

moisture content, organic carbon, nitrogen, potassium, calcium, magnesium, phosphorous, iron and boron. The total organic carbon (TOC) was calculated using the partial oxidation method (Nelson and Sommers, 1983), followed by pH using pH electrode model no. 9618-10D (Shimadzu, Japan) in a saturated colloidal solution of deionized water and the moisture content using the oven-dry method (Zheng *et al*., 2009). Nitrogen content in the soil was determined using the micro Kjeldahl method and total phosphorus was measured colorimetrically (Jm and Mulvaney, 1982). Determination of iron, boron, calcium, potassium, and magnesium was carried out using an Agilent Varian 715-ES ICP-AES instrument using the following conditions: plasma flow: 15 L/min; sample uptake: 30 sec; pump rate: 15 rpm; nebulizer pressure: 200 kPa; auxiliary flow: 1.5 L/min (Hagen and Howard, 2011). 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 the 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 ND1000 version 3.8 (Thermo Fisher Scientific) and Qubit Fluorometer 2.0 (Thermo Fisher Scientific) after tenfold dilution in triplicate. Finally, the DNA quality was assessed using agarose gel electrophoresis in 1.2% agarose gel (Nair *et al*., 2016).

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 in 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 2× master mix (NEB). The PCR products were purified by using 1× Ampure XP beads (Beckmann Coulter, USA). Purified PCR amplicons from each sample were pooled at equimolar concentration. These pooled barcoded samples were then subjected to sequencing adapter ligation using a 16S Barcoding Kit (SQK-RAB204). Sequencing was performed on MinION Mk1b (Oxford

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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Nanopore Technologies, Oxford, UK) using SpotON flow cell (FLO-MIN107) in a 48h sequencing protocol on MinKNOW 1.10.11 (Juul *et al*., 2015; Kilianski *et al*., 2015; Benítez-Páez *et al*., 2016).

Read 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) (Volden *et al*., 2018). Basecalled reads from each sample were subjected to microbial identification using an EPI2ME desktop agent. 16S rRNA analysis was carried out using the 16S rRNA pipeline from the EPI2ME database (Quick *et al*., 2015; Cao *et al*., 2016). The raw reads were also processed in parallel using the 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 (Bhatia *et al*., 2015; Suzuki *et al*., 2017). The Alpha diversity parameters of the four contrasting climatic zones like Shannon-Weiner diversity index (H'), Simpson's 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 as 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 Genbank database's Sequenced Read Archive (SRA) service maintained by the NCBI server under the accession numbers SRR8003384 to SRR8003387.

RESULTS

Chemical parameters 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 pH and moisture content values. The moisture content percentage was recorded as highest (in TWCZ and lowest in ACZ. The pH was recorded as the lowest in TWCZ compared to ACZ and HSCZ, which were mildly alkaline. MCZ showed a mildly acidic nature with a moderate pH value (Table 2). Total organic carbon (TOC) values did not show any drastic difference and were highest for MCZ. Nitrogen, calcium, magnesium, phosphorus, iron and boron values were recorded as 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 calcium and magnesium values, which were recorded lowest in HSCZ compared to the other climatic zones (Table 2).

Read characteristics and bacterial diversity parameters

A total of 201615 high-quality reads were obtained from the four contrasting climatic zones that were assigned to 16556 bacterial OTUs (Table 3). 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 of OTUs, whereas ACZ had the

Table 2: Chemical parameters of soil samples from four contrasting climatic zones.

Soil properties	TWCZ	ACZ	HSCZ	MCZ
Moisture (%)	13.06	0.07	0.30	7.70
pH	3.94	8.05	8.13	5.44
TOC (mg/kg)	103.07	93.10	94.60	112.27
N (%)	14.11	17.00	11.03	10.90
K (%)	2.90	1.86	2.90	1.88
Ca (%)	4.30	6.07	1.80	8.01
Mg (%)	1.22	2.43	0.40	1.22
P (%)	0.05	0.08	0.07	0.12
Fe (%)	1.09	1.26	1.04	1.13
B (ppm)	10.04	15.12	12.80	11.20

TOC, total organic carbon; N, total nitrogen; K, total potassium; Ca, Calcium; Mg, Magnesium; P, total phosphorous; Fe, Iron; B, Boron.

Table 3: Alpha diversity analysis of four contrasting climatic zones.

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

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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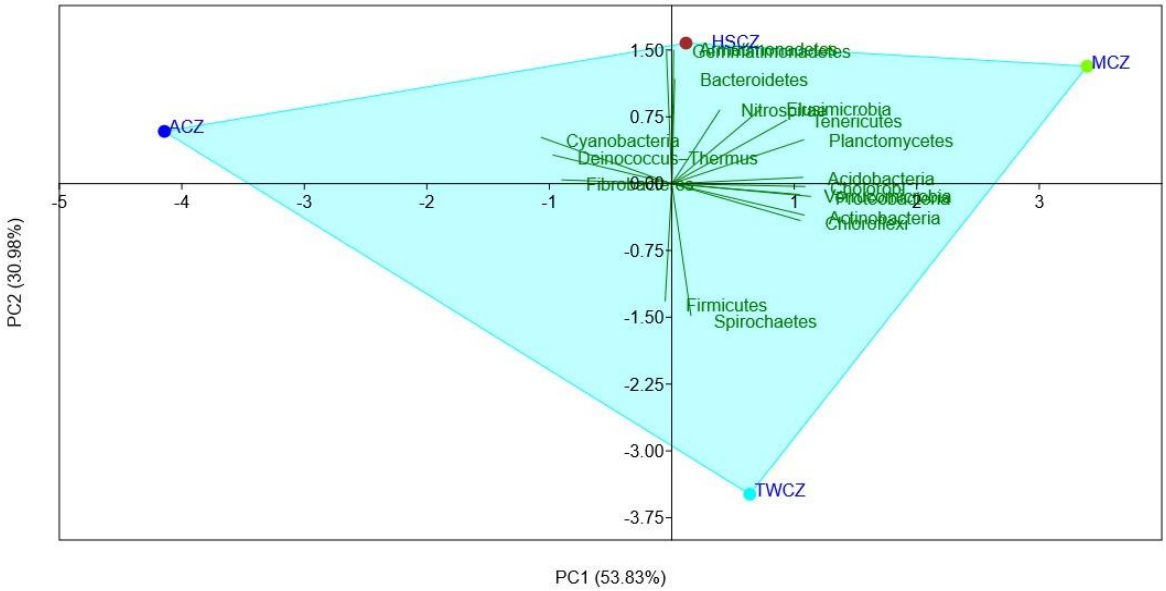

Figure 1: Principal component analysis (PCA) plot of bacterial abundance (OTUs) in four contrasting climatic zones. In this plot, the principal component axes 1 and 2 explain most of the variance in the data cumulatively (PC1 = 53.832% and PC2 = 30.98). Soil metagenomes are represented as blue circular dots.

lowest number of OTUs among the zones. The alpha-diversity parameters, such as 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. There was not much difference in the diversity indices (H', D) among the zones except for ACZ (Table 3). The betadiversity was shown using a principal component analysis (PCA) plot, which revealed a high degree of variance among the bacterial OTUs among the four climatic zones. The first axis of the PCA plot explained 53.832% of the variation in bacterial diversity, while the second axis explained 30.98% of the variance (Figure 1).

Relative abundance of bacterial community composition

The classified sequences (known taxonomy) obtained from all the four contrasting climatic zones were affiliated with eighteen different phyla, which were 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 (Figure 2). Amongst these eight phyla, Phylum Firmicutes appeared as the most dominant phylum, followed by phyla 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 for all these eight phyla compared to other zones. Contrastingly, Cyanobacteria abundance was higher in ACZ than in the other zones. Firmicutes mostly dominated TWCZ with a lower percentage of unclassified bacterial phyla, which was seen as high in MCZ. 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 (Figure 2).

Bacilli and Alpha Proteobacteria 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 Alpha Proteobacteria, Beta Proteobacteria, Planctomycetia and Phycisphaerae were considerably high in MCZ, whereas Bacilli, Actinobacteria and Delta Proteobacteria were less compared to other zones. Similarly, at the order level, the predominance of Bacillales was observed in TWCZ, ACZ and HSCZ compared to MCZ, in which Rhizobiales showed more predominance. In family level distribution, Bacillaceae was predominant in all the zones except in the zone MCZ, where Gemmataceae, Hyphomicrobiaceae, Gaiellaceae, Bradyrhizobiaceae were predominant over Bacillaceae. 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 prevalent 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

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 175-187
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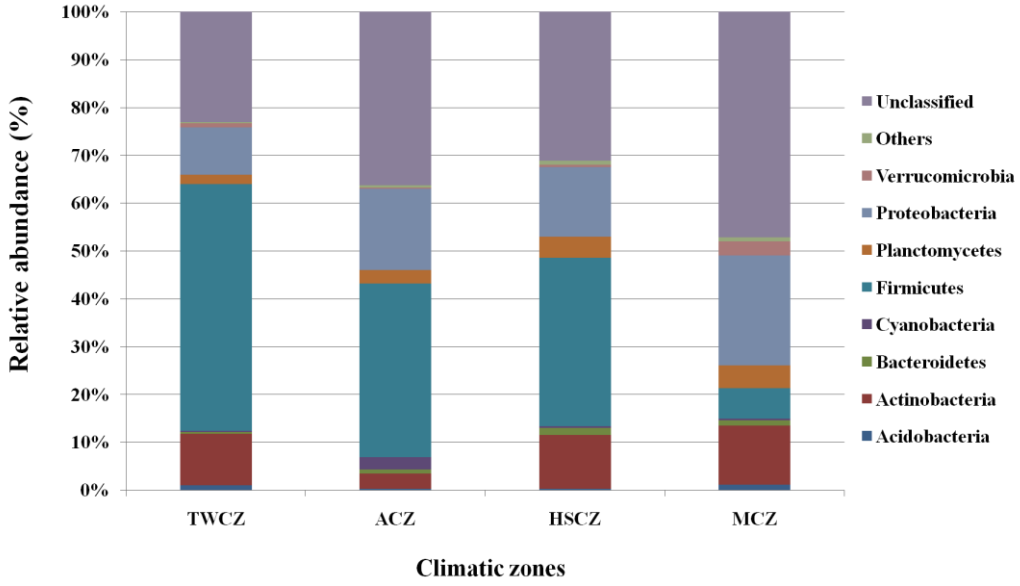

Figure 2: Relative abundance (%) of the major phyla of bacterial community detected in soils collected at four contrasting climatic zones *viz.* TWCZ (tropical wet climatic zone); ACZ (Arid climatic zone); HSCZ (humid subtropical climatic zone); MCZ (Mountain climatic zone). Firmicutes and Proteobacteria were the most dominant phyla among all. "Others" contains the sum of all the minor phyla.

Table 4: Significance of correlation analysis between the soil properties and climatic factors on the Shannon diversity index (H') and OTU abundance at a 95% confidence level.

		No. of OTU's		Shannon diversity index (H')	
		R^2	P	R^2	P
Soil parameters	Moisture	0.391670217	> 0.05	0.20013	> 0.05
	pH	0.365546085	> 0.05	0.176204	> 0.05
	OC	0.286084436	> 0.05	0.141417	> 0.05
	N	0.600331121	> 0.05	0.670161	> 0.05
	K	0.447594506	> 0.05	0.552124	> 0.05
	Ca	0.096543731	> 0.05	0.223924	> 0.05
	Mg	0.738880268	> 0.05	0.898851	≤ 0.05
	P	0.017629721	> 0.05	0.031382	> 0.05
	Fe	0.850538457	> 0.05	0.968315	< 0.05*
	B	0.803720973	> 0.05	0.606002	> 0.05
Climatic factors	MAT	0.331539777	> 0.05	0.293027	> 0.05
	MAP	0.366976302	> 0.05	0.199345	> 0.05

*Significance was observed for Fe at *p*-value<0.05.

abundant genera were 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. A significant share of bacterial communities in the climatic zones was not identifiable at the species level and was categorized as an unidentified bacterium (Figure 3). *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 a high prevalence in MCZ compared to the other three zones. 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.

Correlation analysis with soil profile and climatic factors

The correlation analysis revealed that H' (Shannon diversity) index, S (species richness) and OTU abundance were positively correlated with moisture, TOC, K and MAP (mean annual precipitation). On the other hand, a negative correlation was observed with pH, Ca, N, B. Fe, P, Mg and MAT (mean annual temperature)

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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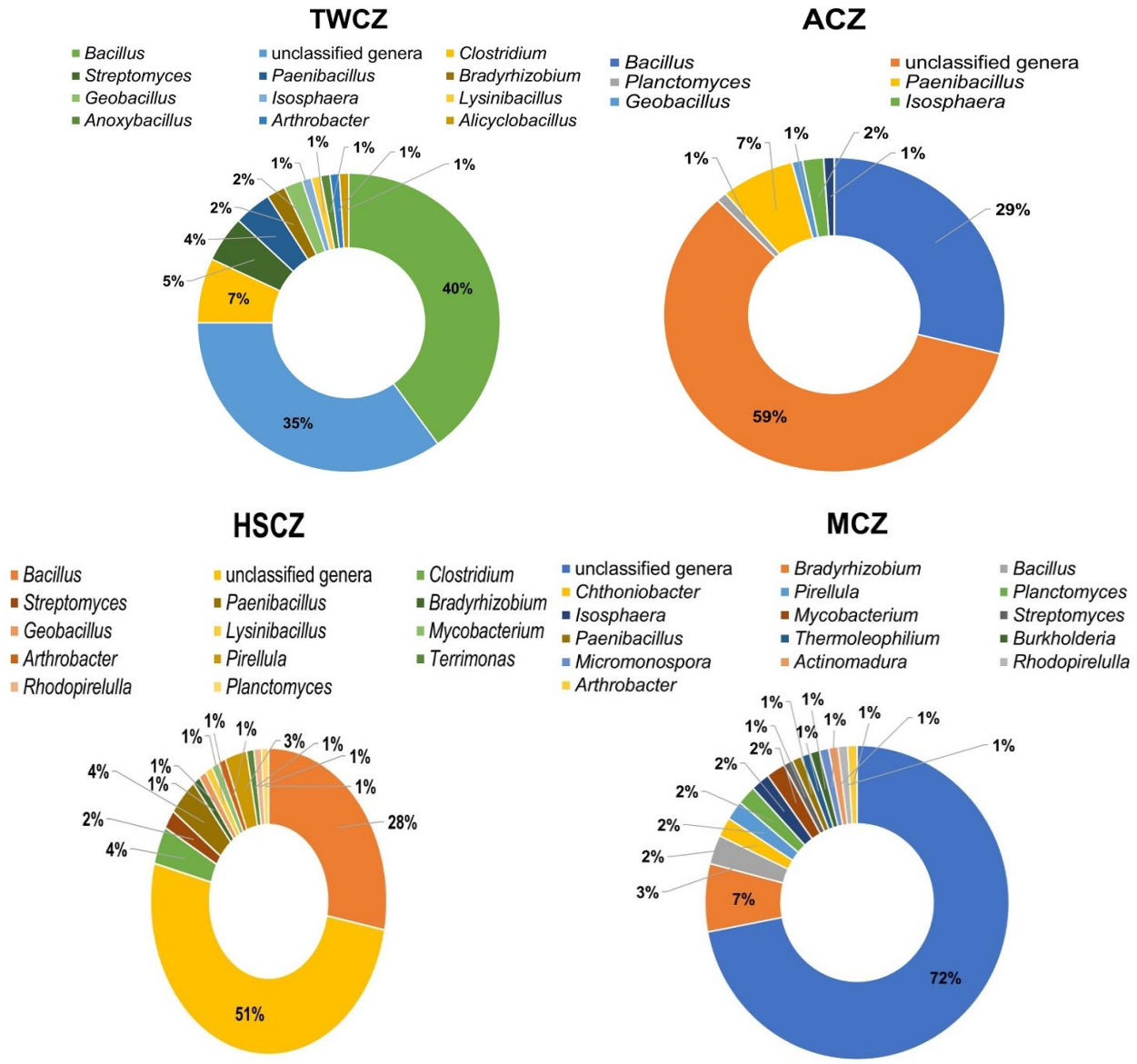

Figure 3: Relative abundance (%) of bacterial genera detected in the four contrasting climatic zones. *viz.* TWCZ (tropical wet climatic zone); ACZ (Arid climatic zone); HSCZ (humid subtropical climatic zone); MCZ (Mountainous climatic zone). *Bacillus* was the most abundant genus in all the zones except MCZ, which was dominated by *Bradyrhizobium*. Major proportion was unclassified.

(Figure 4). The significance of the correlation was tested using a multiple regression analysis in which a significant correlation was observed for only Iron values (p>0.05) against Shannon diversity (Table 4).

DISCUSSION

The community composition and change in the pattern of bacterial diversity in soil microenvironments located in different Köppen climatic zones of the 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 analysing 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 (Table 2). It is evident from several reports that soil pH has a substantial influence on soil diversity and bacterial community composition, suggesting that a neutral pH favours maximum bacterial diversity compared to acidic and alkaline pH (Bartram *et al*., 2014; Cho *et al*., 2016; Kim *et al*., 2016). The pH of soil present in the four

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	No. of OTU's	H'	S	Moisture	pH	TOC	N	K	Ca	Mg	P	Fe	B	MAT	MAP
No. of OTU's	1	0.975497	0.979447	0.625836	-0.6046	0.534869	-0.77481	0.669025	-0.31071	-0.85958	-0.13278	-0.92225	-0.8965	-0.57579	0.605786
H'	0.975497268	1	0.995063	0.447359	-0.41977	0.376055	-0.81863	0.74305	-0.47321	-0.94808	-0.17715	-0.98403	-0.77846	-0.54132	0.446481
S	0.979447339	0.995063	1	0.50144	-0.47057	0.356935	-0.75902	0.785563	-0.4938	-0.92115	-0.25694	-0.97677	-0.80624	-0.47346	0.515949
Moisture	0.625835615	0.447359	0.50144	1	-0.99809	0.698934	-0.1368	0.263727	0.245147	-0.14098	-0.15649	-0.30454	-0.90294	-0.23254	0.97467
pH	-0.604604073	-0.41977	-0.47057	-0.99809	1	-0.73392	0.138978	-0.20642	-0.30293	0.112751	0.099653	0.27059	0.893919	0.262891	-0.9604
TOC	0.534868615	0.376055	0.356935	0.698934	-0.73392	1	-0.52132	-0.23927	0.635949	-0.19014	0.579843	-0.21376	-0.72952	-0.79551	0.523114
N	-0.774810377	-0.81863	-0.75902	-0.1368	0.138978	-0.52132	1	-0.28637	0.149607	0.872855	-0.3809	0.796623	0.527817	0.877917	-0.02847
K	0.669025042	0.74305	0.785563	0.263727	-0.20642	-0.23927	-0.28637	1	-0.86801	-0.70871	-0.77678	-0.80474	-0.46658	0.155888	0.413696
Ca	-0.310714871	-0.47321	-0.4938	0.245147	-0.30293	0.635949	0.149607	-0.86801	1	0.588692	0.743378	0.609977	-0.01777	-0.34179	0.07102
Mg	-0.859581449	-0.94808	-0.92115	-0.14098	0.112751	-0.19014	0.872855	-0.70871	0.588692	1	0.111013	0.978154	0.544596	0.542001	-0.14223
P	-0.13277696	-0.17715	-0.25694	-0.15649	0.099653	0.579843	-0.3809	-0.77678	0.743378	0.111013	1	0.252518	0.09676	-0.72929	-0.37289
Fe	-0.922246419	-0.98403	-0.97677	-0.30454	0.27059	-0.21376	0.796623	-0.80474	0.609977	0.978154	0.252518	1	0.662947	0.456014	-0.32871
B	-0.896504865	-0.77846	-0.80624	-0.90294	0.893919	-0.72952	0.527817	-0.46658	-0.01777	0.544596	0.09676	0.662947	1	0.495942	-0.86339
MAT	-0.575794909	-0.54132	-0.47346	-0.23254	0.262891	-0.79551	0.877917	0.155888	-0.34179	0.542001	-0.72929	0.456014	0.495942	1	-0.04459
MAP	0.60578569	0.446481	0.515949	0.97467	-0.9604	0.523114	-0.02847	0.413696	0.07102	-0.14223	-0.37289	-0.32871	-0.86339	-0.04459	1

Figure 4: Correlation matrix showing the relationship between soil properties and climatic factors with diversity indices. OTU's, Operational taxonomic units; H', Shannon diversity index; S, Species richness index; TOC, Total organic carbon; MAT, Mean annual temperature; MAP, Mean annual precipitation.

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) 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 acidic soils (Xue *et al*., 2018). 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 (Table 3). In contrast, the effect of pH was not seen much in the relatively abundant profiles of predominant phyla Firmicutes and Proteobacteria in the four contrasting climatic zones (Figure 2). The amount of moisture present in the soil depends upon the water-holding capacity of the soil and it has a direct and direct 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 Gamma Proteobacteria 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 (Schnürer *et al*., 1986; Rawat *et al*., 2012; Borowik and Wyszkowska, 2016). 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 a deficient moisture level (0.07%) and TWCZ soil with the highest moisture level (13.06%) (Table 3). In contrast to the previous studies, which reported a decrease in Cyanobacterial abundance in the soil during drought, the present finding showed a higher relative abundance of Cyanobacteria in ACZ than in TWCZ. Having said this, the study only analyzed the soil bacterial diversity present in these zones during the pre-monsoon season. The analysis of the 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 except iron, like TOC (total organic carbon), nitrogen, calcium, magnesium, potassium, phosphorous and boron, do not seem to show considerable influence on bacterial abundance and diversity since no significant correlation was observed with the H' (Shannon diversity index) and OTU abundance (Table 4).

A greater read length and read number would increase sequencing depth, generating more sample information. This depends, to a great extent, on the sequencing methodology used and the MinION sequencer from Oxford Nanopore produces a longer read length, which allows detailed bacterial community characterization down to the family or even genus level at low sequencing cost (Nair and Raja, 2017). However, the accuracy and sequencing output is limited compared to reads obtained using a shorter-read platform like Illumina (Brown *et al*., 2017; Meisner *et al*., 2018). 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 (Table 3). A low-quality sequence could result from different factors but primarily from the quality of DNA obtained during isolation (Sanderson *et al*., 2018). Beta-diversity metrics thus assess the differences between microbial communities. The beta-diversity of the four contrasting climatic zones was analyzed by performing a Principal component analysis (PCA) of the bacterial OTUs, which is a dissimilarity measure for analyzing variation in the samples. The PCA plot of bacterial OTUs showed high variance among the four contrasting climatic zones (Figure 1). This high level of variance and the distinct positioning of the four contrasting climatic zones in the plot indicated that the zones had greater differences in the distribution of different members of bacterial communities.

Considering the taxonomic abundance and bacterial diversity, it was observed that the two major phyla, Firmicutes and Proteobacteria, were predominating in

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

four contrasting Köppen climatic zones (Figure 2). This points out that the soil's prevailing climatic conditions and chemical nature 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 in the climatic zones were Actinobacteria and Planctomycetes, which showed little variation in abundance pattern within and among the zones. *Actinobacteria* constitute high G + C content bacteria, the richest source of bioactive molecules, primarily antibiotics (Mahajan and Balachandran, 2012; Zaheer *et al*., 2018). 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 (Buckley *et al*., 2006). Cyanobacteria are the photosynthetic carbon fixers and are among the members of biological soil crusts present in arid and semi-arid ecosystems, which play an important role in fixing carbon in low vegetative areas as well as in 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 (Tirkey and Adhikary, 2005; Kumar and Adhikary, 2015). 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 (Bergmann *et al*., 2011). The members of the genus *Bacillus* are Gram-positive rods with the ability to produce spores that enable 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, the diversity of its species varies in different climatic zones (Nair and Raja, 2018). 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 (VanInsberghe *et al*., 2015). Studies have also found the presence of *Bradyrhizobium* spp. in deep soil and other soil bacteria inhabited by leguminous trees (Dupuy and Dreyfus, 1992; Thomas *et al*., 1994). The prevalence of this genus in MCZ (Figure 3) 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; the rest remained as unclassified groups (Figure 2). These unclassified groups treasure novel bacteria, isolation and identification, which demands improvised culture techniques.

A correlation analysis was carried out to establish the relationship between soil parameters and bacterial diversity (Figure 4). A positive correlation leads to an increase in community diversity if one of the soil parameters or climatic factors increases, whereas in a negative correlation, this trend is the opposite, and in zero correlation, the parameters have no effect on community diversity. The diversity parameter H' (Shannon diversity) index was positively correlated with TOC (+0.376055), K (+0.74305), moisture (+0.447359) and MAP (0.446481), which tend to increase the diversity of the soil bacterial community in these climatic zones. These observations were also true for the diversity parameter S (Species richness index) (Figure 4). Most of the other parameters had a negative correlation on the diversity profile, in which iron values showed a strong negative correlation (-0.98403) with significance (p>0.05) (Fierer and Jackson, 2006; Lauber *et al*., 2009) (Table 4).

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 abundance pattern among and within the four zones. However, 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 the Indian subcontinent. However, these minor variations in the diversity and composition of bacterial species in different climatic zones may reflect the combined effect of climate, MAT, MAP, some soil parameters and other factors. Although many biotic and abiotic factors are responsible for shaping the soil bacterial community in these climatic zones, the interdependency of the bacterial community, soil parameters and microclimate is certain and could vary among different geographical regions. A detailed temporal study including more samples for more than 2 years could give us a broader picture of the effect of climate and soil factors on specific groups of bacterial communities in these Köppen climatic zones.

ACKNOWLEDGEMENTS

The authors acknowledge the Council of Scientific and Industrial Research (CSIR), India, for the financial support. The funding body had no role in the design of the study and collection, analysis, and interpretation of data, writing the manuscript and the decision to publish. The authors also sincerely thank Dr. R. Vijayakumar, Assistant Professor, Department of Microbiology, Government Arts and Science College, Perambalur, Tamil Nadu, India, for his honest guidance and support.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538