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Diversity of soil bacteria in some sacred patches of Purba Bardhaman District, West Bengal, India

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ABSTRACT

Sacred patches are remnants of prehistoric land that some local populations preserve as a holy place. The diversity of bacterial population was studied from soil samples of five different sacred patches of Purba Bardhaman district in West Bengal. The sites explored were Baba Borthakur Tola at Paraj, Buro Raj Tola at Bhaghason, Dighi Par at Rayna, Maer Tola at Mohindar and Sahadol Tola at Narugram. The aerobic heterotrophic, starch-hydrolyzing, phosphate and lipid solubilizing, spore-forming, Gram negative, nitrate-reducing bacterial populations ranged from 4.66 to 6.79× 10^6, 1.57 to 7.19× 10^6, 2.76 to 5.14× 10^6, 2.30 to 5.65 × 10^6, 1.18 to 5.31 × 10^6, 0.22 to 2.30 × 10^6 and 1.69 to 4.26 × 10^6 cfu/g dry soil respectively. The physical properties of soil samples from the study sites were also evaluated. The organic carbon content, available nitrate nitrogen and phosphate was found to vary from 0.38% to 0.75%, 34.08 to 213.02 mg/kg and 102.25 to 477.18 mg/kg respectively. The bacterial population was found to vary significantly ($p < 0.05$) among the soil samples from the sacred patches studied as was shown by the results of one-way ANOVA. Three components with Eigenvalues of 2.893, 1.503 and 0.548 respectively were extracted from principal component analysis (PCA). Hierarchical classical clustering between the different groups of soil bacteria aided in deciphering the similarities between them. The Rényi diversity profiles helped to understand the diversity, richness and evenness of the soil bacterial population in the sacred patches. The results showed that Buro Raj Tola was the most diverse while Baba Borthakur Tola was the least diverse. The Berger-Parker index was, however, highest for Maer Tola (0.2553) indicating that the dominant species account for the majority of the total bacterial population in the area. The variations observed was probably because of the differences in the microhabitat in the different sacred patches studied.

Key words: *Diversity, Soil bacteria, Sacred patch, Rényi Diversity profiles, Principal component analysis (PCA), Hierarchical classical clustering.*

Introduction

In the recent years high rates of deforestation all over the world has led to ecological degradation. Surprisingly, the sacred patches have been able to survive these destructive activities over a long pe-

riod of time. The sacred patches are considered to be the abode of Gods and Goddesses and their origins are associated with religious beliefs. These small areas of natural vegetation have been preserved since ages mainly out of fear and superstitions that dominate among the local communities associated with them. As such, they form important sites for the study of interactions between different components of the ecosystem that contribute to the conservation of biodiversity (Edwards *et al.,* 1998; Kayang, 2006). Research activities have been undertaken from time to time to study the floral and faunal diversity of these sacred patches but the soil microbiota of sacred patches have rarely been explored (Devi and Dkhar, 2014). The soil microbiota are pivotal members of the biotic community in a forest ecosystem because they play important roles in regulating them primarily through nutrient mobilizations (Hackl *et al.,* 2004). Earlier studies have highlighted the importance of the understanding of microbial diversity in relation to soil ecology and the associated ecosystems (Atlas, 1984). The soil microbial diversity also determines the resilience to soil ecosystem disturbances (Devi and Dkhar, 2014).

The forest floor of sacred patches have often been observed with certain formations of biological soil crusts which influence the structure of the soil and the activities of the soil microbiota (Vinoth *et al.,* 2017). The soil microbial processes are also responsible for providing the plants with nutrients thereby regulating the natural ecosystem's net primary productivity (Paul and Clark, 1997). The variation in the diversity of soil microbiota depends on species composition of plants, seasonal fluctuations, temperature, type of the soil and its organic matter composition (McCulley and Burke, 2004). It also depends upon the physicochemical characteristics of the soil (Sathe *et al.,* 2018). Alterations in soil microbial populations may also serve to indicate alterations in soil quality as they are very sensitive and respond more readily than soil carbon or nitrogen (Kennedy and Papendick, 1995). Soil may be considered to be an aggregation of diverse microhabitats with significant variations of environmental conditions in them (Sathe *et al.,* 2018). Thus, soil offers diversified ecological niches (Sathe *et al.,* 2018) for the microbiota to flourish differentially depending on their specific requirements. The microbial population of soil consists of five major groups, namely, fungi, algae, bacteria, protozoa and actinomycetes (Holt, 1986). The bacterial population is comparatively higher than others (Alexander, 1978). Therefore, a knowledge of the bacterial population in the soil samples from the sacred patches helps in the holistic understanding of their ecological functioning.

The district of Purba Bardhaman is home to a number of such sacred patches all of which are, in a way, associated with nature worship springing from religious beliefs. Over the years they have offered protection to several important plant and animal species which might have been eroded from the adjoining areas. Thus, these patches are a storehouse of genetic information of various organisms in their original environs that speak of their ecological positions and levels of interactions (McNeely, 1994; Campbell *et al.,* 1996). However, the soil microbiota of these patches has rarely been explored. The current investigation was undertaken to study the diversity of bacterial population and the physicochemical characteristics of the soil samples collected from some sacred patches of Purba Bardhaman district, West Bengal, India.

Materials and Methods

Study sites

Five sacred patches were selected from the district of Purba Bardhaman, West Bengal, India. These were Baba Borthakur Tola, Paraj (23°23'53.95"N 87°36'13.91"E); Buro Raj Tola, Bhaghason (23°25' 22.55"N 88°03'36.88"E); Dighi Par, Rayna (23°03'51.91"N 87°53'37.15"E); Maer Tola, Mohindar (23°04'18.11"N 88°03'17.06"E) and Sahadol Tola, Narugram (23°08'27.09"N 87°53'44.18"E)(Figure 1).

Collection of soil samples

Soil samples were collected from November, 2019 to February, 2020. About 100 g of soil was collected from each sacred patch after scrapping off the topmost soil layer (1 cm depth). They were then transferred to sterile polythene bags and sealed with rubber bands. Following this, they were carried to the Microbiology and Parasitology Research Laboratory, The University of Burdwan, for physicochemical and microbial analyses.

Analysis of soil samples

For isolation of bacterial population serial dilution was done. To determine the heterotrophic viable aerobic bacterial population, soil samples were diluted upto 10^{-5} and a 10 µl soil suspension (10^{-5}) was

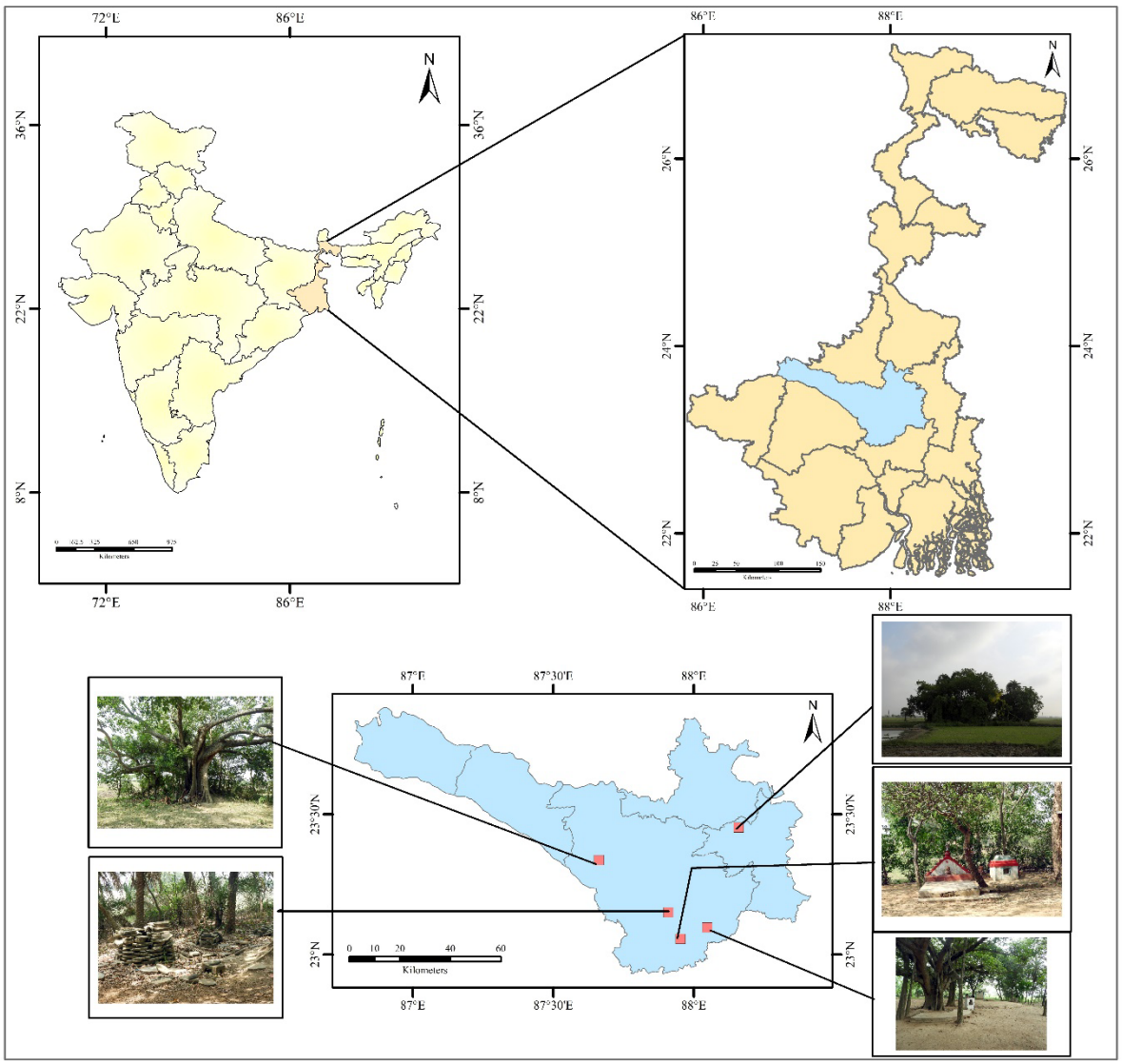

Fig. 1. Location map of the five sacred patches of Purba Bardhaman, West Bengal, India

mixed with 100 ml nutrient agar (peptone 5 g/l, beef extract 3 g/l, agar 2 g/l, pH 7). The mixture was then incubated at 30 ± 1°C in a BOD incubator. In order to determine different bacterial groups separately, 10 µl of soil suspension (10^{-4}) was mixed with 100 ml of different specific media in five different plates and incubated at 30 ± 1°C in a BOD incubator. The soil suspension was pasteurized for 30 minutes at 60 °C to stimulate the growth of the spore formers.To identify the starch hydrolyzing bacteria the soil samples were incubated on starch agar media for 24 hours followed by flooding the colonies with Gram's iodine. The bacterial colonies that gave a halo zone (i.e. positive result) were counted and recorded. All other groups of bacterial populations were counted after an incubation period of 3 days. The aerobic heterotrophic and spore forming bacteria were studied using nutrient agar media following the standard methods. The Gram-negative bacteria were made visible by adding crystal violet to the medium (peptone 5g/l, beef extract 3 g/l, lactose 10 g/l, crystal violet 0.0033 g/l, agar 15 g/l, pH 6.8±0.1) before plating. Formation of halo zone around the colonies on insoluble phosphate [$Ca_3(PO_4)_2$] containing medium helped to identify the inorganic phosphate-solubilizing bacteria. The Colony Forming Unit (cfu) per gram soil was calculated on dry weight basis. Standard methodologies were followed to determine the physico-chemical parameters of the soil samples (Sumner and Miller,

1996).

Statistical analyses

Visual and quantitative comparison of multiple aspects of diversity can be studied by using a technique called diversity ordering (Patil and Taillie, 1977) which yields diversity curves, known as diversity profiles (Kindt and Coe, 2005). In this sudy, the exponential Rényi diversity profiles have been used to understand the diversity, richness and evenness of the bacterial population in the soil samples collected from the sacred patches of Purba Bardhaman. The values of Rényi diversity profiles are based on alpha parameters (Lupatini *et al.*, 2013). The Rényi diversity index is denoted by H-alpha (H). Alpha (α) is a scale function with a value varying from zero to infinity. The calculations of the diversity profile values (H-alpha) are based on the formula (Eq. 1).

$$H_\alpha = {\ln\left(\sum p_i^\alpha\right)}/{1-\alpha} \quad \text{.. (Eq. 1)}$$

where p_i stands for proportional adundances (or, p_i = abundance of species i/total abundance) (Tóthmérész, 1995). The commonly studied four diversity indices from Rényi's entropy formula show relation to the corresponding values of α as H_0 = species richness, H_1 = Shannon Diversity Index, H_2 = Simpson Diversity Index and H_∞ = Berger-Parker Index (Kindt *et al.*, 2006).

To identify the various classes of bacteria found in soil samples from sacred patches, Hierarchical classical clustering was performed using a single linkage algorithm with a Morisita similarity index and 10,000 bootstraps between sites. Principal component analysis (PCA) was performed to represent a relationship among the different bacterial groups (Manly, 2004). Quadrant distribution of the sacred patches studied was also done.

All the statistical analyses were performed using the PAST software (Version 4.03). The Quadrant distribution of the sacred patches was done using STATA 14.

Results

In the soil samples, collected from different sacred patches of Purba Bardhaman (Figure 1), the aerobic heterotrophic, starch hydrolyzing, phosphate solubilizing, lipid solubilizing, spore forming, Gram negative and nitrate reducing bacterial populations ranged from 4.66 to 6.79× 10^6, 1.57 to 7.19× 10^6, 2.76 to 5.14× 10^6, 2.30 to 5.65 × 10^6, 1.18 to 5.31 × 10^6, 0.22 to 2.30 × $10^6$and 1.69 to 4.26 × 10^6cfu/g dry soil, respectively (Table 1). The aerobic heterotrophic and nitrate reducing bacterial population were higher in the soil samples collected from Baba Borthakur Tola compared to that of other sacred patches(Table 1). Similarly, the spore forming and Gram-negative bacterial population were found to be the highest at Buro Raj Tola (Table 1). The lipid solubilising bacterial population was the highest at Dighi Par, Rayna (Table 1). The starch hydrolyzing bacterial population was found to be the highest in the soil samples of Sahadol Tola (Table 1). Phosphate solubilising bacterial population was the highest at Maer Tola (Table 1).

The organic carbon content of the soil samples collected from the sacred patches ranged from 0.38 to 0.75 % with the highest being recorded from Baba Borthakur Tola (0.75 %) (Table 2). The available nitrate nitrogen content of the soil samples from the study areas varied from 34.08 to 213.02 mg/kg with the highest being obtained from Baba Borthakur Tola (213.0 mg/kg) (Table 2). The available ammo-

Table 1. Bacterial population (cfu/g dry soil) of different sacred patches of Purba Bardhaman, West Bengal, India.

Bacterial population [(Mean± SE)× 10^6)]	Sacred Patches				
	Baba Borthakur Tola	Buro Raj Tola	Dighi Par, Rayna	Maer Tola	Sahadol Tola
Heterotrophic	6.79±0.020	6.08±0.136	4.66±0.08	6.63±0.017	5.73±0.029
Starch-hydrolyzing	3.66±0.032	2.14±0.083	5.83±0.052	1.57±0.128	6.19±0.074
Phosphate solubilising	5.05±0.034	4.68±0.032	2.76±0.024	5.14±0.090	3.14±0.020
Lipid solubilising	2.93±0.045	2.30±0.081	5.65±0.020	3.24±0.037	5.42±0.056
Spore forming	1.18±0.032	5.31±0.043	2.15±0.034	2.25±0.120	3.21±0.035
Gram-negative	0.22±0.028	2.30±0.040	0.59±0.020	0.83±0.035	1.16±0.170
Nitrate-reducing	4.26±0.026	2.16±0.054	1.69±0.137	3.84±0.028	1.83±0.057

Results are means of nine replications
cfu= colony-forming unit

nium nitrogen in the soils of the study area varied from 102.25 to 222.04 mg/kg and was the highest at Buro Raj Tola (222.04 mg/kg) (Table 2). The available phosphate content in the soil samples of the sacred patches ranged from 102.25 to 477.18 mg/kg (Table 2). The phosphate content recorded from Maer Tola was 477.18 mg/kg which was the highest among all the sacred patches (Table 2). The available potassium content of the soil samples from the sacred patches chosen for the study varied from 2726.76 to 3340.28 mg/kg (Table 2). It was the highest at Dighi Par (3340.28 mg/kg) (Table 2). The soil pH of the sacred patches studied ranged from 6.0 (at Maer Tola) to 7.6 (at Baba Borthakur Tola) (Table 2).

The one-way ANOVA findings revealed major differences ($p = 0.001159$) in microbial diversity with regard to the relevant sacred patches. Comparative study of the diversity profiles for the sacred patches showed that they could not be clearly separated based on species richness (H_0) (Figure 2, Table 3). Since the diversity profile curves of Dighi Par and Maer Tola cross each other they could not be compared in terms of diversity (Figure 2). Close inspection of Shannon's Diversity Index (H_1) for all the sacred patches showed that Buro Raj Tola has the highest value (1.849) while Baba Borthakur Tola (1.724) has the lowest (Table 3). However, the dominance indices (i.e. H_2 and beyond) showed Maer Tola to have the highest value (0.2553) followed by Baba Borthakur Tola (0.2491), Buro Raj Tola (0.2403), Sahadol Tola (0.2249) and Dighi Par (0.2143) (Figure 2, Table 3).

In the Principal Component Analysis (PCA) three

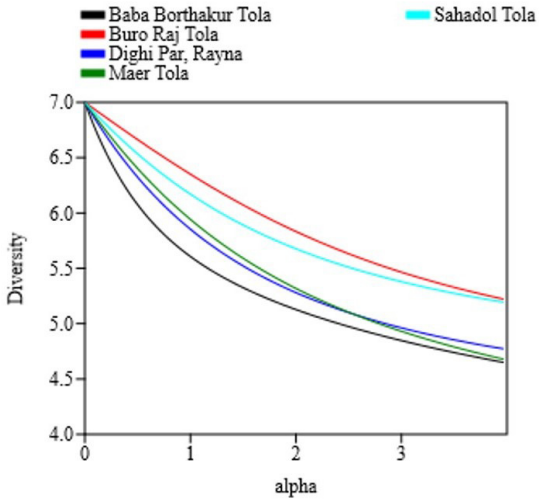

Fig. 2. Rényi diversity profiles of soil bacterial population from different sacred patches of Purba Bardhaman, West Bengal, India. [The Rényi formula value is shown on the x-axis, and the Rényi diversity profile values (Hα) are shown on the y-axis. α values at the scales of 0, 1 and 2 are associated with species richness, Shannon diversity index and Simpson diversity index respectively.]

Table 2. Physicochemical parameters of soil samples collected from different sacred patches of Purba Bardhaman, West Bengal, India.

Sacred Patches	Organic carbon (%)	Ammonium Nitrogen (mg/kg)	Nitrate nitrogen (mg/kg)	Phosphate (mg/kg)	Potassium (mg/kg)	Soil pH
Baba Borthakur Tola	0.75	127.81	213.02	434.57	2982.39	7.6
Buro Raj Tola	0.57	222.04	51.12	340.84	3297.67	7.5
Dighi Par, Rayna	0.38	102.25	34.08	102.25	3340.28	7.4
Maer Tola	0.72	187.46	170.42	477.18	3084.65	6.0
Sahadol Tola	0.55	144.85	42.60	178.94	2726.76	7

Table 3. Hα values for some important scales of α (obtained from Rényi diversity profiles of soil bacterial population of different sacred patches of Purba Bardhaman, West Bengal, India). [H_0, H_1, H_2 and H_∞ are associated with species richness, Shannon diversity index, Simpson diversity index and Berger-Parker index respectively.]

Study areas	H_0	H_1	H_2	H_∞
Baba Borthakur Tola	0.845	1.724	0.805	0.2491
Buro Raj Tola	0.845	1.849	0.8286	0.2403
Dighi Par, Rayna	0.845	1.767	0.8106	0.2143
Maer Tola	0.845	1.782	0.812	0.2553
Sahadol Tola	0.845	1.82	0.8239	0.2249

components with Eigenvalues of 2.893, 1.503 and 0.548 respectively were extracted that explained more than 98% (component 1—57.87%, component 2—30.07 % and component 3—10.97 %)of the variance of the microbial abundance in the soil samples collected from the sacred patches of Purba Bardhaman (Figure 3, Table 4). The five sacred patches were plotted on quadrant plot and among the four quadrants Buro Raj Tola, Maer Tola and Baba Borthakur Tola were in the second quadrant while Sahadol Tola and Dighi Par were in the fourth quadrant (Figure 4). Hierarchical cluster analysis of the different bacterial groups in the soil samples from different sacred patches showed that the heterotrophic, starch hydrolyzing, phosphate solubilising, lipid solubilising, spore forming, Gram negative and nitrate reducing bacteria formed clusters in respect of their distribution pattern (Figure 5). The spore forming and Gram-negative bacterial population showed a close association with 91% similarity (Figure 5). Starch-hydrolyzing and lipid solubilising bacteria showed an association with 82% similarity (Figure 5). Phosphate solubilising and heterotrophic bacterial groups formed a cluster having 72% similarity and this cluster linked with

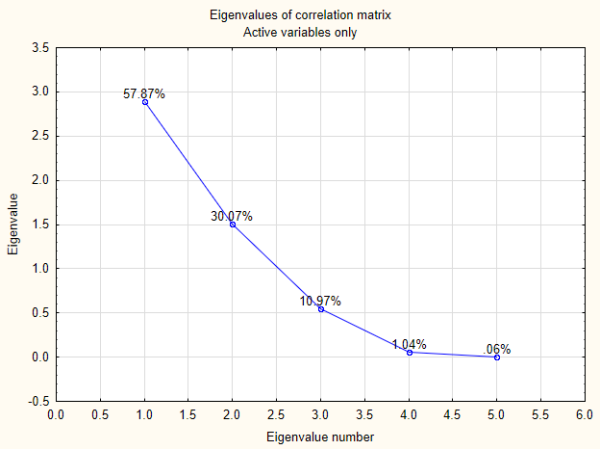

Fig. 3. PCA scree plot of the bacterial isolates of the sacred patches in Purba Bardhaman, West Bengal, India.

Table 4. Summary of Principal Component Analysis (PCA)

	Eigen value	Variance (%)	Cumulative (%)
F1	2.893	57.865	57.865
F2	1.503	30.069	87.934
F3	0.548	10.973	98.908

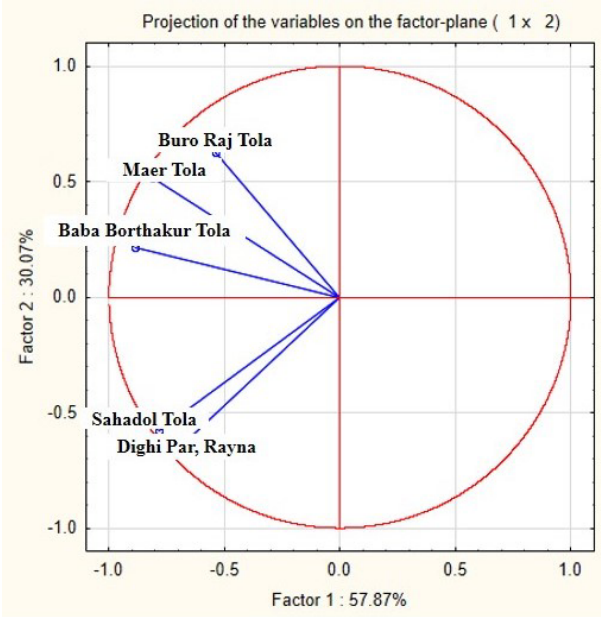

Fig. 4. Quadrant distribution of different sacred patches of Purba Bardhaman, West Bengal, India.

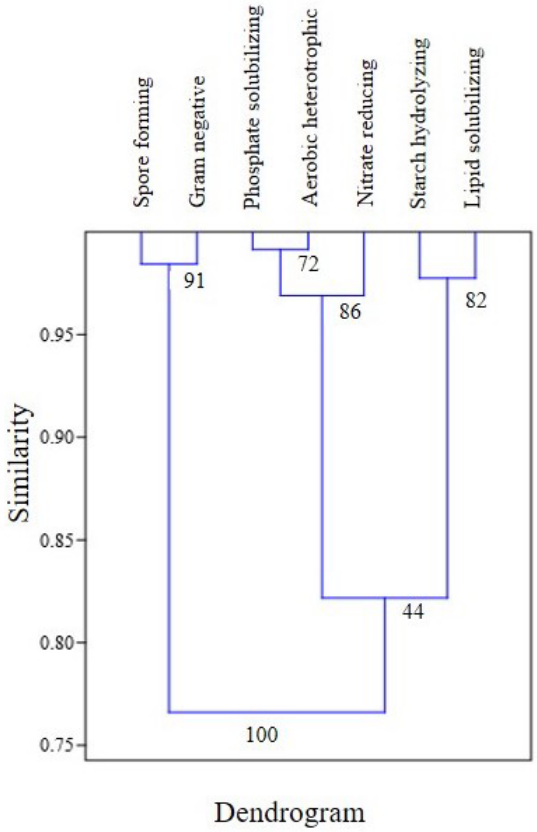

Fig. 5. Agglomerative hierarchical cluster analysis (AHC) of the soil bacterial isolates of different sacred patches of Purba Bardhaman, West Bengal, India.

nitrate reducing bacterial group with 86% similarity (Figure 5). Correlation matrix among different groups of bacterial population and physiochemical parameters of the soil samples showed strong positive corelation between organic carbon and aerobic heterotrophic bacteria (0.987), phosphate and aerobic heterotrophic bacteria (0.944), phosphate and phosphate solubilising bacteria (0.986), nitrate nitrogen and nitrate-reducing bacterial group (0.996) (Figure 6, Table 5).

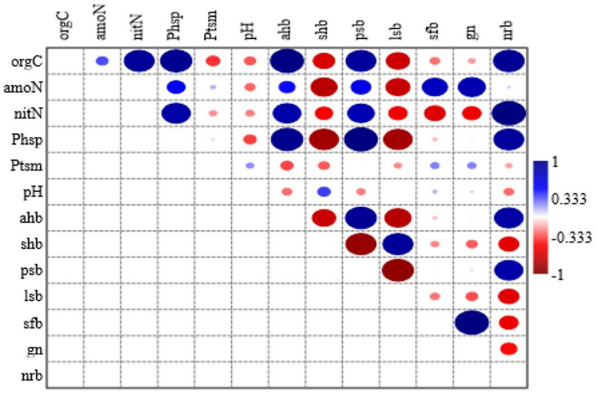

Fig. 6. Pearson correlations plot ($p < 0.05$ boxed) of soil bacterial population and soil physiochemical parameters from some sacred patches of Purba Bardhaman, West Bengal (**ahb** - aerobic heterotrophic bacteria; **shb**- starch-hydrolyzing bacteria; **psb** - phosphate-solubilising bacteria; **lsb** - lipid solubilizing bacteria; **sfb** - spore-forming bacteria; **gnb** - Gram-negative bacteria; **nrb** - nitrate-reducing bacteria; **orgC** - organic carbon; **amoN** - ammonium nitrogen; **nitN** - nitrate nitrogen; **Phsp** - phosphate; **Ptsm** - potassium).

Discussion

The soil microorganisms cause decomposition of the organic matter leading to the release of minerals which in turn enhance the nutrient content of the soil (Liu *et al.*, 2006). Thus, they play important roles in nutrient cycling (Liu *et al.*, 2006) which in turn inflences the soil fertility (Yao *et al.*, 2000). Besides exerting their effects on the belowground biodiversity, the microorganisms inhabiting the soil also affect the aboveground biodiversity through their roles in the formation of soil structures (Dodd *et al.*, 2000), plant health (Smith and Goodman, 1999) and plant nutrition (George *et al.*, 1995). Therefore, the characterization of the soil samples, to a large extent, depends upon the qualitative and quantitative assay of soil bacteria (Arunachalam and Arunachalam, 2000). The composition of the soil microbial population depends upon the physicochemical parameters of the soil and the differences in the microhabitats (Sathe *et al.*, 2018). Though the soil microbial diversity of sacred patches have rarely been studied some of them include works by Vinoth *et al.* (2017) and Lyngwi and Joshi (2015). The study on the nutrient status of soil samples from some sacred patches of Maharashtra by Sathe *et al.* (2018) is also worth mentioning. However, similar studies on soil microbial diversity from different areas, other than sacred patches, include those by Das *et al.* (2013), Radhakrishnan and Varadharajan (2016), Azmi and Chatterjee (2016), Sharma *et al.* (2019);

Table 5. Correlation matrix among soil bacterial population and soil physiochemical parameters.

	orgC	amoN	nitN	Phsp	Ptsm	pH	ahb	shb	psb	lsb	sfb	gn	nrb
orgC													
amoN	0.350												
nitN	0.894	-0.001											
Phsp	0.934	0.530	0.846										
Ptsm	-0.404	0.150	-0.220	-0.076									
pH	-0.333	-0.297	-0.240	-0.373	0.224								
ahb	0.987	0.479	0.825	0.944	-0.364	-0.287							
shb	-0.450	-0.551	-0.501	-0.739	-0.611	0.205	-0.495						
psb	0.891	0.591	0.791	0.986	0.024	-0.252	0.921	-0.791					
lsb	-0.702	-0.710	-0.552	-0.854	-0.222	-0.006	-0.783	0.813	-0.927				
sfb	-0.280	0.749	-0.624	-0.133	0.248	0.137	-0.124	-0.083	-0.026	-0.272			
gn	-0.198	0.808	-0.553	-0.041	0.245	0.086	-0.041	-0.156	0.064	-0.348	0.996		
nrb	0.914	0.087	0.996	0.890	-0.185	-0.287	0.856	-0.563	0.830	-0.612	-0.559	-0.483	

ahb - aerobic heterotrophic bacteria; **shb**- starch-hydrolyzing bacteria; **psb** - phosphate-solubilising bacteria; **lsb** - lipid solubilizing bacteria; **sfb** - spore-forming bacteria; **gnb** - Gram-negative bacteria; **nrb** - nitrate-reducing bacteria; **orgC** - organic carbon; **amoN** - ammonium nitrogen; **nitN** - nitrate nitrogen; **Phsp** - phosphate; **Ptsm** - potassium.

Kumar *et al.* (2019); Dhakar and Pandey (2020); Kavitha *et al.* (2020) and Liu *et al.* (2020).

The viable plate count method was applied to study the bacterial diversity of the soil samples in the current investigation. The aerobic heterotrophic bacterial population from the sacred patches were quite high which indicates high composition of organic matters in the soil. This result is in agreement with Kaur and Singh (2000) who opined that the heterotrophic bacterial population was low in sandy soils because of its poor organic content compared to that of clay or humus soils. The organic carbon was found to be the highest in the soil of Baba Borthakur Tola (0.75%) followed by Maer Tola (0.72%) which were also the sites where the aerobic heterotrophic bacterial population were comparatively higher than those of other scared patches. The results corroborates the findings of Dinesh *et al.* (2004) who stated that organic carbon influences soil microbial growth. The ability of the Gram-positive bacteria to withstand stress and produce endospores allows it to flourish over the Gram-negative bacterial population (Hecker *et al.*, 2007). Aerobic endospore-forming bacteria regulate the soil nutrient cycles like the nitrogen cycle. The spore forming bacteria can degrade hemicellulose, cellulose and pectin thereby playing pivotal roles in mineralizing plant and humic materials (Mandic-Mulec and James, 2011). Additionally, their chitinase activities may aid in the breakdown of fungal cell walls and insect exoskeletons (Mandic-Mulec and James, 2011). In the current study, the spore forming bacteria was found to cluster with Gram negative bacteria. Probably the spore forming bacteria provide nutrients that support the survival and growth of other species. The microbial population of soils has also been found to be influenced by various abiotic factors (Bever, 1994). Elevated levels of available phosphate and potassium may help the bacterial population to flourish or, the other way round, higher concentration of these nutrients may result from increased turnover rates due to mircobial activity (Bever, 1994). Soil microorganisms like bacteria and fungi have pivotal roles in augmenting the bioavailability of certain nutrients like potassium, phosphorus and iron by mobilizing them (Rashid *et al.*, 2016). This has an important impact on soil fertility and plant growth. The nutrients also help the plants to adapt themselves to various biotic and abiotic stresses and prevent invasion by pathogens (Wang *et al.*, 2020). The phosphate content of the soil collected from Maer Tola was the highest (477.18 mg/kg)among all the scared patches studied. This explains the high population of phosphate solubilising bacteria in the area. Similarly, the high nitrogen content in the soil samples from Baba Borthakur Tola might be due to the high density of nitrate reducing bacteria in them. The ANOVA outcome showed that the relative density of the microbes varied in the soil samples collected from the sacred patches considered in this study ($p<0.05$). The reason for such variation may be attributed to the differences in the microhabitats of the areas studied which, in a way, may be correlated to the physico-chemical parameters of the soil from these sacred patches as well. These results again support the views of earlier workers like Bever (1994), Mandic-Mulec and James (2011), Azmi and Chatterjee (2016), Rashid *et al.* (2016) and Wang *et al.* (2020). The correlation among the bacterial groups depending on their relative density was highlighted by clustering analysis (Fig. 5). The principal component analysis (PCA) further helped to understand the association among the bacterial groups depending on their abundance in the soil samples collected from different sacred patches of Purba Bardhaman. Earlier studies have shown that microbial population varies with the depth of soil which in turn is related to the availability of organic matters, mineral nutrients, temperature and moisture (Kayang, 2006). It has also been suggested that these parameters vary with seasons as a result of which the microbial growth also varies accordingly (Kayang, 2006). Thus, the microbial population of soil not only depends on its physio-chemical parameters but also the climatic regime and vegetation type that dominates the area (Zeller *et al.*, 2001; Kayang, 2006).

In diversity studies, it has been observed that, single indices-based ranking is often erroneous because the order of ranking frequently alters with different indices (Tüthmérész, 1995). Therefore, diversity ordering techniques have been found to be more suitable (Tüthmérész, 1995). In the current investigation, the Rényi diversity profiles helped to discern the diversity of the soil bacterial communities in the sacred patches studied. It is a well known fact that Shannon's Diversity index combines evenness and richness while Berger-Parker and Simpson's indices represents the dominance or evenness (Oldeland *et al.*, 2010). Comparative analysis of the Shannon's Diversity index (H_1), for the sacred patches being studied, showed that Buro Raj Tola has the most di-

verse composition of soil bacteria while Baba Borthakur Tola has the least diverse composition. Similar results were also indicated by the Simpson's Diversity index (H_2). The reason for such high diversity of Buro Raj Tola may be because of the combination of physicochemical factors of the soil like organic carbon, available nitrogen, etc. in the region that favoured the growth of diverse bacterial population. The Berger-Parker index implies the dominance of the most abundant species compared to the sum total of all the available species in the sample being studied (Caruso *et al.,* 2006). Therefore, high values of Berger-Parker index indicates an uneven distribution of species in an assemblage. However, this index is influenced by environmental factors and other disturbances (Noti *et al.,* 2003; Lindo and Visser, 2004). In the current study, Maer Tola show the highest Berger-Parker index (H_∞) value which means that the dominant species of soil bacteria are the ones most commonly encountered and accounts for the total number of bacteria at the study site. Again, the physicochemical parameters of the soil may be responsible for this variation though the dominant vegetation of the sacred patches may also influence the species composition of the soil bacteria (Osborne *et al.,* 2011). The similarities or dissimilarities between the diversities of the sacred patches studied donot, however, indicate the same species composition of the bacterial communities at different sites (Lupatini *et al.,* 2013). The species composition may change with time due to environmental factors whereby certain species may be replaced by others which are better adapted to the changing conditions (Lupatini *et al.* 2013).

Conclusion

Sacred patches are small undisturbed areas harbouring a rich diversity of flora and fauna which is richer than the surrounding areas. They may be considered as natural laboratories where the environmental factors have played their roles over a long period of time without any hindrance from anthropogenic activities. Undoubtedly, soil is an important part of such sacred patches along with its microorganisms which play multifarious roles in determining the soil fertility, mobilization of nutrients, soil-health and thereby plant growth. However, there is a dearth of information on soil bacteria from sacred patches. The current investigation showed that the soil bacterial groups are quite diverse and varies among the sacred patches. Further studies may be conducted to identify the distribution of individual species of soil bacteria in these patches.Thus, databases may be generated and comparative analyses may be carried out between different areas to develop a better understanding about the diversity in these sacred patches. The abundance of PGPB (plant growth promoting bacteria) in the soil samples from sacred patches have been highlighted by Lyngwi and Joshi (2015). They have important roles in agriculture as they promote plant growth (Lyngwi and Joshi, 2015). Therefore, the sacred patches may also be an important source of numerous economically important microbes which also needs to be explored.

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