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Variations in bacterial population numbers and enzyme activities in different land-use systems of Brahmaputra valley, Assam

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Abstract
A culture-based approach was used, in the present investigation, to assess the soil bacterial diversity, while enzyme activities such as dehydrogenase, phosphatase and urease were estimated using standard protocols. Degraded forest (DF) and relatively less disturbed forest (RLDF) were the two different land-use systems used, here, to assess the soil bacterial diversity and enzymatic activity. Soil samples were collected randomly at different depths, in four different seasons. A total of 76 bacterial isolates were isolated and characterized based on their morphological and biochemical properties. Occurrence of Gram-negative bacteria were more over Gram-positive bacteria. More bacterial population numbers and enzyme activities were recorded at surface soil that decreased with increase in vertical depths. Maximum bacterial populations as well as activities of different enzymes were recorded during spring season at RLDF while the value was minimum during winter season at DF. Bacterial genera like *Actinobacteria* sp., *Burkholderia* sp., *Chromobacterium* sp., *Escherichia coli*, *Enterobacter* sp., *Micrococcus* sp., *Pseudomonas* sp., *Serratia* sp., showed their restricted distributions at particular land-use system. Significant positive correlation was observed between the bacterial population number and soil pH at both the surface and subsurface soils (R^2=0.993 and 0.954 respectively) in RLDF. The data are significantly different from each other at significance level of $P< 0.05$. The results of the present investigation indicated that difference in soil depth, seasonal variation and land-use systems has a potential influence on the existence of bacterial population numbers and soil enzyme activities.

Keywords: Culture-based approach, degraded forest, enzyme activity, seasonal variation, relatively less disturbed forest, soil bacterial diversity, vertical depths.

1. Introduction
Soil is the unique natural environment [1] representing good reservoir of bacteria. However, unfortunately, very little information is available on true bacterial life in soil since most of the studies [2] related to soil bacteriology have generally been focused exclusively on the surface soil of 10-40 cm. To explore more bacterial diversity we need to study the pristine soil locations like subsurface soil [3]. Subsurface soil environments are both physically and chemically heterogeneous [4]. The fact that bacteria are essential for the entire ecosystem since they perform numerous functions like maintenance of biogeochemical cycles has spurred keen interest in scientists for the exploration of the vast resource of soil bacterial diversity [5]. Soil bacteria are also known to influence the soil physical, chemical and biological properties [6], the knowledge on which is important to develop the technologies like microbial bioprospecting, molecular biology and biotechnology.
Soil enzyme activities are considered as natural indicators of soil health [7]. Soil enzyme activities basically include the activities of dehydrogenase, urease and phosphatase enzymes. Dehydrogenase is considered to play an essential role in the oxidation of soil organic matter [8]. Enzyme urease is responsible for the breakdown of urea into CO_2 and NH_3 [9], thus, it is important for maintaining the N_2-economy of soil. While, the intensity of phosphatase is crucial as it affects the rate of phosphorus cycling [10]. Soil enzyme activities, however, might respond differently to factors like land-use system, soil depth, season, vegetation types etc. [3].
North-east India is the bio geographical gateway of greater India that can be considered as one of the richest biodiversity hot-spot zone [11, 12]. The state of Assam has been regarded as an active centre of evolution of many novel gene pools [13].

The Brahmaputra valley of Assam with an average elevation of 50 to 120 m above mean sea level (msl) represents a unique landscape that is considered as one of the important priority ecoregion in India among 200 global priority ecoregions [14]. However, unfortunately, due to inaccessibility of some tough terrains, the soil bacteria of this geologically important region have not so far been explored properly.

The present work has, therefore, been designed to investigate the seasonal and depth-wise variations in bacterial population numbers using a cultivation-based approach and two different land-use systems. Enzymatic activity associated along with nutrient acquisition and decomposition pathway was also estimated during the course of investigation.

2. Materials and methods

2.1 Description of the study site

The study was conducted at two different study locations in Brahmaputra valley, Assam, varying in their degree of disturbance. Nameri reserve forest (26º57/ 30.8//N latitude and 92º47/19.4//E longitude, representing relatively less disturbed forest (RLDF) and Nodoar forest (26º54/ 49.4//N latitude and 92º58/54.2//E, representing degraded forest (DF) are the two sampling areas.

The climate of the study area is tropical monsoon. *Alstonia scholaris* (L.)., *Artocarpus heterophyllus* Lamk., *Castanopsis indica* (Roxb.) DC., *Dillenia indica* L., *Dipterocarpus retusus* Blume., *Ficus benghalensis* L., *Mangifera indica* L., *Mesua assamica* Kosterm., *M. ferrea* L., *Michelia champaca* L., *Tamarindus indica* L., *Pteridium aquilinum* (L.) Kuhn., *Pteris biaurita* L., *Tectona grandis* L. f. *Terminalia arjuna* (DC) W. & A., etc. basically represented the major vegetation patterns in RLDF while they were not usually present in DF.

2.2 Collection of the soil samples

Soil was sampled randomly from three different spots, from each of the study locations. Sampling was made at seven different depths such as 1-9 cm, 10-15 cm, 16-30 cm, 31-50 cm, 51-100 cm and 101-200 cm using a sterilized hand auger. Presterilized polythene bags were used for the collection of soil samples and composite sample was prepared in the laboratory. The soil samples were kept in a refrigerator at 4 ±1 °C till isolation procedure was completed.

2.3 Analysis of soil physico-chemical properties

The pH of the soil samples were observed on an electrical digital pH meter in 1: 5 (w/v) soil- water suspensions. Soil temperature was recorded with the help of a soil thermometer in field at different depths, during sampling. The soil moisture content was determined by drying 10 g fresh soil in a hot air oven at 150 °C for 24h.

For chemical analysis (estimation of C, N, P and K), samples were air dried, ground and sieved through 0.2 mm sieve. Wakley and Black's rapid titration method [15] was followed for determining organic carbon (C_{org}). Total N (N_{tot}) was estimated by Indophenol blue method. The molybdenum blue method was followed to determine the available soil P. Soil K was extracted in an ammonium acetate solution (pH-7) and was measured with a digital flame photometer.

2.4 Isolation and characterization of bacteria

Bacterial population was estimated by serial dilution plate method [16] using nutrient agar medium and 10^6 dilutions. In nutrient agar slants, the abundance of growth, pigmentation and optical characteristics were observed. Bacterial isolates were characterized by using morphological and biochemical characters [17]. Bergey's Manual of Systematic Bacteriology [18] was followed for bacterial identification.

2.5 Determination of enzyme activity

Dehydrogenase activity of the soil samples were estimated by 2, 3, 5 triphenyl tetrazolium chloride reduction technique (TTC) as suggested [19]. Tabatabai and Bremner's [20] was followed to assess phosphatase activity. McGariety and Meyer's [21] was followed to determine the urease activity of soil samples.

2.6 Data analysis

The regression equation and simple linear correlation co-efficient graphs between bacterial population number with soil physico-chemical variables (pH, temperature, moisture, C_{org}, N_{tot}, exchangeable P and available K) at both the study locations were calculated [22]. *P* values <0.01 and <0.05 were considered as significant at each case.

3. Results

3.1 Physico-chemical properties of soils

The physico-chemical properties of soils like pH, moisture, temperature, C_{org}, N_{tot}, exchangeable P and available K varied at both the study locations in different seasons and depths. The surface soil (1-9 cm) was more acidic than the subsurface soils at RLDF, while the subsurface soil in DF, recorded more acidic (pH 5.1) in nature (Fig 1a-b).

Maximum surface soil temperature (35.2 °C) was recorded in summer at RLDF. The C_{org} and N_{tot} were highest in the RLDF surface soil (2.5% and 2.9% respectively). Further, the C/N ratio and exchangeable P was observed as highest in the organic layer of 1-9 cm than the deeper horizons irrespective of the land-use systems. Available K decreased with increase in vertical depths of soil, irrespective of the land-use systems.

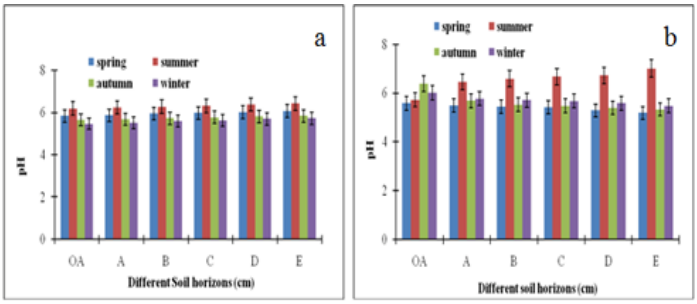

Fig 1(a–b): Seasonal variation in Soil pH at different horizons i.e. OA (1–9 cm), A (10–15 cm), B (16–30 cm), C (31–50 cm), D (51–100 cm) and E (101–200 cm) in a. RLDF b. DF

* Vertical bars represent standard errors.

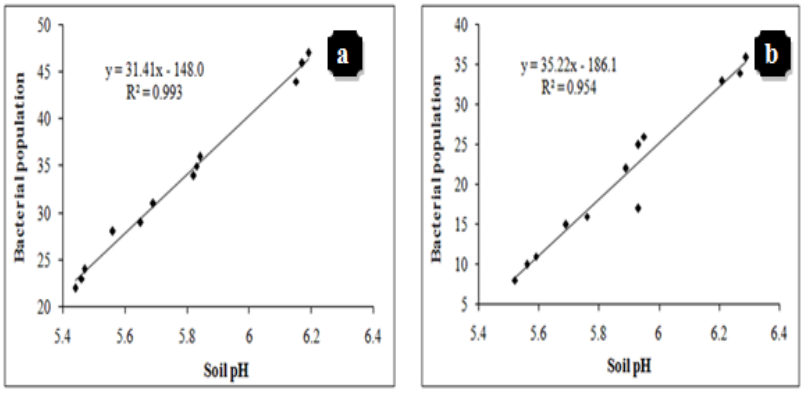

Fig 2(a-b): Correlation between bacterial population number and soil pH at a. surface soil of RLDF soil; b. subsurface soil of RLDF soil.
* RLDF; Relatively less disturbed forest.

3.2 Variations in soil bacterial population numbers

A total of 76 bacterial isolates were recovered from both the land-use systems. Microbial counts were generally higher in surface soil that gradually declined with increasing vertical depths. Maximum bacterial population numbers were, however, recorded in the RLDF surface soil irrespective of the season. Occurrence of Gram-negative bacteria was more over Gram-positive bacteria. In RLDF forest, the surface bacterial population ranged from (79.0±3.0) x 10^6 to (236.3±2.7) x 10^6 cfu/g dry soil and in the subsurface soil it ranged from (36.6±0.13) x 10^6 to (82.7±4.3) x 10^6 cfu/g dry soil. Gram reactions and agar slant culture characteristics of some isolated bacterial species as regards to their occurrence are shown in table 1. The percentage of isolation of *Pseudomonas* was more in RLDF (65%). Although in general, the bacterial population decreased with increase in vertical depths of soil, higher bacterial population was recorded in 51-100 cm depth as compared to 31-50 cm, when isolation media was diluted to 100-200 times using phosphate buffer.

Correlation was observed between the bacterial population number and soil pH in surface and subsurface soils at RLDF (R^2=0.993 and 0.954) (Fig 2a-b). RLDF soil showed positive correlations between the bacterial population number and C_{org} at both the soil horizons, while the same was not observed in DF. RLDF and DF soils at both the soil horizons indicated significant positive correlations (R^2=0.982 and 0.853, and R^2=0.784 and 0.567), when correlation was examined between the bacterial population number and N_{tot}.

Table 1: Gram reactions, culture characteristics and relative distribution of soil bacterial isolates in different land-use systems of Brahmaputra valley, Assam

Bacterial isolates	Gram stain	Agar slant character	RLDF*	DF*
Escherichia coli	(-), Rod	White, moist	+	--
Bacillus sp.	(-), Rod	White, abundant	++	+
Pseudomonas fluorescence	(-), Rod	Green, moist	++	+
Micrococcus sp.	(+), Cocci	Soft, yellow	+	--
Enterobacter sp.	(-), Rod	Thick, white shinning	--	++
Azotobacter sp.	(-), Rod	White, soft	++	+
A. chroococcum	(-), Rod	Milky, flat	+	+
Azospirillum. sp.	(-), Rod	Moist, white	+	--
Bacillus polymyxa	(-), Rod	Thin growth	++	+
Staphylococcus sp.	(+), Cocci	Slimy, white	--	+
Streptococcus sp.	(+), Cocci	White, abundant	+	++

* RLDF; Relatively less disturbed forest, DF; Degraded forest.
Where, - = Absent
++ = High bacterial population density (07-12 isolates)
+ = Low bacterial population density (01- 6 isolates)

3.3 Variations in enzyme activities

Seasonal and depth-wise variations in activities of enzymes i.e. dehydrogenase, phosphatase and urease in soils collected from the two land-use systems are presented in table 2. Maximum dehydrogenase activity was recorded in RLDF soil at both the surface and subsurface soil horizons during summer season. A significant difference in phosphatase and urease activity was also observed throughout the study locations with increasing vertical depths of soil. In the present investigation, the data are significantly different from each other at significance level of $P< 0.05$.

Table 2: Seasonal variation in dehydrogenase, phosphatase and urease activities in surface and subsurface soils of different land-use systems

Locations/ Seasons	Dehydrogenase activity (Formazon released mg g^{-1} dry soil 24 h^{-1})		Phosphatase activity (P-Nitrophenyl released µg g^{-1} dry soil h^{-1})		Urease activity (NH_4^+-N released mg^{-1} dry soil 3 h^{-1})	
	S	SS	S	SS	S	SS
RLDF*						
Spring	0.108±0.01	0.057±0.01	0.458±0.02	0.356±0.0	0.459±0.06	0.421±0.01
Summer	0.208±0.0	0.069±0.0	0.417±0.07	0.342±0.0	0.632±0.0	0.531±0.0
Autumn	0.149±0.0	0.065±0.06	0.395±0.0	0.327±0.0	0.584±0.2	0.547±0.0
Winter	0.089±0.02	0.043±0.0	0.344±0.0	0.294±0.09	0.351±0.0	0.318±0.0
DF*						
Spring	0.115±0.0	0.037±0.0	0.362±0.01	0.280±0.02	0.303±0.0	0.233±0.0
Summer	0.154±0.0	0.073±0.0	0.342±0.01	0.245±0.0	0.430±0.02	0.086±0.0
Autumn	0.079±0.01	0.04±0.02	0.322±0.0	0.175±0.0	0.145±0.01	0.056±0.0
Winter	0.067±0.04	0.039±0.01	0.267±0.0	0.070±0.0	0.225±0.04	0.070±0.02

* RLDF; Relatively less disturbed forest, DF; Degraded forest.
Data are the mean of three replicates ±SD

4. Discussion

In the present investigation, bacterial population was higher in surface soil that decreased with increase in vertical depths irrespective of the land-use systems. This might be due to more amounts of soil organic carbon, higher aeration, soil temperature, exchangeable P and favourable moisture in surface soil. There are reports of decline in microbial population numbers along with increasing soil depths [6]. The depth-wise increase in soil pH might be due to incomplete decomposition of the leaf litter in surface soil followed by subsequent accumulation of organic acids in grater depths [23]. Summer followed by spring season recorded maximum bacterial population numbers as compared to autumn and winter seasons. Higher microbial density during spring and summer in present investigation might be due to increased soil moisture and exchangeable P that eventually stabilized the organic matter of decomposing leaves as most of the leaf litters were added to soil during those seasons [24].

Out of the two forest stands, low microbial population numbers were recorded in the DF soil. This might be due to decreased mineralization of soil nutrients like C, P, N, K which eventually slowed down the microbial succession process [25] there. However, abundant quantity of plant residues accumulating in RLDF soil might be the possible reason for maximum occurrence of bacterial populations there [26].

Gram-negative bacteria were more dominant over Gram-positive bacteria in the present investigation, which is in accordance with Joshi *et al* [27]. This is mainly because of their ability to efficiently use the nutrients through root exudates [28].

Maximum dehydrogenase activity recorded in RLDF surface soil during summer season might be due to more bacterial population numbers, availability of organic carbon and increased moisture in that particular soil region [29]. Human activities in the buffer zone of the DF were mainly responsible for significant reduction in enzyme activities there [30].

5. Conclusion

The present investigation indicated the analysis of soil bacterial diversity along with its community composition in the surface and pristine subsurface soil horizons of Brahmaputra valley, Assam in relation to seasonal and habitat change. The present work is important to fulfil the aim of basic and applied scientific research and natural resource management that would better facilitate their application as a reliable component in sustainable agricultural system and future human welfare in need.

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7. References

1. Ghazanfar S, Azim A, Ghazanfar. Metagenomics and its application in soil microbial community studies: biotechnological prospects. JAPS. 2010; 6(2):611-622.
2. Jia X, Han S, Zhao Y, Zhou Y. Comparisons of extraction and purification methods of soil microorganism DNA from rhizosphere soil. J Forestry Res. 2006; 17(1):31-34.
3. Bhattacharyya PN. Diversity of microorganisms in the surface and subsurface soil of the Jia Bharali river catchment area of Brahmaputra plains. 2012. PhD Thesis, Gauhati University, Guwahati, Assam, India, 6.
4. Zhou J. Microbial diversity and heterogeneity in sandy subsurface soils. Appl Environ Microbiol. 2004; 70(3):1723-1734.
5. Madsen EL. Microorganisms and their roles in fundamental biogeochemical cycles. Curr Opin Biotechnol. 2011; 22(3):456-464.
6. Tangjang S, Arunachalam K, Arunachalam A, Shukla AK. Microbial population dynamics of soil under traditional agro forestry systems in Northeast India. Res J Soil Biol. 2009; 7:1-7.
7. Pajarej S. Enzyme activity as an indicator of soil quality changes in degraded cultivated acrisols in the Mexican trans-volcanic belt. Land Degrad Develop 2011; 22:373-381.
8. Makoi J, Ndakidemi P. Selected soil enzymes: Examples of their potential roles in the ecosystem. Afr J Biotechnol. 2008; 7:181-191.
9. Karplus PA, Pearson MA, Hausinger RP. 70 years of crystalline urease: what have we learned? Acc Chemical Res 1997; 30(8):330-337.
10. Huang W, Liu J, Zhou G, Zhang D, Deng Q. Effects of precipitation on soil acid phosphatase activity in three successional forests in Southern China. Bio geosciences Discuss 2011; 8:157-183.
11. Myers N, Russell AM, Cristina GM, Gustavo AB. da Fonseca, Kent J. Biodiversity hotspots for conservation priorities. Nature 2000; 403:853-858.

12. Bhattacharyya PN, Baruah C, Jha DK, Sharma DK. Potential application of microbial resources in North-East India: Future prospects and challenges. NeBIO 2011; 2(3):12-18.
13. Bhattacharyya PN, Tanti B, Barman P, Jha DK. Culture-independent metagenomic approach to characterize the surface and subsurface soil bacterial community in the Brahmaputra valley, Assam, North-East India, an Indo-Burma mega-biodiversity hotspot. World J Microbiol Biotechnol. 2013; 30(2):519-528.
14. Chatterjee S. Biodiversity Significance of North East India. Forests Conservation Programme, WWF-India, 172 B Lodi Estate, New Delhi 110003, 2006.
15. Walkey AE, Black JA. An examination of the Degtiga Vett. Method for determining soil organic matter and proposed modification of the chromic acid titration method. Soil Sci, 1934, 37:29.
16. Johnson LF, Curl EA. Methods for research on the ecology of soil-borne plant pathogens. Burgess Publishing Co., Minneapolis, MN, 1972, 247.
17. Cappucino JG, Sherman N. Microbiology – A Laboratory Manual. 2004; 7th edn., Pearson Education, Dorling Kindersley (India) Pvt. Ltd.
18. Holt JH. Bergey's manual of determinative bacteriology, 9th ed., Lippincott, Williams & Wilkins, Philadelphia, PA. 1994.
19. Casida LE. Microbial metabolic activity in soil as measured by dehydrogenase determinations. Appl Environ Microbiol. 1977; 34(6):630-636.
20. Tabatabai MA, Bremner JM. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1969; 1(4):301-307.
21. McGarity JW, Mayers MG. A survey of urease activity in soil of Northern New South Wales. Plant Soil 1967; 27(2):217-238.
22. Gomez KA, Gomez AA. Statistical procedures for agricultural research. John Wiley and Sons, New York, USA. 1984, 680.
23. Bhattacharyya PN, Jha DK. Seasonal and depth-wise variation in microfungal population numbers in Nameri forest soil, Assam, Northeast India. Mycosphere 2011; 2(4):297-305.
24. Arunachalam A, Arunachalam K. Influence of gap size and soil properties on microbial biomass in a subtropical humid forest of north-east India. Plant Soil 2000; 223:185-193.
25. Nannipieri P. Microbial diversity and soil functions. Eur J Soil Sci. 2003; 54:655-670.
26. Abbasi MK, Zafar M, Sultan T. Changes in soil properties and microbial indices across various management sites in the mountain environments of Azad Jammu and Kashmir. Comm Soil Sci Plant Anal 2010; 41(6):768-782.
27. Joshi P, Tyagi V, Bhatt AB. Characterization of rhizobacteria diversity isolated from *Oryza sativa* cultivated at different altitude in North Himalaya. Adv Appl Sci Res 2011; 2(4):208-216.
28. Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol. 2012; 28:1327-1350.
29. Jha DK, Sharma GD, Mishra RR. Soil microbial population numbers and enzyme activities in relation to altitude and forest degradation. Soil Biol Biochem 1992; 24(8):761-767.
30. Ralte V, Pandey HN, Barik SK, Tripathi RS, Prabhu SD. Changes in microbial biomass and activity in relation to shifting cultivation and horticultural practices in subtropical evergreen forest ecosystem of north-east India**.** Acta Oecologica 2005; 28(2):163-172.