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Research Article

Impact Of Natural And Anthropogenic Disturbances On The Microbial Diversity In The Critical Zone Of Pranmati Basin In North-West Himalaya, Uttarakhand

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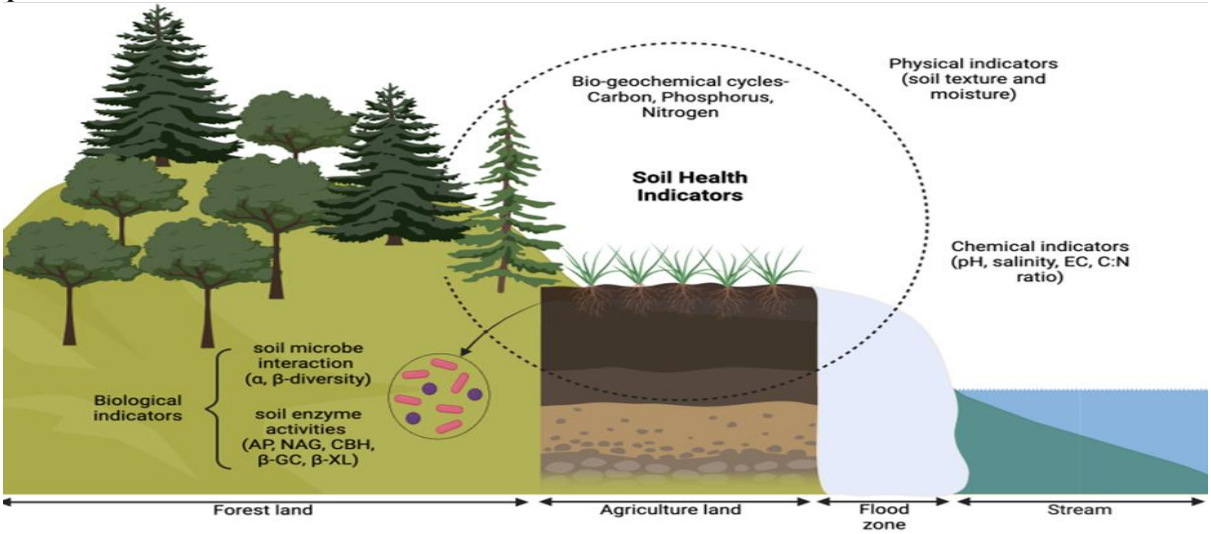
Abstract
Bacterial community, an indispensable component of the soil in the critical zones, plays a significant role in maintaining soil health and ecological balance. This study focuses on assessing soil health by utilizing the bacterial diversity and enzymatic activity of the bacterial community in natural environments such as forest land, disturbed environments like flood zone land, and human-managed environments like agricultural fields in the unexplored Himalayan region. The activity of acid phosphatase and β-glucosidase was consistently high in soils from all landforms, the highest being in slightly acidic (pH 6.39 - 6.87) forest soil. Actinobacteriota was the dominant phylum in agriculture (28.97%) and flood zone lands (34.37%), whereas Proteobacteria (29.44%) was predominant in forest land. The beta diversity analysis indicated similarity in the species composition of forest and agricultural land, while differential abundance analysis showed maximum species abundance in forest land. Phylogenetic studies based on 16S rRNA sequences revealed lipopolysaccharide biosynthesis as a major metabolic function among all land covers. Principal component analysis shows that forest soil and agricultural soil are connected in terms of abiotic and biotic parameters. However, these parameters are not the same in flood zone soil, even though the soil is physically connected. The present work provides valuable insights into soil health and environmental management in different natural and human-managed settings by understanding how the native bacteria interact and function with respect to their surroundings, aiding landscape restoration and terrestrial ecosystem functioning of the Pranmati Basin, a critical zone in the Himalayas, Uttarakhand.

Graphical Abstract



Impact Of Natural And Anthropogenic Disturbances On The Microbial Diversity In The Critical Zone Of Pranmati Basin In North-West Himalaya, Uttarakhand

The interconnectedness of both natural (forest land and flood zone) and human-managed (agricultural field) environment in terms of abiotic and biotic parameters to predict soil health of the Pranmati Basin, a critical zone in the Himalayas, Uttarakhand.

Keywords: Agricultural land, Bacterial diversity, Critical zone, Enzymatic activities, Flood zone, Forest land, Soil health.

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1. Introduction

In mountainous regions, anthropogenic activities such as the construction of huge dams, mining operations, and heavy metal pollution in water bodies, together with overpopulation and climate change, have resulted in the reduction of forest cover, depletion of natural resources, extinction of biodiversity, degradation of soil, and other environmental issues (Sharma et al., 2021; Kour et al., 2023). The mountainous habitats are characterized by varying topography, climate, and vegetation, resulting in distinctive soil characteristics and microbial biodiversity (Sidhu & Surya, 2014).

Soil health refers to the soil's ability to function as a vital living system that supports biological productivity, enhances the quality of water and air, and protects the health of humans, plants, and animals within the ecosystem (Doran and Safley, 1997). People widely use the texture and moisture content of soil as physical indicators of soil health, while chemical indicators include pH, carbon (C), hydrogen (H), nitrogen (N), sulphur (S), salinity, and conductivity of soil (Basu et al., 2021). The composition and diversity of the microbial communities and the activities of their enzymes in soil can also act as biological indicators of soil health (Kour et al., 2023).

Soil in mountainous regions is often thin and fragile, and struggles to retain moisture and nutrients due to various factors such as steep slopes and harsh climatic conditions which restricts soil formation and increases soil erosion in mountains (Kour et al., 2023). These soils are susceptible to degradation and soil erosion, especially during periods of rainfall and snowmelt (Sen et al., 2002). Human activities, like deforestation or agriculture, can exacerbate these processes by further destabilizing the soil. Therefore, sustainable land management practices are important to maintain soil health and stability in physical and chemical factors categorizing abiotic conditions, which regulate soil health or its degradation (Kour et al., 2023). The physical factors, like texture, structure, temperature, and water, along with the soil reaction, acidity, alkalinity, and salinity, and changes in the level of soil nutrients, affect soil quality and health. These factors play a crucial role in soil productivity (Basu et al., 2021).

Microbes are essential components of the soil and play a critical role in maintaining the soil's health and ecology. We cannot overstate the ecological value of bacterial communities in the soil of hilly areas. Bacteria act as a key component of soil microbiota with a major impact on the flora of a particular geographical area. Microbes help in plant growth promotion through their mutual relationships with the plant rhizosphere and mycorrhizal associations, thereby enhancing the soil's health (Chen et al., 2024). Bacteria are also involved in many essential processes, including the production of soil, removal of toxins, and the necessary biogeochemical cycles, which are important for the maintenance of healthy soil and ecological stability (Wang et al., 2020). Seasonal changes in the vegetation, surrounding landscape activity, and nutrient availability can influence the microbial community of soil. Studying soil microbial diversity and its complex interactions is essential for developing sustainable applications to preserve and manage our natural resources (Kour et al., 2023). The diversity of soil microbes can be studied by cultural and non-cultural methods. However, less than 1% of the entire bacterial population is culturable; therefore, cultural techniques do not fully represent the soil microbial community. Metagenomics, an advanced method, eliminates the need to culture bacteria from different sources. Its capacity to offer genetic insights into microbial diversity has fundamentally changed the knowledge of soil ecosystems and their ecological functions (Sidhu and Surya, 2014).

The present study uses the Illumina sequencing platform to explore and compare structural and functional bacterial diversity from natural environments such as forests and flood zones, as well as human-managed agricultural land. The metagenomics analysis of microbial communities present in the Pranmati Basin is important to estimate, evaluate, and record the microbial taxa before they change or are lost due to environmental stressors. We aimed to comprehend the intricate relationships between soil microbes and their environment, and their reactions to environmental issues

such as land degradation, climate change, agricultural intensification, cloudbursts, flash floods, landslides, nutrient loss, and urbanization, particularly in mountainous areas. These issues are causing habitat loss, altering the composition of endemic native flora and fauna, and impeding the proper functioning of the Pranmati Basin, also known as the Himalayan Critical Zone. Therefore, soil health assessment and environment management strategies thus become crucial in such biodiversity-rich hotspots.

2. Materials and methods

2.1. Study area

The area selected for the present study was Pranmati Basin (30° 5´ – 30° 13´ N and 79° 29´ – 79° 37´ E) located in the district of Chamoli, Uttarakhand, India. Pranmati basin has an area of 94 km^2. A wide disparity was visible in the basin with reference to elevation, slope, and types. The elevation of the basin ranged from 1154 m to 4020 m with an average elevation of 2425 m. It majorly envelopes 2000 - 2500 m of basin area with an average slope of 25.7° (Sarkar and Singh, 2022). The Pranmati stream flows through the basin in the southwest direction, covering a distance of 17.7 km, and merges with river Pinder, a tributary of river Alaknanda which is one of the headwaters tributaries of river Ganga. The basin has approximately 80 % forest cover, 9.6 % barren land, 6.9% agriculture fields, 1.6% grassland, 1.2% waterbody, and 0.5% built up area (Sarkar and Singh, 2022). Mixed vegetation of coniferous trees like *Pinus*, and broadleaf species such as *Rhododendron arboreum*, *Myrica esculenta*, *Quercus leucotricophora* were present in the forest (Rana et al., 2025), while in agricultural fields, crops like wheat, paddy, barley, potato, and mustard were grown. In the flood zone, boulders of size 1 to 6 m were present. During monsoon (July-September), the width of the river increases due to heavy rainfall and flooding (Sen et al., 2002).

For the current study, Pranmati basin was divided into three segments based on the elevation viz. Lower-segment (1100 m to 1500 m), Mid-segment (1500 m to 2000 m) and Upper-segment (2000 m to above). The upper segment had very harsh terrain that had very low accessibility due to steep slopes and dense forest. Therefore, sampling was restricted to forest, agriculture, and flood zone in the mid and lower-segment along the Pranmati stream. Sampling was done in April (2022) and a total of six sites were selected, from the mid-segment (S1, S2, S3) and lower-segment (S4, S5, S6) (Fig.1). The latitude, longitude, and elevation of sampling sites are listed in table 1.

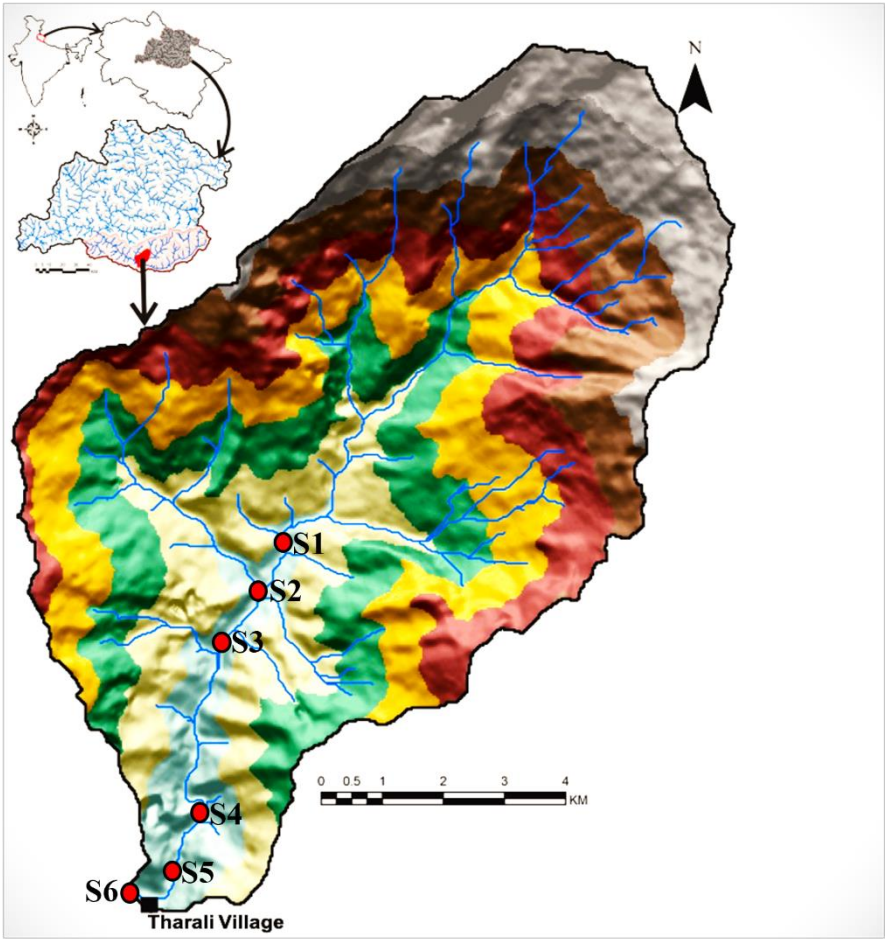


Fig. 1. Geographical location of the sampling sites and schematic representation of the collected samples from different landforms of Pranmati Basin, Chamoli, Uttarakhand, India.

Table 1. Latitude, longitude, and elevation of sampling sites in forest land, agriculture land, and flood zone land.

Site Name	Forest Land (Longitude Latitude Elevation)	Agriculture Land (Longitude Latitude Elevation)	Flood Zone (Longitude Latitude Elevation)
Site 1	30° 8' 4.54" N 79° 31' 43.85" E 1718 m	30° 8' 5.46" N 79° 31' 47.05" E 1716 m	30° 8' 4.10" N 79° 31' 44.81" E 1711 m
Site 2	30° 7' 50.51" N 79° 31' 12.75" E 1677 m	30° 7' 49.78" N 79° 31' 13.25" E 1672 m	30° 7' 48.31" N 79° 31' 16.37" E 1647 m
Site 3	30° 7' 26.99" N 79° 31' 5.85" E 1630 m	30° 7' 23.83" N 79° 31' 7.95" E 1600 m	30° 7' 21.77" N 79° 31' 5.76" E 1590 m
Site 4	30° 5' 8.72" N 79° 30' 21.22" E 1317 m	30° 5' 7.54" N 79° 30' 22.48" E 1352 m	30° 5' 9.58" N 79° 30' 18.22" E 1300 m
Site 5	30° 4' 36.02" N 79° 30' 4.26" E 1252 m	30° 4' 33.16" N 79° 30' 6.33" E 1249 m	30° 4' 33.56" N 79° 30' 4.30" E 1238 m
Site 6	30° 4' 35.68" N 79° 29' 48.76" E 1229 m	30° 4' 37.12" N 79° 29' 44.31" E 1213 m	30° 4' 36.79" N 79° 29' 42.68" E 1207 m

2.2. Sample collection

Three types of soil samples were collected from each site for the analysis of physico-chemical parameters, enzyme activity, and microbial diversity. Sampling was done next to the stream from three different land use types i.e. forest, agriculture, and flood zone land. Flood zone samples were collected a few meters (15-25m) from the bank of the stream (flooded soil). Agriculture land samples were collected 50-150m away from the stream. Forest land samples were collected 50-200m on either side of the stream. Total 108 soil samples were collected from three different land use types at six sites. Six replicates (n=6) of soil samples collected from one landform at a site were composited to form one indicative sample for each site. The soil samples were collected from a depth of 1 - 10 cm from the surface using a sterile trowel, after removal of the top layer of litter the samples were stored in sterile zip pouches and cryovials.

2.3. Physico-chemical parameters

Soil sample (1 Kg) was collected in the sterilized zip pouches, stored at room temperature, and transported to laboratory, used for the soil texture analysis and Carbon (C), Hydrogen (H), Nitrogen (N), Sulphur (S) analysis. The sample was air-dried and sieved through 2 mm mesh for soil texture and chemically treated with sodium carbonate (2 %), sodium acetate (5 %), sodium bicarbonate (1M), sodium citrate dihydrate (0.3M) solutions, KCl (1N), and CaCl2 (1N) for the removal of iron, salts, and the organic matter followed by sieving with 53-micron mesh to separate sand. The silt and clay were separated by sedimentation process and expressed as percentages following the US Department of Agriculture (USDA) standard method and analyzed using a soil texture triangle (Toogood, 1958). Soil (5 gm) was grounded using a mortar pestle and powdered soil was used for CHNS analysis (CHNSO analyzer Elementar Analysensysteme Germany Vario Micro Cube Serial no. 19171021).

Soil samples (50 g) sieved with a 2 mm sieve were collected in sterilized pouches, stored at 4° C, and transported to the laboratory were used for the evaluation of soil moisture, pH, salinity, and electric conductivity (EC). Soil moisture content was measured using the R. Ohlinger method (Schinner et al., 2012). The moist soil suspension was made in 1:5 (w/v), left undisturbed overnight and the next day pH, salinity, and EC were calculated using multi-parameter tester 35 series (Eu-tech Instruments).

2.4. Soil Enzyme Assays

Enzyme assays from the moist soil samples stored at 4 °C were conducted within a week of sample collection. The extracellular enzyme activity of Carbon (β-1, 4-Xylosidase, Cellobiohydrolase, β-1, 4-Glucosidase) Phosphorus (Acid Phosphatase) and Nitrogen (β-1, 4-N-acetyl-β-Glucosaminidase) cycling enzymes, were examined according to Wang et al. (2020). Fluorescence was measured at 365 λ excitation and 450 λ emission filters using a microplate fluorometer (Synergy H1, BioTek). The enzyme activity was measured in nmol $g^{-1}h^{-1}$ after negative controls and quenching corrections.

2.5. Metagenomic DNA extraction and 16S Amplicon Sequencing

Soil samples (5 g, n = 6) were collected in cryovials from each site in all three environments, and stored in liquid nitrogen in the field. In the laboratory, the samples were transferred to -80° C and then to -20 ° C to prevent heat shock to the DNA. The genomic DNA was extracted from 250 mg of soil using DNeasy PowerSoil Kit (Qiagen), following the manufacturer's protocol. Six replicates were pooled together as one indicative sample

of a landform at a site. Extracted DNA was visualized on 0.8% agarose gel. The purity and concentration of DNA were measured at 260/280 nm using a NanoDrop (ND-ONE, Spectrophotometer, Thermo Fisher Scientific). The PCR Amplification of the hypervariable V3-V4 region of 16S rRNA gene (read length 2 x 250 bp) was done in 25 µl reaction mixture using the universal primer pair [Forward Primer 341(FP): 5' "CCTACGGGNGGCWGCAG" 3'; Reverse Primer 805(RP) 5' "GACTACHVGGGTATCTAATCC"] (Liu et al., 2020). PCR was performed in a total of 25 µl of reaction containing metagenomic DNA (2.5 µl of 25 ng/µl), 5 µl of 'FP' (1 µM), 5 µl of 'RP' (1 µM), and 12.5 µl of 2x KAPA HiFi HotStart Ready Mix made up the reaction mixture. PCR cycler thermal condition involves initial denaturation at 95° C (3 minutes), 25 cycles of denaturation at 95° C (30 seconds), annealing at 55° C (30 seconds), extension at 72° C (30 seconds), followed by a final extension at 72° C (5 minutes). The PCR products were eluted using Thermo Scientific GeneJET gel extraction kit by following the manufacturer's protocol. The eluted PCR products were sequenced by Illumina NovaSeq PE250 sequencing (Anuvanshiki (OPC) Pvt. Ltd.) and 250 bp paired-end raw reads were produced. The library was examined using a Qubit fluorometer and a bioanalyzer to detect the size distribution.

2.6. Bioinformatics analysis

The paired-end generated by Illumina NovaSeq PE250 sequencing was assigned to samples based on their unique barcodes further, truncated the barcode and primer sequences. Reads were combined using FLASH software (v1.2.11, http://ccb.jhu.edu/software/FLASH/). The quality control of the raw reads was performed by fastq software (v0.11.9) and clean tags were acquired. Using the DADA2 (v1.22.0) pr deblur module in the QIIME2 software, the effective tags with less than 5 abundance were filtered out as OTUs (Operational Taxonomic Units). OTUs were compared with the SILVA138 database (https://www.arb-silva.de/documentation/release-138/) and species annotation of each OTUs were procured using the Classify-sklern moduler in QIIME2 program (Bolyen et al., 2019). QIIME2 software (v2022.11.1) was used to determine the alpha diversity indices (Chao1, Observed OTUs, Shannon, Simpson) and beta diversity of the landforms from each site. To examine the community structure difference between landforms, QIIME2 software (adonis and anosim function) was employed. LefSe software calculated the significant difference at the species level. For functional OTU prediction, PICRUSt2 software (v2.5.0) and ggpicrust2 for comparison analysis and visualization were employed. Linda and Aldex2 methods were used to compute the pathway's differential abundance across different landforms with a p-value cut-off of less than 0.02. On the basis of the OTUs tree and the SILVA database, the functional spectrum of the common ancestor was inferred.

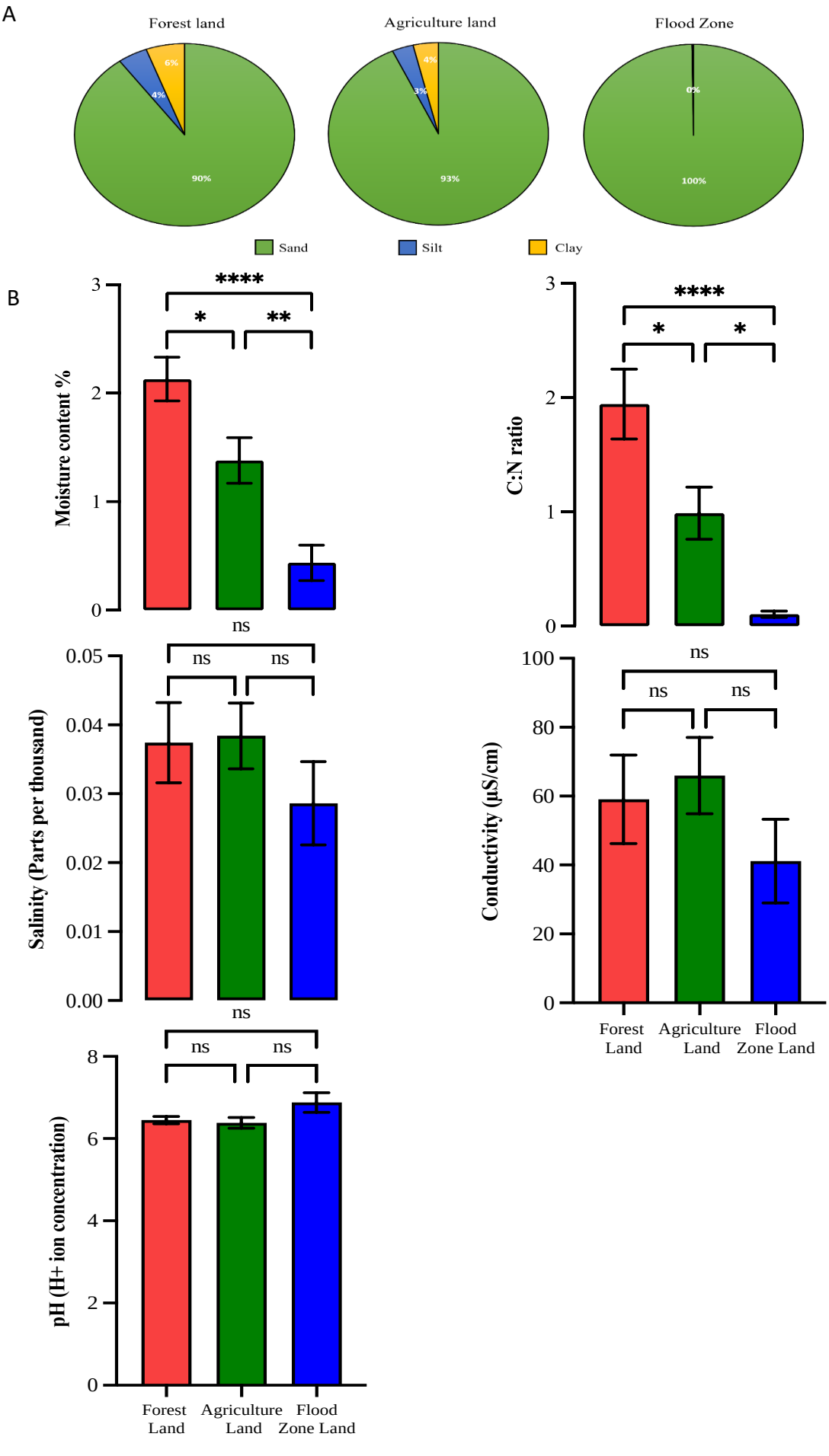
2.7. Statistical analysis

GraphPad Prism software (version 10.3.1) was used to calculate the central tendency in the data. One-way ANOVA followed by Tukey's multiple comparison test was used to determine the significance of differences at p-value < 0.05 in soil physico-chemical properties, enzyme activities, and alpha diversity indices between different landforms. Graphs and Plots were prepared using R (version 4.1.1) packages ggplot2, ggpubr, and Ade4. R software was also used to execute MetaStat and t-test to compare samples up to the species level at p-value < 0.05 to obtain a significant difference. Principal component analysis among biotic and abiotic factors was also performed in R studio (version R 4.4.1).

3. Results

3.1. Physico-chemical properties of Soil

Soil texture analysis indicates that forest and agricultural land have a maximum percentage of sandy soil while flood zones have 100% sandy soil (Fig. 2A). For calculating the average, we have taken six replicates (n=6). The average C/N ratio was significantly higher (p-value 0.0001) in forest land (1.94 ± 0.4) followed by agriculture land (0.99 ± 0.3) and least in the flood zone land (0.11 ± 0.04). Average moisture content was significantly different in all three landforms (p = 0.0001) and was highest in forest land (2.13 ± 0.2 %) and lowest in flood zone land (0.44 ± 0.1 %). The pH was slightly acidic in all the landforms. The average pH in forest land was 6.45 ± 0.09, agriculture land was 6.39 ± 0.13 and flood zone land was 6.87 ± 0.24. The average soil salinity in forest, agriculture, and flood zone soil were 0.040 ± 0.006 ppt, 0.040 ± 0.005 ppt, and 0.030 ± 0.005 ppt respectively. EC is reported maximum in agriculture fields (66 ± 11 µS/cm), followed by forest soil (59.1 ± 12.8 µS/cm), and minimum (41.1 ± 12.2) in flood zone. However, the difference was non-significant in the values of salinity, pH, and EC among the three landforms (Fig. 2B).


A
Forest land
Agriculture land
Flood Zone
6%
4%
90%
4%
3%
93%
0%
100%
Sand
Silt
Clay
B
Moisture content %
****
*
**
ns
C:N ratio
****
*
*
ns
Salinity (Parts per thousand)
ns
ns
ns
Conductivity (µS/cm)
ns
ns
ns
pH (H+ ion concentration)
ns
ns
Forest Land
Agriculture Land
Flood Zone Land

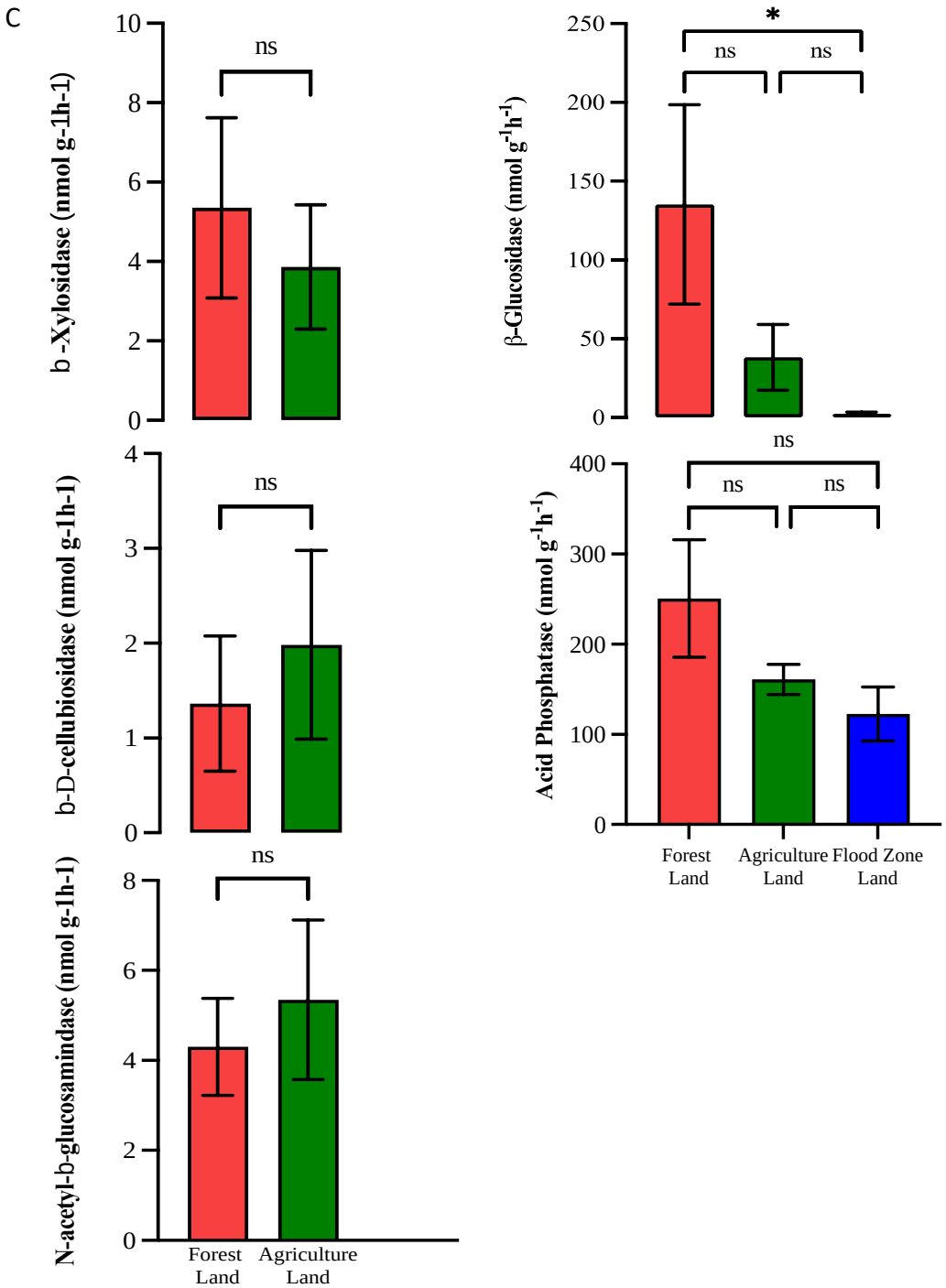


Fig. 2. Physico-chemical parameters and soil enzyme activities in three landforms (Forest, Agriculture, Flood Zone) of Pranmati basin. **A** Pie charts showing the percentage distribution of sand, silt and clay in three landforms. **B** Physico-chemical parameters- Moisture content, Salinity, pH, C: N ratio, Electric conductivity. **C** Soil enzyme activities in different landforms. Statistical analysis was performed using One way ANOVA at $p < 0.05$ followed by Tukey's multiple comparison test (n=6). Bars with different superscripts (ns) non-significant and (*) represents a significant difference.

3.2. Soil Enzyme Activities

Carbon (β-Glucosidase, β-Xylosidase, Cellobiohydrolase), Nitrogen (N-acetyl glucosaminidase) and Phosphorus (Acid Phosphatase) cycling enzyme activities are illustrated in Fig. 2C. Soil enzyme activity depicted that Acid Phosphatase and β-Glucosidase were the dominant enzymes present in the soil of Pranmati basin. Acid Phosphatase enzyme activity was present in all the environment, but relatively higher in forest land followed by agriculture land and lowest in flood zone. β-Glucosidase activity was found to be maximum among the three carbon cycling enzymes. It shows a significant difference between forest and flood zone land (p-value 0.03) whereas no significant difference was observed between forest and agriculture land, as well as between flood zone and

agriculture land. β-Xylosidase activity was found more in forest land compared to agricultural land and zero activity in flood zone with no significant difference between the landforms ($p < 0.05$). Cellobiohydrolase activity was slightly higher in agricultural land in comparison to forest land with no significant difference among them ($p < 0.05$) and no activity was observed in flood zone land. N-acetyl glucosaminidase activity was higher in agricultural land as compared to forest land with no significant difference among the environments ($p < 0.05$) whereas zero activity was observed in flood zone land.

3.3. Metagenomic Sequencing Data and Comparative Analysis across landforms

A total of 1350561 reads, representing 49849 unique Amplicon Sequence Variants (ASVs) exhibiting 97% nucleotide identity with the SILVA138 database were obtained across the different landforms. The total number of ASVs per sample are enlisted in Table 2. The raw sequencing file has been submitted to the NCBI database with BioProject ID: PRJNA1097364. The rarefaction curves initially grew exponentially and approached a plateau phase later indicating that the OTUs/ASVs included in the analysis were appropriate and sufficient for bacterial diversity analysis (Fig. 3A). These effective reads were used further for bacterial diversity analysis.

Table 2. Statistical outcome of data processing. The column displays raw reads, filtered, merged, nonchim, GC percentage, number of bases in effective tags, and number of OTUs/ASVs present in forest, agriculture, and flood zone.

Samples	Raw Reads	Filtered	Merged	Non-chim	GC%	Mean Length	No. of ASVs/OTUs
			FOREST LAND				
S1-FC	103981	95176	71966	66144	56	225	3468
S2-FC	104405	95430	70281	63869	56	224	3717
S3-FC	102684	92604	67210	61000	55.5	224	3447
S4-FC	104436	96911	76254	69637	56.5	225	3296
S5-FC	103261	93593	69114	62604	56	224	3398
S6-FC	103272	93103	68928	63408	55.5	224	3121
							20447
			AGRICULTURE LAND				
S1-AL	102166	94623	70715	64256	56.5	225	3300
S2-AL	103485	95820	73195	67560	57	225	3436
S3-AL	103303	95519	68804	62488	56	225	3398
S4-AL	103335	93958	72906	67343	56.5	224	3124
S5-AL	103276	95691	72960	67458	56.5	225	3316
S6-AL	79247	72118	53363	48966	54.5	224	1771
							18345
			FLOOD ZONE				
S1-FZ	102617	95839	89631	87804	56	225	738
S2-FZ	105945	98153	82866	78436	55	225	1709
S3-FZ	174301	156866	144139	141812	52.5	224	1653
S4-FZ	116356	104712	93441	89490	55.5	225	1749
S5-FZ	106468	99113	87672	83540	55	225	1306
S6-FZ	146420	132115	111294	104746	56.5	224	3902
							11057

Venn diagram was employed to calculate the number of shared or unique taxa among three different landforms (Fig. 3B). Forest land, agriculture land, and flood zone land have 12430, 10176, 7692 unique ASVs suggesting these bacterial strains were only present in the respective landforms. Forest land and agriculture land have 1992 ASVs in common, whereas agriculture land and flood zone land share 606 ASVs in common, flood zone land and forest land share 397 ASVs in common. All three landforms have 905 common ASVs suggesting similar bacterial strains.

3.4. Bacterial composition and relative abundance

Taxonomic hierarchy from phylum to genus level showing relative abundance percentage (greater than 2%) in different landforms (forest, agriculture, and flood zone) is depicted in Table 3. Two phyla, Actinobacteriota and Proteobacteria, were predominant viz. Actinobacteriota, (28.97%) in agriculture and (34.37%) flood zone lands, whereas Proteobacteria (29.44%) was predominant in forest land. *Bacillus* in forest (8.14%) and agriculture land (9.3%), whereas *Lactobacillus* (18.19%) in flood zone were the two top most abundant genera observed. The percent relative frequency of bacterial communities at phylum and genus level are shown in the form of stacked bar plots in Fig. 3C. It indicates the differences in bacterial composition across three different landforms and less than 2% have been categorized in another group named others.

Table 3. The taxonomic hierarchy from phylum to genus level depicting relative abundance percentage in different land form (forest, agriculture, and flood zone land) of Pranmati Basin. The table include the taxa having greater than or equal to 2% relative abundance.

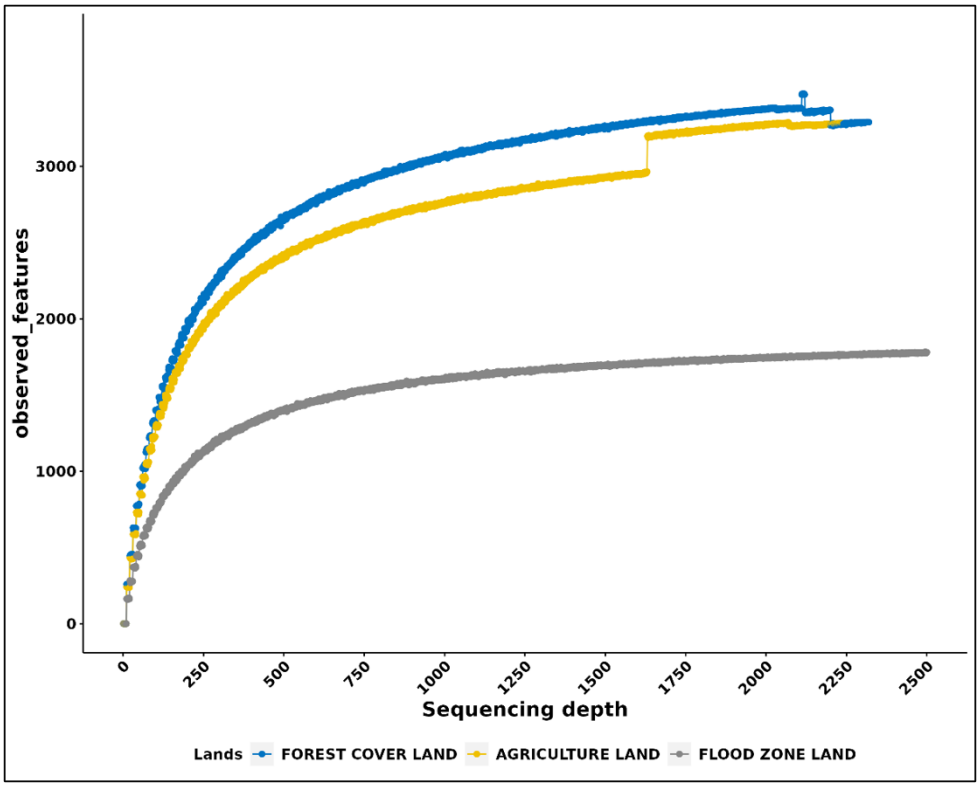
FOREST LAND	AGRICULTURE LAND	FLOOD ZONE
	Phylum	
Proteobacteria (29.44)	Actinobacteriota (28.97)	Actinobacteriota (34.37)
Actinobacteriota (20.1)	Proteobacteria (23.16)	Firmicutes (25.05)
Acidobacteriota (14.45)	Firmicutes (18.78)	Proteobacteria (23.25)
Firmicutes (13.08)	Acidobacteriota (9.64)	Bacteroidota (5.44)
Verrucomicrobiota (5.16)	Chloroflexi (3.76)	Chloroflexi (3.39)
Bacteroidota (4.47)	Myxococcota (3.29)	Acidobacteriota (2.11)
Chloroflexi (3.17)	Bacteroidota (2.75)	
Myxococcota (2.56)	Gemmatimonadota (2.47)	
Gemmatimonadota (2.14)	Verrucomicrobiota (2.06)	
	Class	
Alphaproteobacteria (19.65)	Bacilli (15.45)	Actinobacteria (30.5)
Gammaproteobacteria (10.16)	Actinobacteria (14.38)	Bacilli (21.93)
Bacilli (10.04)	Alphaproteobacteria (13.75)	Alphaproteobacteria (14.75)
Thermoleophilia (9.1)	Thermoleophilia (11.78)	Gammaproteobacteria (8.56)
Actinobacteria (7.63)	Gammaproteobacteria (9.63)	Bacteroidia (5.43)
Verrucomicrobiae (5.21)	Acidobacteriae (4.34)	Clostridia (2.94)
Acidobacteriae (4.85)	Clostridia (3.25)	Thermoleophilia (2.89)
Vicinamibacteria (4.69)	Bacteroidia (2.74)	
Bacteroidia (4.49)	Vicinamibacteria (2.4)	
Clostridia (3.11)	Gemmatimonadetes (2.36)	
Blastocatellia (2.9)	Polyangia (2.22)	
Acidimicrobiia (2.26)	Verrucomicrobiae (2.06)	
	Acidimicrobiia (2.04)	
	Order	
Rhizobiales (14.86)	Rhizobiales (9.25)	Lactobacillales (16.21)
Burkholderiales (6.48)	Bacillales (8)	Micrococcales (11.31)
Bacillales (5.95)	Gaiellales (6.44)	Propionibacteriales (7.76)
Gaiellales (4.91)	Burkholderiales (6.38)	Corynebacteriales (6.52)
Vicinamibacterales (4.7)	Solirubrobacterales (5.67)	Sphingomonadales (5.68)
Solirubrobacterales (4.49)	Micrococcales (3.89)	Rhizobiales (4.97)
Chthoniobacterales (3.5)	Lactobacillales (2.75)	Burkholderiales (4.68)
Acidobacteriales (2.54)	Frankiales (2.66)	Frankiales (3.13)
Pyrinomonadales (2.21)	Gemmatimonadales (2.46)	Bacillales (2.81)
Chitinophagales (2.19)	Vicinamibacterales (2.37)	Chitinophagales (2.5)
Gemmatimonadales (2.09)	Acidobacteriales (2.36)	
	Erysipelotrichales (2.21)	
	Family	
Xanthobacteraceae (11.45)	Bacillaceae (7.54)	Lactobacillaceae (16.62)
Bacillaceae (6.04)	Xanthobacteraceae (5.92)	Micrococcaceae (8.78)
Chthoniobacteraceae (3.78)	Solirubrobacteraceae (3.83)	Nocardioidaceae (8.05)
Solirubrobacteraceae (2.76)	Micrococcaceae (3.02)	Sphingomonadaceae (5.97)
Pyrinomonadaceae (2.65)	Gemmatimonadaceae (2.88)	Nocardiaceae (3.82)
67-14 (2.61)	67-14 (2.77)	Chitinophagaceae (2.6)
Chitinophagaceae (2.5)	Erysipelotrichaceae (2.55)	Oxalobacteraceae (2.39)
Gemmatimonadaceae (2.5)	Paenibacillaceae (2.27)	Mycobacteriaceae (2.38)
Nitrosomonadaceae (2.36)	Lactobacillaceae (2.2)	Planococcaceae (2.38)
Sphingomonadaceae (2.31)	Lachnospiraceae (2.11)	Caulobacteraceae (2.04)
Pedosphaeraceae (2.19)		Exiguobacteraceae (2.02)
Pseudomonadaceae (2.12)		
	Genus	

Bacillus (8.14)	*Bacillus* (9.3)	*Lactobacillus* (18.19)
Candidatus udaeobacter (4.54)	*Arthrobacter* (2.53)	*Nocardioides* (7.49)
Bradyrhizobium (3.6)	*Gaiella* (2.35)	*Sphingomonas* (4.94)
RB41 (3.59)	*Solirubrobacter* (2.29)	*Arthrobacter* (4.36)
Pseudomonas (2.81)	*Bradyrhizobium* (2.18)	*Rhodococcus* (4.2)
Sphingomonas (2.2)	*Conexibacter* (2.08)	*Pseudarthrobacter* (2.65)
Rhodoplanes (2.19)		*Mycobacterium* (2.64)
Gaiella (2.11)		*Exiguobacterium* (2.24)
		Planomicrobium (2.21)

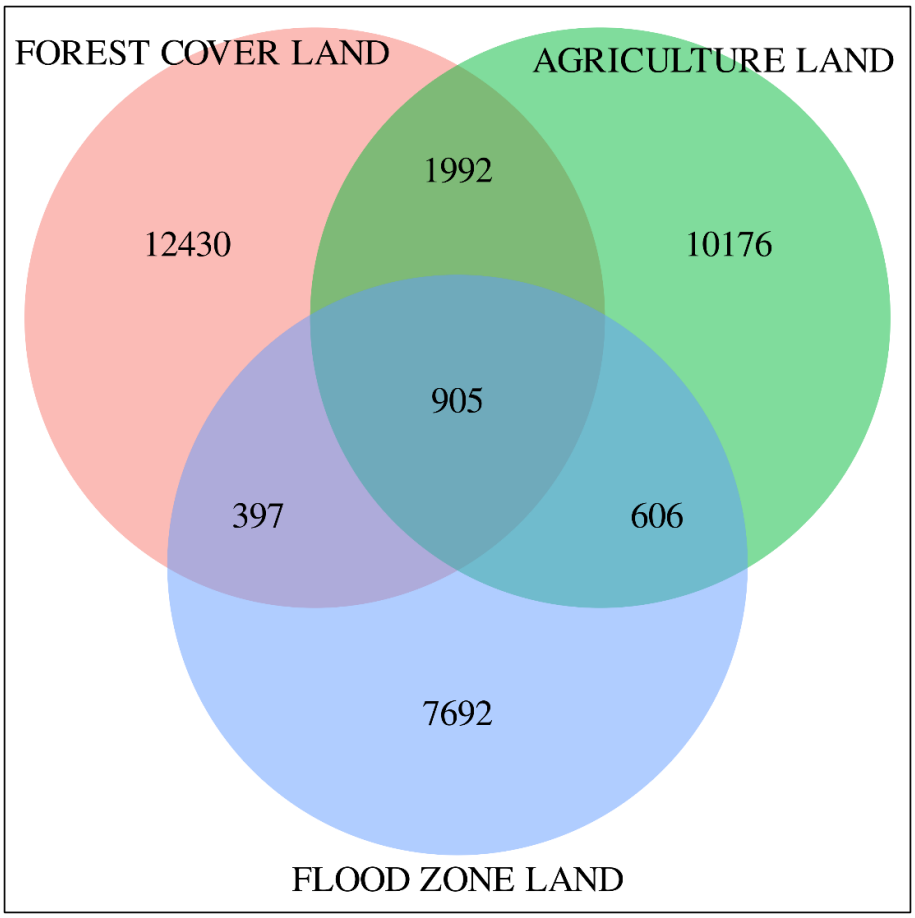
note: number indicating the relative abundance of taxa in percentage.

The similarity and grouping in the microbial diversity among three different landforms are illustrated in clustered heat maps using 30 most abundant bacterial phyla and genera (Fig. 3D). Proteobacteria (forest land) and Actinobacteriota (agriculture land and flood zone) were two of the most abundant phyla present in the basin. *Bacillus* (forest and agriculture) and *Nocardioides* (flood zone) belonging to phylum Firmicutes and Actinobacteriota respectively were the dominant genera present in the landforms. The flood zone land was characterised by the absence of Verrucomicrobiota, Myxococcota and Gemmatimonadota phyla which were part of forest and agriculture land. Almost different genera were present in all the landforms. Forest land includes *Bacillus, Sphingomonas, Candidatus udaeobacter, Bradyrhizobium, RB41, Pseudomonas, Gaiella,* and *Rhodoplanes*. Further, *Solirubrobacter* and *Conexibacter* were unique to agriculture land whereas *Bacillus, Bradyrhizobium,* and *Gaiella* were common with forest cover. Flood zone includes a maximum number of distinct genera such as *Lactobacillus, Nocardioides, Sphingomonas, Rhodococcus, Pseudarthrobacter, Mycobacterium, Exiguobacterium* and *Planomicrobium*. Only one genus, *Arthrobacter*, was common between flood zone land and agriculture land. Some of the OTUs were not identified at genus level and included order Solirubrobacterales, family 67-14, order Gaiellales and family Xanthobacteraceae were present in forest and agriculture land, order Vicinamibacterales only in forest land.

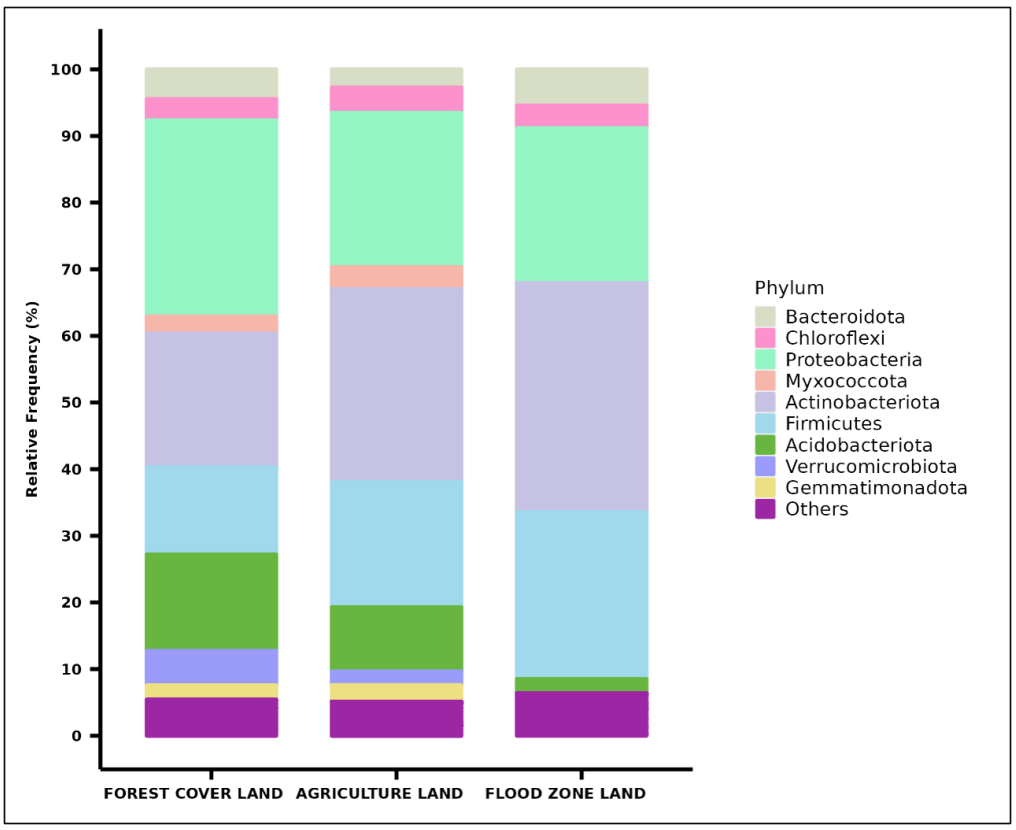
A

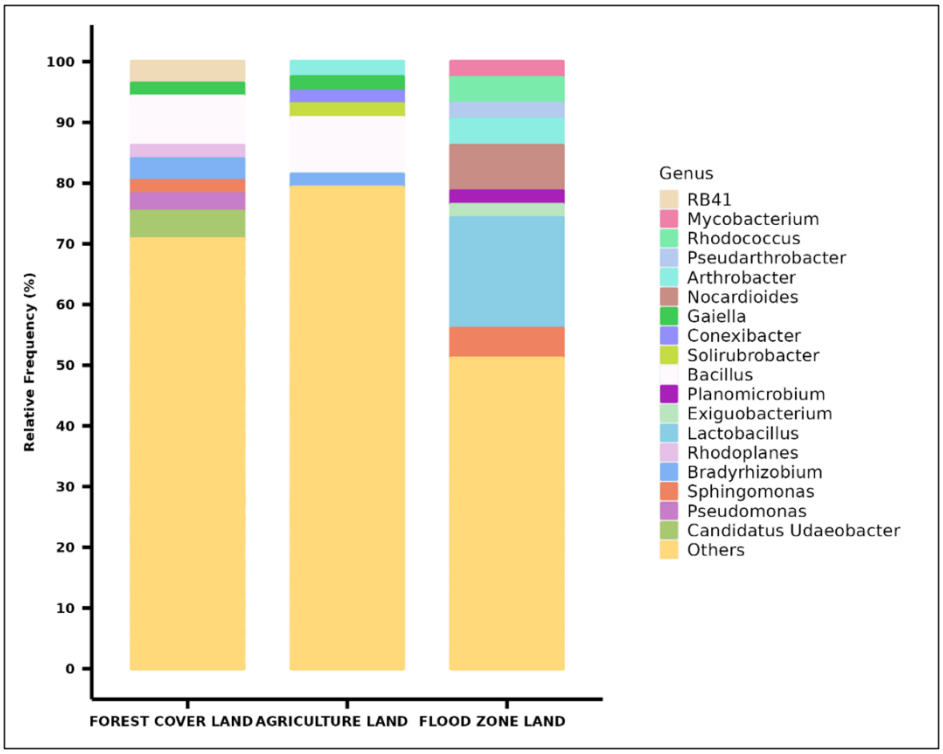


B


FOREST COVER LAND
AGRICULTURE LAND
FLOOD ZONE LAND
12430
1992
10176
905
397
606
7692

C


Relative Frequency (%)
100
90
80
70
60
50
40
30
20
10
0
FOREST COVER LAND
AGRICULTURE LAND
FLOOD ZONE LAND
Phylum
Bacteroidota
Chloroflexi
Proteobacteria
Myxococcota
Actinobacteriota
Firmicutes
Acidobacteriota
Verrucomicrobiota
Gemmatimonadota
Others



D

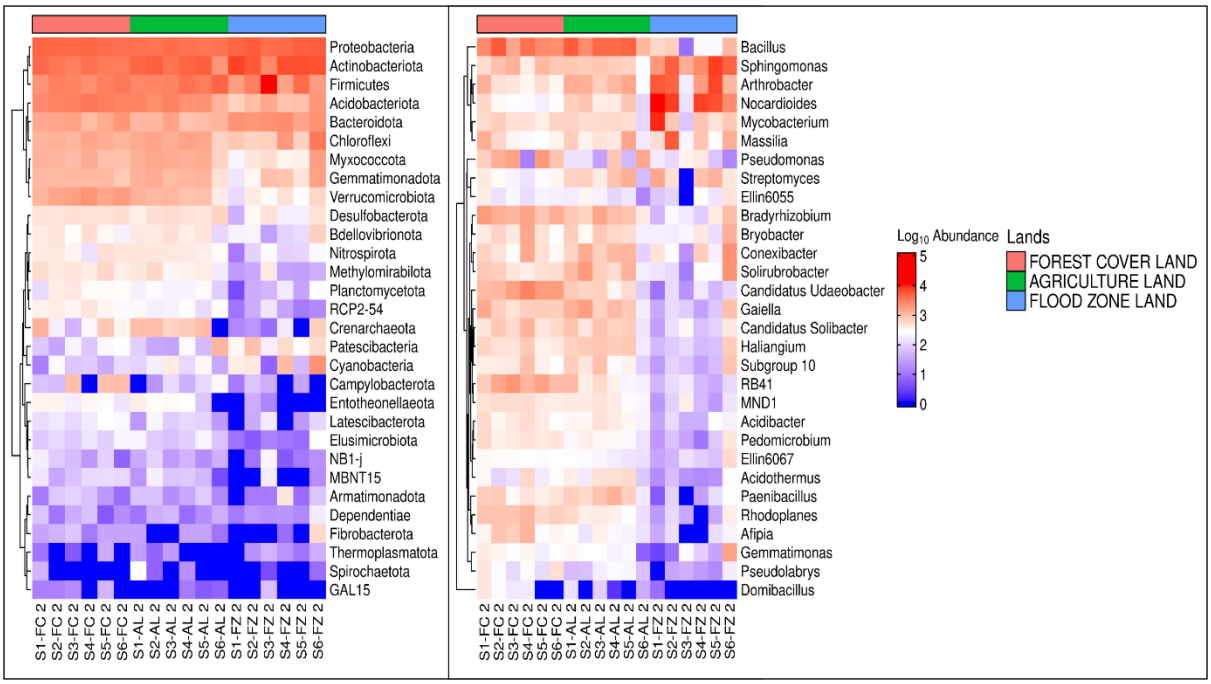


Fig. 3. Rarefaction curves based on the number of OTUs/ASVs represent the bacterial diversity within samples by using sequencing depth observed features. **B** Venn diagram demonstrating the number of taxa commonly shared (overlapped region) and unique (non-overlapped region) among three land covers. **C** Barplot of average relative frequency (%) of taxa across landforms of bacterial phyla and genera whereas less than < 2% relative frequency are grouped in others. **D** Abundance heatmap of top 30 bacterial phyla and genera, at log 10 abundance.

3.5. Bacterial diversity and functional analysis

The alpha diversity, including community richness (Chao1), observed richness, and community diversity indices (Shannon, Simpson) are listed in Table 4. The average Chao1 value (p-value 0.0008) showed the highest species richness (3677.3 ± 90.4) in the forest land as compared to agriculture land (3290.6 ± 280.8) and lowest in flood zone (1894.7 ± 458.8) suggesting soil resilience through true species richness with the presence of several undetected species. The forest had higher average observed richness (3295.7 ± 78.6) than agriculture land (2953.2 ± 240.7) followed by flood zone

(1712.5 ± 418.5) depicted total number of species present in a landform (p-value 0.001). Shannon diversity was found to be maximum in forest land (10.53 ± 0.06) followed by agriculture land (10.18 ± 0.39) and minimum in flood zone (7.82 ± 0.8) suggesting the relative abundance of each species by taking into account both species richness and evenness, including species which are less common (p-value 0.002). The forest has higher average Simpson diversity (0.99 ± 0.0001) than agriculture land (0.99 ± 0.002) and lower flood zone land (0.94 ± 0.04) depicts species dominance (p-value 0.02). Significant difference was observed between forest and flood zone, also among agriculture land and flood zone whereas no significant difference was observed between forest and agriculture land based on alpha diversity indices (Fig. 4A). Forest land had significantly high alpha diversity indices compared to agriculture land whereas the lowest in flood zone.

Table 4. Comparison of alpha diversity indices of microbial diversity in forest, agriculture, and flood zone.

Samples	Chao1	Observed features	Shannon entropy	Simpson
		FOREST		
S1-FC 2	3807.89	3349	10.56	0.998
S2-FC 2	3985.56	3585	10.74	0.998
S3-FC 2	3699.43	3363	10.47	0.998
S4-FC 2	3559.45	3185	10.64	0.998
S5-FC 2	3678.59	3282	10.45	0.998
S6-FC 2	3332.73	3010	10.32	0.997
		AGRICULTURE		
S1-AL 2	3575.5	3181	10.58	0.998
S2-AL 2	3625.24	3302	10.65	0.998
S3-AL 2	3710.49	3286	10.67	0.998
S4-AL 2	3307.75	3000	10.39	0.998
S5-AL 2	3610.19	3180	10.53	0.998
S6-AL 2	1914.61	1770	8.26	0.986
		FLOOD ZONE		
S1-FZ 2	732.5	683	6.37	0.960
S2-FZ 2	1799.17	1609	8.59	0.991
S3-FZ 2	1720.57	1428	5.33	0.733
S4-FZ 2	1725.1	1627	8.56	0.988
S5-FZ 2	1351.21	1246	7.17	0.969
S6-FZ 2	4039.81	3682	10.9	0.998

Beta diversity analysis based on the Bray-Curtis distance matrix showed the maximum mapping contribution rate. Closer the two sample distances, more similar the species composition structure. For a two-dimensional PCoA plot, the first and second primary coordinates were chosen. Two principal coordinates (PCs) explained 30.76 % of the total variance in bacterial communities among the land cover types, with PC1 and PC2, respectively contributing 19.74 % and 11.02 % of the total variability (Fig. 4B). Comparison of the PCs showed the species composition of forest and agriculture land were highly similar and overlapped whereas flood zone samples were distant and not homogeneously distributed and hence have different community structures with reference to PC1. The distribution of six replicates of each landform on the eclipse of the bi-plot led to the establishment of three groups of land covers.

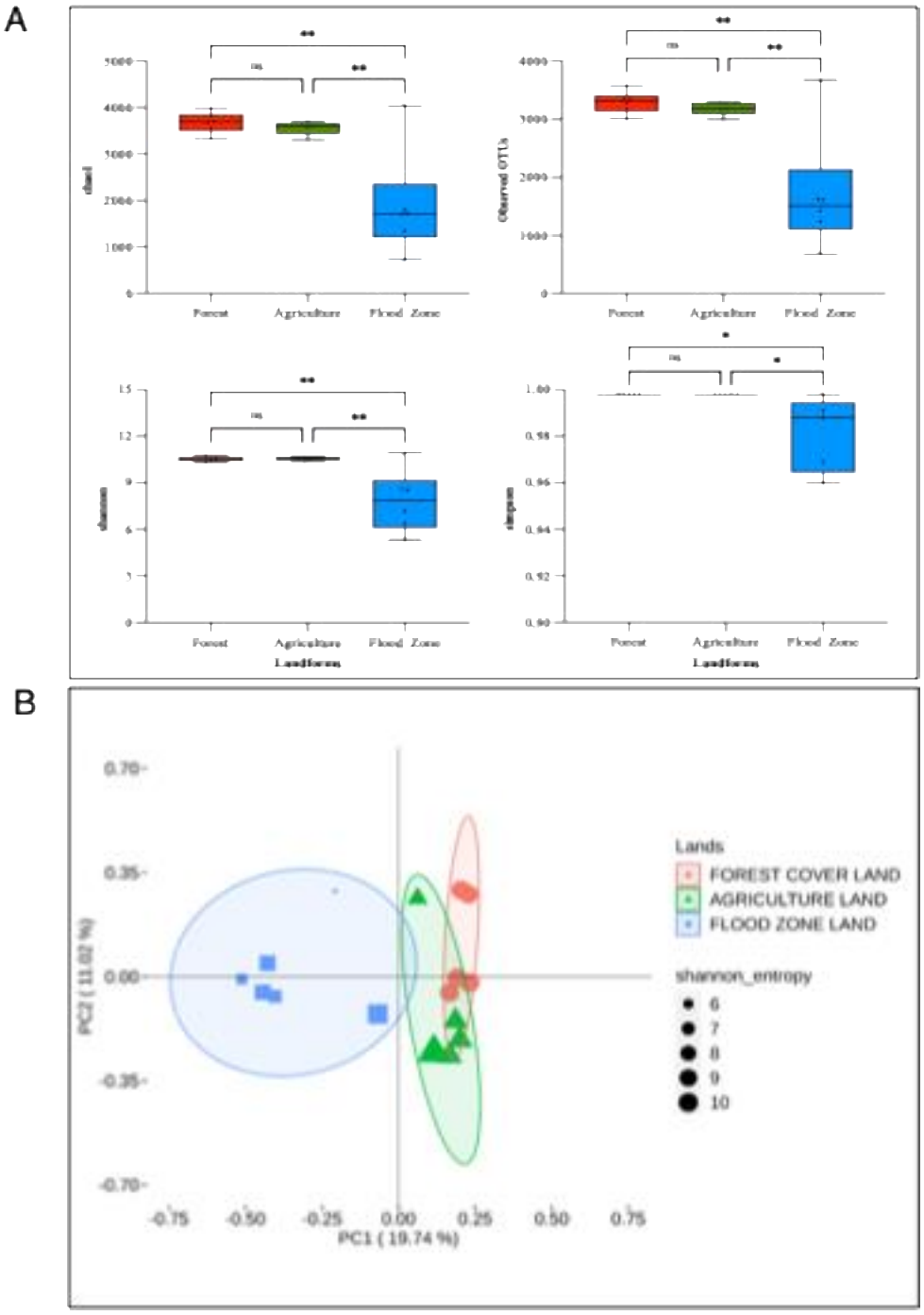


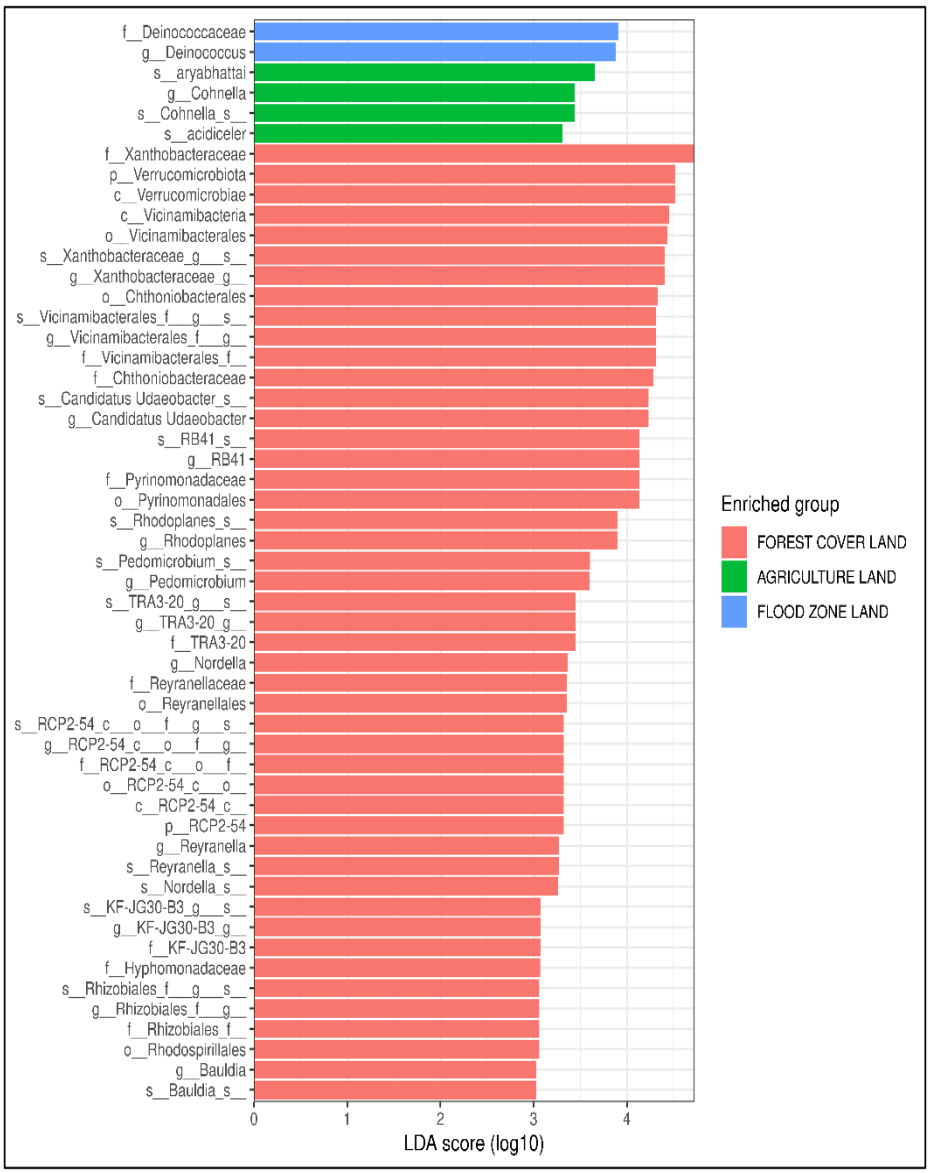
Fig. 4. Comparative bacterial diversity analysis among forest, agriculture and flood zone of Pranmati basin. **A** Boxplot of alpha diversity indices determined by Chao1, observed OTUs, Shannon entropy and Simpson. The line in box depicts the median value, box covers 95% confidence intervals, vertical lines reach to farthermost samples within 1.5 x box height and any outliers are specified as points. **B** Principal Coordinate Analysis (PCoA) based on the correlation of the most abundant ASVs across land covers using the Bray Curtis distance matrix. The percentage represents the variability of samples in PC1.

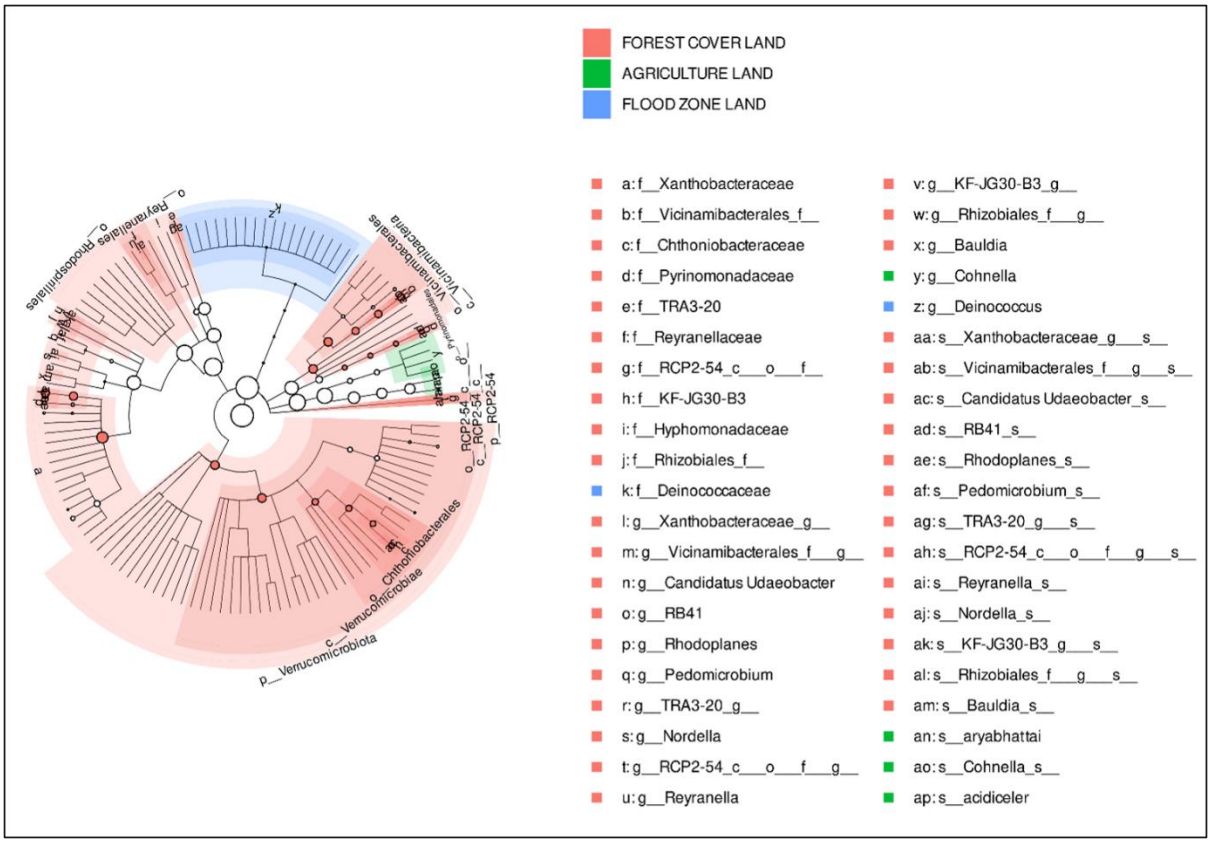
Differential abundance analysis using linear discriminant analysis effect size (Lefse) LDA bar plot among the land cover types and the influencing degree of species was expressed by LDA score ≤ 3 or the length of bar in histogram (Fig. 5A). Forest land was the most enriched group with maximum species abundance including family Xanthobacteraceae and Vicinamibacterales, genus *Candidiatus udaeobacter*, *RB41*, *Rhodoplanes,* etc. followed by agriculture land including species *Aryabhattai*, *Cohnella* and *Acidiceler* and minimum in flood zone land which includes family Deinococcaceae and genus *Deinococcus*. In the cladogram, the circle extended inside out illustrated the classification from phylum to species (Fig. 5A). Each small circle at a different classification represents a taxon and the diameter of the circle is proportional to the relative abundance. It showed the core bacterial species with prominent differences at all levels and this indicates forest is having higher species abundance when compared to agriculture and flood zone.

The functional contributions across different landforms suggests various soil microbial activities in multiple

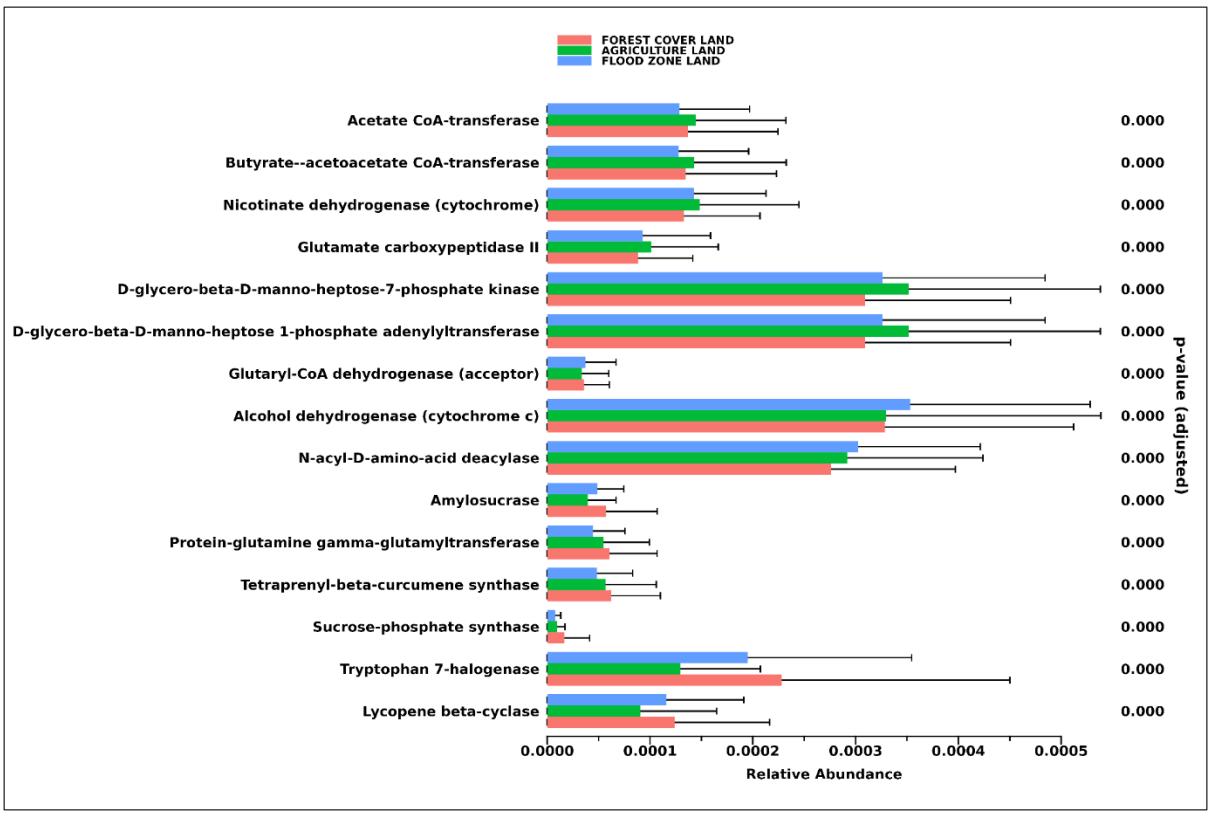
metabolic pathways and biosynthetic processes. Enzyme Commission (EC) illustrated D-glycero-beta-D-manno-heptose-7-phosphate kinase and D-glycero-beta-D-manno-heptose-1-phosphate adenylyltransferase were the most abundant enzymes in all the landforms, involved in the biosynthesis of lipopolysaccharide (LPS) which constitutes the outer membrane in gram negative bacteria and helps them in sustaining the stressed environmental conditions. Alcohol dehydrogenase activity is not specific to any bacterial group and plays a major role in detoxification, energy production, and metabolic pathways (Fig. 5B). The Kyoto Encyclopedia of Genes and Genomes (KEGG) prediction showed (Fig. 5C) an abundance of functions such as heptosyltransferase and glycerol-3-phosphate cytidylyltransferase aids in LPS biosynthesis, ethanolamine utilization protein (carbon and nitrogen nutrient source for bacteria) helps bacteria in nutrient cycling processes. KEGG Orthology (KO) bar plot taking forest land as reference (Fig. 5D) mainly belonging to metabolism involving LPS biosynthesis, environmental information processing implying signal transduction pathway, human diseases involving immune disease across the landforms.

A

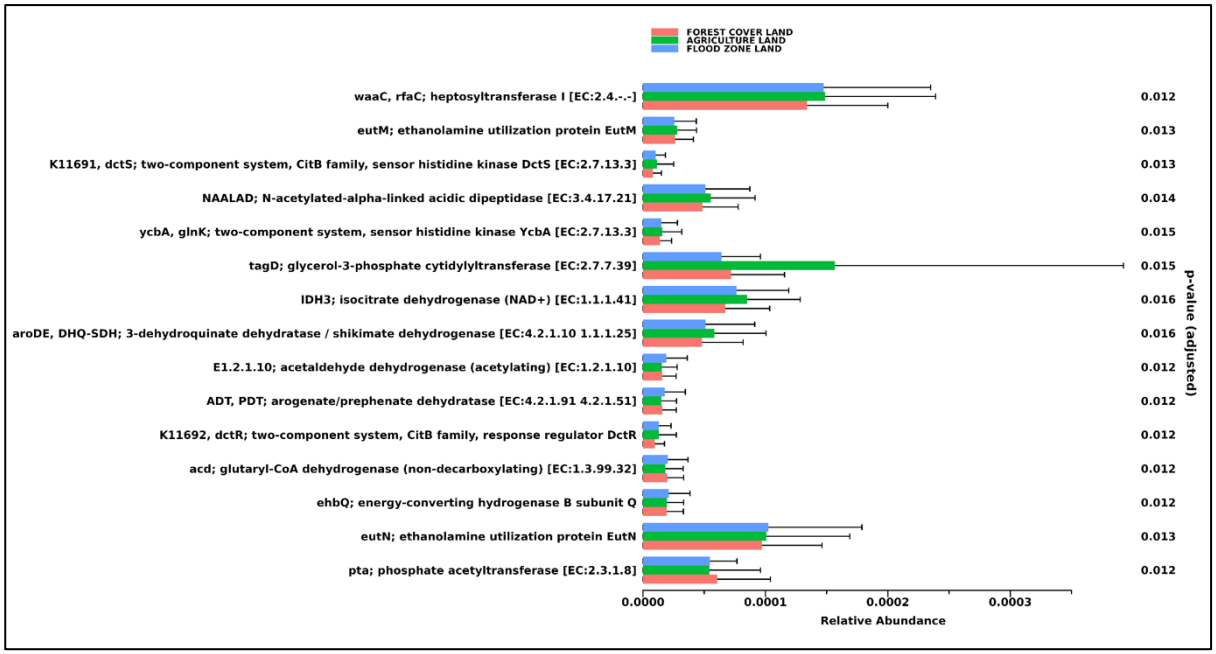



FOREST COVER LAND
AGRICULTURE LAND
FLOOD ZONE LAND
a:f__Xanthobacteraceae
b:f__Vicinamibacterales_f__
c:f__Chthoniobacteraceae
d:f__Pyrinomonadaceae
e:f__TRA3-20
f:f__Reyranellaceae
g:f__RCP2-54_c___o___f__
h:f__KF-JG30-B3
i:f__Hyphomonadaceae
j:f__Rhizobiales_f__
k:f__Deinococcaceae
l:g__Xanthobacteraceae_g__
m:g__Vicinamibacterales_f___g__
n:g__Candidatus Udaeobacter
o:g__RB41
p:g__Rhodoplanes
q:g__Pedomicrobium
r:g__TRA3-20_g__
s:g__Nordella
t:g__RCP2-54_c___o___f___g__
u:g__Reyranella
v:g__KF-JG30-B3_g__
w:g__Rhizobiales_f___g__
x:g__Bauldia
y:g__Cohnella
z:g__Deinococcus
aa:s__Xanthobacteraceae_g___s__
ab:s__Vicinamibacterales_f___g___s__
ac:s__Candidatus Udaeobacter_s__
ad:s__RB41_s__
ae:s__Rhodoplanes_s__
af:s__Pedomicrobium_s__
ag:s__TRA3-20_g___s__
ah:s__RCP2-54_c___o___f___g___s__
ai:s__Reyranella_s__
aj:s__Nordella_s__
ak:s__KF-JG30-B3_g___s__
al:s__Rhizobiales_f___g___s__
am:s__Bauldia_s__
an:s__aryabhattai
ao:s__Cohnella_s__
ap:s__acidiceler
p__Verrucomicrobiota
c__Verrucomicrobiae
Chthoniobacterales
Vicinamibacteraceae
Rhodoplanes
Reyranellales
RCP2-54
p__RCP2-54

B


FOREST COVER LAND
AGRICULTURE LAND
FLOOD ZONE LAND
Acetate CoA-transferase
Butyrate--acetoacetate CoA-transferase
Nicotinate dehydrogenase (cytochrome)
Glutamate carboxypeptidase II
D-glycero-beta-D-manno-heptose-7-phosphate kinase
D-glycero-beta-D-manno-heptose 1-phosphate adenylyltransferase
Glutaryl-CoA dehydrogenase (acceptor)
Alcohol dehydrogenase (cytochrome c)
N-acyl-D-amino-acid deacylase
Amylosucrase
Protein-glutamine gamma-glutamyltransferase
Tetraprenyl-beta-curcumene synthase
Sucrose-phosphate synthase
Tryptophan 7-halogenase
Lycopene beta-cyclase
0.000
p-value (adjusted)
0.0000
0.0001
0.0002
0.0003
0.0004
0.0005
Relative Abundance

C



D

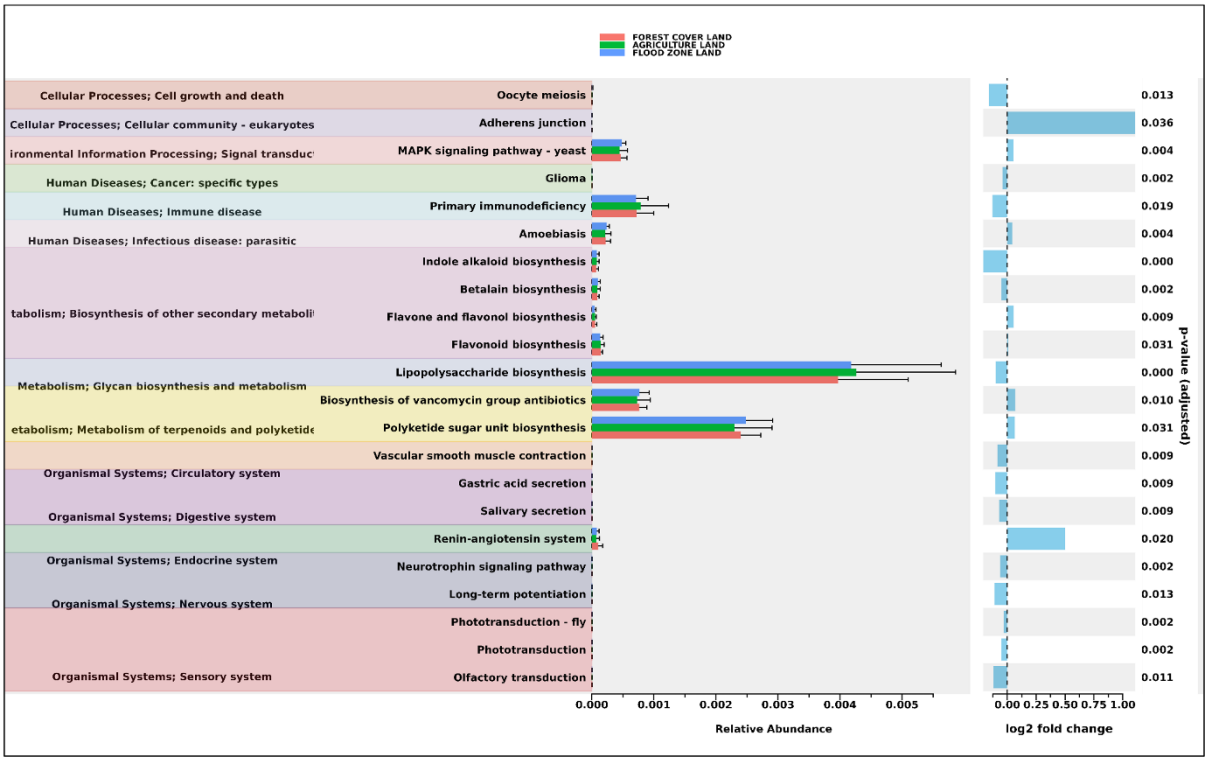


Fig. 5. The LEfSe (LDA Effect Size) analysis and predicted functional groups of OTUs in three landforms. **A** The histogram of the LDA score showed the biomarkers with statistical difference between land covers and in cladogram the circle extended inside out illustrated the classification from phylum to species. **B** Enzyme Commission (EC) **C** Kyoto Encyclopedia of Genes and Genomes **D** KEGG Orthology (KO) barplot taking forest cover as reference using PICRUSt.

3.6. Correlation among biotic and abiotic factors

Principal component analysis was performed among abiotic and biotic factors of soil. Two principal components (PC1 37.5% and PC2 14.4%) explained 51.9 % of the total variance. The score plot of the landforms and loading plot of physicochemical parameters, bacterial genera and enzyme activity are depicted in Fig. 6. The score plot depicts the abiotic and biotic factors present in forest and agricultural land shows positive correlation and negatively correlated to flood zone land (Fig. 6A). Loading plot shows three groups, among them group I and group II are positively correlated whereas group III is negatively correlated. All the physicochemical parameters (except pH) show positive correlation with the soil enzyme activity.

The correlations among different bacterial genera show that while many Proteobacteria (*Pseudomonas*, *Rhodoplanes*, *Bradyrhizobium*), Firmicutes (*Bacillus*), Verrucomicrobiota (*Candidatus Udaeobacter*), and Acidobacteriota (RB41) are positively associated with

healthy soil conditions, some genera (e.g., *Sphingomonas*, *Lactobacillus* and *Planomicrobium*) are not. Genera belonging to phylum Actinobacteriota show both negative (*Nocardioides*, *Arthrobacter*, *Pseudarthrobacter*, *Rhodococcus*, *Mycobacterium*) and positive (*Solirubrobacter*, *Conexibacter* and *Gaiella*) correlation. Mostly the genera present in flood zone are showing negative correlation to biotic and abiotic factors, resulting in disturbed or poor soil health conditions whereas forest and agriculture land were positively correlated hence, having healthy soil (Fig. 6B).

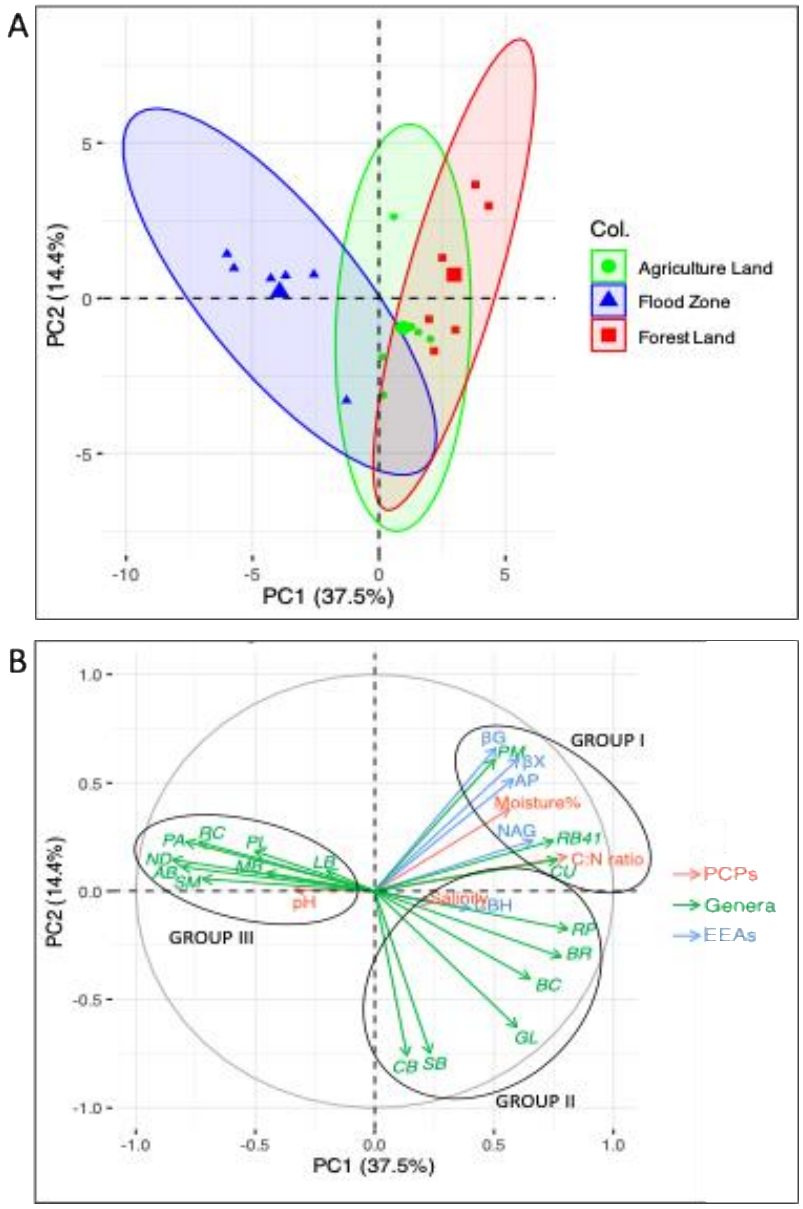


Fig. 6. Principal Component Analysis (PCA) of physico-chemical parameters (PCPs), Bacterial genera (PM- *Pseudomonas*, RP- *Rhodoplanes*, BR- *Bradyrhizobium*, SM- *Sphingomonas*, SB- *Solirubrobacter*, CB- *Conexibacter*, GL- *Gaiella*, ND- *Nocardioides*, AB- *Arthrobacter*, PA- *Pseudarthrobacter*, RC- *Rhodococcus*, MB- *Mycobacterium*, LB- *Lactobacillus*, PL- *Planomicrobium*, BC- *Bacillus*, CU- *Candidatus Udaeobacter*, RB41) and (EEAs) extracellular enzyme activity (βX- β-1, 4-Xylosidase, CBH- Cellobiohydrolase, βG- β-1, 4-Glucosidase, AP-Acid Phosphatase, NAG- β-1, 4-N-acetyl-β-Glucosaminidase) of three landforms. **A** PCA score plot **B** PCA loading plot.

4. Discussion

The physicochemical characteristics determine the nutrient status of soil, which varies depending on the bedrock type, vegetation, physiographic position, climate, etc. (Sharma et al., 2010). Physical and chemical indicators of soil health include soil texture, soil moisture, pH, salinity, EC, and CHNS components. In the current investigation, biotic and abiotic factors affecting soil health has been assessed and a relationship has been established among forest, agriculture and flood zone land. The Pranmati basin soil belongs to the sandy class based on soil texture analysis. Sandy soil is primarily composed of sand-sized particles ranging from 0.05 to 2mm and is known for its high permeability, low water retention, and low nutrient-holding capacity (Huang and Hartemink, 2020).

The moisture content of the soil samples ranged between 0.44% to 2.13%, with forests having the highest moisture. Summer sampling may have contributed to the low moisture content in all locations. According to Huang and Hartemink (2020) and Herawati et al. (2021), sandy soil has the least capacity to hold water, resulting in low moisture content and the current study classifies the soil texture as sandy soil. Huge trees in forests aid in retaining water, while agricultural land with 93% sand and crops also retains water, albeit to a lesser extent than forest land with 90% sand (Ellison et al., 2017). Flood zones with the lowest moisture content may have less water-holding capacity due to the presence of large coarse particles in 100% sand (Ferro, 2017). In a similar study, Bargali et al. (2019) also observed the lowest moisture content in the hill region of the Central Himalayas among all the land use systems. In present study, a significant difference in the C/N ratio between the samples, ranging from 0.11 to 1.94 was observed. It was maximum in forest land, followed by agricultural land, and it was minimum in the flood zone. It might be due to the huge biomass/organic matter in forests and in agricultural land due to the addition of manure and was negligible in flood zone land due to no vegetation or source of carbon/nitrogen (Ellison et al., 2017). Bargali et al. (2019) had observed a C/N ratio of 7.24–10.28, highest in the Tarai region and lowest in the Bhabhar region of Nainital, in all the agricultural fields.

The pH of the soil samples was slightly acidic across the landforms, which aligns with the findings of Rana et al. (2025). This is likely due to the presence of the phylum Acidobacteria and pine forests, as the pine needles release acid (Turner and Newman, 2005). Non-saline soil was present in the pranmati basin which corresponds to the result reported by Siwach et al. (2021). High soil salinity has negative effects on plant growth, bacterial communities, and their activities, like reduction in carbon dioxide generation, microbial biomass, or enzyme activity (Zhang et al., 2019). All the soil samples had an EC ranging from 41.1 to 66 S/cm, indicating fertile and healthy soil. Siwach et al. (2021) reported a similar observation in the Garhwal Himalayas, where the EC ranged from 40.80 to 228 S/cm. Shifts in the parameters of pH and EC can demonstrate the impact of variations in pH or EC on specific microbial-mediated processes.

The enzymes that help with cycling carbon (C), nitrogen (N), and phosphorus (P) find substrates and work efficiently. The structure of the bacterial population and changes in the chemical makeup of the soil due to different land use contribute to this (Wang et al., 2020). In the present study, acid phosphatase and β-glucosidase (P- and C-cycling enzymes) have the most consistent and potentially high soil enzyme activity in all the samples. Weathering rocks, soil erosion, dead animal bones, and other factors may contribute to high phosphorus enzyme activity (Lal and Stewart, 2016). Haynes (1982) states that soil with a pH between 6 and 7.2 typically contains more phosphorus availability. In this study, the pH of soil samples ranged from 6.39 to 6.87 across all land use patterns, indicating a change in soil enzyme activity that directly impacts plants. An earlier study revealed that vegetation succession significantly influences soil enzyme variation (Wang et al., 2020). Forest land had the highest levels of P- and C-cycling enzyme activities. This might be because plant roots can hold on to nutrients and pine needle litter is acidic, which helps break down carbon and phosphorus molecules (Turner and Newman, 2005; Rana et al., 2021).

Nitrogen was found in trace amounts but it was more in agricultural land compared to forest land, possibly due to the use of manure to increase crop yield (Lal and Stewart, 2016; Ferro, 2017). Subsistence farming practices that rely on manure and abstain from pesticides have a beneficial impact on microbial flora compared to conventional farming, which uses synthetic fertilizers and pesticides. Studies have shown that manure promotes soil health by enhancing diverse and stable microbial communities, elevating nutrient cycling, improving soil structure, promoting aeration, and retaining water. This contrasts with the heavy use of chemical inputs like fertilizers and pesticides, which leads to a shift in microbial diversity favoring resistant strains, decreased microbial flora, and decreased fertility in agricultural fields (García-Orenes et al., 2016; Zhang et al., 2019; Liu et al., 2022).

Many phosphate-solubilizing bacteria (PSB), like *Pseudomonas*, *Bacillus*, *Rhizobium*, etc., play a vital role in phosphorus mineralization (Billah et al., 2019). The highest activity of phosphorus cycling enzymes was found in forests, where *Bacillus* (8.14%) and *Pseudomonas* (2.81%) were present, making up 10.95% of the total. In contrast, only *Bacillus* (9.3%) was found in agricultural land, and no such genus was found in the flood zone. As reported by Sarkar and Singh (2022), the rocks present in the Pranmati basin are sericite schist, augen gneiss, ultramylonite, and protomylonite. When these rocks weather or erode due to soil erosion, they contribute phosphorus in the form of apatite and organic matter, which may indirectly contribute to the soil's carbon content. Actinobacteria and genera like *Bacillus, Pseudomonas, Streptomyces, Cellulomonas, Clostridium,* and *Actinomycetes* are involved in soil organic matter decomposition and are the key source of carbon (Bin, 2021). These enzymes were most active in forestland due to the presence of Actinobacteria (7.63%), followed by *Bacillus* (8.14%) and *Pseudomonas* (2.81%). In agricultural land, Actinobacteria (14.38%) and *Bacillus* (9.3%) were most common, while in the flood zone, only Actinobacteria (30.5%) was found. Continuous disturbance events such as flash floods, cloudbursts, and deglaciation alter the microflora in naturally disturbed areas, preventing them from residing for longer durations, resulting in low microbial activity in the flood zone.

All three sampling locations of the Pranmati Basin have identified a total of 49 phyla, 156 classes, 367 orders, 626 families, and 1465 genera. Two phyla, Actinobacteriota and Proteobacteria, contributed 50–58% of the total bacterial abundance, which proves their dominant status among bacterial populations in all three locations. Actinobacteriota is very important for breaking down organic matter, recycling and cycling

nutrients, keeping the soil stable, and maintaining the ecological balance of microbial communities. Proteobacteria, on the other hand, helps with biodegradation, bioremediation, plant symbiosis, and cycling nutrients in soil ecosystems (Ansari et al., 2023; Rana et al., 2025). Hence, for agricultural practices, environmental conservation initiatives, and soil health, it is essential to comprehend their ecological responsibilities (Dindhoria et al., 2021; Ansari et al., 2023). Reports from the past (Dindhoria et al., 2021; Viruel et al., 2022; Ansari et al., 2023; Jia et al., 2023; Rana et al., 2025) agree that Proteobacteria are more common in forests than Actinobacteriota in farmland and flood zones.

Two genera, i.e., *Bacillus* in forest and agricultural land, constitutively contribute 17.44% abundance, while *Lactobacillus* has 18.19% abundance in the flood zone. Researchers recognize *Bacillus* for its ability to encourage plant development, prevent infections, and participate in the nutrient cycle (Wongkiew et al., 2022). Biopesticides, biofertilizers, and other agricultural products increasingly incorporate *Bacillus* to reduce chemical use, increase crop yields, encourage environmentally friendly practices, and enhance soil health (Hashmi et al., 2020; Viruel et al., 2022). The ability of these bacteria to produce endospores that have a high resilience to heat, chemicals, and dehydration enables them to endure harsh environments (Mandic-Mulec et al., 2016). *Lactobacillus* helps with pH regulation, organic matter breakdown, nutrient cycling, carbohydrates, and amino acids metabolism (Hashmi et al., 2020). In combination, they support the soil ecosystem's general health and fertility due to high resilience and resistance from environmental stress and natural disturbances (Wongkiew et al., 2022).

The alpha diversity indices reflect the richness and evenness of species, which include Chao1 and observed features (species richness), Simpson diversity, and Shannon entropy (richness and evenness). The alpha diversity indices show a robust bacterial diversity, with a higher presence of species in forest land, followed by agricultural land, and the lowest diversity in flood zones. The forest's intricate ecosystem and comparatively undisturbed environments are the home to a wide variety of plants, animals, and microbes and serve as the hotspot for biodiversity (Zuo et al., 2023). Compared to agricultural land, the forest exhibits significantly higher alpha diversity, indicating single species dominance, high resistance, and a resilient soil ecosystem. The flood zone exhibits the lowest alpha diversity, leading to a dispersed distribution of species and a diverse community due to regular disturbances.

The beta diversity analysis reveals that forest and agricultural land have the highest number of overlapping ASVs due to their proximity, resulting in an extremely similar species composition. Flood zones displayed the fewest ASVs, dispersed and not evenly distributed, leading to distinct community structures. Griffin et al. (2017) reported a similar high overlap between soil and sediment samples, which led to community homogenization among them. Differential abundance analysis (Lefse) also shows that the core bacterial species were noticeably different at every level of the hierarchy. This indicates that there are significantly more species in forest land compared to flood zones and farmland.

Principal component analysis suggested the interconnectedness between biotic and abiotic factors of soil health. The score plot suggests that abiotic and biotic factors present in forest and agricultural land show positive correlation; thus, these two locations share similar soil health characteristics (Griffin et al., 2017). The physical boundaries of the forest sample sites abut both the agricultural fields and flood zones. The rainwater carrying organic matter and nutrients flows from the forest floor to terraced agricultural fields and has a comparatively longer resting time there (Ferro, 2017). Farmers procure fodder from the forests to feed the livestock and then apply the manure to the agricultural fields. Despite the physical link between forests and flood zones, the soil health dynamics suffer from frequent disturbances, nutrient leaching, and a lack of organic matter (Rawat et al., 2022; Rana et al., 2025). The loading plot indicates all the physics. The loading plot shows a positive relationship between all physicochemical parameters (except pH) and enzyme activity in the soil. This means that when certain soil conditions (soil moisture, salinity and C:N ratio) get better, enzyme activities that are important for nutrient cycling (βX, CBH, βG, AP, NAG) increases (Wang et al., 2020), while high acidity or alkalinity may hinder enzyme activity.

Current study found a positive association between different bacterial genera of phylum Proteobacteria, Firmicutes, Verrucomicrobiota, and Acidobacteriota and healthy soil environments. Conversely, Actinobacteriota exhibited both positive and negative correlations, while some genera, including *Sphingomonas*, *Lactobacillus*, and *Planomicrobium*, demonstrated negative associations. This suggests a complex interaction within the microbial community, where some bacteria may flourish under specific conditions while others might not (Rana et al., 2025). Mostly the genera present in the flood zone are showing negative correlation to biotic and abiotic factors, whereas forest and agricultural land were positively associated due to similar soil health characteristics. This implies that we can enhance management practices in agriculture and forest land while we need to take action in flood zones to mitigate the negative impact on soil health (Wongkiew et al., 2022).

The metabolism plays a central role in all landforms, primarily involving the biosynthesis of lipopolysaccharide (LPS), alcohol dehydrogenase activity, and the ethanolamine utilization protein. These processes play a significant role in energy synthesis and metabolic pathways (Ahmed and Yonus, 2019; Giordano et al., 2020; Zeng et al., 2021). LPS biosynthesis is essential for soil bacteria to survive, adapt, and interact with each other and with the complex and dynamic soil ecology (Giordano et al., 2020). Microbes in these areas are involved in many biogeochemical cycles and adapted to survive harsh environments (Rana et al., 2025). This is supported by

the fact that it changes how microbes interact with each other and makes bacteria more resistant to environmental stressors.

In addition, the presence of several unclassified genera in the soil of the Pranmati basin presents an opportunity for finding novel bacteria and elucidating their functions. The identification of such diversity is important to determine the changes happening in the soil microflora due to changes in climate and land use patterns (Griffin et al., 2017; Dindhoria et al., 2021). To use microbes in the restoration of degraded environments, it is essential to assess microbial diversity and the detailed interactions of biotic and abiotic factors in pristine environments (Rawat et al., 2022; Rana et al., 2025).

5. Conclusion

The bacterial community and their impact on soil health was observed in three different landforms of Pranmati Basin, Himalayan Critical Zone, Uttarakhand, India. The relationship between biotic and abiotic factors, land use patterns and composition of bacterial communities was studied. It was observed that bacterial functions were influenced by land use patterns, e.g. enzyme activities related to Phosphorus and Carbon cycling were maximum in forest land and minimum in flood zone. The alpha diversity analysis showed that the forestland had the highest bacterial abundance and species richness, followed by agricultural land suggesting physical as well as material flow interconnectedness, whereas flood zone despite having physical interconnectedness have low bacterial diversity due to repeated disturbances. Beta diversity analysis indicated the closeness of forest and agricultural land whereas microbial diversity was dispersed and distinct in flood zone. Functional analysis showed that the biosynthesis of lipopolysaccharide was the prime metabolic function in all the landforms. The study gives insight to the endemic diversity residing in this ecologically sensitive area, before it shifts or changes due to climate change, flash floods, deglaciation, cloud bursts, land degradation etc. Therefore, strategies need to be made for managing farmlands and natural disturbances sustainably, and reducing the effect of anthropogenic activities by restricting human perturbation in the critical zone. To solve these environmental issues and promote sustainable land management, it's essential to comprehend these soil microbial interactions and their roles.

Author Contributions

Nitika Sharma: Writing - original draft, Visualization, Methodology, Investigation, Formal analysis; **Rishikesh Krishan Laxmi:** Writing - Review & Editing, Visualization, Resources; **Mohit Kumar:** Writing - Review & Editing, Validation, Conceptualization; **Varunendra Singh Rawat:** Writing - Review & Editing, Validation, Conceptualization; **Dileep Kumar Singh:** Writing - Review & Editing, Validation, Conceptualization, Supervision, Funding Acquisition, Project Administration; **Neeta Sehgal:** Review & Editing, Validation, Conceptualization, Supervision, Project Administration, Funding Acquisition.

Conflict of Interest Statement

None of the authors have any kind of conflict of interest.

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Data Availability Statement

The data generated during the current study will be made available on reasonable request.

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