



Biogeographic Distribution of Soil Bacteria across Precipitation Gradient in Soil Profiles

Shumaila Memon ^a and Emmanuel Kazuva ^{b*}

^a Department of Education and Literacy, Sindh Government, Sindh 76040, Pakistan.

^b Department of Geography, Open University of Tanzania, Dar es Salaam, Box 23409, Tanzania.

Authors' contributions

This work was carried out in collaboration between both authors. Author SM designed the study, managed literature review, prepared the tools, guided and fieldwork and wrote the first draft. Author EK conducted data analysis, involved in designing the study, managed literature review, wrote the study protocol, involved in data collection, involved in writing the first draft and revised the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The biogeographical distribution of soil microbial diversity, driven by climate change, notably precipitation, significantly influences soil functioning in diverse ecosystems. Land-use changes can alter ecosystem services mediated by microbial diversity, particularly in near-surface soil. In this study, we investigated soil bacterial diversity across precipitation gradients in various land uses. We focused on two soil layers (10-20 cm and 40-50 cm) in seven sampling areas along the Northeast China Transect. Utilizing 16S rRNA sequencing, we obtained 897,776,803 optimized sequences. The results reveal a significant shift in bacterial community composition with changing precipitation levels. As precipitation increases, the shifts in dominant bacterial phyla were generally observed. Additionally, soil depth played a role in structuring bacterial communities, with variations observed between surface and subsurface layers. Dominant taxa (Proteobacteria, Acidobacteria, Actinobacteria, Choloroflexi) exhibited changes in the 10-20 cm layer due to human activity's

*Corresponding author: E-mail: ekazuva@gmail.com;

influence, while the 40-50 cm layer showed more similarity, unaffected by human activities. Notably, parent material significantly affected soil bacterial diversity patterns, underscored by distinct habitats. Such results offer insights into climate change effects and parent material influences on soil bacterial diversity, highlighting the intricate interplay between precipitation, soil depth, and bacterial distribution; enhancing our understanding of ecosystem functioning and responses to environmental changes. This study contributes to the broader comprehension of microbial ecology and underscores the importance of considering multiple factors when studying soil bacterial dynamics.

Keywords: *Soil microbial biodiversity; agriculture; forest; climate change; high-throughput sequencing; human activity.*

1. INTRODUCTION

“Soil microbial biodiversity represents a significant challenge for better validity of soil resources and to implement more sustainable management of agricultural soil. Knowledge of the biogeographical pattern of microbial diversity across the different types of soil in different land uses and identification of potential biotic indicators of changes are critical to better understanding the land-use effects on soil microbial biodiversity. These indicators help to manage device strategies for examined microbial biodiversity” [1]. “Local to continental studies showed that soil properties (e.g., Soil pH) are diverse in the diversity and structure of microbial community patterns, with land-use and plant diversity as a secondary confounded correlate” [2,3]. “Land use plays a primary role in bacterial communities; biodiversity varies among soils as shown by a bio-geographical study [4]. Furthermore, different types of soil found in land use are essential for sustainable soil management to better understand the impacts of land use on soil microbial biodiversity specific to soil type. Soil microbes are an essential component in biogeochemical cycling” [5,6]. “The structure and biodiversity of microbial diversity are predominantly limited in soil horizons. As a result of microbial diversity that exists in the depth of the soil, the biomass of microbes often exhibits an exponential decrease with the increases in depth” [7-10]. “The spatial distribution characteristics determined the environmental interaction between soil-vegetation, functional characteristics and biodiversity patterns of soil microbes have substantial effects on the growth of the above parts of the plants” [11,12]. Although previous studies [13-15] indicate that “different ecosystems have different bio-geographical patterns of microbial biodiversity within different soil profiles, their relationship between microbial-mediated nutrient cycling”. Numerous studies, in

next-generation developments high throughput sequencing, have made whole soil microbial diversity patterns affordable at various scales [16-19]. In comparison to previous studies, “comprehensive information on the biogeographical pattern of soil bacterial communities of soil in China is limited, but recently some hard work in gathering some information was made mono ecosystems of the northeast parts of China” [18,20]. “In the previous years, the use of modern biological techniques particularly high-throughput sequencing and supplied powerful technical support for studying spatial distribution patterns of bacterial communities were limited” [5,6]. The recent findings showed “regular changes in bacterial community patterns, individual abundances, or biodiversity with environmental changes” [21]. “At the regional scale, critical environmental factors such as precipitation and temperature become more critical for the pattern of bacterial communities along precipitation gradients of both soil profile and habitat location” [22,2,23]. “The regional scale effect of soil pH and precipitation in shaping and pattern of microbial community composition has been well documented in a wide variety of habitats” [24-27].

However, the spatial scale effect for the distribution of microbial assemblage in distinctive soil profiles remains poorly understood, as most studies focus only on those communities that are found near the surface horizons. Expanding our knowledge of bacterial diversity and distribution from the soil profile to habitat ingredients will improve our understanding of biodiversity and the functioning of environmental variables.

In this study, we evaluated the biogeography of soil bacterial pattern and diversity structure along with the gradients of soil profile and precipitation for different habitats in a large number of the soil samples collected and characterized by the Northeast China Transect. The sampling sites

represented three major land uses (Cropland across all transect, grassland from the west side, and primary and secondary forest from the east side). The study aimed to determine how soil bacteria diversity patterns respond to the precipitation gradient across a wide variety of taxonomic groups. It determined the pattern of bacterial diversity in different land uses between 10-20cm and 40-50cm soil horizons and the similarities between the three land use horizons. We hypothesized that different climatic factors and soil horizons affect the soil bacterial community among different land uses.

2. MATERIALS AND METHODS

2.1 Study Area and Soil Sampling

The study used seven main study sites in the Northeast China transect, the areas experiencing precipitation changes at various gradients. The amount of precipitation ranges from 400 to 1000mm in the elevation of 140 meters West to 700 meters East above sea surface. This is the area covered by Grasslands and Forests with a total length of 500 Km from West to East. The study selected Alkali-saline soil, Black soil (chernozem) and Mollisol, and Dark-brown soil in the entire area (West to East). Seven sampling sites along the transect were selected, choosing three habitats (cropland grassland and forested land; from West to East) While grasslands were considered the primary habitats, forestland formed both primary and secondary habitats. Croplands that were translated from local natural habitats were dominated by corn that have been planted for more than 30 years in every site. In each habitat, three soil profiles of 50cm depth were collected, and the profile was divided into five sub-samples of 10cm each. The fresh samples were taken in the lab and stored at -80°C for DNA extraction. The seven sites' localization, their climate and soil types, temperature and mean annual precipitation are summarized in Table 1.

2.2 Soil DNA Extraction

The total genomic DNA was extracted from triplicate soil samples, each consisting of 0.5g soil (wet weight) using a soil DNA isolation Kit (MO BIO- Laboratories) Shanghai Majorbio Biopharm Technology Co., Ltd, each soil sample was extracted according to the manufacturer's instructions. The extracted soil DNA samples were stored at -20 °C for further subsequent metagenomic analysis.

2.3 16S rRNA Gene Amplification, Processing, and Data Sequencing

DNA was extracted from the soil samples (0.5g wet weight) using the Power Soil DNA Isolation Kit (MO BIO Shanghai LTD). Each sample was three times amplified and then combined in one sample for high throughput sequencing [18]. The V3-V4 hypervariable region of bacterial 16S rRNA was amplified using the primers 338F: ACTCCTACGGGAGGCAGCA, 806R: GACTA-CHVGGGTWTCTAAT. Functions of PCR were performed in 20uL system, containing 4 uL x 5 FastPfu Buffer, 2uL dNTPs (2.5 mmol/L), 0.8uL Forward/Reverse primer (5 umol/L), 0.4 uL FastPfu Polymerase, and 10ng Soil DNA as a template; some ddH₂O was added to the system reaching 20uL. Thermal cycling conditions; 95°C for 3min, followed by 27 cycles of 95° for 30 sec; 55°C for 30 sec; 72°C for 45 sec, then an extension at 72°C for 10 minutes.

We identified raw total sequences reads 48,874,738, Quality-filtered raw reads were clustered into Operational Taxonomic Unit (OTU) at 97% sequencing similarity using the QIIME pipeline. The species diversity indices like Shannon and Chao diversity index (richness) and ACE diversity index (evenness) were estimated at 97% similarity sequencing using Mothur [28,29].

2.4 Statistical Analysis

Principal Components Analysis (PCA) was used to compare the differences in soil bacterial diversity patterns in different habitats and PCoA was used to analyze the community differences between two layers of different soil profiles. In this study, the similarity of soil bacterial biodiversity was defined as the OTUs shared in all the soil samples relative abundance < 0.1% share in all the soil samples.

3. RESULTS

3.1 Soil Bacteria Biodiversity Pattern in Soil Depth

As shown in Table 1, a total of 897,776,803 sequences of bacteria diversity were extracted from 78 soil samples, and the length of sequencing varied from 255.466 to 256.482 base pairs (bp). Soil bacterial diversity pattern (Shannon index) evenness (ACE index) and richness (Chao Index) 97% species similarity presented. Shannon diversity index was increased along with the depth with the highest

value (6.81) at (10_20cm surface soil) and the lowest value (2.17) at (40_50). Besides, the evenness index declined with depth, with the highest values (5382) at 10_20cm respectively (Table 2).

3.2 Soil Bacteria Biodiversity Pattern on the Top Layer

The relative abundance of soil bacterial diversity on phylum level 97% similarity presented inconsistent changing patterns along with the precipitation (Fig. 1). The results showed 75% similarity as well as differences between bacterial community patterns in soil depth with PCA (Principal component analysis) levels in 10-20cm and 40-50cm depth levels in all samples. The differences in farmland, grassland, primary and secondary forest were identified. The effect of human activities was notable on natural habitats. It has the highest value in cropland at 10-20cm as compared to grassland and forest due to land-use effects. Nevertheless, at 40-50cm they have the lowest value due to precipitation impact in this depth of soil. The total result of bacterial community patterns in soil profiles from West to East, which is 75%, are similar and the pattern of biodiversity is low in the depth of cropland.

3.3 Soil Bacteria Diversity under Different Land Uses

At the phylum level, soil bacterial diversity patterns in all the samples at transect areas were studied as well and results were obtained. There were eight phyla and relative abundance over 1% in the 17 common phyla Proteobacteria (31.97%), Acidobacteria (16.27%), Actinobacteria (15.40%), Chloroflexi (10.88%), Verrucomicrobia (5.13%), Gemmatimonadetes (4.82%), Bacteroidetes (2.66%), Rokubacteria (2.02%), and accounting for 89.06% of the total relative abundance in different land uses as vindicated in Fig. 2. The community analysis pie plot showed that Proteobacteria, Acidobacteria, Actinobacteria, and verrucrombia are the most prominent phyla. The relative abundance of Acidobacteria exhibited major differences along the precipitation gradient only.

4. DISCUSSION

4.1 Soil Bacteria Biodiversity Pattern in Soil Depth

Assessing the effects of four land-use types (grassland, cropland, primary forest, and secondary forest), and investigating the pattern

of soil bacterial community and the soil depths effects; The study hypothesized that (1) the land-use type affects soil bacterial diversity pattern and substrate the quality and quantity of the soil (2) land-use effects will be most pronounced in the upper layer of the horizon (10-20cm) because of human activity, and at the depth near parent material (in this case 40-50cm) the effect would be due to soil bacterial diversity along a precipitation gradient. (3) Thus, the difference between land uses declined with the increase in soil depths because there are no human activity effects in this zone.

"It was found that the land uses had the strongest effects on soil bacterial diversity patterns in upper soil horizons and deeper soil horizons. As shown in the results, all bacterial biomass is in topsoil layers. This makes the differentials in available resources for agricultural practices and vegetation composition and tillage, causing low microbial biomass in cropland due to the effect of land use type or natural trend" [30]. "On another hand, the effect of precipitation, land use, and depths of soil on bacterial community pattern does not have a direct impact on the upper layer of horizons but it is effective at the regional of parent material (lower layers). This behaviour not only decreases the pattern but also shifts soil bacterial community structure" [28,31].

The obtained results are consistent with recent studies making reliable concluding that there is a direct relationship between bio-geographical patterns and bacterial diversity along the precipitation gradient. Various patterns, such as monotonic decreasing humpbacked or hollow, were reported. For instance, the study by Cong et al. [32] which investigated soil bacterial diversity of four typical vegetation types (cropland, grassland, primary, and secondary forest) reported that a pattern of monotonic decrease in soil bacterial diversity along with a depth of soil. Likewise, Margesin et al. [33] reported that bacterial diversity decreases with increasing depth. In this study, soil bacterial community patterns decrease with increasing depth of soil profile, and the pattern and similarity between the cropland compared to grassland are the same. However, in the primary and secondary forests, the pattern of bacterial diversity is changing with the influence of bacterial function and general environmental influences. For insistence, Actinobacteria increased in Primary and Secondary forests than how it is in the crop and grassland. This is shown in cluster analysis as displayed in Fig. 3.

Table 1. Geographical characteristics of the seven sampling sites

Sites No.	Area	Latitude	Longitude	Altitude	Soil Type	MAT*	MAP*
01	Changling	44° 35' 40"	120° 33' 44"	140m	saline-alkali soil	4.9 °C	400mm
02	Sijianfang	44° 18' 35"	124° 07' 34"	190m	chernozem	4.8°C	430mm
03	Nongan	44° 11' 33"	124° 37' 54"	250m	chernozem	4.9 °C	480mm
04	Changchun	43°59'59"	125°23'46"	200m	black soil	5.0°C	520mm
05	Dongliao	42° 54' 14"	125° 25' 37"	310m	dark brown soil	5.1°C	600mm
06	Longwan	42°22'42"	126°26'49"	670m	dark brown soil	5.0°C	650mm
07	Baihe	42° 23'04"	128° 5' 38"	780m	dark brown soil	4.9 °C	710mm

*MAT - mean annual temperature

*MAP - mean annual precipitation

Table 2. Sequencing results and diversity data from soil samples

Habitats	Differences in depths	Shannon	Chao	Ace
Cropland	10-20cm_40-50cm	6.813 _ 2.170	5234_1711	5382_1768
Grassland	10-20_40-50cm	6.620 _ 5.821	4947_3146	4947_3008
P. Forest	10-20cm_40-50cm	6.941 _ 4.960	4980_2802	4980_3327
S. Forest	10-20cm_40-50cm	6.923 _ 5.001	5180_3564	5129_4430

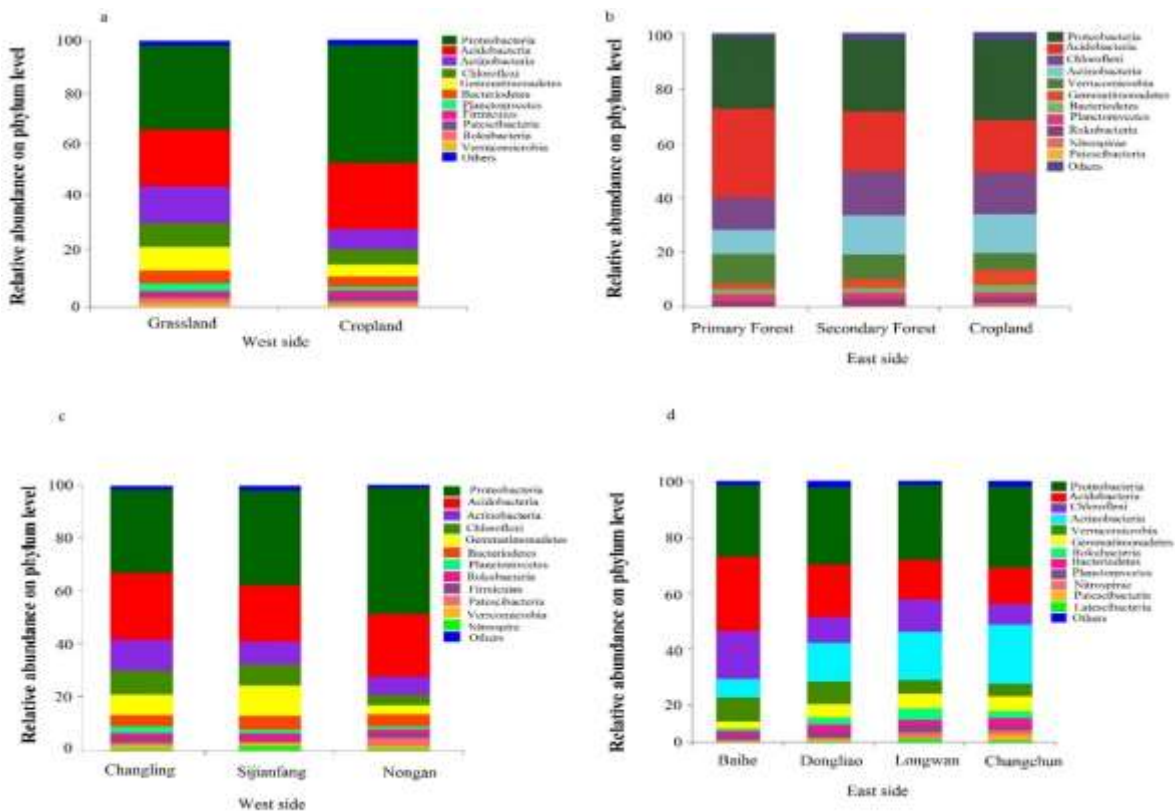


Fig. 1. Relative abundance % of soil bacteria diversity from east to west at different land-use changes (a & b); at different sampling sites (c & d)

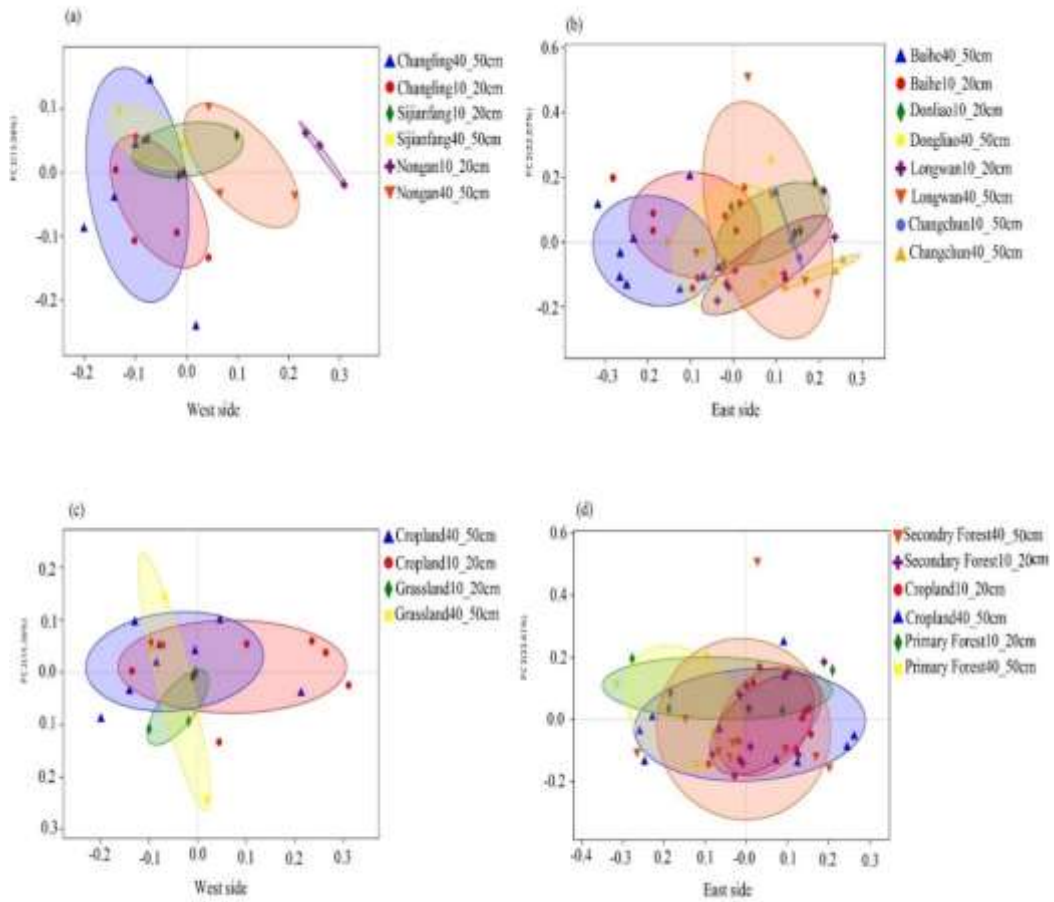


Fig. 2. Differences in sampling areas and land uses in two soil layers of the OTU level

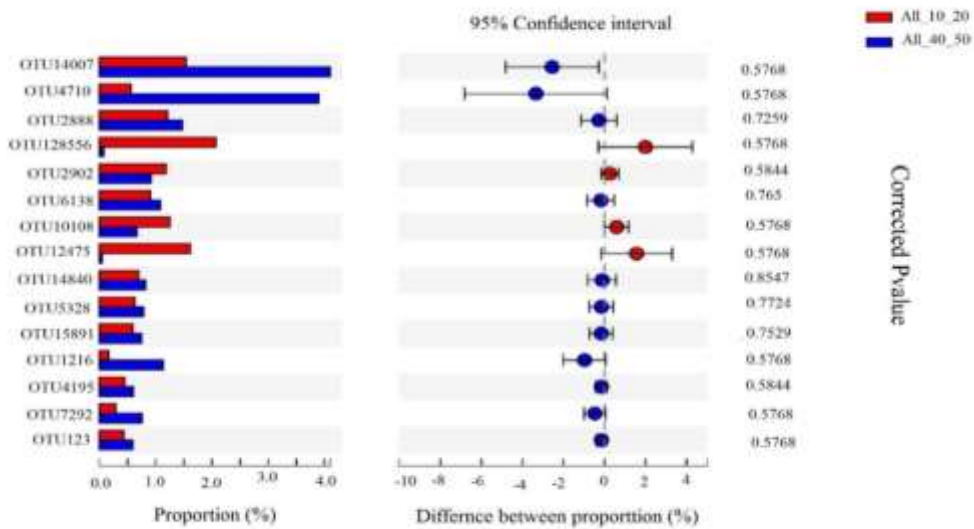


Fig. 3. Variation in the relative abundance of bacterial diversity patterns across soil depths along a precipitation gradient of two soil layers (10-20cm and 40-50cm)

4.2 Soil Bacterial Diversity Patterns and Precipitation Gradient Relationship

Precipitation, Temperature, and moisture are the main environmental factors. The northeast China transect (NECT) has a variation of mean annual temperature of 4.9 -5.1°C. On the other hand, there is also a difference in annual precipitation with a major variation of 400mm and 710mm. There is a steady trend of foliage projective cover and decreasing structure density toward the dry end of the transect. The annual precipitation changes along the elevation i.e., high elevation tends to have low precipitation and vice versa. Other major results were on soil pH which slightly increased with the depth of the soil profile but remained at a value of 5.5 in most of the measured cultivations layered (Fig. 4).

4.3 Land use Effect on Soil Bacteria Biodiversity Pattern in the Soil Profile

There is a relative abundance of soil bacterial diversity patterns on the phylum level. The percentage of the bacterial community on the phylum level showed significant differences

between the 10-20cm and 40-50cm depth of the soil profiles. In the 10-20cm soil profile (the depth dominated affected by agricultural activities) there are higher bacterial communities than at 40-50cm. However, the later depth was discovered to have special bacteria community (Chloroflexi) due to the high pH and precipitation.

According to the habitats, the bacterial community patterns are also different. Our analysis showed different graphs to describe the bacterial community pattern along the precipitation gradient and effects of the land use. Principle component analysis showed differences between land uses (west cropland and grassland) that showed 95% similarity between them. The highest values of in farmland, and lowest value in secondary forest and other species of bacterial community are also different in different habitats and the highest value of all the species are in farmland respectively primary forest, grassland, and secondary forest. The differences between the 10-20cm and 40-50cm profile depth of lands are mostly evident in all the layers of farmland grassland and primary, and secondary forest due to the effect of precipitation in the lower layers of the horizons. This does not affect the upper layers of the horizon in the

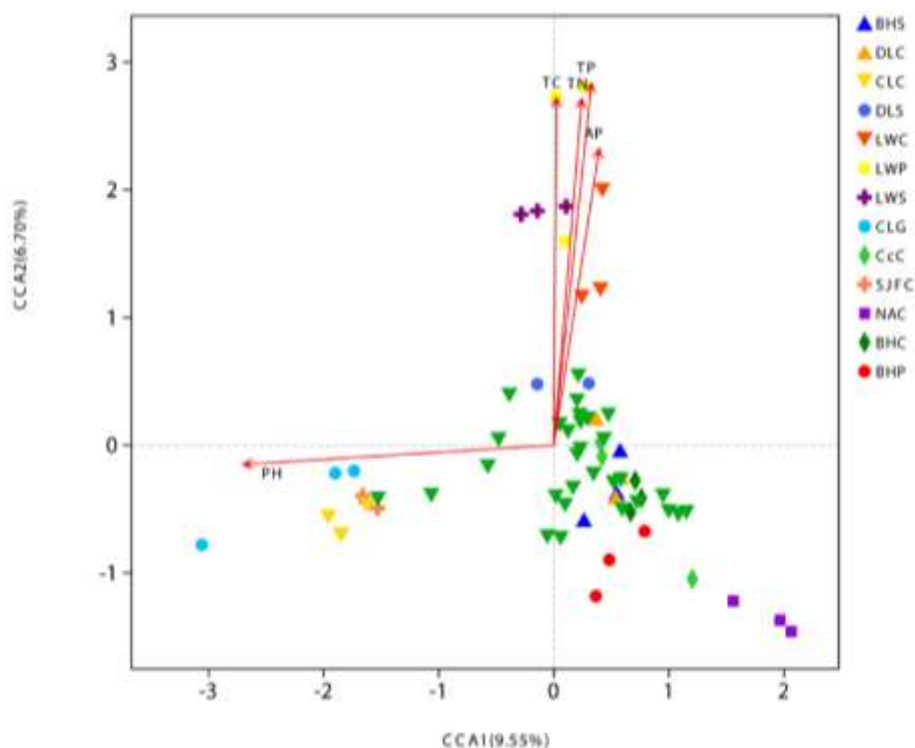


Fig. 4. The relationship between soil bacterial diversity patterns and environmental variables in soil profiles

community component. As the results showed the relationship between soil bacterial diversity with environmental factors between the seven sampling areas is significantly different. From further analysis, the west cropland and west grassland which have 95% similarity between them also showed different land use effects on the soil bacterial community along with precipitation in which bacterial biomass increased from farmland to forest to grassland in the horizons 10-20cm whereas within the 40-50cm no differences were found (ref. Fig. 1). According to the differences of communities in all seven cities, Changling, Sijianfang Nongan, Longwan, Changchun, Baihe, and Dongliao, the highest pattern is Proteobacteria in all the other species of soil bacteria diversity.

“Soil pH is a universal factor for predicting bacterial diversity and community structure” [2,34]. “The critical role of pH in the structure of bacterial diversity patterns is well characterized” (Fierer and Jackson [2,35-38]. Moreover, due to the relatively small growth tolerance exhibited by most bacterial taxa, soil microbial diversity was found influenced primarily by soil pH, making the strong connection between the soil microbes and soil pH. Furthermore, each type of microbes has an optimal pH value whereby, the increases in pH and decreases in abundance of acidobacteria were also observed in different soil types [39].

At the phylum level, there is a significant difference in soil bacterial diversity patterns in sample areas at different depths. Janssen [40] reviewed the bacterial communities of various soil types (cropland, grassland, forest, meadow, organic soil, mineral soil, and deserts) in Italy, Norway, Russia, Germany, Netherlands, United States and found that despite these regions having different soil types, proteobacteria and Acidobacteria were the central bacterial communities in all the studied regions, accounting for 39% and 20%, respectively; Actinobacteria, chloroflexi, Verrucomicrobia, forming the secondary phyla. In the current study, Proteobacteria, Acidobacteria, Actinobacteria, and Verrucomicrobia were the most prominent phyla.

From the above results, it is concluded that “at the phylum level, the dominant populations of bacteria are generally consistent in different soil types and soil depths as well as an ecosystem” [40,41]. All the microbial activity and its biomass differed strongly with the cropland, grassland, and forest in 10-20cm and are similar in four different types of land uses.

The study aimed to assess the effect of land use and precipitation on soil bacterial community patterns in seven soil sampling areas from east to west at two different land-use soil depths along a precipitation gradient. The study results have shown how land use at different soil depths has a significant impact on soil bacterial biodiversity patterns in the soil profile. Different forms of land use can alter the physical, chemical, and biological properties of the soil, influencing the composition and diversity of soil microbial communities. With all the aforementioned ways in which land use affects soil bacteria biodiversity patterns; nutrient inputs and soil fertility (agricultural practices), organic matter input, land cover changes, pollution and climate change aspects- to mention, but few, influence the types of bacteria that can thrive in the soil, resulting into bacteria biodiversity patterns, including bacterial communities. The results of this study are very important and valuable in a larger biogeographic context for agricultural soils under the common land uses that were studied [42].

5. CONCLUSION

The current research revealed that the biogeographical pattern of soil bacterial diversity under different land uses between two soil layers along a precipitation gradient in soil profiles of the Northeast China transect has a significant effect on soil bacterial diversity in soil horizons. The sample was taken from seven sampling sites of different land use (from Westside grassland to Eastside primary and secondary forest and the cropland) making 78 soil samples. There are significant differences in bacterial diversity pattern, evenness, and richness between the two layers 10-20cm and 40-50cm. Similarly, the bacterial community between the sample plots decreased with increased precipitation in profile depth. The soil's physical and chemical characteristics and land-use effect on the near-surface horizon and lower horizon of soil profile along the precipitation were the main driving forces that directly affected the pattern of the bacterial community. This study showed that land use strongly affected soil bacterial diversity in upper soil horizons but in the depth of 40-50cm, pedological conditions are very important as drivers. This research also showed 35% of bacterial biomass and their activities are found in 40-50cm near the parent material. This indicates that the parent material does not affect the 40–50cm soil horizon when studying the soil depth of ecosystem services. They also influence soil

functions through the land-use changes along precipitation gradient with limited effects on soil bacterial diversity pattern in two layers along different land-use.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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