Original Research Article

Biogeographic Distribution of Soil Bacteria across a Precipitation Gradient in Soil Profiles

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 and land uses, focusing 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. Our findings reveal significant shifts in bacterial community composition with changing precipitation levels. As precipitation increases, we observed shifts in dominant bacterial phyla and genera. Additionally, soil depth played a role in structuring bacterial communities, with variations observed between surface and subsurface layers. Dominant taxa (Proteobacteria, Acidobacteria, Actinobacteria, Cholloroflexi) exhibited changes in the 10-20 cm layer due to human activity's influence, while the 40-50 cm layer showed more similarity, unaffected by human activity. Notably, parent material significantly affected soil bacterial diversity patterns, underscored by distinct habitats. Such findings offer insights into climate change effects and parent material influences on soil bacterial diversity, highlightingthe intricate interplay between precipitation, soil depth, and bacterial distribution; enhancing our understanding of ecosystem functioning and responses to environmental changes. This research 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

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1. Introduction

Soil microbial biodiversity represents a significant challenge for better validity of soil resources and to implement more sustainable management of agricultural soil (Bender *et al*., 2016). 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 will help to manage device strategies for examined microbial biodiversity (Ranjard *et al*., 2010). 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 (Fierer and Jackson, 2006; Thomson *et al*., 2015). Landuse has a primary diver of bacterial communities; biodiversity varies among soils as shown by a bio-geographical study (Ranjard *et al*., 2013). Furthermore, different types of soil found in landuse, it is essential for sustainable soil management to better understand the impacts of landuse on soil microbial biodiversity specific to soil type. Soil microbes are an essential component in biogeochemical cycling (Ali et al., 2019; Ali et al., 2019). 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 is often exhibited exponential decreases with increases the depth (Fierer et al., 2003; Hartmann et al., 2009). Its 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 (Chabrerie et al., 2003; Gömöryová et al., 2009). Although previous studies (Xiong et al., 2012; Liu et al., 2015;Xia et al., 2016) across the different ecosystems have different bio-geographical patterns of microbial biodiversity, within different soil profiles their biodiversity and 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 (Kuang et al., 2013; Wu et al., 2013; Liu et al., 2014; Zhang et al., 2018). In a comparison of previous studies, comprehensive information on the bio-geographical pattern of soil bacterial communities in Chinese soil is limited, but recently some hard work in gathering some information was made mono ecosystems of the northeast parts of China (Liu et al., 2014; Wang et al., 2015). In the previous years, modern biological techniques particularly high-throughput sequencing and supplied powerful technical support for studying spatial distribution patterns of bacterial communities (Ali et al., 2019). The recent finding showed regular changings of bacterial community pattern, individual abundances, or biodiversity with environmental changes (Ge et al., 2008). At the regional scale, critical environmental factors such as precipitation and temperature become more criticalfor the pattern of bacterial communities along precipitation gradients of both soil profile and habitat location (Ettema and Wardle, 2002; Fierer and Jackson 2006; Lauber et al., 2008). The regional scale effect of soil pH and precipitation in shaping and pattern of microbial community composition have been well documented in a wide variety of habitats. (Nilsson et al., 2007; Baker et al., 2009; Rousk et al., 2010; Chen et al., 2013).

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 habitant 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 the 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 landuses (Cropland across all transect, grassland from the west side, and forest Primary and secondary from the east side). This study aimed to determine how soil bacteria diversity pattern respond to the precipitation gradient across a wide variety of taxonomic groups and thepattern of bacterial biodiversity in different land uses between the two horizons in 10-20cm and 40-50cm and what are the similarities between three land uses horizons, and they have regularly distributed along with the soil profile and habitat. We hypothesized thatdifferent climatic factors and soil horizons affect the soil bacterial community amongdifferent landuses.

2. Material and Methods

2.1 Study area and soil sampling

We used seven main study sites along with precipitation gradient change on the Northeast China transect. The size range of precipitation is 400 to 1000mm in the elevation of 140 meters from (West) to 700 meters (East) above sea surface; and has covered the Grassland and Forest, with a total length of a transect of 500 Km from West to East. We choose Alkali-saline soil Black soil (chernozem), Black soil (Mollisol), and Dark-brown soil from (W) to (E). There are seven sampling sites along with a transect and in every sampling site, we choose three habitats such as grassland (W) and forest (E). Grassland is a secondary one and forest includes primary and secondary forests. Croplands were translated from local natural habitats, and corn has 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 storedat -80 for DNA extraction.

The~~se~~ seven siteslocalization, their climate and soil type characteristics are in table 1.~~are situated in Changling, (44°35'40"N, 123°30'44"E), Sijianfang (44° 18′ 36”, 124° 07′ 34”),Nongan (44° 11′ 33”,124° 37′ 54”), Changchun (43°49'1.46" 125°19'24.76"), Dongliao (42° 54′ 14” 125° 25′ 37”), Longwan (27° 55' 59.99" N 120° 48' 25.19" E) Baihe (42° 23′ 4”,128° 5′ 38”); details in table 1~~.

Table 1: ~~Microbial diversity~~Geographical, climate and soil type characteristics along the gradientprecipitation~~from~~of the seven sampling sites ~~research sites in this study.~~

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

*MAT - mean annual temperature
*MAP - mean annual precipitation

2.2 Soil DNA extraction

The total genomic DNA was extracted from triplicate soil samples, each consisting of 0.5 g soil (wet weight) using a soil DNA isolation Kit (MO BIO Laboratories) Shanghai Majorbio Bio-pharm Technology Co., Ltd, each soil sample was extracted according to the manufacturer’s instructions. The extracted soil DNA samples were stored at -20 ^{0}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 (Liu et al., 2014). The V3-V4 hypervariable region of bacterial 16S rRNA was amplified using the primers 338F: ACTCCTACGGGAGGCAGCA, 806R: GGACTACHVGGGTWTCTAAT. 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 uLFastPfu Polymerase, and 10ng Soil DNA as a template; some ddH_2O was added to the system reaching 20uL. Thermal cycling conditions; 95^{0}C for 3min, followed by 27 cycles of 95^{0}for 30 sec; 55^{0}C for 30 sec; 72^{0}C for 45 sec, then an extension at 72^{0}C for 10 minutes.

We identified raw total sequences reads 48,874738, 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(Xu et al., 2012; Miera et al., 2014).

2.4 Statistical Analysis

Principal Components Analysis (PCA) was used to compare the differences in soil bacterial diversity patterns on 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

- ~~***Soil bacteria biodiversity pattern on the top layer (10-20cm)***~~

All the soil sampling sites ~~microbial diversity characterized in this study~~is 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 256.482 to 255.466 base pair (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).

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

3.2 Soil bacteria biodiversity pattern on the top layer (10-20cm) and (40-50cm)

The relative abundance of soil bacterial diversity on phylum level 97% similarity presented inconsistent changing patterns along with the precipitation that is shown in (Figure 1), they showed 75% similarity and the similarity as well as differences between bacterial community patterns in soil depth with PCA (Principal component analysis) level between 10-20cm and 40-50cm in all the samples and differences between in farmland, grassland, primary and secondary forest but the effect of the human activity only on natural habitats, and we can see the highest value in cropland in (10-20cm) as compared to grassland and forest due to land-use effects but in (40-50cm) they have the lowest value due to high precipitation in the 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.

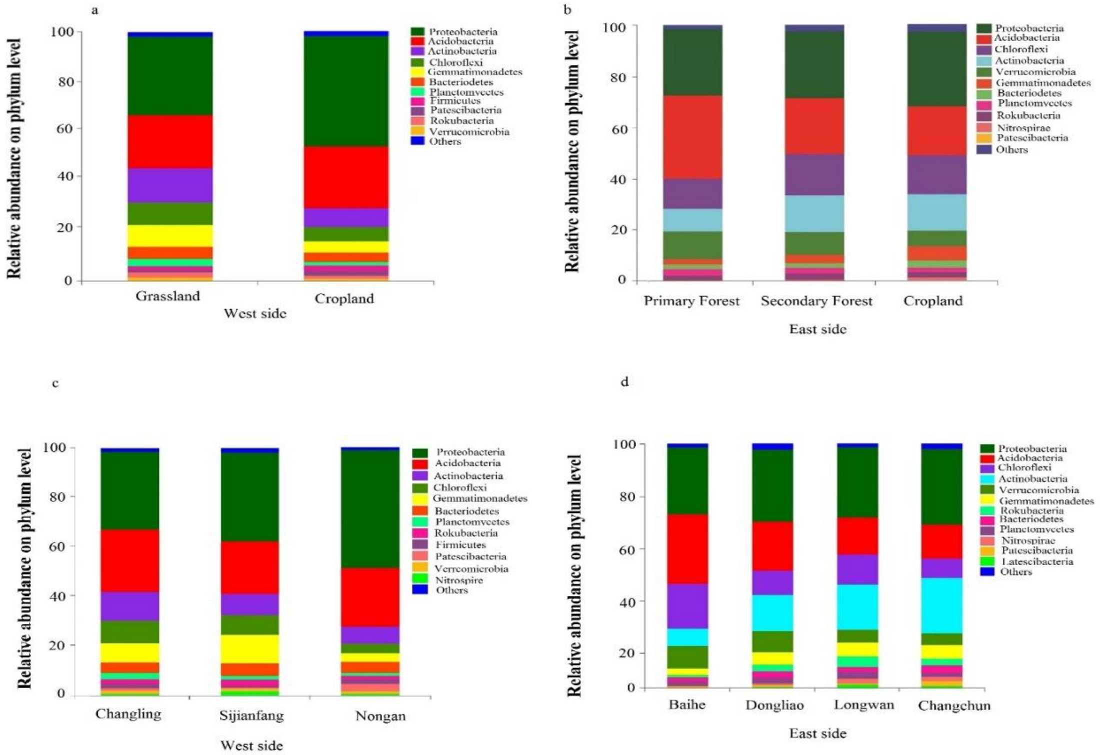

Figure 1: Relative abundance % of soil bacteria diversity from east to west at different land-use changes (a & b); at different sampling sites (c & d)

3.3 Soil bacteria biodiversity under different landuses

At the phylum level, soil bacterial diversity pattern in all the samples at transect areas. 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%),Verrucomicobia (5.13%), Gemmatimonadetes (4.82%), Bacteriodetes (2.66%),Rokubacteria(2.02%), and accounting for total 89.06% of the total relative abundance in different land uses which shows in (Figure 2). The community analysis pie plot showed Proteobacteria, Acidobacteria, Actinobacteria, and verrucrombia were the most prominent phyla. The relative abundance of Acidobacteria exhibited major differences along the precipitation gradient only.

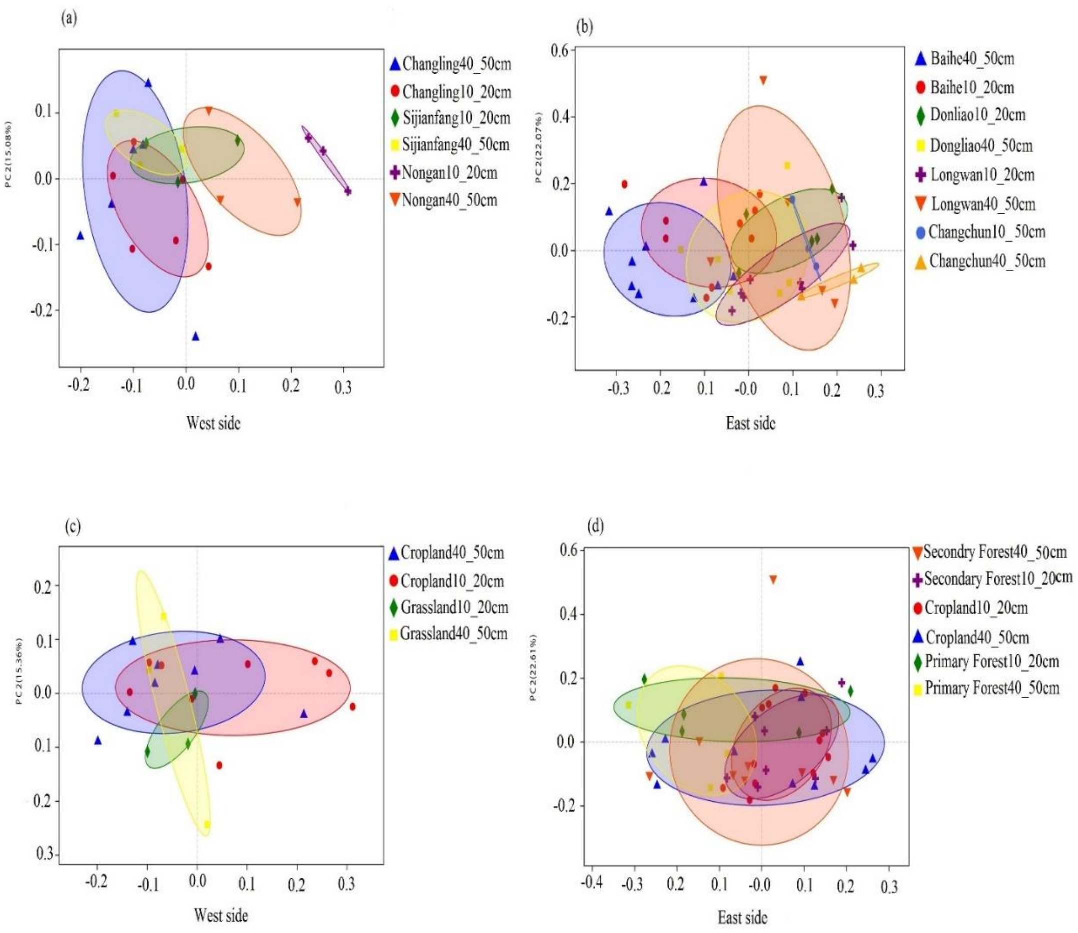

Figure 2: Differences in sampling areas and land uses in two soil layers of the OTU level

4. Discussion

4.1 Soil bacteria biodiversity pattern in soil depth

The study objectives were to assess the effects of four land-use types from the west side. These are grassland, cropland, primary forest, and secondary forest. It investigated the pattern of soil bacterial community and the soil depths effects. We 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 of 40-50cm near parent material the effect is due to soil bacterial diversity along a precipitation gradient. (3) Thus, the difference between landuses declined with the increase in soil depths (40-50cm) 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 (40-50cm).

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 (Six et al., 2006). In 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 behavior not only decreases the pattern but also shifts soil bacterial community structure (Xu et al, 2012).

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., (2013) 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., (2009) 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 that how it is in the crop and grassland. This is shown in cluster analysis as displayed in Figure 3

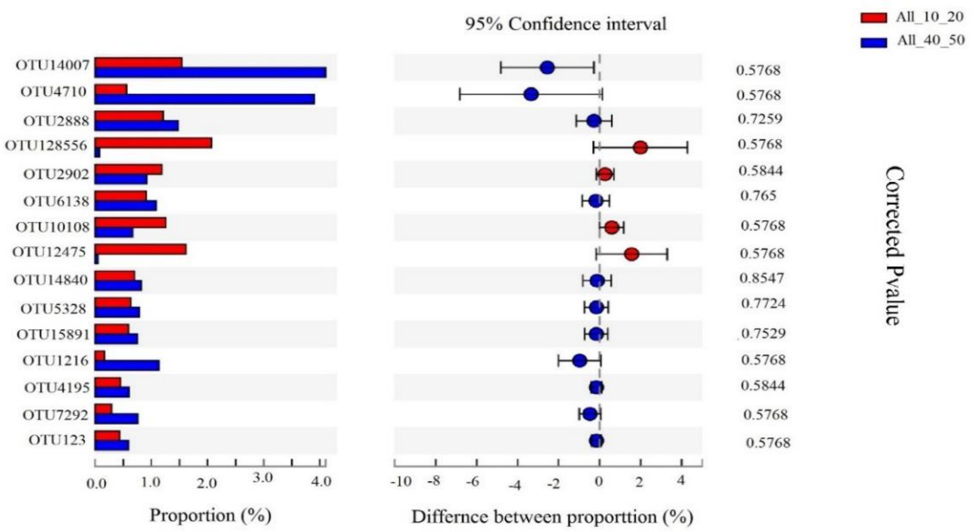

Figure 3: Variation in the relative abundance of bacterial diversity patterns across soil depths along a precipitation gradient of two soil layers (10-20cm and 40-50cm)

4.2 Relationship between soil bacterial diversity patterns with precipitation gradient

Precipitation, Temperature, and moisture are the main environmental factors. The northeast China transect (NECT) is the variation of mean annual temperature (4.9-5.1^{0}C) but the major variation in the annual precipitation (400-710mm). There is a steady trend of foliage projective cover and decreasing structure density toward the dry end of this transect. The annual precipitation is increasing and decreases along the elevation the elevation is high then the precipitation is automatically low there. In general, soil pH slightly increased with the depth of the soil profile but remained at a value of 5.5 in most of the measured cultivations and layered (Figure 4)

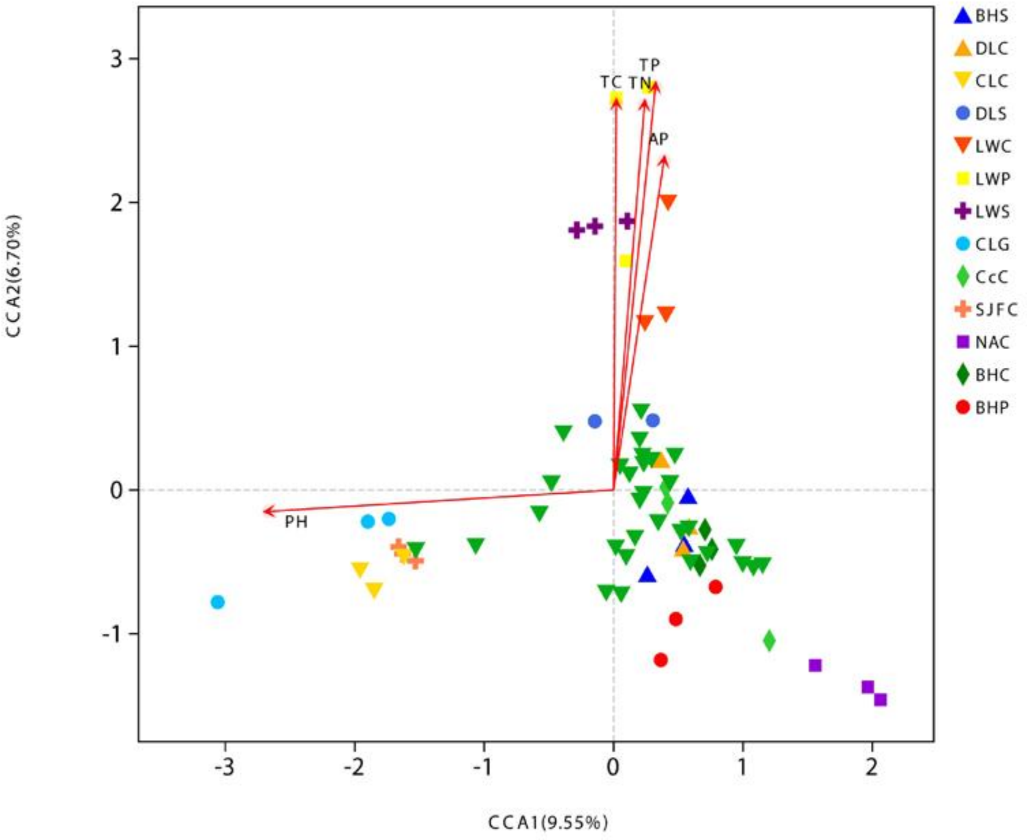

Figure 4: The Relationship between soil bacterial diversity patterns and environmental variables in soil profiles

4.3 The effect of different landuse on soil bacteria biodiversity pattern in the soil profile

The relative abundance of soil bacterial diversity pattern on the phylum level, according to the percent of the bacterial community on the phylum level that the differences between 10-20cm and 40-50cm depth of profiles in 10-20cm the bacterial community percent have high then 40-50cm but the Chloroflexihas different in different depths due the high pH and precipitation.

According to the habitats, the bacterial community patterns are also different; we 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 explained 95% similarity between them in and 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. And the differences between 10-20cm and 40-50cm profiles depth of lands, all are mostly different in all the layers of farmland grassland and primary, secondary forest due to the effect of precipitation in the lower layers of the horizons but did not effect on upper layers of the horizon in community component analysis showed the relationship between soil bacterial diversity with environmental factors between seven sampling area (west cropland and east forest and cropland is everywhere) showed that significant differences between west cropland and west grassland which have 95% similarity between them it also showed different land use effect 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 in (Figure1) and according to the differences of communities in all seven cities, Changling, SijianfangNongan, Longwan, Changchun, Baihe, and Dongliao the highest pattern have Proteobacteria in all the other species of soil bacteria diversity.

Soil pH is a universal factor for predicting bacterial diversity and community structure (Fierer and Jackson 2006; Singh et al., 2012). The critical role of pH in the structure of bacterial diversity patterns is well characterized (Fierer and Jackson 2006; Lauber et al., 2009; Chu et al., 2010; Griffiths et al., 2011; Ling et al., 2016). Moreover, due to the relatively small growth tolerance exhibited by most bacterial taxa, soil microbial diversity was found influenced primarily by soil pH, 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 (Eichorst et al., 2007).

At the phylum level, there is a significant difference in soil bacterial diversity patterns in sample areas at different depths. Janssen, (2006) 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 these regions have different soil types. Proteobacteria and Acidobacteria were the central bacterial communities in those regions accounting for 39% and 20% respectively. Actinobacteria, chloroflexi, Verrucomicrobia, were 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 (Janssen 2006; Wang et al., 2014). All the microbial activity and its biomass are differed strongly between cropland, grassland, and forest in 10-20cm and are similar in four different types of land uses. The aim was 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 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.

5. Conclusion

The current research revealed that the biogeographical pattern of soil bacterial diversity under different land uses between two soils layers along a precipitation gradient in soil profiles of northeast China transect are of significant effect on soil bacterial diversity in soil horizons. The sample was taken from seven sampling sites of different landuse (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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