



### Full Length Article

## Diversity of Soil Acidobacterial Community of Different Land use Types in the Sanjiang Plain, Northeast of China

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### Abstract

Different perturbation regimes, including disturbance caused by cultivation or the process of natural restoration can have significant effects on the soil acidobacterial community in wetland. The relationship between the community composition and diversity of acidobacteria and perturbation in wetland to quantify the extent of such disturbance related changes in northeast China. We assessed the diversity of acidobacterial communities in nine samples of wetland soil collected from pristine marsh, neighboring cropland and a restoration forest land. High-throughput sequencing of a acidobacteria-specific genomic sequence the internal transcribed spacer (16S rRNA) region was used to identify acidobacterial taxa. We obtained 1,523,313 sequences that represented 7881 acidobacterial operational taxonomic units across the three types of sampling sites. Of these, 2847 OTUs occurred at all three site types, 814 were shared between farmland and pristine wetland, 206 were shared between forestland and pristine wetland 1459 were shared between farmland and forestland. All sites also hosted unique acidobacterial OTUs with 639 OTUs exclusive to farmland 1616 exclusive to pristine wetland and 300 exclusive to forestland. Sequences were affiliated to 25 acidobacterial subgroups throughout the dataset. Sequence abundance showed that members of Acidobacteria\_Gp1, Acidobacteria\_Gp3, Acidobacteria\_Gp7, Acidobacteria\_Gp6, Acidobacteria\_Gp2, Acidobacteria\_Gp4, Acidobacteria\_Gp13 (relative abundance > 1%), which represented an overwhelming proportion of the soil acidobacterial communities and another 18 subgroups with an average relative abundance were < 1%. The dominant subgroups that showed the greatest variation among habitat types. The soil bacterial community diversity decreased from a maximum in farmland, through the wetland, to a minimum in forestland. Canonical correlation analyses demonstrated that land use patterns changing, significantly altered the bacterial community composition of soil. The  $\alpha$ -diversity of the soil acidobacterial community was most affected by soil physical and chemical properties, such as pH, soil organic carbon (SOC), total nitrogen (TN), available nitrogen (AN) and available phosphorus (AP). Meanwhile, the soil acidobacterial community composition was significantly affected by pH available phosphorus, nitrogen and organic carbon. Overall, the results from our study showed that the acidobacterial community composition of wetland were affected by SOC, TN, TP and AN. In addition, our results also indicate that cultivation and natural restoration influenced the acidobacterial community structure and diversity. Cultivation can significantly enhance the acidobacterial diversity; however, once the composition of the wetland bacterial community has been altered by cultivated disturbance, it might be difficult to restore to its original state. These findings highlight the importance of effectively managing the soil acidobacterial community to maintain a naturally functioning soil ecosystem. © 2017 Friends Science Publishers

**Keywords:** High-throughput sequencing; Soil acidobacteria;  $\beta$ -diversity; Community structure

### Introduction

Acidobacteria, which is the most widely distributed and diverse in various natural ecosystems, play an important role in maintaining soil ecological processes (Liu *et al.*, 2016). Acidobacteria, account for 10–50% of total soil bacterial communities by thorough analysis of the 16S rRNA gene (Lee *et al.*, 2008). But there are still rarely known about the ecological functions of this phylum of bacteria because they

are hard to culture (Jones *et al.*, 2009). In recent years, we have known more about the Acidobacterial subgroups composition by using the molecular biological technique, for example, the number of Acidobacterial subgroups were increased from previous 4 or 5 into 26 recent (Ludwig *et al.*, 1997; Lauber *et al.*, 2009). Different acidobacteria would be affected by different soil environmental conditions hence, the research on the diversity and community composition of acidobacteria under various

ecosystems and environmental conditions will give a well understanding for the roles of acidobacteria.

Sanjiang Plain is one of the main Marsh wetland (Marsh) distribution areas in Northeastern of China and play an key role in local ecological balance (Liu *et al.*, 2014a,b). The soil in this region contends high humus and low number and variety of microorganisms due to the special climatic conditions and geographical location. Therefore, the ecological systems are very easy to be damaged because its fragile ecological environment (Liu *et al.*, 2014a, b). The marsh wetland in northeastern China has been a certain degree degradation under the influence of global warming and human reclamation and deforestation (Song *et al.*, 2011; Wang *et al.*, 2017). Hence, the ecosystem functions, such as plant diversity, soil nutrition materials processes are seriously affected, resulting the soil become thinning, barren, lower groundwater levels and finally changing the community structure and composition of soil microbes (Zhou *et al.*, 2009). Recently, this phenomenon has been attracted the concern of many Chinese scientists. The impacts of wetland cultivation (Zhou *et al.*, 2009) the rules of wetland greenhouse gas emission (Song *et al.*, 2009) wetland microbial participation in carbon and nitrogen cycle has become hot topics recent years (Li *et al.*, 2013; Sui *et al.*, 2017). The people have realized that soil microbes are the most sensitive organisms in the soil ecological environment and are constantly explored ways to promote soil environmental remediation by controlling soil microbes (Liu *et al.*, 2014a). In the former study, our work indicated that Acidobacteria was the most plentiful bacterial phylum in the Sanjiang plain wetland (Sui *et al.*, 2017). The Acidobacterial numbers are most, also accounting for 20–50%, and distributed most widely in the soil, involving in plant residue matter decomposition, ferrikinetics process, single carbon compounds metabolism and some important ecological functions on the ecological environment (Wang, 2010). Its community structure and composition can reflect the changes in soil nutrients and even the impact of environmental changes, and can directly affect the soil functions (Arroyo *et al.*, 2015). Therefore, the more classification and the function of soil acidobacteria will be better to understand the decomposition of organic matter, nitrogen fixation, and control plant growth and degradation of organic pesticide regulation mechanism deeply.

Until now, there were still no suitable methods to analyze the diversity and community composition of acidobacteria in each environment. Some studies have tried to design suitable primers for applying the acidobacteria by using the high through sequence method (Zhang *et al.*, 2014). In these studies, The universal bacterial primers such as 31F and 1492R were used to amplify the acidobacteria (Barns *et al.*, 1999). However, the universal bacterial primers cannot be efficiently amplify all the acidobacterial subgroups (Barns *et al.*, 2007; Jones *et al.*, 2009) and use this primer to amplify Acidobacteria in soil were mainly using T-RFLP and clone library method (Silvana *et al.*,

2008; Jones *et al.*, 2009), none of these use the high through sequence method. In 2016, Liu (2016) used the primer ACIDO to study the distribution patterns of acidobacterial communities in the black soil zone of northeast China (Liu *et al.*, 2016). The result showed a larger coverage for acidobacteria in black soil. Therefore, in this study, we select the special primer to investigate the acidobacterial community diversity and distribution in the different land use patterns of Sanjiang plain of northeast China.

Additionally, this study can deeply understand the bacterial community diversity when original marshes altered to other land patterns. Through our research, we would like to understand that what are the diversity and community composition of acidobacteria across original marshes to forest and farmland? Secondly, what environmental factors affect the diversity and community composition of acidobacteria in different land types?

## Materials and Methods

### Site Description and Soil Sampling

We selected our research site at the Sanjiang Wetland Experimental Station (47°35'N and 133°31'E) (Fig. 1). The ranges of local average temperature are 1.9°C, which the temperature is highest in July and lowest in January. During the May to October the precipitation account for 80% for the whole year, the average annual precipitation is 560 mm. Three sites (wetland, forest and farmland) inside the station were selected for this study.

The approximate 1 kg top soils (0–20 cm) were collected by using the Five-point sampling method on 15 October, 2016. The soils were immediately transferred into an ice-cooled box. When arrived, the soils were divided into two parts: one, approximately 10 g soil was placed in a sterile microcentrifuge tube and stored in -80°C for molecular analysis; the remaining of the soils were air-dried to detect the soil physiochemical properties. These were measured as described before (Sui *et al.*, 2017) and included the pH of the soil mixed with water (1:5 w/v); total organic carbon (TC), total nitrogen (TN) and total phosphorus (TP) content, as well as available nitrogen (AN) and available phosphorus (AP). The physiochemical properties of the soil samples from the three different land uses are summarized in Table 1. Statistical analysis was performed by using SPSS 17.0 for Windows.

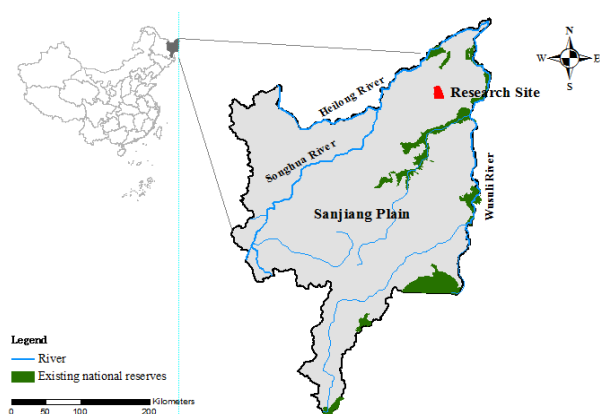
### Soil DNA Extraction and Acidobacterial 16S rRNA Gene Amplification and Illumine Miseqsequencing

DNA was extracted from 0.5 g of each frozen soil sample as previously described using the MOBIO PowerSoil DNA Isolation Kit (USA) (Sui *et al.*, 2017) and stored at -20°C until use. The Acidobacterial region was selected ACIDO/342r for PCR amplification and pyrosequencing (Lee and Cho, 2011).

**Table 1:** Physicochemical properties of soil from three different land use types

Type	pH	Total Carbon (g/kg)	Total Nitrogen (g/kg)	Available Nitrogen (mg/kg)	Total Phosphorus(mg/kg)	Available Phosphorus (mg/kg)
Wetland(B)	5.47±0.04 <sup>a</sup>	52.67±1.34 <sup>b</sup>	4.29±0.79 <sup>c</sup>	455.25±29.58 <sup>c</sup>	6.36±1.17 <sup>b</sup>	26.34±1.82 <sup>a</sup>
Farmland(L)	5.77±0.06 <sup>b</sup>	25.60±1.41 <sup>a</sup>	3.13±0.63 <sup>b</sup>	197.61±7.48 <sup>b</sup>	3.71±0.72 <sup>a</sup>	26.65±3.59 <sup>a</sup>
Forest(M)	7.35±0.05 <sup>c</sup>	25.13±1.52 <sup>a</sup>	1.80±0.32 <sup>a</sup>	143.75±7.20 <sup>a</sup>	3.17±0.51 <sup>a</sup>	32.71±4.46 <sup>a</sup>

Statistical significance ( $p < 0.05$ ) is indicated by different superscript letters in the same column. Identical superscripts indicate there was no significant difference

**Fig. 1:** Map of the Research Site in the Sanjiang Plain, China

High-throughput sequencing was performed by the Shanghai Majorbio Biotechnology Company, Shanghai, China. Barcoding was used to simultaneously sequence multiple samples. Sequence reads shorter than 150 bp were discarded as were sequences with imprecise base calling or reads starting with primer sequences that had two or more mismatches.

### Data Analysis

QIIME Pipeline Version 1.7.0 (<http://qiime.org/tutorials/tutorial.html>) and FLASH (fast length adjustment of shortreads) software were selected to analyze the high-throughput sequences. The trimmed sequences were detected and removed by chimera and Uchime algorithm software. The remaining unique sequences were clustered at 97% similarity to build operational taxonomic units (OTUs). The unique sequence reads were used to identify operational taxonomic units (OTU) at 97% similarity. The Python Nearest Alignment Space Termination (PyNASt) tool was select to build a neighbor-joining tree for representative sequence from each phylotype. The sequences, which not matched to the Acidobacteria were removed from the sequences in order to next analysis.

### Statistical Analysis

The diversity of acidobacteria was tested by using SPSS 17.0 for Windows, significance for 0.05. The effects of environmental factors on Acidobacteria  $\beta$ -diversity were

used Mantel test. The correlation of acidobacterial community composition with soil environmental factors has been done by Canonical Correspondence Analysis (CCA) and BioEnv. All analysis were accomplished by using the “vegan” package in the R environment.

## Results

### Analysis of Different Land use Patterns by High Throughput Sequencing Technology

A total of 1,523,313 sequences, which the sequences of acidobacteria were 822 and 744 belong to 25 subgroups from 9 soil samples of three plots by the Miseq and optimized. We selected sequences and the numbers of OTU to build the rarefaction curve (Fig. 2). It can be seen that the rarefaction curve was tend to be flat indicating that the sequencing data was reasonable and that more sequencing data will only produce a small amount new OTU.

### The Distribution of Soil Acidobacterial Community in Different Land use Types

The Venn chart is used to count the number of common and unique OTUs in multiple samples and the number of OTUs can be more intuitive to show the similarity and overlapping of all samples. The total OTUs numbers were 7881, which the common OTUs were 2847 accounting for 36.12% (Fig. 3). The OTUs of forest land was least (300) accounting for 3.81% and the OTUs of wetland was most (1616), accounting for 20.51% and the OTUs of farmland was 639, accounting for 8.18%. Moreover, the OUTs that were common in wetland and farmland were 10.33%; the OUTs that were common infarmland and forest land were 18.51%; the OUTs that were common in forestland and wetland were 2.61%. It can be seen that the distribution of OTUs in the forestland and farmland soil is the most significant different.

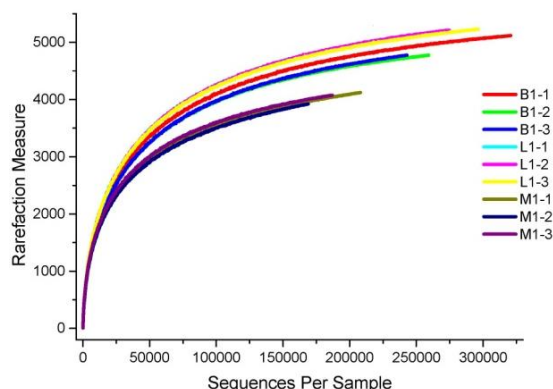
### The $\alpha$ -acidobacterial Diversity of Different Land use Patterns

The  $\alpha$  acidobacterial diversity was used to analyze the three different land use patterns (Table 2). The results showed that the soil acidobacterial community diversity of wetland was lower compared with those of the other land use types (Table 2). Farmland significantly increased the soil bacterial community diversity but forest land did not increase significantly compared with that of wetland.

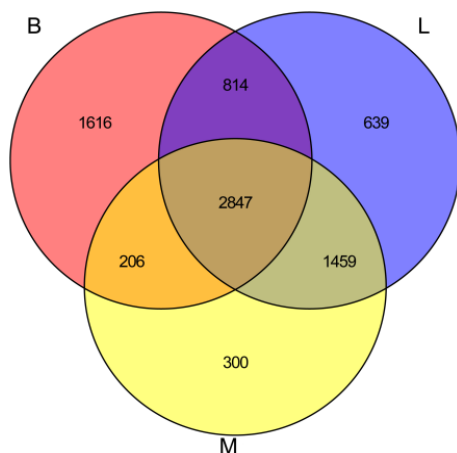
**Table 2:** The acidobacterial  $\alpha$ -diversity indices of three different land use types

Types	Chao	Observed_species	Shannon	Simpson
Wetland	5375.18±92.06b	4889.27±114.27b	9.21±0.02a	0.99±0.00b
Farmland	5705.44±19.62c	5184.97±36.74c	9.44±0.02b	0.99±0.00ab
Forest	4641.07±24.82a	4040.40±59.92a	9.23±0.04a	0.99±0.00a

Note: the lowercase mean the significant value is  $P < 0.05$

**Fig. 2:** Rarefaction curves showing the extent of OTU detection at the different quadrats

Note: B is wetland; L is farmland; M is forest land. The same as below

**Fig. 3:** Venn diagram showing the numbers of shared and exclusive OTUs of soil acidobacteria identified at different land use types

### Composition of Soil Acidobacterial Community in Different Samples

At the similar level of 97% the representative sequences of OTUs were analyzed by taxonomy (Fig. 3). The results showed that 7881 OTUs were detected in 9 soil samples, belonging to 25 subgroups that the Acidobacteria\_Gp1, Acidobacteria\_Gp3, Acidobacteria\_Gp7, Acidobacteria\_Gp6, Acidobacteria\_Gp2, Acidobacteria\_Gp4, Acidobacteria\_Gp13 (relative

abundance > 1%) were the main acidobacterial subgroups. According to the beta diversity distance matrix the similarity and the difference of soil acidobacterial community were compared using the non-weighted method to construct the tree structure. The similarity of soil flora structure in the wetland was high and different with farmland and forest land of which was similarity of soil flora.

### The Relationships of Acidobacterial Diversity and Soil Physical and Chemical Properties

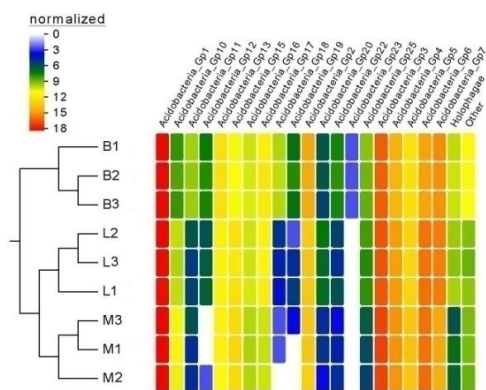
The OTU (97%) composition of soil bacteria was analyzed by CCA and the contribution rate of the two axes was 86.14% (Fig. 5). The wetland was distributed in the positive direction and forestland and farmland was distributed in the negative direction of the first ordinal axis, indicating that the wetland was the main influencing factors of the first sorting axis. AN, TN, TP and TOC are positively correlated with the first ordinal axis and pH Available P are negatively correlated with the first ordinal axis. The results identified that pH, AN, TN, TP and TOC had the most significant effect on soil acidobacterial community structure.

The correlation analysis of each subgroup was assessed in relation to the total dataset. This analysis identified significant differences of where the soil physical and chemical properties affected acidobacterial subgroups. Fig. 6 shows where the relative differences were mainly observed, irrespective of the absolute abundance of that acidobacteria subgroups. There were three parts divided according the relevance levels. Ap and pH were positive correlated with Acidobacteria\_Gp1, Gp6, Gp4, Gp10, Gp3, Gp5 and Gp15 meanwhile TP, TC, TN and AN were negative correlations; Ap, TP and pH were negative with Gp16, Gp20, Gp7 and TC, TN, AN were positive with Gp16, Gp20, Gp7; the Ap and pH were negative with Gp2, Gp12, Gp22, Holophagae, Gp18, Gp25, Gp17, Gp19, Gp23, Gp13 and Gp11 and TC, TN, AN were positive with them (Fig. 6).

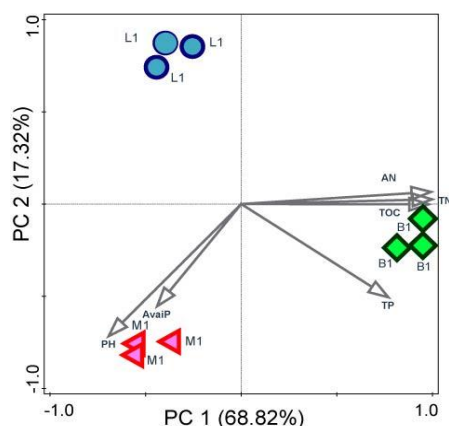
### Discussion

At present, the research on soil bacterial community is not deep, so soil bacteria are called to "biological dark matter" (Staff, 2014) and the scientific problems such as the mechanism of land use on soil bacterial community need to be solved urgently (Singleton *et al.*, 2001; Liu *et al.*, 2014a). In this study, high-throughput sequencing technology was used to determine the soil acidobacterial community in three different land use types. The results showed that there were significant differences in soil acidobacterial community structure between different land use types. From the perspective of clustering the soil acidobacterial community structures of the farmland and the forest land was similar but the vegetation type was different, indicating the effect of vegetation type on the soil acidobacterial community structure is not obvious.

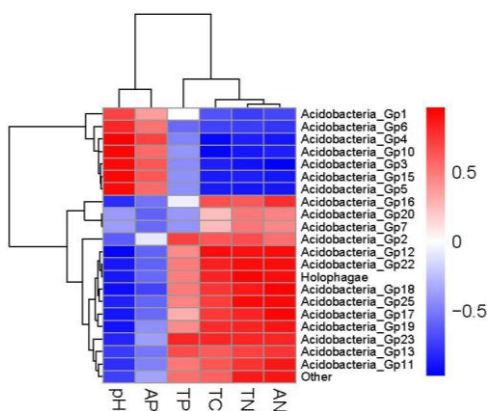




**Fig. 4:** Histogram and cluster tree of soil bacterial community in different land use patterns, for three wetland samples (B1, B2, B3), three forestland samples (L1, L2, L3) and three farmland samples (M1, M2, M3)



**Fig. 5:** CCA of soil acidobacterial community and environmental factors



**Fig. 6:** the correlation analysis of dominant acidobacteria subgroup with soil physical and chemical properties of three different land use types

The soil acidobacterial community structure of original wetland is different to those of others soil types. It may be due to the fact that the soil environment was completely

changed from wetland to the other two types and the different soil properties lead to the soil acidobacterial structure varied.

Changes in soil acidobacterial community structure based on the change of community composition the difference of soil acidobacterial community composition in different land use types indicates that environmental changes provide specific survival conditions for these acidobacteria, so as to select flora. The soil acidobacterial community of three different habitats of Sanjiang Plain belonged to 25 subgroups. Land use have significantly changed the acidobacterial composition, such as the acidobacteria\_Gp10, acidobacteria\_Gp11, acidobacteria\_Gp16, acidobacteria\_Gp17, acidobacteria\_Gp18, acidobacteria\_Gp22 were obviously different in wetland; acidobacteria\_Gp13, acidobacteria\_Gp24 were obviously different in farm land; acidobacteria\_Gp23, acidobacteria\_Gp24 were obviously different in forest land (Fig. 3). The variation of acidobacterial composition indicated that the land use process destroyed the ecological balance of the original soil acidobacterial community and changed the original soil Habitat conditions resulting some acidobacteria population increased and/or decrease and the new flora provided suitable habitat conditions and changed acidobacterial community diversity (Kielak *et al.*, 2008).

There are many specific high relative abundance and low abundance acidobacterial species in different habitats (Liu *et al.*, 2014b). For example, the relative abundance of the acidobacteria\_Gp11 and acidobacteria\_Gp18 in the wetland was significantly higher than that of the forest land and farmland, while the relative abundance of the Bacteroidetes and Tenericutes in the forest was significantly higher as well as Planctomycetes and Gemmatimonadetes in the farmland was significantly higher. The soil environment such as pH, organic carbon, total nitrogen etc has important effected on soil acidobacterial community composition (Figs. 4 and 5). The high diversity of acidobacteria in the farmland indicated that there was a complex soil acidobacterial community structure. Understanding the soil acidobacterial communities played an important role to regulate the soil ecosystem balance and promoting the sustainable of farmland (Zimmermann *et al.*, 2005).

It is a very effective method to use the diversity indices to analyze soil acidobacterial communities (Lee *et al.*, 2011). In this study, we analyzed the soil alpha diversity and found that land use increased the soil acidobacterial diversity that is similar with the study of Mendes who found that the soil acidobacterial abundant was higher in the forest than that in the other land (Mendes *et al.*, 2015). Wang, however, found that the acidobacterial diversity in fresh wetland was highest than other land types (Wang *et al.*, 2012). This was inconsistent with this study, suggesting that the complex

of soil microbial community diversity in different land use types. The soil environment is complex in the Sanjiang Plain. Soil carbon, total nitrogen and pH can be improved the soil microbial diversity by through supplying the soil nutrient. The diversity of farmland had the high diversity because the soil nutrient supplied by external input, hence, soil nutrient conditions is the key factor to control the soil microbial community diversity.

The process from wetland to farmland to forest can indirectly affect soil acidobacterial community structure by changing the soil hydrothermal conditions, soil structure and soil nutrient conditions (Sheik *et al.*, 2012). Changes in soil bacterial community diversity are a direct manifestation of adaptation to land use changed, which accelerate the decomposition of soil organic matter and also promote plant uptake of soil nutrients (Staff *et al.*, 2014). Changes in land use patterns resulted the soil physical and chemical properties such as organic carbon, total nitrogen, available phosphorus and phosphorus decrease but the soil microbial diversity increased, inferring that the carbon and nitrogen decreased could improve the soil diversity. But this result seemed unreasonable because the contents of TC, TN, AN and TP significantly decreased from wetland to forestland, this phenomenon may be due to the original swamp soil organic matter after perennial accumulation, although the soil nutrient content is very high but its natural form cannot be used by majority of soil bacteria. The land use patterns changed the original form of soil nutrients in the swamp wetland, which provided favorable conditions for the utilization of carbon and nitrogen for soil acidobacteria. Therefore, the content of carbon and nitrogen is not the key limiting factor of soil acidobacterial diversity. We studied the effects of six environmental factors on soil acidobacterial community diversity, but some of important factors such as soil temperature and soil oxygen content were lack the mechanism of its impact remains to be further studied.

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