Analysis of the relationship between soil microbial ecological characteristics and soil physical and chemical properties based on molecular technology

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Keywords: Molecular technology; Soil microorganism; Physical and chemical properties of soil

Abstract: Taking different soil samples of natural secondary forests in the study areas as research materials, the quantitative characteristics of microorganisms and their correlation with soil physical and chemical parameters were studied by using high-throughput sequencing in molecular technology. The results showed that the affect of slope aspect on soil microbial characteristics was complicated. The activity of rhizosphere microorganisms on the sunny slope is stronger than that on the other two slopes. According to the characteristics of soil chemical properties and microbial biomass, the non-rhizosphere soil microorganisms on the shady slope are more abundant and active. The content of total carbon and total nitrogen in soil sample II was significantly higher than that in soil sample I; Soil pH value, total carbon, total nitrogen and available nitrogen are the main factors affecting the differences of soil bacterial community structure in Quercus mongolica forest in this area, while soil pH value and total carbon content are the main controlling factors affecting the differences of fungal community structure in natural secondary forest in this area.

1. Introduction

As decomposers and producers, microorganisms support the material circulation on the whole earth, and make life continue. It is the place with the richest microbial diversity, and only a small part of it has been studied by human beings, and most of it is still in an unknown state [1].

In recent years, popular research methods are molecular techniques based on molecular biology, such as denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism analysis. With the development of high-throughput sequencing technology, its application in the field of soil microbial diversity is becoming more and more extensive [2-3]. In this study, the natural secondary forest was taken as the research object, and the high-throughput sequencing was used to study its soil microbial flora composition and corresponding environmental characteristics, so as to reveal the spatial distribution differences of soil microbial flora composition and its main control factors in the study area, and further clarify the response mechanism of soil microorganisms to changes in soil environmental factors, aiming at providing scientific basis for the study of soil microbial community structure diversity in the study area.

2. Materials and methods

2.1. Overview of study area

The study area is located in a nature reserve, which belongs to temperate monsoon climate, with four distinct seasons, annual average temperature of 6.7℃, average rainfall of 936~1200mm and frost-free period of 128~145d. The soil is dark brown forest soil and brown forest soil, and the main forest types include oak forest, broad-leaved miscellaneous forest, Korean pine forest, larch forest, etc.

  DOI: 10.25236/icbcme.2021.025

2.2. Material

Multi-point sampling, the soil depth is 0 ~ 25 cm, and about 6 kg of fresh mixed soil samples are collected. Usually, one or two points are selected in a natural village, and soil samples of natural secondary forests with different years, textures and fertility levels are collected. After the soil samples are collected, 1 kg of fresh soil is taken according to the quartering method and passed through a 2mm sieve, and various microbial indicators are determined.

2.3. Method

2.3.1. Determination of physical and chemical properties of soil

The total nitrogen in soil is semi-micro Kelvin (K_2SO_4 - $CuSO_4$ - Se distillation method). Alkaline hydrolysis diffusion method is adopted for effective nitrogen. Molybdenum antimony colorimetric method ($HClO_4$ - H_2SO_4 method) was used for total phosphorus. Available phosphorus was extracted with 0.5 $mol \cdot L^{-1}$ sodium bicarbonate-molybdenum antimony colorimetric method. The pH value is measured by potential method, and the soil moisture content is measured by drying method.

2.3.2. Microbial culture and counting

Take the soil sample out of the refrigerator at-20℃ and thaw it at 4℃. Take 2g of the soil sample from the ultra-clean table and put it in a sterilized triangular flask containing 18ml of normal saline (0.85%) and glass beads. Shake it for 15min at 250 $r \cdot min^{-1}$. Dilute the suspension by gradient dilution method. Take 200μ l of the diluted soil suspension with 10^{-3} and 10^{-4} concentration and coat it on the oligotrophic medium PYGV plate at 25.

Take about 0.5g of soil sample and dilute it to $10^{-1} g \cdot mL^{-1}$ with sterile normal saline (0.85%) according to the mass ratio. At the same time, add several sterile glass beads, put it in a shaking table for 250 $r \cdot min^{-1}$ minutes, take 1ml of suspension and add it to 9ml of sterile normal saline for gradient dilution. When $10^{-4} g \cdot mL^{-1}$. counts, count at least 30 randomly selected fields of vision for each sample, and calculate the average number of cells. Calculate the total number of cells in the soil sample according to the following formula:

$$E = X \times \frac{S_1}{S_2} \times \frac{1}{V} \times 10^4$$

In which, E is the total number of cells in the sample ($cell \cdot g^{-1}$); X is the average value ($cell$) of the total number of fine bacteria in 30 visual fields; S_1 is the area of filter membrane (mm^2); S_2 is the visual field area of microscope (mm^2); V is the volume of filtered sample (mL).

2.3.3. Amplification and sequencing of bacterial 16SrRNA and fungal ITS

The ITS region of the fungus was amplified with primers ITS1F (5' -CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5' -GCTGCGTTCTTCATCGATGC-3'). The PCR reaction system is the same as that of bacteria [8-9]. The PCR amplification conditions are:

Pre-denatured at 98℃ for 2min, denatured at 98℃ for 30s, annealed at 52℃ for 30s, extended at 72℃ for 45s, 28 cycles, and finally extended at 72℃ for 5min.

PCR amplification products were detected by 2% agarose gel electrophoresis, and the target fragments were cut and recovered by gel recovery kit of AXYGEN company.

Truseq nano DNA lt library prep kit (il-Lumina company) was used to prepare sequencing library.

2.3.4. Microbial community structure

The diversity of microbial community structure was measured by phospholipid fatty acid analysis (PLFA).

3. Results and analysis

3.1. Soil microbial community structure in different slope directions

Fig. 1 shows the characteristics of soil microbial community in different slope directions.

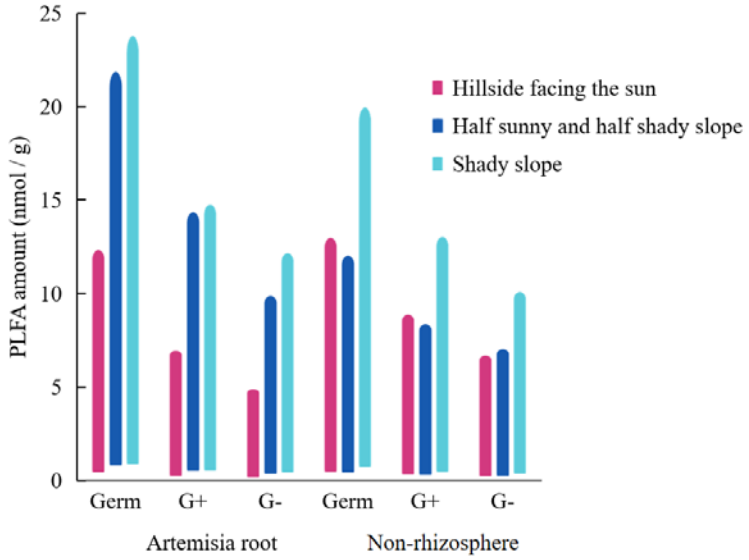

Figure 1 Characteristics of soil microbial community in different slope directions

Bacterial content in rhizosphere soil of Artemisia scoparia was in the order of negative slope > semi-positive and semi-negative slope > positive slope. There was significant difference between positive slope and negative slope ($p<0.05$), but there was no significant difference between semi-positive and semi-negative slope and positive and negative slope ($p>0.05$).

Non-rhizosphere soil: shady slope > semi-sunny and semi-shady slope > sunny slope. There is no significant difference between sunny slope and semi-sunny and semi-shady slope, but there is a significant difference with shady slope ($p<0.05$).

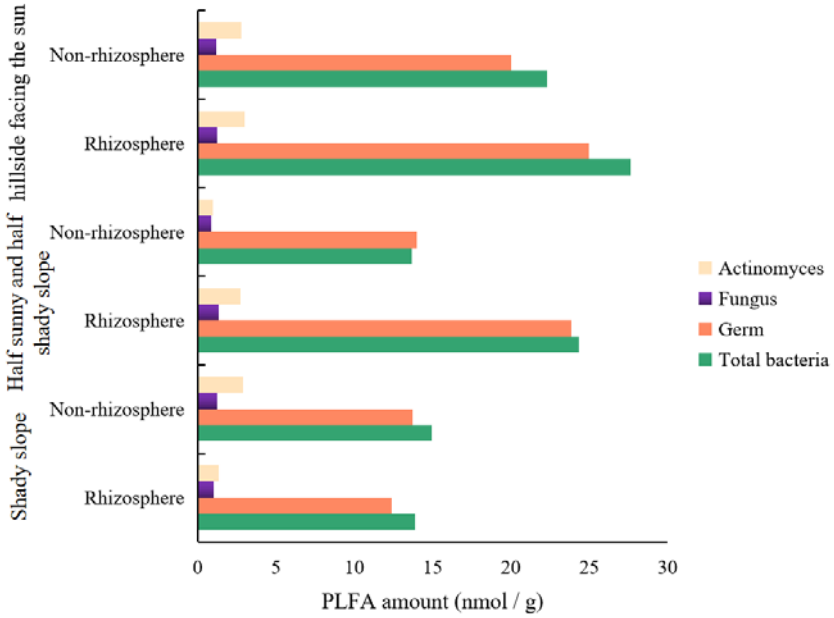

Figure 2 Composition of soil microbial community in different slope directions

As shown in Figure 2, from the composition of strains, bacteria account for the absolute

advantage of the total amount of microorganisms. In the same slope direction, there are significant differences between rhizosphere and non-rhizosphere on semi-sunny and semi-shady slopes ($p<0.05$), but there are no significant differences between rhizosphere and non-rhizosphere on sunny and shady slopes.

3.2. Physical and chemical properties of natural secondary forest soil

There is no significant difference in soil available nitrogen, total phosphorus, available phosphorus content and C/N ratio between soil sample I and soil sample II. The total carbon and nitrogen contents of soil sample II were 84.56 $g \cdot kg^{-1}$ and 7284.01 $mg \cdot kg^{-1}$, and the pH value of soil sample II was 4.83, which was significantly lower than that of soil sample I (Table 1).

Table 1 Physical and chemical properties of natural secondary forest soil

Project	Soil sample II	Soil sample I
pH value	4.83±0.12*	5.88±0.16**
Total carbon / $g \cdot kg^{-1}$	84.56±3.39	56.88±14.79*
Total nitrogen / $mg \cdot kg^{-1}$	7284.01±1207.28**	4396.57±1103.87**
Available nitrogen / $mg \cdot kg^{-1}$	48.91±5.47*	32.09±7.16
Carbon nitrogen ratio	10.26±1.34**	12.57±0.26**
Total phosphorus/ $g \cdot kg^{-1}$	2.44±0.87*	2.36±0.92**
Rapidly available phosphorus / $mg \cdot kg^{-1}$	0.77±0.01*	0.56±0.24**

Note: "*" indicates significant difference ($P < 0.05$); "* *" indicates that the difference is extremely significant ($P < 0.01$).

3.3. Correlation between dominant fungal flora and soil physical and chemical properties

The relative abundance of dominant fungi pHylum and soil pHysical and chemical properties were analyzed by Person correlation. the results showed that ascomycete phylum was positively correlated with soil ph value ($r=0.88$, $p<0.01$), while basidiomycete phylum was negatively correlated with soil ph value ($r=-0.73$, $p<0.01$) (Table 2).

Table 2 Correlation analysis between dominant fungal groups in natural secondary forest and soil physical and chemical properties

Project	Ascomycetes phylum	Basidiomycete phylum	Zygomycota
pH value	0.88*	-0.91	0.59
Total carbon	-0.73*	0.77	-0.05
Total nitrogen	-0.65	0.63	-0.33
Available nitrogen	-0.51	0.52	-0.14
Carbon nitrogen ratio	0.37	-0.34	0.69
Total phosphorus	-0.28	0.31	-0.55
Rapidly available phosphorus	0.21	-0.26	0.36

4. Discussion

Bacterial PLFA is an absolute advantage in the total amount of microorganisms. Whether in rhizosphere or non-rhizosphere, the shady slope is larger than the other two slopes and has significant difference with them. G- is slightly more than G+. Because of the good water condition in the shady slope, the soil carbon turnover rate is fast, so the bacterial PLFA in the shady slope is more than that in the other two slope directions [4]. Relevant studies have shown that the lower the PLFA ratio of soil bacteria to fungi, the more stable the soil ecosystem is [5], and the bacteria/fungi in rhizosphere soil and non-rhizosphere soil are the smallest on sunny slope, that is to say, the

ecosystem on sunny slope should be more stable.

The results showed that all rhizosphere effect values were greater than 1, indicating that the overall rhizosphere habitat was better than that of non-rhizosphere. On the basis of the research results of basic physical and chemical properties, the trend of rhizosphere effect is the highest in sunny slope.

Soil microorganisms can participate in various biochemical reactions, which is the driving force of plant nutrient transformation, organic matter metabolism and pollutant degradation. The decomposition of soil microorganisms to plant litter and soil organic matter can provide mineral nutrients needed by plant growth. Soil microorganisms play the role of storage and source for plant effective nutrients, and have a profound impact on the plant availability of soil carbon, nitrogen, phosphorus and sulfur [6]. Ascomycota and Basidiomycota are the main decomposers in the soil, and Ascomycota is the key decomposing community in the soil, which can decompose many refractory organic matters, such as lignin and keratin [7-8].

PH value plays an significant role in soil biogeochemical cycle, which is related to vegetation type, soil type and management measures [9]. In this study, in the small-scale habitat, the pH value affects the fungal community structure by changing the physical and chemical properties of soil. Among them, soil pH value has the greatest influence on saprophytic fungi. Studies have shown that compared with bacteria, fungal communities have a wider range of adaptation to soil pH value, and soil with pH value between 5 and 9 will not inhibit the growth of fungi [10]. Literature [11] thinks that a good soil should have good biological activity and stable microbial species composition, and that both soil nutrient surplus and unbalanced nutrient ratio will cause the decrease of soil microbial activity and the change of community structure. Therefore, attention should be paid to the application amount and ratio of fertilizer in vegetable production, so as to create a good ecological environment for soil microorganisms and facilitate the sustainable development of agriculture.

5. Conclusions

The activity of rhizosphere microorganisms on the sunny slope is stronger than that on the other two slopes. According to the characteristics of soil chemical properties and microbial biomass, the non-rhizosphere soil microorganisms on the shady slope are more abundant and active. Soil pH value, total carbon, total nitrogen, available nitrogen content and C/N ratio are the main factors affecting the differences of soil bacterial community structure in natural secondary forests in this area, while soil pH value is the main factor affecting the differences of soil fungal community structure in natural secondary forests in this area.

Acknowledgements

Fund Project:2021 scientific research fund project for young teachers of Laiwu vocational and technical college, application of green circular agriculture model in traditional Chinese medicine planting and livestock and poultry breeding (Project number: 2021qnzx03)

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