RESEARCH ARTICLE

Relationship between changes of forest soil microbial community structure and disturbance of tourism activities

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The structure of microbial communities in forest soil can have an impact on the growth of local forests, and human tourism activities can also affect the microbial community in soil. To protect forest resources better, it is imperative to further understand the relationship between changes in microbial community structure and disturbances resulting from human tourism activities. This study briefly introduced the soil microbial community and then took Yuanshan National Forest Park in Zibo, Shandong, China as the subject of the case study. The areas with or without tourism interference were divided according to whether they were opened to tourists. The physical and chemical properties of the soil, the number of microbial communities, and functional diversity were then tested. The results found that the soil without tourism interference had better physical and chemical properties, and the microbial community in it was more diverse. This research laid the foundation of using field investigations to analyze the physicochemical properties of forest soil and microbial communities under the interference of tourism activities, providing an effective reference for studying the relationship between soil microbial community structure and tourism activity disturbance.

Keywords: forest; soil; microbial community; tourism disturbance.

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Introduction

With the advances of the economy, tourism and many natural scenic spots have also been developed, which attracts more tourists [1]. Scenic areas that promote "ecotourism" usually have a good natural environment, and forests are one of them. For forest vegetation, the soil environment is crucial for good growth in addition to sufficient light [2]. The structure and function of soil microbial communities are essential for maintaining healthy soil and vegetation growth. Some microorganisms can promote plant growth, while others can cause plant diseases [3]. There is a vast number and variety of microorganisms in the soil, and their functions in the soil include oxidation, nitrification, nitrogen fixation, *etc*. [4]. As a natural culture medium for microorganisms, soil exhibits variations in its composition due to different soil types in various regions. Consequently, soil microbial communities also exhibit variations in structure. However, the development of tourism not only brings economic benefits, but also brings negative impacts on scenic spots. In the process of tourism activities, tourists will inevitably have an impact on scenic spots such as garbage generated by tourists, waste generated in the operation process of scenic spots, and even the movement of tourists during the tour, which will have a physical impact on the soil [5]. The microorganisms in soil constitute an important part of the whole ecosystem and usually act as decomposers in the process of the ecological cycle [6]. When the soil environment such as pH, moisture, and organic matter contents are affected, the structure of the microbial community will also be affected, and finally the chain reaction will affect the ecological balance of vegetation and the entire tourist attraction [7]. Once the ecological balance is broken, the vegetation scenery in the scenic spot is bound to be negatively affected, which will not only affect the ecological environment but also reduce the tourism value. The study on the relationship between forest soil microorganisms in scenic spots and the disturbance of tourism activities can provide effective support for protecting the environment of tourist scenic spots [8].

Jurburg *et al*. used soil microenvironment experiments and multiple model disturbances to discuss the short-term effects of the same or new stresses on restoring soil microbiota [9]. The results showed that the compounded disturbance affected the restoration of the bacterial community by changing its structure, thus changing the response of the community in the process of succession. Bora *et al*. analyzed and compared the soil microbial community structure and functional characteristics of unpruned and pruned tea trees using metagenomics [10]. The principal component analysis revealed unique metagenomes of two plots in key microbial groups and metabolic pathways. Fabienne *et al*. analyzed the microbial communities of 31 formerly corn-grown agricultural wheat fields in Brittany, France, using the meta-barcode method [11]. The results clearly highlighted the importance of tillage to bacteria and fungi, and although the core microbiomes were similar under both tillage practices, soil species richness and uniformity were remarkably higher under minimum tillage than under conventional tillage. Those previous studies have analyzed the microbial community in soil. Some used control experiments to analyze factors influencing the structure of microbial communities. Some studies started from a genetic perspective to study the functionality of microbial communities, while others analyzed the effect of cultivation methods on soil microbial communities. All these studies have analyzed factors that can affect the structure of soil microbial communities.

This study briefly introduced the soil microbial community and then took Yuanshan National Forest Park in Zibo, Shandong, China as a case study to analyze the impact of tourism activities on forest soil microbial communities with two investigated areas of open to tourists and not open to tourists. The soil microbial communities in these two areas were analyzed to compare the differences in soil and soil microbial community, thus analyzing the influence of tourism activities on forest soil microbial community structure by using field investigation to analyze the physicochemical properties of forest soils with and without tourism disturbances, as well as their microbial communities. The results would provide an effective reference for studying the relationship between soil microbial community structure and tourism activity disturbances.

Materials and methods

Study area overview

Yuanshan National Forest Park in Zibo City, Shandong, China (117.82°E, 36.44°N) was selected for this study. The park locates in a warm temperate zone with a semi-humid and semi-arid continental climate with the average annual precipitation of 650 mm, the average annual temperature ranged from 12.5℃ to 14.2℃, the average annual sunshine duration between 2,209 and 2,523 hours, and the average annual frost-free period about 190 to 210 days. The location is rich in vegetation resources with hundreds of woody and herbaceous plants.

Study site selection and sample collection

Table 1. 31 carbon sources for testing the diversity of microflora.

Saccharides	Amino acids	Carboxylic acids	Amines	Phenolic acids	Polymers
β-methyl -D-glucoside	L-arginine	γ-hydroxybutyric acid	Phenethylamine	2-hydroxybenzoic acid	Tween 40
D-xylose	L-asparagine	α-ketobutyric acid	Putrescine	4-hydroxybenzoic acid	Tween 80
i-erythritol	L-phenylalanine	D-gluconic acid			α-cyclodextrin
D-mannitol	L-serine	D-malic acid			Glycogen
N-acetyl-D glucosamine	L-threonine	D-galacturonic acid			
D-cellobiose	glycyl-L-glutamic acid	Methyl pyruvate			
α-D-lactose		Itaconic acid			
D-galactonic acid γ -lactone					
D, L-α-phosphoglycerol					
1-glucose phosphate					

The study areas were divided into two forest scenic spot parts with one as the open forest scenic spot centered on tourist hotels or other tourist facilities and another area as the closed spot to tourists. Eight sampling sites with a size of 10 × 10 m^2 were randomly assigned in each area. Soil samples were collected along the S-type route in the sampling sites, and the distance between consecutive adjacent points was 0.2 m [12]. 200 g of soil was collected at each sampling point. The soil samples obtained from the sampling sites in the same area were uniformly mixed, screened by 2 mm mesh, and then properly stored for subsequent experiments.

Testing physical and chemical properties of soil

An acidity meter (Yidian Scientific Instrument Co., Ltd., Shanghai, China) was employed to measure the pH of the soil. The weight difference of soil samples before and after drying at 120°C was measured to calculate the soil moisture content. The soil carbon and nitrogen contents were determined using TS-9600 total carbon and nitrogen analyzer (Kanchang Scientific Instrument Co., Ltd., Nanjing, Jiangsu, China). The alkaline melt soil sample was dissolved with dilute sulfuric acid, and the phosphorus content was determined after adding a chromogenic agent (Sigma-Aldrich, Saint Louis, MO, USA). The potassium content was determined by flame photometry after dissolving alkaline molten soil sample with dilute hydrochloric acid at a mass fraction of 5% [13].

Testing of soil microbial community

The number of soil microorganisms was determined by dissolving 1.0 g of grounded soil sample with 10 mL of sterilized normal saline (0.9%) and being cultured at 37°C, 180 rpm for 30 min. The suspension was then gradient diluted, and 100 µL of diluted suspension was evenly coated on the solid medium plate with 10 g/L tryptone, 5 g/L yeast extract, 10 g/L sodium chloride, and 10 g/L agar powder. The number of colonies was counted after culture at 37°C for three days, and the number of soil microorganisms was calculated as follows.

$$X = A \times Y \times 100 \quad (1)$$

where X was the number of soil microorganisms. A was the average number of colonies on the agar medium plate. Y was the dilution ratio. The community functional diversity

Table 2. Soil physical and chemical properties of two areas.

	With tourism interference	Without tourism interference	*P* value
Moisture content (%)	24.53 ± 2.36	35.25 ± 1.48	0.011
pH	6.32 ± 0.24	7.58 ± 0.12	0.012
Nitrogen content (g/kg)	4.13 ± 0.21	6.54 ± 0.18	0.010
Carbon content (g/kg)	55.89 ± 1.35	88.96 ± 1.23	0.009
Phosphorus content (g/kg)	1.05 ± 0.08	2.57 ± 0.14	0.011
Potassium content (g/kg)	16.32 ± 0.87	25.78 ± 1.23	0.010

Table 3. The microbial community number in the soil.

Microbial species	With tourism interference	Without tourism interference	*P* value
Bacteria × 10^4/g	42.300 ± 1.100	75.600 ± 0.900	0.001
Actinomycetes × 10^4/g	2.890 ± 0.870	4.120 ± 0.790	0.001
Fungus × 10^4/g	0.598 ± 0.011	0.912 ± 0.008	0.001

was measured by dissolving 10.0 g of soil sample with 100 mL of sterilized normal saline before the sample was cultured at 37°C, 180 rpm for 30 min. The supernatant was then diluted 100 times after the insoluble precipitated. A total of 31 single carbon sources (Sigma-Aldrich, Saint Louis, MO, USA) were mixed with the diluted supernatants [14] (Table 1). The plate was incubated at 37°C before the absorbance was measured at 590 nm every 24 h for total of 144 h. The measurement was calculated as follows.

$$\begin{cases} AWCD = \dfrac{\sum (C-R)}{31} \\ H = -\sum P_i (\ln P_i) \\ D = 1 - \sum P_i^2 \end{cases} \tag{2}$$

where $AWCD$ was the average well color development, which indicated the overall capacity of microbial communities in the soil to utilize carbon sources. C was the absorbance of each well of the plate inoculating sample solution. R was the absorbance of the control well (sterile physiological saline of equal volume). H was the Shannon index. D was the Simpson index. Both Shannon index and Simpson index were used to measure microbial community diversity.

Statistical analysis

Microsoft Excel (Microsoft, Redmond, WA, USA) and SPSS (IBM, Armonk, New York, USA) were used to analyze the collected data [15]. The data was expressed as mean ± standard deviation. The independent t-test was conducted to compare the differences of soil and microbial communities in areas with or without tourism disturbance.

Results and discussion

The physical and chemical properties of soil in the two studied areas with or without tourism interference demonstrated that the moisture content, nitrogen, carbon, phosphorus, and potassium content of the soil in the forest area without tourism interference were significantly higher than that in the forest area with tourism interference (Table 2). In addition, in terms of soil acidity and alkalinity, the soil in the forest area with tourism interference was significantly more acidic. The number of soil microorganisms in the two areas with or without tourism showed that, among the soil microorganisms, the content of bacteria was the highest, the content of fungi was the least, and the soil without tourism interference had significantly more microbial numbers (Table 3).

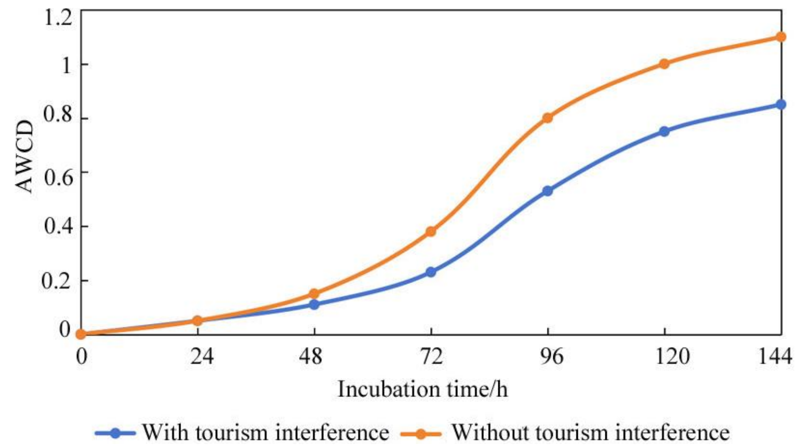

Figure 1. The AWCD of soil microorganisms in the two areas.

Table 4. Functional diversity of soil microbial communities in two areas.

Indicators of microbial community diversity	With tourism interference	Without tourism interference	*P* value
Shannon index	2.8 ± 1.1	4.5 ± 0.9	0.001
Simpson Index	0.6 ± 0.3	1.1 ± 0.6	0.002

The average well color development (AWCD) of soil microorganisms in the two areas with or without tourism interference was shown in Figure 1. The results showed that, with the passage of culture time, the AWCD of soil microorganisms in both areas increased and tended to be stable. During the rising process, the AWCD of soil microorganisms in the area without tourism interference increased faster and was higher after stabilization. The functional diversity of microbial communities after AWCD stabilization was calculated (Table 4). The Shannon index and Simpson index both reflected the functional diversity of soil microorganisms. The soil microbial community of the area without tourism interference had significantly higher species diversity and more dominant species.

Tourism develops with the improvement of social and economic levels. In this process, tourism activities will inevitably disturb the natural environment of the tourist place. As a kind of scenic spot, forest parks have enough vegetation, and the growth state of vegetation is affected by many factors, among which soil environment is one of the important influencing factors. The tourism activities brought by tourism will have an impact on the soil environment of the scenic spot. Changes in the soil environment are bound to have an impact on vegetation. For forest parks, once vegetation changes, its tourism value will be affected. Therefore, the study of the relationship between soil microbial community and tourism interference can provide effective support for protecting the environment of tourist scenic spots. In this study, the forest park was first divided into two areas with and without tourism interference according to whether it was open to tourists or not. The physical and chemical properties of the soil samples collected from the two areas were measured, and the number of microbial communities and functional diversity in the soil samples were determined. In terms of physical and chemical properties, the soil with tourism interference had lower water content, stronger acidity, and less carbon, nitrogen, phosphorus, and potassium content. In terms of microbial communities, the area with tourism interference had fewer microbial communities and lower functional diversity. The reasons causing the above phenomena were possibly that the soil was compacted, and the porosity of the soil was reduced due to the trampling of tourists in tourism activities, which reduced the water retention capacity of the soil and decreased the water content. The weakened water retention ability led to rapid water loss in the soil, and in the process of water loss, a large amount of alkaline salt was taken away, making the soil acidified. Pollutants produced by tourism activities led to the increase of harmful substances in the soil, and nutrients like carbon, phosphorus, nitrogen, and potassium were reduced due to water loss caused by low water retention. In the analysis of soil microbial community, the soil without any interference from tourism had a considerably greater abundance of microbial communities compared to the soil with tourism interference. The microbial community in the soil without tourism interference had significantly higher species diversity and more dominant species.

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