The effect of Chinese fir planting on soil microbial community in artificial garden

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As a tree with high economic value, Chinese fir has been widely used, and its planting scale has gradually increased. However, in the process of planting, operators found that the increase of successive planting rotations of Chinese fir would affect the output of Chinese fir. This study sampled and tested the soils of the first, second, and third rotations of continuously planted Chinese fir and related soil microorganisms in the state-owned Fushan Forest Farm of Yantai, Shandong Province. The results showed that the continuous planting of Chinese fir in the land reduced the nutrient contents of the land and the overall number of microorganisms, resulted in community imbalance and the loss of diversity reacted upon soil environment, which deteriorated the land environment and formed a vicious circle. In conclusion, the continuous planting of Chinese fir in the same area will lead to the decrease of soil fertility, the imbalance of microbial community structure, and the diversity decrease of microbial community. Therefore, in order to avoid these adverse effects, the continuous planting of Chinese fir in the same area should be avoided.

Keywords: Chinese fir; continuous planting; microbial community; diversity.

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Introduction

In order to reduce the impact of the consumption of forest resources, people began to plant trees and return farmland to forest. Moreover, for the purpose to ensure the sufficiency of wood resources, a batch of fast-growing forests were selected from the perspective of economy for artificial planting to form artificial garden [1]. One of the characteristics of fast-growing forest is its rapid growth. It usually takes only a few years from seedling to harvesting, and its commercial quality as wood is good enough. Because of the advantages of fast-growing forest, such as rapid growth, high yield, and high economic value, large area of fast-growing forest is often planted in the artificial garden. However, the continuous planting of fast-growing forest will affect the local soil and the microbial community in the soil and eventually lead to a reduction in production [2]. The microbial community in the soil is closely related to the soil fertility. The study on the influence of fast-growing forest planting on the soil microorganisms in the artificial garden has a reference value for improving the yield of fast-growing forest. Sheng *et al.* [3] studied the effects of continuous cropping of *Pseudostellariae heterophylla* on soil microorganisms and found that *Pseudostellariae heterophylla* could accumulate toxicity in the soil during continuous cropping, which made the microbial community unbalanced. Li *et al.* [4] studied the effect of continuous cropping of *Artemisia annua* on soil microbial community and found that allelochemicals released from the rhizosphere of *Artemisia annua* could inhibit the metabolism of microorganisms in the rhizosphere soil. This study focused on the

Table 1. Overview of sampling sites.

Sample plot	The first generation of Chinese fir (FCF)	The second generation of Chinese fir (SCF)	The third generation of Chinese fir (TCF)
Tree age (years)	59	32	16
Gradient (degree)	21	22	21
Average tree height (m)	30.1	16.2	10.3
Average tree diameter (cm)	29.3	14.1	9.4
Distribution density (number/hectare)	1,023	1,125	1,136
Area (hectare)	1.25	1.15	1.35

Chinese fir and its soil microorganisms. By analyzing the chemical properties of soil and the diversity of microbial community, the soil that was cropped with continuously planted Chinese fir and its microbial community were explored.

Materials and methods

Overview of the study area

In this study, the experimental plot was set in the state-owned Fushan Forest Farm (121° 20′ E, 37° 22′ N) in Yantai City, Shandong Province, China covering an area of 22.5 km^2.

The general situation of the sample plot is shown in Table 1. According to the survey, the horizontal distance between the areas of the first and the second rotations of Chinese fir was about 0.5 km, and the horizontal distance between the second and the third rotations of Chinese fir was about 1.1 km. The horizontal distance was defined as along the horizontal plane, where gradient represents the gentle degree of the ground surface, usually the ratio of the vertical height of the slope to the horizontal distance. The first rotation of Chinese fir forest was planted in 1960. The second rotation was planted in 1987 after removing some of the first rotation, and the third rotation was planted in 2003 after removing some of the second rotation.

Setting up sample plots and sampling

The Chinese fir forest of the first to third rotations occupied a large area. It was impossible to sample everywhere in the area. Therefore, three sampling sites were randomly set up in each rotation area with the sampling specifications as (1) three soil samples were taken along a route of "S" shape in the sampling sites [5], and (2) the soil samples were mixed evenly and sieved using holes with a diameter of 2 mm [6]. The screened soil samples were stored for later experiments.

Measurement of chemical properties of soil

The soil pH value was measured by mixing soil and potassium chloride solution at a ratio of 1:2.5 [7]. The contents of soil carbon and nitrogen were determined by using CN 802 Carbon Nitrogen Elemental Analyzer (VELP Scientifica, Usmate, Italy). The content of phosphorus in the soil after alkali fusion was detected through molybdenum antimony colorimetry method by using 722S Spectrophotometer (Juchuang Environmental Co., Ltd., Qingdao, Shandong, China). The content of potassium in the soil after alkali fusion was measured by using Sherwood M410 Flame Photometer (Zhongke Keer Instrument Co., Ltd., Beijing, China).

Measurement of functional diversity of soil microbial community

The 96-well plate was employed in this study as the ecological plate to detect the functional diversity of microorganisms. There were 31 types of single carbon sources with a single carbon source in each well on the plate [8], which include Methyl pyruvate, Tween 40, Tween 80, α-cyclodextrin, Glycogen, D-cellobiose, α-D-lactose, β-methyl-D-glucoside, D-xylose, i-erythritol, D-mannitol ,N-acetyl-D-glucosamine, D-glucosamine acid, α-D-glucose-1-phosphoric acid, D,L-α-phosphogly cerol, D-galactono lactone, D-galacturonic acid, 2-hydroxybenzoic acid, 4-hydroxybenzoic acid, γ-hydroxybutyric acid, Itaconic acid, α-ketobutyric acid, D-malic acid, L-arginine, L-asparagine, L-phenylalanine, L-serine, L-threonine, Glycyl-L-glutamine, Phenylethylamine, Putrescine (Hubei Ruitai Pharmaceutical Technology Co. Ltd., Huangshi, Hubei, China). On each 96-well plate, 32 wells were assigned to one experimental group with three groups on each plate. Each well contains 300 µL of one type of single carbon source (0.1 g/mL) and tetrazolium violet at the same dose except the control well. Briefly, 10 g of soil sample was weighed and put into a triangular flask and dissolved by 100 mL of sterilized saline [9]. The sample suspension was shaken at 27℃, 140 rpm for 30 min, and then, put aside for 25 min for the insoluble substance precipitation. The supernatant was collected and diluted with sterile water at a ratio of 1:100. The ecological plate was preheated to 25℃ before 120 µL of sample solution was inoculated to the plate. The inoculated plate was incubated at 28℃, while the absorbance of each well was measured by using SpectraMax iD5 Microplate Reader (Molecular Devices, San Jose, CA, USA) under the wavelength of 590 nm every 24 h, up to 168 h. The experiment was set as three repeats for each soil sample. The average values were calculated as the results by using the following formulations.

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

Where *AWCD* was the average well color development, which was used to describe the overall ability of microbial community to utilize carbon sources in soil. *C* was the absorbance of each well on the plate which was inoculated with the sample solution. *R* referred to the absorbance of the control well that had the same carbon source, but no soil sample solution. *H* was the Shannon index. *D* was the Simpson index. P_i was the comprehensive ratio of the relative absorbance of the i^{th} hole to the relative absorbance of the whole plate.

Detection of diversity of microbial community structure in soil

The diversity of structure of microbial community in the soil was detected by using phosphor lipid fatty acid method [10] which could determine the type of microorganism based on different phospholipid fatty acids in microorganism under different environments.

Statistical analysis

The experimental data were analyzed by using Microsoft Excel and SPSS software (IBM Company, Armonk, New York, USA). The calculated results were expressed as Mean ± SD. The difference between the groups was determined by using t-test with $P < 0.05$ as statistically significant.

Results

The chemical properties of sampled soils

The measurement results of the chemical properties of sampled soils were shown in Table 2. The data showed that the pH values and the contents of nitrogen, carbon, phosphorus, and potassium in the soils demonstrated decrease tendency in the regions with the increase of planting rotations of Chinese fir. All the differences showed statistically significant ($P < 0.05$)

The functional diversity of microorganisms

Figure 1 showed that the average well color development (AWCD) of soil microbial community in three rotations of Chinese fir increased with the increase of culture time, which indicated that the overall activity of microbial community increased gradually, and the overall activity gap between the three rotations of soil microbial communities was more obvious in the later stage of culture. At the ends of 24 h and 48 h, due to the early stage of culture, the number of microorganisms was small while

Table 2. Chemical properties of soil under the first, second and third rotations of Chinese fir planting.

Type of sample soil	The first rotation of Chinese fir planting (FCF)	The second rotation of Chinese fir planting (SCF)	The third rotation of Chinese fir planting (TCF)
pH value	3.89±0.03	3.76±0.04*	3.69±0.05*
Total nitrogen content (g/kg)	2.264±0.36	1.788±0.35*	1.734±0.25*
Total carbon content (g/kg)	21.184±0.14	14.776±0.33*	14.408±0.23*
Total phosphorus content (g/kg)	0.37±0.04	0.32±0.03*	0.25±0.05*
Total potassium content (g/kg)	26.34±0.58	23.08±0.28*	21.24±1.06*

Note: * indication of significant difference ($P < 0.05$).

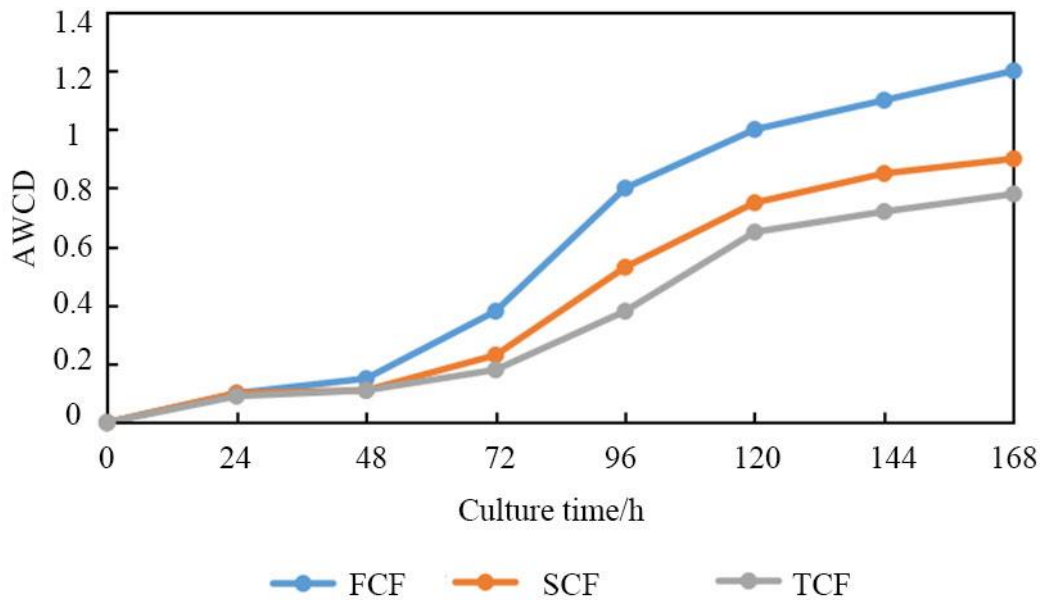

Figure 1. AWCD changes of microorganisms in the soils of the first, second, and third rotations of Chinese fir planting.

the use of carbon sources was few. Therefore, the difference of AWCD was not large. After 48 h, the AWCD gap between microorganisms of the three kinds of soils gradually increased, and the activity of the first rotation of soil was the highest, followed by the second rotation of soil and the third rotation of soil.

Figure 2 demonstrated the Shannon and Simpson indexes of microorganisms of three rotations of Chinese fir planting. After 96 hours of incubation, the Shannon indexes of microorganisms of the first rotation of Chinese fir planting (FCF), the second rotation of Chinese fir planting (SCF), and the third rotation of Chinese fir planting (TCF) were 3.634 ± 0.021, 3.238 ± 0.027, and 3.002 ± 0.021, respectively, while the Simpson indexes of FCF, SCF, and TCF were 0.888 ± 0.007, 0.799 ± 0.008, and 0.734 ± 0.006, respectively. The results showed that the diversity of microorganisms in FCF soil was the highest one followed by SCF and TCF soils with significant differences ($P < 0.05$).

The distribution of different microorganisms in the soil was shown in Figure 3. The microflora of

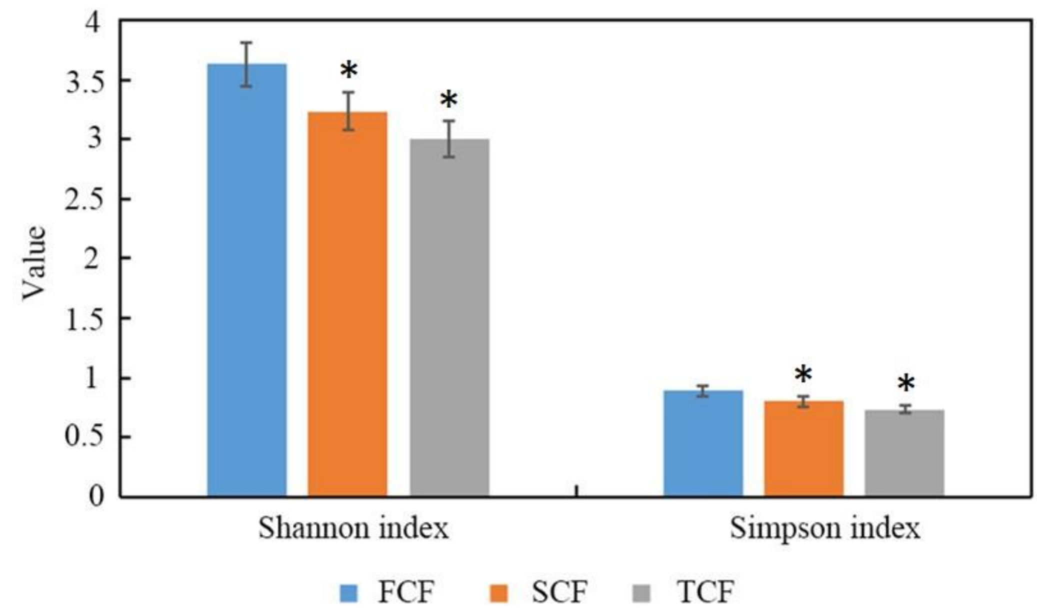

Figure 2. The functional diversity of soil microbial community under different rotations of Chinese fir planting after 96 hours of culture. (* indicates significant difference (*P* < 0.05)).

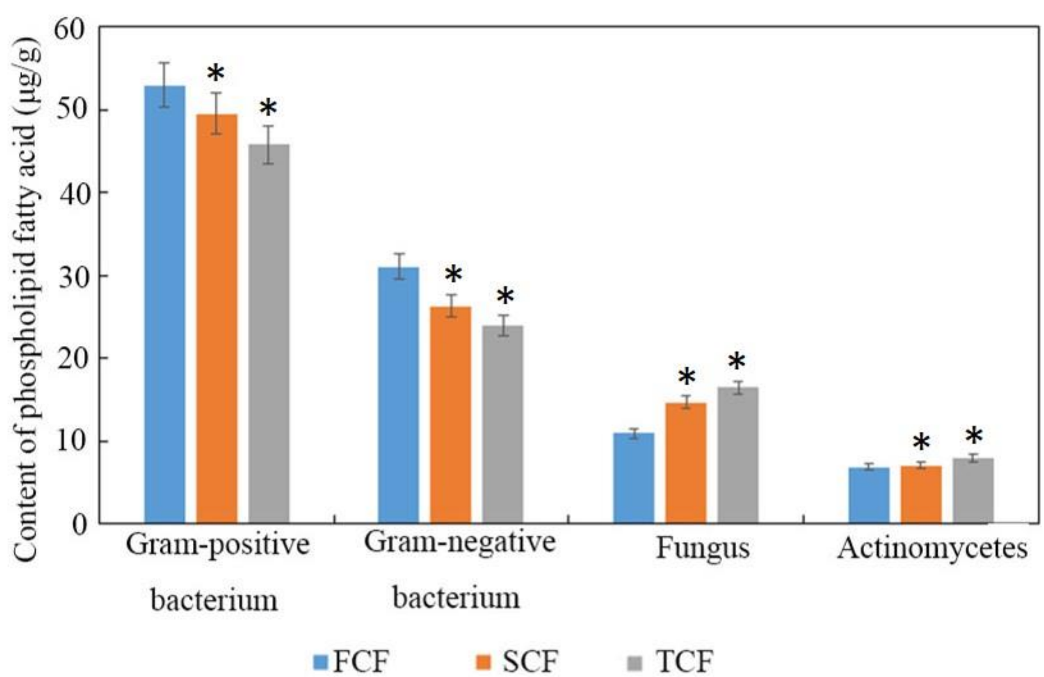

Figure 3. The structural diversity of microflora in the soils of different rotations of Chinese fir planting. (* indicates significant difference (P < 0.05)).

FCF contained 52.96 ± 0.51 μg/g of gram-positive bacteria, 31.05 ± 0.34 μg/g of gram-negative bacteria, 10.93 ± 0.10 μg/g of fungi, and 6.83 ± 0.08 of actinomycetes. The microflora of SCF contained 49.49 ± 0.35 μg/g of gram-positive bacteria, 26.25 ± 0.18 μg/g of gram-negative bacteria, 14.66 ± 0.15 of fungi, and 7.04 ± 0.06 of actinomycetes. The microflora of TCF contained 45.81 ± 0.21 μg/g of gram-positive bacteria, 23.93 ± 0.15 of gram-negative bacteria, 16.41 ±

0.19 of fungi, and 7.94 ± 0.11 of actinomycetes. The results showed that there were large bacteria populations among the soil microflora of the same rotation of Chinese fir, followed by fungi and actinomycetes with the significant differences ($P < 0.05$). The population of bacteria in the soil decreased with the increase of the planting rotations, while the fungi and actinomycetes amount increased.

Discussion

In this study, the chemical properties of the soil which was continuously planted with different rotations of Chinese fir were tested. The results showed that the soil acidification increased while the contents of carbon, nitrogen, phosphorus, and potassium decreased with the increase of the rotations of Chinese fir. Therefore, the continuous planting of Chinese fir will decrease the soil nutrient contents. It is different to animals that general plants cannot get organic molecules directly from the outsources to transform them into their own nutrients. Instead, plants need to use photosynthesis to generate organic molecules to supply themselves. Soil fertility often refers to the presence of recombinant carbon, nitrogen, phosphorus, and potassium in the soil. However, carbon, nitrogen, and phosphorus usually exist in the form of organic molecules. Since Chinese fir cannot directly absorb organic molecules, it is necessary for microorganisms in the soil to decompose organic molecules into inorganic molecules.

The results of this study indicated that the continuous planting of Chinese fir reduced the functional diversity of soil microbial community, which might be caused by the secretion of allelochemicals from the roots of Chinese fir to inhibit the utilization of carbon sources in soil by microorganisms, and thus, reduce the functional diversity. In the soil of the first rotation of Chinese fir forest, the soil itself is nutritious and has strong microbial function. Even though the Chinese fir root system secretes allelochemicals, the concentration is low, which is difficult to inhibit the functional diversity of microorganisms. However, such inhibition property will be transformed gradually during the growth process. After the second rotation of continuous planting of Chinese fir, the allelochemicals secreted by the previous rotation remain in the soil and tend to have increased concentration under the accumulation. The increased allelochemicals will bind to microorganisms in the soil to inhibit their diversity of functions and make the physical and chemical properties of soil worse than it before, which will eventually lead to the yield reduction of Chinese fir.

Conclusion

This research tested the soils of the first, second, and third rotations of continuously planted Chinese fir forest in the state-owned Fushan Forest Farm of Yantai, Shandong, China. The results showed that (1) with the increase of rotations of continuous planting, the soil pH value and the contents of carbon, nitrogen, phosphorus, and potassium gradually decreased; (2) the diversity of microbial function decreased with the increase of culture time; (3) in the soils of all three rotations, the content of Gram-positive bacteria was the highest population followed by Gram-negative bacteria, fungi, and actinomycetes. With the increase of the rotations of planting, bacteria populations decreased while fungi and actinomycetes increased. This study provided solid evidence of influences to the soil properties and microflora in the soil by continuous planting of Chinese fir, which can guide the scientific planning of Chinese fir and give considerations of planting benefits vs. environmental protection. This study also provides references to the continuous planting of economic trees in tree farm and the environmental protection of the planting land.

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