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Effects of Nitrogen and Phosphorus Combined Fertilization on Fine Roots and Soil Microorganisms of Machilus Pauhoi

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Abstract

Machilus pauhoi is a precious tree species and timber forest in the subtropical forest system of China, and the increase in soil nitrogen input will have a profound impact on soil microorganisms. However, it is still unclear about the relative growth rate, fine root traits, and the optimal nitrogen to phosphorus ratio of soil microorganisms of Machilus pauhoi. The essay taking the 3-year-old artificial wood-shaven Machilus pauhoi forest as the research object, five fertilization treatments with different nitrogen and phosphorus ratios were set up (Including control group). Based on the investigation and analysis of Machilus pauhoi's growth in different seasons, fine root characteristics, soil physical and chemical properties and soil microorganisms in 0-20cm soil layer, the influence mechanism of nitrogen and phosphorus combined fertilization on Machilus pauhoi's relative growth rate, fine root characteristics, soil physical and chemical properties and microorganisms was preliminarily revealed, and the synergistic influence mechanism of fine root, soil and microorganisms on Machilus pauhoi's growth was discussed. In September, the content d of soil (TN), soluble organic nitrogen (DON), soil organic matter (SOM) and TP reached the maximum level, and the comprehensive effects of combined fertilization of nitrogen and phosphorus on them should be kept at the best N: P ratio, with the range of 10~12. Bacteria, fungi, actinomycetes, microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) all reached the maximum in September. Different combined fertilization of nitrogen and phosphorus is beneficial to the growth of the three major flora and the retention of soil carbon and nitrogen nutrients by microorganisms, so it is necessary to keep the overall N: P ratio between 10 and 12. SRL and RTD of 0-1mm fine roots are the main fine root factors that affect the relative growth rate (RGR) of whole plant biomass of Machilus pauhoi. MBN is the main microbial factor affecting RGR; Soil TN, DON and SOM are the main soil factors affecting RGR. Through stepwise regression analysis of fine roots, soil and microbial factors, it is found that MBN, DON and soil TP are the main environmental factors that can most affect RGR.

Keywords: Soil microorganism; Physical and chemical properties of soil; Machilus pauhoi.
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1. Introduction

After Europe and North America, China has become the third largest nitrogen deposition area in the world, and the nitrogen deposition is gradually increasing. The relative excess of soil nitrogen will cause soil acidification and a series of ecological problems such as biodiversity reduction,[1] it may also indirectly cause the change of soil phosphorus cycle by affecting soil physical and chemical properties, aggravate phosphorus limitation, and lead to the imbalance of soil nitrogen and phosphorus ratio. The area of soil with phosphorus deficiency in China is about 6.72107 hm^2, especially in most red soil distribution areas in southern China. The problem of phosphorus deficiency is very serious, and soil N and P elements are two essential elements for the growth of most plants. Nutrient addition may alter competitive interactions among species, while also shifting mixed-species communities from symmetric competition for both above- and below-ground resources toward asymmetric competition dominated by aboveground light acquisition.[2] Studies have demonstrated that nitrogen (N) addition may reduce,[3,4] increase,[5,6] or have no effect on.[3] interspecific complementarity. It may also selectively impact complementarity of individual tree species rather than simultaneously affecting both species in a mixture.[3,7] Phosphorus (P) addition has been found to enhance interspecific complementarity.[8] Overall, the effects of N and P additions on complementarity vary with species identity,

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nutrient type,[8] and addition rate.[4,9,10] Current research on N/P addition and mixed-species interactions primarily focuses on single-nutrient manipulations,[3,4] with outcomes diverging across species combinations.[3,11] How specific tree species mixtures respond to coupled N-P additions remains to be elucidated. Therefore, under the background of increasing global nitrogen deposition and the lack of subtropical phosphorus in southern China, it is of great significance to explore the influence mechanism of nitrogen and phosphorus combined fertilization on subtropical biological communities in China, which can provide theoretical reference for us to formulate strategies to deal with environmental change and soil nutrient shortage.

Fine roots are the main nutrient absorption organs of plants. Plants mainly obtain, transport and store nutrients and water through fine roots, and adapt to the changes of the external environment by adjusting the morphological structure of fine roots. Fine roots can sensitively perceive and indicate the changes of the environment, so they can also indicate the health level of trees and even the whole ecosystem to a certain extent.[12] Soil microorganism is an important part of underground ecosystem, which undertakes many important functions such as the decomposition and formation of soil organic matter, the flow and circulation of soil nutrients,[13] and plays an important role in underground ecosystem, which is not only closely related to soil health status, but also sensitively reflects the slight changes of soil environment, so it has an important indication for the dynamics of soil ecosystem.[14] The increase of soil nitrogen input will have a series of effects on underground ecosystem, especially on soil microorganisms. Therefore, studying the influence mechanism of combined application of nitrogen and phosphorus on soil microorganisms will help us to predict the changes of soil environment earlier and improve the utilization rate of forest soil nutrients by precise fertilization. N and P addition had a great direct effect on soil bacterial community and an indirect effect on it mainly by changing the litter biomass. Our findings highlighted that severe niche differentiation was induced by P along with a high-level N, further emphasizing the importance of simultaneously evaluating response of soil bacterial community to N and P addition, especially in the context of increasing anthropogenic nutrient additions.[15] Underground biome is an important part of forest ecosystem. Previous studies have paid too much attention to aboveground parts and neglected the important role of underground biome.[16] Fine roots and soil microorganisms are the main components of underground biological communities, fine roots are the main nutrient absorption organs of plants, and soil microorganisms are regarded as the storage and important source of nutrients needed by plants,[17] both of which play a very important role in plant growth. However, environmental changes (such as combined application of nitrogen and phosphorus) will directly or indirectly cause changes in the two plants and the whole plant, and even eventually lead to changes in the entire forest ecosystem. Machilus pauhoi is a precious tree species and timber forest in China's subtropical forest system. Therefore, it is of great significance to study the effects of nitrogen and phosphorus combined fertilization on Machilus pauhoi's fine roots and soil microorganisms, explore their adaptation strategies to environmental changes, and find out the most suitable proportion of nitrogen and phosphorus combined fertilization for Machilus pauhoi's growth, which will improve fertilizer efficiency and utilization rate, and then improve China's subtropical forest productivity, and also have practical significance and application value in the management and management of artificial young forests. In addition, revealing the interaction and feedback mechanism among Machilus pauhoi fine roots, soil microorganisms and soil physical and chemical properties, and exploring how their synergistic development affects Machilus pauhoi growth can provide theoretical basis for further exploring the mechanism of underground nutrient flow and distribution, improving soil natural fertility and maintaining the stability of forest ecosystem, and also provide theoretical reference and basic data for further understanding the underground physiological and ecological process of subtropical arbor forests in China.

2. Materials and methods

2.1 Natural overview of the study area

The experimental plot is located in Dagan Town, Shunchang County, Nanping City, Fujian Province, which belongs to subtropical monsoon climate with abundant sunshine and rainfall. The annual average temperature is 19℃, the coldest January is about 8℃, and the hottest July is about 28 ℃. Precipitation is generally concentrated in 99February-September, and the average annual rainfall is usually between 1600 and 1900 mm; The average annual frost-free period is about 305d, and the average annual sunshine hours are about 1741h. The experimental forest is an artificial young forest under the forest, which is located under the mixed forest of 7-width and 3-width Chinese fir. Michelia macclurei, Cunninghamia lanceolata and other tree species are the dominant species in the mixed forest, with a canopy density of 0.4, an average DBH of 16.36cm and a stand density of 470 plants /hm^2. The experimental young plantation is a 3-year-old Machilus pauhoi plantation, which is located in the middle and lower slopes of low mountains. The geographical coordinates are about 26º59′34"-26º59′35"N, 117º40′45"-117º40′46"E, the altitude is about 570m, and the average slope is about 35. The soil in the experimental area is red soil, and its water content, bulk density and pH value are about 30.30±1.6, 0.93 0.23g/cm3,4.57±0.15 respectively. The specific basic survey information is shown in Table 1.

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Table 1: Basic information of Machilus pauhoi stand.

Stand characteristics			Soil nutrient		
Average ground diameter(mm)	Average tree height(cm)	Standing tree density(trees/mu)	TC(mg/g)	TN(mg/g)	TP(mg/g)
12.65	95.24	180	32.8±0.26	2.02±0.01	0.60±0.11

In the 3-year-old Machilus pauhoi plantation, 15 samples of 10×10m were set, and a buffer zone of 2m was set around. This experiment is based on the previous study of Ling Fei on the effect of nitrogen and phosphorus spraying on nutrient reabsorption efficiency of Machilus pauhoi leaves,[18] and set up fertilization treatment on this basis. Fertilization amount is fixed according to N addition amount, and the standard of pure nitrogen addition amount is 100 kg▪hm^{-2}▪a^{-1}, the addition amount of P is determined according to different N:P ratios, and five treatments are set up in total: Control group: CK, No fertilization.

NP1: N:P=8:1, N=100 kg▪hm-2▪a-1, P=12.5 kg▪hm-2▪a-1

NP2: N:P=10:1, N=100 kg▪hm-2▪a-1, P=10 kg▪hm-2▪a-1

NP3: N:P=12:1, N=100 kg▪hm-2▪a-1, P≈8.33 kg▪hm-2▪a-11

NP4: N:P=15:1, N=100 kg▪hm-2▪a-1, P≈6.67 kg▪hm-2▪a-1

There are 3 duplicate quadrats for each treatment, which are sprayed from April to September in 2016 and 2017 respectively, and applied once in the middle of each month for 6 times/year. Fertilizer N uses NH_4NO_3 and fertilizer P uses P_2O_5. According to Table 2, weigh the amount of NH_4NO_3 and P_2O_5 required by the quadrat, dissolve them in 20L distilled water, and spray the seedlings in the whole quadrat evenly from top to bottom with a sprayer by foliar spraying method. The treatment (CK) of the control group was also sprayed with 20L distilled water, and the method was the same as that of spraying fertilizer. See Table 2 for the specific amount of fertilization:

2.2 Sampling method

Four forest surveys and sampling were conducted in the experimental area from December 11 to 15, 2016 (Winter), March 11 to 15, 2017 (Spring), June 11 to 15, 2017 (Summer) and September 11 to 15, 2017 (Autumn), among which June and September are required before spraying. Every survey measures the crown width, plant height and ground diameter of each tree in the quadrat.

Eight young trees of medium size should be selected as fine roots in each quadrat, and the ground litter and weeds should be cleaned up. One of the four directions of the selected sample trees should be sampled at a time, which requires that the sampling direction of each sample tree is different in each season, and it is also necessary to ensure that samples are taken in all four directions within one year. The sampling of fine roots is divided into two soil layers (0-10cm and 10-20cm). According to the root distribution law of Machilus pauhoi parvifolius obtained in the previous experiment, the clods of 10×10×10cm are dug at a position 30cm away from the trunk, and packed into self-sealed bags and numbered. Washing and sieving with deionized water to take out the fine roots, selecting the fine roots of Machilus pauhoi parvifolius according to the shape, smell and color of the fine roots, eliminating other roots, and then dividing the selected fine roots into two grades according to their diameters, namely, 0-1mm and 1-2mm with vernier calipers. Soil sampling is divided into two soil layers (0-10cm and 10-20cm) in each quadrat to take soil around the roots. After fully mixing the soil samples of each soil layer in the same quadrat, take out about 400g and divide it into two parts, one part is naturally air-dried for the determination of soil physical and chemical properties, and the other part is stored in the refrigerator at MINUS 20 degrees for the determination of soil microbial related indicators.

3. Determination method

3.1 Determination of C and N contents in fine root samples

The fine root samples brought back indoors were dried to a constant weight at 65 °C, and then weighed. Then, they were ground into powder by a pulverizer and passed through a 100-mesh sieve. The total carbon (TC) and total nitrogen (TN) of fine roots were determined by an Elemental Analyzer Vario ELIII made in Germany, and the total phosphorus (TP) was extracted by sulfuric acid-perchloric acid digestion and determined by a continuous flow analyzer (See the literature: Bao Shidan, 2000 for all the above methods).

3.2 Determination of basic physical and chemical properties of soil samples

Mainly refer to the literature of Shidan Bao to determine the physical and chemical properties of soil.[19] After the air-dried soil samples were ground, the samples were screened by 100 mesh sieve, and the total carbon (TC) and total nitrogen (TN) in the soil were determined by vario MAX carbon and nitrogen analyzer. Total phosphorus (TP) was extracted by sulfuric acid-perchloric acid digestion method, and the determination of TP was completed by continuous flow analyzer. After 0.5mol hydrochloric acid was used to treat soil organic matter, the soil organic matter was determined by vario MAX carbon and nitrogen element analyzer. The moisture content of soil was determined by aluminum box drying method. Use portable pH meter to measure the pH value of soil. The ratio of soil to water is 2.5:1(V/V), and the DOC and DON of soil are extracted by 0.5 mol▪L^{-1} K_2SO_4, and determined by total organic carbon analyzer and continuous flow analyzer respectively.[20]

Table 2: Application amounts of NH_4NO_3 and P_2O_5.

Treatment	Fertilization amount of each square per month (g)		Total fertilization amount of each plot in the whole year (g)	
	NH_4NO_3	P_2O_5	NH_4NO_3	P_2O_5
CK	0	0	0	0
NP1(N:P=8)	476.19	47.715	2857.14	286.29
NP2(N:P=10)	476.19	38.17	2857.14	229.02
NP3(N:P=12)	476.19	31.81	2857.14	190.86
NP4(N:P=15)	476.19	25.45	2857.14	152.70

3.3 Determination of soil microbial biomass carbon and nitrogen

Soil microbial biomass carbon and nitrogen were determined by chloroform fumigation -K_2SO_4 extraction method,[21] and 5g fresh soil was weighed, put into 50ml beaker, fumigated with chloroform in vacuum dryer for 24h in the dark, and taken out. At the same time, 5g fresh soil was weighed as the control group, and the other treatment methods were the same except chloroform fumigation. Then it was leached with 0.5 mol ▪ L-1 K2SO4, and the carbon in the leaching solution was determined by TOC-V total organic carbon analyzer, and the nitrogen in the leaching solution was determined by continuous flow analyzer. The contents of MBC and MBN are obtained by dividing the difference of total organic carbon and total nitrogen in the extracted liquid of fumigated and non-fumigated soil samples by the conversion coefficient of 0.45[22] and 0.54.[21]

3.4 Determination of fatty acid content of soil microbial phospholipids

The determination of phospholipid fatty acid content in soil microbial community refers to the method proposed by Zelles and other scholars in 1992, and the specific methods are as follows: Sieving (2 mm) the collected soil samples, and freeze-drying the soil samples. Extract 3g of freeze-dried sample soil and put it in a 50ml test tube, add 2ml of extract, shake it for 20 seconds, perform ultrasonic treatment for 10 minutes, repeat the shaking and ultrasonic treatment for 3 times, perform vortex treatment for 10 seconds, perform centrifugal treatment for 10 minutes, control the temperature at 25 ℃ and centrifuge rate at 2500r, and transfer the supernatant. Add 5 ml of extract to the original soil sample, shake for 20s, perform ultrasonic treatment for 10min, centrifuge for 10min, control the temperature at 25℃ and centrifuge rate at 2500r, and transfer the supernatant. Mix the supernatants for two times, add 3.1 ml chloroform and 3.1 ml phosphate buffer, vortex for 20 s, centrifuge for 10 minutes, control the temperature at 25 ℃ and the centrifugation rate at 2500r, take off the supernatant and blow dry with nitrogen. Add 1ml of chloroform to the extraction column for wetting and washing, add 200 microliters of chloroform to the nitrogen-dried test tube, shake for 10 seconds, transfer to the extraction column, complete self-leakage, repeat the treatment twice, and add 5ml of chloroform, 6ml of acetone and methanol to the extraction column to wet and wash the bottom of the column. Put a test tube at the bottom of the column, add 5ml of methanol to the column, and dry it with nitrogen. Add 1 ml of methanol and toluene, vortex for 10 seconds, 1ml of KOH vortex for 20 seconds, and treat in water bath for 15 minutes at 37℃. After the above work is completed, 2 ml of imported n-hexane, 1ml of glacial acetic acid and 1ml of deionized water are added in sequence, and the temperature is controlled to be 25 ℃ and the centrifugal rate is 2,500r, and then the supernatant is extracted, and 2ml of imported n-hexane is added to the remaining samples, and then the centrifugal treatment is carried out for 5min, and the temperature is controlled to be 25℃ and the centrifugal rate is 2,500r, and then the supernatant is extracted twice. Then it was dissolved in 200 microliters of n-hexane, with esterification C19:0 as internal standard. Agilent 6890 N gas chromatograph produced in Germany was used for determination.

The single fatty acid type was expressed by nmol/g dry soil, and the concentration of each fatty acid was calculated based on the concentration of carbon internal standard of 19:0.[23-24]

In this paper, bacterial biomass is characterized by the total PLFAs of i14:0, i15:0, a15:0, 16:0, 16:19c, 16:17c, i17:0, a17:0, cy17:0, 18:17c, 18:15c, cy19:0;[25] Fungal biomass is characterized by the total PLFAs of 18:1 ω 9c, 18:2 ω 6/9c;[26] The biomass of 232 actinomycetes is characterized by the PLFAs content of 10Me16:0, 10Me17:0, 10Me18:0.[27]

3.5 Data processing methods

Excel 2010 was used for basic data calculation and processing, SPSS19.0 was used for covariance analysis, multivariate analysis of variance, multiple regression analysis and correlation analysis, and Origin 9.0 and Canoco 5.0 were used for drawing.

4. Effects of nitrogen and phosphorus combined fertilization methods on soil physical and chemical properties and microorganisms of Machilus pauhoi

Table 3: Soil physical-chemical properties of different treatments in different months ($\bar{x} \pm s$).

Season	Soil layer	Treatment	TN(mg/g)	TC(mg/g)	TP(mg/g)	C/N	N/P	C/P
December	0-10cm	CK	2.19±0.22b	31.98±3.27c	0.38±0.04a	14.61±0.82c	5.81±0.94ab	84.88±13.69b
		NP1	2.34±0.37b	34.14±2.16b	0.42±0.01a	14.59±0.83c	5.62±0.74b	82.02±3.67b
		NP2	2.52±0.26a	38.27±1.74a	0.41±0.02a	15.16±0.62b	6.06±0.38a	91.87±8.01a
		NP3	2.59±0.31a	39.34±3.08a	0.42±0.02a	15.20±0.99b	6.10±0.50a	92.80±13.03a
		NP4	2.47±0.28a	39.03±3.36a	0.43±0.02a	15.80±1.16a	5.74±0.46b	90.66±11.23a
.	10-20 cm	CK	2.07±0.19b	28.98±1.44c	0.29±0.04b	13.99±1.09c	7.07±0.46a	98.95±16.14b
		NP1	1.96±0.19c	29.36±2.00bc	0.37±0.04a	14.98±0.79b	5.23±0.22c	78.40±5.22d
		NP2	2.13±0.22b	31.55±2.47b	0.36±0.05a	14.83±0.61b	5.95±0.22b	88.29±12.49c
		NP3	2.22±0.32ab	32.91±1.86b	0.35±0.03a	14.86±0.69b	6.36±1.20b	94.42±11.75b
		NP4	2.30±0.13a	35.53±7.41a	0.34±0.03a	15.42±0.53a	6.86±0.57a	105.80±29.07a
March	0-10 cm	CK	1.86±0.11c	27.47±7.33d	0.54±0.03c	14.76±1.12c	3.45±0.09b	50.86±13.72b
		NP1	2.08±0.16b	31.95±1.15c	0.78±0.13a	15.34±1.19b	2.68±0.37d	41.12±7.40c
		NP2	2.14±0.10b	34.10±1.54bc	0.68±0.07b	15.94±0.84b	3.15±0.43c	50.18±6.80b
		NP3	2.31±0.15a	36.07±3.35ab	0.57±0.04c	15.63±0.44b	4.04±0.45a	63.20±10.10a
		NP4	2.25±0.32a	37.07±4.05a	0.61±0.03c	16.47±0.64a	3.70±0.61ab	60.92±8.60a
	10-20cm	CK	1.77±0.89b	25.03±3.29b	0.51±0.03c	14.17±1.10a	3.49±0.25b	49.44±7.55b
		NP1	2.03±1.03a	27.28±2.49b	0.71±0.06a	13.44±0.92b	2.87±0.36c	38.62±5.20c
		NP2	1.91±0.95b	26.25±0.87b	0.64±0.28b	13.75±0.77b	2.97±1.06c	40.86±6.96c
		NP3	2.15±0.92a	30.35±3.69a	0.59±0.02b	14.15±0.57a	3.62±0.52b	51.15±6.23b
		NP4	2.14±1.01a	31.57±1.43a	0.52±0.05c	14.78±1.47a	4.08±0.97a	60.22±4.81a
June	0-10cm	CK	1.89±0.04c	27.91±3.31c	0.62±0.05c	14.78±1.18c	3.03±0.18b	44.73±8.87b
		NP1	2.04±0.07b	32.16±6.37b	0.83±0.04a	15.77±1.55b	2.46±0.06c	38.77±7.24b
		NP2	2.22±0.04a	33.21±1.48b	0.77±0.03a	14.93±0.90c	2.89±0.08b	43.09±3.59b
		NP3	2.29±0.12a	36.10±4.37a	0.64±0.05c	15.78±0.90b	3.58±0.11a	56.51±3.01a
		NP4	2.17±0.14a	37.84±4.06a	0.70±0.04b	16.53±0.86a	3.09±0.12b	53.84±9.01a
	10-20cm	CK	1.65±0.13c	25.84±4.49b	0.60±0.06c	15.71±1.80a	2.76±0.14a	43.38±9.23ab
		NP1	1.88±0.10b	27.68±2.67b	0.64±0.07bc	14.73±0.94b	2.94±0.26a	43.30±5.77ab
		NP2	2.00±0.41ab	28.22±3.59b	0.72±0.05a	14.16±0.64b	2.78±0.45a	39.43±5.52b
		NP3	2.04±0.11a	31.50±2.26a	0.66±0.05b	15.46±0.27a	3.10±0.08a	47.95±5.86a
		NP4	2.07±0.13a	32.29±2.29a	0.69±0.06ab	15.62±0.60a	3.02±0.09a	47.14±7.74a
September	0-10cm	CK	2.17±0.21c	32.08±4.12c	0.72±0.06b	14.77±1.01a	3.03±0.24b	44.70±8.38b
		NP1	2.61±0.19b	37.98±2.09b	0.84±0.05a	14.54±0.65b	3.10±0.04b	45.08±2.57b
		NP2	2.49±0.19b	37.49±2.16b	0.90±0.06a	15.07±0.87a	2.76±0.16c	41.59±5.13b
		NP3	2.78±0.25ab	41.46±2.39a	0.74±0.05b	14.93±0.62a	3.74±0.08a	55.81±6.39a
		NP4	2.89±0.32a	43.03±4.41a	0.79±0.05b	14.87±1.44a	3.67±0.40a	54.61±8.22a

Season	Soil layer	Treatment	TN(mg/g)	TC(mg/g)	TP(mg/g)	C/N	N/P	C/P
	10-20cm	CK	2.19±0.31c	28.29±5.18c	0.71±0.05b	12.95±1.14b	3.09±0.41b	40.05±7.71b
		NP1	2.20±0.38c	32.28±3.76b	0.83±0.06a	14.68±1.68a	2.64±0.25c	38.81±3.64b
		NP2	2.28±0.40bc	32.66±4.32b	0.75±0.05b	14.33±0.70a	3.04±0.50b	43.61±9.22b
		NP3	2.44±0.35b	35.35±5.05ab	0.81±0.06a	14.50±0.97a	3.03±0.22b	43.90±8.10b
		NP4	2.67±0.20a	37.55±5.15a	0.77±0.08ab	14.05±0.39a	3.46±0.26a	48.61±11.18a

Note: TN- soil total nitrogen, TC- soil total carbon, TP- soil total phosphorus, C/N- Soil carbon-nitrogen ratio, N/P-soil nitrogen-phosphorus ratio, C/P-soil carbon-phosphorus ratio. Different letters indicate that there are significant or extremely significant differences between different treatments of the same soil layer in the same month at the level of 0.05 or 0.01).

Table 4: Variance analysis of the effects of treatment, soil layer and their interaction on soil physicochemical properties (n=60).

	Soil layer	Month	Treatment	Month×Soil layer	Month×Treatment	Soil layer×Treatment	Month×Soil layer×Treatment
TN	25.80**	24.43**	12.18**	0.63	0.66	0.67	0.29
TC	54.48**	13.07**	19.17**	0.10	0.23	0.73	0.11
TP	15.13**	201.43**	14.51**	0.71	1.41	1.90	1.20
SOM	24.39**	16.34**	17.01**	0.10	0.34	0.54	0.22
DON	7.67*	21.62**	5.86**	1.58	0.84	0.52	0.07
DOC	32.43**	107.51**	12.10**	3.87*	1.33	0.40	0.12
C/P	0.82	132.73**	7.89**	1.65	0.40	0.85	0.53
N/P	0.45	215.22**	8.75**	2.20	0.79	1.61	1.18
C/N	2.39	0.57	0.64	0.33	0.14	0.10	0.14
pH	0.02	76.35**	20.54**	2.45	1.46	0.10	0.30
SW	13.25**	298.76**	7.43**	0.30	1.28	0.58	0.31

Notes: TN- soil total nitrogen, TC- soil total carbon, TP- soil total phosphorus, C/N-soil carbon-nitrogen ratio,N/P- soil nitrogen-phosphorus ratio, C/P-soil carbon-phosphorus ratio, DOC- soluble organic carbon, DON- soluble organic nitrogen, SOM- soil organic matter, PH- soil pH value, SW- soil. ** means P<0.01, * means P<0.05, and n means the sample number of each index.

4.1 Effect on physical and chemical properties of woodland soil

The contents of soil organic matter (SOM), total nitrogen (TN) and total carbon (TC) in the two soil layers were higher in September (autumn) and December (winter), and lower in March (spring) and June (summer). However, the content of soil total phosphorus (TP) increased with the increase of fertilization time, that is, it reached the minimum in December and the maximum in September. The contents of SOM, TN, TC and TP in soil were increased in different degrees by all nitrogen and phosphorus combined fertilization treatments. According to Table 3, there was no significant difference in C/N between months in the 0-10cm soil layer (P>0.05), and it was the lowest in March in the 10-20cm soil layer, and the N/P and C/P in both soil layers were significantly highest in December (P<0.05), and the responses of N/P and C/P in both soil layers to each treatment showed a trend of first decreasing and then increasing in four months.

Different letters indicate that there are significant or extremely significant differences between different treatments of the same soil layer in the same month at the level of 0.05 or 0.01.) Soil moisture content has obvious seasonal law, which is larger in March and June and smaller in September and December. As far as fertilization treatment is concerned, among the four fertilization treatments, the high nitrogen and phosphorus treatment (NP4) is the largest, while the other three treatments are relatively small, and the water content of 0-10cm soil layer is slightly higher than that of 10-20cm soil layer (Table 3). Soluble organic carbon (DOC) and soluble organic nitrogen (DON) in soil were higher in September and December, and were the highest in NP2 and NP1 treatments, respectively, and were lower in March and June, and were the

Table 5: The correlation among soil physical-chemical properties and relative growth rate (n=60).

y-x	TN	TC	TP	C/N	N/P	C/P	DOC	DON	SOM	pH	SW	RGR
TN	1											
TC	0.79**	1										
TP	0.51*	0.48	1									
C/N	-0.87*	0.86*	-0.24	1								
N/P	0.40	0.30	-0.59*	0.28	1							
C/P	0.37	0.77*	-0.63*	0.78*	0.68**	1						
DOC	0.67*	0.55*	-0.35	0.71*	0.46	0.41	1					
DON	0.76*	0.60*	0.53*	-0.60*	0.49*	0.31	0.83**	1				
SOM	0.90**	0.89*	0.49	0.35	0.30	-0.48	0.76**	0.72**	1			
pH	-0.83**	-0.76*	-0.26	-0.42	-0.49	-0.42	-0.53*	-0.64*	-0.77**	1		
SW	-0.34	0.28	-0.56*	0.35	0.29	0.59*	-0.41	-0.47	-0.37	-0.45	1	
RGR	0.55*	0.23	0.64*	-0.26	0.19	-0.20	0.64**	0.59*	0.56*	-0.31	0.35	1

Notes: TN- soil total nitrogen, TC- soil total carbon, TP- soil total phosphorus, C/N- soil carbon-nitrogen ratio, N/P- soil nitrogen-phosphorus ratio, C/P- soil carbon-phosphorus ratio, DOC- soluble organic carbon, DON- soluble organic nitrogen, SOM- soil organic matter, pH- soil pH value, SW- soil. ** means P<0.01, * means P<0.05, and n means the sample number of each indicator.

highest in NP3 treatment. The response trends of the two treatments are basically the same, and both of them have increased in different degrees compared with the control. In terms of soil layers, the 0-10cm soil layer is larger than the 10-20cm soil layer (Table 3).

Except for soil C/N, month and treatment methods have significant effects on other soil physical and chemical properties (P<0.01), while soil layer has significant effects on other soil physical and chemical properties except soil C/N, C/P, N/P and pH (P<0.05). The interaction of the three factors basically has no significant influence on the physical and chemical properties of soil (P>0.05), but only the month and soil layer have significant interactive influence on DOC (P<0.05) (Table 4).

From Table 5, it can be seen that there is a close relationship between soil physical and chemical properties, among which SOM is positively correlated with TN, TC, DOC and DON (P<0.01), which reflects that SOM is one of the important indicators indicating soil nutrient status, and DON and DOC, as important sources of soil available nutrients, are significantly correlated with soil TN, TC and TP (P<0.05), and soil pH is significantly correlated with TN. These different correlations between soil physical and chemical properties, such as TN, TC, SOM and pH, indicate that the physical and chemical properties of soil are mutually influenced and interacted, and jointly affect the soil fertility.

4.2 Effect on the main flora of soil microorganisms

The three main microbial groups in soil are bacteria, fungi and actinomycetes, and their responses to combined fertilization of nitrogen and phosphorus have many similarities. As can be seen from Fig. 1, bacteria, fungi and actinomycetes all show the seasonal law of September (Autumn) > March (Spring) > December (Winter) ≈ June (Summer). In terms of soil layer, the number of the three major flora decreased with the increase of soil layer, and the change trend in the two soil layers was basically the same, that is, the fluctuation amplitude in response to different treatments was basically that the 0-10cm soil layer was larger than the 10-20cm soil layer. In terms of fertilization treatment, the responses of the three major flora to different nitrogen and phosphorus combined fertilization treatments in four seasons all showed a trend of increasing at first and then decreasing, with the maximum in NP2 and NP treatments in September and December, and the maximum in NP3 treatment in March and June, and the high nitrogen and phosphorus treatment (NP4) inhibited the three major flora to varying degrees. The interaction between month and soil layer has a significant effect on the three major flora (P<0.05), while

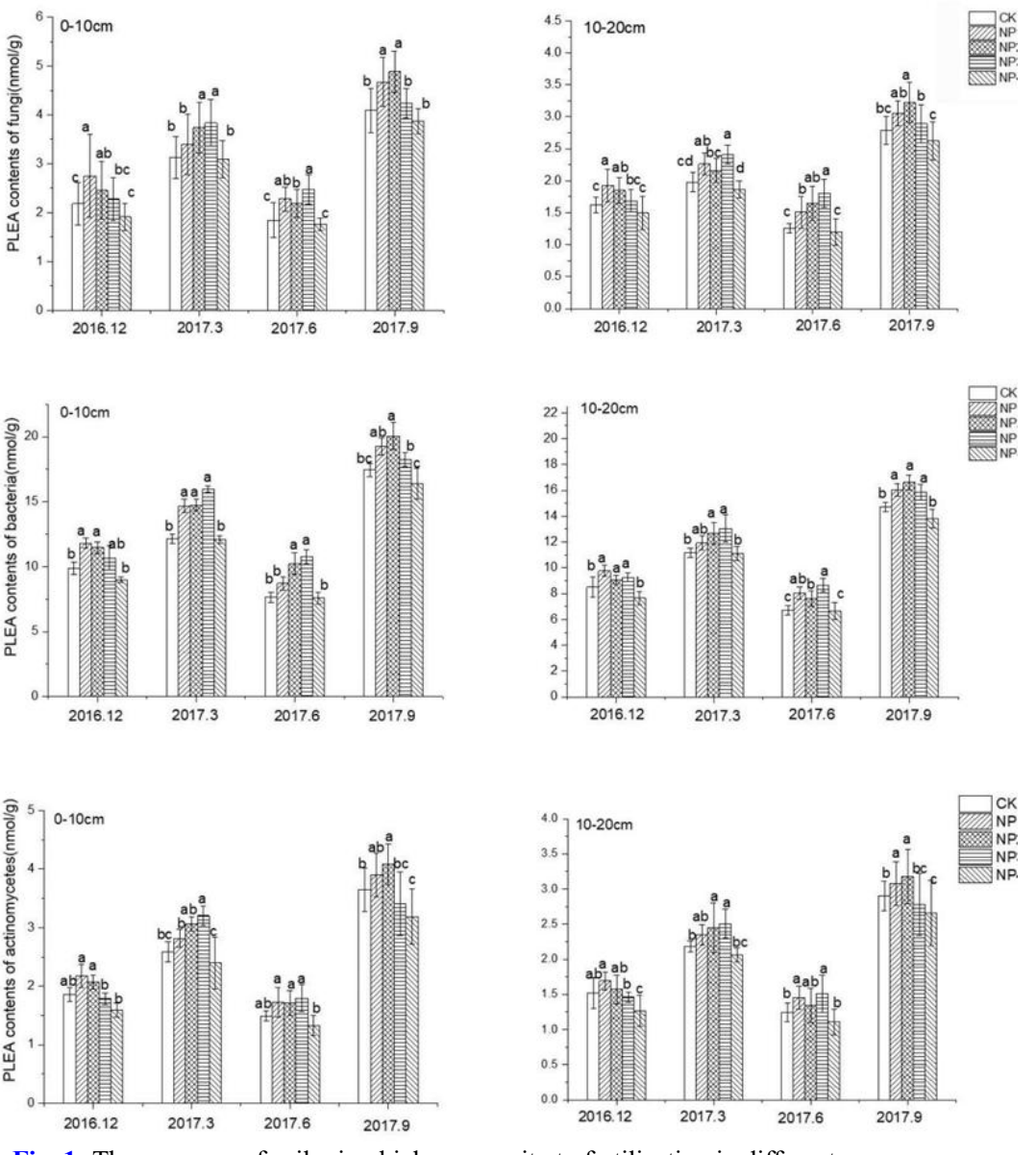

Fig. 1: The response of soil microbial community to fertilization in different seasons.

Note: Different letters indicate that there are significant differences at the level of 0.05 or 0.01 between different treatments in the same season.

Table 6: Variance analysis of the effects of treatment, soil layer and their interaction on soil microorganism (n=60).

	Soil layer	Month	Treatment	Month×Soil layer	Month×Treatment	Soil layer×Treatment	Month×Soil layer×Treatment
Germ	8.62*	162.84**	89.18**	10.60**	7.70**	6.22**	1.08
Fungus	37.37**	45.79**	12.83**	12.30**	2.60*	0.44	0.21
Actinomyces	10.66*	156.69**	10.69**	2.45*	1.46	0.33	0.12
MBC	48.43**	33.77**	27.16**	2.81*	1.97*	0.55	0.13
MBN	25.28**	38.71**	9.41**	0.86	0.95	0.84	0.17
MBC:MBC	1.37	4.63	3.72*	1.49	0.68	0.48	0.35

Note: MBN- soil microbial biomass nitrogen, MBC- soil microbial biomass carbon. ** means $p<0.001$, * means $p<0.05$, and n means the sample number of each indicator.

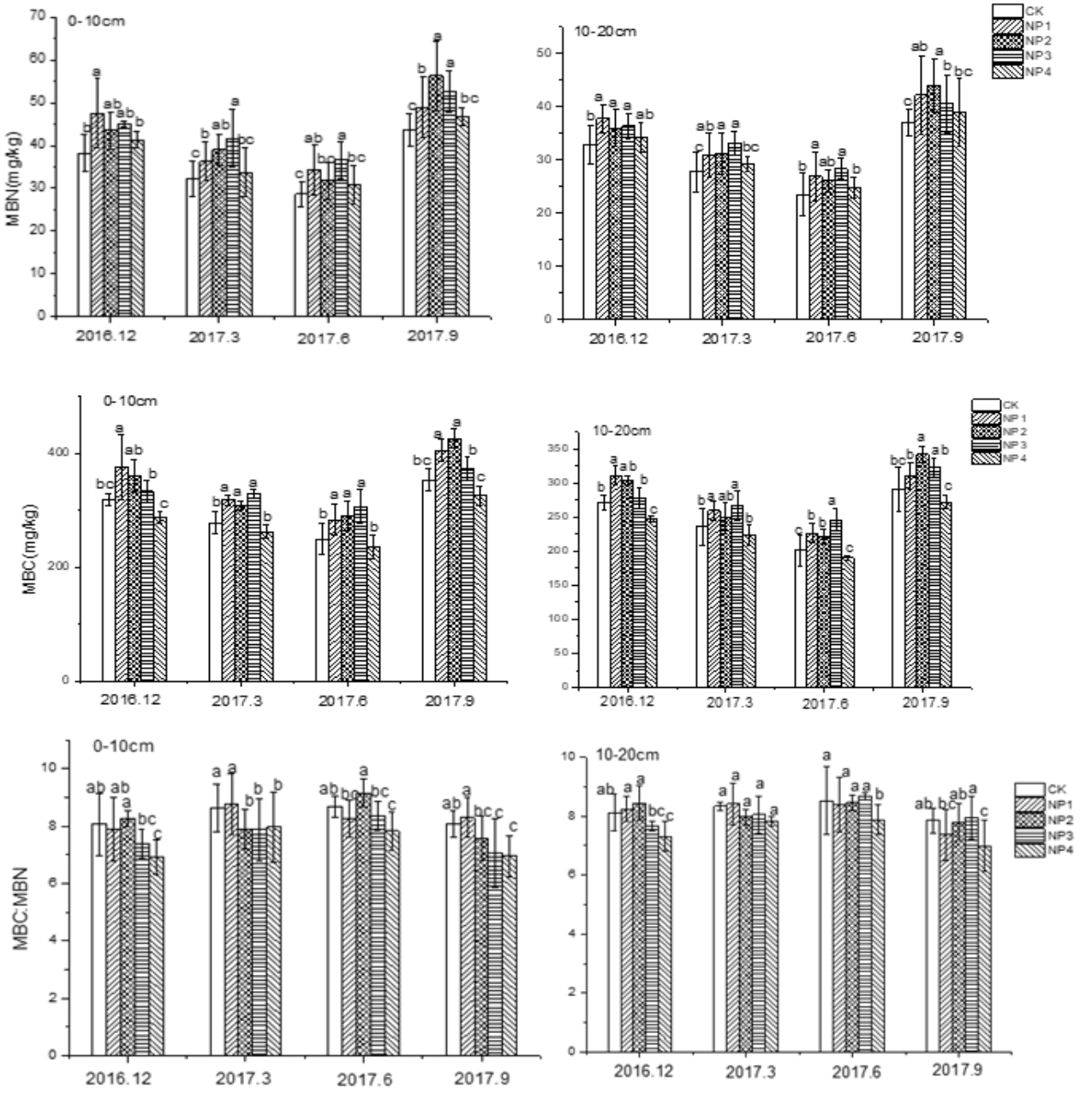

Fig. 2: The response of MBN and MBC to fertilization in different seasons.
Note: Different letters indicate that there are significant differences at the level of 0.05 or 0.01 between different treatments in the same season.

the interaction between month and treatment has a significant effect on bacteria and fungi ($P<0.05$), and the interaction between soil layer and treatment only has a significant effect on bacteria ($P<0.05$), while the interaction between month, soil layer and treatment has no significant effect on the three major flora ($P>0.05$) (Table 6).

4.3 Effect on soil microbial biomass carbon and nitrogen

As shown in Fig. 2, soil microbial biomass carbon (MBC) and soil microbial biomass nitrogen (MBN) show obvious seasonal rhythm in response to nitrogen and phosphorus fertilization, both of which are larger in September (Autumn) and December (Winter), and smaller in March (Spring) and June (Summer). As far as different soil layers are concerned, MBC and MBN decrease with the increase of soil layers, and the response trends and seasonal rhythms of MBC and MBN in the two soil layers are basically the same. In terms of different treatments, the MBC and MBN of the two soil layers were the largest in NP2 and NP1 treatments in September and December, respectively, and the largest in NP3 treatment in March and June. The MBC: MBN of the two soil layers decreased significantly ($P<0.05$) in the treatment of NP4, but there was no significant difference between months and between the two soil layers ($P>0.05$).

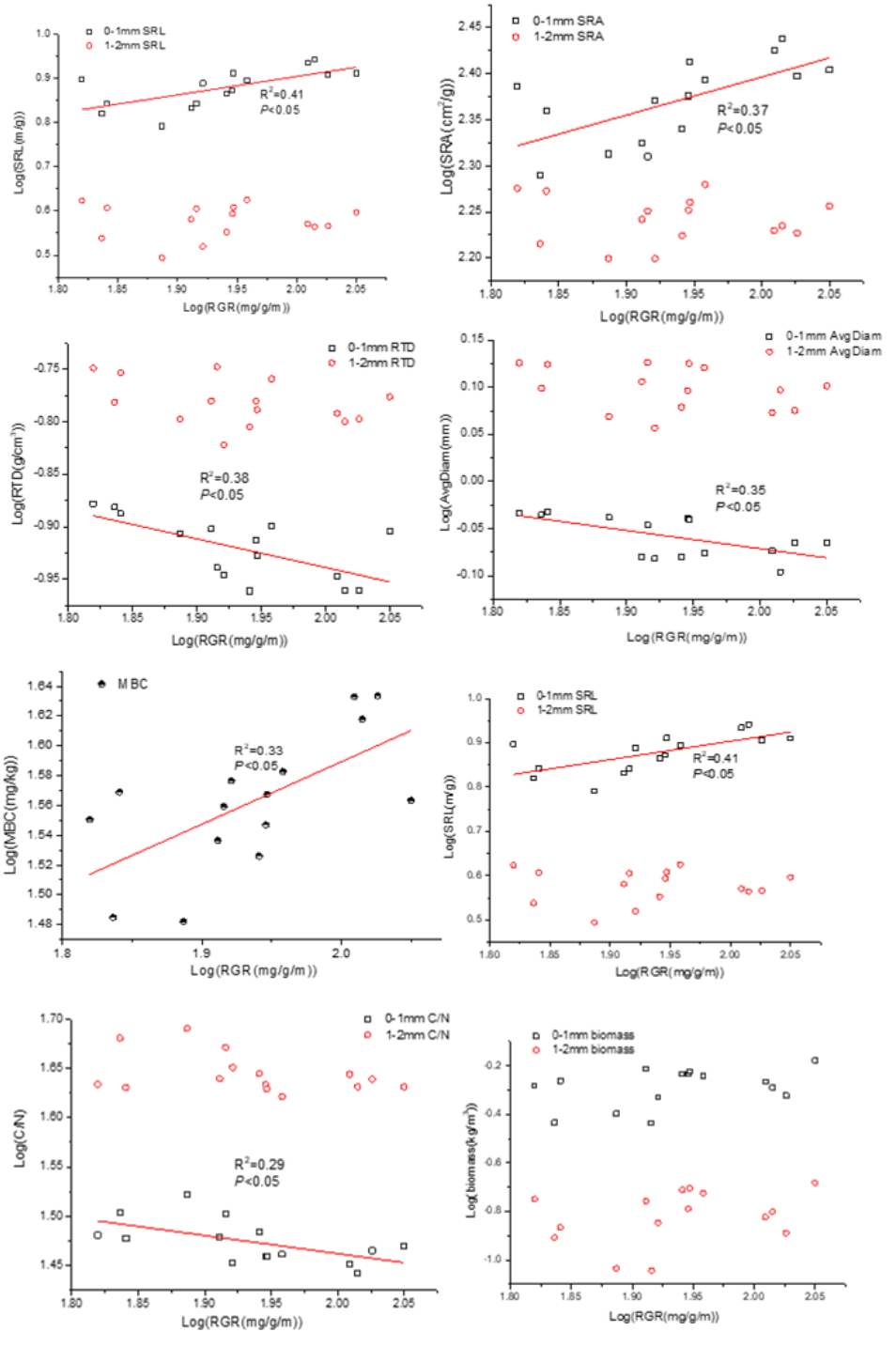

Fig. 3: The correlation between fine root traits and the relative growth rate of Machilus pauhoi.

The interaction between month and soil layer, and between month and treatment have significant effects on MBC (P<0.05), but not on MBN and MBC: MBN. The interaction between soil layer and treatment and the interaction between soil layer, treatment and month have no significant effects on MBC, MBN and MBC: MBN (P>0.05). (Table 6).

4.4 Relationship between fine roots, soil physical and chemical properties, microorganisms and growth rate of Machilus pauhoi

The fine roots of 0-1 mm, which contained SRL, SRA and TN,were significantly positively correlated with the relative growth rate of the whole plant biomass of Machilus pauhoi (P<0.05). However, the fine roots of 0-1 mm, which contained AvgDiam, RTD and C/N, were negatively correlated with the relative growth rate of the whole plant biomass of Machilus pauhoi (P<0.05). As shown in Fig. 3.

Bacteria and fungi were positively correlated with the relative growth rate of whole plant biomass of Machilus pauhoi (P<0.05), but actinomycetes were not significantly correlated with them (P>0.05). In addition, MBC and MBN are also positively correlated with the relative growth rate of

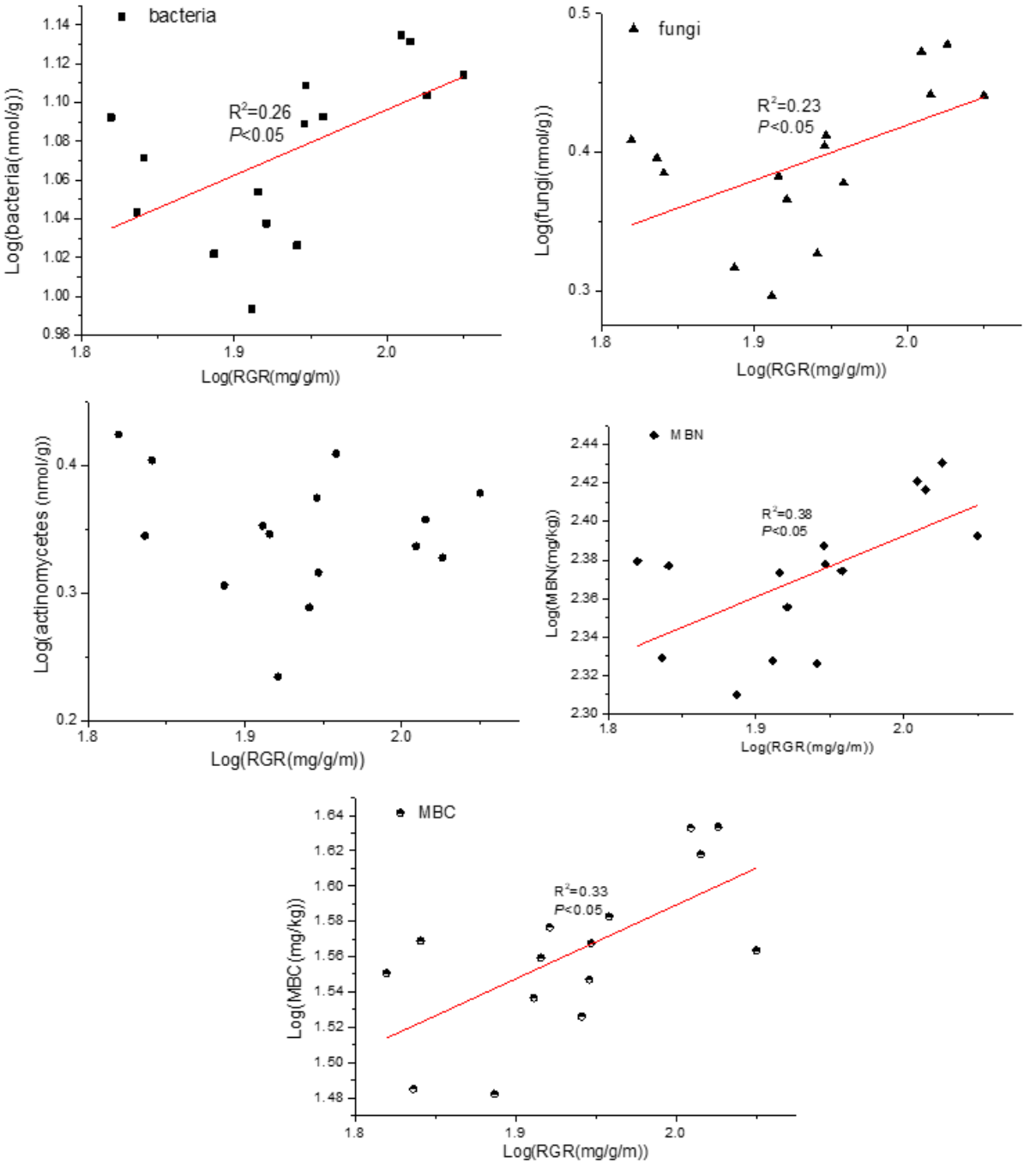

Fig. 4: The correlation between soil microbes and the relative growth rate of Machilus pauhoi.

the whole plant biomass of Machilus pauhoi (P<0.05), as shown in Fig. 4.

4.5 Stepwise regression analysis of fine roots, soil physical and chemical properties, microorganisms and growth rate

The fine root characters studied in this paper were used as independent variables and the relative growth rate of the whole plant biomass of Machilus pauhoi was analyzed by stepwise regression. The results showed that RGR = 1.795*SRL1-1.229*RTD1 (Adjusted R2=0.76, AIC=12.93, P=0.033), where RGR is the relative growth rate of the whole plant biomass of Machilus pauhoi. SRL1 and RTD1 are the specific root length and tissue density of 0-1mm fine roots, respectively, indicating that SRL and RTD of 0-1mm fine roots are the main fine root factors affecting the relative growth rate of Machilus pauhoi biomass, in which SRL of 0-1mm fine roots has a positive effect on it, while RTD has a negative effect on it.

Taking bacteria, fungi, actinomycetes, MBC and MBN as independent variables and the dependent variable relative growth rate of whole plant biomass (RGR), the results showed that RGR=0.669*MBN (Adjusted R2=0.404, AIC=10.82, P=0.006), which indicated that MBN in microbial factors affected the relative biomass of whole plant of Machilus

pauhoi.

The physical and chemical properties of soil also have different effects on the relative growth rate of whole plant biomass of Machilus pauhoi. By stepwise regression analysis of the soil indexes studied in this paper and the relative growth rate (RGR) of whole plant biomass of Machilus pauhoi, it is obtained that: RGR=1.83*DON+0.46*SOM+0.42*TN (Adjusted R2=0.84, AIC=10.82, P=0.012). The results showed that DON, SOM and TN were the main soil factors affecting the relative growth rate of the whole plant biomass of Machilus pauhoi.

Taking the main fine root traits, soil physical and chemical properties and soil microbial factors studied in this paper as independent variables and the dependent variable relative growth rate (RGR) of whole plant biomass, we can get the following results: RGR=1.191*MBN+0.596*DON+0.762*TP (Adjusted R2=0.804, AIC=18.02, P=0.01). The results showed that MBN, DON and TP in soil were the most closely related environmental factors to the relative growth rate of Machilus pauhoi's whole plant biomass. At the same time, it also showed that the influence of fine roots and microorganisms on the relative growth rat of Machilus pauhoi's whole plant biomass was weaker than that of soil physical and chemical properties, and fine roots and soil microorganisms ultimately affected the growth of Machilus pauhoi by affecting soil physical and chemical properties to some extent.

5. Discussion

There are many minerals needed for plant growth in soil, but many of these minerals are difficult to dissolve and need to be transformed by microorganisms before they can be directly absorbed and utilized by plants. Their enzymatic weathering of minerals and hydrolysis of organic matter liberate immobilized nutrients (N, P, Ca^{2+}), which are then translocated to host plants via hyphal networks in exchange for photosynthates.[28,29] Most of the bacteria of soil fungi are branched and filamentous, and soil fungi are also the main decomposers in forest soil, which plays an important role in the development and formation of forest soil.[30,31] Ectomycorrhizal fungi, endophytic fungi, arbuscular mycorrhizal fungi and saprophytic fungi are all important components of the internal community structure of fungi, which are closely related to the growth and development of plants. Studies have found that fungi have a stronger ability to release phosphorus than other flora.[32] Blum *et al.* found that ectomycorrhiza can release Ca^{2+} through weathered apatite, providing plants with needed calcium.[33] Hodge showed that the infection of arbuscular mycorrhizal fungi was helpful to improve the nitrogen absorption capacity of plants.[34] Hui Liu *et al.* found that endophytic fungi can significantly increase the total biomass of Leymus chinensis.[35] These studies show that fungal communities can improve the utilization rate of nitrogen, phosphorus and other nutrients, and have positive feedback on plant growth.[36] In this study, there is a significant positive correlation between fungi and the relative growth rate of the whole plant biomass of Machilus pauhoi, which is not only related to the above functions of fungi, but also related to some characteristics of fungi. Fungal hyphae are relatively developed, which can swim and adhere to the surface of soil animal and plant residues or extend to areas lacking nutrients,[37] helping to improve the nutrient absorption efficiency of plants. Bacteria also play an important role in the growth and development of plants. Potassium-solubilizing bacteria and phosphorus-solubilizing bacteria can release potassium and phosphorus from minerals for plant growth and development,[32] and phosphorus-solubilizing bacteria can also secrete organic acids such as acetic acid and citric acid during their activities, thus alleviating the decrease of local soil pH.[28] It is found that the phosphorus solubilization of phosphate-solubilizing bacteria is one of the important factors to promote plant growth in soil with low fertility.[32] In this study, bacteria were significantly positively correlated with the relative growth rate of the whole plant biomass of Machilus pauhoi, but also with the indicators indicating nutrient absorption efficiency of fine roots such as SRL and SRA of 0-1mm, and soil nutrient factors such as TN, SOM and TP, indicating that the soil patches where bacteria were active had good nutrient conditions, and there was positive feedback with the nutrient absorption efficiency of fine roots, which finally promoted plant growth. In this paper, MBN and MBC are also significantly positively correlated with the relative growth rate of the whole plant biomass of Machilus pauhoi, which is mainly due to the fact that soil microbial biomass such as MBN and MBC is one of the reservoirs and important sources of plant nutrients.[38]

After fertilization, soil nutrients such as TN, TP, SOM and DON were significantly positively correlated with the relative growth rate of the whole plant biomass of Machilus pauhoi, but the negative correlation between soil pH and the relative growth rate was not significant, which indicated that fertilization eased the nutrient limitation of Machilus pauhoi to some extent and contributed to its growth, and appropriate phosphorus addition at the same time alleviated the negative impact of soil PH reduction to some extent.

To sum up, fine roots, soil physical and chemical properties and soil microorganisms have different effects on the growth of Machilus pauhoi from different aspects, which is the result of the coordinated development of fine roots, soil physical and chemical properties and soil microorganisms.[38] Fertilization affects soil physical and chemical properties, while fine roots and soil microorganisms perceive the changes in soil physical and chemical properties and adjust their growth methods and survival strategies. At the same time, fine roots and microorganisms will also cause a series of changes in soil physical and chemical properties during their growth activities. While the current study design was optimized for capturing empirical relationships, we fully agree that targeted mechanistic experiments are needed to validate these theoretical links. Such work is planned as a follow-up to this

observational study.

Declarations

Ethics approval and consent to participate

This study is unrelated to ethical approval.

Consent for publication

Written consent to publish this information was obtained from study participants.

Availability of data and materials

The data supporting the findings of this study are available within the article. Additional datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Conflicts of Interest

The authors declare no conflicts of interest.

Supporting Information

Not applicable.

CRediT Statement

Danhong Yin: Writing—original draft preparation, **Quanlin Zhong:** Conceptualization and methodology.

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