

Original Research

Effects of Raising Chickens under *Pinus massoniana* Forest on Soil Physicochemical Properties and Microbial Community

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Abstract

Soil physical and chemical properties are important indicators to measure soil quality, and soil microorganisms can sensitively reflect changes in the soil environment. The impact of raising chickens under the forest on the soil environment under the forest can be fed back through the changes in both. However, previous studies on the effects of chicken breeding in Masson pine forests on soil physical and chemical properties and the diversity of soil microbial communities are rare. Thus, the design of this study was centered on the chicken coop, within 50 m, and every 10 m is a sample point. A five-point sampling method was used to study the impact of chicken flock activities on soil physical and chemical properties and microbial communities at different distances. The results showed that soil pH and nutrient content presented a decreasing trend with increasing distance, with significant differences among groups. Soil bulk density, water content and organic carbon content had opposite trends. The diversity of the soil microbial community showed a decreasing trend with increasing distance, in which bacterial diversity was higher than fungal diversity; *Proteobacteria*, *Acidobacteria* and *Actinobacteria* were the dominant bacteria; and *Arthropoda* and *Ascomycota* were the dominant phyla. This study shows that raising chickens under the forest has improved the forest environment, and a certain frequency of chicken activity or breeding density may be the key factor affecting the forest environment. When applying and promoting raising chickens under the forest, we should pay attention to the appropriate breeding density.

Keywords: aising chickens, pine forest, soil properties, soil microbial

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Introduction

Soil nutrients are the necessary material basis for plants to survive and are important indicators to measure soil fertility standards. In the whole process of plant growth, soil provides nutrients such as nitrogen, phosphorus and potassium for plant growth. In turn, plants provide the soil environment with such things as surface litter and plant and animal residues back to the soil, which become an important supplement to soil nutrients through the decomposition of microorganisms in the soil. Soil microorganisms play an important role in maintaining the stability of soil ecosystems, such as material decomposition and nutrient cycling, and have a significant impact on plant health and growth, soil quality and function maintenance [1].

Soil microorganisms are very sensitive to changes in soil nutrients and pH values and can respond to changes in soil quality and soil ecosystems in a timely manner [2]. Therefore, they are often used to predict changes in soil nutrients and environmental quality [3]. Many previous studies have shown that soil microbial community diversity and structure are susceptible to soil physicochemical properties, soil management practices, and other factors [4-5]. Since most bacteria exhibit relatively narrow growth tolerance, their diversity levels are directly influenced by soil pH [6]. The pH value affects soil fertility, structure and plant growth [2]. Thus, it indirectly alters the soil bacterial community structure, and it is the most important soil factor affecting the microbial community structure [7]. The change in the fungal community may be a response to changes in environmental factors, such as pH and amino acids. The addition of poultry manure increases the content of soil C, which can improve soil aggregates, thus improving the energy source and habitat of fungi and increasing the diversity and abundance of fungi [8]. Therefore, the study of soil microbial community characteristics is of great significance for the study of soil ecological environment change and soil environment protection.

Masson pine (*Pinus massoniana*) is a gymnosperm of Pinaceae with developed roots that are typically *ectomycorrhizal* [9]. It is one of the main pioneer trees and important native species for reforestation in southern China because of its excellent characteristics of rapid growth, drought tolerance, adaptability, versatility and economic value. Masson pine can grow to 45 m, and its fast-growing final cutting period is generally 20 years. Because of its tall trees and long growth cycle, to achieve maximum economic benefits, some regions in Guizhou have carried out chicken raising activities under Masson pine forests. In this mode, on the one hand, chickens can freely move under the forest and eat insects and weeds under the forest; on the other hand, along with the activities of chickens, they also excrete chicken manure, which provides a certain nutrient base for plant growth. Chicken manure can be used as an organic fertilizer, which has rich

and complete nutrients, rich biological macromolecules such as proteins and amino acids, and nutrient elements such as calcium, phosphorus and potassium. Studies have shown that chicken manure can be used as a potential source of nutrition for plant growth. The application of poultry manure can improve soil nutrients, improve the forestland soil environment, promote plant growth, and increase crop yield [10-12]. Fertilization changes the physical and chemical properties of soil through the input of nutrients, thus affecting soil microorganisms [13]. Organic fertilizer can improve soil structure and fertility, and the addition of organic fertilizer can help to increase the abundance and diversity of the soil microbial community [14-15].

However, some studies have shown that excessive feces can significantly reduce microbial diversity [16]. In recent years, some studies have shown that under the model of raising chickens under the forest, the density of chickens is an important factor affecting the environment under the forest. Because of the excessive density of chickens, soil hardening and a reduction in biodiversity under the forest have occurred [17]. However, some studies have shown that raising chickens in different forests will have different impacts on the environment under the forest, but generally speaking, it will increase the content of soil nutrients, improve the soil environment and increase the number of soil microorganisms [18-20]. However, in these reports, there are few reports about the impact of raising chickens under the masson pine forest on the environment under the forest. In addition, previous research on the soil microbial community under the environment of raising chickens under the forest is not deep enough, and the understanding of the composition structure and species of the soil microbial community is still shallow. Therefore, the impact of raising chickens under forests on the soil environment and soil microbial community needs further study, especially on the diversity and composition of the microbial community. In recent years, with the update of research methods, high-throughput sequencing technology has become a hot spot in soil microbial research because of its high throughput, high precision and ability to analyze a large number of samples at the same time [21]. Therefore, this study selected Masson pine chicken breeding forestland. By taking the soil at different locations from the chicken coop, it is assumed that the soil nutrients are relatively high and the soil microorganisms are relatively rich in the nearby places where the chicken flocks are relatively frequent. Then, the physical and chemical properties of soil were determined, and the diversity and community structure of soil bacteria and fungi were analyzed through high-throughput sequencing technology to verify this hypothesis. The aim of this study was to investigate the effects of chicken raising activities on soil physicochemical properties and the changes in soil microbial communities in the understory of Masson pine forest, with a view to providing some theoretical references for the better development of the business

model of chicken raising in the understory and the protection of the ecological environment.

The purpose of this study is to explore the impact of chicken raising activities on the physical and chemical properties of soil and the changes in the soil microbial community under a pine forest to provide theoretical reference for better development of the management mode of chicken raising under the forest and the protection of the ecological environment.

Materials and Methods

Experimental Sites

The experimental site was Tianzhu County, Guizhou Province (108.70°E, 26.50°N). Tianzhu County is located in the subtropical climate zone, with an annual average temperature of 14~22°C, abundant rainfall and a frost-free period of 281 days. It has a typical subtropical monsoon warm and humid climate. The test forestland was a 40-year-old Masson pine forest with a density of 400 birds/hm² and two years of raising chickens. In the process of breeding, there is no restriction on the activities of chicken flocks, and chicken manure is excreted by the natural activities of chicken flocks, with no interference or cleaning. The soil type was yellow soil.

Experimental Design

Taking the chicken coop as the center, within the range of 50 m, and in combination with the range of chicken activity frequency, each 10 m range is a sample point, which is numbered 10 m (M10), 20 m (M20), 30 m (M30), 40 m (M40) and 50 m (M50). Then, the five-point sampling method was adopted at the corresponding distance to dig the soil from 0~20 cm in the understory root zone. Finally, the rocks, dead leaves and fine roots in the soil sample were removed, mixed evenly, stored in seal and brought back to the laboratory. One part was placed in a refrigerator at 4°C to measure soil microbial diversity, and the other part was ground and screened through a 2 mm sieve after natural air drying to measure soil physical and chemical indexes.

Physical and Chemical Parameters of Soil Samples

The soil pH was measured with a ZLJC/YQ-025 pH meter; soil bulk density (SBD) was measured by the ring knife method; soil water content (SWC) was determined by drying at 105°C [22]; soil organic carbon (SOC) was oxidized by potassium dichromate and heated externally; total nitrogen (TN) was determined by the Kjeldahl method; total phosphorus (TP) was determined by sodium oxide melting molybdenum antimony anti colorimetry; total potassium (TK) was determined

by the sodium oxide melting flame photometer method [23]; alkali hydrolyzed nitrogen (AN) was determined by the alkali hydrolysis diffusion method; available potassium (AK) was determined by the ammonium acetate extraction flame photometric method [24]; and available phosphorus (AP) in soil was determined by the hydrochloric acid ammonium chloride extraction method [25]. Each sample had 3 replicates, and the data are the mean±standard deviation.

Sequencing Analysis of Soil Microorganisms

Soil samples were extracted for microbial DNA. The American Fast DNA[®] Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) kit was used, and 0.5 g of soil was used for total DNA extraction according to the instructions. NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA, USA) was used to evaluate the DNA concentration and purity, and then 1% agarose gel electrophoresis was used to detect the extracted genomic DNA [26]. The V3-V4 region of the 16S rRNA gene of bacteria was amplified with primers 338F (5'-ACTCCTACGGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and the V4 region was selected by fungi [27]. After the total DNA of the sample was extracted, the primers were designed according to the conserved region. A sequencing connector was added at the end of the primer, PCR amplification was performed, and the products were purified, quantified and homogenized to form a sequencing library. Library quality inspection was carried out for the constructed library first, and the qualified library was sequenced with Illumina HiSeq 2500. The original image data file obtained by high-throughput sequencing was converted into the original sequenced reads by base calling analysis. The results were stored in FASTQ (fq for short) file format, which contains the sequence information of the sequenced reads and the corresponding sequencing quality information [28].

Data Processing

The data statistics were completed in Excel 2019, and the IBM SPSS Statistics 26 statistical software used the least significant difference method (LSD) to judge the significant difference level ($p < 0.05$).

After the high-throughput sequencing data were downloaded from the computer and the original data were obtained, Trimmomatic (version 0.33) was first used to filter the quality of the original data [29]. Second, Cutadapt (version 1.9.1) was used to identify and remove primer sequences [30], and FLASH (version 1.2.11) was used to splice double-ended reads and remove chimeras UCHIME (version 8.1) [31-32]. Finally, Usearch software was used to cluster and divide OTUs of reads at a 97.0% similarity level [33].

Taking SILVA and Unite as reference databases [34-35], we used a naive Bayesian classifier to perform

taxonomic annotation on feature sequences, obtained the species classification information corresponding to each feature, and then performed statistics on the composition of each sample community at each level. QIIME software was used to generate species abundance tables at different classification levels, and then R language tools were used to draw the community structure diagram of samples at each taxonomic level. QIIME2 software was used to evaluate the alpha diversity index of the samples. Mothur software and R language tools were used to draw a Shannon diversity index dilution curve according to the Shannon index (the index reflecting microbial diversity in the sample) of each sample's sequencing quantity at different sequencing depths to reflect the microbial diversity of each sample at different sequencing quantities.

QIIME software was used to conduct beta diversity analysis to compare the similarity of species diversity among samples. Based on the four distance matrices (binary, bray, weighted, unweighted) obtained from the feature table and beta diversity analysis, species clustering was carried out, and a heatmap of the samples was drawn by R language. The differences between samples can be seen intuitively according to the changes in the color gradient.

Results and Discussion

Effect of Raising Chickens under Forests on Soil Physical and Chemical Properties

The physical and chemical properties of soils are important indicators of soil quality and an important embodiment of the impact of chicken raising activities

under forests on the soil environment. The results showed that the soil pH showed a decreasing trend with increasing distance from the chicken coop, and there was a significant difference among the groups ($p < 0.05$), among which the highest pH was at 30 m, and the pH was 4.63 (Table 1). However, it is still in a weakly acidic ($4 < \text{pH} < 6$) environment as a whole, which is the most suitable acidic condition for the growth of Masson pine [36]. TN, TK and available nutrients also showed a decreasing trend with increasing distance. Moreover, the average content of TN was 4.61 g/kg, TK was 2.12 g/kg, AN was 123.91 mg/kg, AP was 76.96 mg/kg and AK was 204.59 mg/kg at 10 m~30 m, and there was a significant difference between the content at 10 m~30 m and that at 40 m~50 m ($p < 0.05$). The content of TP was the highest at 20 m, reaching 3.26 g/kg, and then decreased again. However, the content near the chicken coop was also higher than that at 40 m and 50 m. This may be due to the rich alkaline nutrients in fresh chicken manure, which is weakly alkaline. Moreover, the nitrogen, phosphorus and potassium contents in chicken manure are relatively high, 1.63%, 1.54% and 0.85%, respectively [37-39]. The closer to the chicken coop, the more frequently the chickens move, and the more chicken excrement is discharged, which causes an increase in soil pH. The nutrients in chicken manure accumulate in the soil through degradation, leaching and other processes. The application of chicken manure will improve the total soil nutrients and soil fertility [40]. Therefore, in places close to the chicken coop and with high activity frequency of chicken flocks, there will be more chicken manure, and the soil nutrient content will be relatively high. Nitrogen is one of the essential elements for life to synthesize protein and maintain life activities. Soil nitrogen is an important

Table 1. Analysis of physical and chemical properties of soil.

Samples	pH	SBD (g/cm ³)	SWC (%)	SOC (g/kg)	TN (g/kg)
M10	4.74±0.01a	1.18±0.06c	44.88±1.31b	41.26±0.48c	4.61±0.15a
M20	4.63±0.01b	0.60±0.06e	37.12±1.69c	35.00±0.53e	4.54±0.03a
M30	4.54±0.03c	1.07±0.05d	34.14±1.36d	37.35±0.55d	4.06±0.04b
M40	4.43±0.07d	1.32±0.04b	51.31±0.06a	43.09±0.22b	3.48±0.03c
M50	4.37±0.01d	1.44±0.02a	52.32±0.38a	45.77±0.10a	2.94±0.05d
Samples	TP (g/kg)	TK (g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)
M10	2.75±0.08b	2.12±0.05a	123.91±1.48a	76.96±0.79a	204.59±0.60a
M20	3.26±0.00a	1.93±0.01b	118.33±1.43b	74.02±0.66b	191.98±1.16b
M30	2.27±0.15c	1.73±0.04c	106.72±0.53c	72.39±0.32c	177.35±1.26c
M40	2.47±0.05c	1.59±0.08d	95.66±1.19d	69.73±0.30d	166.30±1.76d
M50	1.48±0.23d	1.54±0.01d	84.10±1.22e	69.24±0.14d	150.65±4.53e

Note: SWC, soil water content; SBD, soil bulk density; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; and AK, available potassium. The results are given as the mean±SD (standard deviation). Different lowercase letters within a column indicate significant differences at the $P = 0.05$ level among the different treatments, with Duncan's test.

element that affects plant growth and yield and is also an essential element required by plants [24]. Uric acid in chicken manure is the main source of nitrogen, which can be converted into ammonium nitrogen in a very short time, which is one of the reasons why the soil is rich in nitrogen. Soil available phosphorus is the main indicator used to judge the abundance and deficiency of soil phosphorus, and the growth of Masson pine is often limited by the lack of phosphorus. When chickens are raised in forests, the content of soil available phosphorus is high, which can provide sufficient nutrition for the growth of Masson pine. Soil potassium is usually transformed into different forms, and the dynamic balance between total potassium and available potassium is maintained to jointly maintain the growth of plants.

The SBD, SWC and SOC had the opposite trend, all of which were lowest at 20 m and then showed a tendency to increase again and were lower at 10 m~30 m than at 40 m~50 m. SBD is an important index to measure soil compactness [41]. Generally, the smaller the SBD is, the looser the soil quality is, which is conducive to the development of plant roots and the interaction between water and nutrients in the soil. In this study, the SBD and SWC were the lowest at 20 m, and the SBD was small, but the SWC was low. Because chickens like to hunt in groups, the diversity of undergrowth herbs near the chicken coop is greatly reduced due to long-term trampling and gnawing. Lacking the water holding function of herbaceous plants, although the SBD is reduced, the SWC is also greatly reduced compared with that far away from the chicken coop. Similarly, SOC is considered by most people to be the most important indicator to measure soil fertility and is an important source of nitrogen, phosphorus and potassium in soil. In this study, although the content of nitrogen, phosphorus and potassium near the chicken coop was higher than that in the distance, the content of organic carbon was lower than that in the

distance, which may be related to the source of SOC in the forestland. On the one hand, the frequency of chicken activities near the chicken coop will increase the emissions of nitrogen, phosphorus and potassium, thus supplementing the organic carbon. However, due to the influence of the activity characteristics of the chickens themselves, the activity frequency of the chickens is relatively low at 40 m~50 m, and the litter in the distance can be accumulated, so the SOC content is relatively high. The application of organic fertilizer, such as animal manure, can increase the nutrient content and SOC content in the soil and can improve the soil pH [42-43]. This is consistent with the results of this study. Soil nutrients and pH value change with the distance from the chicken coop, which is the result of the influence of chicken manure organic fertilizer on soil physical and chemical properties.

Diversity of Soil Microbial Community in Underforest Chicken Breeding

The dilution curve was constructed by sequencing depth and species number, OTU sequences were randomly selected from the measured sequences, and the species number was counted. The results showed (Fig. 1) that when the number of bacterial OTUs reached approximately 1000 and the number of fungal OTUs reached approximately 400, the dilution curve tended to be stable, indicating that the sequencing amount was sufficient to cover the microbial groups in the soil at this time.

OTU classification of all sequences of samples at 5 distances can be carried out, and the abundance of fungi and bacteria at each level can be calculated with reference to the Silva and UNITE databases. The Venn diagram can be used to compare the number of common and unique OTUs in different test samples. The results showed that there were 1190 OTUs of soil bacteria. There were 839 OTUs in 5 samples, accounting for

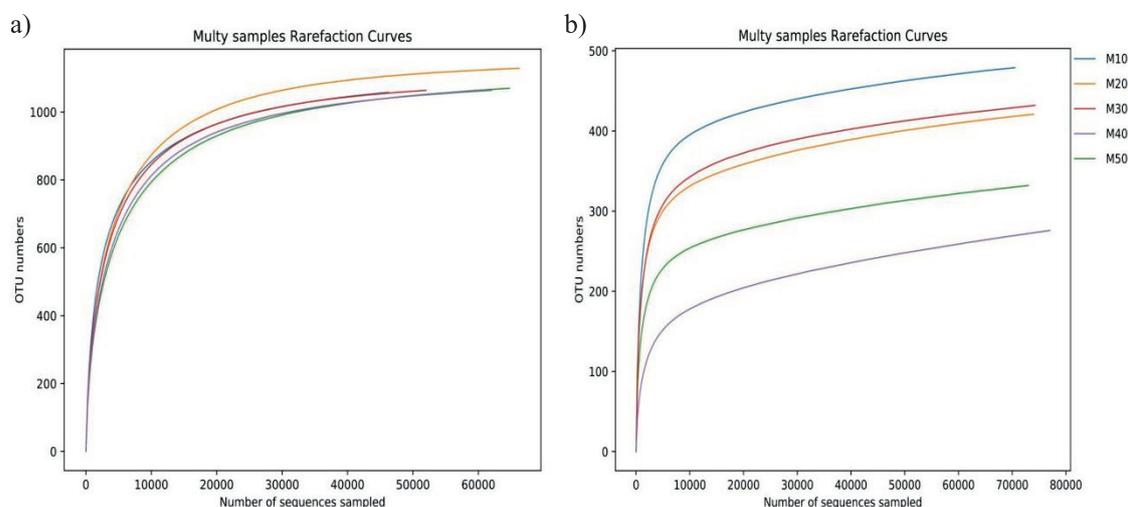


Fig. 1. Rarefaction curves of the soil bacteria a) and soil fungi b).

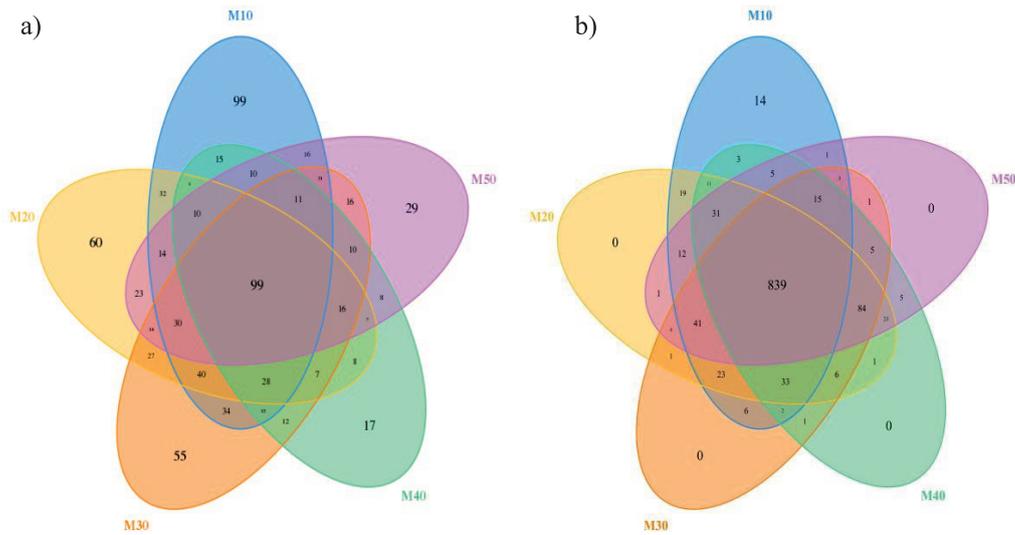


Fig. 2. Venns of the soil bacteria a) and soil fungi b).

70.5% of the total bacterial OTUs. Except for 14 unique OTUs at 10 m, accounting for 7% of the total bacterial OTUs, the other groups had no unique OTUs (Fig. 2a). Fungal results showed that soil fungi had 786 OTUs, less than bacteria. The number of OTUs shared by the 5 samples was 99, which was approximately 12.6% of the total number of fungi. Different from bacteria, each sampled fungus had its own OTUs, including 99 OTUs at 10 m, 60 OTUs at 20 m, 55 OTUs at 30 m, 17 OTUs at 40 m and 29 OTUs at 50 m. The unique OTUs

of bacteria and fungi were the most abundant at 10 m, and the unique OTUs of fungi decreased with distance (Fig. 2b).

The notes of soil microorganisms at different levels are shown in Fig. 3. that there are 340 species of bacteria belonging to 23 phyla, 52 classes, 122 orders, 205 families, and 321 genera. There are 138 species of fungi in 137 genera, 135 families, 121 orders, 65 classes, and 25 phyla. Among them, the soil microbial community at 10 m was the most abundant, and the

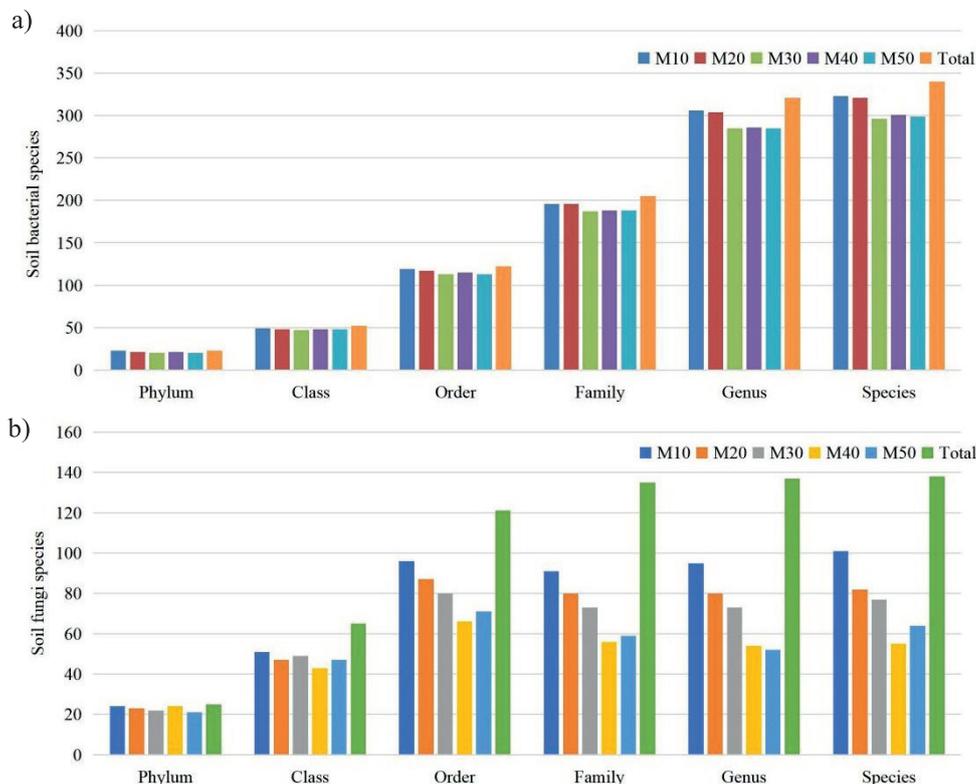


Fig. 3. Statistics of the soil bacterial a) and soil fungal b) species at different levels.

bacterial community annotated 323 species of 306 genera, 196 families, 119 orders, 49 classes, and 23 phyla. There were 101 species in the fungal community in 95 genera, 91 families, 96 orders, 51 classes, and 24 families.

Alpha diversity can reflect the species abundance and diversity of a single sample, mainly including the Chao1 index, ACE index, Simpson index, Shannon index, etc. Among them, the Chao1 and Ace indexes measure the species abundance, that is, the number of species. Shannon and Simpson indexes are used to measure species diversity, which is affected by species abundance and species evenness in the sample community. In the case of the same species abundance, the greater the evenness of each species in the community, the greater the diversity of the community. The greater the Shannon index and Simpson index, the higher the species diversity of the sample. As shown in Table 2, at the same distance α , the diversity index was higher than that of fungi. The diversity index showed that the abundance and diversity of the bacterial community were higher than those of fungi in the chicken breeding environment under the forest, and the soil microorganisms were dominated by the bacterial community. At different distances, the bacterial Chao1 index and ACE index had little difference, but the Shannon and Simpson indexes were slightly greater than those at 40 m and 50 m within 30 m. Fungi were different, and the alpha diversity index was greater than that at 40 m and 50 m within 30 m. From the above analysis, the abundance and diversity of bacteria and fungi at different distances decreased with increasing distance, indicating that the soil microorganisms were positively affected at places with relatively high soil nutrients. This may be because chicken manure in forestland has a certain impact on the soil microbial community, and the impact on fungi is greater than that on bacteria. Bacteria and fungi have different response strategies and tolerance to changes in environmental

factors. Compared with bacteria, fungi can live in soil with higher organic carbon and total nitrogen contents due to their advantages in living strategies [44]. The application of organic fertilizer improves soil nutrients, thus improving the diversity of soil microorganisms, which is consistent with the results of this study [45].

Characteristics and Differences in the Soil Microbial Community under Pine Forest

The influence of raising chickens under the pine forest on the bacterial community structure can be seen from the phyla level species abundance that the relative abundance of soil bacteria is more than 1%, including *Acobacteria*, *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, *Verrucomicrobia*, *Chloroflexi*, *WPS-2*, *Bacteroides*, *Gemmatimonadetes* and *Firmicutes* (Fig. 4a). In general, *Proteobacteria*, *Acidobacteria* and *Actinobacteria* are the dominant bacteria in the chicken breeding soil under the Masson pine forest, which is similar to the bacteria found in other organic fertilizer soil [46-47]. Moreover, at 10 m, *Proteobacteria* 41.7%, *Acidobacteria* 22.4% and *Actinobacteria* 11.4% were higher than the relative abundance at other distances and showed a downward trend with increasing distance. *Proteus* had the highest abundance among soil bacteria, which may be related to the high content of soil organic carbon and total nitrogen. *Proteus* belongs to symbiotic microorganisms (r-strategic type) and is more adaptable to environmental conditions with high organic carbon content [48]. Acid bacilli are related to soil pH and are common and dominant in acid red yellow tropical soil [49]. Actinomycetes are suitable for living in an environment with sufficient carbon sources and play an important role in the geochemical cycle of organic carbon [48]. Similarly, at 10 m, the relative abundances of *Bacteroidetes* and *Firmicutes* were 4.9% and 9.7%, respectively, which were higher than those at other

Table 2. Alpha diversity index statistics.

Samples	Microbial	Feature	ACE	Chao1	Simpson	Shannon
M10	Bacteria	1,058	1,094.93	1,091.74	0.99	8.38
	Fungi	479	531.06	528.04	0.97	6.74
M20	Bacteria	1,129	1,145.93	1,156.01	0.99	8.09
	Fungi	421	484.88	474	0.97	6.59
M30	Bacteria	1,064	1,089.98	1,095.04	0.99	8.13
	Fungi	432	517.65	514.88	0.96	6.4
M40	Bacteria	1,064	1,091.87	1,104.08	0.98	7.83
	Fungi	376	480.16	418.00	0.62	3.11
M50	Bacteria	1,070	1,099.62	1,112.04	0.98	7.79
	Fungi	332	467.54	427.40	0.96	5.82

Note: Feature is the number of features (OTUs); Chao1, Ace, Shannon, Simpson represent each index.

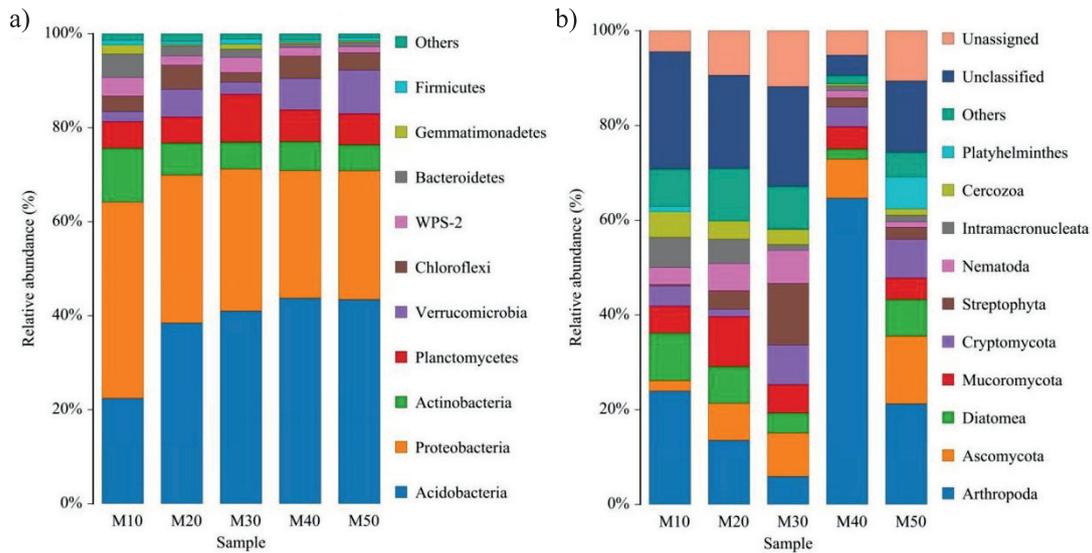


Fig. 4. Characteristics of the soil bacterial community a) and soil fungal community b) structure at the phylum level.

distances, and the farther the distance was, the smaller the relative abundances were. Gandolfi et al. found in a poultry manure composting experiment that during the composting process, the composition of bacteria changed from *Alphaproteobacteria* and *Gammaproteobacteria* to *Bacteroidetes* and *Firmicutes* [50]. This may be due to the bacteria carried by the chicken manure itself, which also shows that the activities of chickens at 10 m have the greatest impact on the soil microbial community. It can be seen from the order level species abundance that the bacteria with a relative abundance of more than 1% in the soil are *Subgroup_2*, *Solibacterales*, *Acidobacteriales*, *Rhizobiales*, *Elsterales*, *Chthoniobacterales*, *Gemmatales*, *Betaproteobacteriales*, *Isosphaerales*, and *Gammaproteobacteria_Incertae_Sedis* (Fig. 5a). In general, *Subgroup_2*, *Solidicacterales*, *Acidobacteriales*, *Rhizobiales* and *Beta proteobacteriales* were the dominant orders of bacteria in chicken soil

under Masson pine forests. The relative abundances of *Subgroup_2*, *Solicharacters* and *Betaproteobacteriales* at 10 m were 6.5%, 5.4% and 4.6%, respectively, which were higher than those at other distances and showed a downward trend with increasing distance. In contrast, the relative abundance of *Acidobacteriales* was the lowest at 8.7% at 10 m and increased with increasing distance. From the results of bacterial community composition at different levels, it can be seen that raising chickens under forests has a great impact on the composition of the soil microbial community. This further verified that the diversity of soil microorganisms was affected by different distances, namely, different frequencies of chicken flock activities.

The influence of raising chickens under the forest on the fungal community structure is shown by the phyla level species abundance (Fig. 4b). The relative abundance of soil fungi was greater than 1% for *Arthropoda*,

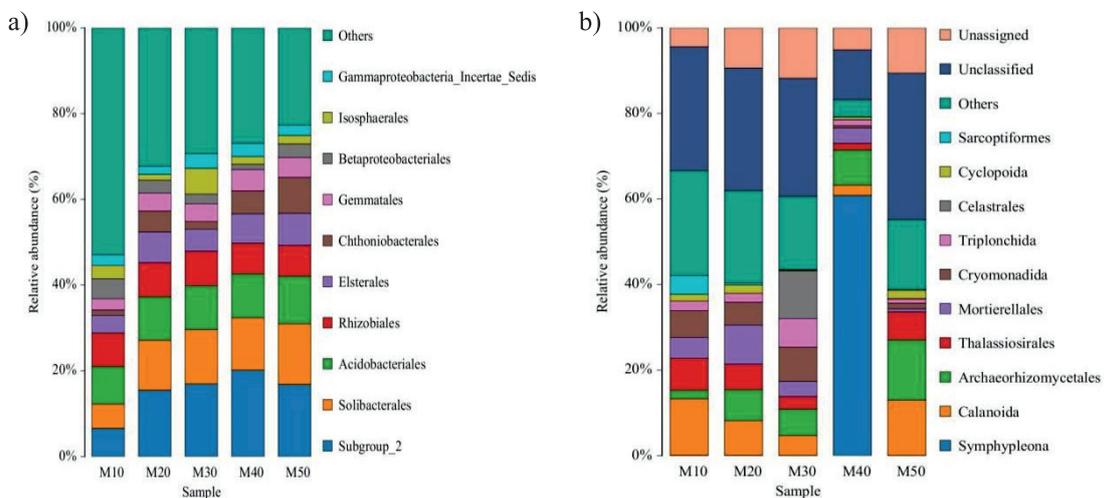


Fig. 5. Characteristics of the soil bacterial community a) and soil fungal community b) structure at the order level.

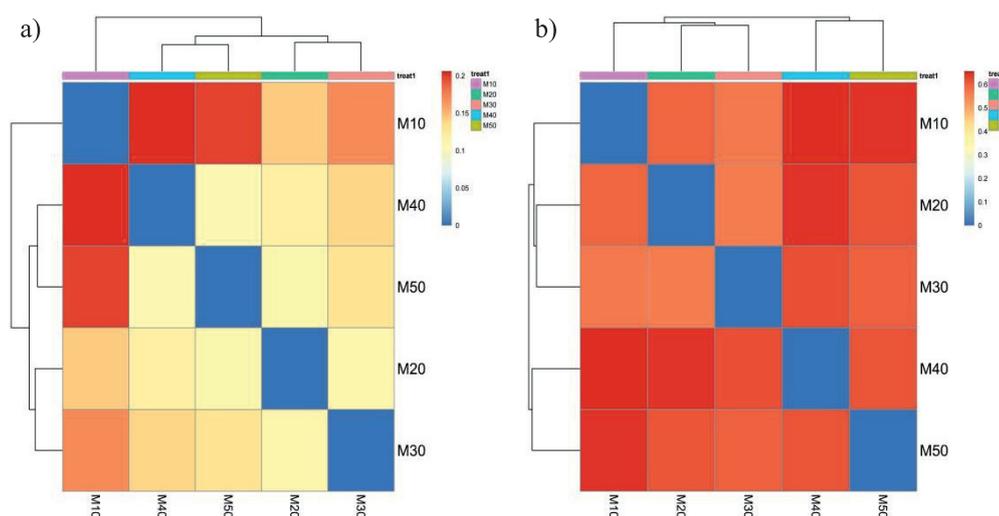


Fig. 6. Soil bacterial a) and soil fungal b) clustering heatmap.

Note: Color gradient from blue to red indicates the distance between samples from near to far

Ascomycota, *Diatomea*, *Mucoromycota*, *Cryptomycota*, *Streptophyta*, *Nematoda*, *Intramacronucleata*, *Cercozoa*, and *Platyhelminthes*. Among them, *Arthropoda* and *Ascomycota* were the dominant phyla. *Nematoda*, *Intramacronucleata* and *Cercozoa* decrease with increasing distance. However, *Streptophyta* showed the opposite trend, being highest at 50 m, and its relative abundance reached 26%. *Ascomycota*, on the other hand, shows fluctuation. With increasing distance, it decreases first and then increases. The relative abundance at 50 m was 14.2% but lower than that at 10 m (21.9%). At the order level, the relative abundance of species was more than 1%, including *Symphyteona*, *Calanoida*, *Archaeorhizomycota*, *Thalassiosirales*, *Mortierella*, *Cryomonadida*, *Triplonchida*, *Celastrales*, *Cyclopoida*, and *Sarcopositives* (Fig. 5b). In general, *Calanoida*, *Archaeorhizomycota*, *Thalassiosirales* and *Mortierella* are the dominant fungi in chicken breeding soil under *Pinus massoniana* forests. Except for *Archaeorhizomycota*, the relative abundance of other dominant fungi decreased with increasing distance. Soil fungi play an important role in regulating soil ecosystems. Some fungi can form mutually beneficial relationships with the plant rhizosphere, which is called mycorrhizal symbiosis. The study shows that *Ascomycetes* play a leading role in the decomposition of organic carbon. In this study, *Ascomycetes* were the dominant bacteria in the soil and were most abundant at 10 m, indicating that *Ascomycetes* play a key role in the decomposition of chicken manure [51].

Beta diversity analysis can compare the similarity of different samples in terms of species diversity. Among them, a heatmap can compare the differences and similarities in species composition among different groups through color gradient changes. The clustering results show that soil bacteria (Fig. 6a) and soil fungi (Fig. 6b) have the same rule, that is, soil microbial communities at different distances are grouped into

three categories, including M10, M20 and M30, and M40 and M50. The clustering results showed that the composition of soil microbial communities had differences and similarities at different distances. The composition of the soil microbial community at 10 m was different from that at other distances, but there was similarity at 20 m and 30 m and similarity at 40 m and 50 m. This shows that raising chickens under the forest has a greater impact on the composition of the soil microbial community at different distances from the chicken coop, which may be related to the activity frequency of chickens at different distances. The results of cluster analysis further verified the impact of chicken-raising activities under forests on the soil microbial community; the diversity of soil microbial communities was higher where chicken activities were more frequent.

Conclusion

In this study, the effects of raising chickens under forests on soil physical and chemical properties and soil microbial community diversity were explored through the experimental measurement of soil physical and chemical properties and high-throughput sequencing of soil microorganisms under forests with different distance gradients near the chicken coop.

Within 10 m of the chicken coop, the activities of chicken flocks are more frequent, while at greater distances, the activities of chicken flocks are less frequent. The results show that in the range of 20 m~30 m from the chicken coop, where the frequency of chicken flocks is relatively moderate, the diversity and richness of soil nutrients and soil microbial communities are relatively high, which is basically consistent with the hypothesis. Raising chickens under forests can improve soil pH and soil nutrient

content, improve soil structure, activate soil microbial activity, and increase the diversity of the soil microbial community. This study shows that under the appropriate density, raising chickens under the forest is not only conducive to economic development but also conducive to the growth of trees. Combined with the local forestry environment, it is worth further promotion and application to reasonably carry out cultivation activities under the forest.

This study is a field survey on the pine forestland where local farmers are raising chickens. Sampling on the spot takes into account the impact of raising chickens under the forest on the ecological environment under the forest in real life, as well as the protection of the environment under the forest and biodiversity. However, the influence of other factors, such as chicken age, season, weather, and temperature, on the forest environment has not been considered. The results under this natural condition are highly random and accidental. However, under natural conditions where no one intervenes, it may be the best model for raising chickens under forests, which can better reflect the impact of most of the models on the soil environment. This study can provide a theoretical reference for revealing the effects of raising chickens under forests on soil fertility and the soil microbial community.

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Conflict of Interest

The authors declare no conflict of interest.

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