Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Effects of Different Fermented Fertilizer Formulations on Soil Nutrient Status and Microbial Communities in Morchella sextelata Cultivation

Yongmei Miao

mym416@163.com

Anhui Science and Technology University

Wangyang Wu

Anhui Science and Technology University

Qiong Wu

Anhui Science and Technology University

Tao Han

Anhui Science and Technology University

Huaqi He

Anhui Science and Technology University

Research Article

Keywords: Morchella sextelata, fermented fertilizer, soil physicochemical properties, soil microbial community, high-throughput sequencing

Posted Date: September 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10285617/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Fermented fertilizers are widely used in morel cultivation, yet their effects on soil nutrient status and bacterial-fungal community responses remain unclear. This study compared one unfertilized control (CK) and six representative fermented fertilizer treatments (Ms1–Ms6) in *Morchella sextelata* cultivation. Cultivation performance was evaluated using fruiting time, yield-related traits, and nutritional quality, while soil physicochemical properties, bacterial and fungal diversity, community composition, and their relationships with environmental factors were analyzed. Comprehensive evaluation ranked the treatments as Ms1 > Ms2 > Ms4 > Ms3 > Ms5 > Ms6 > CK, with Ms1 and Ms2 showing better overall performance. Compared with CK, fermented fertilizer treatments generally increased soil pH, total potassium (TK), and available potassium (AK). Ms2 showed the highest organic carbon (OC), total nitrogen (TN), pH, and available nitrogen (AN), indicating improved carbon and nitrogen supply under this formulation. Bacterial and fungal alpha diversity varied among treatments, but higher cultivation performance was not consistently associated with higher microbial diversity. PCoA and ANOSIM showed clear differences in bacterial and fungal community structures between CK and fertilized soils. Dominant bacterial phyla included Pseudomonadota, Bacillota, Bacteroidota, Actinomycetota, Acidobacteriota, and Chloroflexota, whereas dominant fungal phyla included Ascomycota, Mortierellomycota, and Basidiomycota. Spearman correlation and RDA indicated that pH, OC, TN, TK, AK, and available phosphorus (AP) were closely associated with microbial community variation. These findings suggest that suitable fermented fertilizer formulations may improve morel cultivation performance by creating favorable nutrient conditions and shaping bacterial–fungal community structures.

1. Introduction

Morels (*Morchella* spp.) belong to the phylum Ascomycota and are among the most highly valued rare edible mushrooms worldwide. Their fruiting bodies have a distinctive flavor and are rich in proteins, polysaccharides, amino acids, mineral elements, and various bioactive compounds, giving them high nutritional value, medicinal potential, and market value [1, 2]. In recent years, with advances in artificial domestication and facility-based cultivation technologies, black morels represented by *Morchella sextelata* and *M. importuna* have gradually been cultivated on a large scale, driving the rapid development of China's morel industry [3, 4]. However, artificial morel cultivation remains highly unstable. Problems such as uneven fruiting, large yield fluctuations, marked differences in quality, and continuous cropping obstacles are still common in production, and these have become key factors limiting the sustainable development of the morel industry [5, 6, 7].

The growth and development of *M. sextelata* are closely related to the soil environment. Unlike most edible mushrooms, field cultivation of morels relies on soil as an important ecological interface. Mycelial growth, nutrient bag decomposition, primordium formation, and fruiting body development are all affected by factors such as soil pH, organic carbon, nitrogen forms, phosphorus and potassium supply, mineral elements, and moisture conditions [8, 9, 10]. Previous studies have shown that morel cultivation can alter the levels of available phosphorus, available nitrogen, potassium, pH, and various mineral elements in soil,

and may change the soil nutrient balance through nutrient uptake, mycelial translocation, and microbial metabolism [11, 12]. In addition, mineral elements such as Fe, Zn, Mn, and Cu are not only involved in morel mycelial growth and spore formation, but may also affect the structure and function of soil microbial communities [13]. Therefore, analyzing the response mechanisms of morel cultivation from the perspective of soil nutrient changes is an important way to understand the basis for achieving high and stable yields.

In addition to soil physicochemical properties, the soil microbial community is also an important ecological factor affecting morel growth. Soil bacteria and fungi are involved in processes such as organic matter decomposition, nitrogen, phosphorus, and potassium transformation, mineral element activation, and pathogen suppression, making them key components in maintaining soil fertility and ecological stability [14]. Previous studies have shown that morel yield is closely associated with soil fungal diversity, microbial community evenness, and specific functional microbial groups. Certain bacterial taxa, such as *Pseudomonas*, Pedobacter, Stenotrophomonas, and Flavobacterium, may be involved in nutrient bag degradation and fruiting body development [15, 16]. Meanwhile, continuous cultivation or improper management can alter soil fungal communities, lead to the accumulation of harmful microbes, and disrupt the soil microecological balance, thereby triggering continuous cropping obstacles or disease outbreaks [17, 18]. Therefore, clarifying how different cultivation practices affect soil microbial diversity and community structure is of great importance for achieving high-yield and high-quality morel cultivation.

Exogenous nutrient bags and organic fertilizer application are key steps in morel cultivation. Different carbon sources, nitrogen sources, C/N ratios, and fertilizer application rates can change soil carbon and nitrogen supply, mineral nutrient release, and microbial niches, thereby affecting morel mycelial growth, fruiting body formation, and quality accumulation [19]. The available carbon and nitrogen released during the decomposition of organic materials can provide a nutritional basis for morels and their associated microorganisms. However, an excessively high or low C/N ratio, unsuitable fertilizer type, or unreasonable application rate may lead to asynchronous nutrient release, stronger microbial competition, or pathogen enrichment, ultimately affecting cultivation performance [20]. At present, studies on soil microorganisms in morel cultivation have mainly focused on different morel species, different cultivation years, or continuous cropping obstacles. In contrast, research on the relationships among “soil physicochemical properties–microbial community structure–overall cultivation performance” under different exogenous fertilizer treatments remains relatively limited.

Based on this, and building on a previous comprehensive evaluation of the effects of different exogenous fertilizer treatments on the agronomic traits and nutritional quality of morels, this study selected fertilizer treatments with different performance levels, together with CK, and analyzed soil physicochemical properties, bacterial and fungal community diversity, community composition, and their relationships with environmental factors during the peak fruiting stage. The aims of this study were to: (1) determine the effects of different fertilizer treatments on soil physicochemical properties, including pH, carbon, nitrogen, phosphorus, and potassium, in morel cultivation; (2) reveal the changes in bacterial and fungal community

diversity and composition under different fertilizer treatments; and (3) analyze the relationships between soil physicochemical factors and microbial communities, and clarify the possible mechanisms by which exogenous fertilizers regulate the soil microecology of morel cultivation. The results may provide a reference for optimizing fertilizer formulations, regulating soil microecology, and developing high-yield and high-quality cultivation systems for morels.

2. Materials and Methods

2.1 Experimental Site and Design

The cultivation experiment of *M. sextelata* was conducted from December 2024 to April 2025 in a plastic greenhouse at the Edible Fungi Base of Anhui Science and Technology University. A total of seven treatments were set up, including one control treatment without fermented fertilizer application (CK) and six representative fermented fertilizer treatments selected based on the results of a previous comprehensive evaluation (Ms1–Ms6). Each treatment included three biological replicates, giving a total of 21 samples. The detailed treatment settings are shown in Table 1.

Table 1
Representative treatments selected based on the previous comprehensive evaluation

Treatment	Overall performance level	Fertilizer type	C/N	Application rate ($kg \cdot m^{-2}$)
CK	None	None	None	None
Ms1	Better-performing	Corncob + cow manure	35:1	1.5
Ms2	Better-performing	Corncob + cow manure	35:1	1.8
Ms3	Moderate-performing	Sawdust + rabbit manure	35:1	1.5
Ms4	Moderate-performing	Sawdust + rabbit manure	35:1	1.8
Ms5	Poor-performing	Sawdust + rabbit manure	45:1	1.5
Ms6	Poor-performing	Sawdust + rabbit manure	45:1	1.8

Note: CK refers to the control treatment without fermented fertilizer application, while Ms1–Ms6 refer to representative fermented fertilizer treatments selected based on the results of the previous comprehensive evaluation. Basic information such as fertilizer type, C/N ratio, and application rate was retained for each treatment. However, in this study, these treatments were mainly compared as independent representative treatments. The focus was on evaluating their effects on soil nutrients and microbial communities in *Morchella sextelata* cultivation, rather than separately analyzing the single-factor effects of C/N ratio or fertilizer application rate.

2.2 Cultivation Conditions

M. sextelata was cultivated in a plastic greenhouse. During the cultivation period, shading, temperature control, and moisture management were applied. Soil temperature was maintained at 15–18°C, and the relative air humidity was kept at 80%–90% to provide suitable conditions for mycelial growth and fruiting body formation. Except for the different fermented fertilizer treatments, all treatments were managed in the same way in terms of sowing, soil covering, irrigation, ventilation, and fruiting management.

2.3 Soil Sample Collection and Processing

Soil samples were collected from the mushroom beds around the fruiting bodies after harvest during the peak fruiting stage of *M. sextelata*. Each treatment included three biological replicates, and soil samples for each replicate were collected using a five-point sampling method. During sampling, the top 2–3 cm of soil was first removed, and then topsoil samples were collected using a soil auger. The soil collected from the five sampling points within each replicate was thoroughly mixed to form one composite sample.

The collected soil samples were immediately transported back to the laboratory. Stones, visible plant residues, animal remains, and other impurities were removed, and each sample was divided into two parts. One portion of fresh soil was used for soil microbial community analysis. It was thoroughly mixed, placed into sterile centrifuge tubes, and stored at − 80°C. The other portion was air-dried naturally, ground, and sieved for the determination of soil physicochemical properties. The air-dried soil samples were mainly used to measure organic carbon, total nitrogen, total phosphorus, total potassium, pH, alkali-hydrolyzable nitrogen, available phosphorus, and available potassium.

2.4 Determination of Soil Physicochemical Properties

Soil physicochemical properties were measured by Chengdu Baihui Biotechnology Co., Ltd. Soil organic carbon (OC) was determined using the potassium dichromate oxidation with external heating method. Total nitrogen (TN) was measured using the sulfuric acid–catalyst digestion method. Total phosphorus (TP) was determined using the molybdenum–antimony colorimetric method. Total potassium (TK) was measured by flame photometry. Soil pH was determined using the electrode method. Alkali-hydrolyzable nitrogen (AN) was measured using the alkali diffusion–Kjeldahl method. Available phosphorus (AP) was determined using the molybdenum–antimony colorimetric method, and available potassium (AK) was measured by flame photometry.

2.5 Soil Microbial Community Analysis

Soil microbial community analysis was performed by Shanghai Majorbio Bio-pharm Technology Co., Ltd. After total soil microbial DNA was extracted, high-throughput sequencing was carried out on the Illumina MiSeq PE300 platform. The raw paired-end reads were quality-filtered using fastp (v0.23.4), and paired-end reads were merged using FLASH (v1.2.11). The quality-controlled valid sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using USEARCH (v11), and chimeric sequences were removed.

For the bacterial community, taxonomic annotation was performed using the RDP Classifier (v2.11) against the Silva 138.2 database. For the fungal community, taxonomic annotation was performed using

the RDP Classifier (v2.11) against the Unite 9.0 database. The confidence threshold for classification was set at 70% in both cases. Before analysis, chloroplast and mitochondrial sequences were removed, and the sequence number of each sample was rarefied to reduce the effect of differences in sequencing depth on downstream analyses.

Alpha diversity indices, including the Chao, Shannon, and Simpson indices, were calculated using Mothur (v1.30.2). The relative abundances of dominant bacterial and fungal taxa were summarized at the phylum and genus levels, and community composition bar plots were generated. Principal coordinate analysis (PCoA) was performed based on Bray–Curtis distances, and ANOSIM was used to test differences in microbial community structure among treatments. Spearman correlation analysis was used to examine the relationships between soil physicochemical properties and major microbial taxa. Redundancy analysis (RDA) was used to evaluate the effects of soil nutrient factors on microbial community distribution. All related microbial data analyses and figure generation were completed on the Majorbio Cloud Platform.

2.6 Data Processing and Statistical Analysis

The experimental data were organized using Excel. Soil physicochemical properties, microbial alpha diversity indices, and the relative abundances of dominant taxa were expressed as mean values. Differences among treatments were tested using one-way analysis of variance, with the significance level set at $P < 0.05$. PCoA analysis was performed based on Bray–Curtis distances, and ANOSIM was used to test differences in bacterial and fungal community structures among treatments. Spearman correlation analysis was used to examine the relationships between soil physicochemical factors and microbial taxa, while RDA was used to evaluate the effects of major soil environmental factors on bacterial and fungal community structures. Microbial community analysis and visualization were mainly completed on the Majorbio Cloud Platform.

3. Results and Analysis

3.1 Effects of Different Fertilizer Treatments on Soil Physicochemical Properties

As shown in Table 2, different fertilizer treatments significantly changed the physicochemical properties of morel cultivation soil. Compared with CK, all fertilizer treatments generally increased soil pH, TK, and AK levels, indicating that the application of exogenous fertilizers clearly improved the soil nutrient environment. Among the treatments, Ms2 showed the most notable performance in terms of OC, TN, pH, and AN. Its OC and TN contents were significantly higher than those of the other treatments, suggesting that the combination of corncob + cow manure, a C/N ratio of 35:1, and an application rate of 1.8 $kg \cdot m^{-2}$ may be more favorable for soil carbon and nitrogen accumulation and nutrient supply. Ms4 and Ms6 had relatively high AK contents, indicating that high application rates of sawdust + rabbit manure may help improve soil potassium supply. In contrast, Ms5 showed relatively low levels of OC, TN, AN, and AK, which may be related to its poorer overall performance in the previous evaluation. In addition, AP levels in all fertilizer treatments were lower than those in CK, suggesting that morel growth or microbial activity may

have promoted the uptake, utilization, or transformation of AP. Overall, different fertilizer treatments created distinct soil nutrient environments by regulating soil carbon, nitrogen, phosphorus, potassium, and pH levels, which may provide an important basis for subsequent changes in bacterial and fungal community structures.

Table 2
Physicochemical properties of morel cultivation soil under different fertilizer treatments

Treatment	OC (g/kg)	TN (g/kg)	TP (g/kg)	TK (g/kg)	pH	AN (g/kg)	AP (mg/kg)	AK (mg/kg)
CK	11.145 ± 0.560e	1.338 ± 0.105d	1.205 ± 0.115d	13.961 ± 0.095d	5.583 ± 0.448d	0.149 ± 0.013bc	295.669 ± 73.800a	150.806 ± 46.224f
Ms1	14.529 ± 0.061c	1.558 ± 0.060bc	1.256 ± 0.057cd	14.213 ± 0.008c	7.397 ± 0.045ab	0.165 ± 0.003b	116.385 ± 0.004b	206.946 ± 0.047e
Ms2	23.604 ± 0.019a	2.105 ± 0.013a	1.416 ± 0.007b	14.507 ± 0.006a	7.647 ± 0.025a	0.197 ± 0.001a	126.910 ± 0.104b	311.546 ± 0.010bc
Ms3	15.097 ± 0.015c	1.619 ± 0.006b	1.580 ± 0.008a	14.563 ± 0.006a	6.680 ± 0.010c	0.161 ± 0.002b	172.256 ± 0.006b	266.358 ± 0.030cd
Ms4	14.709 ± 0.008c	1.606 ± 0.010b	1.388 ± 0.006bc	14.577 ± 0.006a	7.077 ± 0.029bc	0.185 ± 0.003a	176.791 ± 0.023b	425.076 ± 0.086a
Ms5	13.360 ± 0.062d	1.469 ± 0.006c	1.254 ± 0.003cd	14.319 ± 0.023b	6.737 ± 0.047c	0.144 ± 0.003c	130.166 ± 0.026b	248.738 ± 0.020de
Ms6	16.648 ± 0.009b	1.623 ± 0.012b	1.512 ± 0.007ab	14.532 ± 0.005a	6.963 ± 0.021bc	0.154 ± 0.007bc	173.921 ± 0.034b	360.046 ± 0.063b

3.2 Effects of Different Fertilizer Treatments on Alpha Diversity of Soil Bacterial and Fungal Communities

As shown in Table 3, different fertilizer treatments affected the alpha diversity of bacterial and fungal communities in morel cultivation soil to varying degrees. In the bacterial community, the Chao index was highest in Ms5 and lowest in Ms2, indicating that different fertilizer treatments had a certain effect on bacterial community richness. The Shannon index was highest in CK, followed by Ms5, while Ms2, Ms4, and Ms6 showed relatively lower values, suggesting that some fertilizer treatments reduced bacterial community diversity. The Simpson index showed a pattern generally consistent with the Shannon index. CK and Ms5 had lower Simpson index values, whereas Ms1, Ms2, and Ms6 had higher values, indicating that bacterial community diversity was relatively higher in CK and Ms5, while some fertilizer treatments may have promoted the enrichment of specific dominant bacterial taxa. The fungal community showed a

more pronounced response to fertilizer treatments. CK had relatively high Chao and Shannon index values, indicating higher fungal richness and diversity. In contrast, Ms2 and Ms6 had lower Chao index values, and Ms6 had the lowest Shannon index and the highest Simpson index, suggesting that the Ms6 treatment significantly reduced fungal community richness and diversity. Overall, different fertilizer treatments altered the alpha diversity of soil bacterial and fungal communities. However, the better-performing treatments did not necessarily show the highest microbial diversity, suggesting that differences in the overall performance of morels may be more closely related to the enrichment of specific microbial taxa and changes in community structure, rather than simply depending on the level of alpha diversity.

Table 3
Alpha diversity indices of soil bacterial and fungal communities in morel cultivation under different fertilizer treatments

Treatment	Bacteria			Fungi		
	Chao	Shannon	Simpson	Chao	Shannon	Simpson
CK	3386.32 ± 29.53 ab	6.52 ± 0.11 a	0.0057 ± 0.0012 c	376.04 ± 26.48 a	3.77 ± 0.26 a	0.0691 ± 0.0342 e
Ms1	3350.33 ± 240.34 ab	5.98 ± 0.32 bc	0.0193 ± 0.0057 a	346.50 ± 48.20 ab	2.74 ± 0.33 bc	0.1397 ± 0.0416 cde
Ms2	2608.86 ± 232.81 c	5.75 ± 0.19 c	0.0168 ± 0.0042 a	262.88 ± 13.88 c	2.31 ± 0.20 bc	0.1987 ± 0.0475 abc
Ms3	3293.38 ± 185.98 ab	6.24 ± 0.12 ab	0.0090 ± 0.0018 bc	367.26 ± 25.65 a	2.68 ± 0.22 bc	0.1643 ± 0.0392 bcd
Ms4	2978.65 ± 42.39 bc	5.84 ± 0.10 c	0.0130 ± 0.0018 ab	340.32 ± 27.39 ab	2.84 ± 0.10 a	0.1123 ± 0.0071 de
Ms5	3475.48 ± 350.39 a	6.37 ± 0.36 ab	0.0076 ± 0.0044 bc	373.41 ± 72.85 a	2.48 ± 0.35 bc	0.2447 ± 0.0477 ab
Ms6	3011.86 ± 245.18 b	5.82 ± 0.14 c	0.0177 ± 0.0014 a	281.86 ± 21.27 bc	2.00 ± 0.16 c	0.2783 ± 0.0727 a

3.3 Effects of Different Fertilizer Treatments on Beta Diversity of Soil Bacterial and Fungal Communities

PCoA based on Bray–Curtis distances at the genus level showed clear separation of soil bacterial community structures among the different fertilizer treatments (Fig. 1A). PC1 and PC2 explained 49.04% and 11.45% of the variation, respectively, with a cumulative explanation rate of 60.49%. ANOSIM further showed that the differences in bacterial community structure among treatments were highly significant (R = 0.88713, P = 0.001), indicating that fertilizer treatments significantly reshaped the bacterial community composition in morel cultivation soil. The CK samples were located on the negative side of PC1 and were clearly separated from all fertilized treatments, suggesting that exogenous fertilizer input was an important factor affecting bacterial community structure. The better-performing treatments, Ms1

and Ms2, were also somewhat separated from the other treatments, suggesting that they may have formed relatively distinct bacterial community structures. At the same time, the moderate treatments (Ms3/Ms4) were relatively close to the poorer-performing treatments (Ms5/Ms6), indicating that bacterial community structure could not be completely distinguished according to overall performance level alone. The microecological differences among treatments may also have been jointly influenced by fertilizer type, C/N ratio, application rate, and fungal communities.

PCoA based on Bray–Curtis distances at the OTU level showed a clear separation of soil fungal community structures among the different fertilizer treatments (Fig. 1B). PC1 and PC2 explained 36.09% and 24.01% of the variation, respectively, with a cumulative explanation rate of 60.10%. ANOSIM results showed that the differences in fungal community structure among treatments were also highly significant (R = 0.86546, P = 0.001), indicating that fertilizer treatments had a significant effect on the fungal community composition in morel cultivation soil. The CK samples were clearly separated from all fertilized treatments, further showing that exogenous fertilizer input was an important factor shaping soil fungal community structure. The better-performing treatments, Ms1 and Ms2, were located close to each other in the ordination plot but were separated from the other treatments, suggesting that the corncob + cow manure treatment with a C/N ratio of 35:1 may have formed a relatively characteristic fungal community structure. Meanwhile, Ms3 was relatively close to Ms5, and Ms4 was relatively close to Ms6, suggesting that fungal community structure may also have been jointly affected by fertilizer type, C/N ratio, and application rate.

3.4 Effects of Different Fertilizer Treatments on the Bacterial Community Composition in Morel Cultivation Soil

Figure 2A shows the distribution of soil bacteria at the phylum level under different treatments. Overall, the six most abundant phyla were Pseudomonadota, Bacillota, Bacteroidota, Actinomycetota, Acidobacteriota, and Chloroflexota, accounting for 23.61%–46.80%, 12.58%–18.96%, 5.36%–14.79%, 5.66%–13.56%, 1.85%–18.31%, and 2.57%–13.04% of the total abundance, respectively. These phyla were the dominant bacterial groups, together accounting for 81.79%–92.47% of the total abundance. The relative abundance of bacterial communities differed among the seven treatments. Pseudomonadota had the highest relative abundance in Ms6 (47.03%) and the lowest in CK (23.61%). The secondary dominant phyla also varied among treatments. Acidobacteriota was the secondary dominant phylum in CK, with a relative abundance of 18.31%. Bacillota was the secondary dominant phylum in Ms1, Ms2, Ms3, Ms4, and Ms5, with relative abundances of 13.85%, 18.66%, 17.97%, 18.96%, and 13.85%, respectively. In Ms6, Bacteroidota was the secondary dominant phylum, with a relative abundance of 14.79%.

Compared with the phylum level, the genus-level results showed greater specificity. As shown in Fig. 2B, the dominant genus in CK was *Neobacillus*, with a relative abundance of 3.85%. The dominant genus in Ms3 and Ms5 was *Pseudarthrobacter*, with relative abundances of 5.91% and 4.39%, respectively. In the other four soil samples, the dominant genus was *Pseudomonas*, with relative abundances of 8.60% in Ms1, 8.35% in Ms2, 7.60% in Ms4, and 10.41% in Ms6. The secondary dominant genera also differed

among samples. In CK, Ms1, Ms2, Ms4, and Ms6, the secondary dominant genus was *Pseudarthrobacter*, with relative abundances of 0.90%, 8.24%, 7.98%, 4.98%, and 5.44%, respectively. In Ms3 and Ms5, the secondary dominant genus was *Pseudomonas*, with relative abundances of 4.60% and 4.17%, respectively. Overall, the relative abundance of secondary dominant genera varied only slightly among samples and showed a relatively even distribution, with no absolute secondary dominant genus observed.

Note

A, bacterial community composition at the phylum level; B, bacterial community composition at the genus level. The bar charts show the relative abundances of dominant bacterial taxa under different treatments.

3.5 Changes in Soil Fungal Community Composition Under Different Fertilizer Treatments

As shown in Fig. 3A, at the phylum level, the fungal communities in morel cultivation soil under different fertilizer treatments were mainly composed of Ascomycota, Mortierellomycota, and Basidiomycota. These phyla accounted for 62.38%–89.28%, 2.88%–33.73%, and 0.16%–16.24% of the total abundance, respectively, and their combined relative abundance was higher than 85% in all samples. The relative abundance of microbial communities differed among soil samples, indicating differences in microbial community structure across treatments. Among them, Ascomycota had the highest relative abundance in all treatments, accounting for 64.40%, 62.38%, 73.84%, 88.36%, 65.09%, 89.28%, and 79.83%, respectively, and was the primary dominant phylum across all treatments. In Ms5, the secondary dominant phylum was Basidiomycota, with a relative abundance of 5.07%. In the other treatments, the secondary dominant phylum was Mortierellomycota, with relative abundances of 17.88%, 33.73%, 23.40%, 5.99%, 16.75%, and 10.54%, respectively.

At the genus level (Fig. 3B), the fungal communities showed greater specificity among soil samples. In CK, *Alternaria* had the highest relative abundance, at 12.69%. In Ms1 and Ms4, the dominant genus was *Mortierella*, with relative abundances of 29.98% and 16.03%, respectively. In Ms2, the dominant genus was *Schizothecium*, with a relative abundance of 41.02%. In Ms3, Ms5, and Ms6, the dominant genus was *Sordaria*, with relative abundances of 36.42%, 46.92%, and 43.47%, respectively. The secondary dominant genera also differed among treatments. In CK and Ms2, the secondary dominant genus was *Mortierella*, with relative abundances of 12.35% and 20.07%, respectively. In Ms1, Ms3, and Ms6, the secondary dominant genus was *Schizothecium*, with relative abundances of 20.05%, 5.98%, and 19.62%, respectively. In Ms4, the secondary dominant genus was *Coprinellus*, with a relative abundance of 13.66%. In Ms5, the secondary dominant genus was *Oedocephalum*, with a relative abundance of 11.27%. Overall, the dominant genera differed markedly among treatments and showed clear absolute dominance, whereas the differences in secondary dominant genera were relatively small, with no absolute secondary dominant genus observed.

Note

A, fungal community composition at the phylum level; B, fungal community composition at the genus level. The bar charts show the relative abundances of dominant fungal taxa under different treatments.

3.6 Relationships Between Soil Physicochemical Properties and Bacterial and Fungal Communities

3.6.1 Correlation Analysis

The Spearman correlation heatmap (Fig. 5A) showed clear correlations between the bacterial community at the phylum level and soil physicochemical properties in morel cultivation soil. Among these factors, pH, OC, TN, TK, and AK were important environmental factors affecting bacterial community composition at the phylum level. Overall, dominant bacterial phyla such as Pseudomonadota, Bacillota, Bacteroidota, and Actinomycetota were mostly positively correlated with nutrient indicators such as soil OC, TN, TK, and AK. This suggests that, after fertilizer treatments increased soil carbon, nitrogen, and potassium supply, they may have promoted the enrichment of bacterial groups associated with organic matter decomposition, nutrient transformation, and soil fertility improvement. In contrast, Acidobacteriota, Chloroflexota, Verrucomicrobiota, Patescibacteria, and Nitrospirota were mostly negatively correlated with pH or most nutrient factors, suggesting that these groups may be better adapted to relatively low-nutrient, acidic, or specific niche environments. Together with the soil physicochemical results, the fertilizer treatments generally increased soil pH, OC, TN, and AK, while CK had higher soil available phosphorus. Therefore, the correlation pattern of AP with some bacterial phyla may differ from that of other nutrient factors, indicating that available phosphorus may have a selective effect on bacterial communities at the phylum level. Overall, different fertilizer treatments may promote the relative enrichment of copiotrophic bacterial phyla while reducing the relative abundance of some acid-adapted or low-nutrient-adapted bacterial phyla by regulating soil pH, carbon and nitrogen supply, and potassium levels, thereby reshaping the bacterial community structure in morel cultivation soil.

Spearman correlation analysis also showed clear correlations between the fungal community at the phylum level and soil physicochemical properties, with different fungal groups responding differently to environmental factors. Overall, pH, AN, OC, TN, TK, and AK were important factors affecting fungal community composition. *Schizothecium*, *Mortierella*, *Trichocladium*, *Pseudogymnoascus*, unclassified_f__Chaetomiaceae, and Thelebolaceae_gen_Incertae_sedis were mostly positively correlated with pH, AN, OC, and TN, suggesting that these groups may be better adapted to the higher carbon and nitrogen supply created by fertilization and may participate in organic matter decomposition and nutrient transformation. In contrast, *Alternaria*, *Curvularia*, *Plectosphaerella*, *Acremonium*, *Neocosmospora*, *Cladosporium*, and *Rhodotorula* were mostly negatively correlated with nutrient factors such as OC, TN, TK, and AK, suggesting that these fungi may be more adapted to relatively low carbon and nitrogen conditions or specific phosphorus environments. *Sporormiella*, *Coprinellus*, *Sordaria*, and *Lophotrichus* showed certain positive correlations with potassium-related indicators, indicating that potassium supply may influence the distribution of some saprotrophic fungal groups. Overall, different fertilizer treatments may selectively

reshape the fungal community structure in morel cultivation soil by regulating soil pH, carbon and nitrogen supply, and potassium levels.

A

B

Note

The X-axis and Y-axis represent environmental factors and taxa, respectively. The *R* values are shown using different colors. 0.01 < $p \leq 0.05$, *; 0.001 < $p \leq 0.01$, **; $p \leq 0.001$, ***.

3.6.2 RDA Analysis

As shown in Fig. 5A, RDA1 and RDA2 together explained 54.15% of the variation in the bacterial community. The results showed that AP had a significant effect on bacterial community distribution. The AP arrow pointed in the positive direction of RDA1 and was closely associated with the distribution of CK samples. This indicates that the unfertilized soil had a higher available phosphorus content, which led to the enrichment of specific bacterial groups. In contrast, the fertilized treatments, such as Ms2, Ms3, Ms4, and Ms6, were mostly distributed on the negative side of RDA1, suggesting that fertilization significantly altered bacterial community structure by increasing soil nutrients such as OC, TN, and AK. Under fertilized conditions, the bacterial community showed clear changes, mainly driven by the increases in OC and TN.

In the RDA analysis of the fungal community (Fig. 5B), RDA1 and RDA2 together explained 37.84% of the variation. The results showed that soil AP, OC, and TN had relatively strong effects on fungal community structure. Fertilized treatments, such as Ms1, Ms2, and Ms3, were mainly distributed on the negative side of RDA1, whereas the unfertilized CK samples were concentrated on the positive side of RDA1. This suggests that differences in AP, OC, and TN among treatments were closely associated with the distribution of fungal communities.

4. Discussion

4.1 Different Fertilizer Treatments Altered the Soil Nutrient Environment in Morel Cultivation

Morels are typical soil-dependent edible fungi, and their mycelial growth, primordium formation, and fruiting body development are closely related to soil physicochemical properties [21]. Previous studies have shown that exogenous nutrient input during morel cultivation can change soil organic carbon, nitrogen, phosphorus, potassium, and pH levels, which in turn affects microbial community structure and yield performance [22]. In this study, all fertilizer treatments generally increased soil pH, TK, and AK compared with CK, indicating that exogenous organic fertilizers improved the nutrient supply conditions of morel cultivation soil. Among the treatments, Ms2 showed relatively strong performance in OC, TN, pH,

and AN, suggesting that the combination of corncob + cow manure, a C/N ratio of 35:1, and an application rate of 1.8 kg·m⁻² may be more favorable for soil carbon and nitrogen accumulation and nutrient supply. Previous studies on exogenous nutrient bags have also shown that different substrate formulations can significantly affect available nutrients in morel cultivation soil and further influence yield and economic benefits [23]. In this study, Ms4 and Ms6 had relatively high AK contents, indicating that high application rates of sawdust + rabbit manure helped improve soil potassium supply. However, their overall performance was not necessarily better than that of Ms1 and Ms2, suggesting that although potassium is an important factor in morel cultivation, higher potassium levels do not always lead to better results. Huang et al. found that wood ash could improve morel yield and quality by increasing soil pH, improving soil fertility, and regulating microbial communities, but its effect depended on an appropriate application rate [24]. Yue et al. also pointed out that soil potassium can indirectly regulate morel yield by affecting fungal richness [25]. Therefore, fertilizer management in morel cultivation should focus on the coordination among the C/N ratio, potassium supply, and microbial communities, rather than simply pursuing an increase in a single nutrient indicator. In addition, AP was lower in all fertilizer treatments than in CK, which may be related to the uptake, fixation, or transformation of available phosphorus by morel mycelia and soil microorganisms. Feng et al. reported that soil factors such as pH, AK, AP, and TP can significantly affect the fungal community structure in morel cultivation soil [26]. Therefore, the decrease in AP does not necessarily indicate a decline in soil fertility, but may also reflect the rapid utilization or transformation of phosphorus during morel cultivation.

4.2 Fertilizer Treatments Reshaped Soil Microbial Community Structure

The soil microbial community is an important factor affecting stable morel production. Previous studies have shown that exogenous nutrients, soil type, and continuous cropping can significantly change the structure of bacterial and fungal communities during morel cultivation[27, 28]. In this study, different fertilizer treatments affected the alpha diversity of both bacterial and fungal communities. However, the better-performing treatments did not necessarily show the highest microbial diversity. For example, CK and Ms5 had relatively high bacterial Shannon index values, whereas Ms2, Ms4, and Ms6 had lower values. In the fungal community, Ms6 had the lowest Shannon index and the highest Simpson index, indicating that some fertilizer treatments may have promoted the enrichment of specific dominant taxa, thereby reducing community evenness. These results suggest that the quality of morel cultivation soil cannot be evaluated simply by the level of microbial diversity. What matters more is whether a functional community favorable for morel mycelial growth, nutrient transformation, and pathogen suppression has been established. Zhang et al. found that continuous cultivation can lead to an imbalance in the soil fungal community, accompanied by the expansion of saprotrophic fungi and a decline in primordium yield [29]. Liu et al. compared soils with normal fruiting, no fruiting, and mushroom death, and found that normally fruiting soil had higher bacterial and fungal richness and diversity, as well as a more stable microbial co-occurrence network, indicating that microecological stability is important for successful morel cultivation[30]. In this study, although Ms1 and Ms2 did not show the highest alpha diversity, they were clearly separated from the other treatments in the PCoA ordination, suggesting that they may have

formed characteristic microbial community structures more suitable for morel growth. Beta diversity analysis further showed clear separation of bacterial and fungal community structures among different fertilizer treatments, and ANOSIM results reached a highly significant level. This indicates that exogenous fertilizer input was an important driver of microbial community changes in morel cultivation soil. In particular, CK was clearly separated from all fertilizer treatments, suggesting that fertilization not only changed soil nutrient levels, but also shifted the ecological selection of microbial communities.

4.3 Changes in Dominant Microbial Groups Reflected Nutrient Transformation and Ecological Competition

In this study, the bacterial communities were mainly composed of Pseudomonadota, Bacillota, Bacteroidota, Actinomycetota, Acidobacteriota, and Chloroflexota. Among them, the relative abundance of Pseudomonadota generally increased after fertilizer application. At the genus level, *Pseudomonas* was the dominant genus in Ms1, Ms2, Ms4, and Ms6, while *Pseudarthrobacter* was dominant in Ms3 and Ms5. *Pseudomonas* is a common potentially beneficial bacterial group in morel cultivation. Previous studies have suggested that it may promote mycelial growth and primordium formation and may also be involved in pathogen suppression [25]. Li et al. reported that soil nitrate and microbial components involved in denitrification are closely related to morel growth [30]. Yu et al. also found nitrogen-fixing and nitrifying bacterial groups in high-yield morel soils [32]. Therefore, the enrichment of *Pseudomonas*, *Pseudarthrobacter*, and related taxa in Ms1 and Ms2 may be associated with their better overall performance. Although Ms6 had the highest abundance of *Pseudomonas*, it was not the best-performing treatment. This suggests that the high abundance of a single beneficial genus alone cannot fully determine morel cultivation performance. Stable morel production may depend on an overall balance among beneficial microbial enrichment, pathogen suppression, and microbial community stability. Chen et al. found that dazomet treatment reduced the abundance of soil-borne fungal pathogens such as *Paecilomyces*, *Trichoderma*, *Fusarium*, *Penicillium*, and *Acremonium*, while improving morel yield under continuous cropping [33]. This indicates that microecological regulation should focus on both beneficial groups and potential pathogens. In terms of fungal communities, this study found that Ascomycota, Mortierellomycota, and Basidiomycota were the main dominant phyla, with Ascomycota being the primary dominant phylum in all treatments. Previous studies have shown that morel cultivation usually has a stronger effect on soil fungal communities than on bacterial communities, and that changes in fungal communities are closely related to mineral element regulation and yield performance [34]. At the genus level, *Alternaria* was dominant in CK, *Mortierella* was dominant in Ms1 and Ms4, *Schizothecium* was dominant in Ms2, and *Sordaria* was dominant in Ms3, Ms5, and Ms6. *Mortierella* is generally associated with organic matter decomposition and nutrient cycling, and its higher abundance in Ms1 and Ms4 may indicate more active decomposition of organic materials under these treatments. *Schizothecium* and *Sordaria* are mostly saprotrophic or coprophilous fungi and can utilize environments rich in cellulose and animal-manure-derived organic matter. The marked enrichment of *Sordaria* in Ms3, Ms5, and Ms6 suggests that saprotrophic fungal competition may have been stronger under these treatments. If such fungi occupy too much ecological space, they may compete with morel mycelia for nutrients and space, thereby affecting subsequent growth performance.

4.4 Soil Physicochemical Factors Were Important Drivers of Microbial Community Changes

Correlation analysis and RDA results showed that pH, OC, TN, TK, AK, and AP were important environmental factors affecting the structure of bacterial and fungal communities in morel cultivation soil. In the bacterial community, dominant phyla such as Pseudomonadota, Bacillota, Bacteroidota, and Actinomycetota were mostly positively correlated with OC, TN, TK, and AK. This suggests that, after fertilization increased soil carbon, nitrogen, and potassium supply, copiotrophic bacterial groups may have been enriched. In contrast, Acidobacteriota, Chloroflexota, Verrucomicrobiota, and other groups were negatively correlated with most nutrient factors, indicating that they may be better adapted to relatively low-nutrient conditions or specific ecological niches. In the fungal community, *Schizothecium*, *Mortierella*, *Trichocladium*, and *Pseudogymnoascus* were mostly positively correlated with pH, AN, OC, and TN, suggesting that they may be involved in organic matter decomposition and carbon and nitrogen transformation. In contrast, *Alternaria*, *Curvularia*, *Plectosphaerella*, *Acremonium*, and *Neocosmospora* were negatively correlated with most nutrient factors, indicating that different fungal groups responded differently to the nutrient environment created by fertilization. Tan et al. used a semi-synthetic substrate system and found that microbial succession and nutrient accumulation jointly drove the formation of morel fruiting bodies, further showing the close relationship among soil substrates, nutrient supply, and microbial processes [35].

Overall, different fertilizer treatments significantly reshaped soil microbial communities in morel cultivation by regulating soil physicochemical properties. Suitable organic material combinations and C/N levels may help form a more stable soil nutrient environment and microecological structure, whereas excessive or unbalanced nutrient input may lead to the dominance of saprotrophic fungi and stronger ecological competition. Therefore, morel cultivation management should shift from simply “supplying nutrients” to the coordinated optimization of nutrient supply, microbial regulation, and disease risk control, so as to achieve high-yield, stable, and sustainable morel cultivation.

5. Conclusion

This study showed that different exogenous fertilizer treatments significantly changed the physicochemical properties and microbial community structure of morel cultivation soil. Compared with CK, all fertilizer treatments generally increased soil pH, TK, and AK levels. Among them, Ms2 showed relatively strong performance in OC, TN, pH, and AN, indicating that the combination of corncob + cow manure, a C/N ratio of 35:1, and an application rate of 1.8 $kg \cdot m^{-2}$ was more favorable for soil carbon and nitrogen accumulation and nutrient supply.

Different fertilizer treatments altered the alpha diversity of bacterial and fungal communities, but the better-performing treatments did not necessarily have the highest microbial diversity. This suggests that differences in the overall performance of morels may depend more on the enrichment of specific functional groups and the optimization of community structure. PCoA and ANOSIM analyses showed that

fertilization significantly reshaped bacterial and fungal community structures, with CK clearly separated from the fertilized treatments. The bacterial communities were mainly composed of Pseudomonadota, Bacillota, Bacteroidota, Actinomycetota, Acidobacteriota, and Chloroflexota, while the fungal communities were mainly composed of Ascomycota, Mortierellomycota, and Basidiomycota. The dominant genera differed markedly among treatments.

Correlation and RDA analyses showed that pH, OC, TN, TK, AK, and AP were important factors affecting changes in soil microbial communities. In summary, different fertilizer treatments selectively reshaped bacterial and fungal communities in morel cultivation soil by regulating soil carbon, nitrogen, and potassium supply, pH, and available phosphorus levels, forming distinct “fertilizer–soil nutrient–microbial community” interaction patterns. This study provides a theoretical basis for optimizing exogenous nutrition, regulating soil microecology, and optimizing fermented fertilizer formulations and regulating soil microecology in *M. sextelata* cultivation.

1. **PatentsAuthor Contributions**: Conceptualization, Yongmei Miao; methodology, Wangyang Wu and Yongmei Miao; validation, Wangyang Wu, Qiong Wu and Tao Han; formal analysis, Wangyang Wu; investigation, Wangyang Wu, Qiong Wu and Tao Han; resources, Wangyang Wu and Tao Han; data curation, Qiong Wu; writing—original draft preparation, Wangyang Wu; writing—review and editing, Yongmei Miao and Huaqi He; visualization, Wangyang Wu; supervision, Yongmei Miao; project administration, Huaqi He; funding acquisition, Yongmei Miao. All authors have read and agreed to the published version of the manuscript.**Funding**: This research was supported by Anhui Provincial Department of Education Collaborative Innovation Project for Higher Education Institutions (Competitive Cagegory)(GXXT-2023-080)**Data Availability Statement**:

- The raw sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) database under BioProject accession number PRJNA1508730. The corresponding BioSample accession numbers are SAMN62238882–SAMN62238902, and the SRA run accession numbers are SRR40041394–SRR40041435. These data are now publicly available. All other data supporting the findings of this study are included in the article; further inquiries can be directed to the corresponding author.

Declarations

Conflicts of Interest:

The authors declare no conflicts of interest.

Author Contribution

Author Contributions: Conceptualization, Y.M.; methodology, W.W. and Y.M.; validation, W.W., Q.W. and T.H.; formal analysis, W.W.; investigation, W.W., Q.W. and T.H.; resources, W.W. and T.H.; data curation,

Q.W.; writing—original draft preparation, W.W.; writing—review and editing, Y.M. and H.H.; visualization, W.W.; supervision, Y.M.; project administration, H.H.; funding acquisition, Y.M. All authors have read and agreed to the published version of the manuscript.

References

1. Li JL, Cao XL, Chen Y, Xie LY, Liu SS, Tian C, Liu LX, Tang J, Hu RP (2026) Comprehensive Evaluation of Textural Properties and Nutritional Quality in 18 Morchella Germplasm Accessions, Science and Technology of Food Industry 1–21. https://doi.org/10.13386/j.issn1002-0306.2025100260
2. Wu H, Chen J, Li J, Liu Y, Park HJ, Yang L (2021) Recent advances on bioactive ingredients of Morchella esculenta. Appl Biochem Biotechnol 193:1–17. https://doi.org/10.1007/S12010-021-03670-1
3. Du XH, Yang ZL (2021) Mating systems in true morels (Morchella). Microbiol Mol Biol Rev 85:e00220–e00220. https://doi.org/10.1128/MMBR.00220-20
4. Liu Q, Ma H, Zhang Y, Dong C (2018) Artificial cultivation of true morels: current state, issues and perspectives. Crit Rev Biotechnol 38:259–271. https://doi.org/10.1080/07388551.2017.1333082
5. Du XH, Zhao Q, Yang ZL (2015) A review on research advances, issues, and perspectives of morels. Mycology 6:78–85. https://doi.org/10.1080/21501203.2015.1016561
6. Liu WY, Guo HB, Bi KX, Sibirina LA, Qi XJ, Yu XD (2022) Determining why continuous cropping reduces the production of the morel *Morchella sextelata*. Front Microbiol 13:903983. https://doi.org/10.3389/FMICB.2022.903983
7. He XL, Peng WH, Miao RY, Tang J, Chen Y, Liu LX, Wang D, Gan BC (2017) White mold on cultivated morels caused by Paecilomyces penicillatus. FEMS Microbiol Lett 364:fnx037. https://doi.org/10.1093/femsle/fnx037
8. Lohberger A, Spangenberg JE, Ventura Y, Bindschedler S, Verrecchia EP, Bshary R, Junier P (2019) Effect of organic carbon and nitrogen on the interactions of Morchella spp. and bacteria dispersing on their mycelium. Front Microbiol 10:124. https://doi.org/10.3389/fmicb.2019.00124
9. Liu QY, Liu HM, Chen CQ, Wang JM, Han Y, Long ZF (2017) Effects of element complexes containing Fe, Zn and Mn on artificial morel's biological characteristics and soil bacterial community structures. PLoS ONE 12:e0174618. https://doi.org/10.1371/journal.pone.0174618
10. Zhang FS, Long L, Hu ZY, Yu XR, Liu QY, Bao JK, Long ZF (2019) Analyses of artificial morel soil bacterial community structure and mineral element contents in ascocarp and the cultivated soil. Can J Microbiol 65:738–749. https://doi.org/10.1139/cjm-2018-0600
11. Tan H, Kohler A, Miao RY, Liu TH, Zhang Q, Zhang B, Jiang L, Wang Y, Xie LY, Tang J, Li XL, Liu LX, Grigoriev IV, Daum C, LaButti K, Lipzen A, Kuo A, Morin E, Drula E, Henrissat B, Wang B, Huang ZQ, Gan BC, Peng WH, Martin FM (2019) Multi-omic analyses of exogenous nutrient bag decomposition by the black morel Morchella importuna reveal sustained carbon acquisition and transferring. Environ Microbiol 21:3909–3926. https://doi.org/10.1111/1462-2920.14741

12. Zhang Y, Li Q, Li YX, Xiao JC, Yang J, Zhang ZX (2023) Analysis of Nutrition and Soil Environment of *Morchella sextelata* Cultivated in Greenhouse. Edible Fungi China 42:74–79. https://doi.org/10.13629/j.cnki.53-1054.2023.06.012
13. Li X, Fu T, Li H, Zhang BX, Li W, Zhang BG, Wang X, Wang J, Chen Q, He X, Chen H, Zhang Q, Zhang Y, Yang R, Peng Y (2023) Safe production strategies for soil-covered cultivation of morel in heavy metal-contaminated soils. J Fungi 9:765. https://doi.org/10.3390/JOF9070765
14. Longley R, Benucci GNM, Mills G, Bonito G (2019) Fungal and bacterial community dynamics in substrates during the cultivation of morels (Morchella rufobrunnea) indoors, FEMS Microbiol. Lett 366:fnz215. https://doi.org/10.1093/femsle/fnz215
15. Tan H, Liu TH, Yu Y, Tang J, Jiang L, Martin FM, Peng WH (2021) Morel production related to soil microbial diversity and evenness. Microbiol Spectr 9:e00229–e00221. https://doi.org/10.1128/SPECTRUM.00229-21
16. Orlofsky E, Zabari L, Bonito G, Masaphy S (2021) Changes in soil bacteria functional ecology associated with Morchella rufobrunnea fruiting in a natural habitat. Environ Microbiol 23:6651–6662. https://doi.org/10.1111/1462-2920.15692
17. Yin Q, Chen Z, He PX, Liu W, Zhang WY, Cao XM (2024) Allelopathic effects of phenolic acid extracts on Morchella mushrooms, pathogenic fungus, and soil-dominant fungus uncover the mechanism of morel continuous cropping obstacle. Arch Microbiol 206:55. https://doi.org/10.1007/S00203-023-03790-8
18. Li PX (2024) Analysis of soil microbial community and construction of synthetic microbial community in morels cultivation, dissertation, Chinese Academy of Agricultural Sciences. https://doi.org/10.27630/d.cnki.gznky.2024.000478
19. Li Q, Xiong C, Huang W, Li X (2017) Controlled surface fire for improving yields of Morchella importuna. Mycol Prog 16:1057–1063. https://doi.org/10.1007/s11557-017-1350-9
20. Liu TH, Yang SQ, Liu FP, Miao RY, Yu Y, Wu X, Tang J, Wang Y, Peng WH, Tan H (2022) Effects of Organic Fertilizers Fermented with Wheat Straw and Chicken Manure on the Continuous Cultivation of *Morchella sextelata*. Biotechnol Bull 38:263–273. https://doi.org/10.13560/j.cnki.biotech.bull.1985.2022-0123
21. Liao YT, Wang CQ, Wei JJ, Zhao CG, Huang SL, Luo YL, Yan Y (2026) Changes in Soil Physicochemical Properties and Microbial Communities Under Continuous Cropping of Morchella and Their Associated Mechanisms. Biotechnol Bull 1–13. https://doi.org/10.13560/j.cnki.biotech.bull.1985.2025-1093
22. Xu L, Zhang Y, Li HJ, Li J, Xu J (2024) Challenges and strategies for continuous cropping of Morchella spp.: a review. Horticulturae 10:1288. https://doi.org/10.3390/horticulturae10121288
23. Yan Q, Zhang WD, Wang Q, Yang TH, Wang P, Yu Y, Tan X, Kang XP, Wen JW (2025) Screening of substrates and optimization of formulations for exogenous nutrient bags of *Morchella sextelata* (black morel), Horticulturae 11 863. https://doi.org/10.3390/horticulturae11070863

24. Huang K, Li L, Wu WJ, Pu KL, Qi W, Qi JZ, Li ML (2024) Enhancing Morchella mushroom yield and quality through the amendment of soil physicochemical properties and microbial community with wood ash. Microorganisms 12:2406. https://doi.org/10.3390/microorganisms12122406
25. Yue YH, Hao HB, Wang Q, Xiao TT, Zhang YC, Chen Q, Chen H, Zhang JJ (2024) Dynamics of the soil microbial community associated with Morchella cultivation: diversity, assembly mechanism and yield prediction. Front Microbiol 15:1345231. https://doi.org/10.3389/fmicb.2024.1345231
26. Feng WL, Wang JW, Jin QL, Guo ZE, Cai WM (2025) Analysis of soil fungal community characteristics of *Morchella sextelata* under different rotations and intercropping patterns and influencing factors. Agriculture 15:823. https://doi.org/10.3390/agriculture15080823
27. Zhang Y, Zhao Q, Uroz S, Gao TP, Li J, He FQ, Rosazlina R, Martin F, Xu L (2023) The cultivation regimes of *Morchella sextelata* trigger shifts in the community assemblage and ecological traits of soil bacteria. Front Microbiol 14:1257905. https://doi.org/10.3389/fmicb.2023.1257905
28. Zhao J, Zeng R, Zhang CM, He B, Zhang Q, Zhou QH, Gong ZK, Liu HL, Liu SQ (2025) Comparative analysis of soil properties before and after *Morchella sextelata* cultivation across various soil types. Front Microbiol 16:1700246. https://doi.org/10.3389/fmicb.2025.1700246
29. Zhang Y, Sun SF, Luo DD, Mao P, Rosazlina R, Martin F, Xu L (2023) Decline in morel production upon continuous cropping is related to changes in soil mycobiome. J Fungi 9:492. https://doi.org/10.3390/jof9040492
30. Liu XH, Yin B, Meng LQ, Zhao XY, Wang JL, Liu R, Hu LN, Wang XX, Liu Y, Ma YP (2025) Investigation of the impact of soil physicochemical properties and microbial communities on the successful cultivation of Morchella in greenhouses. Horticulturae 11:356. https://doi.org/10.3390/horticulturae11040356
31. Li YJ, Lin W, Chen J, Lin JB, Feng RC, Yan JJ, Miao RY, Gan BC (2024) Nitrates and microbiome components engaged in denitrification within soil regulate Morchella spp. growth. Horticulturae 10:905. https://doi.org/10.3390/horticulturae10090905
32. Yu FM, Jayawardena RS, Thongklang N, Lv ML, Zhu XT, Zhao Q (2022) Morel production associated with soil nitrogen-fixing and nitrifying microorganisms. J Fungi 8:299. https://doi.org/10.3390/jof8030299
33. Chen B, Shao GG, Zhou T, Fan QH, Yang NL, Cui M, Zhang JW, Wu XL, Zhang BX, Zhang RY (2023) Dazomet changes microbial communities and improves morel mushroom yield under continuous cropping. Front Microbiol 14:1200226. https://doi.org/10.3389/fmicb.2023.1200226
34. Fu YW, Fan MX, Qin HY, Zhang ZY, Liu SJ, Wu SW, Wang Y, Yuan X (2025) Interactions between morel cultivation, soil microbes, and mineral nutrients: impacts and mechanisms. J Fungi 11:405. https://doi.org/10.3390/jof11060405
35. Tan H, Yu Y, Tang J, Liu TH, Miao RY, Huang ZQ, Martin FM, Peng WH (2021) Build your own mushroom soil: microbiota succession and nutritional accumulation in semi-synthetic substratum drive the fructification of a soil-saprotrophic morel. Front Microbiol 12:656656. https://doi.org/10.3389/fmicb.2021.656656

Figures

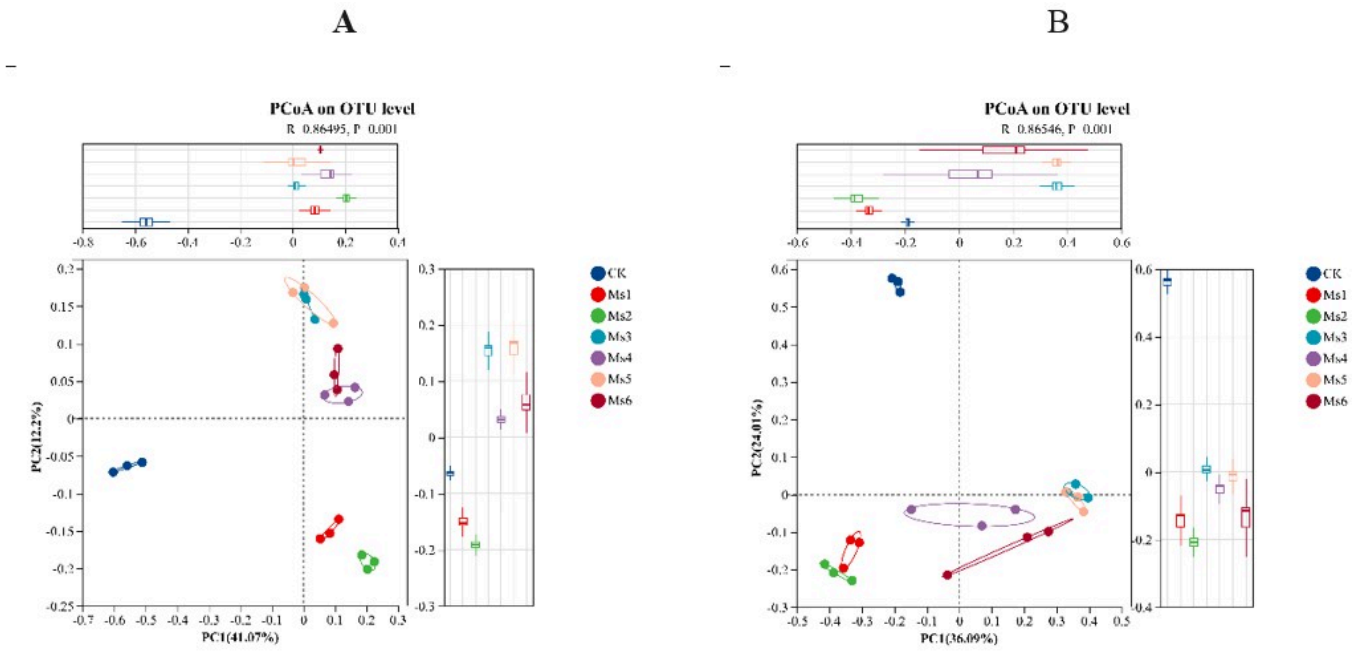


Figure 1

Beta diversity analysis of bacterial and fungal communities in morel cultivation soil under different fertilizer treatments

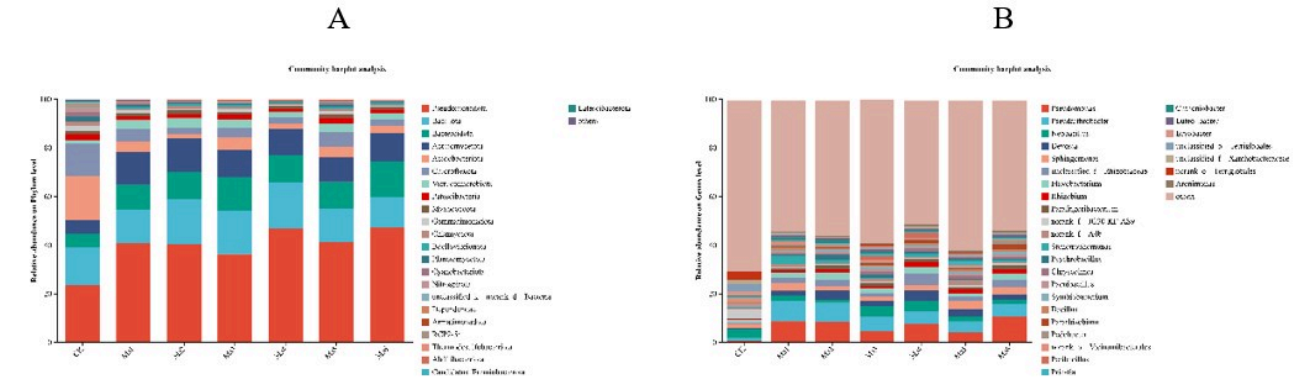


Figure 2

Bacterial community composition in morel cultivation soil under different fertilizer treatments

Note: A, bacterial community composition at the phylum level; B, bacterial community composition at the genus level. The bar charts show the relative abundances of dominant bacterial taxa under different treatments.

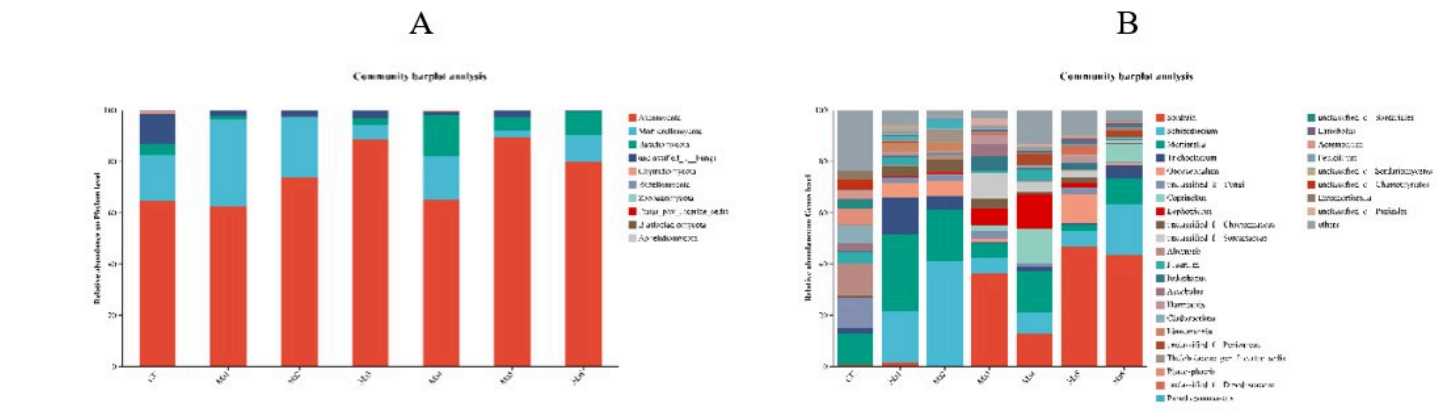


Figure 3

Fungal community composition in morel cultivation soil under different fertilizer treatments

Note: A, fungal community composition at the phylum level; B, fungal community composition at the genus level. The bar charts show the relative abundances of dominant fungal taxa under different treatments.

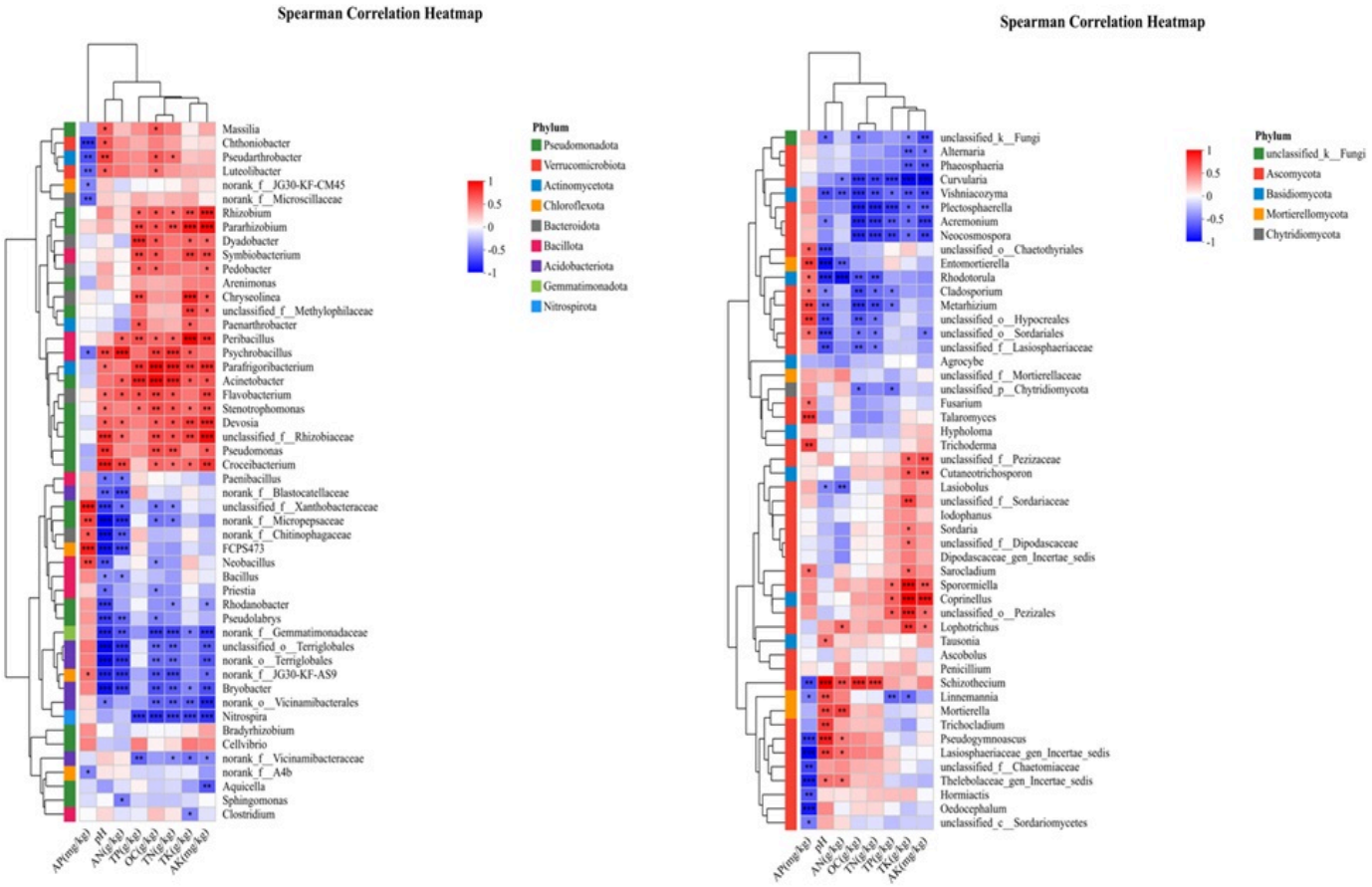


Figure 4

Spearman correlation heatmap showing the relationships between soil physicochemical properties and bacterial and fungal communities at the phylum level under different fertilizer treatments

Note: The X-axis and Y-axis represent environmental factors and taxa, respectively. The *R*values are shown using different colors. 0.01 < *p* ≤ 0.05, *; 0.001 < *p* ≤ 0.01, **; *p* ≤ 0.001, ***.

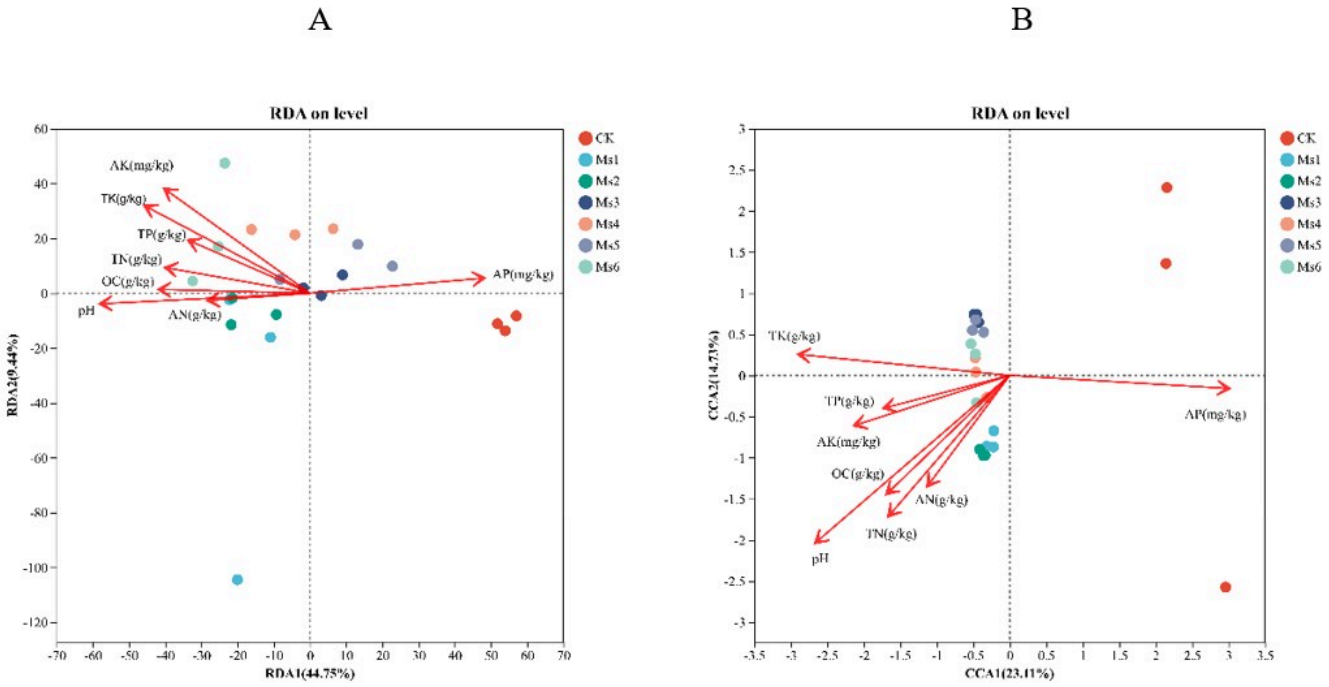


Figure 5

RDA analysis of bacterial and fungal communities and soil physicochemical properties

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx