RESEARCH ARTICLE

Effects of different fertilization methods on fungal community structure and diversity in strawberry rhizosphere soil

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Soil fungi are critical components of rhizosphere microecology, regulating soil nutrient cycling, plant root development, and soil-borne pathogen inhibition. Fertilization is the most important agricultural measure to regulate soil fertility and microbial community structure. Rational fertilization management is essential for alleviating continuous cropping obstacles and maintaining soil ecological health in strawberry greenhouse cultivation. However, the systematic comparative study on how single and combined applications of chemical fertilizer, organic fertilizer, and biochar regulate rhizosphere fungal community is still insufficient. This research used "Benihoppe" strawberry variety to investigate the effects of different fertilization methods on the fungal community structure and diversity in strawberry rhizosphere soil. Nine fertilization treatments were applied including single applications of chemical fertilizer, organic fertilizer, and biochar, as well as their combinations and reduced chemical fertilizer treatments. The ITS1 region of 18S rRNA in rhizosphere soil was sequenced. The α-diversity, OTU abundance, and distribution characteristics of rhizosphere soil fungi under different treatments were analyzed to clarify the species composition and relative abundance differences of the community. Cluster analysis was performed using PCA and UPGMA methods. The results showed that, compared to the control treatment without fertilization or biochar, the combined application of organic fertilizer and biochar significantly increased the Chao1, ACE, and Shannon indices of rhizosphere soil fungi with a fungal abundance increase of 10.77%. Compared to single chemical or organic fertilizer treatments, the addition of biochar generally improved the Chao1, ACE, and Shannon indices, enhancing the diversity and richness of rhizosphere fungal communities. Community composition analysis revealed that *Ascomycota* was the dominant phylum in strawberry rhizosphere soil with *Mortierella* as the core dominant genus. The relative abundances of phyla and genera exhibited differential responses to fertilization treatments. The combined application of chemical fertilizer, organic fertilizer, and biochar significantly reduced the relative abundance of *Ascomycota* by 22.6%, while other single and combined fertilization treatments increased its abundance by 8.4 – 35.2%. The combined application of chemical fertilizer and biochar significantly reduced the relative abundance of *Mortierella* by 26%, and the treatment with halved chemical fertilizer combined with biochar reduced it by 18%. These two combined fertilization modes showed significant inhibitory effects on *Mortierella* abundance, whereas other treatments promoted the enrichment of this dominant genus. Different fertilization treatments had distinct regulatory effects on the fungal community in strawberry rhizosphere soil. The combined application of organic fertilizer and biochar effectively optimized the fungal community structure, enhanced diversity and richness, and promoted healthier soil microecology. This fertilization mode not only met the nutrient requirements for strawberry growth but also avoided soil pollution caused by single chemical fertilizer application, aligning with the industry's demand for green, high-quality strawberry production and continuous cropping soil improvement, making it suitable for long-term strawberry cultivation.

Keywords: fertilization; strawberry; rhizosphere soil; high-throughput sequencing; microbial diversity.

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Introduction

The soil in the rhizosphere of plants serves as a pivotal hub for material cycling, linking the inorganic soil environment with the plant biological system. It mediates the transformation and utilization of nutrient elements and the migration and fate of pollutants, acting as a critical interface for material exchange in the soil-plant micro-ecosystem [1]. Rhizosphere microorganisms as the core functional groups of soil microecology participate in key biochemical processes such as soil organic matter decomposition, nitrogen fixation, and nutrient transformation, which play a vital regulatory role in plant growth and development with their community structure and activity exhibiting high sensitivity to changes in soil environmental factors [2]. Soil fungi as an essential component of rhizosphere microorganisms colonize and proliferate in the soil as single-celled or multicellular hyphae. Their community composition, diversity, and functional characteristics directly affect soil physicochemical properties and fertility levels, making them a core factor in maintaining soil microecological stability [3]. Strawberry as a perennial herbaceous fruit with significant economic benefits in both protected and open-field cultivation has high demands for soil fertility and rhizosphere microecological conditions. The composition and activity of rhizosphere fungal communities affect strawberry root growth, nutrient uptake, fruit quality, and yield [4].

Currently, continuous cropping obstacles are prominent in intensive strawberry cultivation. Imbalanced soil microbial communities, pathogen accumulation, and deterioration of soil physicochemical properties are major contributors to continuous cropping obstacles with disrupted rhizosphere fungal community structure being a key driving factor [5]. Fertilization as a critical measure in agricultural production to regulate soil fertility influences the community assembly and functional expression of strawberry rhizosphere fungi by altering soil nutrient supply and physicochemical conditions, thereby indirectly regulating soil nutrient availability and overall fertility [6]. Numerous studies have focused on the response of soil microbial communities to fertilization measures. Fertilizer type, combination patterns, and functional fertilizer applications can significantly regulate fungal community structure, diversity, and functional group distribution by modifying the soil microenvironment [7]. Lu *et al*. showed that attapulgite compound microbial fertilizer and bio-organic fertilizer significantly increased the microbial diversity in continuous cropping strawberry rhizosphere. Biochar could regulate the fungal community structure in strawberry rhizosphere, significantly increasing the abundance of biocontrol-functional genera, thereby enhancing plant disease resistance [8]. Ma *et al*. demonstrated that employing metagenomic sequencing technology to study the impact of fertilization on bacterial, fungal, and archaeal community composition and structure provided scientific basis for the sustainable and healthy development of facility vegetable fields [9]. Zhao *et al*. found that slow-release fertilizer application through basal and top-dressing methods could improve the diversity and abundance of cassava rhizosphere fungal communities. Fertilization, time, and rhizosphere factors all significantly affected fungal community structure and a few functional genes. Correlation analysis suggested that cassava rhizosphere fungi might participate in the cycling of soil available nutrients and the function of functional genes, offering scientific

insights for further understanding cassava rhizosphere microecological processes [10]. Semenov *et al*. revealed that the influence of potential plant species on rhizosphere microbial abundance and diversity was controlled by long-term fertilization. Therefore, fertilization management could be used to regulate rhizosphere fungal communities and suppress soil-borne pathogens [11]. Chen *et al*. showed that, under legume-grass intercropping, fertilization and fungal communities exhibited significant differences in the effects of various fertilization strategies on rhizosphere fungal diversity and soil fertility indicators [12]. In fertilizer combination studies, treatments involving fenaminosulf disinfection followed by organic or microbial fertilizer application, although reducing the overall diversity of strawberry rhizosphere soil fungi, significantly increased the abundance of beneficial genera such as *Pseudomonas* and *Streptomyces*, thereby inhibiting soil-borne diseases. In contrast, chemical fertilizer alone tended to disrupt fungal community balance and increase the risk of pathogen enrichment. Additionally, combined organic-inorganic fertilization has been widely proven to maintain the stability of strawberry rhizosphere fungal communities, whereas sole chemical fertilizer application may induce soil acidification, indirectly affecting fungal diversity and the balanced distribution of functional groups. It is urgent to clarify the differences of fungal community richness, diversity, and species composition under different fertilization treatments [13]. Currently, studies on fertilization-mediated soil microbial effects primarily focus on major crops such as watermelon [14], corn [15], rice [16], rapeseed [17], cabbage [18], and pakchoi [19], while systematic studies on strawberry rhizosphere soil fungal diversity remain relatively scarce.

This study explored the regulatory effects of different fertilization methods on fungal community structure and diversity in strawberry rhizosphere soil, screened optimal fertilization strategy for alleviating continuous cropping obstacles and maintaining soil microecological stability. Fungal OTU composition, α diversity, species relative abundance, and community similarity under nine fertilization treatments were analyzed using high-throughput sequencing technology [20]. The results of this study provided theoretical basis and practical reference for mitigating strawberry continuous cropping obstacles, maintaining soil fertility, managing rational fertilization, improving soil microecology, and achieving high-quality, high-yield cultivation of strawberries [21, 22].

Materials and methods

Sample collection

The research was carried out in Luoshui Town, Xuanwei, Yunnan, China (26.12°N, 104.08°E) using “Benihoppe” strawberry variety. 45 – 50 days old, virus-free plug seedlings with a plant height of 12 – 15 cm, a root-collar diameter of 0.8 – 1.0 cm, 4 – 5 functional leaves, well-developed white roots, and no signs of pests or diseases were selected from the local strawberry nursery base. During the peak fruiting period, rhizosphere soil samples were collected using the root-shaking method [23]. Each fertilization treatment was assigned to an independent sampling unit. The collected soil samples were promptly mixed into composite samples, passed through a 2 mm sieve to remove residual roots, plant debris, and other impurities, placed in sterile sealed bags, and immediately transported to the lab and stored at -80°C.

Different fertilizer treatments

The experiments were conducted in a continuous-cropping strawberry greenhouse. Uniform planting specifications, irrigation, fertilization, and field management were implemented to ensure experimental consistency. The tested fertilizers included potassium nitrate with $N + K_2O \geq 60\%$, potassium dihydrogen phosphate with $P_2O_5 + K_2O \geq 99\%$, commercial mature organic fertilizer with organic matter ≥ 45% and $N + P_2O_5 + K_2O \geq 5\%$, and biochar prepared from corn straw pyrolysis with the particle size of 2 – 5 mm and organic matter

≥ 80%. Elongated plastic pots with dimensions of 72 cm × 15 cm × 25 cm were employed as experimental containers with drainage holes evenly distributed at the bottom of each pot. The pot was filled with an equal amount of air-dried, sieved, and continuous-cropping soil to a height of 20 cm. A completely randomized block design was adopted with a total of 9 (A to I) fertilization treatments including "A" with single chemical fertilizer, "B" with single organic fertilizer, "C" with chemical fertilizer combined with organic fertilizer, "D" with chemical fertilizer combined with biochar, "E" with organic fertilizer combined with biochar, "F" with conventional dosage of chemical fertilizer + organic fertilizer + biochar, "G" with reduced chemical fertilizer combined with organic fertilizer and biochar, "H" with reduced chemical fertilizer combined with biochar, and "I" as blank control without fertilization. The application rates of potassium nitrate, potassium dihydrogen phosphate, organic fertilizer, and biochar per plant were designed based on the results of previous study regarding strawberry fertilization [24]. The conventional application rate of potassium nitrate was 13.86 g/plant, and the reduced rate was 6.93 g/plant. The conventional rate of potassium dihydrogen phosphate was 4.2 g/plant, and the reduced rate was 2.1 g/plant. Organic fertilizer was applied at 500 g/plant, and biochar was applied at 270 g/plant. Each treatment consisted of 10 pots with 3 strawberry seedlings planted per pot at uniform spacing. The replicates were arranged randomly.

Polymerase chain reaction (PCR) amplification

Soil total microbial genomic DNA was extracted using FastDNA® SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) following manufacturer's instructions. Fungal ITS1 region 18S rRNA universal forward primer (5'-GCA TCG ATG AAG AAC GCA GC-3') and reverse primer (5'-TCC TCC GCT TAT TGA TAT GC-3') were designed. PCR amplification was performed by using Premix Taq™ PCR Kit (Takara Biomedical Technology Co., Ltd., Dalian, Liaoning, China) on an ABI Veriti 96-well Thermal Cycler (Applied Biosystems, Foster City, CA, USA) following kit instructions. PCR products were purified and recovered using AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and sent to Novogene Bioinformatics Technology Co., Ltd. (Beijing), Beijing, China for high-throughput sequencing.

Quality control and analysis of sequencing data

Raw sequence quality filtering was performed by using Trimmomatic (v0.39) (http://www.usadellab.org/cms/?page=trimmomatic). Paired-end reads were assembled using FLASH (v1.2.11) (https://ccb.jhu.edu/software/FLASH/). OTU clustering at 97% similarity was conducted using UPARSE v7.0 (http://drive5.com/uparse/), and chimeric sequences were removed with UCHIME (v4.2) (http://drive5.com/usearch/manual/uchime_algo.html). Taxonomic annotation was performed against the Silva (v138) database (https://www.arb-silva.de/) using RDP Classifier (v2.11) (https://rdp.cme.msu.edu/classifier/) [25]. Alpha diversity indices were calculated by Mothur (v1.46.1) (https://www.mothur.org/). R (v4.3.2) (https://www.r-project.org/) with ggplot2 and pheatmap packages was used for visual plotting. PCoA analysis was carried out using Canoco (v5.1) (https://www.canoco5.com/), and UPGMA clustering tree was constructed by using FastTree (http://www.microbesonline.org/fasttree/).

Statistical analysis

SPSS 26.0 (IBM, Armonk, NY, USA) was employed for statistical analysis of this research. One-way analysis of variance (ANOVA) was performed. Duncan's multiple range test was adopted to evaluate significant differences among treatments with P value less than 0.05 as statistically significant difference.

Results

Sequencing results and sequence depth validation

The results showed that, when the sequencing volume reached 60,000 reads, the rarefaction curves of all nine treatments gradually plateaued

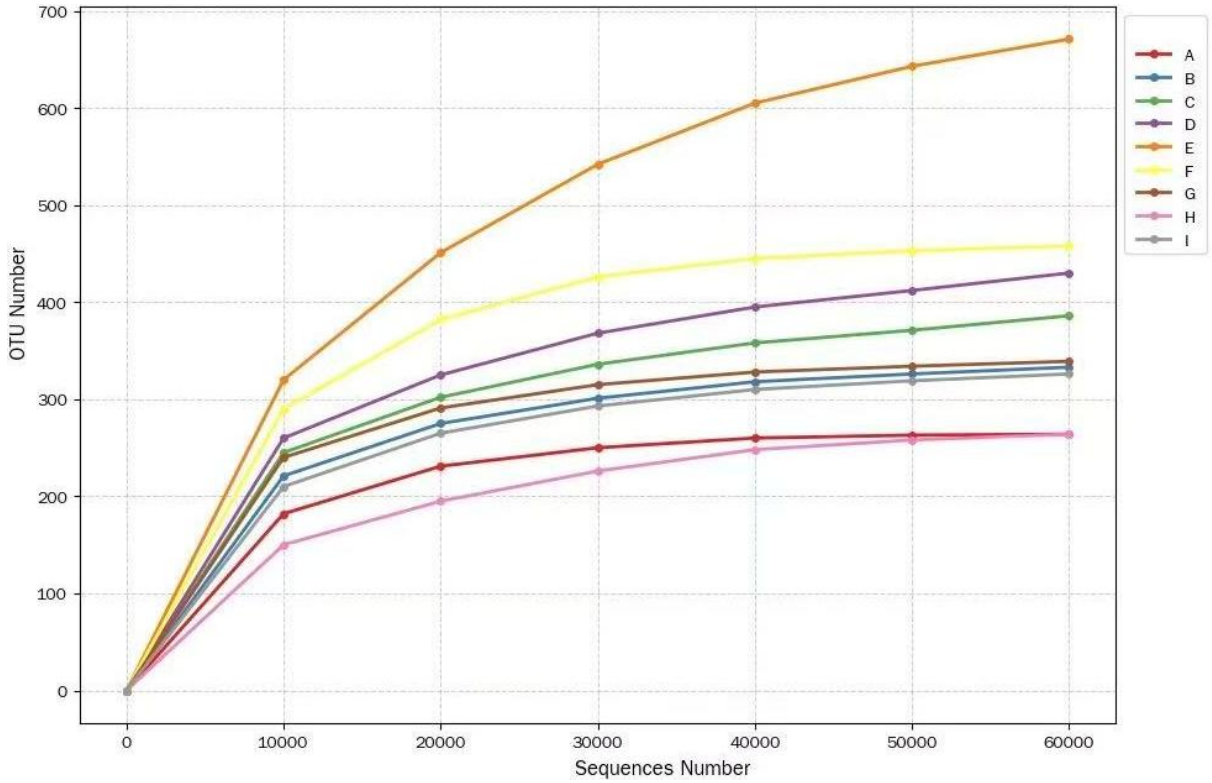

Figure 1. Rarefaction curves of fungal OTUs in strawberry rhizosphere soil under different fertilization regimes.

with increasing sequencing depth, indicating sufficient sequencing coverage and reasonable sequencing volume in this study (Figure 1). A total of 672,851 valid gene sequences were obtained and clustered into 4,559 fungal OTUs. Coverage of all samples exceeded 99.7%, indicating that the sequencing data could comprehensively and accurately reflect the fungal community composition in strawberry rhizosphere soil. Statistical analysis showed that OTU number, Chao1, ACE, and Shannon indices differed significantly among different treatments. Increasing the data volume would only yield to a small number of new species OTUs, suggesting that the obtained OTU count had approached the maximum measurable quantity. Further increasing the sequencing depth would hardly provide additional insights into the true fungal community structure in the environment. The current sequencing volume was reasonable and could comprehensively and accurately reflect the composition and diversity characteristics of fungal communities in strawberry rhizosphere soil under nine different fertilization treatments. The alpha diversity indices of fungal communities in strawberry rhizosphere soil under different fertilization treatments showed that the number of fungal OTUs across treatments ranged from 264 to 671 with treatment E having the highest count and treatment H demonstrating the lowest. The Chao1 and ACE indices followed the same trend, both peaking in treatment E as 772.28 and 757.00, respectively, and reaching their lowest values in treatment H as 274.80 and 283.70, respectively. The Shannon index ranged from 3.83 to 5.83 with treatments D, G, and H exhibiting relatively higher values, indicating greater fungal community diversity under these treatments. The results demonstrated that the number of OTUs ranked from high to low was E > G > D > A > B > C > I > F > H (Table 1).

OTU abundance and α diversity of soil fungi under different fertilization methods

The richness and diversity of soil fungal

Table 1. Alpha diversity indices of fungi in strawberry rhizosphere soil under different fertilization methods.

Treatment	OTUs	Chao1 index	ACE index	Shannon index	Coverage
A	543	694.68	668.9	4.59	99.7%
B	530	655.30	649.0	4.96	99.7%
C	524	617.19	606.0	5.37	99.8%
D	544	608.19	594.2	5.88	99.8%
E	671	772.28	757.0	5.42	99.7%
F	451	555.21	569.2	3.83	99.7%
G	567	576.75	588.4	5.82	99.9%
H	264	274.80	283.7	5.83	99.9%
I	465	591.29	562.3	4.09	99.7%

communities demonstrated that the Chao1 index ranked as E > G > D > A > B > C > I > F > H, indicating that treatments with chemical fertilizer + organic fertilizer + biochar (F) and half-dose chemical fertilizer + biochar (H) significantly reduced rhizosphere fungal richness, while other treatments enhanced fungal richness. Among them, organic fertilizer + biochar (E) showed the most significant improvement, whereas treatment H exhibited the strongest inhibitory effect. The ACE index ranked as E > A > B > C > D > G > F > I > H. The results showed that only treatment H reduced the enrichment level of fungal communities, while all other treatments improved it to varying degrees. Treatment E demonstrated the most pronounced enhancement of fungal enrichment, whereas treatment H significantly suppressed it. The Shannon index ranked as D > H > G > E > C > B > A > I > F, indicating that only the treatment with chemical fertilizer + organic fertilizer + biochar (F) had no significant promoting effect on rhizosphere fungal diversity. All other fertilization treatments significantly improved fungal community diversity, exhibiting a clear positive regulatory effect on fungal community structure.

Composition and abundance of fungal communities in strawberry rhizosphere soil at the phylum level

At the fungal phylum level, the dominant fungal groups in the strawberry rhizosphere soil across different treatments included *Ascomycota*, *Mortierellomycota*, *Chytridiomycota*, *Basidiomycota*, *Rozellomycota*, *Zoopagomycota*, *Olpidiomycota*, *Glomeromycota*, and *Mucoromycota* aside from the "Others" category. Under treatment A, the relative abundances of *Ascomycota* and *Mortierellomycota* were 10.00% and 38.00%, respectively, making them the dominant phyla. Compared to treatment A, B reduced the relative abundance of *Mortierellomycota* to 28.00% and increased that of *Ascomycota* to 10.80%. Treatment D decreased the relative abundance of *Ascomycota* to 8.50%, while treatment F increased the relative abundance of *Ascomycota* and *Mortierellomycota* to 10.50% and 50.54%, respectively. Treatment H significantly raised the relative abundance of *Ascomycota* to 16.00%. In contrast, blank treatment I reduced the relative abundance of *Ascomycota* to 6.50%, while increasing that of *Mucoromycota* by 10.00%. The combined application of chemical fertilizer and biochar treatment G and H both significantly reduced the relative abundance of *Mortierellomycota* to 37.24% and 25.84%, respectively. Additionally, compared to treatment G, treatment H increased the relative abundances of *Ascomycota* and *Basidiomycota* while decreasing that of *Mortierellomycota*. Compared to treatment F, blank treatment I elevated the relative abundances of *Mucoromycota* and *Olpidiomycota* but reduced that of *Mortierellomycota* (Figure 2). Different fertilization regimes significantly altered the fungal community structure in strawberry rhizosphere soil. Among them, the combined application of organic fertilizer and biochar

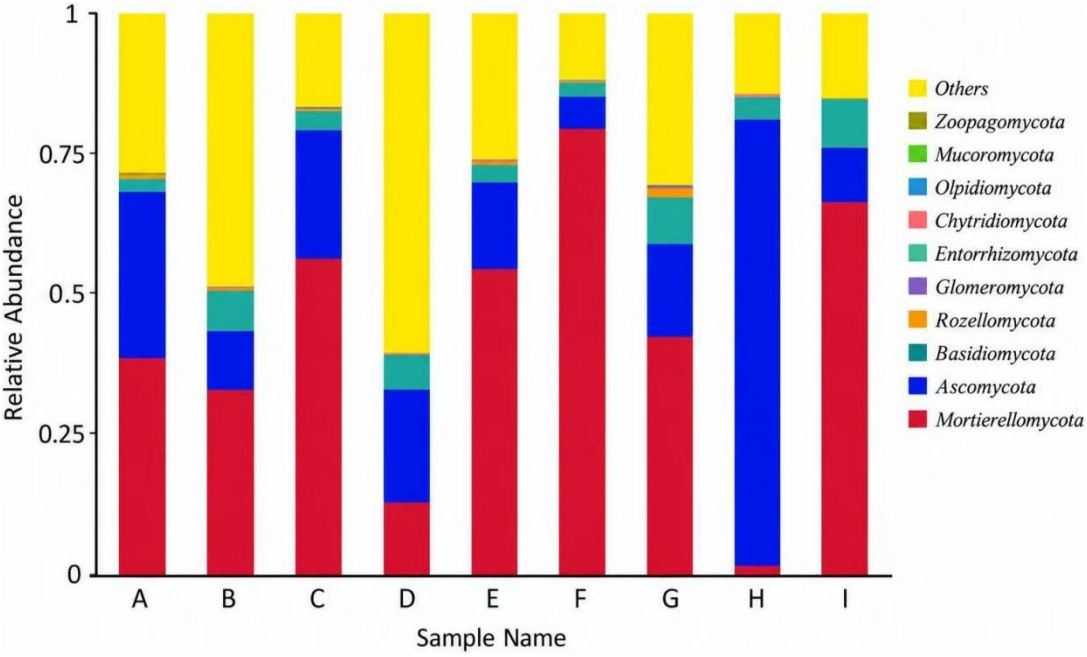

Figure 2. Relative abundance of fungi at the phylum level in strawberry rhizosphere soil under different fertilization regimes.

demonstrated notable regulatory effects in optimizing rhizosphere fungal communities and reducing the abundance of pathogen-related phyla.

Composition and abundance of fungal communities in strawberry rhizosphere soil at the genus level

At the fungal genus classification level, the dominant groups in strawberry rhizosphere soil across different treatments included *Mortierella*, *Stomiopeltis*, *Alternaria*, *Archaeorhizomyces*, *Cladosporium*, *Fusarium*, *Talaromyces*, *Solicoccozyma*, *Aspergillus*, *Saitozyma*. Under treatment F, the relative abundance of *Mortierella* was 20.00%, while under treatment E, it was 32.00%. Compared to treatment F, the combined treatment increased the relative abundance of *Mortierella* by 12.00%. Under treatment A, the relative abundance of *Mortierella* was 8.00%, while under treatment G, it was 22.00%, and under treatment H, it was 0.00%. Compared to treatment F, the single chemical fertilizer treatment reduced the relative abundance of *Mortierella* by 12.00%, the combined chemical fertilizer and biochar treatment reduced it by 10.00%, and the half-dose chemical fertilizer with biochar treatment reduced it by 32.00%, indicating that the combined application of chemical fertilizer and biochar effectively suppressed the enrichment of *Mortierella*. Compared to treatment A, treatment H increased the relative abundance of *Stomiopeltis* by 15.00%, *Alternaria* by 3.00%, and *Archaeorhizomyces* by 5.00%. In contrast, treatment F reduced the relative abundance of *Stomiopeltis* by 15.00%, *Alternaria* by 5.00%, and *Archaeorhizomyces* by 5.00%, suggesting that organic fertilizer application regulated the distribution of biocontrol-related genera. Under reduced chemical fertilizer conditions, treatment B increased the relative abundance of *Aspergillus* by 1.00% and *Saitozyma* by 3.00% compared to treatment A with no significant effect on *Mortierella*. Meanwhile, treatment D reduced the relative abundance of *Mortierella* by 6.00%, increased *Fusarium* by 1.00%, and increased *Talaromyces* by 3.00% compared to the single chemical fertilizer treatment. Additionally, treatment I significantly reduced the abundance of all detected fungal genera compared to treatment A, retaining only minimal levels of

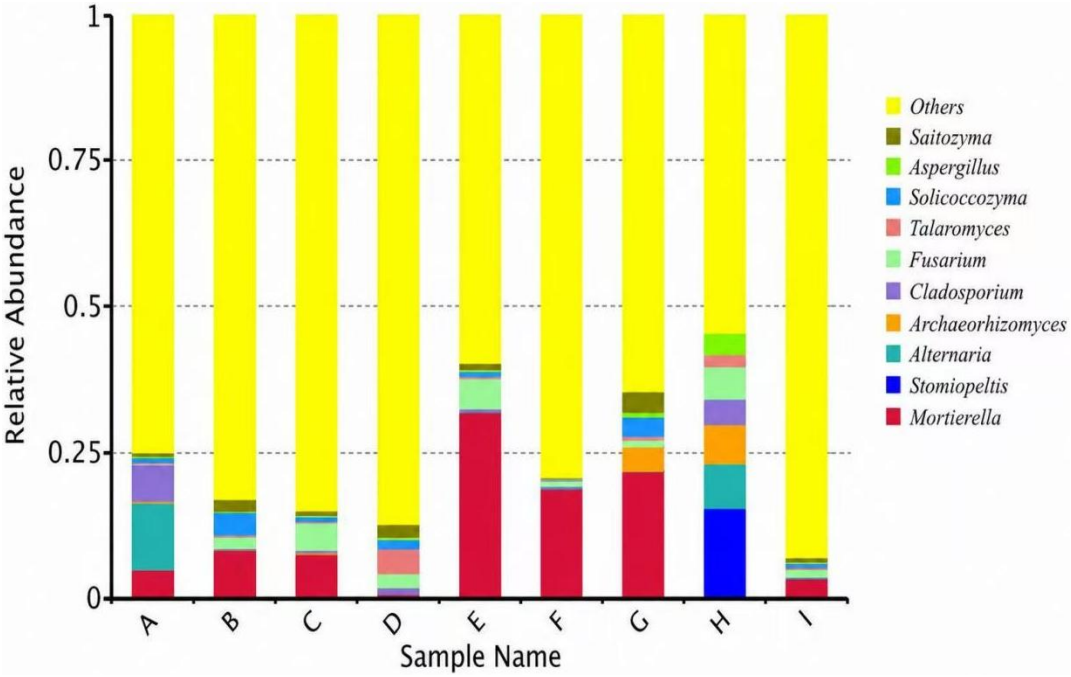

Figure 3. Relative abundance of fungi at the genus level in strawberry rhizosphere soil under different fertilization regimes.

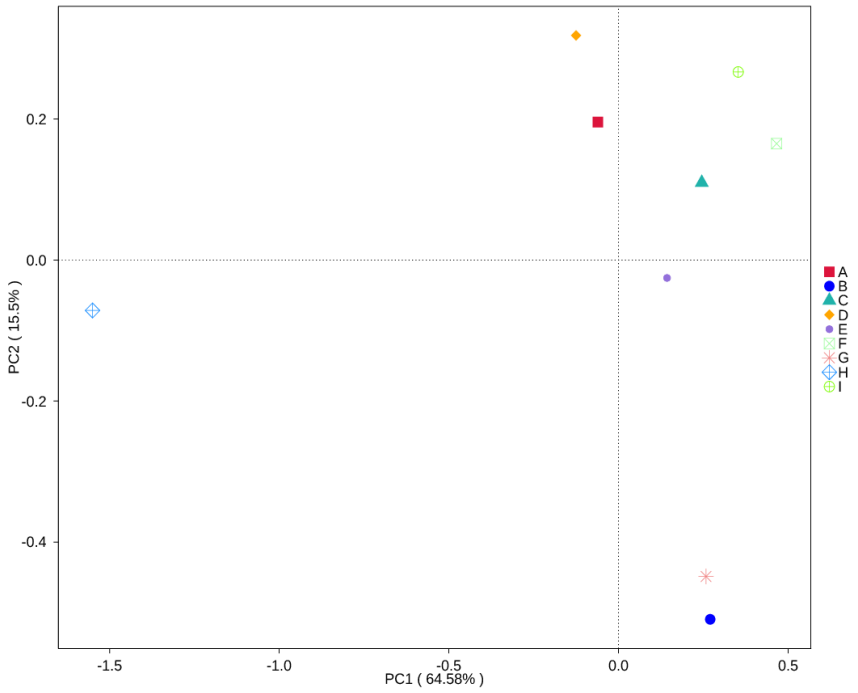

Figure 4. PCoA analysis of fungal communities in strawberry rhizosphere soil.

fungal communities (Figure 3). The results indicated that biochar application alone exerted a strong inhibitory effect on the abundance of fungal genera in the strawberry rhizosphere. Different fertilization regimes could markedly regulate the distribution of fungal genera in

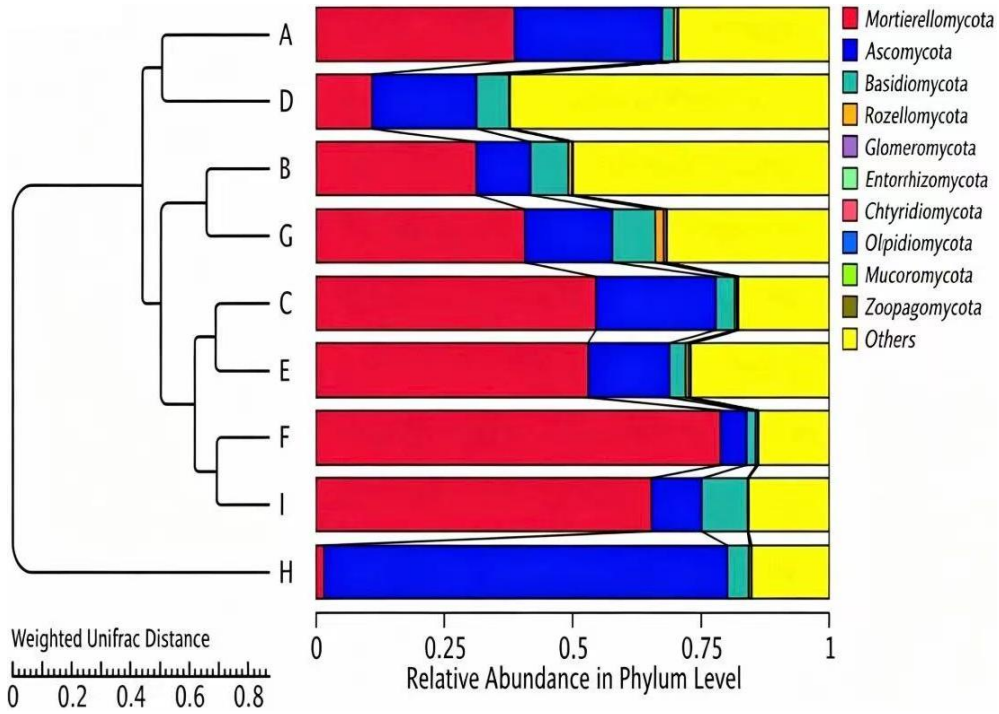

Figure 5. UPGMA cluster analysis of fungal communities at the phylum level in strawberry rhizosphere soil.

strawberry rhizosphere soil. Among them, the combined application of reduced chemical fertilizers with organic fertilizers and biochar demonstrated a clear regulatory effect in optimizing rhizosphere fungal community structure and reducing the abundance of potential pathogenic genera. In contrast, the sole application of chemical fertilizers or biochar tended to disrupt the balance of fungal community structure.

Similarity analysis of fungal communities in strawberry rhizosphere

PCoA results indicated that PC1 and PC2 explained 64.58% and 15.5% of the total variation, respectively, with a cumulative contribution rate of 80.08%, which could well reflect the overall difference of fungal community structure among treatments. All samples presented obvious spatial clustering distribution in the two-dimensional coordinate system. Samples with similar fertilization regimes gathered closely, while treatments with different fertilization patterns showed obvious spatial separation (Figure 4). UPGMA clustering analysis based on Weighted Unifrac distance further verified the community differentiation rule. The results demonstrated that different fertilization methods significantly changed the community similarity and phylogenetic relationship of strawberry rhizosphere soil fungi (Figure 5).

Discussion

As a core functional group in farmland ecosystems, soil fungi play a critical role in processes such as carbon sequestration [26], organic matter decomposition, nutrient cycling and transformation, and soil microenvironment improvement. The stability of their community structure and diversity serves as a fundamental basis for maintaining soil ecological functions and high-quality crop production. The results of this study demonstrated that, compared to the control treatment without fertilizer or biochar application, the combined application of organic fertilizer and biochar significantly increased the

ACE index, Chao1 index, and Shannon index of strawberry rhizosphere soil fungi. The fungal population increased, and the diverse characteristics of the community composition became more pronounced. These findings aligned with the research conclusions of Huang *et al*. [27] and indicated that the active organic matter and diverse nutrients in organic fertilizers could trigger complex biochemical reactions in the soil system [28], activate microbial metabolic activity, and thereby optimize fungal community diversity [29]. The enhancement of fungal diversity could improve the stability and resilience of farmland ecosystems, promote efficient transformation and utilization of soil nutrients, and provide a favorable microenvironment for strawberry growth. The combined application of chemical fertilizer, organic fertilizer, and biochar treatment reduced the diversity of the rhizosphere soil fungal community [30], which might be closely related to changes in soil physicochemical properties induced by chemical fertilizer application. Previous studies have confirmed this observation. Guo *et al*. demonstrated that excessive nitrogen application reduced aggregate stability, increased soil bulk density, deteriorated pore structure, and limited oxygen supply, significantly inhibiting the growth of aerobic fungi and altering community composition [31]. Zhang *et al*. found that long-term excessive nitrogen application led to increased soil bulk density, poor aeration, a significant decline in the abundance and diversity of aerobic fungi, and a rise in the proportion of anaerobic fungi [32]. Meanwhile, Wang *et al*. revealed that long-term chemical nitrogen fertilization was the primary cause of black soil acidification. The decline in soil pH drove shifts in fungal community structure and function by altering their living environment [33]. Deng *et al*. reported that fungal communities in treatments with single chemical fertilizers differed significantly from the control with pH and nitrate nitrogen being identified as key factors driving community changes, highlighting the indirect restructuring effect of acidification [34]. Xu *et al*. systematically reviewed the acidification mechanisms induced by chemical fertilizers, especially nitrogen fertilizers, confirming that acidification reshaped microbial ecology by promoting the proliferation of acidophilic harmful fungi while reducing beneficial fungi, thereby restructuring communities and influencing soil-borne disease occurrence [35]. Additionally, the half-dose chemical fertilizer + biochar treatment exhibited a distinct pattern of "increased diversity but reduced abundance", likely due to altered nutrient supply from reduced fertilizer application and the regulatory effects of biochar. Fertilizer reduction could mitigate soil acidification and structural degradation, creating favorable conditions for fungal community diversity recovery [36]. However, biochar's adsorption and slow-release effects on soil nutrients might shift competitive dynamics among dominant fungal taxa, leading to decreased abundance of certain dominant groups and an overall reduction in fungal abundance [37]. This phenomenon aligned with the findings of Yang *et al*. who concluded that carbon source additions such as biochar and straw could optimize microbial community structure and enhance nutrient use efficiency by improving microbial habitats and regulating nutrient availability [38]. However, the synergistic effects of such amendments with fertilizers required comprehensive consideration of application ratios. From the resulting characteristics of dominant phyla and genera, the most dominant phylum in strawberry rhizosphere soil in this study was *Ascomycota*, followed by *Mortierellomycota* with the core dominant genus being *Mortierella*. The results aligned closely with findings from studies on rhizosphere fungal communities of crops such as black wolfberry, mulberry [39], morel mushroom [40], and pepper [41], suggesting that this dominant taxonomic composition might be a common feature of horticultural crop rhizosphere fungal communities. *Ascomycota*, a key functional group in soil organic matter decomposition, exhibited a negative correlation between its abundance and soil pH and played a central role in carbon and nitrogen cycling. In contrast, *Mortierellomycota* could form

mycorrhizal symbiotic systems with plants, enhancing stress resistance such as drought tolerance by improving soil water retention and synthesizing osmoregulatory substances, thereby promoting nutrient uptake. In this study, the combined application of chemical fertilizer, organic fertilizer, and biochar led to a decrease in *Ascomycota* abundance but an increase in *Mortierellomycota* abundance. This differential response might be related to field irrigation levels. Recent research found that arbuscular mycorrhizal fungal abundance initially decreased and then increased with reduced irrigation, suggesting that the symbiotic advantages of *Mortierellomycota* became more pronounced under water stress, potentially offsetting the functional impact of declining *Ascomycota* abundance [42]. Additionally, treatments involving chemical fertilizer + biochar and half-dose chemical fertilizer + biochar resulted in reduced *Mortierella* abundance, which warranted attention. As an important functional genus within *Ascomycota*, *Mortierella* enhanced pest resistance through synergistic interactions with plants, and its decline might weaken strawberry resistance to diseases and pests. The shifts in dominant microbial abundances induced by the combined application of chemical fertilizer and biochar essentially reflected the combined effects of optimized nutrient supply and microenvironmental improvements in soil. Biochar enhanced the availability of soil nutrients [43], promoted microbial community diversity, and thereby reduced the competitive advantage of single dominant taxa, leading to a more stable community structure. This result was consistent with recent conclusions that biochar optimized the soil microenvironment and stimulated microbial proliferation. It not only met the diverse nutrient demands of strawberry growth but also reduced the environmental pollution risks associated with excessive fertilizer input. Additionally, it enhanced the stability and sustainability of the soil ecosystem, making it an optimal fertilization strategy for long-term continuous strawberry cultivation [44].

Acknowledgements

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