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Organic Fertilizer with High Nutrient Levels Affected Peanut-Growing Soil Bacteria More Than Fungi at Low Doses

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Abstract: The breeding of laying hens and broilers in China has increased tremendously. Wet organic fertilizer prepared from hen manure using high-temperature container fermentation preserves high levels of nutrients and a diverse microbial community. We applied low doses of organic fertilizer to peanuts in the black soil area of China's northeastern region. Based on the calculation of nitrogen content, treatments were set as follows: chemical fertilizer (PCF), organic fertilizer (POF, 4500 kg·ha^{-1}), and replacement of 50% chemical fertilizer with organic fertilizer (PR, 2250 kg·ha^{-1}). Compared to the plots with chemical fertilizers, the use of organic fertilizer and replacing 50% of the chemical fertilizers with organic fertilizer significantly increased peanut yields. Both the organic fertilizer and replacing 50% of the chemical fertilizers with organic fertilizer did not significantly affect the activities of the most tested soil enzymes related to carbon transformation and the absolute abundance of microorganisms. However, they did significantly enhance soil dehydrogenase and α-glucosidase. The community abundance ratio of fungi/bacteria trended downward, leading to soil with a high-fertility bacterial composition. The replacement of 50% chemical fertilizer with organic fertilizer significantly enhanced the species richness and diversity of the bacterial and fungal communities. Organic fertilizer treatment significantly increased the relative abundance of *Gemmatimonas* and *Sphingomonas*. The relative abundance of Mycobacterium in the treatment where 50% of the chemical fertilizers were replaced with organic fertilizer was significantly lower than that in the organic fertilizer treatment. PCoA results showed that the low-dose organic fertilizer treatment, replacing 50% of the chemical fertilizers with organic fertilizer, had a significant impact on the composition of soil bacterial communities.

Keywords: hen manure; organic fertilizer; soil physicochemical property; soil enzyme; microbial community

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1. Introduction

Over the past 70 years, exploitative practices have been implemented in the black soil of Northeast China, including rotary tillage, single chemical fertilizer application, straw cleaning, and straw burning [1,2]. High outputs of intensive agricultural activity without organic material incorporation has led to organic matter reduction, black soil layer thinning, cultivation layer shallowing, and a sharp decline in effective nutrient contents.

Arable soil has encountered degradation issues such as nutrient imbalance, properties deteriorating, and biological health declining [3–5]. The pool and utilization potential of soil nutrients has also decreased. These issues have hindered the sustainable development of the region's nature, society, and economy, jeopardizing food security and the ecological environment [4,6,7]. Organic fertilizer (OF) application is essential for sustainable farmland management and green agriculture [8–10].

The livestock and poultry industry in China has developed rapidly and extensively in the past decade, and substantial manure has accumulated [11]. The large quantities of manure have not only caused environmental pollution but also facilitated pathogen spread. It is urgent and important to convert manure into fertilizers effectively and quickly in China. Using the China National Knowledge Infrastructure (CNKI, from July 2013 to July 2023), we found 1227 articles about OF substitution for chemical fertilizer technology. Research on OF substitution has increased over the last 10 years, and particularly rapidly in the last 5 years (Figure 1a). Based on a statistical analysis of the literature, we found that OF substitution for chemical fertilizer technology constituted a large proportion of the basic trial category. OF substitution technology is a growing and dominating research direction and hotspot (Figure 1b). Most research has focused on the study of crop yields, fertilizer utilization, soil fertility, and environmental responses with the partial or total replacement of chemical fertilizers with OFs [12,13]. Many studies confirmed that OF application enhanced the health of agricultural systems and ultimately achieved better economic and environmental benefits [14–16]. OF applications were found to promote organic carbon storage and sequestration rates; regulate soil C/N ratios; stabilize soil pH levels; decrease soil bulk density; enhance soil porosity; increase soil nutrient holding capacity; improve soil fertility; enhance fertilizer efficiency; improve the root microbial environment; stimulate crop root systems and secreted organic acids, amino acids, and sugars; and boost crop nutrient uptake (e.g., nitrogen, phosphorus, potassium, etc.) [17–19]. OF application also enhanced enzymatic reactions and microbial activity related to soil carbon, nitrogen, and phosphorus transformations; facilitated the spread of beneficial microorganisms; and improved soil microbiota reproduction [20–22].

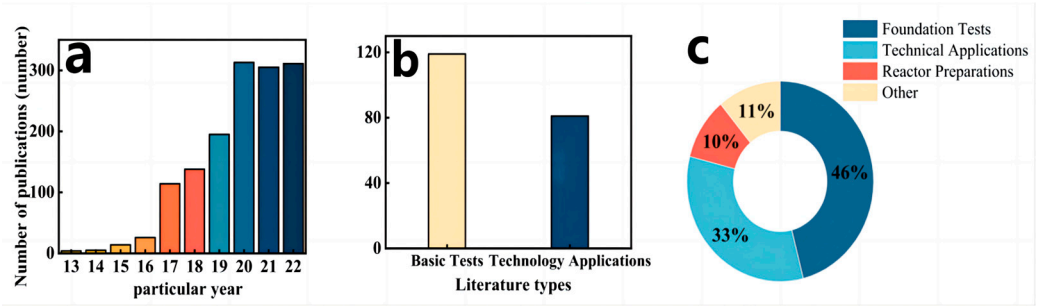

Figure 1. Distribution of the literature on organic fertilizer substitution (**a**); status of literature classification during last 10 years (**b**,**c**).

More attention has been paid to the potential for reducing chemical fertilizers through technological advancements. Various devices have been widely used to manage livestock and poultry manure in China [23,24]. OF prepared through different techniques varies widely. Using "Web of Science" and the China National Knowledge Infrastructure (CNKI), we found that few studies reported the preparation of organic fertilizers using reactors. Further research on OF preparation by reactors is necessary (Figure 1c). Compared to the traditional static composting technology (CCT), the high-temperature aerobic fermentation technique (HTC) has a broader range and offers more advantages [8,25]. HTC involves exposing livestock and poultry manure and microbial agents to ample oxygen at elevated temperatures above 60 °C, which facilitates decomposition rapidly and thoroughly. Automated high-temperature composting (HTC) technology improves compost maturity, speeds

up organic matter degradation, preserves more nitrogen, enhances the inactivation rate of pathogenic microorganisms, and stabilizes the quality of organic fertilizer products [8–10]. At the same time, HTC maintains a stable temperature, accurately shortens the fermentation cycle, occupies a small land area, and maximizes the efficiency of fermentation. The superiority of HTC technology includes its low cost, environmental protection, and high-efficiency automation, which have promoted its application. Compared to static compost products, OF produced using HTC technology contained more NPK nutrients and microbial flora. Soil microbial population, activity, and bio-prophylaxis functions were enhanced by applying wet organic fertilizers [26–28]. It is necessary to comprehensively study the effects of low-dose wet organic fertilizers with high nutrient levels on crops and soils.

As is well known, the impact mechanism of organic fertilizer on different soils varies greatly [29–31]. Peanut is an oilseed crop with the highest yield potential and holds a significant position in the national economy [32]. The degradation of soil in Northeast China seriously constrains peanut yield and quality [33,34]. The present study aimed to investigate the effect of organic and chemical fertilizer application with different ratios on soil characteristics in the peanut system of the northeast region. Three treatments included using chemical fertilizer alone, a combination of chemical fertilizer and wet organic fertilizers prepared with HTC, and wet organic fertilizers without any additional fertilizer. We investigated peanut yield, soil physicochemical properties, and microbial activity, quantity, and community composition. We hypothesized that OF substitution for chemical fertilizer at low doses has the potential to enhance peanut yield, maintaining soil quality and restoring soil health. We also predicted that our data should provide a theoretical basis for regulating the degraded arable land quality in China's black soil region.

2. Materials and Methods

2.1. Experimental Site Overview and Experimental Design

The experiment was conducted in the spring of 2022 in Fujia Town, Changtu County, Tieling City, Liaoning Province. The location is in the northwestern part of Changtu (43°30′ N, 123°79′ E), with an average annual temperature of 7 °C, an average annual precipitation of 639.2 mm, and an elevation of approximately 500 m above sea level. Additionally, the region experiences an annual frost-free period of about 143 days. The soil type is aeolian sandy soil with a sandy loam texture.

The present study involved three treatments: chemical fertilizer (PCF), organic fertilizer replacing 50% of the chemical fertilizer (PR), and organic fertilizer alone (POF). Each treatment set had three replicates, and each experimental area was 500 m^2. Fertilizers were applied as base fertilizers before seeding. The PCF treatment was applied using a commercially available potassium sulfate compound fertilizer (12–18–15) at a rate of 750 kg·ha^{-1}: Urea, 200 kg·ha^{-1}; P_2O_5, 300 kg·ha^{-1}; and K_2O, 250 kg·ha^{-1}. The PR organic substitution treatment involved a combination of chemical fertilizers and organic fertilizers derived from chicken manure sources produced through high-temperature aerobic fermentation. In this case, potassium sulfate compound fertilizers were used at a treatment rate of 375 kg·ha^{-1}, while organic fertilizers were applied at a rate of 2250 kg·ha^{-1}. POF plots were applied with organic fertilizers at a rate of 4500 kg·ha^{-1}. Specific fertilizer application rates are shown in Table 1.

Table 1. Fertilizer rates in each treatment (kg·ha^{-1}).

Treatment	N (kg·ha^{-1})		P_2O_5 (kg·ha^{-1})		K_2O (kg·ha^{-1})	
	F	OM	F	OM	F	OM
PCF	200	0	300	0	250	0
PR	100	106.9	150	123.9	125	68.6
POF	0	213.8	0	247.8	0	137.2

NOTE: F: fertilizer; OM: organic material.

The tested peanut variety was "308". Chemical fertilizers were purchased from local agricultural stores. Organic fertilizers were provided by Liaoning ChengNuo Agricultural Development Technology Co., Tieling, China. The experimental site was managed using local peanut cultivation practices. The chemical properties of the tested soils and organic fertilizers are shown in Table 2.

Table 2. Chemical properties of tested soil and organic fertilizer.

Treatment	pH	SOC	TN	TP	TK	AN	AP	AK
		$g \cdot kg^{-1}$				$mg \cdot kg^{-1}$		
Soil	6.38	5.00	//	//	//	41.06	51.85	104.55
Organic Fertilizer	8.1	393.0	47.5	55.1	30.5	//	//	//

NOTE: SOC: organic carbon; TN: total nitrogen content; TP: total phosphorus content; TK: total potassium content; AN: alkali-hydrolyzed nitrogen; AP: available phosphorus content; AK: available potassium content.

2.2. Sample Collection and Processing

We collected peanut samples and soil samples in October 2022.

Peanut Sample Collection: Three sample squares were randomly selected in each treatment plot, and a sample area of 1 m × 1 m was designated for collecting peanut samples. Peanut samples were weighed after removing impurities. The moisture content of the peanuts was determined using a moisture meter. The yield was calculated as follows: Theoretical yield ($kg \cdot ha^{-1}$) = Yield per plant × Number of plants ÷ 666.7.

Soil Sample Collection: We randomly collected soil at approximately 10 points from each plot and combined it to create a soil sample. After removing peanut crop residues from the soil surface, soil samples (0–20 cm depth) were collected using a 3 cm diameter soil auger.

We removed stones, stalks, and root fragments from the soil samples, and then homogenized and sieved the soil samples (<2 mm). Soil samples were divided into three parts. The first part was placed in a self-sealing bag and stored in a box filled with dry ice (CO_2). It was then promptly sent to the laboratory for the extraction of soil microbial DNA and the determination of microbial abundance and diversity. The second part was stored in a refrigerator (4 °C) for the determination of soil enzyme activity within two weeks. The third part was air-dried and then divided into two subsamples. One subsample was prepared for the determination of soil physiochemical properties, while another subsample was ground using a ball mill and sieved to <0.15 mm for the analysis of soil total nitrogen, phosphorus, and potassium.

2.3. Analysis of Soil Physical and Chemical Properties

Soil pH was measured (water–soil ratio: 2.5:1) using a pH meter. The total carbon and total nitrogen content in the soil were determined using an elemental analyzer (Vario MACRO cube, Elementar, Germany). The soil organic carbon (SOC) content was determined using the $K_2Cr_2O_7$ oxidation method [35].

A total of 0.1 g of air-dried soil (<0.149 mm) was mixed with 2 mL hydrofluoric acid, 1.5 mL hydrochloric acid, and 4.5 mL nitric acid, and the digest was prepared using an inductively coupled plasma–optical emission spectrometer (Milestone ETHOS UP Microwave Dissolver, Nanjing, China) [36]. The total phosphorus content was determined using the molybdenum blue colorimetric method at 880 nm [37]. The total potassium content was determined using a flame photometer.

Soil alkali-hydrolyzable nitrogen was determined using the Alkaline Dissolution Diffusion Method [38]. The available phosphorus content of the soil was determined using the molybdenum blue colorimetric method at 880 nm [37]. The available potassium content in the soil was determined with a flame brightness meter [39].

2.4. Analysis of Soil Enzyme Activity

We investigated the activities of several soil extracellular enzymes closely related to soil carbon accumulation, including α-1,4-glucosidase, β-1,4-N-acetylaminoglucosidase, β-1,4-glucosidase, polyphenol oxidases (PODs), and peroxidases (PPOs). Enzymes play a crucial role in the bodies of microorganisms. They break down organic matter, synthesize biomolecules, and regulate the intracellular environment. Dehydrogenase (DHA) activity was determined using the 2,3,5-triphenyl tetrazolium chloride (TTC) method as a substrate. The samples were incubated at 37 °C for 24 h, and the colorimetric analysis was performed at 485 nm [40]. Soil β-1,4-glucosidase catalyzes the terminal reactions in soil organic matter (SOM) decomposition. α(β)-glucosidase (αG, βG), α(β)-galactosidase (αGal, βGal), and N-acetyl-β-D-amino glucosidase were tested using p-nitrophenyl-α(β)-D-glucoside, p-nitrophenyl-α(β)-D-galactoside, and β-N-acetyl-aminoglucoside as substrates, respectively. The enzymes were incubated at 37 °C for 1 h, and the colorimetry was determined using a colorimetric method at 400 nm. Cellulase activity was measured at 710 nm using a colorimetric assay with carboxymethyl cellulose sodium salt as the substrate, and incubated for 24 h at 37 °C. Xylanase (XYL) was measured using colorimetry at 710 nm after incubation at 50 °C for 24 h with xylan as the substrate [39]. Polyphenol oxidase and peroxidase (POD) were measured using colorimetry at 485 nm. The enzymes were incubated with L-3,4-dihydroxyphenylalanine and acetate buffer at 25 °C for 0.5 h [41].

2.5. DNA Extraction and High-Throughput Sequencing

Microbial DNA was extracted from cryopreserved soil samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocols. The V4-V5 region of the bacteria 16S rDNA was amplified by PCR (95 °C for 2 min; followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min) using primers 515F (5′-barcode-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′), where the barcode was an eight-base sequence unique to each sample. Fungi ITS was amplified using the primers ITS1 (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-TGCGTTCTTCATCGATGC-3′).

PCR reactions were performed in triplicate using a 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) following the manufacturer's instructions.

2.6. Statistical Analyses

We used Tukey's test to conduct a one-way ANOVA. Multiple comparisons were conducted using the LSD method to identify statistical differences among various treatments; a significance level of $p < 0.05$ was considered. All statistical analyses were performed using SPSS 21.0 software (SPSS, Chicago, IL, USA).

Microbial communities were represented based on stacked bar plots and histograms, which provided significant insight into the variability between species. The alpha diversity index was calculated using the R package "vegan". PCoA was conducted using the "ape" package in R. To assess the impact of organic fertilizer on microbial beta diversity, we employed alternative principal components derived from distances other than the Euclidean distance to detect variations in the composition of the sample communities through dimensionality reduction. The correlation analysis heatmap was generated using the "pheatmap" package in the R language. Correlation thermograms were more effective in analyzing the relationships between soil physicochemical properties, soil enzyme activities, and microbial diversity.

3. Results

3.1. Effects of Low-Dose Organic Fertilizer on Soil Microbial Activities and Absolute Abundances

Compared to the PCF treatment, the POF and PR treatments showed similar effects on soil dehydrogenase activity. The soil dehydrogenase activity of the PR treatment was significantly higher, by 1.6%, compared to the POF treatment (Figure 2a). Compared with the PCF treatment, the PR and POF treatments tended to decrease the absolute abundance ratio of fungi and bacteria (Figure 2b), although there were no significant differences in the absolute abundance of soil bacteria and fungi among the three treatments (Figure 2c,d).

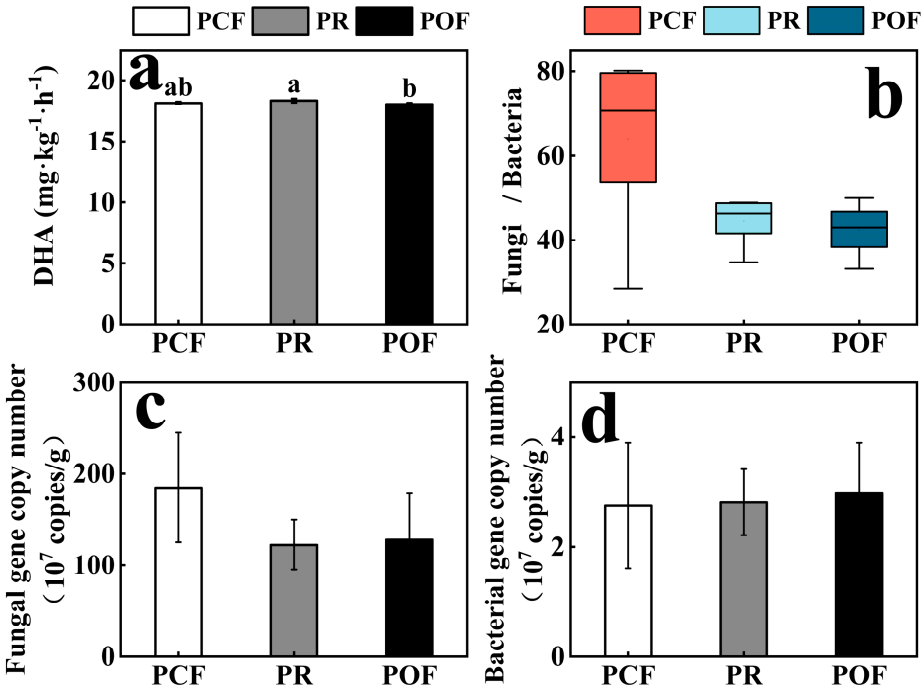

Figure 2. Effects of low-dose organic fertilizer on soil microbial activities, and gene copy number of soil bacteria and fungi. Note: PCF: chemical fertilizer application; POF: organic fertilizer application; PR: organic fertilizer replaces 50% chemical fertilizer. DHA: dehydrogenase. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

The activity of the most tested soil enzymes related to carbon transformation did not vary significantly under the PR and POF treatments, including β-glucosidase, β-N-acetyl-glucosaminidase, α-galactosidase, β-galactosidase, polyphenol oxidase, peroxidase, xylanase, and cellulase (Figure 3b–i). The activity of α-glucosidase in the PR plots was significantly higher than that in the POF plots. There were no significant differences in soil α-glucosidase activity between the PCF and PR or POF plots.

3.2. Effects of Low-Dose Organic Fertilizer on Soil Microbial Diversity and Structures

Both the POF and PR treatments significantly enhanced the α-diversity of the bacterial and fungal communities. Compared to the chemical fertilizer application, the PR and POF applications significantly increased the α-diversity index of the soil bacteria and fungi (Figure 4). The PR treatment increased the Chao1 index, the Shannon index, the observed species index, and the PD faith index of the soil bacteria, as well as the observed species index of the soil fungi community. The POF treatment increased the observed species index and PD faith index of the soil bacteria, as well as the observed species index of the soil fungi.

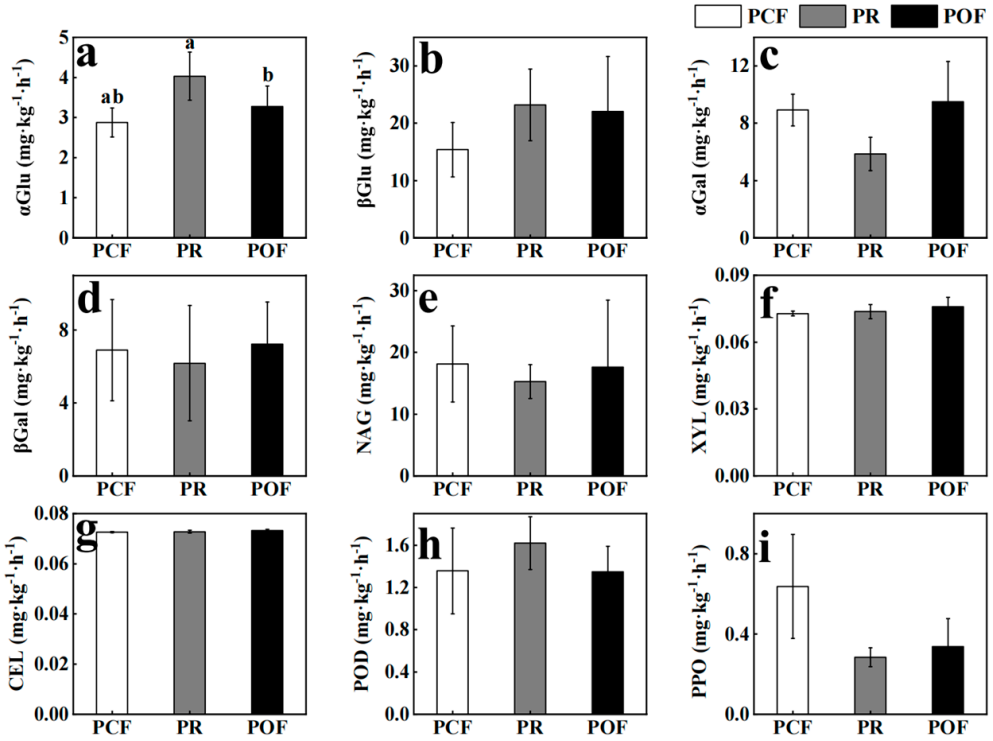

Figure 3. Effects of low-dose organic fertilizer on soil enzyme activity. Note: PCF: chemical fertilizer application; POF: organic fertilizer application; PR: organic fertilizer replaces 50% chemical fertilizer; αGlu: α-glucosidase; βGlu: β-glucosidase; NAG: β-N-acetyl-glucosaminidase; αGAL: α-galactosidase; βGAL: β-galactosidase; XYL: xylanase; CEL: cellulase; POD: peroxidase; PPO: polyphenol oxidase. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

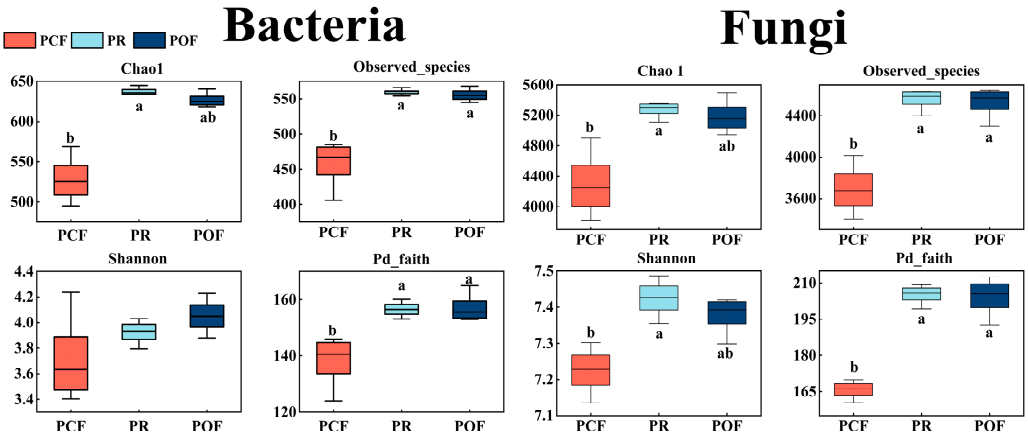

Figure 4. Effects of low-dose organic fertilizer on alpha diversity of soil bacterial and fungal communities. Note: PCF: chemical fertilizer application; POF: organic fertilizer application; PR: organic fertilizer replaces 50% chemical fertilizer. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

The predominant phylum of soil bacteria in the three treatments was similar. The main bacterial communities at that time included Proteobacteria, Acidobacteria, Actinobacteriota, Chloroflexi, Bacteroidetes, Gemmatimonadota, Myxococcota, Firmicutes, Verrucomicrobiota, and Patescibacteria (Figure 5a). Compared with the PCF treatment, the PR treatment significantly increased the relative abundance of Chloroflexi ($p < 0.05$), and the POF treatment significantly increased the relative abundance of Gemmatimonadetes ($p < 0.05$). The

soil bacteria genera in the test treatments mainly included *Bradyrhizobium*, *Mycobacterium*, *Gemmatimonas*, *Sphingomonas*, *Haliangium*, *RB41 bacterial genus*, and *Candidatus Solibacter* (Figure 5b). The POF treatment significantly increased the relative abundance of *Gemmatimonas* and *Sphingomonas*. The PR treatment significantly decreased the relative abundance of *Mycobacterium* compared to the POF treatment ($p < 0.05$) (Figure 5b).

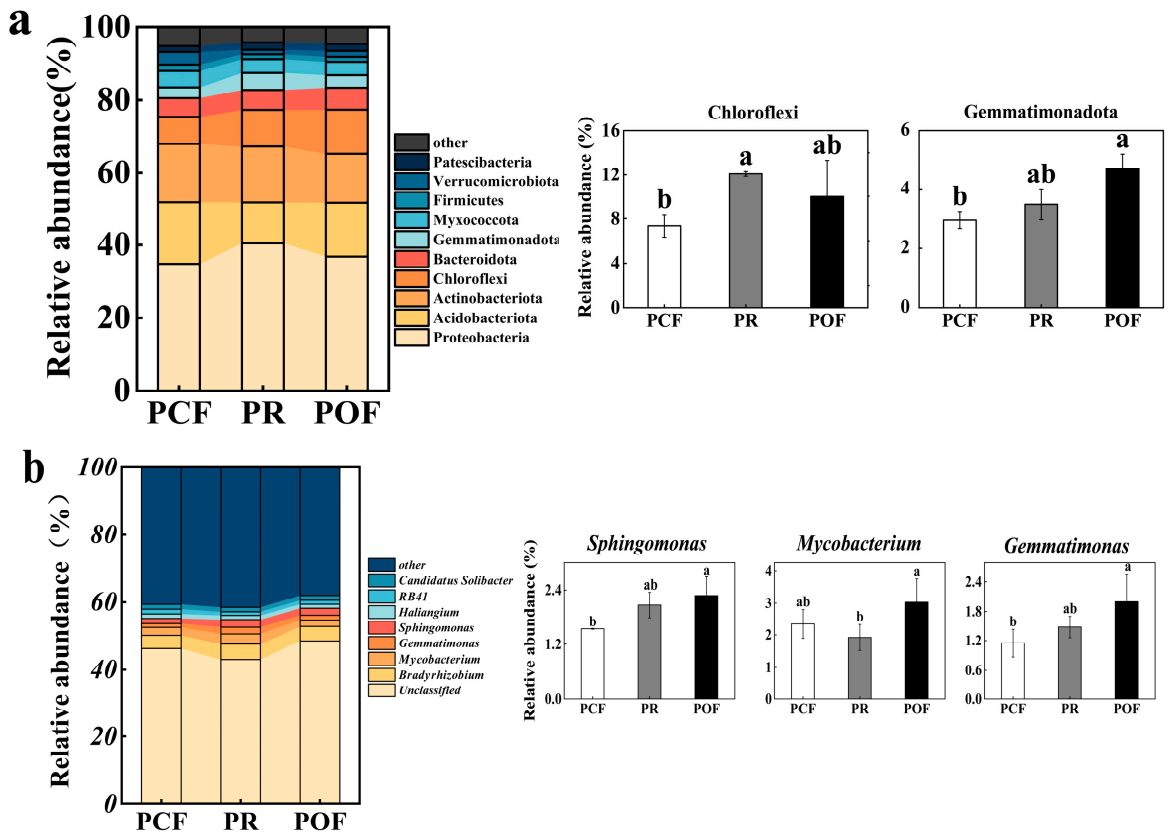

Figure 5. Effects of low-dose organic fertilizer produced by high-temperature aerobic fermentation on the relative abundance of soil bacteria at the phylum (**a**) and genus (**b**) levels. Note: PCF: chemical fertilizer application; POF: organic fertilizer application; PR: organic fertilizer replaces 50% chemical fertilizer. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

There were three dominant phyla in the fungi community in the PCF, PR, and POF treatments, including Ascomycota, Basidiomycota, and Mucoromycota (Figure 6a). The POF treatment decreased the relative abundance of soil Mucoromycota and Basidiomycota significantly. The PR treatment also decreased the relative abundance of soil Mucoromycota. The PR and POF treatments significantly increased the relative abundance of Ascomycota ($p < 0.05$) (Figure 6a). At the genus level, there were eight species of dominant fungal genera, including *Tausonia*, *Ascobolus*, *Mortierella*, *Leptosphaerulina*, *Fusarium*, *Trichocladium*, *Cryptococcus*, *Epicoccum*, and *Pseudombrophila* (Figure 6b). The PR and POF treatments significantly decreased the relative abundance of soil *Trichocladium* ($p < 0.05$). The POF treatment decreased the relative abundance of *Mortierella*. The PR treatment significantly decreased the relative abundance of *Cryptococcus* and increased the relative abundance of soil *Leptosphaerulina* ($p < 0.05$). The POF and PR treatments did not distinctly affect *Ascobolus* and *Fusarium*.

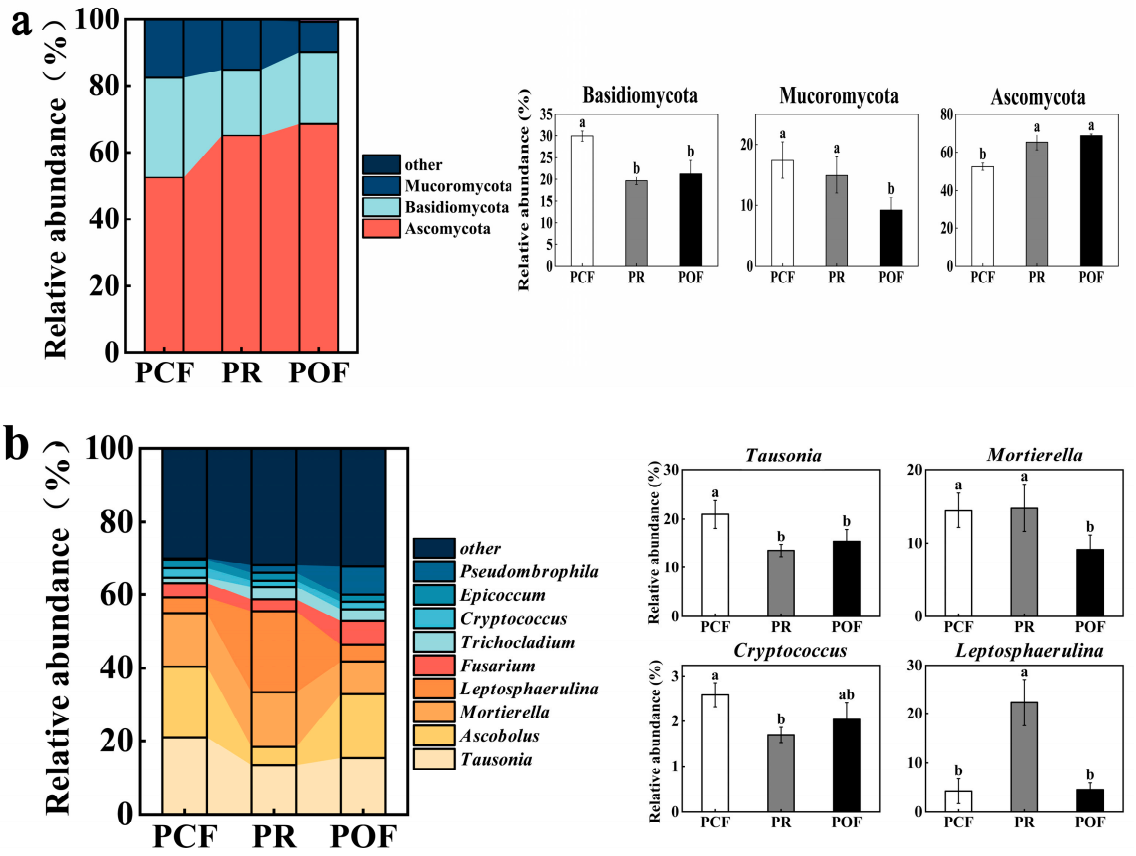

Figure 6. Effects of low-dose organic fertilizer produced by high-temperature aerobic fermentation on the relative abundance of soil fungi at the phylum (**a**) and genus (**b**) levels. Note: PCF: chemical fertilizer application; POF: organic fertilizer application; PR: organic fertilizer replaces 50% chemical fertilizer. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

3.3. Correlation Analysis between Soil Microbial Communities, Chemical Properties, and Enzyme Activities

According to the heatmap of the correlation analysis, the results revealed that soil DHA activity was significantly positively affected by the total potassium content, while it was negatively affected by pH (Figure 7a). The activities of soil α-Gal, peroxidase, and polyphenol oxidase were significantly positively affected by the pH, total phosphorus, and available potassium. The activities of soil β-Glucosidase, N-acetyl-glucosaminidase, and xylosidase were positively correlated with available phosphorus. The activities of soil α-Gal, NAG, and XYL were positively correlated with the available potassium content. There were no significant correlations between microbial community diversity and structure, except for a significant negative correlation between the soil organic carbon content and fungal Shannon index ($p < 0.05$) (Figure 7b). A principal coordinate analysis (PCoA) could account for 64% of the variation in the soil bacterial population among the test treatments (Figure 7c). In contrast, the three fertilization treatments were not significantly different on the PC2 axis, which accounted for only 14% of the variation in the bacterial community. On the first principal component axis, a statistically significant separation was observed between the fertilizer treatment and the replacement organic fertilizer treatment. This was confirmed by a *p*-value of 0.017, which is below the 0.05 threshold. The PCoA of the soil fungal communities from the test treatments revealed that the two principal components accounted for 36% of the variation in fungal communities (Figure 7d). The test treatments did not show significant differentiation on either the PC1 or PC2 axes. Moreover, the *p*-value (0.087) was found to be greater than 0.05 (Figure 7d).

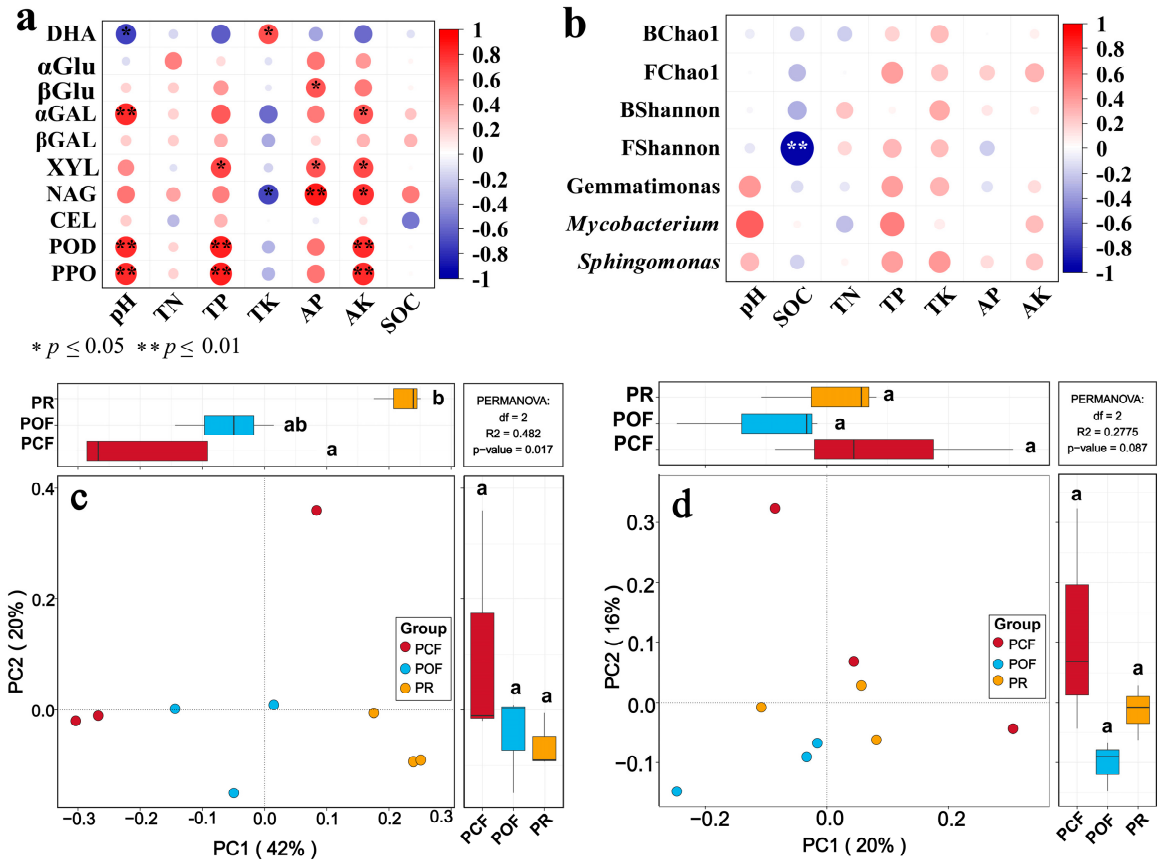

Figure 7. Correlation analysis between soil chemical properties and enzyme activities (**a**). Correlation analysis between soil chemical properties and microbial communities (**b**). Principal coordinate analysis (PCoA) of bacteria and fungi in organic fertilizer-applied soil. Note: PCF: chemical fertilizer application; POF: organic fertilizer application; PR: organic fertilizer replaces 50% chemical fertilizer. SOC: organic carbon; TN: total nitrogen content; TP: total phosphorus content; TK: total potassium content; AP: available phosphorus content; AK: available potassium content; DHA: dehydrogenase; αGlu: α-glucosidase; βGlu: β-glucosidase; αGAL: α-galactosidase; βGAL: β-galactosidase; NAG: β -N-acetyl-glucosaminidase; XYL: xylanase; CEL: cellulase; PPO: polyphenol oxidase; POD: peroxidase; BChao1: Bacterial Chao1; FChao1: Fungus Chao1; BShannon: Bacterial Shannon; FShannon: Fungus Shannon. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

The soil chemical properties and enzyme activities were considered as explanatory variables, while the relative abundance of dominant phyla was used for redundancy analysis (Figure 8). The explanatory rate of RDA 1 in the redundancy analysis of the dominant soil bacteria and chemical properties was 72.79%, while the explanatory rate of RDA 1 in the redundancy analysis was 80.84%. These rates exceeded 70% and are suitable for redundancy analysis. TP exhibited a significant correlation with the predominant bacteria, suggesting that TP exerted a pronounced influence on Gemmatimonadota (Figure 8a). Additionally, α-galactosidase activity was positively correlated with β-N-acetyl-glucosaminidase activity. Both α-galactosidase activity and β-N-acetyl-glucosaminidase activity showed a significant relationship with the dominant bacteria (Figure 8b) ($p < 0.05$).

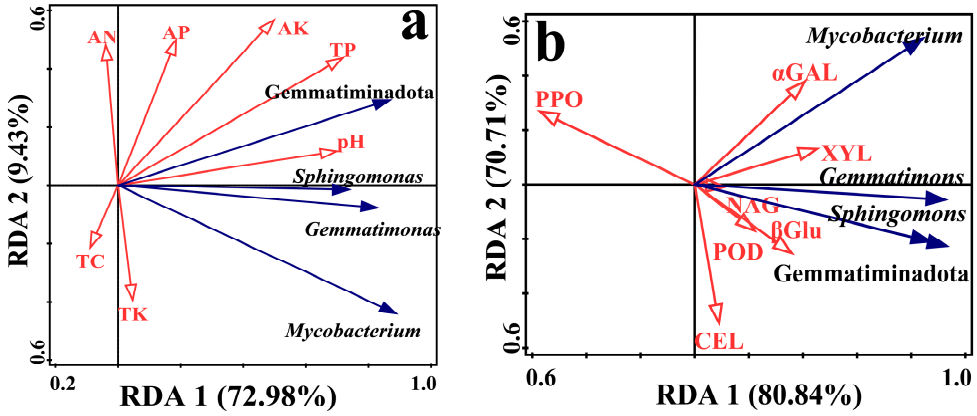

Figure 8. Redundant Disk Array Controller (RDA) of microbial principal components and environmental factors and enzyme activities in organic fertilizer-applied soil. βGlu: β-glucosidase; NAG: β-N-acetyl-glucosaminidase; αGAL: α-galactosidase; XYL: xylanase; CEL: cellulase; POD: peroxidase; PPO: polyphenol oxidase. TC: total carbon content; TP: total phosphorus content; TK: total potassium content; AP: available phosphorus content; AK: available potassium content; AN: alkali-hydrolyzed nitrogen content.

4. Discussion

4.1. Effect of Organic Fertilizer Treatment on Basic Chemical Properties and Enzyme Activities of Soil

Compared to the chemical fertilizer treatment in the present study, the PR and POF treatments significantly increased peanut yield (Figure S2), which was attributed to OF application, which resulted in improvements in soil properties [42–44]. Previous studies indicated that OF treatment improves soil physicochemical properties, creates a favorable soil environment, activates effective soil nutrients, and provides essential nutrients for plant growth and microbial activity [45,46]. The present study showed that both the organic fertilizer (POF) and organic fertilizer replacement (PR) treatments at low doses increased soil pH and TP significantly, while the soil organic matter content increased insignificantly (Figure S1). Both the nutrients provided by the OF treatments and applied for microbial growth resulted in a direct increase in soil TP [47–51]. OFs are rich in organic matter, which generates various organic acids during the decomposition process, contributing to a buffering effect on soil pH [52–54]. Maintaining appropriate soil pH is crucial for the normal growth and development of crops [55–57]. Excessive or insufficient soil pH affects crop nutrient absorption and utilization. As is well known, soil extracellular enzyme activities are closely related to soil fertility. OF treatments increase soil α-glucosidase activities related to soil carbon accumulation even with low-dose applications [58–61]. Soil α-glucosidase plays an important role in soil by catalyzing the hydrolysis of glucosidic bonds, providing energy and essential nutrients for plants, and thereby promoting plant growth and development [62,63]. OF application in our experiment had relatively little impact on most soil enzymes, which was attributed to the low dose of the OF application [64,65]. OF amendments supply specific amounts of available nitrogen, phosphorus, carbon, and other sources to the oligotrophic environment, altering both biotic and abiotic (temperature, moisture, pH, nutrient availability) factors. Correlation analysis revealed that soil pH positively affected the activities of soil α-Gal, POD, and PPO, but negatively affected soil DHA activity. Variations in microbial activity were driven by both abiotic and biotic factors [66]. Essential nutrients required for microbial growth and affecting microbial community composition were provided by enzymatic degradation [67,68]. OF at low doses led to the activation of numerous minor potentially active microorganisms that responded quickly to additional substrate input [69].

4.2. Effect of Organic Fertilizer Treatments on Soil Microbial Community Activity, Abundance, Diversity, and Composition

Soil microorganisms play a crucial role in decomposing organic matter and driving nutrient cycling in agroecosystems [70,71]. Soil microorganisms' activity was characterized as soil dehydrogenase activity in the present study [72–74]. Compared to the chemical fertilizer treatment, the POF and PR treatments did not significantly alter soil dehydrogenase activity, although the soil dehydrogenase activity of the PR treatment was significantly higher than that of the POF treatment. The present study did not reveal significant changes in soil bacterial and fungal populations among the treatments. The absence of significant variations in the abundance of soil fungi and bacteria indicated that OF incorporated at a low dose had an inconspicuous impact on the population of soil microorganisms. The aeolian sandy soil studied in the present experiment is a typical barren soil, which has a large soil particle diameter and small pore space, resulting in poor water-holding and nutrient-holding capacity. Additionally, aeolian sandy soil has relatively low organic matter content, which results in a reduced microbial population [75–77]. The unfavorable conditions hindered microbial reproduction and survival. Fungi convert organic matter into simpler substances in the initial decomposition stage, and bacteria further break down the simpler substances released in the decomposition products of fungi and other microorganisms in subsequent stages. On the other hand, the downward trend of the ratio of fungi/bacteria (F/B) in the POF and PR treatments indicated that the soil organic matter decomposition and nutrient cycling speed were accelerated, leading to enhanced soil fertility [78,79]. OFs provide nutrients, create a suitable environment, and enhance soil management., further promoting microbial survival and activity [80,81].

The present study applied OF at a low dose in aeolian sandy soil, and our results regarding soil microorganisms were consistent with many previous studies [31,82,83]. The POF and PR treatments increased the community richness, observed species index, PD faith index, and α-diversity index of the soil bacteria significantly. Additionally, the POF and PR treatments also increased the observed species index and PD faith index of the soil fungi.

The low-dose OF treatment did not alter soil microbial community composition significantly, but it did vary the main dominant communities after half a year. Proteobacteria had the highest abundance in the present study. Proteobacteria is composed of mesophilic and neutrophilic bacteria. Proteobacteria belong to Gram-negative bacteria and reproduce rapidly [84]. Proteobacteria play a role in the biological cycling of essential mineral nutrients in soil. A high proportion of Proteobacteria in soil is beneficial for maintaining soil fertility and promoting plant growth [85]. Acidobacteria represent about 20% of the total soil bacterial communities. Actinobacteria are widely distributed in arid and semi-arid regions, exhibiting heat and drought resistance. The mycelium of Actinobacteria degrades insoluble substances in soil, provides nutrients for cells, and plays an important role in the cycling of carbon, nitrogen, and other elements, thereby improving soil productivity. Chlorobacteria are involved in soil carbon cycling processes, including CO_2 fixation, CO oxidation, and CH4 oxidation (supported by metagenomic evidence), and the degradation of macromolecules such as cellulose. Chlorobacterium also plays a role in the biogeochemical cycling of elements such as nitrogen (N) and sulfur (S). Post-remediation (PR) treatment notably elevated the relative abundance of Chloroflexi. The Bacteroidetes phylum efficiently utilizes soil organic matter. Organic fertilizers provide the necessary nutrients for the growth and reproduction of Bacteroidetes. The POF treatment significantly increased the relative abundance of Gemmatimonadota, indicating enhanced soil nitrogen fixation, secretion of antibiotics, production of hormone-like substances, and biological control functions, as well as the ability to decompose organic matter [63]. Gemmatimonadetes produce various secondary metabolites that effectively inhibit pathogenic microorganisms [86–88]. Many Firmicutes have good halophilic and alkaline characteristics, produce spores with high salt tolerance, and adapt to drought and saline–alkali stresses. Unlike most

agricultural soils, the test soil contained no Planctomycetes; instead, it had Myxococcota and Patescibacteria.

The genera of the soil bacteria in the test treatments were mainly *Bradyrhizobium*, Mycobacterium, *Gemmatimonas*, *Sphingomonas*, *Haliangium*, *RB41 bacterial genus*, and *Candidatus Solibacte*. The genus Bradyrhizobium of Proteobacteria is best known for its N2-fixing members that nodulate legumes. The POF treatment significantly increased the relative abundance of *Gemmatimonas* and *Sphingomonas*, indicating efficient metabolic regulation mechanisms, enormous potential for environmental remediation, and the promotion of plant growth. The POF treatment significantly increased the relative abundance of *Mycobacterium* compared to the PR treatment.

Ascomycota was the most prevalent major fungal phylum, followed by Basidiomycota and Mucoromycota. Ascomycetes dominated in each treatment, and both the PR and POF treatments significantly increased the relative abundance of Ascomycota, which aligns with the findings of [89]. The ecological adaptability of soil dominated by Ascomycota is strong due to its rapid evolution speed and rich species diversity. The PR and POF treatments significantly decreased the relative abundance of soil Mucoromycota, and the POF treatment also decreased Basidiomycota. Among the dominant fungal genera, *Ascobolus*, *Epicocum*, *Leptosphaerulina*, *Fusarium*, *Cryptococcus*, and *Pseudomycohila* belong to Ascomycota, further indicating that Ascomycota was the main dominant fungal phylum in the study soil. The relative abundance of fungal genera was less affected by the application of organic fertilizers compared to bacteria.

PCoA indicated that both the POF and PR treatments had a significant impact on the composition of soil bacterial communities. However, there were no notable variations in soil fungal community composition following the application of organic fertilizers. The response of soil bacterial communities to low-dose organic fertilizer application was faster than that of soil fungi. The soil is developing towards a eutrophication environment. The low-dose application of organic fertilizer with high nutrient levels improved the structure of the soil bacterial community and enhanced the metabolic activity of soil microorganisms.

Organic fertilizer application enhanced soil enzyme activity to a certain extent, strongly affecting the composition of soil microbial communities, while having a weaker impact on soil physical and chemical properties and microbial activity, quantity, and diversity. Low-dose application of organic fertilizers with high nutrient levels only lasts for one growing season. Further research on organic fertilizers with high nutrient levels produced by high-temperature container fermentation is needed.

5. Conclusions

Our results indicated that low-dose organic fertilizer prepared from the manure of laying hens and broilers activated certain microbial metabolic pathways, positively affecting soil microbial diversity. The application of low-dose organic fertilizer enhanced specific enzyme activity such as α-glucosidase and increased the species richness and α-diversity of soil bacteria and fungi. Low-dose organic fertilizer and its alternative treatment increased peanut yield significantly. The nutrients and other components supplied by organic fertilizers, as well as the nutrients released, promoted the soil microbial communities' activity and proliferation and affected the soil chemical properties and enzyme activities, thereby promoting crop growth. The impact of low-dose organic fertilizer on soil chemical properties, microbial activity, microbial gene copy numbers, most soil enzyme activities, and soil fungal communities was relatively small. However, the overall effect indicated that low-dose organic fertilizer prepared from the manure of laying hens and broiler by high-temperature fermentation technology should be considered as a sustainable soil amendment. Organic fertilizers prepared from the manure of laying hens and broilers have higher nutrient content, play an important role in providing nutrients for crops, and have the potential to enhance soil microbial community activity and improve soil health; therefore, they could largely replace chemical fertilizers.

There are various sources of organic fertilizers, and different techniques should also be used to treat the raw materials. Researchers and farmers should classify and apply organic fertilizers accurately based on their type, crop needs, and soil conditions. Proper application of organic fertilizers is essential to achieve better enhancement effects.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14040765/s1, Figure S1: Effects of low-dose organic fertilizer produced by high-temperature aerobic fermentation on soil physicochemical properties. Figure S2: Effects of low-dose organic fertilizer produced by high-temperature aerobic fermentation on peanut yields.

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