Effects of Vinegar Residue Spent Mushroom Substrate on Soil Microorganisms and Enzyme Activities of Three Crops (Postprint)

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Abstract

To investigate the effects of vinegar residue mushroom bran as a biofertilizer on soil fertility and to provide a scientific basis for addressing environmental pollution from edible fungus residue and its effective utilization, this study conducted field experiments to measure soil urease, sucrase, and catalase activities, populations of bacteria, actinomycetes, and fungi, as well as microbial biomass carbon and nitrogen contents in rhizosphere soil at different growth stages following basal application of vinegar residue mushroom bran to three crops: maize, sorghum, and waxy maize. The results showed that: 1) Vinegar residue mushroom bran significantly increased the populations of bacteria, actinomycetes, and fungi in crop rhizosphere soil. Throughout the entire growth period, the bacterial population in soil amended with vinegar residue mushroom bran increased by 32%~54% compared to the control; the actinomycetes population exhibited a significant increase at the maturity stage, with the maximum increase of 101% observed in maize field soil; and the fungal population displayed an overall trend of initial increase followed by decline. 2) The application of vinegar residue mushroom bran enhanced the activities of urease, catalase, and sucrase in crop rhizosphere soil. The increase rates of urease activity in soils planted with sorghum, maize, and waxy maize were 239%, 189%, and 185%, respectively; soil catalase activity in the three crops peaked at the heading stage, with a maximum increase rate of 40%; soil sucrase activity in the three crops showed different trends across growth stages, with relatively stable changes observed in maize at various growth stages, exhibiting increase rates of 38%, 28%, and 48%, respectively. 3) The application of vinegar residue mushroom bran increased microbial biomass carbon and nitrogen contents in crop rhizosphere soil, with increases in soil microbial biomass carbon ranging from 58.10 to 407.67 mg·kg ^{1} and microbial biomass nitrogen ranging from 11.98 to 27.55 mg·kg ^{1} across different growth stages for the three crops. These results demonstrate that the

application of vinegar residue mushroom bran can enhance the sustainability of soil productivity, thereby achieving the effect of protecting and improving the soil environment. Furthermore, this study provides a scientific basis for the effective utilization of vinegar residue mushroom bran.

Full Text

Preamble

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Effect of Vinegar Residue Fungus Chaff on Soil Microbial Populations and Enzyme Activities in Three Crop Systems

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Abstract

This study investigated the effects of vinegar residue fungus chaff as a biofertilizer on soil fertility, providing scientific evidence for addressing environmental pollution from edible fungus substrate waste and its effective utilization. Through field experiments, we examined soil urease, sucrase, and catalase activities, populations of bacteria, actinomycetes, and fungi, and microbial biomass carbon and nitrogen content in the rhizosphere soils of three crops (maize, sorghum, and waxy maize) at different growth stages following basal application of vinegar residue fungus chaff.

The results demonstrated three key findings. First, vinegar residue fungus chaff significantly increased rhizosphere soil populations of bacteria, actinomycetes, and fungi. Throughout the growth period, soil bacterial counts increased by 32–54% compared to the control. Actinomycete populations showed the most pronounced increase at maturity, with the greatest enhancement (101%) observed in maize fields. Fungal populations exhibited an overall trend of initial increase followed by decline. Second, application of vinegar residue fungus chaff enhanced soil enzyme activities. Urease activity increased by 239%, 189%, and 185% in sorghum, maize, and waxy maize soils, respectively. Catalase activity peaked at the heading stage for all three crops, with a maximum increase of 40%. Sucrase activity showed distinct trends across growth stages, with relatively stable changes in maize soils exhibiting increases of 38%, 28%, and 48%

at different stages. Third, microbial biomass carbon and nitrogen contents increased following fungus chaff application, with carbon content rising by 58.10–407.67 mg · kg ^{1} and nitrogen content by 11.98–27.55 mg · kg ^{1} across the growth periods of the three crops.

These findings indicate that vinegar residue fungus chaff application can enhance the sustainability of soil productivity while protecting and improving soil environmental quality. This research provides a scientific basis for the effective utilization of edible fungus substrate waste.

Keywords: Vinegar residue fungus chaff; Soil enzyme activity; Soil microbial population; Soil microbial biomass carbon and nitrogen; Maize; Sorghum; Waxy maize

Introduction

Vinegar residue, the solid byproduct generated from starch-based vinegar production, is typically discarded as waste, leading to environmental pollution and resource waste when it accumulates and molds. To transform this waste into a valuable resource and mitigate environmental contamination, numerous researchers have investigated the cultivation of edible fungi using vinegar residue, achieving promising results. The spent substrate remaining after mushroom harvesting—referred to as vinegar residue fungus chaff—contains higher crude protein and fat content but significantly lower crude fiber compared to other types of fungus chaff. However, improper disposal of this material continues to pose environmental challenges. Applying vinegar residue fungus chaff as an organic fertilizer represents a viable solution, as the spent substrate has a loose texture and contains various organic compounds and mineral elements that can conserve soil moisture and nutrients, effectively ameliorate nutrient deficiencies, and enhance soil fertility. Consequently, it serves as a functional, high-quality soil amendment and substrate material.

Soil microorganisms constitute a vital component of soil ecosystems, with microbial populations and enzyme activities serving as important indicators for evaluating soil fertility. Previous research has extensively documented soil microbial and enzymatic responses to organic amendments. Studies on rice straw return have demonstrated changes in microbial and enzymatic biological indicators that illustrate soil fertility improvement effects. Research on mushroom residue application has shown significant increases in bacterial populations, reflecting favorable microbial ecological characteristics. Long-term fertilization studies on maize have revealed that soil enzyme activities align with crop growth dynamics, and that long-term application of NPK fertilizers can substantially improve soil biochemical fertility. Investigations on distiller' s grains bio-organic fertilizer have similarly demonstrated significant enhancements in soil microbial populations, enzyme activities, and crop yields.

Most studies indicate that organic fertilizer application significantly increases soil microbial biomass carbon and nitrogen contents and enzyme activities.

Some research suggests that organic or combined organic-inorganic fertilization markedly increases bacterial, fungal, and actinomycete populations, though other studies report that while bacterial numbers increase significantly with organic-inorganic compound fertilizer application, fungal populations may decrease. Soil microorganisms and soil fertility exhibit a mutually promoting and coordinative relationship: high-fertility soils support extensive microbial proliferation, while abundant microorganisms maintain nutrient balance through organic matter decomposition. Applying organic materials improves the soil microbial ecological environment, alters microbial community structure, and enhances soil fertility.

Despite these advances, research on vinegar residue fungus chaff—a potentially valuable secondary organic fertilizer—remains limited, particularly regarding its effects on soil microorganisms and enzymes. Therefore, this study investigated three common crops (sorghum, maize, and waxy maize) to examine how vinegar residue fungus chaff application influences key soil biological indicators, including enzyme activities and microbial population dynamics. The objective was to assess its impact on soil fertility and provide scientific evidence for mitigating environmental pollution from edible fungus substrate waste.

Materials and Methods

1.1 Study Site Description

The field experiment was conducted in 2015 in Jinzhong City, Shanxi Province. The study area has an average annual temperature of 9.9°C, a frost-free period of 171.2 days, and mean annual precipitation of 441.8 mm. The experimental soil was classified as calcic cinnamon soil. On April 8, baseline measurements of the 0–20 cm tillage layer revealed organic matter content of 35.9 $g \cdot kg^{1}$, alkaline hydrolyzable nitrogen of 33.82 $mg \cdot kg^{1}$, available phosphorus of 37.06 $mg \cdot kg^{1}$, available potassium of 278.52 $mg \cdot kg^{1}$, and pH of 7.53.

1.2 Experimental Design

The experiment employed a two-factor split-plot design. The main factor consisted of two levels: with and without vinegar residue fungus chaff application. The subplot factor comprised three crop varieties: maize ('Fushengyuan 55'), sorghum ('Jinzhong 405'), and waxy maize ('Tengnuo 1'). This resulted in six treatments with three replications, totaling 18 plots. Basal fertilization included compound fertilizer (N:P:K = 25:10:16, total nutrients 51%) at 1,000 $kg \cdot hm^{2}$ and chicken manure at 1,500 $kg \cdot hm^{2}$. Vinegar residue fungus chaff, derived from *Pleurotus ostreatus* cultivation on vinegar residue, was applied as organic fertilizer at 25,000 $kg \cdot hm^{2}$. The fungus chaff contained 584.4 $g \cdot kg^{1}$ organic matter, 2.4 $g \cdot kg^{1}$ total nitrogen, 0.33 $g \cdot kg^{1}$ total phosphorus, and 2.2 $g \cdot kg^{1}$ total potassium.

Each plot measured 2 m × 5 m. Before sowing, ridges were prepared and furrows opened with hoes. Compound fertilizer was applied as basal dressing, followed

by the respective fungus chuff treatments. All three crops were sown on April 14. Sorghum was planted at 132,000 plants · hm ² with 0.25 m row spacing and 0.3 m plant spacing. Maize was planted at 65,000 plants · hm ² with 0.4 m row and plant spacing, two seeds per hole, thinned after emergence. Waxy maize was planted at 55,500 plants · hm ² with 0.4 m row spacing and 0.5 m plant spacing. All crops were harvested on August 30. During the growth period, irrigation and regular management practices were implemented. Soil and plant samples were collected during different growth stages: jointing (June 13–20), heading (July 26–August 1), and maturity (August 30) using multi-point sampling methods.

1.3 Laboratory Analyses

1.3.1 Soil Sampling and Preparation Baseline soil samples were collected on April 8 using a diagonal sampling method from the 0–20 cm tillage layer in each plot' s root zone, with approximately 1 kg of soil collected per sample. Samples for nutrient analysis were air-dried in the soil preparation room. Samples for microbial counts, enzyme activity, and microbial biomass carbon/nitrogen determination were placed in sterile sealed plastic bags, thoroughly mixed, and stored at 4°C until analysis.

1.3.2 Soil Microbial Population Determination Microbial populations were determined using the dilution plating method. Soil suspensions were prepared through gradient dilution and enumerated using plate counting techniques. Culture media for quantifying culturable bacteria, fungi, and actinomycetes in fresh soil were beef extract peptone agar, Rose Bengal agar, and modified Gause' s No. 1 medium, respectively.

1.3.3 Soil Microbial Biomass Carbon and Nitrogen Determination Microbial biomass carbon and nitrogen were determined using the chloroform fumigation-extraction method. Contents were calculated as the difference between fumigated and non-fumigated samples, divided by respective recovery coefficients (Kc = 0.38 and Kn = 0.54).

1.3.4 Soil Enzyme Activity Determination Soil urease activity was measured using the indophenol blue colorimetric method and expressed as mg NH -N per gram of soil after 24 hours. Catalase activity was determined using potassium permanganate titration and expressed as mL of 0.1 mol · L ¹ KMnO consumed per gram of soil after 20 minutes. Sucrase activity was measured using the 3,5-dinitrosalicylic acid method and expressed as mg glucose per gram of soil after 24 hours.

1.3.5 Soil Chemical Property Determination Soil organic matter was determined by the potassium dichromate volumetric method. Alkaline hydrolyzable nitrogen was measured by the alkaline hydrolysis diffusion method. Available phosphorus was extracted with sodium bicarbonate, and available potas-

sium was extracted with ammonium acetate and determined by flame photometry.

1.4 Statistical Analysis

Experimental data were analyzed using SPSS 11.5 software.

Results

2.1 Effects of Vinegar Residue Fungus Chaff on Soil Microbial Populations

Soil microorganisms are crucial indicators of soil health and quality. Exogenous microorganisms introduced through amendments interact with indigenous soil microbes in nearly all material cycles and energy metabolism processes. Microbial abundance depends not only on soil texture and fertility status but also on competition for nutrients between crop roots and soil microorganisms.

2.1.1 Effects on Soil Bacterial Populations Bacteria represent the dominant microbial community in crop rhizosphere soils. As shown in , bacterial populations in maize, sorghum, and waxy maize soils exhibited similar fluctuations across growth stages, generally increasing initially then decreasing. The peak bacterial growth occurred at the heading stage, with significant differences between fungus chaff-treated and control plots for maize and sorghum, though not for waxy maize. Bacterial counts were relatively low at the jointing stage, with no significant differences between treatments. At maturity, only sorghum showed significant differences between treated and control plots, while maize and waxy maize did not. Overall, fungus chaff application significantly increased rhizosphere soil bacterial populations, with increases of 32–54% across the three crops at the jointing stage.

2.1.2 Effects on Soil Actinomycete Populations Actinomycetes are the second most abundant soil microbial group after bacteria. shows that actinomycete populations in the rhizosphere soils of all three crops increased continuously throughout the growth period, with the most rapid growth occurring from heading to maturity. Significant differences among crops were observed at each growth stage. Actinomycete counts were low at the jointing stage, with fungus chaff treatments showing lower values than controls for maize and waxy maize. At heading, significant differences between treated and control plots were observed for maize and sorghum, but not for waxy maize. At maturity, actinomycete populations increased substantially with fungus chaff application, with maize showing the greatest enhancement of 101%.

2.1.3 Effects on Soil Fungal Populations Fungal populations were substantially lower than bacterial and actinomycete populations, likely due to inhibitory effects of fungus chaff application on fungal proliferation. As shown in

, fungal populations in the rhizosphere soils of all three crops followed a trend of initial increase followed by decline, rising significantly from jointing to heading then decreasing markedly from heading to maturity. Differences between fungus chaff-treated and control plots varied by stage: no significant differences at jointing for any crop, a significant difference for waxy maize at heading, and a pronounced reduction at maturity with fungus chaff application (decreases of 56.25–76.92%).

2.2 Effects of Vinegar Residue Fungus Chaff on Soil Enzyme Activities

Soil enzymes primarily originate from microbial metabolism, root exudates, and decomposition of plant and animal residues, participating in numerous biochemical processes and material cycles including litter decomposition and synthesis of various organic compounds. They represent important indicators for soil fertility assessment. Catalase activity typically reflects soil purification capacity by preventing toxicity from hydrogen peroxide generated during metabolic processes, while urease activity characterizes soil nitrogen status.

2.2.1 Effects on Soil Urease Activity As shown in , urease activity in fungus chaff-treated plots was significantly higher than in control plots across all growth stages. Without fungus chaff, maize and sorghum soils showed urease activity that increased initially then decreased, while waxy maize soils exhibited continuously decreasing activity. With fungus chaff application, maize and sorghum soils showed continuously decreasing urease activity, with a more pronounced decline in maize than sorghum. Waxy maize soils showed overall decreasing activity but reached maximum values at jointing (7.91 $mg \cdot g^{1}$) and heading (7.65 $mg \cdot g^{1}$). Overall, fungus chaff treatments significantly elevated urease activity, with the greatest increases observed in sorghum at jointing (239%), waxy maize at heading (184%), and waxy maize at harvest (101%).

2.2.2 Effects on Soil Sucrase Activity Fungus chaff application increased soil sucrase activity compared to control plots. Waxy maize soils showed sucrase activity decreasing in the order: jointing > heading > harvest. Although sorghum soils with fungus chaff had higher sucrase activity than controls, the trend across growth stages was opposite, with the lowest increase (30%) at heading. Maize soils exhibited relatively stable sucrase activity across growth stages, with consistent increases of 38%, 28%, and 48%.

2.2.3 Effects on Soil Catalase Activity As shown in , fungus chaff application enhanced catalase activity at jointing and heading stages for all three crops. Without fungus chaff, catalase activity increased gradually across growth stages. Both treatments showed similar activity at jointing and harvest, but significant differences emerged at heading, where fungus chaff treatments were substantially higher than controls, with a maximum increase of 40%.

2.3 Effects of Vinegar Residue Fungus Chaff on Soil Microbial Biomass Carbon and Nitrogen

Soil microbial biomass carbon serves as a sensitive indicator of soil organic carbon, while microbial biomass nitrogen constitutes an important component of soil nitrogen mineralization potential. Analysis of variance ($P < 0.05$) in revealed that fungus chaff application increased soil microbial biomass carbon by 58.10–407.67 mg • kg ^{1} and biomass nitrogen by 11.98–27.55 mg • kg ^{1} across different growth stages of the three crops. Compared to controls, fungus chaff application generally promoted microbial biomass carbon and nitrogen, with significant increases in microbial biomass nitrogen for waxy maize at jointing and heading. Microbial biomass nitrogen peaked at heading for all crops, indicating the most pronounced effect of fungus chaff at this stage. Overall, microbial biomass nitrogen was lowest at jointing, increased subsequently, and decreased again at maturity. The substantial increase from jointing to heading aligned with trends in actinomycete, bacterial, and fungal populations. This pattern likely reflects increasing crop nutrient uptake as growth accelerated, causing nitrogen fixed in microbial biomass to decrease while carbon exudation from roots increased, promoting carbon fixation by microbes and resulting in overall increasing microbial biomass carbon throughout the growth period.

Discussion and Conclusion

Fungus chaff contains abundant microorganisms that can alter microbial populations, potentially promoting beneficial bacteria while inhibiting harmful fungi, thereby protecting soil health. As an organic fertilizer, it further decomposes into humus, increasing soil organic matter content, improving soil fertility and biological activity, and enhancing soil water-holding, nutrient retention, and aeration properties. This study examined microbial populations, enzyme activities, and microbial biomass carbon and nitrogen in three crops following vinegar residue fungus chaff application, providing insights into its effects on soil fertility and offering scientific evidence for mitigating environmental pollution from edible fungus substrate waste.

Consistent with previous research, our results demonstrate that fungus chaff application significantly influences soil microbial populations. Studies have reported that fungus chaff application reduces fungal (harmful) populations while increasing bacterial and actinomycete (beneficial) populations. Our findings align with these results, showing that vinegar residue fungus chaff promoted beneficial bacteria and actinomycetes while inhibiting harmful fungi. Bacterial populations peaked during heading and maturity across all three crops, with lower counts at jointing. This effect likely occurs because fungus chaff application increases soil organic matter, available phosphorus, and available potassium, providing abundant carbon and nitrogen sources for microbial growth while introducing substantial exogenous microbial communities.

Actinomycete populations increased modestly at jointing and heading, then rose

significantly at maturity, possibly due to improved nutrient availability from fungus chaff decomposition that supported both crop growth and microbial proliferation. Fungal populations remained low throughout the growth period, with fungus chaff application further suppressing harmful fungal proliferation.

Soil enzymes originate from root exudates, decomposition of plant and animal residues, and microbial metabolism. Previous research has demonstrated that higher bacterial and actinomycete densities correlate with greater soil fertility. Significant enhancement of urease, sucrase, and catalase activities improves soil microbial conditions. The observed increase in microbial biomass carbon following fungus chaff application indicates its positive effect on carbon availability. Microbial biomass carbon, nitrogen, and soil respiration reflect soil quality changes and serve as biological indicators of soil fertility. Fungus chaff application enhanced soil biological activity, substantially increasing enzyme activities and microbial populations while augmenting soil nitrogen and carbon content. These results demonstrate that vinegar residue fungus chaff, as a nutrient-rich substrate, effectively improves the rhizosphere ecological environment and provides a theoretical reference for addressing edible fungus substrate waste pollution.

Similar studies have reported that fungus chaff fertilizer increases urease, catalase, and sucrase activities, with sucrase showing the most pronounced effect (up to 96.52% increase). Our results corroborate these findings, showing that fungus chaff application elevated all three enzyme activities above control levels throughout the growth period. The most notable changes occurred at heading, indicating that fungus chaff application increased microbial populations and enzyme activities, improved rhizosphere microbial structure, and enhanced soil biochemical functions. Microbial biomass carbon reached maximum values at crop maturity, while microbial biomass nitrogen reached minimum values, likely because increased nitrogen demand during late growth stages caused crops to absorb nitrogen previously immobilized in microbial biomass. Long-term fertilization studies have similarly shown that fertilization treatments significantly increase microbial biomass carbon, nitrogen, and populations compared to controls. Our findings indicate that vinegar residue fungus chaff promotes microbial proliferation, thereby increasing microbial biomass carbon and nitrogen, with its beneficial effects intensifying as crops develop. At maturity, maximum microbial biomass carbon values were observed, while nitrogen values decreased, presumably due to enhanced crop nitrogen uptake.

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Note: Figure translations are in progress. See original paper for figures.