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EFFECT OF NITROGEN APPLICATION ON *PHANEROCHAETE CHRYSOSPORIUM* ENZYME ACTIVITY AND WHEAT STRAW BIODEGRADATION IN DIFFERENT WHEAT GENOTYPES

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ABSTRACT
Wheat straw, a major agricultural byproduct, presents a challenge for disposal and an opportunity for valorization through biodegradation. This study investigates the impact of varying nitrogen application rates on the enzymatic activity of the white rot fungus *Phanerochaete chrysosporium*, specifically Laccase and Manganese Peroxidase (MnP), and their role in the biodegradation of straw from five different wheat genotypes (C306, DPW621-50, HD2967, DBW303, and DM7). The experiment was conducted over a period of 45 days, with measurements taken at 15, 30, and 45-day intervals. Three nitrogen application rates were tested: N0 (0 kg/acre), N1 (120 kg/acre), and N2 (240 kg/acre). The results show that enzyme activities varied significantly across genotypes and were strongly influenced by nitrogen levels and time. Notably, the N2 treatment generally led to the highest enzyme activities for both Laccase and MnP, particularly at the 45-day mark. This suggests that higher nitrogen availability enhances the biodegradative potential of *Phanerochaete chrysosporium* on wheat straw. These findings have significant implications for sustainable agricultural practices and the management of crop residues.

KEYWORDS: White rot fungus, Biodegradability, enzymatic activity.

INTRODUCTION
The global agricultural sector faces a pressing environmental and economic challenge in the management of crop residues, particularly cereal straw. Annually, billions of tons of lignocellulosic biomass, including wheat straw, are produced worldwide.[1] This vast quantity of agricultural byproduct is often disposed of through open-field burning, a practice that, while seemingly convenient, leads to severe environmental degradation. The combustion of wheat straw releases large amounts of greenhouse gases, such as carbon dioxide and nitrous oxide, as well as particulate matter, contributing significantly to air pollution, climate change, and various respiratory health issues in human populations.[2] Furthermore, this destructive disposal method depletes soil organic matter, kills beneficial microorganisms, and removes vital nutrients, thereby diminishing long-term soil fertility and productivity. The search for sustainable and economically viable alternatives for valorizing this biomass has become a critical area of research.

One of the most promising avenues for converting lignocellulosic waste into valuable resources is biological decomposition. Among the various microorganisms capable of this process, white rot fungi are uniquely suited due to their exceptional ability to degrade lignin, the complex and recalcitrant polymer that gives plant cell walls their rigidity.[3] Lignin forms a protective matrix around cellulose and hemicellulose, making these energy-rich polysaccharides inaccessible to most decomposers. White rot fungi, and notably the model organism *Phanerochaete chrysosporium*, are renowned for their non-specific enzymatic systems that can break down this tough lignin barrier.[4] This remarkable capability makes them central players in the natural carbon cycle and potent agents for applications ranging from biofuel production to bioremediation.[5]

The ligninolytic system of *Phanerochaete chrysosporium* is a highly sophisticated network of extracellular enzymes, primarily comprising Laccase and Manganese Peroxidase (MnP). Laccase is a multi-copper oxidase that catalyzes the oxidation of various phenolic compounds, a key step in lignin degradation. It functions by removing electrons from substrates, producing free radicals that can then break down the complex lignin structure. Manganese Peroxidase, on the other hand, is a

heme-containing enzyme that utilizes hydrogen peroxide to oxidize manganese ions (Mn2+) to highly reactive manganese (Mn3+) ions. These manganese ions, often chelated by organic acids, diffuse from the enzyme and act as powerful oxidants, attacking the lignin polymer in a non-specific manner.[6] The synergistic action of these two enzymes allows *Phanerochaete chrysosporium* to effectively dismantle the lignin scaffold, thereby exposing the more readily degradable cellulose and hemicellulose to other cellulolytic enzymes, ultimately facilitating the complete biodegradation of the wheat straw.

While the enzymatic machinery of white rot fungi is well-understood, its efficiency is heavily dependent on environmental conditions, particularly the availability of key nutrients. Nitrogen is one of the most critical factors influencing fungal growth and metabolism. It is a fundamental component of proteins, enzymes, nucleic acids, and other cellular building blocks. The relationship between nitrogen availability and lignin degradation is complex and often referred to as a nitrogen paradox. Fungi require nitrogen for growth, but the production of ligninolytic enzymes is often induced or enhanced under nitrogen-limiting conditions. This is because, under low nitrogen stress, the fungus shifts its metabolic strategy. Instead of relying on easily assimilable nitrogen sources, it activates the lignin-degrading enzymes to access the small amounts of nitrogen that are chemically bound within the lignin structure of the plant biomass. However, if the nitrogen concentration is too low, fungal growth is severely stunted, limiting the overall production of enzymes. Conversely, a high concentration of readily available nitrogen can lead to the catabolic repression of ligninase genes, as the fungus prefers to use the easily accessible nutrient for primary growth rather than investing energy in producing complex lignin-degrading enzymes.[7]

This intricate relationship highlights the need for a precise understanding of the optimal nitrogen levels for maximum enzyme activity and biodegradation efficiency. While previous research has explored the effects of varying nitrogen levels on fungal degradation of different biomass types, there is a significant gap in the literature regarding the interactive effects on specific agricultural residues, such as wheat straw from different genotypes. The chemical composition of wheat straw, including its lignin and cellulose content, can vary significantly between genotypes. This variation could influence how effectively the fungal strain is able to colonize and degrade the straw, and how it responds to different nutrient regimes.[8] For example, a genotype with higher lignin content might require different nitrogen conditions to trigger optimal ligninase production compared to a genotype with a lower lignin content.

This research aims to bridge this knowledge gap by systematically investigating the impact of three different nitrogen application rates on the enzymatic activity of *Phanerochaete chrysosporium* grown on straw from five distinct wheat genotypes. The three nitrogen treatments—N0 (0 kg/acre), N1 (120 kg/acre), and N2 (240 kg/acre)—were selected to represent a range from nitrogen-deficient to nitrogen-rich environments. The chosen wheat genotypes (C306, DPW621-50, HD2967, DBW303, and DM7) are representative of different genetic backgrounds, allowing for a comprehensive comparison of their biodegradability. We hypothesize that an intermediate to high level of nitrogen, specifically the N2 treatment, will stimulate optimal fungal growth and consequently lead to the highest levels of Laccase and MnP activity, ultimately enhancing the overall biodegradation of the wheat straw. We further anticipate that the magnitude of this effect will vary significantly across the different wheat genotypes, highlighting the importance of genotype-specific considerations in the design of future biodegradation systems. The findings of this study will provide crucial data for optimizing conditions for biological valorization of wheat straw, contributing to more sustainable agricultural practices.

MATERIALS AND METHODS

Plant Material and Experimental Design

Straw from five distinct wheat genotypes (C306, DPW621-50, HD2967, DBW303, and DM7) was collected post-harvest. The straw was chopped into approximately 2-3 cm pieces and air-dried. A completely randomized block design was employed with three treatments based on nitrogen application rates: N0 (0 kg/acre), N1 (120 kg/acre), and N2 (240 kg/acre). The experiment was conducted with three replicate blocks for each treatment and genotype combination. Nitrogen was applied as Ammonium Nitrate (NH4NO3) from SIGMA-ALDRICH (St. Louis, MO, USA).

Fungal Strain and Inoculation

The white rot fungal strain used was *Phanerochaete chrysosporium* (ATCC 24725). The strain was maintained on potato dextrose agar (PDA) slants at 4°C. Spore suspensions were prepared and used for inoculation following standard protocols previously described by Smith.[9] For each experimental unit, 100 grams of prepared wheat straw were placed in a sterile container, and 10 mL of the fungal spore suspension were added. The containers were incubated at 28°C for a total of 45 days.

Enzyme Activity Assays

Samples were collected at 15, 30, and 45 days post-inoculation. Enzyme crude extracts were prepared using a previously published method.[10] All enzyme activities are expressed in micromoles per hour per milliliter of enzyme (µmol/h/mL).

- Laccase Activity: Laccase activity was determined by measuring the oxidation of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). The procedure was performed as described by Mishra[11] with minor modifications.

- Manganese Peroxidase (MnP) Activity: MnP activity was measured by following the oxidation of guaiacol. The assay procedure was adapted from Singh.[12]

STATISTICAL ANALYSIS

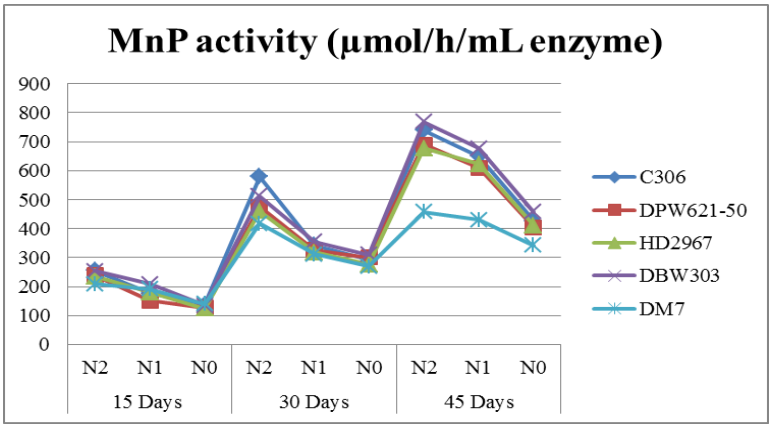
The data were analyzed using a two-way analysis of variance (ANOVA) to determine the effects of nitrogen rates, wheat genotypes, and their interaction on Laccase and MnP activities. Significant differences between treatment means were compared using Tukey's Honest Significant Difference (HSD) test at a significance level of $P < 0.05$. Statistical analysis was performed using SPSS Statistics[13] (IBM Corporation, Armonk, NY, USA).

RESULTS

The application of different nitrogen rates had a significant impact on the activity of both Laccase and MnP across the five wheat genotypes. Enzyme activity for both types was influenced by nitrogen level, genotype, and the duration of the experiment. The highest enzyme activities were consistently observed at the 45-day sampling point.

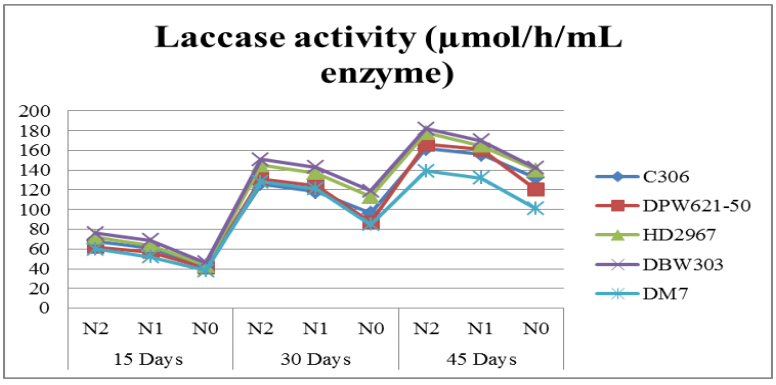
Manganese Peroxidase (MnP) Activity

As shown in the graph below, MnP activity generally increased over the 45-day period for all treatments and genotypes. The N2 treatment consistently resulted in the highest MnP activity, with peak values reaching between 650 and 780 µmol/h/mL. This suggests a positive correlation between higher nitrogen availability and MnP production. The DBW303 genotype displayed the highest overall MnP activity, indicating a strong potential for biodegradation under high nitrogen conditions.



Laccase Activity

Similarly, Laccase activity showed a trend of increasing activity over time, peaking at 45 days. The N2 treatment yielded the highest Laccase activity for all genotypes, with values ranging from 150 to over 180 µmol/h/mL. Like the MnP results, the DBW303 genotype showed the highest Laccase activity, reinforcing its high biodegradative potential. Conversely, the DM7 genotype generally exhibited lower enzyme activity compared to the other genotypes across all treatments.



DISCUSSION

This study's findings provide critical insights into the relationship between nitrogen availability, fungal metabolism, and the biodegradation of different wheat genotypes. The results confirm a strong positive correlation between increased nitrogen application rates and the activity of both Laccase and Manganese Peroxidase (MnP) enzymes, particularly at the highest rate of N2 (240 kg/acre).

This observation is significant and directly relates to the complex "nitrogen paradox" described in the literature.[7]

While some studies suggest that nitrogen limitation is necessary to induce ligninolytic enzyme production, our results indicate that for *Phanerochaete chrysosporium*, the high nitrogen level provided in the N2 treatment was essential to support the vigorous fungal growth and overall metabolic activity required for the high-level synthesis of these enzymes. The abundance of nitrogen likely provided the necessary building blocks for protein synthesis, allowing the fungus to produce and secrete a large quantity of these extracellular enzymes. The increase in enzyme activity over the 45-day period further confirms that the biodegradation process is not immediate but requires time for fungal colonization and the subsequent induction of the lignin-degrading enzymatic system. The peak at 45 days represents the point of maximum metabolic efficiency in the decomposition process under these specific conditions. It is also notable that MnP activity was significantly higher than Laccase activity, a trend that is consistent with the known primary role of MnP in the ligninolytic system of *Phanerochaete chrysosporium.*[14] The effectiveness of MnP is also influenced by the concentration of manganese in the substrate, highlighting the importance of substrate composition in the degradation process.[15]

The differential response among the five wheat genotypes is another key finding. The consistently high enzyme activities observed with the DBW303 genotype suggest that its straw composition is particularly conducive to degradation by *Phanerochaete chrysosporium*. This could be due to a more favorable carbon-to-nitrogen ratio, a lower degree of lignin polymerization, or the presence of specific structural features that enhance fungal attachment and colonization, as documented in previous studies on agricultural residue variability.[16] Conversely, the lower enzyme activity seen with the DM7 genotype may be indicative of a more recalcitrant lignin structure or the presence of compounds that inhibit fungal growth and enzyme production. This highlights a crucial practical implication: the biodegradative potential of crop residues is not uniform and may require genotype-specific management strategies.

In summary, this research demonstrates that the strategic application of nitrogen can serve as an effective tool to enhance the enzymatic activity of *Phanerochaete chrysosporium*, thereby accelerating the biodegradation of wheat straw.[17] The findings challenge the simple view of the nitrogen paradox by showing that a high nitrogen supply can be highly beneficial for overall enzymatic output. This information has direct implications for developing more efficient and sustainable on-farm composting and residue management systems. The effect of nitrogen on fungal metabolism is complex and dependent on both the fungal species and the substrate, warranting a deeper investigation into the underlying mechanisms.[18] Recent studies on MnP activity in white rot fungi further confirm the importance of nitrogen as a key regulator of ligninolysis.[19,20] Future research should focus on a more detailed chemical analysis of the different wheat genotypes to pinpoint the exact factors influencing their biodegradability and to optimize nitrogen application for specific straw types.[21]

CONCLUSION

This study clearly demonstrates that the application of nitrogen, particularly at a rate of 240 kg/acre, significantly enhances the activity of Laccase and Manganese Peroxidase, key enzymes involved in the biodegradation of wheat straw by the fungus *Phanerochaete chrysosporium*. The highest enzymatic activity was observed at 45 days, indicating the peak period of decomposition. The results also show that different wheat genotypes respond differently to the treatments, with DBW303 exhibiting the most significant biodegradative potential under high nitrogen conditions.

The importance and relevance of these findings are twofold. Scientifically, the results provide a nuanced perspective on the "nitrogen paradox," suggesting that under the right conditions, a high nitrogen supply can serve as a catalyst for increased fungal enzyme production and activity, a finding that adds a valuable layer to our understanding of microbial metabolism. From a practical standpoint, this research provides a clear, actionable recommendation for sustainable agricultural management. By optimizing the nitrogen-to-biomass ratio, it is possible to accelerate the decomposition of wheat straw, thereby reducing the need for open-field burning and its associated environmental hazards. This opens the door for more efficient on-farm composting and the conversion of agricultural waste into valuable soil organic matter. Future applications could involve developing tailored biological consortia for specific crop residue types to maximize their decomposition.

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AUTHER CONTRIBUTIONS

AK performed the experiments and design by GCP and PR. GCP, PR and AK wrote and edited the manuscript.

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DECLARATIONS

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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