EXPLORING WHITE ROT FUNGI REMEDIATION OF IMIDACLOPRID-CONTAMINATED SOIL MICROBIAL COMMUNITIES

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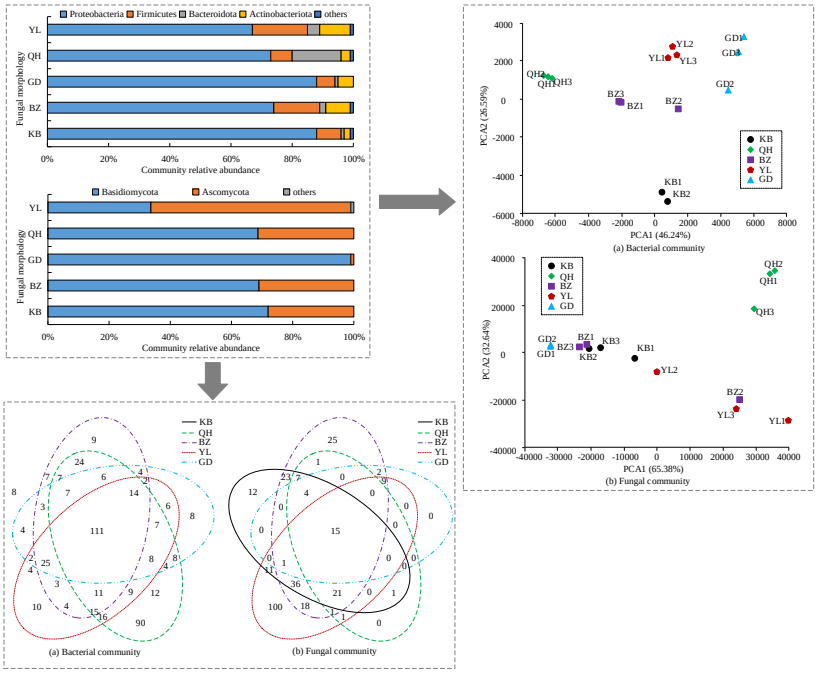
Abstract. Imidacloprid, a widely used pesticide, pollutes soil, disrupts microbial communities, and reduces crop yields. This study investigates the degradation of imidacloprid by white rot fungi and its impact on soil microbial remediation. The experiments revealed IMI removal rates ranging from 82% to 91% across different soil groups. Bacterial community structure changed significantly after inoculation with white rot fungi. Principal component analysis revealed significant differences in soil bacterial and fungal communities in the spore state group. These results reveal that white rot fungi in spore form accelerate the degradation of imidacloprid and restore microbial communities. White rot fungi degrade imidacloprid by secreting enzymes, altering the microbial environment to reduce pollution and restore balance, thereby repairing microbial communities.
Keywords: *white rot fungus, imidacloprid, degradation, microorganisms, access form, soil pollution*

Graphic abstract

This study explores the degradation of imidacloprid by white rot fungi as a biomaterial, as well as the microbial remediation effect and mechanism of imidacloprid contaminated soil. The experimental results showed that the incorporation of white rot fungi in soil containing imidacloprid in spore form could simultaneously accelerate the degradation of imidacloprid and repair the soil microbial community.



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Introduction

In recent decades, the development of agriculture and heavy industry has led to the widespread use of imidacloprid (IMI) as an insecticide. IMI is a neonicotinoid insecticide that is widely used in pest control. Its chemical structure is characterized by the presence of nitroguanidine or cyanamidine molecules. This insecticide targets the central nervous system of insects and binds to the nicotinic acetylcholine receptor, ultimately leading to paralysis and death. However, its accumulation in soil has caused severe environmental issues (Kumar, 2020; Kruisdijk, 2022). IMI's poor biodegradability and high solubility pose environmental and health risks (Aponte, 2020). Its removal has become a focal point for environmentalists, researchers, and policymakers (Wang, 2020; Gao, 2021; Feng, 2021). Current methods for treating IMI contamination in soil include physical, chemical, and biological approaches (Kninger, 2021). However, each of them has its own shortcomings. Physical and chemical methods have high processing costs, may generate secondary pollution, and may alter soil structure and properties (Gao, 2020; Li, 2020; Zeng, 2021). Biological methods are limited by low efficiency, lengthy processing times, and stringent environmental requirements. Therefore, it is imperative to identify an efficient and sustainable remediation method. White rot fungi are basidiomycetous fungi, renowned for their capacity to degrade lignin, a complex and recalcitrant component of plant cell walls. White-rot fungi produce a variety of extracellular enzymes, including lignin peroxidase, manganese peroxidase, and laccase, which enable them to degrade not only lignin but also a diverse array of environmental pollutants. The capacity of white-rot fungi to regulate and remediate soil microbial communities renders them optimal candidates for addressing IMI contamination in soil ecosystems.

This study selected white rot fungi (*Phanerochaete chrysosporium*) as the research object. The reason is that white rot fungi can produce various degrading enzymes and have good degradation ability for environmental pollutants such as IMI. Moreover, they have the ability to adjust the structure of soil microbial communities to achieve a healthy ecological balance and achieve soil remediation. Therefore, using white rot fungi to remove IMI pollution may become a new technological route with clear technical prospects and economic benefits. While the potential of white-rot fungi for environmental remediation is well established, the precise mechanisms by which they degrade IMI and restore microbial community homeostasis remain poorly understood. The objective of the study is to investigate the degradation of IMI by white-rot fungi, with a focus on the degradation process itself and the analysis of the microbial remediation mechanisms involved. This will facilitate comprehension of the manner in which white-rot fungi can be employed to expedite the degradation of IMI and remediate the compromised soil microbial community, thereby offering an efficacious and sustainable strategy for the remediation of pesticide contamination in agricultural soils. This study will focus on exploring this issue and look forward to providing new solutions to address the IMI pollution. The white rot fungi in the study are selected primarily for their ability to degrade IMI contaminants in soil and to repair damaged soil microbial communities. The white rot fungi in the study are not plant pathogens. The application methods and objectives of the experimental system in this study are summarized in *Table 1*.

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Table 1. *Analysis of methods and research objectives used in the study*

Section	Methodology	Objective
2.1.1 Experimental materials and reagents	Listed instruments (e.g., UV sterile workbench, constant temperature shaker, electronic balance, high-pressure steam sterilizer, HPLC, rotary evaporator) and reagents (e.g., anhydrous calcium chloride, sodium chloride, magnesium sulfate heptahydrate, ammonium tartrate, agar, acetonitrile, methanol).	To provide the necessary equipment and reagents for accurate and reproducible experimental conditions.
2.1.2 Cell passage culture and free-state cell culture	Cultured white rot fungal spores using potato dextrose agar medium; Prepared Kirk liquid medium containing KH_2PO_4, anhydrous calcium chloride, ammonium tartrate, glucose, anhydrous sodium acetate, inorganic salt solution, and vitamin solution; Inoculated fungal spores under sterile conditions and cultured with constant temperature shaking.	To cultivate fungal spores and free-state cells for subsequent degradation experiments.
2.1.3 IMI degradation experiments	Designed self-degradation experiments with ultrapure water and Kirk liquid medium under light and dark conditions; Conducted degradation experiments with different IMI concentrations (2 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 50 mg/L) and regular sampling for analysis.	To explore the degradation efficiency of white rot fungi at different IMI concentrations and assess their degradation capabilities.
2.1.4 Activity testing of white rot fungi	Cultured white rot fungi in different IMI concentrations and measured cell activity every 12 hours for 4 days using a UV spectrophotometer.	To analyze the activity changes of white rot fungi in different IMI concentrations and evaluate their tolerance.
2.1.5 Enzyme solution preparation and peroxidase determination	Ground fungal pellets to extract intracellular enzyme solutions; Measured activities of catalase (CAT) and peroxidase (POD) in the enzyme solutions, as well as reactive oxygen species (ROS) levels.	To assess the enzyme activity changes during IMI degradation and reveal the underlying mechanisms.
2.2.1 Soil selection and treatment	Collected surface soil from the Central Plains of China and determined its basic physicochemical properties (e.g., moisture content, pH, organic matter); Mixed soil with straw powder and adjusted the moisture content to 30% after adding IMI solution.	To prepare soil samples for degradation experiments and simulate real-world contaminated soil environments.
2.2.2 Cultivation of different forms of white rot fungi	Cultivated spore, free, immobilized, and enhanced forms of white rot fungi; Inoculated different forms of white rot fungi into soil-straw mixtures for degradation experiments.	To investigate the degradation efficiency of different forms of white rot fungi in soil.
2.2.3 IMI degradation in soil	Added different forms of white rot fungi to sterilized and unsterilized soils with an IMI concentration gradient of 10 mg/kg; Regularly monitored the IMI content in soil samples.	To evaluate the degradation efficiency of white rot fungi under different soil conditions and identify optimal conditions.
2.2.4 Analysis of soil physicochemical properties	Measured soil pH, organic matter content, and sucrase activity; Analyzed changes in soil properties before and after treatment with white rot fungi.	To assess the impact of white rot fungi on soil physicochemical properties and reveal their potential for soil remediation.
2.2.5 Analysis of microbial community changes	Extracted soil DNA, performed PCR amplification, and conducted high-throughput sequencing; Analyzed changes in bacterial and fungal community diversity and composition after treatment.	To investigate the effects of white rot fungi on soil microbial community structure and reveal their ecological impacts.

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Materials and methods

Degradation of IMI contaminated water by white rot fungi

Experimental materials and reagents

In natural environments, soil and water often mix together, and even in IMI contaminated soil, there may be localized IMI contaminated water (Pasieczna, 2021). Therefore, it is necessary to analyze the degradation of white rot fungi in IMI polluted water and IMI soil separately (Shi, 2021). *Table 2* shows the instruments and reagents used in the degradation experiment of IMI contaminated water by white rot fungi.

Table 2. *Instruments and reagents for the degradation experiment of white rot fungi on IMI polluted water bodies*

Type	Name	Model or specification	Manufacturer
Instrument	UV sterile workbench	SW-CJ-2F	Shanghai Hujing Medical Equipment Co., Ltd
	Constant temperature oscillation shaker	ZWY-2112B	Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd
	Electronic balance	BSA124S	Beijing Saiduoli Scientific Instrument Co., Ltd
	High-Pressure Steam Sterilization Pot	LDZM-60KCS	Shanghai Shen'an Medical Device Factory
	Universal induction cooker	DL-1	Beijing Guangming Medical Instrument Co., Ltd
	High performance liquid chromatograph	Agilent1260	Agilent Technology Co., Ltd
Reagent	Anhydrous calcium chloride	Analytial reagent(AR)	China National Pharmaceutical Group Chemical Reagent Co., Ltd
	sodium chloride	AR	China National Pharmaceutical Group Chemical Reagent Co., Ltd
	Magnesium sulfate heptahydrate	AR	China National Pharmaceutical Group Chemical Reagent Co., Ltd
	Ammonium tartrate	AR	Shanghai Jinshaniting New Chemical Reagent Factory
	agar	AR	China National Pharmaceutical Group Chemical Reagent Co., Ltd

Various instruments and reagents are used to conduct degradation experiments of IMI on white rot fungi. The instruments are as follows: UV sterile workbench: SW-CJ-2F type, produced by Shanghai Hujing Medical Equipment Co., Ltd. Constant temperature vibrating screen: ZWY-212B type, manufactured by Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd. Electronic balance: BSA124S model, produced by Beijing Saiduoli Scientific Instrument Co., Ltd. High pressure steam sterilization pot: LDZM-60KCS type, produced by Shanghai Shen'an Medical Equipment Factory. High performance liquid chromatography: Agilent 1260, produced by Limited Company. Rotary evaporator: RE-2000C type, manufactured by Beijing Saiduoli Scientific Instrument Co., Ltd (Shi, 2021).

The instruments are as follows: Anhydrous calcium chloride: analytical reagent (AR) provided by China Pharmaceutical Group Chemical Reagent Co., Ltd. Sodium chloride: AR, provided by China National Pharmaceutical Group Chemical Reagent Co., Ltd.

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Magnesium sulfate heptahydrate: AR, provided by China Pharmaceutical Group Chemical Reagent Co., Ltd. Ammonium Tartrate: AR, provided by Shanghai Jinshanting New Chemical Reagent Factory. Agar: AR, provided by China Pharmaceutical Group Chemical Reagent Co., Ltd. Acetonitrile: provided by China National Pharmaceutical Group Co., Ltd. Methanol: Provided by China National Pharmaceutical Group Co., Ltd (Shi, 2021).

There are significant differences in the physicochemical properties of the microbial community structure between water and soil samples, which may lead to different degradation behaviors of IMI in these two media. IMI in water is prone to diffusion and contact with degrading enzymes due to its high water solubility, resulting in different degradation kinetics. Soil, on the other hand, has a more complex structure, including organic matter, minerals, and micropores. IMI can be adsorbed to soil particles, thereby affecting its bioavailability and degradation rate. Therefore, studying water and soil samples separately can more accurately evaluate the degradation effect and mechanism of white rot fungi in different media. Water is a highly mobile liquid medium with relatively low viscosity and high diffusion ability, while soil is a solid medium with high viscosity and complex particle structure (Gazman, 2023). IMI has a higher solubility in water, making it easier to diffuse and come into contact with microbial degradation enzymes in water. In soil, IMI can be adsorbed by soil particles, reducing its bioavailability. At the same time, the microbial community structure in water bodies is significantly different from that in soil. The microorganisms in water are mostly in a free state with high metabolic activity, and the types of microorganisms are relatively single. In contrast, microorganisms in the soil adhere to soil particles, thereby leading to the formation of a more intricate community structure and an enhanced diversity of species. Furthermore, microorganisms in the soil engage in interactions with organic matter and minerals present in the soil, which can influence the degradation of IMI. There are also differences in environmental conditions such as temperature, humidity, and oxygen concentration between water and soil, which directly affect the growth and enzyme activity of white rot fungi (Chai, 2020).

The research water sample is collected from the Yellow River Basin in Henan Province, China on August 15, 2023. The area is selected for water sampling at relevant locations for studying IMI pollution due to its known history of agricultural activities and pesticide use. The soil samples for the study are taken from the surface soil of the Zhengzhou Plain in Henan Province, China, at a depth of 2-15cm. Sampling is conducted on August 18, 2023. This region represents a typical agricultural soil in central China, where IMI is frequently used.

Cell passage culture and free state cell culture

The subculture plan for fungal spores is as follows. 200 g of peeled potatoes are chopped and mixed with 1L of ultrapure water, boiled and filtered. Then the filtrate is mixed with 20 g of glucose, 18.5 g of agar, 1.5 g of $MgSO_4 \cdot 7H_2O$, and 3g of KH_2PO_4, boiled twice, and divided into sterile bottles. Next, it is sealed and sterilized under high pressure. Afterwards, the culture medium is poured into a sterile culture dish. When it cools and solidifies, the white rot fungus spore powder is applied to it. After the operation is completed, it is sealed and placed in a constant humidity box at 37 ℃ for one week. When the colony matures, it is transferred to the refrigerator for storage (Chai, 2020).

The free state bacterial culture plan is as follows. Firstly, 0.2 g of KH_2PO_4, 0.01 g of anhydrous calcium chloride, 0.221 g of ammonium tartrate, 11.098 g of glucose, 1.641 g

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of anhydrous sodium acetate, 1mL of inorganic salt solution, and 0.5 mL of vitamin solution are prepared. These substances are dissolved in 1 L of ultrapure water to obtain Krik liquid culture medium. 100 mL of culture medium is accurately transferred into sterile bottles and sterilized. Before inoculation, the fungal spores need to be restored at 37°C for 30 minutes. Then in the experiment, the white rot fungus spore powder is dissolved in sterile ultrapure water, adjusted to appropriate turbidity, and the powder suspension is added to Kirk culture medium. Finally, it is cultured in the equipment under constant temperature and shaking for 72 hours to obtain the desired bacterial body. Kirk's liquid medium is the main medium used for cultivation and degradation experiments of white rot fungi. Its composition and concentration are as follows KH_2PO_4 (0.2 g/L), anhydrous calcium chloride (0.01 g/L), ammonium tartrate (0.221 g/L), glucose (11.098 g/L), anhydrous sodium acetate (1.641 g/L), inorganic salt solution (1 mL/L), and vitamin solution (0. 5 mL/L). The above components are dissolved in 1 L of ultrapure water, stirred thoroughly, and filled into sterilization bottles for high-temperature sterilization treatment. After sterilization, it is allowed to cool to room temperature before use.

Degradation and self-degradation experiments of IMI by white rot fungi

The design of the self-degradation experiment is as follows. This experiment mainly consists of four groups to investigate the effects of light and different culture environments on the self-degradation of IMI. They are respectively ultrapure water with light, Krik liquid culture medium system, and ultrapure water without light, Krik liquid culture medium system. To reduce errors, each group has three duplicate samples, and the final result is taken as the average of these three samples.

The production method of Krik liquid culture medium is as follows. Firstly, various chemicals required are accurately weighed, dissolved in 1L of ultrapure water, and inorganic salt solution and vitamin solution are added to prepare Krik liquid culture medium (Miao, 2020).

Then 100 mL of culture medium is loaded into six sterile bottles (Li, 2023). The next step is to take three bottles each under light and no light conditions and set them as two types of Krik system groups. Similarly, 100mL of ultrapure water is loaded into six other sterile bottles and classified according to light conditions (Chen, 2020). These sterile bottles are then subjected to a high temperature sterilization treatment. After sterilization is complete, all sterile bottles are irradiated with a UV lamp for 30 minutes on a sterile table (Borello, 2020). Subsequently, 2 mL of pre-prepared IMI standard solution is added to each of the four groups of samples, resulting in an IMI concentration of 10mg/L in each system. Finally, the bottle is transferred to a constant temperature and humidity light incubator, shaken and cultured, and samples are taken regularly for testing. The reason for selecting an IMI concentration range of 2 mg/L to 50 mg/L for the experiment is that this concentration range of IMI can simulate the pollution level of IMI in soil and water caused by agricultural activities in the real environment. Thus, it is possible to systematically study the degradation efficiency of white rot fungi on IMI.

The degradation experiment of IMI by white rot fungi is as follows. This study establishes five sets of IMI concentration experiments, with IMI concentrations of 2 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, and 50 mg/L, respectively, to investigate their effects on the degradation of white rot fungi. Repeat each experiment three times to reduce errors and obtain an average detection results (Vinayak, 2021).

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Then Kirk liquid culture medium needs to be prepared, evenly distributed in 15 sterile conical flasks, and subjected to high-temperature sterilization treatment. Then white rot fungi are inoculated into the culture medium on a sterile platform and shaken for three days in a constant temperature and humidity light incubator (Jiang, 2021) The next step is to add the IMI standard solution to the culture system at various concentrations and continue culturing in a light incubator at constant temperature and humidity while taking timed samples. Finally, the actual concentration of IMI in each sample is determined using Agilent 1260 High Performance Liquid Chromatography (HPLC).

Activity testing of white rot fungi

The experiment designs four IMI concentration test groups of 0mg/L, 2mg/L, 5mg/L, and 50mg/L to analyze the activity changes during the degradation of IMI by white rot fungi. To reduce errors, each group includes three parallel experiments, and the average value is taken as the statistical result. After Kirk's liquid medium is prepared, it must be packed into 15 clean and sterile bottles, sterilized at high temperature, then inoculated with white rot fungi and cultured in the incubator. IMI standard solution is added to the Kirk liquid medium to adjust the concentration of IMI and continue the culture. After the preparation of the test solution, the cell activity of white rot fungi in the solution is measured using a UV spectrophotometer every 12 hours for 4 days (Sun, 2022).

Preparation of enzyme solution and determination of peroxidase

2 grams of free white rot fungal balls are taken and placed in a clean mortar. A small amount of quartz sand is added to cover the balls, and then liquid nitrogen is poured in. After all the fungal balls are frozen, they are crushed to form a homogeneous slurry with no significant particles or solids (Rong et al., 2021). Subsequently, 4 ml of Phosphate Buffered Saline (PBS) is used to wash the slurry in the mortar into a 10 ml centrifuge tube to obtain the intracellular enzyme solution of white rot fungi.

The role of intracellular antioxidant indicators in white rot fungi reflects the strength of their intracellular oxidative stress response when faced with oxidative environments (Wu, 2020). In terms of catalase determination, the 3ml reaction system of Catalase (CAT) from white rot fungi consists of 1ml of ultrapure water, 1.5 ml of PBS (50 mmol/L, pH 7.0), and 0.3 ml of H_2O_2 (0.1 mmol/L). After preheating the reaction system in a 25℃ water bath for 30 minutes, 0.2 ml of intracellular enzyme solution is quickly added to initiate the reaction, and the absorbance change at 240 nm is monitored for 3 minutes using a UV spectrophotometer. Moreover, the control group uses the same volume of PBS as the reaction promoter (Chen, 2022). The rate of decrease in absorbance of the reaction system at 240 nm is used to express the activity of white rot fungus CAT, and a decrease of 0.01 in absorbance at 240nm per minute is defined as one enzyme activity unit (U).

The measurement steps for peroxidase are as follows. The reaction system of Peroxidase (POD) in white rot fungi includes: 0.8ml of a 0.2% solution of guaiacol, 1ml of PBS (50 mmol/L, pH 7.0), and 1 ml of 3% H2O2. 0.2 ml of intracellular enzyme solution is added in the same way to initiate the reaction, and the absorbance changes are detected within 3 minutes at 470 nm. The rate of increase in absorbance at 470 nm represents the activity of white rot fungus POD, and an increase of 0.01 in absorbance value at 470 nm per minute in the reaction system is defined as one U (Yang, 2020).

The method for detection of reactive oxygen species is as follows. An appropriate amount of H2DCF-DA solution is added to the free-standing Kirk's white rot medium so

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that the final concentration of H2DCF-DA in the medium is 5 μmol/L. Then, according to the preparation method of the intracellular enzyme solution of white rot fungi, the enzyme solution is obtained and placed on a 96 well plate dedicated to an Enzyme-linked Immunosorbent Assay (ELISA) reader. The fluorescence intensity of 2',7'-dichlorofluorescin (DCF) in the enzyme solution is detected using an ELISA. The excitation wavelength of ELISA is 485 nm, and the occurrence wavelength is 525 nm. Based on the values of the control group, the Reactive Oxygen Species (ROS) of each sample can be calculated.

Degradation of IMI contaminated soil by white rot fungi

Experimental materials and reagents

Table 3 shows the instruments and reagents used in the degradation experiment of IMI contaminated soil by white rot fungi. The instruments are as follows: The research equipment used is a high-performance liquid chromatography instrument, which is used to detect the concentration of IMI in soil samples (Agilent 1260, produced by Agilent Technologies Co., Ltd). UV sterile operating table is used to provide a sterile environment (SW-CJ-2F, produced by Shanghai Hujing Medical Equipment Co., Ltd). Electronic balance is used for accurate weighing of experimental reagents (BSA124S, produced by Beijing Sadolis Scientific Instrument Co., Ltd). High pressure steam sterilization pot is used for sterilizing experimental equipment (LDZM-60KCS, produced by Shanghai Shen'an Medical Equipment Factory). Rotary evaporator is used for concentrating sample solutions (model RE-2000C, produced by Beijing Sartorius Scientific Instrument Co., Ltd). Constant temperature oscillation shaker is used for cultivating white rot fungi and other experimental operations (ZWY-2112B, produced by Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd). The reagents are as follows: Acetonitrile is an organic solvent (China National Pharmaceutical Group Chemical Reagent Co., Ltd). Methanol is an organic solvent (China Pharmaceutical Group Chemical Reagent Co., Ltd) (Mori, 2021).

Table 3. *Instruments and reagents for the degradation experiment of white rot fungi on IMI contaminated soil*

Type	Name	Model or specification	Manufacturer
Instrument	High performance liquid chromatograph	Agilent1260	Agilent Technology Co., Ltd
	UV sterile workbench	SW-CJ-2F	Shanghai Hujing Medical Equipment Co., Ltd
	Electronic balance	BSA124S	Beijing Saiduoli Scientific Instrument Co., Ltd
	High-Pressure Steam Sterilization Pot	LDZM-60KCS	Shanghai Shen'an Medical Device Factory
	Rotary evaporator	RE-2000C	Beijing Saiduoli Scientific Instrument Co., Ltd
	Constant temperature oscillation shaker	ZWY-2112B	Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd
Reagent	Acetonitrile	/	China National Pharmaceutical Group Chemical Reagent Co., Ltd
	Methanol	/	China National Pharmaceutical Group Chemical Reagent Co., Ltd

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While brown rot fungi and Poria cocos fungi were not subject to any analysis

The culture strategies for spore and free white rot fungi have been mentioned in the above content. The culture strategy for immobilized white rot fungi is as follows. First, sterile water and a suspension of white rot spores with a turbidity of 60-70 Nephrometric Turbidity Units (NTU) are prepared on a sterile processing platform. The prepared 8% sodium alginate solution is then mixed with an equal amount of white rot spore suspension. A clean and sterile needle free syringe is used to take the mixture and drop it into a previously prepared sterile 6% concentration $CaCl_2$ solution, forming a semi-transparent light brown immobilized white rot fungus polymer. After a night of standing, the polymer is filtered and washed with sterile water. Then, it is transferred to a pre-prepared Kirk liquid culture medium and shaken for cultivation on a shaker under constant temperature, humidity, and light conditions at 37℃. The shaking speed of the shaker is 150 r/min. Mature immobilized white rot fungi can form after 72 hours. The cultivation strategy for enhanced white rot fungi is as follows. The straw is crushed and filtered through a 20 mesh sieve. 6.25 g of straw is weighed and placed in a sterile 250 mL beaker. 14.3 mL of pre-made white rot fungal spore suspension is evenly sprayed onto the straw powder using a spray bottle. Then the beaker containing pretreated straw is placed in a constant temperature, humidity, and light incubator at 37℃ and left to stand for 3 days to obtain enhanced white rot fungal spores.

Soil selection treatment and fungal degradation plan

The soil used in this experiment comes from the surface soil of a plain in central China, which is 2-15 cm deep. After air drying and grinding, the soil is sieved through a 60 mesh sieve, and the basic physical and chemical properties of the soil are determined according to the "Conventional Analysis Method for Soil Agricultural Chemistry". The following parameters are obtained. The soil moisture content, pH value, and organic matter are 32.58%, 6.35%, and 24.26 g/kg, respectively.

This experiment is divided into two parts, namely the sterilized and unsterilized soil-straw mixture experiment. The concentration gradient of IMI is 10 mg/kg, and each treatment group has 30 parallel experiments to reduce possible operational errors or experimental errors caused by other irreversible factors. The results obtained are the averages of each parallel group. The researchers first dry the straw at low temperatures, then crush it and pass it through a 20 mesh sieve. Then the researchers mix the straw powder with the sieved soil in a 15:1 ratio and place it in a 250mL beaker to ensure that the total mass of the soil straw mixture is 100 g. Then the beaker is divided into a non-sterile group and a sterile group in the experiment. Four different forms of white rot fungi that are pre-cultured and matured are added to the mixed soil straw powder. Then an IMI solution is added to achieve an IMI concentration of 10 mg/kg, and the moisture content is adjusted to 30% to obtain the non-sterile group. On the day of sample preparation, and on the 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th day, the IMI content of each group of soil is detected using HPLC.

The physical and chemical properties of polluted soil and the process of obtaining IMI

Firstly, the researchers need to thoroughly disperse the contents of the beaker, take out 10 g of the mixture, and place it into a 50 mL centrifuge tube. Then, 5 mL of ultrapure water and 10 mL of acetonitrile are poured in. A vortex mixer is used to mix the samples for 5 minutes. After mixing, they are left static overnight. 1g of NaCl and 4 g of anhydrous

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magnesium sulfate are added, and then a rolling machine is used for 20 minutes of rolling treatment, with a rolling speed of 70 r/min. After completion, the sample is centrifuged at a speed of 8000 r/min for 5 minutes to obtain the supernatant. The purification steps for IMI are as follows. In the experiment, 4 mL of the supernatant is taken out and placed in a 10 mL centrifuge tube. Then 0.2 g of N-propylenediamine (PSA) and 0.15 g of anhydrous magnesium sulfate are added, followed by 1 minute of vortex mixing. The sample is centrifuged again using the same centrifugation method. Next, the researchers inject the supernatant obtained and filter it through a 0.22 μm organic phase filter head. Finally, they place the sample in a high-performance liquid chromatography injection vial and refrigerate it for storage.

The determination of soil physical and chemical properties is shown below. The soil samples selected by the researchers are from the soil on the 1st and 7th day of fungal degradation analysis of IMI contaminated soil. After obtaining the soil, the pH value of the soil is first measured. The soil sample is first sieved through a 10 mesh sieve, then 10 g of the soil sample is taken and stored in a 50 mL beaker, and 25 mL of carbon dioxide free water is poured in. After 1-3 minutes of uniform stirring, the pH value is measured using a pH meter. The soil organic matter content is measured in the experiment, and the sample is sieved through a 100 mesh sieve. Then 1 g of soil is taken and placed in a 50 mL beaker. 3 mL of ultrapure water is added to fully dissolve the soil, followed by 10 mL of potassium dichromate (39.2245 g/L). Then 10 mL of concentrated sulfuric acid is poured in and shaken evenly. It is left to stand for 20 minutes, and then 10 mL of ultrapure water is added and shaken well. It is left to stand overnight. 3 mL is taken from the supernatant and placed in a 10 mL colorimetric tube. The absorbance at 590 nm is measured using a UV spectrophotometer to a constant volume.

The determination of sugarcane enzyme in soil is shown below. The sample for this experiment is the soil on the 7th day of fungal degradation analysis in IMI contaminated soil. Researchers use the soil sucrase activity detection kit provided by Shanghai Beihu Biotechnology Co., Ltd. to determine sucrase in the soil. White rot fungi significantly increase the activity of sugarcane enzymes in soil by regulating the structure of soil microbial communities. In unsterilized soil, this increase in activity is consistent with higher IMI degradation efficiency. The increase in sugarcane enzyme activity is indicative of the activity of soil microbial communities and the improvement of soil health. Furthermore, it creates a more favorable environment for the degradation of IMI. Therefore, sugarcane enzyme activity can be an important indicator for evaluating the effectiveness of soil remediation.

Analysis rules for microbial community changes in polluted soil

The soil samples for this experiment aretaken from the non-sterile group soil on the 7th day of fungal degradation analysis classification in IMI contaminated soil. The extraction of DNA and amplification of PCR are completed by entrusting relevant pharmaceutical technology companies for testing. In the subsequent operation, microbial DNA is initially extracted from soil samples and subsequently amplified by means of PCR technology for the purpose of conducting high-throughput sequencing analysis of microbial community diversity and composition.

In the experiment, a UV-sterilized operating table (SW-CJ-2F) is used to inoculate the white rot fungi and prepare the culture medium. The components of the culture medium, including ammonium tartrate, magnesium sulfate heptahydrate, agar, etc., were accurately weighed using an electronic balance (BSA124S), dissolved in ultrapure water,

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and sterilized using a high-pressure steam sterilization pot (LDZM-60KCS). After sterilization, the culture medium is cooled and solidified on a sterile operating table, inoculated with white rot fungus spore powder, and then cultured on a constant-temperature oscillating sieve (ZWY-212B). In the degradation experiment of IMI, high-performance liquid chromatography (Agilent 1260) is used to detect the concentration changes of IMI in water samples and soil extracts. The standard solution of IMI used in the experiment is concentrated using a rotary evaporator (RE-2000C). In addition, anhydrous calcium chloride and sodium chloride are used to prepare experimental buffer solutions and to optimize the experimental conditions by adjusting the ionic strength of the solution.

The soil optimization process includes adjusting the pH value to neutral, supplementing organic matter, introducing beneficial microorganisms, managing soil moisture and aeration, and controlling the temperature at 25-30°C. In the water bodies, light conditions need to be optimized, nutrients need to be added, dissolved oxygen levels need to be increased, initial pollutant concentrations need to be controlled, and water temperatures need to be adjusted to the appropriate range (25-30°C). This can not only enhance the ability of white rot fungi to degrade contaminants such as IMI, but also help restore soil and aquatic ecosystems.

Statistical analysis results

The objective of this study was to evaluate the efficacy of white rot fungi in degrading IMI and the associated changes in soil properties, enzyme activity, and microbial diversity. The study analyzed the data using analysis of variance (ANOVA), t-test, and correlation analysis to determine the significance of the differences observed under different experimental conditions, with $P<0.01$ indicating significant statistical differences in the comparative results. The results are presented in *Table 4*.

Table 4. *Alpha diversity index of fungal and bacterial communities in soil samples*

Object	Form	Shannon index	Sobs index	Simpson index	Ace index	Chao index	Bootstrap index	Coverage (%)
Germ	QH	3.49	269.88	0.08	318.45	318.49	297.33	99.73
	BZ	2.94	174.01	0.11	269.13	250.38	196.24	99.75
	KB	2.47	156.70	0.16	197.82	207.12	175.04	99.83
	YL	2.98	177.25	0.12	239.75	228.09	196.58	99.81
	GD	2.12	150.12	0.25	222.37	199.42	170.09	99.78
Fungus	QH	1.67	112.85	0.40	115.64	116.04	116.23	99.99
	BZ	0.98	103.17	0.63	112.39	109.63	109.84	99.98
	KB	1.05	85.49	0.54	98.25	99.28	94.27	99.96
	YL	0.69	31.24	0.63	97.08	49.37	35.92	99.98
	GD	0.14	15.96	0.94	35.22	21.62	18.07	99.99

To analyze the degradation rate of IMI, repeated measures ANOVA was used to compare the degradation rate of IMI among different fungal forms (spore, free, fixed, and enhanced) at different time points. There were significant differences ($P<0.01$) between the different fungal morphologies, with the spore morphology having the highest degradation efficiency.

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In the analysis of changes in soil properties, paired t-test was used to evaluate the changes in soil pH and organic matter content before and after treatment. The results showed that after the application of white rot fungi, pH, and organic matter content were significantly reduced (P<0.05), especially in the intensified fungal treatment group.

An independent t-test was used to compare enzyme activity between sterilized and unsterilized soils. The analysis showed that enzyme activity was significantly reduced in sterilized soil (P<0.01), indicating that microbial populations had a significant effect on enzyme production.

In the analysis of microbial diversity and composition, the alpha diversity index was used to evaluate microbial diversity, and ANOVA was used to treat differences between groups. The research showed significant differences in diversity index, with the highest diversity in QH fungal morphology (P<0.01).

In the correlation analysis, Pearson correlation analysis was performed to explore the relationship between IMI degradation rate and factors such as enzyme activity and protein concentration. The results showed a strong positive correlation between IMI degradation and enzyme activity (r=0.85, P<0.01). The surface area of white rot fungal spores or hyphae was estimated using the geometric formula $A = 4\pi r^2$. In addition, the surface area of immobilized fungi could be calculated by measuring the size of immobilized particles.

Results

Experimental results of degradation of IMI polluted water bodies

Self-degradation of IMI

After configuring the IMI solution, the linear equation of the IMI standard curve is measured in *Equation (1)*.

$$y = 49x + 41 \quad \text{(Eq.1)}$$

In *Equation (1)*, y and x represent the peak area and concentration of IMI, respectively. Figure 1 shows the self-degradation of IMI in water under different lighting conditions. Sub-Figures (a) and (b) in *Figure 1* show the experimental data of Kirk culture medium system and ultrapure water system, respectively. Under light and dark conditions in the Kirk medium system and ultrapure water system, the average daily concentrations of IMI are8.1 mg/L, 8.2 mg/L, and 7.9 mg/L, and 8.0 mg/L, respectively. By repeated measures ANOVA, the results show no significant difference in the rate of self-degradation among different treatment groups (P>0.05), indicating that the contribution of self-degradation to changes in IMI concentration is limited.

Degradation of IMI in water by white rot fungi

Figure 2 shows the experimental results of white rot fungi degrading IMI in water. As time increased, the real-time concentration of IMI in water with initial IMI concentrations of 2 mg/L, 5 mg/L, and 10 mg/L decreased with experimental time. However, in water with initial IMI concentrations of 20 mg/L and 50 mg/L, the real-time concentration of IMI did not show a significant decrease with time. Using repeated measures ANOVA, significant differences (P<0.01) were observed in degradation rates between different

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concentration groups. It indicated that high concentrations of IMI had an inhibitory effect on the degradation ability of white rot fungi.

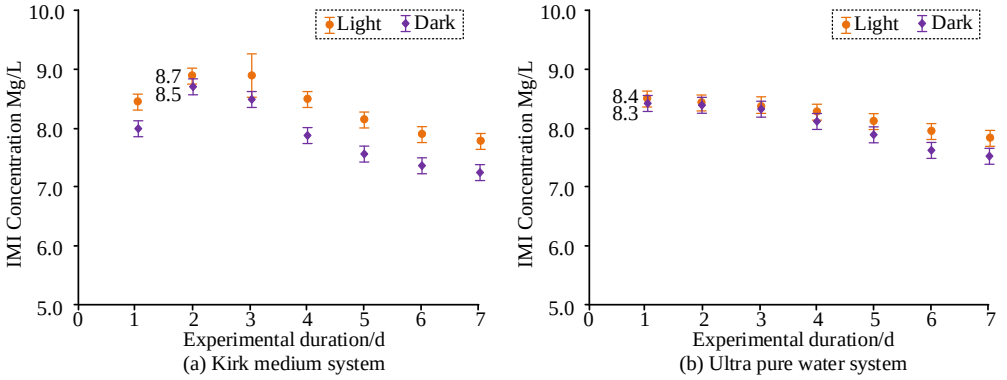

Figure 1. *Self-degradation of IMI in water bodies*

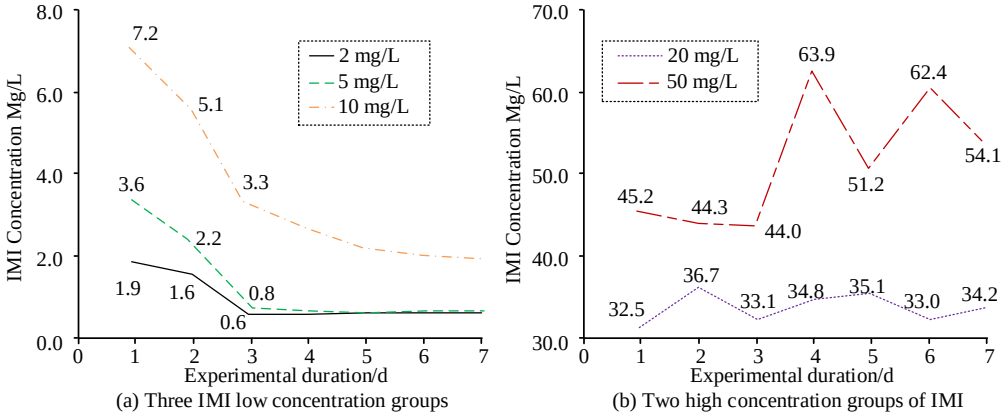

Figure 2. *Experimental results of white rot fungi degrading water IMI*

Cellular activity of white rot fungi in polluted water bodies

Figure 3 shows the cell activity of white rot fungi at different IMI concentrations. The gray area represents the area composed of the range of values for all samples. When the concentration of IMI in water increased from 0 mg/L, the cell activity of white rot fungi increased under the same conditions. However, when the concentration of IMI in water was too high, the cell activity of white rot fungi decreased. For example, after 48 hours of exposure, the white rot fungal activity in samples with IMI concentrations of 0 mg/L, 2 mg/L, 5 mg/L, and 50 mg/L increased to 134%, 146%, 149%, and 194% of the original levels, respectively. From the perspective of exposure time, as the exposure time increased, the activity of white rot fungi in each group of samples showed a trend of increasing and then decreasing. By independent t-test, the results showed a significant difference in cell viability between the low concentration group and the high concentration group (P<0.01), indicating that IMI concentration had a significant effect on the growth and metabolism of white rot fungi.

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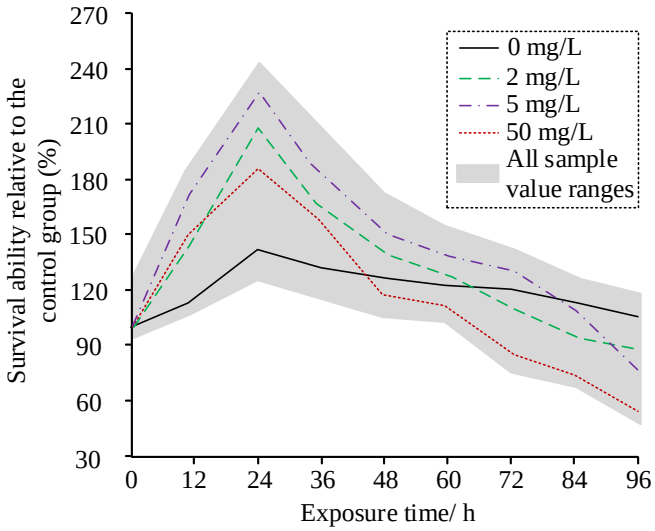

Figure 3. *Cell activity of white rot fungi in different IMI concentrations*

Changes in intracellular antioxidant indicators of white rot fungi

Figure 4 shows the effect of different concentrations of IMI conditions on the ROS of white rot fungi. After being exposed to IMI water for 24 hours, the concentration of white rot fungi was directly proportional to ROS. When the IMI concentration was 50 mg/L, ROS was 346% of the control group with an IMI concentration of 0 mg/L.

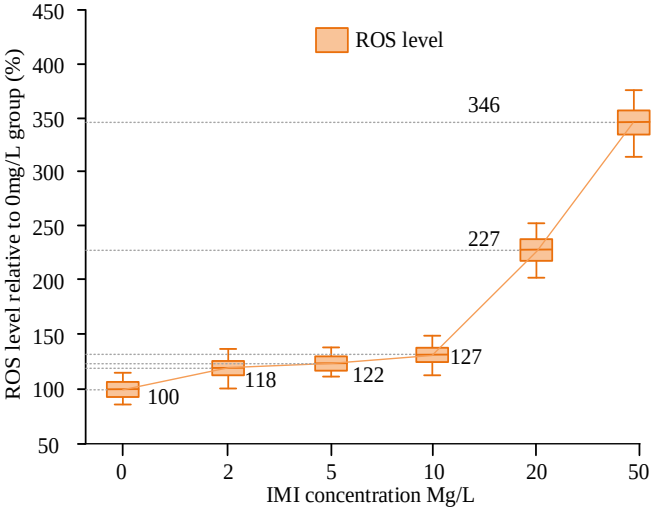

Figure 4. *Effect of IMI concentration in water on white rot fungus ROS*

Figure 5 shows the effect of IMI concentration on the CAT and POD content of white rot fungi. Different fill styles represented different observation times. For the three observation times, as the IMI concentration increased, the CAT and POD content of each white rot fungus sample showed an overall downward trend. Through ANOVA, significant differences ($P<0.01$) were observed in ROS levels and antioxidant enzyme content between different concentration groups, indicating that high concentrations of IMI had a significant inhibitory effect on the antioxidant capacity of white rot fungi.

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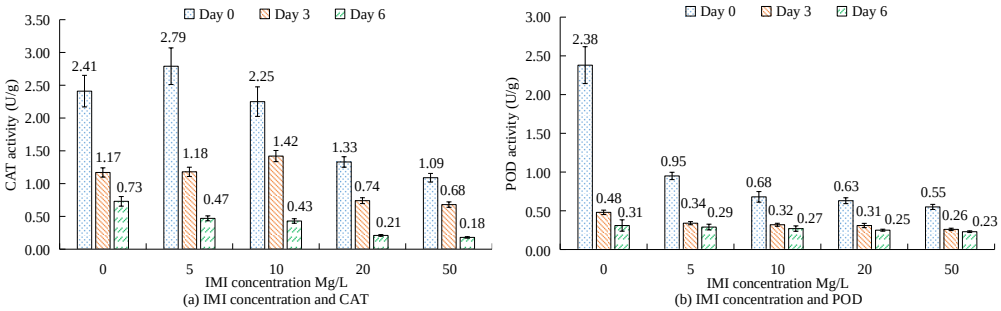

Figure 5. *Effect of miR-155 on apoptosis of Y79 human retinoblastoma cells detected by flow cytometry*

Experimental results of IMI contaminated soil degradation

Remediation effects of different forms of white rot fungi on polluted soil

Figure 6 shows the changes in IMI degradation rate in polluted soil with different forms of white rot fungi. KB, YL, BZ, GD, and QH represented the control, free, spore, immobilized, and enhanced groups, respectively. Under the same conditions, the removal rate of IMI in unsterilized soil was higher than that in sterilized soil. Moreover, as the experimental time increased, the removal rate of soil IMI under the same conditions continued to increase. When the experiment started for 7 days, the IMI removal rates of KB, QH, BZ, YL, and GD in unsterilized and sterilized soil were 84%, 87%, 91%, 82%, 81%, and 49%, 43%, 56%, 58%, and 57%, respectively. *Figure 6* shows that different forms of white rot fungi have varying effects on the degradation of IMI in polluted soil. The spore form of white rot fungi showed the highest IMI removal rate, reaching 91% in unsterilized soil by the 7th day of the experiment. The larger surface area contact between fungal spores and IMI pollutants was conducive to more effective degradation. In contrast, the degradation efficiency of immobilized forms was slightly lower, which may be attributed to the restricted diffusion of IMI in the immobilized matrix, thereby limiting the entry of fungal enzymes. Using repeated measures ANOVA, significant differences (P<0.01) were observed in the degradation efficiency of different forms of white rot fungi, indicating that spore-forming white rot fungi had a significant advantage in soil remediation.

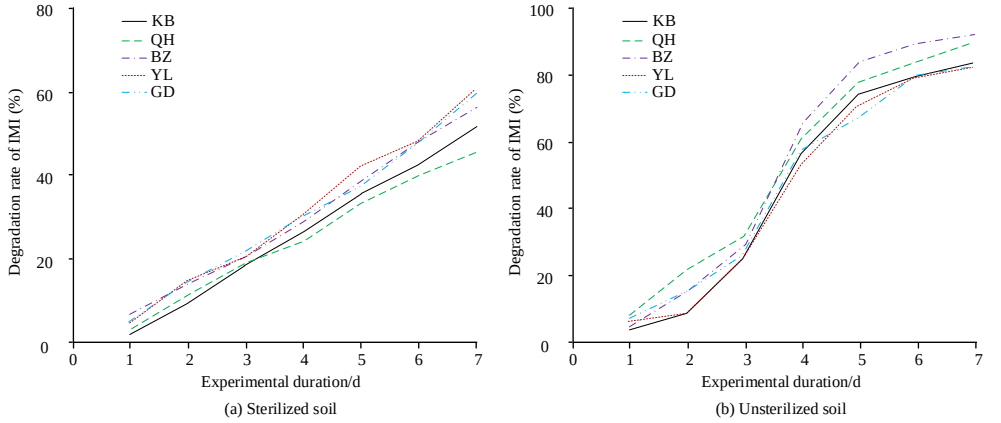

Figure 6. *Changes in IMI degradation rate in polluted soil with different forms of white rot fungi*

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Changes in soil physical and chemical properties during the remediation process

Figure 7 shows the variation of pH value in soil. In both soil forms, the overall sample pH values were higher than those on day 0 of the experiment until day 7. Moreover, within the same soil environment and experimental observation time, there was little difference in the distribution of pH values between different forms of white rot fungi. Compared to sterilized soil, samples of various forms of white rot fungi in unsterilized soil had an overall higher pH on day 0 and a lower pH on day 7, indicating that the overall values were closer to the acid-base neutral range.

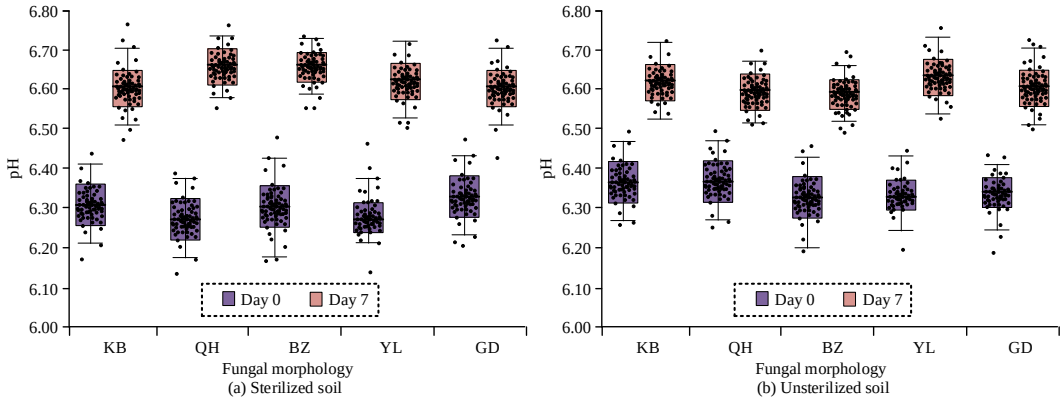

Figure 7. *Statistics of pH changes in soil*

Figure 8 analyzes the changes in organic matter content in different soil environments. The organic matter content in sterilized and unsterilized soil on day 0 and day 7 was 24.3 g/kg, 24.4 g/kg, and 22.6 g/kg, 22.4 g/kg, respectively. This indicated that on the 0th day, sterilization had a minimal impact on the overall organic matter content in the soil. On the 7th day, regardless of sterilization, the overall soil organic matter was lower than on the 0th day.

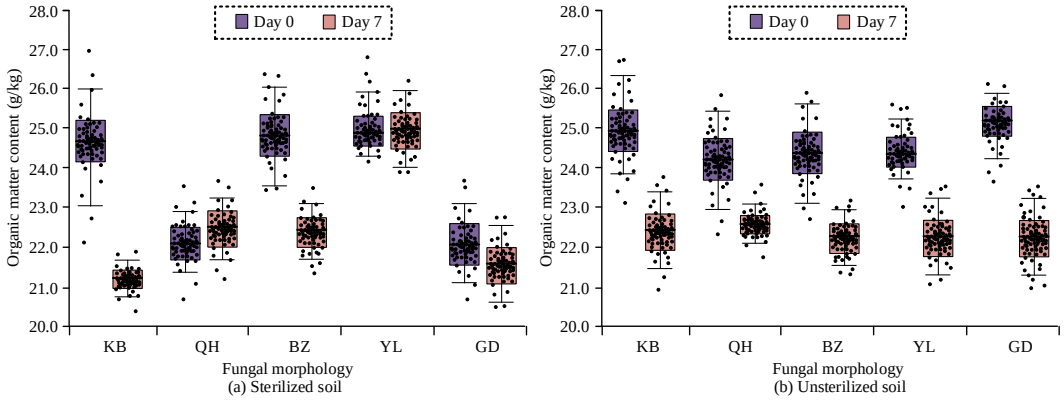

Figure 8. *Statistics of changes in organic matter content in soil*

As shown in *Figures 7 and 8*, during the 7-day experiment, the introduction of white rot fungi into disinfected and unsterilized soil resulted in a significant decrease in soil pH and organic matter content. The decrease in pH indicated that the metabolic activity of

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the fungi, which produce organic acids during decomposition, may lead to soil acidification. A transition of the pH value to a more neutral range was of great importance for the restoration of the microbial balance of soil, as extreme pH values had the potential to inhibit the growth of beneficial soil microorganisms. Furthermore, the reduction in organic matter content suggested that white rot fungi were utilizing this organic matter as a carbon source to support their growth and enzyme activity, thereby accelerating the degradation of IMI. The pH and organic matter content of the treated soil were significantly reduced (P<0.05). By paired t-test, significant differences (P<0.05) were observed in soil pH and organic matter content before and after treatment, indicating that the metabolic activity of white rot fungi had a significant effect on soil properties.

Changes in sugarcane enzymes in polluted soil

Figure 9 shows the sucrase content under different soil and fungal morphology conditions on the 7th day of the experiment. At this time, the sucrase content of various fungal forms in the soil was much lower than the corresponding fungal form data of unsterilized soil. The sucrose enzyme content of YL and BZ fungal forms in sterilized soil and unsterilized soil were 3.3 mg/g, 3.0 mg/g, and 7.8 mg/g, 8.7 mg/g, respectively. The sucrose enzyme content of these two forms was significantly higher than that of other fungal forms under the same conditions. As shown in *Figure 9*, the sucrase activity in the soil of the sterilized sample was significantly reduced compared to the unsterilized sample. This indicated that soil sterilization could inactivate some local microorganisms responsible for sucrase production, thereby reducing the overall enzyme activity in the soil. However, in non-sterilized soils, the presence of active microbial communities could support higher sucrase activity, which was important for the degradation of complex carbohydrates and the maintenance of soil health. Using independent t-test, significant differences (P<0.01) were observed in sucrose enzyme content between different treatment groups, indicating that white rot fungi had a significant effect on soil enzyme activity.

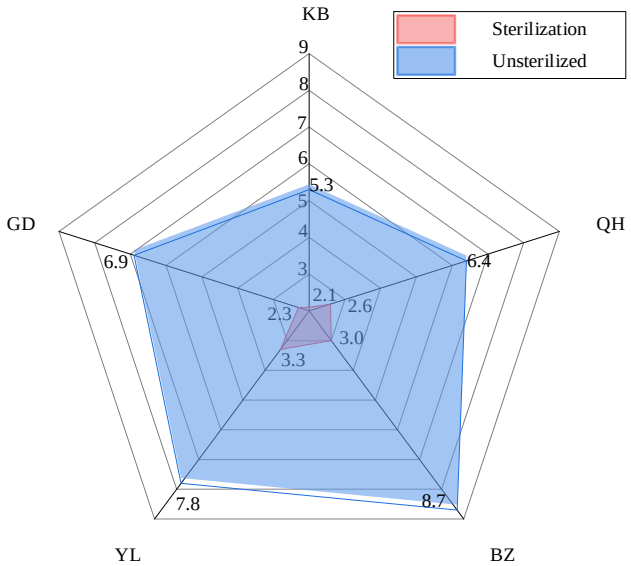

Figure 9. *Sucrase content in different soil and fungal forms on the 7th day of the experiment (unit: mg/g)*

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Analysis of microbial diversity in polluted soil

Table 5 shows the Alpha diversity index of fungal and bacterial communities in soil samples. In bacterial and fungal communities, the Shannon indices of QH morphology were 3.49 and 1.67, respectively, higher than those of other forms. The Shannon indices of GD morphology in bacterial and fungal communities were 2.12 and 0.14, respectively, which were lower than those of other forms. In Ace and Chao indices, the above pattern also existed. The alpha diversity index in *Table 5* showed that the bacterial and fungal diversity in the soil treated with white rot fungi was significantly higher compared to the control group. The increase in microbial diversity indicated that the introduction of white rot fungi not only contributed to contaminant degradation, but also promoted a more diverse and resilient microbial community. ANOVA showed significant differences (P<0.01) in microbial diversity between treatment groups, indicating that white rot fungi had a significant effect on soil microbial community structure.

Table 5. *Research statistical results*

Analysis	Test Used	Significant Results	Key Findings
IMI degradation rate	Repeated measures ANOVA	0.0024 (P<0.01)	Spore form had the highest degradation efficiency.
Soil pH and organic matter	Paired t-test	0.0260 (P<0.05)	Significant reduction in pH and organic matter post-treatment.
Sucrase activity in soil	Independent t-test	0.0084 (P<0.01)	Lower enzyme activity in sterilized soil, indicating the role of native microbes.
Microbial diversity (Shannon Index)	ANOVA	0.0068 (P<0.01)	QH fungal form showed the highest microbial diversity.
Microbial community structure (PCA)	PCA	/	Distinct microbial community clustering based on fungal treatments.
Correlation (IMI degradation & enzyme activity)	Pearson Correlation	r = 0.85, p=0.0026 (P<0.01)	Positive correlation between IMI degradation and enzyme activity.

Analysis of microbial community composition in polluted soil

Figure 10 shows the relative abundance of bacterial and fungal communities in non-sterilized soil after 7 days of inoculation with white rot fungi. Community abundance was calculated in units of species phylum. By observing *Figure 10(a)*, the Proteobacteria phylum was the species with the highest community advantage in unsterilized soil. The relative abundance of Proteobacteria phyla corresponding to different forms of white rot fungi ranged from 67% to 88%, while the relative abundance of other phyla was significantly lower than the former. For the fungal community inoculated with white rot fungi in unsterilized soil for 7 days, except for the YL form, the relative abundance of Basidiomycota phyla in other forms ranged from 68% to 99%, which was higher than that of other phyla. Ascomycota was the second largest phylum, with relative abundance ranging from 1% to 31%, except for YL. The increase in the relative abundance of Proteobacteria in unsterilized soil in *Figure 10* suggested that these bacteria could be involved in the degradation process, or that by-products of fungal metabolism may increase the rate of bacterial colonization. In most fungal forms, the

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Basidiomycoceaefamily increased while the Ascomycota family decreased, indicating that white rot fungi could be more competitive than other fungal species, thereby altering the microbial landscape of the soil.

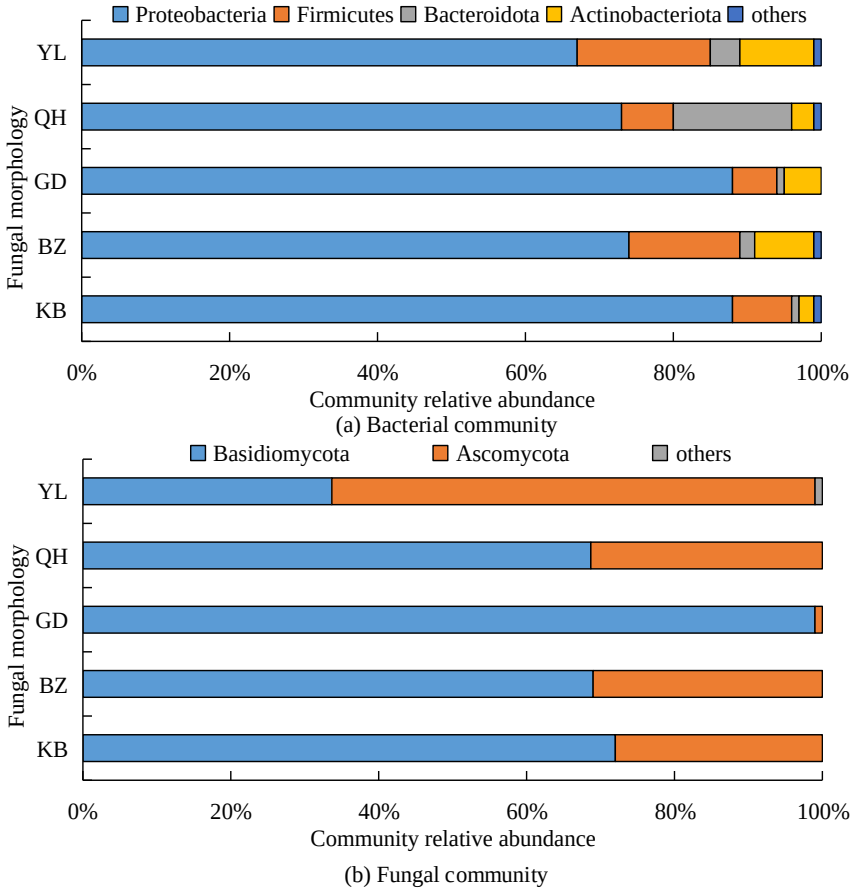

Figure 10. *Relative abundance of bacterial and fungal communities after inoculation with white rot fungi in unsterilized soil*

Microbial species analysis and principal component analysis of polluted soil

Figure 11 shows the Venn plot of bacteria and fungi in soil. From *Figure 11(a)*, the total number of bacterial species in BZ and QH was the highest at 24, followed by YL and QH with 16, while KB and QH had the lowest total number of bacterial species at 12. From *Figure 11(b)*, the total number of fungi in KB and BZ was the highest at 23, followed by the BZ and QH groups at 18, and KB and QH were the least at 11. Moreover, the fungal species overlap between YL and BZ was very high, with only one species being different.

Principal Component Analysis (PCA) was performed on the bacterial and fungal microbial communities in soil in *Figure 12*. In *Figure 12(a)*, there were slight differences within QH, YL, and KB. By observing *Figure 12(b)*, there was a significant difference within YL and BZ.

Figures 11 and 12 further illustrate the impact of white rot fungi on microbial communities in contaminated soils. The significant overlap of bacterial species between the BZ type and the control group suggested that certain bacterial species were resistant

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to IMI contamination and can coexist with white rot fungi. In contrast, the unique fungal species observed in YL and GD morphologies suggested that these fungi introduced new dynamics into soil ecosystems, potentially replacing less competitive species.

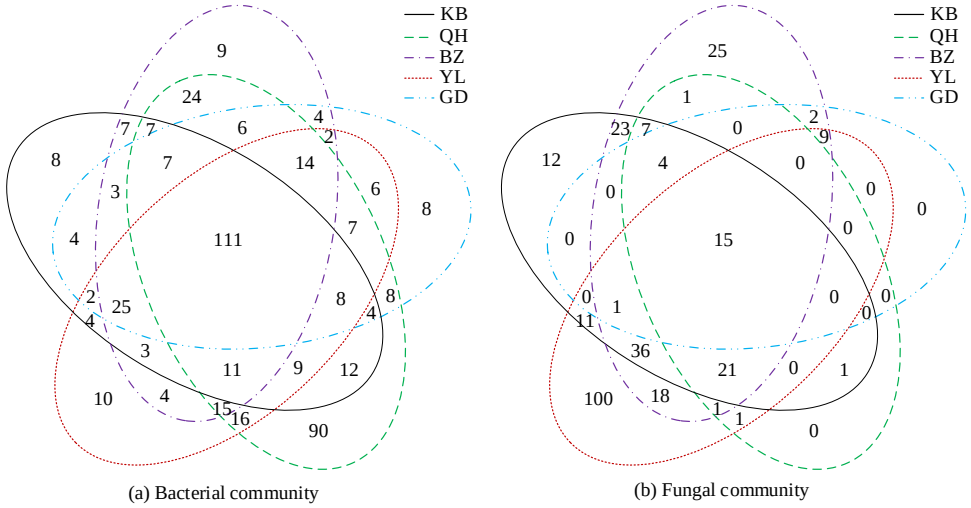

Figure 11. *Venn plot of bacteria and fungi in soil*

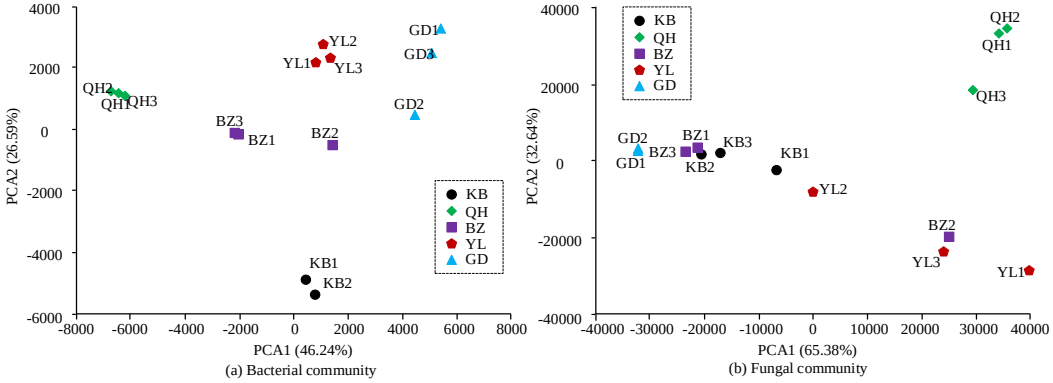

Figure 12. *PCA results of bacterial and fungal microbial communities in soil*

Discussion

Due to the rapid development of modern agriculture and heavy industry, some chemical pollutants were widely present in the environment, disrupting the structure of soil microbial communities and affecting soil ecological stability. IMI was considered a common and challenging environmental pollutant due to its high stability and poor biodegradability. To repair this type of pollution, researchers were searching for effective methods to deal with IMI. White rot fungi, a species of fungi with high efficiency in degrading lignin, have attracted attention due to their unique degradation pathways and diverse degradative enzyme systems. Although the degrading ability of white rot fungi has been widely recognized, there has been relatively little research on their mechanisms for soil microbial community remediation. Therefore, the main objective of this study was to investigate the microbial community mechanism of white rot fungi in the

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remediation of IMI-contaminated soil and the degradation rate of IMI after the addition of white rot fungi to the soil.

The coexistence of soil and water in nature was exemplified by the formation of swamps, wet soil, and puddles in certain geographical landforms. Consequently, it was imperative to assess the impact of white rot fungi on IMI in water bodies. The statistical results in *Figure 1* confirmed that the influence of light intensity and the liquid system used to cultivate white rot fungi on the self-degradation of IMI was minimal and cannot change the trend of self-degradation. In the analysis of *Figure 2*, there was a significant difference in the degradation effect of white rot fungi on different concentrations of IMI in the water. Moreover, when the IMI concentration in the water was not higher than 10 mg/L. After 7 days, the degradation amplitude of IMI concentration generally exceeded 60%. Moreover, as the concentration of IMI in the water increases, the effectiveness of white rot fungi in degrading IMI gradually deteriorates. This was because the high concentration of IMI in the water affected the life activities of white rot fungi, hindering their growth and reproduction, and reducing the degradation efficiency of IMI. The research results of Mori T et al. also demonstrated this pattern (Emmerich, 2021). In *Figure 3*, after the start of the experiment, IMI brought a toxic excitation effect on white rot fungi in a short period of time, thereby briefly enhancing the cell activity of white rot fungi. However, as time goes on, the cell activity of white rot fungi will decrease below the initial value. The results in *Figure 4* indicated that after exposure of white rot fungi to IMI water for a certain period of time, the oxidative pressure inside the cells increased, thereby increasing ROS content and causing fungal oxidative damage. The results of Emmerich et al. using brown rot fungus and Poria cocos fungus for pollution treatment also showed that after being placed in water containing acetyl compounds for more than 4 days, the oxidative damage of fungi significantly increased (Alberoni, 2020). In the analysis of *Figure 5*, at all selected time points, the CAT and POD contents of white rot fungi exposed to IMI water showed an overall decreasing trend, which were lower than the control group. This indicated that the ability of white rot fungal cells to mitigate oxidative stress response and antioxidant stress was attenuated in the IMI environment. However, short-term exposure to IMI water results in an increase in antioxidant enzymes and cell activity of white rot fungi compared to the control group. This was mainly due to the toxic excitation effect of fungal cells affected by low concentrations of contaminants.

The decline of white rot fungi was generally triggered by an increase in intracellular ROS content, and CAT and POD play a role in preventing oxidative damage caused by increased ROS in the fungal body (Abedinifar, 2020). As the compression time of IMI prolongs, the increase in ROS content may make the defense of intracellular antioxidant enzymes unable to resist oxidative damage caused by IMI. This may further trigger an imbalance in the internal ecological balance of cells and the subsequent inhibition of antioxidant enzyme secretion.

The results of a series of soil experiments were analyzed. In *Figure 6*, on the 4th day, the IMI removal efficiency of YL and BZ was superior to the non-microbial degradation mode of the control group. Moreover, YL had the best IMI degradation effect. On the 7th day, YL's IMI degradation effect remains the best. In the non-sterilization treatment experiment, BZ had the highest removal efficiency of IMI on the fourth and seventh days. Therefore, the microorganisms in the soil can indeed played a role in enhancing the degradation efficiency of white rot fungus IMI. Abedinifar et al. also demonstrated similar conclusions in their pollution degradation experiments on brown rot fungi and Poria cocos

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fungi. That is, in unsterilized soil, under the same conditions, the degradation rate of pollutants by brown rot fungus and Poria cocos fungus was faster than that of the two fungi in sterilized soil. The reason why different inoculation states of white rot fungi had an impact on the degradation efficiency of IMI in soil environment may be related to that the inoculation form can change the surface area, volume, and hyphal development of the fungal balls. These factors exert a direct influence on the degradation capacity of white rot fungi. The enhanced white rot fungi may restrict the utilization of carbon and nitrogen sources due to their dependency on straw for nutrients, which may consequently impact mycelial growth. The immobilization of white rot fungi was influenced by the mineralization of sodium alginate and calcium chloride, which may reduce the contact area with IMI, thereby reducing degradation efficiency. Moreover, due to their good growth and larger contact area with IMI, spores and free states had higher degradation efficiency. In addition, soil sterilization treatment may alter the microbial community structure and organic matter content, which was also one of the reasons affecting degradation efficiency. Zhou (2022) 's research also supported this.

A review of *Figures 7 and 8* indicated that the application of an inactivation treatment to the soil had a negligible effect on the pH level of the soil. When white rot fungi were added to the soil, regardless of whether the soil had been inactivated or not, the total organic matter content showed a downward trend. This is because IMI in the soil undergoes adsorption reactions with humic colloids. The reason for the difference in sucrase content between the sterilized and unsterilized groups in *Figure 9* was that soil sterilization under high temperature and pressure could inactivate sucrases belonging to proteins, which (Guo, 2022; Gazman, 2023) also agree with. In the study by Li (2024) it was found that white rot fungi had a significant enhancing effect on the degradation rate of composite organic polluted soil. White rot fungi could effectively degrade mixed polycyclic aromatic hydrocarbons (PAH) pollutants under different levels of pollution, indicating that white rot fungi had a significant effect on the degradation of organic matter. The use of fungal remediation technology could degrade diesel pollution in soil. In Bai et al.'s (2024) study, the fungal solid-state fermentation (SSF) bioremediation method was used for bioremediation of soil pollution. This technology could effectively reduce the problem of diesel pollution in soil. This indicated that the use of fungal remediation technology could effectively degrade soil pollution (Bai et al., 2024). In Zhang et al.'s (2023) study, white rot fungi were used to improve the degradation rate of PAHs. The results showed that white rot fungi could effectively enhance the biodegradation rate of PAHs, indicating that white rot fungi also had good application effects on the biodegradation efficiency of different organic compounds (Zhang et al., 2023).

Based on the above content, the relevant experimental results of soil microbial communities were analyzed. Alpha diversity was suitable for evaluating species diversity in specific regions. The results of soil bacterial diversity testing confirmed that QH had the highest Shannon diversity values, and the Shannon indices of BZ, QH, and YL were higher than those of the control group, indicating that these three white rot fungi could play a role in enriching the soil microbial community. The analysis results based on Ace and Chao indices were consistent with the above. The results of soil fungal diversity testing confirmed that the soil fungal diversity of YL and GD was lower than the other three forms, while the Shannon diversity index of QH form was 1.67. It indicated that the corresponding fungal diversity was still better than all other forms. From the perspective of microbial community, after inoculation with white rot fungi in BZ and QH forms, there was no significant change in the fungal community structure in the soil. After inoculation

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with white rot fungi in BZ and QH forms, the bacterial community structure in the soil undergoes significant changes. Although the largest microbial community was still Proteobacteria, the relative abundance of Proteobacteria in the BZ and QH groups decreased from 0.88 in the KB group to 0.74 and 0.73, indicating a more balanced microbial community structure. Although the soil bacterial structure of YL became more balanced, the fungal structure was disturbed compared to the control group. The soil fungal structure of GD was actually more concentrated compared to the control group. Venn plot analysis confirmed that the common bacteria in the morphology of white rot fungi and KB group accounted for more than 70% of the total number of bacteria in both groups. PCA confirmed that the bacterial diversity of KB, YL, and QH was minimal, while the fungal diversity of KB, QH, and GD was minimal. The differences in bacteria and fungi between BZ and YL were significant, indicating that the ecology of bacteria and fungi in these two groups was more abundant.

In summary, white rot fungi can play a role in degrading IMI in water, and the degradation effect is far superior to light factors. However, high concentrations of IMI can also inhibit the activity of white rot fungi, thereby reducing their IMI degradation ability. In soil, the introduction of white rot fungi in BZ form can accelerate the degradation of soil IMI in both sterilized and unsterilized states. Access to white rot fungi will not have a significant effect on soil pH, but it will reduce the organic matter content of the soil. The microbial communities of bacteria and fungi in the soil become more balanced and diverse only after the incorporation of BZ forms. Therefore, this study suggests that incorporating white rot fungi into soils containing IMI in the form of BZ can simultaneously accelerate IMI degradation and repair soil microbial communities.

The main purpose of the study is to investigate whether white rot fungi have a significant effect on degrading soil and water contaminated with IMI. The research showed that white rot fungi could effectively degrade IMI and significantly improved the microbial community structure in contaminated soil by secreting multiple degradation enzymes. Under different forms of white rot fungus treatment, the removal rate of IMI in soil ranged from 82% to 91%. In addition, white rot fungi could balance the microbial environment in soil and restore damaged soil microbial communities by changing the microbial living environment, reducing or eliminating IMI pollution.

The research revealed that in real environments, the concentration of IMI varied depending on specific usage and environmental conditions. Generally speaking, the application of IMI in agricultural activities may result in concentrations ranging from several milligrams per kilogram (mg/kg) to tens of milligrams per kilogram (mg/kg) in soil. In the study, the initial concentration range of IMI set in the experiment was 2 mg/L to 60 mg/L, which was used to simulate the degradation effect of white rot fungi under different levels of pollution.

The research displayed that white rot fungi had a significant effect on the degradation of IMI pollution under laboratory conditions. However, in real environments, the effectiveness of white rot fungi may be affected by multiple factors, such as the physical and chemical properties of soil, the complexity of microbial communities, environmental temperature and humidity, etc. Therefore, white rot fungus had the functions of environmental regulation and biodegradation in practical applications. Especially, when the environmental conditions were appropriately adjusted and optimized, it could be used as an effective bioremediation method to deal with IMI pollution.

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Data availability. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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