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Article

The Effects of Long-Term Application of Stabilized and Coated Urea on Soil Chemical Properties, Microbial Community Structure and Functional Genes in Paddy

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Abstract: The soil microbial community serves as a crucial indicator for assessing soil fertility and health. The composition and activity of soil microorganisms reflect the soil's well-being and its ability to provide proper nutrients for crops. Fertilization plays a prominent role in enhancing soil fertility, and novel types of fertilizers, such as stabilized fertilizers with nitrification/urease inhibitors and coated fertilizers, have gained popularity in rice and maize cultivation. However, the long-term effects of these stabilized and coated urea fertilizers on soil chemical properties, microbial diversity and community structure, as well as nitrogen cycling functional genes, remain unclear. Therefore, it is essential to investigate the impact of extended use of stabilized and coated urea fertilizers on soil fertility and microbiota in rice paddy fields. This research will provide scientific and theoretical support for the development and promotion of stabilized and coated urea fertilizers. To examine the effects of long-term application of these stabilized and coated urea fertilizers on soil chemical properties and microorganisms in rice paddy fields, soil samples were collected from brown soil rice paddy fields treated with various urease and nitrification inhibitors, stable urea, sulfur-coated urea (SCU), and resin-coated urea (PCU). The study revealed that 16 years of long-term use of conventional urea nitrogen fertilizer led to a considerable reduction in soil TP. On the other hand, NBPT and conventional urea fertilizers, when applied for an extended period, significantly increased soil organic matter (SOM). Moreover, except for HQ and NBPT+DMPP, the prolonged application of new urea fertilizers also significantly enhanced soil total potassium (TK). Notably, among all the treatments, PCU treatment had higher values for various soil chemical properties. In the case of SCU fertilizer used in brown soil rice paddy fields, it resulted in a significant decrease in soil pH over time. However, this change in pH did not affect the population of ammonia-oxidizing bacteria (AOB), as it was primarily influenced by soil available nitrogen. DMPP, HQ+DCD, NBPT+DMPP, SCU, and PCU significantly reduced the copy number of bacterial 16S rRNA genes in the soil, with the coated urea fertilizers (SCU and PCU) having a greater impact. The long-term use of stabilized urea fertilizers containing HQ significantly reduced the bacterial community in rice paddy soil. Conversely, HQ+DCD stable urea fertilizer significantly increased the population structure and abundance of ***Basidiomycota*** fungi while decreasing the population structure and abundance of ***Rozellomycota*** fungi. DMPP stabilized urea fertilizer notably increased the population structure and abundance of ***Ascomycota*** fungi while decreasing the population structure and abundance of ***Rozellmycota*** and ***Chytridiomycota*** fungi. Furthermore, HQ stabilized urea fertilizer significantly reduced the population structure and abundance of ***Chytridiomycota*** fungi. It is important to note that SCU fertilizer is not suitable for long-term application in neutral to slightly acidic meadow brown soil with a background pH. On the other hand, stable urea fertilizers and resin-coated urea fertilizers containing HQ, NBPT, DCD, DMPP, and their combinations are suitable for long-term application in neutral to slightly acidic meadow brown soil.

Keywords: urease inhibitor; nitrification inhibitor; stabilized urea; coated fertilizer; paddy; nitrogen conversion functional genes; AOA; AOB;

1. Introduction

Urea nitrogen fertilizer has historically served as the primary nitrogen source for crop cultivation. The application of urea fertilizer supplies an adequate amount of nitrogen nutrients for crop growth, thereby enhancing or sustaining crop yield. Nevertheless, extensive research indicates that conventional urea nitrogen fertilizer exhibits low utilization efficiency, with plants absorbing and utilizing only a fraction of it. Most of the nitrogen residual in the soil post-application dissipates through processes like ammonia volatilization[1], nitrate leaching[2], nitrification, and denitrification[3], resulting in issues such as soil acidification[4], heightened greenhouse gas emissions[5], and water body eutrophication[6]. Consequently, scholars are dedicated to discovering approaches that boost the utilization efficiency of nitrogen fertilizers, aiming to conserve resources, uphold an environmentally sustainable agricultural production, and safeguard the well-being and stability of agroecosystems. These approaches encompass enhancing field management practices, innovating new fertilizer types, and stable-coated urea fertilizer, which stands as the forefront of emerging efficient and eco-friendly nitrogen fertilizers. stabilized urea fertilizer represents an innovative fertilizer type supplemented urea with nitrification inhibitors, urease inhibitors, or a combination thereof[7]. Coated fertilizers encompass the application of one or more layers of continuous film material onto the fertilizer's surface, effectively regulating nutrient release through physical means. Consequently, this minimizes nutrient losses from soluble fertilizers and enhances fertilizer utilization efficiency. Diverse kinds of coated fertilizers exist, such as sulfur-coated, resin-coated, and fertilizer-encapsulated fertilizers. Both stabilized and coated urea fertilizers have demonstrated notable effectiveness in their implementation, and their utilization is steadily growing. Short-term experimental studies have revealed the capability of nitrification inhibitors to efficiently impede the ammonia oxidation process through the suppression or hindrance of growth in ammonia-oxidizing archaea (AOA) or ammonia-oxidizing bacteria (AOB)[8]. As a result, soil nitrate reductase and denitrification enzyme activities decrease, leading to a reduction in nitrate leaching and N_2O emissions[9]. Furthermore, research has shown that the use of nitrification inhibitors like DMPP markedly decreases the abundance[10] and transcript activity of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB)[11,12], which in turn alters the composition of AOB communities with respect to NH_4^+-N, NO_3^--N, N_2O, and pH[13]. By temporarily inhibiting urease-mediated urea decomposition, urease inhibitors extend the soil's retention time for ammonium ions, resulting in reduced ammonia volatilization and increased availability of NH_4^+ for plant absorption[14]. Additionally, in alkaline soils, urease inhibitors can mitigate N_2O emissions[14]. Several short-term experimental studies indicate that urease inhibitors have negligible effects on the abundance and community structure of soil microorganisms[15]. Nevertheless, additional studies have demonstrated that the inclusion of NBPT can alter both the abundance and community structure of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB)[16]. Moreover, these differences are noticeable in soils with varying pH levels. Furthermore, short-term experiments have indicated that the application of stabilized fertilizers leads to substantial improvements in crop yield[17], nitrogen fertilizer utilization efficiency[18,19], and a reduction in methane emissions[20]. Controlled-release fertilizers, also referred to as coated fertilizers, consist of granular fertilizers coated with polymer or resin materials. These coated fertilizers, diminish nitrate leaching, decrease emissions of CH_4, NH_3, and N_2O gases, and concurrently enhance nitrogen fertilizer utilization efficiency[21]. Although the long-term impacts of applying coated urea fertilizer remain uncertain, short-term investigations have revealed that the degradation of such fertilizers in the soil fluctuates in response to factors like temperature, humidity, and soil biological activity[22]. Consequently, this unpredictability in urea release rate may potentially impose detrimental consequences on crops. In addition, short-term investigations have indicated that the utilization of coated urea results in greater soil microbial diversity during the crop growth period, including the later stages[23]. Conversely, a

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separate study highlights that using both urea and coated urea together leads to reduced biodiversity of soil bacteria and fungi compared to the application of urea alone[24].

Previous investigations have primarily relied on conclusions derived from indoor cultivation or short-term field experiments. Nevertheless, present research on these two categories of fertilizers is restricted to their short-term impact on soil and crops. Our understanding of the prolonged repercussions of employing stabilized and coated urea fertilizer on soil remains limited. Furthermore, there exists an insufficiency of research investigating the implications of enduring utilization of stabilized urea and coated urea fertilizers on soil chemical properties, along with soil microbial composition and function. Specifically, there remains a dearth of systematic and comprehensive investigations gauging alterations in soil pH, nitrogen-related functional genes within soil microorganisms, and the attributes of the soil microbial community structure arising from extended usage of stabilized and coated urea fertilizer. Short-term experimental studies fail to elucidate the enduring consequences of consistent utilization of novel fertilizers on soil, the environment, and crop productivity. Consequently, it is imperative to evaluate the influence stemming from long-term persistent application of such fertilizers on soil and crops. This evaluation plays a critical role in appraising the relative merits and demerits of fertilizer technologies, fostering scientific and technological progress in the field of fertilizers, and refining research objectives. The objective of this experiment is to explore the ramifications arising from the prolonged utilization of stabilized /coated urea fertilizers on the chemical properties and microbial composition of paddy soil. The intended outcome is to establish a solid scientific foundation for the advancement of stabilized urea and coated urea fertilizer technologies.

Previous investigations have primarily relied on conclusions derived from indoor cultivation or short-term field experiments. Nevertheless, present research on these two categories of fertilizers is restricted to their short-term impact on soil and crops. Our understanding of the prolonged repercussions of employing stabilized and coated urea fertilizer on soil remains limited. Furthermore, there exists an insufficiency of research investigating the implications of enduring utilization of stabilized urea and coated urea fertilizers on soil chemical properties, along with soil microbial composition and function. Specifically, there remains a dearth of systematic and comprehensive investigations gauging alterations in soil pH, nitrogen-related functional genes within soil microorganisms, and the attributes of the soil microbial community structure arising from extended usage of stabilized and coated urea fertilizer. Short-term experimental studies fail to elucidate the enduring consequences of consistent utilization of novel fertilizers on soil, the environment, and crop productivity. Consequently, it is imperative to evaluate the influence stemming from long-term persistent application of such fertilizers on soil and crops. This evaluation plays a critical role in appraising the relative merits and demerits of fertilizer technologies, fostering scientific and technological progress in the field of fertilizers, and refining research objectives. The objective of this experiment is to explore the ramifications arising from the prolonged utilization of stabilized /coated urea fertilizers on the chemical properties and microbial composition of paddy soil. The intended outcome is to establish a solid scientific foundation for the advancement of stabilized urea and coated urea fertilizer technologies.

2. Materials and Methods

2.1. Experimental Location and Climatic

The long-term field experiment on rice cultivation is situated within the National Field Station of Agro-ecosystem(43°31′N, 123°22′E), Shenyang, Liaoning Province, China. The experimental site encompasses typical paddy soil in meadow brown soil. This station is situated at the southern terminus of the Song Liao Plain, positioned within the central region of the Liao He Plain—a representative agricultural production hub. The prevailing climate exhibits characteristics of a warm temperate semi-humid continental climate. The average annual temperature ranges between 7-8℃, with accumulated temperatures above 10℃ varying from 3310 to 3400℃. Total solar radiation amounts to approximately 5409.9 to 5598.9 KJcm-2. Annual rainfall ranges from 650 to 700mm, with

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a dryness index of 0.9, while the frost-free period persists for 147 to 164 days. Prior to initiating the long-term field experiment on rice cultivation, the area had been subject to rice cultivation over an extended period, thus rendering it a representative rice-growing region demonstrating a continuous rice cropping system. Commencing from spring 2007, the long-term field experiment on rice cultivation utilizing stable and coated urea fertilizers has continued uninterrupted for a duration of 16 years, culminating in 2022.

Table 1. Basic chemical properties of pre-test soil at 2007.

Organic matter (g/kg)	Total N (g/kg)	Total phosphorus (g/kg)	Total potassium (g/kg)	Available N (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)	pH
23.76	1.25	0.58	25.36	112.75	19.31	70.28	6.20

2.2. Experimental Design

The experiment comprises 10 treatments, involving the application of 6 distinct stabilized urea fertilizers: N-butylthiophosphoric triamide (NBPT), hydroquinone (HQ) as a urease inhibitor, 3,4-dimethylpyrazole phosphate (DMPP), dicyandiamide (DCD) as a nitrification inhibitor, various combinations thereof, and additionally sulfur-coated urea (SCU) and resin-coated urea (PCU). The urea utilized in the experiment originates from the China National Pharmaceutical Group, boasting a nitrogen content of 46%.

The experimental treatments include the following: (1) no fertilizer (CK); (2) Conventional granular urea (N), containing 46% nitrogen; (3) Urea fertilizer supplemented with 1% hydroquinone (HQ), containing 45.55% nitrogen; (4) Urea fertilizer supplemented with 0.5% N-butylthiophosphoric triamide (NBPT), containing 45.77% nitrogen; (5) Urea fertilizer supplemented with 3% dicyandiamide (DCD), containing 44.66% nitrogen;(6) Urea fertilizer supplemented with 1% 3,4-dimethylpyrazole phosphate (DMPP), containing 45.55% nitrogen; (7) Urea fertilizer supplemented with 1% HQ and 3% DCD(HQ+DCD), containing 44.23% nitrogen; (8) Urea fertilizer supplemented with 0.5% NBPT and 1% DMPP(NBPT+DMPP), containing 45.32% nitrogen; (9) Sulfur-coated urea fertilizer(SCU) (120-day release period) produced by Han Feng Company in Canada, containing 34.00% nitrogen; (10) Resin-coated urea fertilizer(PCU) (120-day release period) produced by Shandong Jin Zheng Da Group, containing 43.00% nitrogen. Each treatment is replicated three times, with a plot area of 20 m^2. The plots are randomly arranged in the field, and each plot receives an equal amount of nutrients. The fertilizer application rates adhere to the local standards, with an annual application rate of N 225.00 kg hm^{-2}, P_2O_5 120.00 kg hm^{-2}, and K_2O 150.00 kg hm^{-2}. Nitrogen fertilizers are utilized to supplement nitrogen in each treatment, phosphorus fertilizer consists of calcium superphosphate produced by Yun Tian Hua Company, with a P_2O_5 content of 43.00%, and potassium fertilizer comprises potassium chloride sourced from Russia, offering a K_2O content of 60.00%. Fertilizers are applied as a one-time basal application to the soil before transplanting rice seedlings in spring, without any further application during the rice growing period. Field management adheres to the traditional rice cultivation practices in the local area.

2.3. Sample Collection and Measurement Methods

The collection of soil samples in the experimental field took place after the mature harvest of rice in autumn 2022. The sampling depth ranged from 0 to 20 cm in the topsoil layer. Nine sampling points were selected in each plot, and the samples were meticulously mixed to obtain a representative composite sample. The collected soil samples were cleaned to eliminate impurities and fine roots. A portion of the fresh soil samples was stored at 4°C for the determination of indicators of soil biological activity. Another portion was air-dried for determining the basic chemical properties. Soil NH_4^+-N and NO_3^--N were determined by extracting the samples with a 2 mol L^{-1} KCl solution right after sampling, and the concentrations were measured using a 3-AA3 flow analyzer. Urease activity was determined using the residual urea method, while nitrate reductase activity was determined using

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the Kandeler method. Nitrification potential was determined using the chloride inhibition method. Microbial carbon and microbial nitrogen were extracted using the chloroform fumigation-extraction method and measured with a TOC analyzer. The remaining basic chemical properties were determined using standard methods.

Bacterial and archaeal 16S rRNA genes, as well as fungal ITS rDNA genes, were amplified using quantitative PCR (qPCR). The abundance of ammonia-oxidizing bacteria, ammonia-oxidizing archaea, and fungi was estimated by quantifying the amoA gene expression in ammonia-oxidizing bacteria and by performing qPCR amplification of nirH, nirS, and nirK genes associated with microbial nitrogen transformation. The extraction of DNA for soil microbial composition followed the protocol provided by the Power Soil DNA Isolation Kit (Omega Stool DNA Kit). The extracted DNA underwent analysis for quality and concentration using 1% agarose gel electrophoresis and spectrophotometry. Samples that met the quality criteria were stored at -20°C for future experiments. Bacterial and fungal amplification were performed using the primers 338F-806R and ITS1F-ITS2, respectively. The sequencing process was conducted at Beijing Ovison Gene Technology Co., Ltd. using the high-throughput Illumina Miseq PE300 sequencing platform.

2.4. Data Analyses and Statistics

The analysis and processing of soil chemical properties, microbial community, and gene abundance data were performed using Excel 2021, Origin 2023, R4.2.2, Canoco 5.0, and Past 4.09 software packages. Data analysis techniques applied included the Turkey test (P=0.05), principal component analysis, correlation analysis, and redundancy analysis. Microbial data processing involved using the Vsearch software (v2.7.1) and the UPARSE algorithm to cluster operational taxonomic units (OTUs) with a 97% similarity threshold. OTUs were classified by comparing their similarity to the Unite database using the BLAST algorithm with an e-value set to 1e-5 to obtain species classification information for each OTU. Alpha diversity indices, such as Shannon, Simpson, and Chao1, were analyzed using the QIIME1 software (v1.8.0). Species composition bar plots, considering species annotation and relative abundance results, were generated using the R software (v3.6.0).Beta diversity distance matrices were calculated using the QIIME1 software (v1.8.0), and clustering heatmaps and PCoA analysis based on Weighted Unifrac distances were performed using the R software (v3.6.0).

3. Results

3.1. The Effects of Basic Chemical Properties in Paddy Soil with Long-Term Application of Stabilized and Coated Urea Fertilizers

3.1.1. The Differences in Soil Chemical Properties Between the Long-Term Application of Stabilized and Coated Urea Fertilizer Treatments and the Initial Soil Conditions before the Experiment

Based on Figure 1, there were no significant differences in soil TN (total nitrogen), TP (total phosphorus), and AP (available phosphorus) between the initial soil conditions in 2007 and after 16 years of applying stabilized and coated urea fertilizer in the paddy fields. In contrast, long-term nitrogen fertilizer application resulted in a significant reduction in soil TP compared to the initial soil conditions. The application of stabilized and coated urea fertilizer resulted in a moderate improvement in soil SOM (soil organic matter), with treatments containing NBPT (urease inhibitor) and N showing significant increases in SOM levels. Treatments with NBPT, DCD (nitrification inhibitor), DMPP (nitrification inhibitor), HQ+DCD (nitrification inhibitor), SCU (sulfur-coated urea), and PCU (polymer-coated urea) significantly increased soil TK (total potassium), with the PCU treatment showing the highest increase. Soil AK (available potassium) significantly increased in all treatments, with the PCU treatment showing the highest increase. The SCU treatment resulted in a significant reduction in soil pH, whereas all other treatments led to significant increases in pH, with the DCD treatment showing the highest increase. In general, the PCU treatment exhibited positive performance in various soil indicators.

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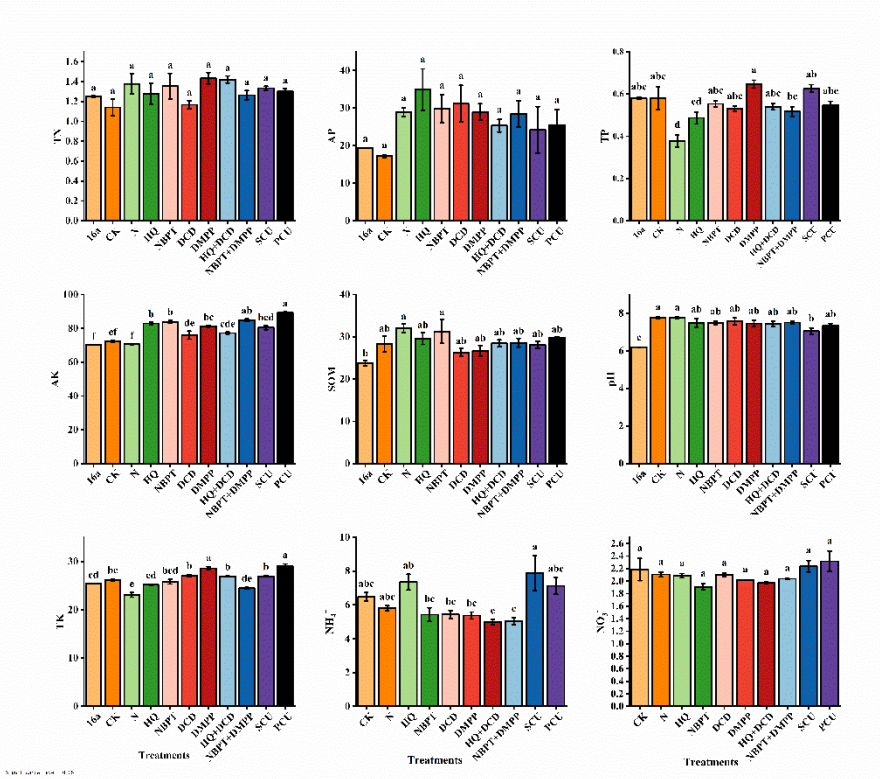

Figure 1. The basic chemical properties of rice field soils in different treatments. Different letters indicate significant differences between different treatments at $P < 0.05$ by Tukey test. (Ammonium and nitrate nitrogen contents were not measured in the soils before the experiment).

3.1.2. Characteristics of Changes in Soil Chemical Properties of Treatments after 16 Years of Application of Stabilized and Coated Urea Fertilizers

Figure 1 indicates that the soil ammonium nitrogen content did not exhibit significant differences ($P<0.05$) between the various treatments and the treatment with N after 16 years of applying different types of urea fertilizers. The HQ, SCU, and PCU treatments exhibited higher ammonium nitrogen content compared to the N treatment. Specifically, the HQ and SCU treatments significantly exceeded the levels observed in the HQ+DCD and NBPT+DMPP treatments, while the PCU treatment showed a significant increase over the HQ+DCD treatment. The SCU treatment displayed the highest ammonium nitrogen content among all the treatments, and both coated urea types exhibited higher ammonium nitrogen content compared to the stabilized urea treatments, with the exception of HQ. The soil nitrate nitrogen content did not differ significantly ($P>0.05$) between the various treatments and the N treatment. The SCU and PCU treatments exhibited higher nitrate nitrogen content compared to the other fertilization treatments, whereas the nitrate nitrogen content of the other fertilization treatments was lower than that of the N treatment. The PCU treatment displayed the highest nitrate nitrogen content. The soil organic matter content did not exhibit significant differences ($P>0.05$) between the various treatments and the N treatment. The organic matter content in all treatments was lower than that of the N treatment, with the DCD and DMPP treatments showing the lowest content, and the HQ and NBPT treatments exhibiting the highest organic matter content among the stabilized urea and coated urea treatments. The organic matter content of both coated urea types was lower than that of the HQ and NBPT treatments. With the exception of the HQ treatment, the soil total phosphorus content in all treatments was significantly higher than that of the N treatment. The N treatment exhibited the lowest soil total phosphorus content, whereas the SCU and PCU coated urea treatments displayed higher total phosphorus content compared to most stabilized urea treatments, with the exception of the DMPP treatment. The soil available phosphorus content did not differ significantly ($P>0.05$) between the various treatments

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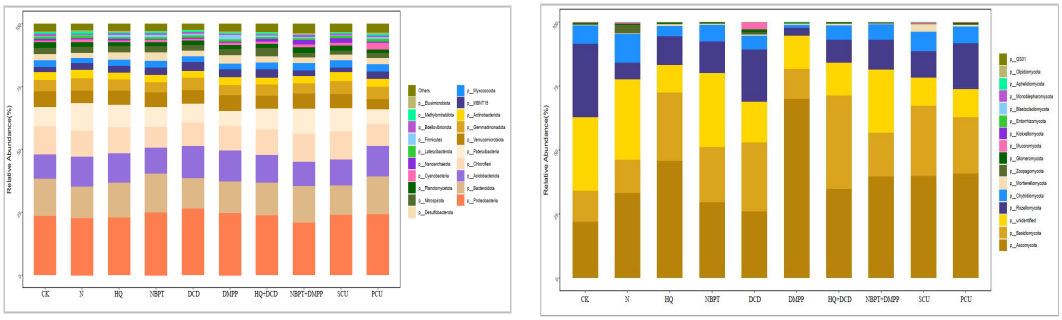
and the N treatment. The HQ treatment exhibited the highest available phosphorus content, while the stabilized urea treatments showed higher levels compared to the two coated fertilizers, with PCU having a higher content than SCU. All fertilization treatments, excluding the NBPT+DMPP treatment, resulted in significantly higher soil total potassium content compared to the N treatment ($P<0.05$). The total potassium content of the two coated urea types was relatively similar to that of the stabilized urea, with PCU showing higher levels than SCU, and DMPP exhibiting higher levels compared to other stabilized urea treatments. Both stabilized and coated urea treatments demonstrated a significantly higher available potassium content in the soil compared to the N treatment ($P<0.05$). The coated urea and stabilized urea treatments exhibited similar potassium content, but the DCD, HQ+DCD, and SCU treatments displayed lower available potassium content compared to other new urea types. The soil pH in the SCU treatment showed a significant decrease compared to the control (CK) and N treatments ($P<0.05$). Additionally, no significant differences ($P>0.05$) were observed between the other treatments and the CK and N treatments, with negligible differences among them.

3.2. Characteristics of Soil Bacterial and Fungal Communities in Rice Fields with Long-Term Application of Stabilized and Coated Urea Fertilisers

At the phylum level, the predominant bacterial phyla found in paddy field soil under different treatments were Proteobacteria (21%-27%), ***Bacteroidota*** (12%-15%), ***Acidobacteriota*** (9%-13%), ***Chloroflexi*** (8%-11%), and ***Patescibacteria*** (6%-11%). ***Proteobacteria***, ***Bacteroidota***, ***Acidobacteriota***, and ***Chloroflexi*** were the dominant phyla, and no significant differences were observed among the treatments (Fig. 2a). Among fungi (Fig. 2b), ***Ascomycota*** exhibited the highest relative abundance in all treatments (20%-70%), followed by ***Basidiomycota*** (12%-36%), ***unidentified fungi*** (11%-31%), ***Rozellomycota*** (7%-29%), and ***Chytridiomycota*** (1%-11%). The relative abundance of ***Ascomycota*** in the DMPP treatment showed a significant increase compared to all treatments except HQ. The abundance of ***Basidiomycota*** in the HQ+DCD treatment exhibited a significant increase compared to the CK, N, DMPP, and NBPT+DMPP treatments, with no significant differences observed among the other treatments. No significant differences were observed for the ***unidentified*** fungi among the treatments. The relative abundance of ***Rozellomycota*** in the DMPP and HQ+DCD treatments exhibited a significant decrease compared to the CK and N treatments, with no significant differences observed among the other treatments. The abundance of ***Chytridiomycota*** in the DMPP treatment showed a significant decrease compared to the CK, N, and SCU treatments. Additionally, the ***Chytridiomycota*** abundance in the HQ treatment exhibited a significant decrease compared to the N treatment, with no significant differences observed among the other treatments. These fungal phyla were present in all samples, as depicted in Fig. 2.



(**a**) (**b**)

Figure 2. The different treatments of rice field soil at the phylum level microbial composition ((a) bacteria, (b)fungi).

3.3. Abundance of Functional Genes of Microorganisms Involved in Nitrogen Cycling in Rice Field Soils with Long-Term Application Stabilized and Coated Urea Fertilizers

According to Fig. 3, the gene copy numbers of soil ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in the various fertilization treatments ranged from 1.48×10^5 to

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1.47×10^6 copies·g^{-1} of dry soil and from 1.60×10^5 to 4.1×10^5 copies·g^{-1} of dry soil, respectively. The highest AOB gene copy number was observed in paddy soil treated with SCU, whereas the lowest AOB gene copy number was found in soil treated with N. The SCU treatment exhibited a 50.78% and 60.88% increase in AOB gene copy numbers compared to the CK and N treatments, respectively. This suggests an augmentation of the AOB community in paddy soil under SCU treatment, while the remaining treatments did not demonstrate any significant differences. The NBPT+DMPP treatment exhibited the highest AOA gene abundance in paddy soil, with an 84.44% increase compared to CK and a 72.04% increase compared to the N treatment. Furthermore, the DMPP treatment demonstrated a relatively high AOA gene abundance in paddy soil, whereas the HQ and PCU treatments displayed lower AOA gene abundances compared to the N treatment. No significant differences were observed in fungal ITS among the various treatments in paddy soil. The highest fungal ITS gene copy number was recorded in the NBPT treatment, with a 49.51% increase compared to CK and a 32.50% increase compared to N. Conversely, the SCU treatment exhibited the lowest fungal ITS gene copy number. Additionally, the DCD, HQ+DCD, and SCU treatments demonstrated lower fungal ITS gene copy numbers compared to the N treatment. The N treatment exhibited the highest bacterial 16S gene copy number in paddy soil, displaying a 62.20% increase compared to CK. In comparison to the N treatment, the DMPP, HQ+DCD, NBPT+DMPP, SCU, and PCU treatments exhibited significantly lower ($p<0.05$) bacterial 16S gene copy numbers. Moreover, the bacterial 16S gene copy numbers for both encapsulated urea treatments were lower compared to long-term application of conventional urea and stabilized urea. The NBPT treatment exhibited the highest nifH gene copy number, whereas the HQ+DCD treatment displayed the lowest. The NBPT treatment demonstrated a significant increase compared to the DMPP, HQ+DCD, and PCU treatments, while no significant differences were observed among the other treatments. In general, the two encapsulated urea treatments did not exhibit any significant differences compared to long-term application of conventional urea fertilizer. However, long-term application of NBPT and NBPT+DMPP resulted in an increase in the nifH gene copy number in soil. No significant differences were observed in nirS gene copy number among the different soil fertilization treatments, with the DCD treatment displaying the lowest counts and the NBPT treatment exhibiting the highest counts. Compared to CK and N treatments, the NBPT treatment exhibited differences of 27.24% and 30.68%, respectively, whereas the differences among the remaining treatments were comparatively minor. The nirK gene abundance in paddy soil treated with HQ+DCD exhibited a significant increase compared to CK, N, HQ, DCD, and PCU treatments, with HQ+DCD representing the highest values and the N treatment displaying the lowest values. The disparities between HQ+DCD and CK and N treatments amounted to 65.89% and 79.82%, respectively.

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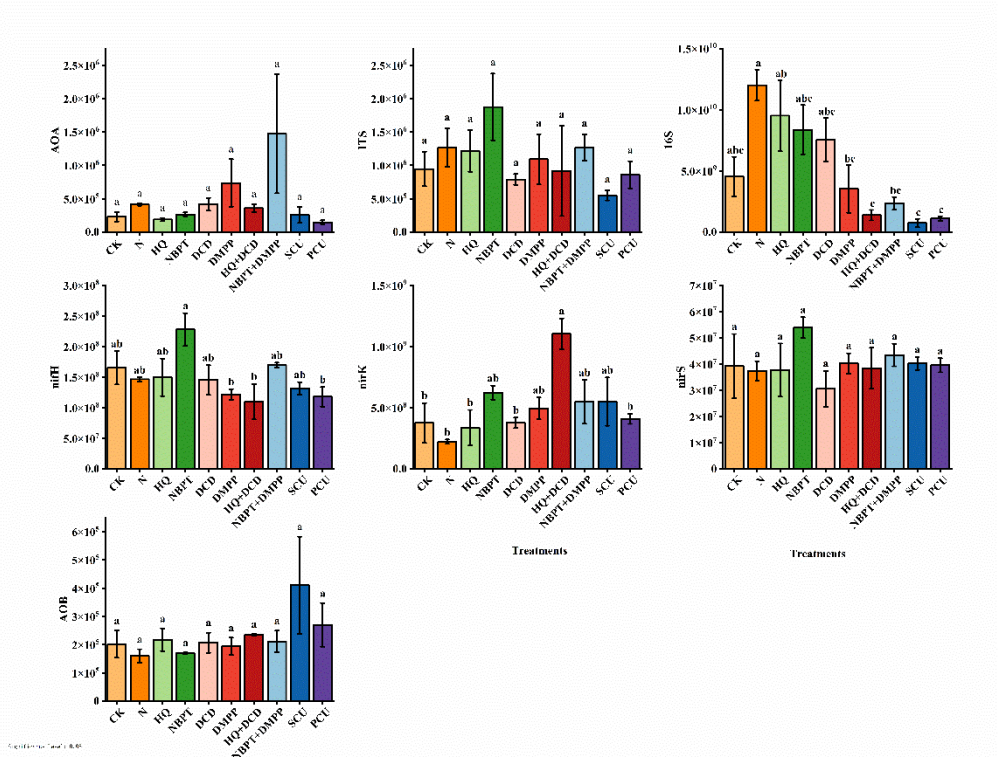

Figure 3. The abundance of AOA, AOB and nitrogen cycling microbial functional genes in soils of different treatments**.**

3.4. The Relation to Microbial Composition and Basic Soil Chemistry of Rice Field Soils with Long-Term Application Stabilized and Coated Urea Fertilisers

Redundancy analysis (RDA) was conducted to analyze the basic chemical properties and phylum-level composition of the microbial community in various soil treatments. The results indicated a significant impact ($p<0.05$, Fig. 4) of long-term application of different types of urea fertilizers on the microbial community. In rice field soil, the pH exhibited a positive correlation with bacterial phyla ***Chytridiomycota***, ***Rozellomycota***, and unidentified species, with ***Chytridiomycota*** displaying the strongest correlation. The bacterial phyla ***Basidiomycota*** and ***Ascomycota*** displayed a negative correlation with pH, with ***Ascomycota*** exhibiting the highest correlation (Fig. 4a). Soil organic matter (SOM) demonstrated a positive correlation with unidentified fungi and the bacterial phylum ***Chytridiomycota***, with unidentified species displaying the strongest correlation. The fungal phyla ***Rozellomycota***, ***Basidiomycota***, and ***Ascomycota*** exhibited a negative correlation with SOM, with ***Ascomycota*** displaying the strongest correlation (Fig. 4b). These findings suggest that the long-term application of stabilized urea and encapsulated urea fertilizers primarily affects bacterial communities through pH, whereas SOM predominantly influences fungal communities. The combined variation of soil pH and SOM content accounted for 15.5% of the variation in bacterial communities and 17.7% of the variation in fungal communities (Fig. 4).

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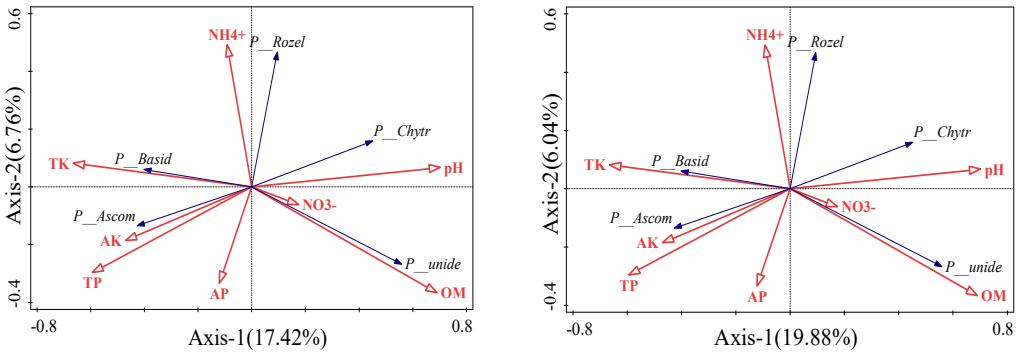

Figure 4. The RDA analysis of the relationship between soil's basic chemical properties and the composition of bacterial communities at the phylum level under different treatments(a). RDA analysis of the relationship between soil's basic chemical properties and the composition of fungal communities at the phylum level under different treatments(b).

3.5. The Correlation Analysis of Soil Basic Chemical Properties and Abundance of Functional Genes of Nitrogen-Cycling Microorganisms in Soil with Long-Term Application Stabilized and Coated Urea Fertilizers

Long-term application of various types of urea fertilizers in rice field soil revealed a significant negative correlation between ammonium nitrogen content and the gene abundance of ammonia-oxidizing archaea (AOA) and nirK. Nitrate nitrogen in the soil exhibited a significant negative correlation with nirK. Soil organic matter demonstrated a significant positive correlation with gene abundances of fungal internal transcribed spacer (ITS), bacterial 16S, nitrogenase reductase (nifH), and nitrite reductase (nirS). Both total potassium and available phosphorus in the soil exhibited a significant negative correlation with bacterial 16S. Soil pH displayed a significant positive correlation with bacterial 16S (Fig. 5).

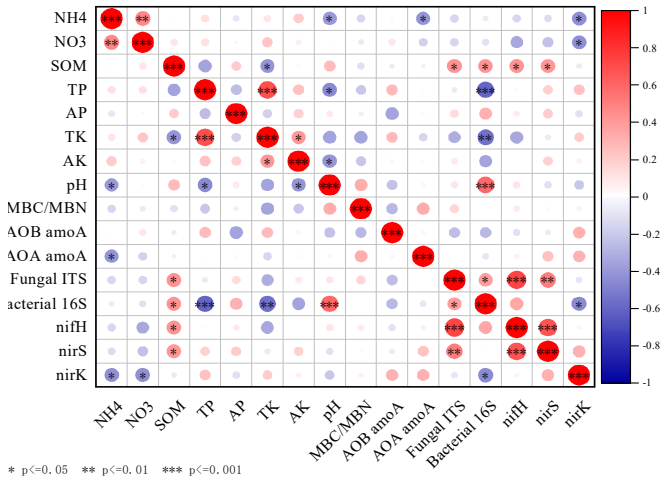

Figure 5. The correlation analysis between the basic chemical properties of soil and the abundance of soil AOA, AOB, and nitrogen cycling functional microbial genes under different treatments.

3.6. The RDA Analysis of the Relationship Between Soil Basic Chemical Properties and Abundance of Functional Genes of Nitrogen-Cycling Microorganisms in Soil with Long-Term Application of Stabilized and Coated Urea Fertilisers

Redundancy analysis (RDA) revealed a significant relationship ($p<0.05$, Fig. 6) between gene copy numbers of ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), fungal

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internal transcribed spacer (ITS), bacterial 16S, nitrogenase reductase (nifH), nitrite reductase (nirS), and nitrite reductase (nirK) in brown soil of rice fields subjected to long-term application of various urea nitrogen fertilizers. Total phosphorus (TP), nitrate (NO3-), and soil organic matter (SOM) were identified as the primary factors influencing gene abundance of bacterial 16S, fungal ITS, nifH, AOA, AOB, nirS, and nirK.

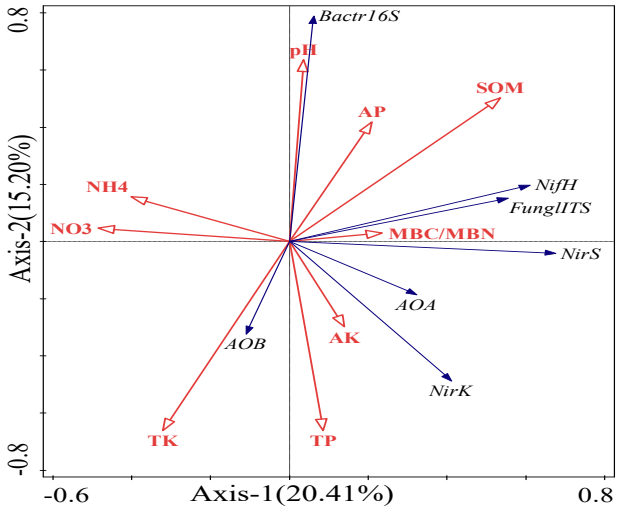

Figure 6. The RDA analysis was conducted to examine the relationships between the basic chemical properties of soil under different treatments and the microbial functional genes related to nitrogen cycling, as well as AOA and AOB.

3.7. Alpha Diversity of Bacterial in Soil

Before calculating the alpha diversity index and conducting ordination analysis, it is essential to mitigate biases arising from variations in sequence counts. After rarefying the raw OUT data, the dilution curve shows a marginal initial increase that eventually plateaus (Fig. 7), indicating the adequacy and appropriateness of the sequencing data for further analysis.

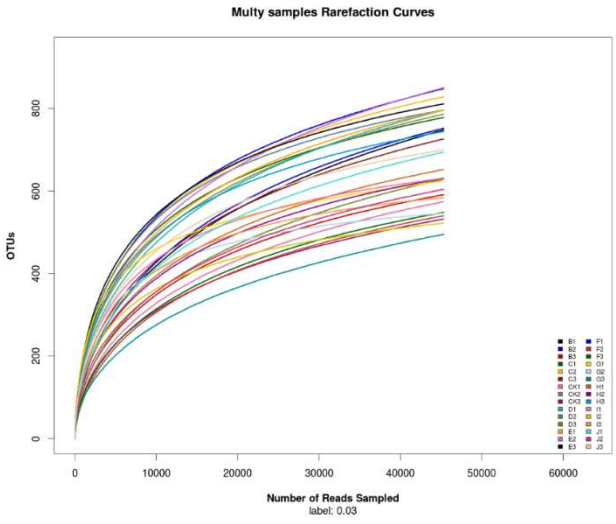

Figure 7. The dilution curves of bacterial communities in different treated soil samples.

We calculated the Pielou evenness index, Chao1 index, and Ace index to assess the diversity of bacterial communities across various soil fertilization treatments. Based on the findings from Fig. 6, we observed no statistically significant differences in the diversity indices of bacterial communities across different soil fertilization treatments ($p<0.05$) (Fig. 8).

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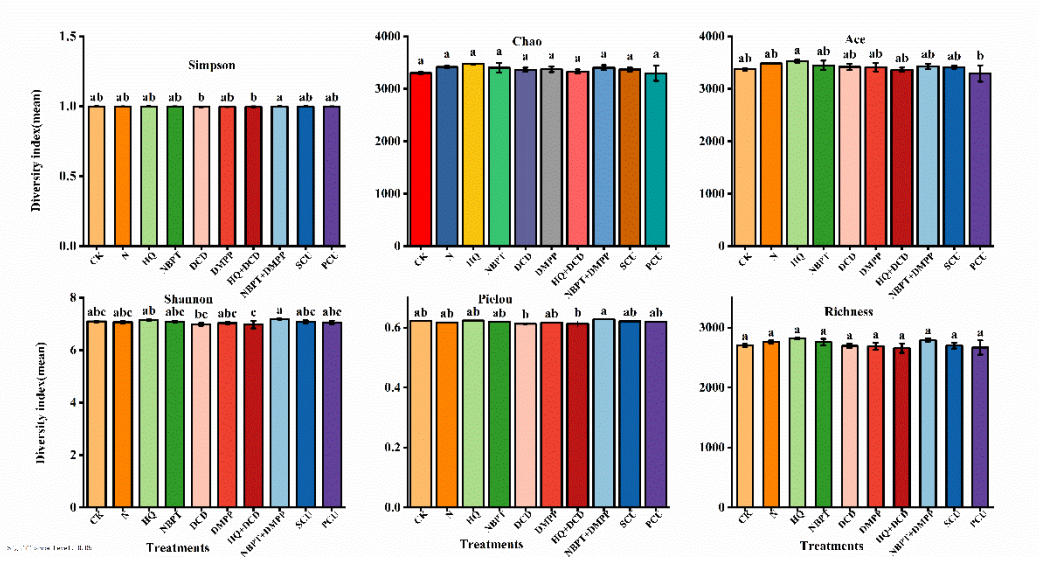

Figure 8. The diversity indices of bacterial communities in different treated soil samples.

3.8. Alpha Diversity of Fungi in Soil

The analysis of Figure 9 indicates a gradual increase in the dilution curve, which eventually levels off, signifying the reliability and appropriateness of the sequenced data for further analysis.

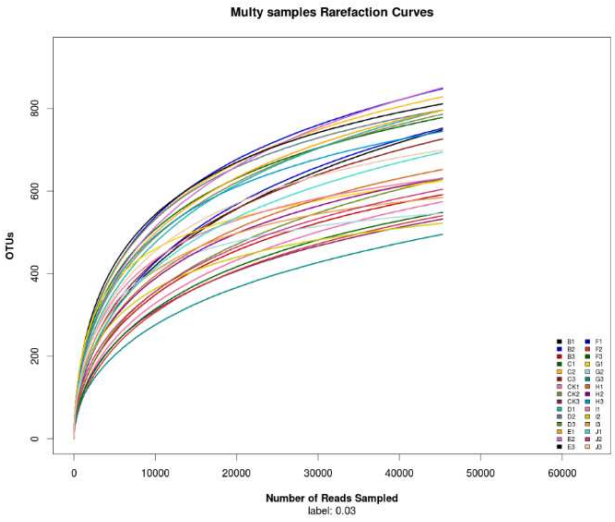

Figure 9. The dilution curves of fungal communities in different treated soil samples.

No significant differences were observed in the Shannon-Wiener, Simpson, and Pielou indices across the treatments. Among the treatments, the DMPP treatment exhibited the lowest soil index, while the remaining treatments displayed higher values compared to both CK and N treatments (Fig. 10). Regarding richness indices, the HQ+DCD diversity index demonstrated the lowest value, and the fungal abundance in soils treated with the urease inhibitor and nitrification inhibitor combination, DMPP, and sulfur-coated urea were all lower compared to CK and N treatments. The Chao index of the SCU treatment exhibited the lowest value, which was significantly lower than the DCD treatment, and the indices for the DMPP, HQ+DCD, and SCU treatments were all lower compared to CK and N treatments (Figure 10). In the Ace index, SCU exhibited a significantly lower value compared to N, HQ, and DCD treatments ($p<0.05$), whereas HQ+DCD showed a significantly lower value compared to the DCD treatment. Although there were no significant differences between the urease inhibitor and nitrification inhibitor mixture treatment and individual application treatments, the Ace index in the former was lower than that of the single inhibitor treatments (Fig. 10).

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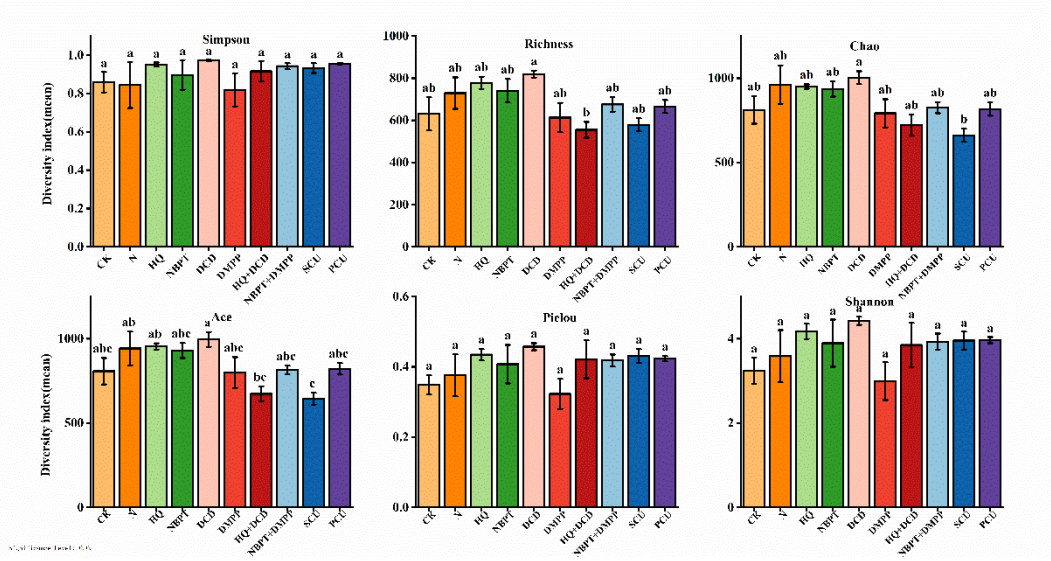

Figure 10. The diversity indices of fungal communities in different soil treatment. Different letters indicate significant differences between different treatments at P < 0.05 by Tukey test.

3.9. The Relationship Between Soil Chemistry and Alpha Diversity of Microbial Communities in Paddies Soil with Long-Term Application of Stabilized and Coated Urea Fertilizers

Figure 11 reveals that the bacterial Chao and Shannon indices display complete independence from the soil's basic chemical properties. However, a significant positive correlation with the microbial biomass C/N ratio ($p<0.05$) is evident. This finding suggests that an increase in the microbial C/N ratio within the soil corresponds to an enrichment in bacterial diversity. Results presented in Figure 12 depict a significant negative correlation ($p<0.01$) between the fungal Chao and Shannon indices and TP. This implies a significant reduction in fungal community diversity within high-phosphorus soil.

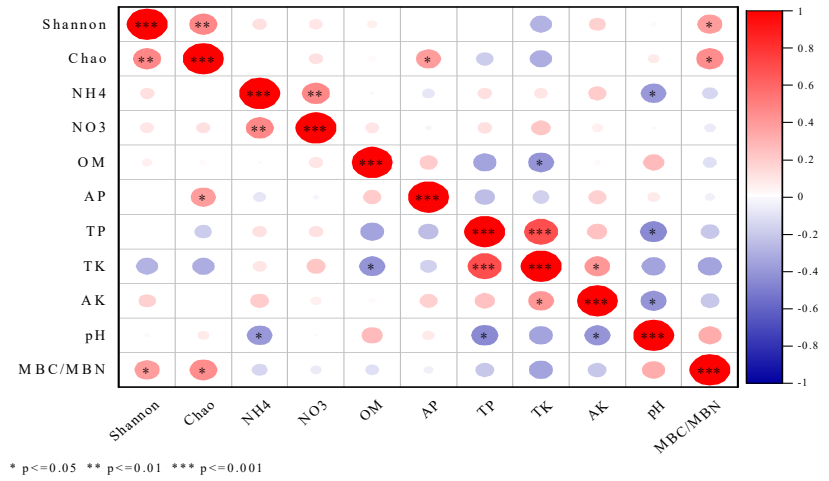

Figure 11. The correlation analysis of soil chemical properties and bacterial diversity in different treatments.

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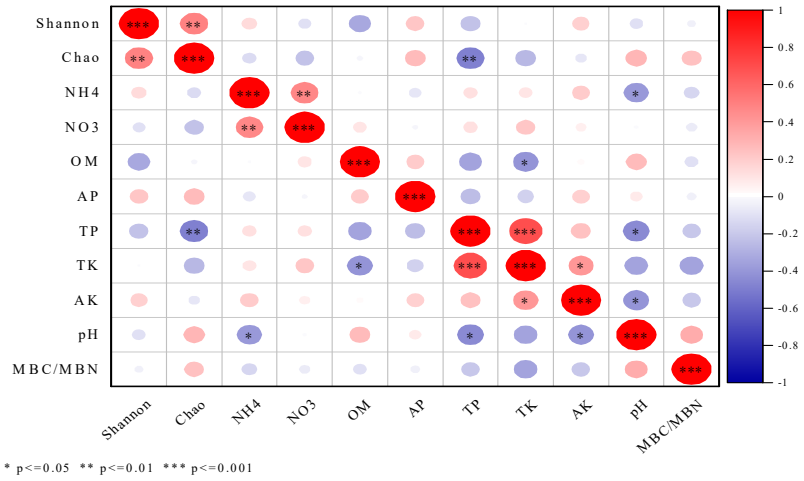
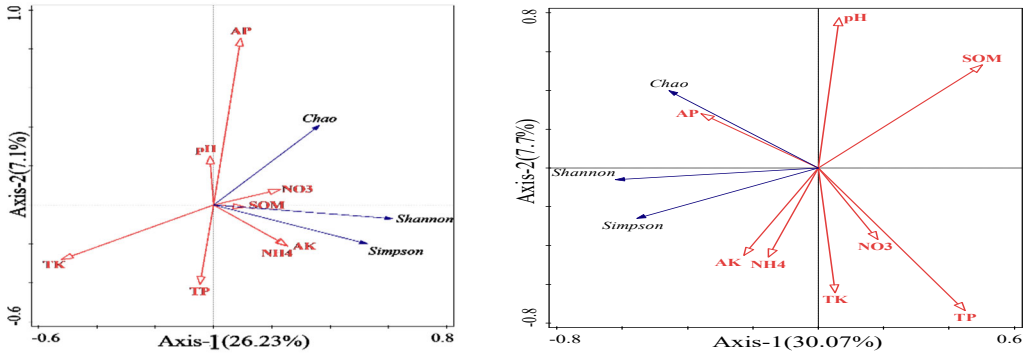


Figure 12. The correlation analysis of soil chemical properties and fungal diversity in different treatments.

Long-term application of different urea fertilizers in paddy soil showed no significant impact on bacterial α-diversity ($p>0.05$). In contrast, fungal α-diversity demonstrated a significant influence from soil organic matter (SOM) ($p<0.05$), as illustrated in both Figure 13 and Figure 13. More specifically, the fungal Shannon and Simpson indices displayed a negative correlation with SOM, whereas the Chao index demonstrated a positive correlation (Figure 13).



(**a**) (**b**)

Figure 13. The RDA analysis examines the relationship between different treatments of soil chemical properties and bacterial α-diversity(a). The RDA analysis explores the relationship between different treatments of soil chemical properties and fungal α-diversity(b).

3.10. The β-Diversity Indices of Soil Bacterial and Fungal Communities in Rice Fields with Long-Term Application Stabilized and Coated Urea Fertilizers

The PCoA analysis utilizing the Bray-Curtis method revealed significant differences in the soil microbial composition at the OTU level across various treatments (Fig. 14). The two principal coordinates accounted for 43.66% of the variation in soil bacteria, where PC1 accounted for 32.26% and PC2 for 11.4% of the variation. The two principal coordinates explained 36.86% of the variation in soil fungi, where PC1 accounted for 24.76% and PC2 accounted for 12.1% of the variation. Bacterial data points for the HQ treatment exhibited significant differences from CK ($P<0.05$, $fs>1$), suggesting that the prolonged application of HQ fertilizer significantly influenced the structure of the soil microbial community in rice fields, leading to substantial alterations in the original soil microbiota. No significant differences ($P>0.05$) were observed in the fungal composition among the different treatments when compared to CK and N (Fig. 14).

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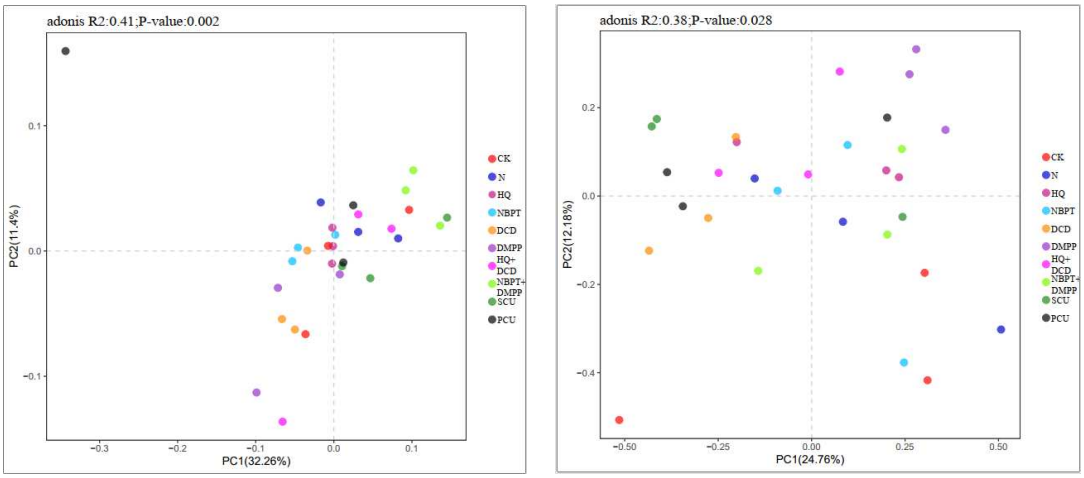

(a) (b)

Figure 14. The Bray-Curtis dissimilarity of bacteria and fungi in soil among different treatments(bacteria(a), fungi (b)).

4. Discussion

4.1. The Effect of Long-Term Application Stabilized and Coated Urea Fertilizers on pH in Rice Field Soils

The pH value of paddy field chestnut soil significantly decreased with the long-term application of SCU, compared to chestnut soil with long-term application of different types of stabilized and PCU urea fertilizers. This decrease in soil pH can be attributed to the oxidation of sulfur elements that enter the soil during the decomposition of the elemental sulfur film[25]. However, long-term application of regular urea nitrogen fertilizer did not cause a significant change in pH, possibly because of the presence of strong carbonates in the paddy field [26]. The long-term application of different types of stabilized urea fertilizers did not cause significant changes in the pH of paddy soil compared to CK. Previous studies have indicated that both short-term and long-term application of stabilized urea fertilizers do not significantly affect the pH value of paddy soil, as short-term application also did not cause substantial changes in soil pH in paddy fields[27]. It can be inferred that the long-term application of different nitrification inhibitors and urease inhibitors in urea fertilizers helped mitigate the soil pH decrease resulting from the nitrification of urea nitrogen fertilizers in paddy soil. This research finding differs significantly from the effect of incorporating various nitrification inhibitors and urease inhibitors in urea fertilizers on soil pH in dryland soil[28]. This disparity can be primarily attributed to the long-term anaerobic conditions and variations in soil microecological environment, soil nutrient metabolism, microbial population structure, and abundance of functional genes between paddy soil and dryland soil.

4.2. The Effects of Long-Term Application Stabilized and Coated Urea Fertilizers on the Abundance of Functional Genes of Nitrogen-Cycling Microorganisms in Rice Field Soils

The abundance of AOA and AOB genes is primarily influenced by soil ammonium nitrogen, total potassium, and organic matter content ($p<0.05$). Previous findings[29] support the notion that long-term application of sulfur-coated urea (SCU) significantly increases the AOB gene abundance in paddy soils. This increase can be attributed to the higher content of ammonium and nitrate nitrogen in the rice field soil, which greatly promotes the gene abundance of AOB. In contrast to related studies[30], our findings reveal a decrease in AOB gene abundance as the pH of acidic soil decreases. This suggests that the AOB community is less susceptible to pH changes in neutral to alkaline conditions. Adding DMPP and NBPT+DMPP to urea fertilizers resulted in an increase in AOA gene abundance in the soil. The correlation analysis revealed a negative relationship between AOA gene abundance and ammonium nitrogen content, suggesting that AOA thrives and reproduces in soil environments with low ammonium nitrogen levels. This finding is consistent with

previous studies[31]. RDA analysis revealed a strong negative correlation between soil nitrate nitrogen content and AOA gene abundance, indicating that AOA tends to proliferate in soil environments with low nitrate nitrogen levels. In conclusion, inorganic nitrogen plays a crucial role in shaping the AOA and AOB populations in neutral paddy soil. However, the population abundance of AOB and AOA remains largely unaffected by the long-term application of nitrification inhibitors, urease inhibitors, resin-coated, and sulfur-coated urea fertilizers over a period of 16 years.

There were no significant differences in fungal ITS among treatments following long-term application of urea fertilizers. However, correlation analysis revealed a strong association between soil organic matter (SOM) and fungal gene abundance. RDA analysis demonstrated a positive correlation between soil organic matter (SOM), total phosphorus (TP), and fungal ITS. This suggests that in paddy soil subjected to long-term application of stabilized and coated urea fertilizers, SOM plays a dominant role in influencing soil fungal population abundance. Previous studies indicate that total phosphorus (TP) significantly influences SOM, and long-term fertilization practices that increase P levels can enhance carbon sequestration in soil, ultimately leading to higher SOM[32]. In paddy soil subjected to long-term application of stabilized and coated urea fertilizers, there was a negative correlation between NO3- and fungal ITS gene abundance, suggesting that higher levels of nitrate nitrogen in the soil are associated with reduced fungal populations. This phenomenon may be attributed to a decrease in soil pH caused by the accumulation of nitrate nitrogen, which subsequently results in a reduction in fungal gene abundance[33]. Soil organic matter (SOM) serves as a nutrient source for fungi, fostering their growth[34] and ultimately leading to an increase in fungal gene abundance.

The long-term application of DMPP, HQ+DCD, NBPT+DMPP, SCU, and PCU urea fertilizers exhibited notable distinctions when compared to the conventional urea fertilizers used for long-term application. This suggests that the utilization of nitrification inhibitors and urease inhibitors can effectively impede bacterial nitrification in the soil for a considerable duration following application. Nonetheless, the prolonged use of urea fertilizers supplemented with nitrification inhibitors and urease inhibitors in brown soil rice fields leads to an accumulation effect that notably diminishes the abundance of bacterial 16S genes. This decline is primarily attributed to the influence exerted by the inhibitors. This reveals that slow-release urea fertilizers featuring resin coating and sulfur coating possess a more pronounced influence on soil microorganisms throughout the process of microbial decomposition in soil. Consequently, this leads to a substantial reduction in the abundance of functional genes within bacteria, as indicated by 16S analysis during prolonged treatment. In comparison, the impact of coated urea fertilizers on microorganisms outweighs that of inhibitors.

The prolonged use of NBPT resulted in an elevation in the gene abundance of nifH, whereas the utilization of DMPP and HQ+DCD led to a reduction in the gene abundance of nifH. The remaining treatments exhibited minimal variations. This suggests that the urease inhibitor NBPT can augment the potential nitrogen-fixing capacity of soil bacteria and archaea harboring the nifH gene. In contrast, the potential nitrogen-fixing ability of bacteria and archaea carrying the nifH gene is diminished by the nitrification inhibitors DMPP and HQ+DCD. These inhibitors adversely impact the activity of soil ammonia-oxidizing enzymes, consequently weakening the potential nitrogen-fixing ability of the nifH gene. However, this experiment did not ascertain whether the prolonged use of NBPT urea fertilizer would lead to an upregulation in the expression of the nifH gene. As DMPP and HQ+DCD suppressed the expression of nifH, the subsequent step should involve evaluating the nitrogen-fixing potential of the nifH gene in soil bacteria and archaea. The utilization of NBPT resulted in an augmentation of the gene abundance of nirS in the soil, whereas the application of DCD led to a decrease in the gene abundance of nirS. The remaining treatments exhibited minimal alterations.

This finding aligns with previous short-term studies and suggests that, in brown soil paddy fields where urease inhibitors, nitrification inhibitors, and coated urea fertilizers have been applied over an extended period, the use of biochemical inhibitors and fertilizer coating does not result in a noteworthy cumulative impact on nirS in the soil[35,36]. Redundancy analysis (RDA) and correlation analysis revealed a significant positive correlation between the nifH and nirS genes and soil SOM. Soil organic matter (SOM) serves as the primary influencing factor for the nifH gene, and the impact

of fertilization on soil organic matter indirectly impacts the gene abundance of nitrogen-fixing bacteria in the soil. The gene abundance of nirK substantially increased in brown soil with paddy fields with prolonged usage of HQ+DCD urea fertilizer. Notably, a significant negative correlation was observed between the gene abundance of nirK and NH_4^+ as well as NO_3^-, which contrasts previous findings in arid soils[37]. Based on RDA analysis, both soil SOM and TP exhibited a significant positive correlation with the nirK gene, supporting previous research[38]. This phenomenon could potentially be attributed to the rise in SOM levels, which stimulates oxygen consumption in paddy soil, creating a more anaerobic environment that fosters the rapid proliferation and reproduction of denitrifying bacteria within the soil. Additionally, the impact of TP on denitrifying bacteria should not be disregarded. Prior research has demonstrated that elevated TP levels can augment the gene abundance of denitrifying bacteria in nutrient-deficient soils, although they do not affect denitrifying bacteria in soils with ample nutrients[39,40]. These findings corroborate our experimental results. In contrast to prevailing knowledge in dryland soils, a notable negative correlation existed between NO_3^- and nirK. This discrepancy might arise from the prolonged flooding conditions within rice fields, resulting in exceedingly low soil oxygen levels that impede the activity of nirK.

4.3. The Effects of Long-Term Application of Stabilized and Coated Urea Fertilizers on Microbial Community Succession in Rice Field Soils

The bacterial and fungal communities in the brown soil of rice paddy fields exhibit divergent responses to the prolonged use of stabilized and coated urea fertilizers. Long-term application of urea fertilizers did not significantly affect the alpha diversity of soil bacteria in rice paddy soils, and no correlation was observed between soil nutrients and bacterial alpha diversity. However, it significantly influenced the alpha diversity of soil fungi. Specifically, the SCU treatment yielded lower Chao and Ace indices relative to the prolonged application of regular urea, thereby signifying a reduction in fungal species richness and diversity in rice paddy fields resulting from the long-term usage of SCU. Prior research on arid soils has indicated that employing SCU diminishes soil bacterial diversity[41], whereas the consequences of SCU treatment on fungal diversity are predominantly dictated by soil organic matter (SOM) in the brown soil of rice paddy fields. Consequently, it implies that the prolonged use of stabilized and coated urea fertilizers can modify the community composition of soil fungi through its influence on SOM content, which constitutes a pivotal factor for fungal reproduction and growth. The Shannon index reflects species evenness and abundance[42], whereas the Simpson index measures species diversity and relative abundance within an ecosystem[43]. Notably, both indices exhibited a positive correlation with SOM, thereby suggesting that higher SOM content leads to a decrease in fungal species but fosters their more equitable distribution. This observation may be attributed to the fact that higher SOM content supplies ample nutrients, conferring a competitive advantage upon specific fungal populations. The Chao index reflects changes in species abundance[44], including rare species, and exhibits a positive correlation with SOM. This implies that higher SOM content in the soil contributes to the identification of previously unknown fungal species. This phenomenon arises due to the enrichment of organic matter, which furnishes additional ecological niches and resources for fungi, thereby facilitating enhanced reproduction and survival of fungal populations. Notable disparities existed in the structure of soil bacterial communities resulting from the prolonged use of HQ urea fertilizer compared to conventional urea in rice paddy fields. HQ exerts physiological toxicity on soil microorganisms[45]; however, no pertinent reports exist on the effects of prolonged application of HQ urea fertilizer on soil microorganisms in rice paddy soils. Our findings demonstrate that the prolonged usage of HQ urea fertilizer may induce toxicity in specific soil bacteria, resulting in diminished bacterial diversity. This is mainly attributed to the comparatively slow decomposition rate of HQ in the brown soil of rice paddy fields, thereby extending its effective duration. Conversely, other biochemical inhibitors did not exert a noteworthy impact on soil bacteria.

Additionally, no significant variations were observed in bacterial composition at the phylum level across the distinct treatments. The RDA analysis revealed that pH exerted a primary influence

on the community composition of soil bacteria in the brown soil of rice paddy fields subjected to prolonged application of stabilized and coated urea fertilizers. Related research findings corroborate that[46] SOM plays a pivotal role in shaping the community composition of fungi. In contrast to multiple other treatments, the HQ+DCD treatment exhibited substantially higher fungal community composition. In the presence of HQ+DCD, ***Basidiomycota*** fungi, participating in the decomposition of organic matter and soil formation processes, demonstrated enhanced species richness. Although SOM content remained unchanged, the species richness of ***Rzellomycota*** was markedly inferior to that of the control (CK) and nitrogen (N) treatments. Other factors, such as the toxic effects of HQ on specific fungal populations, may have contributed to the decline in species richness of ***Rzellomycota***. The enhanced species richness of ***Basidiomycota*** could be attributed to reduced competition from other populations, thereby facilitating their access to a greater pool of available SOM for growth. Despite the insignificant decline in SOM content observed in the DMPP treatment compared to the long-term use of regular urea, a more substantial reduction in quantity was evident. Particularly in rice paddy fields, the species richness of ***Rozellomycota*** and ***Chytridiomycota*** fungi exhibits high sensitivity to variations in the quantity of SOM in the soil. Even slight modifications in SOM content can trigger substantial fluctuations in the abundance of ***Rozellomycota*** and ***Chytridiomycota*** fungi. The noteworthy reduction in species richness of these two fungi in the DMPP treatment could be ascribed to diminished SOM content, consequently leading to a deteriorated fungal habitat and compromised competitive ability.

5. Conclusions

The continuous application of sulfur-coated urea fertilizer for 16 years in neutral to slightly acidic background soil results in a significant reduction in the pH value of the brown soil in paddy soil. The change in pH does not have an impact on the abundance of AOB populations in the brown soil of rice fields. The long-term application of stabilized, resin-coated, and sulfur-coated urea fertilizers does not significantly affect the gene abundance of AOA, AOB, fungal ITS, nirS, nirK, and nirH in the brown soil of rice fields. In contrast, the long-term application of DMPP, HQ+DCD, NBPT+DMPP, SCU, and PCU leads to a significant reduction in the copy number of the bacterial 16S gene in the soil, with resin-coated and sulfur-coated urea fertilizers exhibiting a more pronounced effect. Soil organic matter (SOM) plays a crucial role in influencing the gene abundance of nitrogen cycling microbial functional genes (nirS, nirK, nir), fungal ITS, bacterial 16S gene abundance, and fungal community composition under long-term application of various stabilized, sulfur-coated, and resin-coated urea fertilizers in the brown soil of rice fields. HQ stabilized urea fertilizer greatly diminishes the soil bacterial community in paddy sois, whereas HQ+DCD-stabilized urea fertilizer substantially enhances the population and abundance of ***Basidiomycota*** fungi while reducing those of ***Rozellomycota*** fungi. Prolonged use of DMPP-stabilized urea fertilizer substantially amplifies the population and abundance of ***Ascomycota*** fungi, while notably diminishing the population and abundance of ***Rozellmycota*** and ***Chytridiomycota*** fungi. HQ stabilized urea fertilizer notably diminishes the population and abundance of ***Chytridiomycota*** fungi. It is not recommended to utilize sulfur-coated urea fertilizers for prolonged periods in neutral to slightly acidic meadow brown soil. However, stabilized urea fertilizers containing NBPT, DCD, DMPP, or their combinations, along with resin-coated urea fertilizers, are well-suited for long-term use in meadow brown soil.

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