agronomy

MDPI

Article

Impact of Bio-Organic Fertilizer Incorporation on Soil Nutrients, Enzymatic Activity, and Microbial Community in Wheat–Maize Rotation System

Aamir Ali [1], Xiaoli Liu [1], Wenping Yang [2], Wenguang Li [1], Jie Chen [1], Yuejing Qiao [1], Zhiqiang Gao [1] and Zhenping Yang [1],*

[1] College of Agriculture, Shanxi Agricultural University, Taigu, Jinzhong 030801, China; amshaikh_27@yahoo.com (A.A.)
[2] College of Life Sciences, North China University of Science and Technology, Tangshan 063210, China
* Correspondence: yangzhenping340@163.com; Tel.: +86-139-0344-6521

Abstract: Excessive use of inorganic fertilizers disrupts soil nutrient balance and leads to soil degradation and a decrease in biodiversity. In contrast, bio-fertilizers enhance soil structure and fertility and promote plant growth and sustainable agriculture development. Therefore, this study focused on a rotation system of winter wheat and summer maize and aimed to explore the effects of applying chemical fertilizer (NPK) and bio-fertilizer (BF) in the winter wheat season on the sustainable soil development of current wheat and subsequent maize. Before sowing winter wheat four fertilization treatments were, respectively CK (100% NPK at 750 kg ha^{-1}), A (60% NPK at 450 + 20% BF at 150 kg ha^{-1}), B (60% NPK at 450 + 40% BF at 300 kg ha^{-1}), and C (60% NPK at 450 + 60% BF at 450 kg ha^{-1}), conducted. The results showed that treatment A (60% NPK + 20% BF) replacing the NPK at 300 kg ha^{-1} with BF at 150 kg ha^{-1} significantly soil nutrient contents, enzyme activity, and microbial metabolic activity. The study also found a positive correlation between soil parameters (total nitrogen, alkaline nitrogen, available phosphorus, organic matter, urease, and alkaline phosphatase in the winter wheat and maize cropping season). Furthermore, the soil microbial composition showed significant enrichment of *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Firmicutes*, and variations among treatments. Moreover, the application of biofertilizer enhanced the diversity of soil fungi species, particularly during the winter wheat season. This study highlights the importance of integrating biofertilizers with NPK fertilizer for agricultural system conversion and promoting agricultural production and sustainability.

Keywords: biofertilizer; NPK fertilizer; soil chemical characteristics; soil microbial community; winter wheat–summer maize

Citation: Ali, A.; Liu, X.; Yang, W.; Li, W.; Chen, J.; Qiao, Y.; Gao, Z.; Yang, Z. Impact of Bio-Organic Fertilizer Incorporation on Soil Nutrients, Enzymatic Activity, and Microbial Community in Wheat–Maize Rotation System. *Agronomy* **2024**, *14*, 1942. https://doi.org/10.3390/agronomy14091942

Academic Editor: Maria Angeles Bustamante Muñoz

Received: 1 August 2024
Revised: 21 August 2024
Accepted: 22 August 2024
Published: 28 August 2024

1. Introduction

Agricultural sustainability is essential for addressing the growing global food demand and economic prosperity. In China, the agricultural landscape has undergone significant changes since the 1980s, with the widespread adoption of chemical fertilizers to boost grain production and address food security concerns [1]. However, this practice has resulted in various issues, including imbalances in nutrient supply, reduced soil fertility, and increased environmental risks due to excessive and improper fertilizer application [2–5]. Soil bacteria and fungi, which serve as indicator species, play a significant role in influencing soil structure and plant development. Anthropogenic activities such as intensive agricultural practices and fertilizer use, particularly have a profound impact on soil microbial communities. Therefore, it is crucial to decrease chemical fertilizer application and improve soil microbial community structure.

Scientists have long recognized the importance of technological advancements in improving agricultural production efficiency. One notable innovation is the utilization of organic fertilizers and biofertilizers [6]. Organic fertilizers play a crucial role in addressing the conflict between crop water demand and the soil water supply and have the potential to induce substantial changes in soil microbial populations, leading to increased bacterial and fungal diversity The synergistic application of organic and inorganic fertilizers has a positive impact on crop yield [7,8]. Replacing a portion of the chemical fertilizers with biofertilizers can improve maize yield by delaying senescence and increasing grain weight [9,10]. Many studies have documented the advantages of using organic fertilizers instead of chemical fertilizers in agricultural activities. For instance, organic fertilizers reduced up to 30% greenhouse gas emissions and a 25% decrease in water pollution compared to chemical fertilizers [11]. Similarly, other studies have reported significant cost reductions and yield increases with the substitution of chemical fertilizers [12]. Additionally, there is potential for customized methods of fertilizer substitution. In particular, the ideal substitution ratios for organic fertilizers have been identified for various crops, including maize [13], rice [14], and wheat [15]. Furthermore, another study by [16] showed that adding more organic matter to the soil while lowering soil acidity, especially in acidic soils can enhance soil fertility and structure, particularly in sandy loam soils [17]. However, it is crucial to recognize that finding and using high-quality organic fertilizers can be challenging in developing nations [18]. In order to prevail against those challenges and fully reap the benefits of switching to organic fertilizers in sustainable agriculture, more studies are necessary.

Biofertilizers, a type of microbial inoculant based on organic matter carrier, play a crucial role in integrated nutrient management (INM) strategies by contributing to nutrient mobilization and recovery [19]. This microbial inoculant contains dormant or living cells of specific strains that are useful for fixing nitrogen (N), solubilizing or mobilizing phosphorus (P), and solubilizing potassium (K) [20–22]. They can enhance soil fertility, rhizosphere soil water content, and root activity, thereby mitigating drought effects [23,24]. When combined with chemical fertilizers, microbial fertilizers contribute to improved soil fertility [25]. Substituting biofertilizers with chemical fertilizers effectively maintains and increases soil organic nitrogen storage, reduces soil phosphorus fixation, and enhances soil structure and water retention [5,26,27]. Previous studies have investigated the effects of prolonged sequential use of organic and biofertilizers on soil enzyme activities [28–30]. The application of bacterial fertilizer is associated with increased activities of urease, protease, catalase, and phosphatase in the soil [31].

The region being studied is the dryland of Southern Shanxi Province, located in the eastern part of the Loess Plateau. This region belongs to Huang-Huai-hai middle- and late-maturing winter wheat area, and also is one of the main areas of producing high-yield and high-quality (high-protein, middle- or strong-gluten) wheat, which has significance in ensuring national food security in China. The common cropping system here is a winter wheat–summer maize rotation. However, the impact of different bio-fertilizer substituting ratios for chemical fertilizer during the winter wheat season on soil nutrient content, enzyme activities, microbial communities, and crop yields in the rotation system remains unclear. The study aims to examine and evaluate the impact of the partially substituting NPK compound fertilizer with biofertilizers on the contents of soil nutrients, enzyme activities, and microbial communities, within a crop rotation system in winter wheat and summer maize. The research involves comparing various fertilizer treatments, with a specific focus on key microbial groups. Furthermore, the current research investigates the correlation between soil properties, and crop yields, and the impact of bio-fertilizers on soil microbial diversity. The main goal was to offer insights into the benefits of integrating bio-fertilizers with NPK compound fertilizers to enhance or maintain crop yield in the rotation cropping system, improve soil health, and promote sustainable farming practices.

2. Materials and Methods

2.1. Study Site Description

The experiments were carried out in a rotation field of winter wheat and summer maize from 2016 to 2017 in Yuanqu County (35°14.4′ N, 111°43.3′ E), Shanxi Province, which is located on the Loess Plateau in Northwest China. This region has a subhumid, warm, and continental monsoon climate with a mean frost-free period of 236 days and an average annual precipitation, temperature, and sunshine time of 631 mm, 13.5 °C, and 2026.2 h, respectively.

The soil texture is classified as medium loam and has vertical structural loess with a cinnamon red color. Before 30 September 2016, the soil physiochemical characteristics were assessed in the 0–20 cm deep soil layer of the research field, the soil assessment showed that the pH was 8.0, 10.5 g kg^{-1} soil organic matter (SOM), 0.71 g kg^{-1} total nitrogen (TN), 86.3 mg kg^{-1} alkali-hydrolysable nitrogen (AN), 14.5 mg kg^{-1} available phosphorus (AP), and 112.3 mg kg^{-1} available potassium (AK).

2.2. Experimental Design and Treatments

The field experiments were conducted in a completely randomized block design with three biological repeats and four fertilizer treatments in the winter wheat season. Each treatment was with different ratios of chemical fertilizer (NPK) and bio-fertilizer (BF), via CK (100% NPK at 750 kg ha^{-1}), A (60% NPK at 450 + 20% BF at 150 kg ha^{-1}), B (60% NPK at 450 + 40% BF at 300 kg ha^{-1}), and C (60% NPK at 450 + 60% BF at 450 kg ha^{-1}). There were 12 plots, and the plot size was 660 m^2 (15 m × 44 m). Before sowing winter wheat, commercial biofertilizers and NPK compound fertilizers were applied into the soil at a depth range of 25–30 cm using a rotary machine according to the local tillage practice [32]. The commercial chemical fertilizer (NPK compound fertilizer, $N:P_2O_5:K_2O = 20:10:10$) was provided by Guizhou Kailin Group Co., Ltd., Guiyang, China. The bio-fertilizer (microbial inoculum, powdery), which contains the available living bacterial amount $\geq$ 200 million g^{-1}, and the active organic matter (potassium fulvic acid, etc.), was $\geq$60%, was provided by Anchor Biochemicals Ltd., Maple House 118 High Street, Purely, London, United Kingdom. These living bacteria in bio-fertilizer included *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Yeasts*, EM *photosynthetic microbes*, and *Bacillus thuringiensis*.

2.3. Field Sampling and Processing

The winter wheat cultivar Yannong-21 was sown using a wide ridge (with a base width of 25 cm and a height of 12 cm) and a narrow furrow (with a depth of 8 cm and rows spaced 12 cm apart, sown at the two edges of the furrow) sowing technique on 30 September 2016, and was harvested on 8 June 2017. The wheat seed rate was used at 112.5 kg ha^{-1}. The application dose of NPK compound fertilizer 750 kg ha^{-1} was applied to each plot during the maize cropping season, the space between each plant was spaced about 25 cm, the space in each row was about 50 cm, and the space between blocks and plots was at a distance of 1.2 m. The summer maize cultivar Nongda-372 was directly planted on 12 June 2017 using a seed–fertilizer sowing machine at a seed rate of 112.5 kg ha^{-1} and was harvested on 27 October 2017. Additional field management practices, including irrigation and weeding, were carried out following the local procedures.

Soil samples were collected before harvesting. Three sampling sites were randomly selected in each plot, and five subsamples from each site were collected at a depth ranging from 0 to 20 cm, using a sterilized 4 cm diameter soil drilling sampler, respectively. After sieving through a 2 mm mesh to remove roots, large rocks, and other impurities, five subsamples were mixed into one sample and then divided into two parts for further microbial analysis. One part (fresh soil) was placed in 50 mL centrifuge tube and stored at −80 °C for Illumina sequencing analysis. The other part was air-dried and used to determine the soil nutrient content and enzymatic activity analysis [33,34].

2.4. Soil Characteristics and Microbial Enzymatic Activities

The components of organic matter (SOM) in soil, total nitrogen (TN), and alkaline nitrogen (AN) were calculated following the volumetric method with potassium dichromate, semimicro Kjeldahl method, and alkaline hydrolysis diffusion method, respectively [35,36]. To evaluate the phosphorus (AP) content, 0.5 mol L^{-1} $NaHCO_3$ was determined by atomic absorption and ascorbic acid reduction, respectively [37].

Alkaline phosphatase (ALP) activity was measured as previously described by [38], and the urease (URE) activity was determined by incubating 10 g of soil with 10 mL of a 10% urea solution for 24 h at 37 °C, and the ammonium content was measured by spectrophotometry (UV-2550, Shimadzu, Kyoto, Japan) at 578 nm. Finally, the soil sucrase (SUC) activity was measured according to the procedure outlined in [39]. Each treatment was conducted in triplicates.

2.5. Soil DNA Extraction, Quantitative Real-Time PCR (q-PCR), and Illumina Sequencing

Frozen soil samples of about 0.5 g were used for microbial DNA extraction according to the manufacturer instructions provided with the Fast DNA SPIN Extraction Kit (MP Biomedicals, Santa Ana, California, USA). The determination of the quality of the DNA was conducted following a NanoDrop2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA). The bacterial 16S rRNA gene V3–V4 hypervariable region was amplified using the primer pairs 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) [40]. For multiplex sequencing, the primers contained 7 bp barcodes unique to each sample. The mixture of PCR consisted of FastPfu buffer (5×), primer (5 μM), dNTP mixture (2.5 mM), template DNA, and H_2O, with a total volume of 50 μL. PCR assays were performed as follows: initial denaturation at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min and alumina HiSeq 2500 platform was used to generate the paired-end reads, which totaled 250 nucleotides platform at Personal Biotechnology Co., Ltd., Shanghai, China [32].

2.6. Sequencing Data Processing

Raw sequencing data for wheat–maize underwent quality filtering and double-terminal sequence ligation using QIIME (Quantitative Insight into Microbial Ecology, version 1.9.0, http://www.QIIME.org/). The data filtering criteria were as previously mentioned [32]. Sequences that include ambiguous bases (Ns) or with more than one base mismatch in the 5′-terminal primer regions were removed. Additionally, sequences with more than eight consecutive identical bases and those shorter than 150 base pairs were also excluded. The UCLHIME in MOTHUR software (version 1.31.2, http://www.mothur.org/) was employed to produce high-quality sequencing for further investigation. The UCLUST algorithm in QIIME was employed to group the high-quality sequences based on a 97% sequence similarity threshold. The largest sequence within every group was chosen as the exemplary sequence.

The classification was used to determine richness and diversity indices [41]. The Ribosomal Database Project (RDP) classifier algorithm was formally employed to identify OTU representative sequences of bacteria and fungi. The identification was performed using the RDP Bacterial 16S rRNA database [42] and the Unite Fungal ITS database [43].

2.7. Statistical Analyses

The study analyzed the relationships among microbial diversity, enzyme activities, and soil characteristics. The investigation employed (ANOVA) one-way analysis of variance for the analysis of organic substitution patterns for notable variations ($p < 0.05$) using the SPSS (SPSS 19.0, SPSS Inc., Chicago, IL, USA) software. The least significant difference (LSD) test was used to compare the significant differences among the different organic substitution patterns. We employed Euclidean distance to compute the microbial community, and Haversine distance of soil nutrients, based on their relative abundance at the genus and phylum levels. Using the vegan R package, we computed partial Mantel correlations (with 9999 permutations) between microbial community, functional genes, and soil parameters based on the provided distance matrices.

3. Results

3.1. Impact of Various Fertilization Treatments on Soil Nutrient Levels in Wheat during Different Stages of Growth

The results showed that soil TN content varied significantly across different fertilization treatments from the jointing stage to the mature stage of wheat growth, at the jointing stage, treatment C had a significantly ($p < 0.05$) higher soil TN content, surpassing CK, A, and B. During the heading stage, treatments A and C both demonstrated higher TN content, whereas, at the mature stage, treatment A (60% NPK + 20% BF) had the most significant increase in TN content (Figure 1A). Furthermore, the analysis of AN content revealed that treatment C produced a significant ($p < 0.05$) increase in value at the jointing stage in comparison with CK, A, and B (Figure 1B). Regarding AP content, treatment C outperformed the CK and other treatments at the jointing and heading stages, however, at the mature stage, treatment A recorded the highest AN content, indicating significant differences (Figure 1C). In addition, all treatments increased SOM content when compared to the control, particularly at the heading and mature stages. In particular, treatment A resulted in the most dramatic growth during the mature stage (Figure 1D). These findings suggest that selectively replacing a part of NPK compound fertilizers with biofertilizers can significantly increase soil organic matter content. It is critical to recognize that the efficacy of biofertilizers is not directly proportional to their quantity and that; an ideal threshold for their use exists. Proper calibration and partial substitution of NPK compound fertilizers with these alternatives yielded significant improvements in the soil quality (Figure 1D).

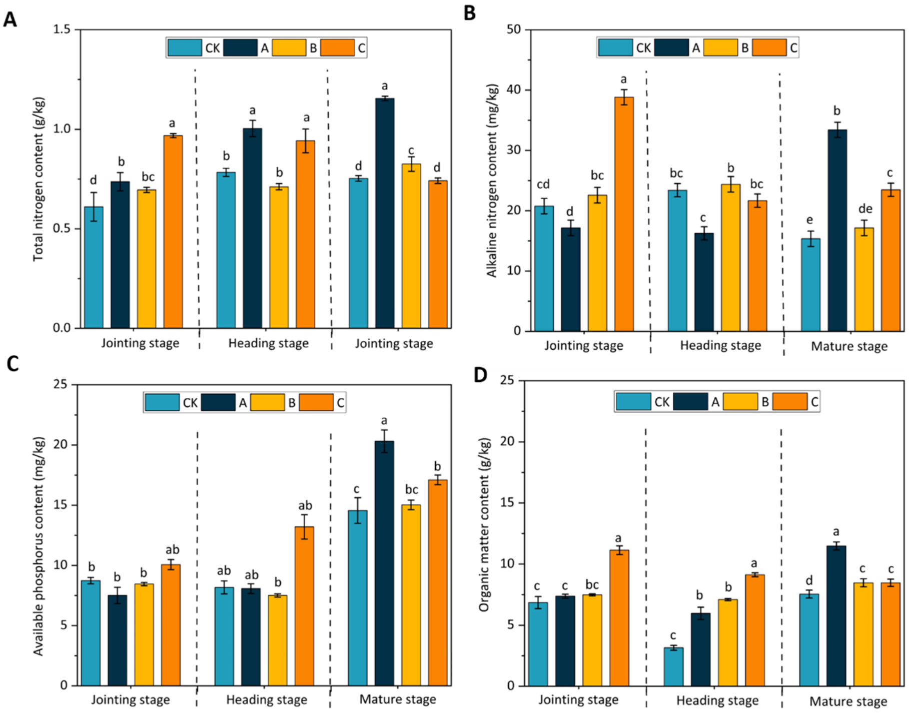

Figure 1. Impacts of various fertilization treatments on soil nutrient levels throughout the different growth phases of wheat. The contents of total nitrogen (**A**), alkaline nitrogen (**B**), available phosphorus (**C**), and organic manure content (**D**). The different lowercase letters indicate statistically significant ($p < 0.05$) variations across treatments. CK stands for (100% NPK at 750 kg ha^{-1}), A (60% NPK at 450 + 20% BF at 150 kg ha^{-1}), B (60% NPK at 450 + 40% BF at 300 kg ha^{-1}), and C (60% NPK at 450 + 60% BF at 450 kg ha^{-1}), respectively.

3.2. Soil Enzymatic Activities at Different Stages of Wheat Growth

The study found that soil urease activities varied significantly from the jointing stage to the mature stage of wheat growth. Substituting NPK compound fertilizers with biofertilizers greatly raised urease activities in the soil in contrast to CK. At the jointing and heading stage, the urease activity was the highest under treatment C compared to B and A. On the other hand, treatment A (60% NPK + 20% BF) appeared more effective in elevating the urease activity only at the maturity stage compared to control and treatments B and C (Figure 2A). Likewise, the AP activity was observed significantly ($p < 0.05$) higher at both jointing and heading stages under treatment C. While, at the maturity stage, treatments A and B appeared more effective than treatment C in elevating the AP activity (Figure 2B). The sucrose enzyme activity was found highest only at the jointing stage under treatments B and C while a negligible but statistically significant ($p < 0.05$) increase was observed at the heading and maturity stage under all treatments compared to control (Figure 2C). These findings indicate that strategic modifications in fertilizer composition can lead to substantial increases in soil enzyme activities, which are essential for enhancing soil health and fertility.

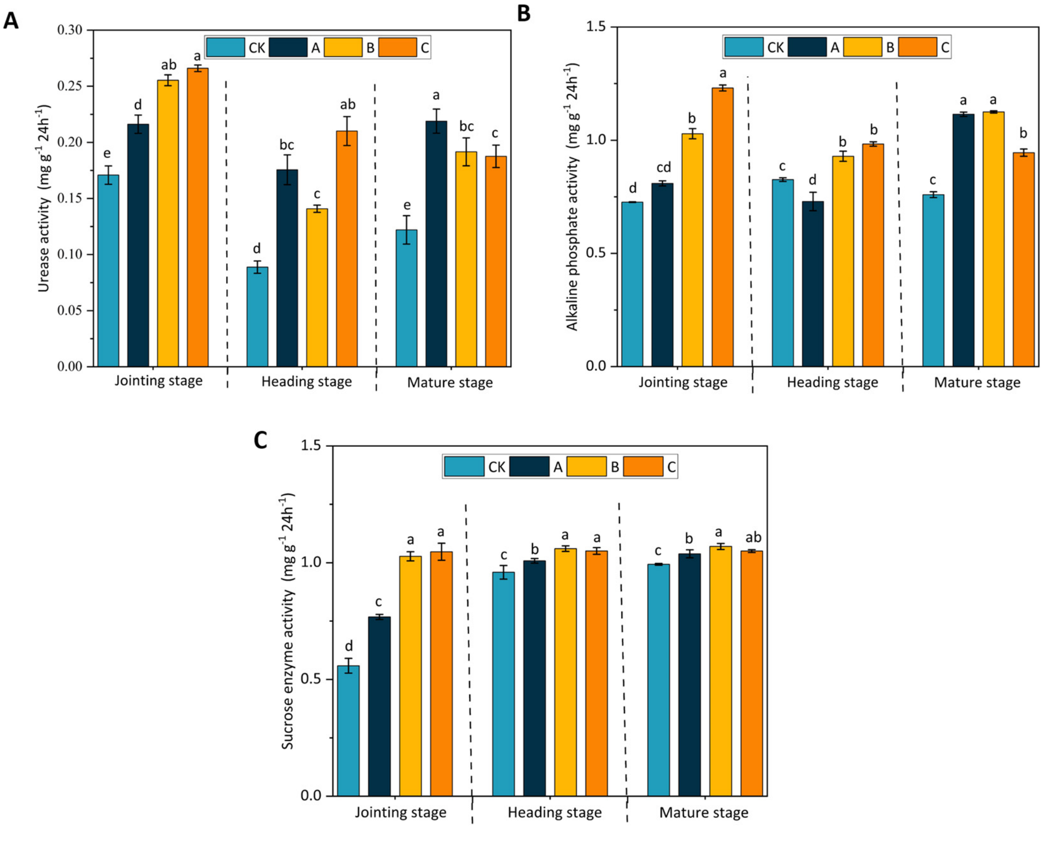

Figure 2. The impacts of various fertilization treatments on soil enzyme activities in wheat at different growth stages. Urease activity (**A**), alkaline phosphate activity (**B**), and sucrose enzyme activity (**C**) at the jointing, heading and mature stages, under treatments, CK (100% NPK at 750 kg ha^{-1}), A (60% NPK at 450 + 20% BF at 150 kg ha^{-1}), B (60% NPK at 450 + 40% BF at 300 kg ha^{-1}), and C (60% NPK at 450 + 60% BF at 450 kg ha^{-1}), respectively. The different lowercase letters indicate statistically significant ($p < 0.05$) variations across treatments.

3.3. Changes in Soil Nutrient Contents under Different Fertilization Treatments during Various Growth Stages in Maize

The effects of various fertilization methods on maize growth showed significant variations in soil nutrient contents across various growth stages. Treatment A (60% NPK + 20% BF) demonstrated a higher soil TN content, in the early and mature stages, and the results were significant ($p < 0.05$) compared to treatments B and C at the mature stage (Figure 3A). Treatment A had the highest AN content in the early and mature stages, with a significant difference ($p < 0.05$). Notably, the study also revealed significant ($p < 0.05$) differences in the AN content (Figure 3B). Additionally, treatment A showed higher available P content in the early and mature stages compared to CK, B, and C (Figure 3C). These findings indicate that substituting NPK compound fertilizers with appropriate amounts of biofertilizers will effectively meet crops' nitrogen uptake needs, as evidenced by the success of treatment A. Furthermore, this partial replacement caused a significant increase in SOM content, particularly at the early stage of treatment A. The results demonstrated a high degree of significance in biofertilizer treatments, with treatment C having the greatest significance, while treatments B and C had a moderate level of significance when compared to CK (Figure 3D). Although biofertilizers are effective, their efficiency is limited. Exceeding this restriction does not result in further advantages. Thus, the strategic replacement of NPK compound fertilizers with biofertilizers can greatly influence soil nutrient dynamics, notably by increasing soil organic matter, which is critical for sustainable agriculture practices.

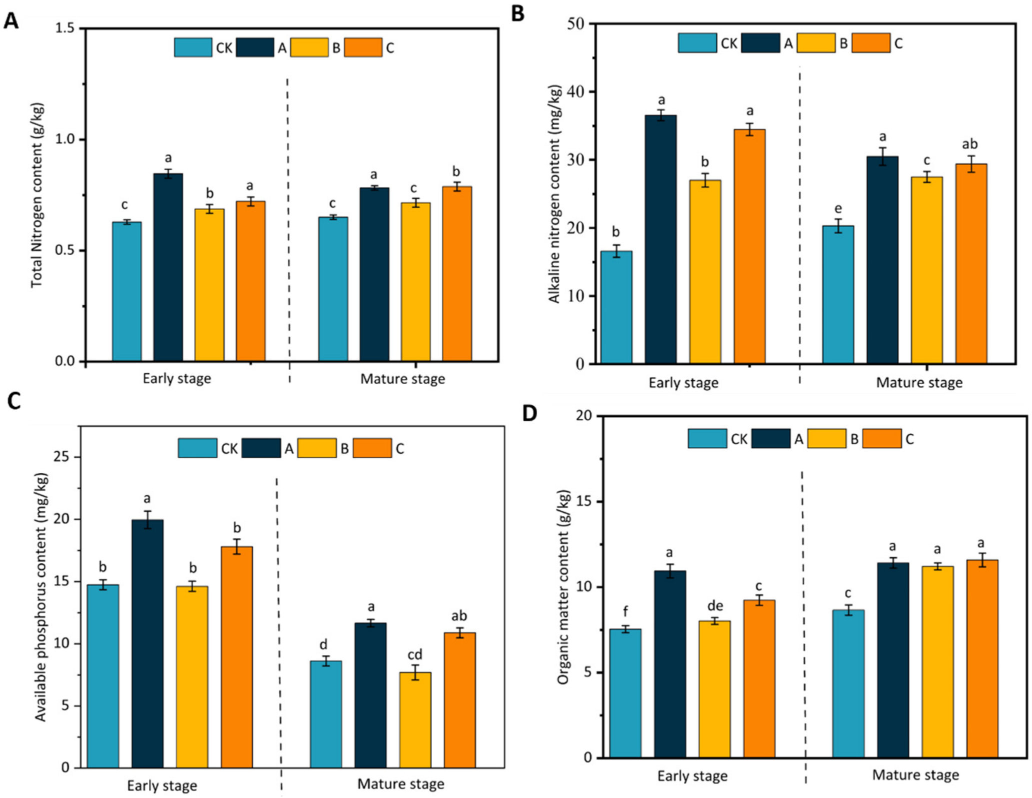

Figure 3. Effects of different fertilization treatments on soil nutrients during different reproductive periods of maize. Total nitrogen content (**A**), alkaline nitrogen content (**B**), available phosphorus content (**C**), and organic manure content (**D**). The CK (100% NPK at 750 kg ha^{-1}), A (60% NPK at 450 + 20% BF at 150 kg ha^{-1}), B (60% NPK at 450 + 40% BF at 300 kg ha^{-1}) and C (60% NPK at 450 + 60% BF at 450 kg ha^{-1}), respectively. The different lowercase letters indicate statistically significant ($p < 0.05$) variations across treatments.

3.4. Alteration in Soil Enzymatic Activities during Different Developmental Phases in Maize

The investigation into soil enzyme activities revealed that the application of treatment A (60% NPK + 20% BF) significantly increased URE activity at an early stage by 28.84%, as compared to treatments B, C, and CK. These increments underscore the efficacy of bio-fertilizers in ensuring an adequate supply of soil nutrients through enhanced nitrogen cycling (Figure 4A). Furthermore, the study found that while AP activity tended to decrease from the early stage to the mature stage, the substitution of a portion of NPK fertilizers with organic and bacterial alternatives led to a substantial rise in the activity of this enzyme when compared to CK. This was particularly evident at both the early and mature stages in treatment A, which displayed the highest level of AP activity, and marked significant differences compared to other treatments (Figure 4B). Sucrose activity in maize soil also exhibited an increasing trend from the early to mature stages, indicating that the capacity of soil to break down sucrose was significantly ($p < 0.05$) enhanced by partial replacement of NPK fertilizers with organic and bacterial alternatives. This trend was pronounced in treatments A and C during the mature stage, which showed a statistically significant ($p < 0.05$) increase in sucrose activity than the CK treatment. The application of bio-fertilizer contributed to this increase by providing an abundant source of energy and microorganisms, which in turn stimulated soil microbial metabolism and subsequently augmented soil enzyme activity (Figure 4C). These findings suggest that integrating organic and bacterial fertilizers into traditional NPK fertilization schemes can substantially enhance soil enzyme activities, which is critical for maintaining soil health and fertility. The improvements in urease, alkaline phosphatase, and sucrose activities reflect the potential of biofertilizers to promote more sustainable soil management practices.

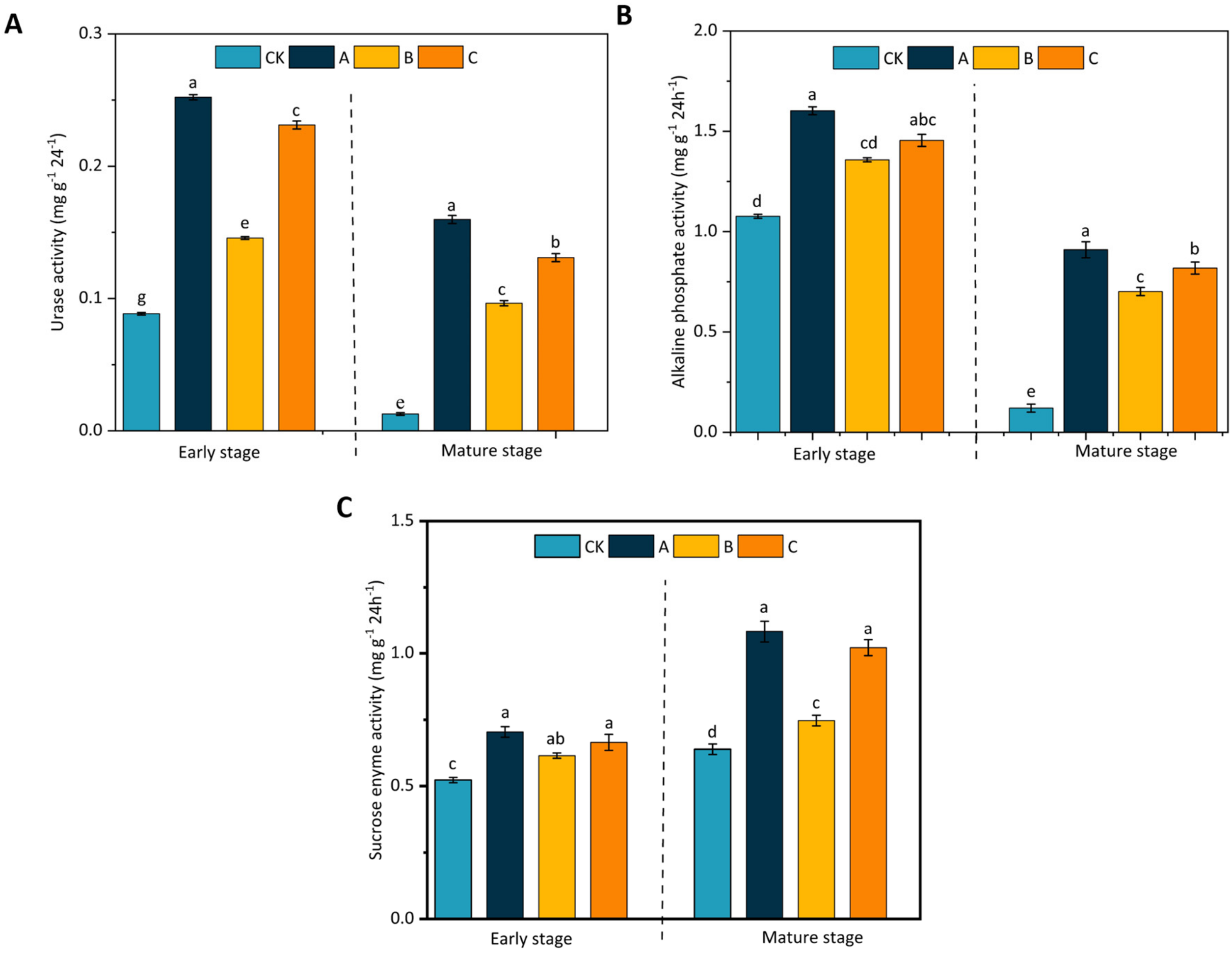

Figure 4. Effects of different fertilization treatments on soil enzyme activities at different maize growth stages. Urease activity (**A**), alkaline phosphate activity (**B**), and sucrose enzyme activity (**C**) at early and mature stages under different treatments, CK (100% NPK at 750 kg ha^{-1}), A (60% NPK at 450 + 20% BF at 150 kg ha^{-1}), B (60% NPK at 450 + 40% BF at 300 kg ha^{-1}), and C (60% NPK at 450 + 60% BF at 450 kg ha^{-1}), respectively. The different lowercase letters indicate statistically significant ($p < 0.05$) variations across treatments.

3.5. Composition and Relative Abundance of Bacterial and Fungal Communities in Soils in Response to Different Fertilization Treatments at Mature the Stage

During the wheat and maize growing season, a comprehensive analysis of the soil bacterial and microbial communities was conducted. At the phylum level, (Figure 5A) illustrates the relative abundances of the top 10 bacteria in summer maize across all treatments. The predominant phyla were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Firmicutes*, and *Gemmatimonadetes*, with *Proteobacteria* consistently exceeding 25%. Notably, *Ascomycota* dominated fungi, particularly in treatment B with an abundance exceeding 80% (Figure 5B). Similarly, in winter wheat, *Proteobacteria* and *Actinobacteria* showed increased relative abundances across all treatments (Figure 5C). Additionally, in wheat fungi, *Ascomycota* showed higher relative abundance levels in all treatments, with the highest abundance in treatments A and C increased at 95% (Figure 5D).

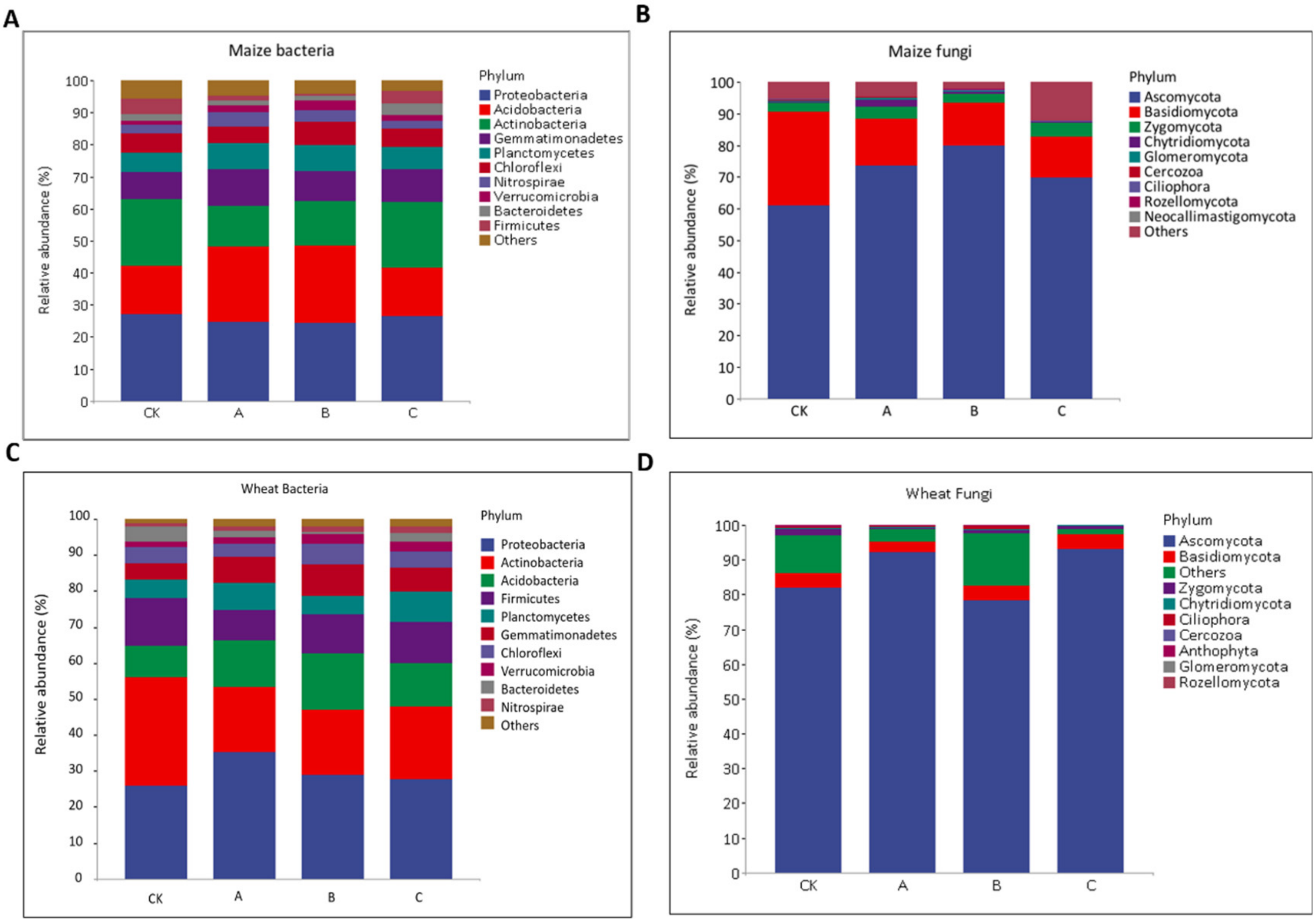

Figure 5. Effects of fertilization on bacterial (**A**,**C**) and fungal (**B**,**D**) taxonomic composition at phylum level under different treatments during winter wheat–summer maize cropping seasons. CK (100% NPK at 750 kg ha^{-1}), A (60% NPK at 450 + 20% BF at 150 kg ha^{-1}), B (60% NPK at 450 + 40% BF at 300 kg ha^{-1}), and C (60% NPK at 450 + 60% BF at 450 kg ha^{-1}), respectively. The numbers of distinct shades in columns show abundance at the phylum level.

Ten bacterial phyla collectively constituted over 98% of the total soil bacterial population, and significant differences were observed when the NPK compound fertilizer was partially replaced with biofertilizers (treatments A, B, and C). Notably, *Proteobacteria* and *Acidobacteria* increased (5.74% to 81.80% and 6.65% to 58.41%, respectively), while *Actinobacteria* and *Firmicutes* decreased (15.74% to 50.26% and 18.67% to 43.82%, respectively).

At the genus level, diversity within bacterial communities was affected, with variations in *Alphaproteobacteria*, *Betaproteobacteria*, *Thermoleophilias*, and others, constituting over 80% of the total soil bacterial composition (Figure 5). The distinct clusters formed by CK and treatments A, B, and C, showed a notable alteration in the soil bacterial genus-level community structure due to the partial substitution of NPK with biofertilizers. Figure 5B highlighted the differences in soil bacterial dominance and relative abundance across the four treatments, emphasizing the effects of fertilization strategies on the soil microbiome.

In maize season, a phylum-level analysis (Figure 5C) highlighted the dominant presence of *Zygomycota* constituting 90% of soil fungi across treatments. Comparative to CK, *Ascomycota* surged by 14.22–30.99%, and *Basidiomycota* maintained 40.17%, varying from 26.38 to 55.30%. At the genus level (Figure 5D), diverse classes represented over 70% of total soil fungi. Treatments substituting NPK with biofertilizers showed an increase in the levels of *Dothideomycetes*, *Saccharomycetes*, and *Agaricomycetes*. Figure 5 revealed differences in soil fungal dominance and relative abundance across the four treatments, suggesting distinct effects of microbial fertilizer substitution on soil fungal communities.

The data suggest that integrating biofertilizers modifies the soil environment, leading to a significant shift in bacterial community dynamics. This shift potentially influences the soil health and nutrient cycling processes that are critical for plant growth, underscoring the influence of fertilization practices on the soil's microbiological ecosystem. These results advocate the benefits of incorporating bio-organic and microbial inputs into conventional fertilization regimes to promote a more diverse and robust soil microbiota.

3.6. Changes in the Relative Abundance of Bacterial and Fungal Composition at Genus Level under Different Bio-Fertilizer Treatments in Wheat–Maize across the Seasons

The cluster analysis of the relative abundance of fungi and bacteria at the genus level in the wheat–maize cropping seasons is presented in (Figure 6). The results of relative abundance at the genus level across all treatments showed that the top 10 most abundant identified bacterial genera in summer maize, (*Solirubrobacter*, *Sphingomonas*, *Nocardioides*, *Lactobacillus*, *Phycicoccus*, *Gemmatirosa*, *Haliangium*, *Rubrobacter*, *Streptomyces* and *Bacillus*), and abundantly identified fungi (*Aspergillus*, *Leucaogaricus*, *Mortierella*, *Themoascus*, *Candida*, *Humicola*, *Seedosporium*, *Chaetomium*, *Thermomyces,* and *Pseudogymnoascus*), were observed in the maize cropping season. In the winter wheat cropping season, the most abundant bacterial and fungal genera were (*Bacillus*, *Lactococcus*, *Burkholderia*, *Gemmatimonadetes*, *Gemmata*, *Solirubrobacter*, *Skermanella*, *Bradyrhizobium*, *Kaistobacter* and *Lysobacter*), and (*Candida*, *Coniolariella*, *Mortierella*, *Staphylotrichum*, *Pyrenochaetopsis*, *Gibberella*, *Ophioceras*, *Humicola*, *Acremonium* and *Chaetomium*), respectively. Notably, biofertilizer treatments exhibited a higher *Solirubrobacter* abundance than the control (CK), and *Solirubrobacter* peaked at 27% in treatment B (Figure 6A). In the maize cropping season, *Aspergillus* remained dominant, particularly in treatment B, which increased by 60% compared with CK (Figure 6B). However, in the wheat cropping season, the bacteria, *Bacillus* and *Lactococcus* decreased in all treatments when compared to CK (Figure 6C). Moreover, in wheat fungi, *Candida* showed the highest response in all treatments, particularly in treatment C, *Candida*, and *Mortierella* increased up to 60% and 68%, respectively, in comparison to CK (Figure 6D).

3.7. Correlation Analysis between Enzymes and Genes Related to Phyla and Genera and Soil Nutrients in Wheat–Maize under Different Treatments

The correlation coefficients demonstrated associations between the composition of the microbial community and the activities of soil enzymes at the phylum and genus levels (Figures 5 and 6). To investigate the correlation between fungal and bacterial differences during wheat–maize cropping seasons, total nitrogen (TN), alkaline nitrogen (AN), available phosphorus content (AP), soil organic matter (SOM), urease activity (URE), alkaline phosphatase (ALP) activity, sucrose enzyme activity (SEA) and yield were analyzed under different treatments during the different stages of wheat and maize (Figure 7). *Lactobacillus*, *Solirubrobacter*, *Gemmatirosa*, *Streptomyces*, *Thermoascus*, *Scedosporium*, and *Thermomyces* were targeted: only *Solirubrobacter* and *Streptomyces* showed positive correlations with TN, AN, AP, SOM, URE, ALP, SEA, and yield during the maize cropping season (Figure 7A). However, *Bascillus*, *Lactococcus*, *Gemmata*, *Gemmatimonadetes*, *Solirubrobacter*, *Skermanella*, *Bradyhizobium*, *Mortierella*, *Staphylotrichum*, *Humicola*, *Acremonium,* and *Chaetomium* showed a significant correlation between soil organic matter (SOM) and URE activity during the wheat cropping season. In contrast, the URE activity and SEA showed significant correlations during the maize cropping season (Figure 7A). *Bacillus* and *Lactococcus* showed mostly positive correlations across the variables, particularly with yield, indicating that their presence might be beneficial for crop productivity. *Gemmata* and *Gemmatimonadetes* exhibit a mix of weak positive and negative correlations without significant impact on most variables. *Bradyrhizobium* has strong, significantly negative correlations with some variables, notably SEA and ALP, suggesting an inhibitory or competitive effect in these contexts. *Chaetomium* showed strong negative correlations across several variables, particularly with yield, indicating potentially harmful effects on soil properties or crop performance (Figure 7B). Moreover, AN and AP showed significant correlations during the maize cropping season, and URE showed a significant correlation during the wheat cropping season (Figure 7C,D).

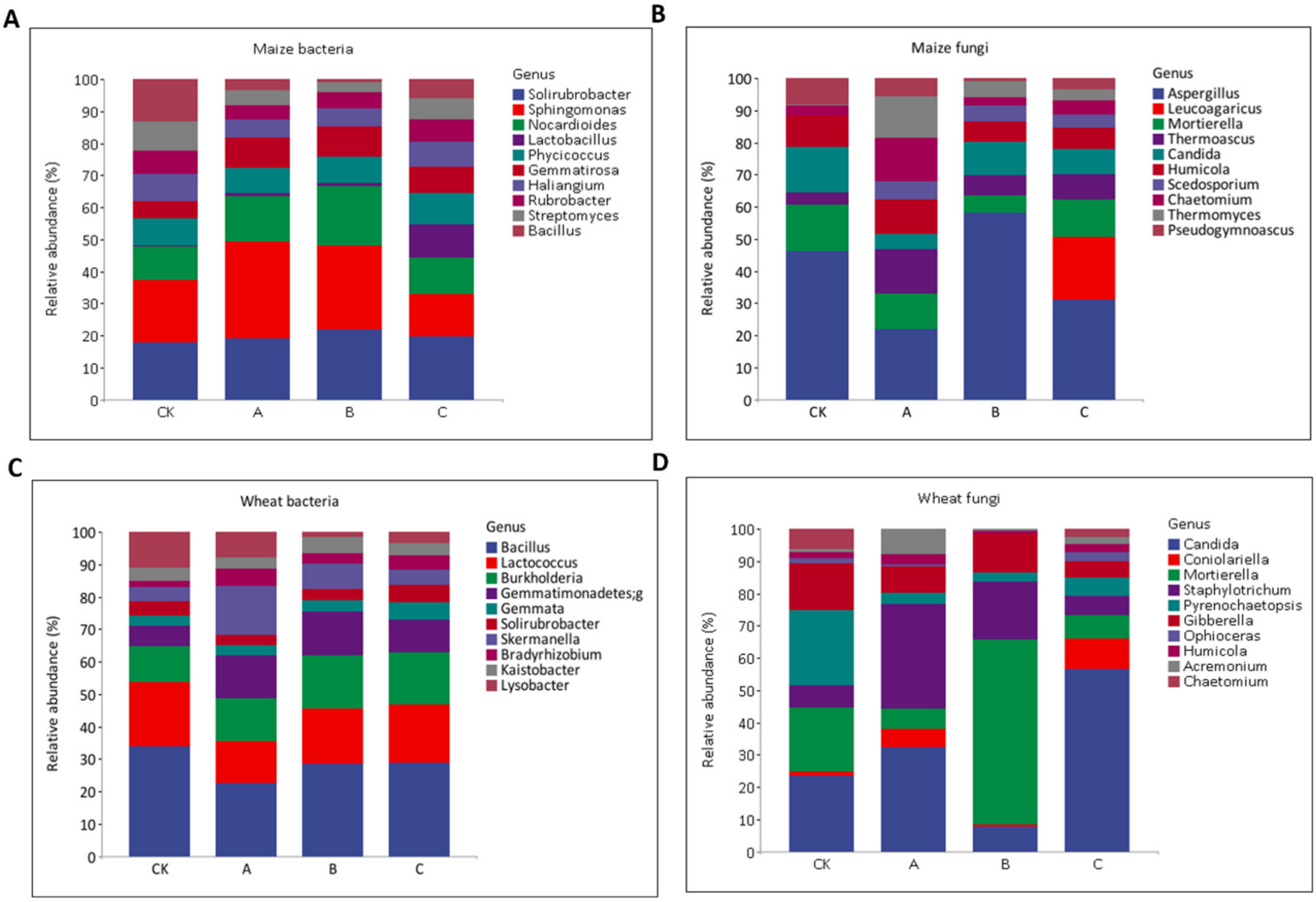

Figure 6. Changes in the relative abundance of soil bacteria (**A**,**C**) and fungi (**B**,**D**) in the maize-wheat cropping season under different treatments. The CK (100% NPK at 750 kg ha^{-1}), A (60% NPK at 450 + 20% BF at 150 kg ha^{-1}), B (60% NPK at 450 + 40% BF at 300 kg ha^{-1}) and C (60% NPK at 450 + 60% BF at 450 kg ha^{-1}), respectively. The numbers of various colors of the columns indicate the abundance at the genus level.

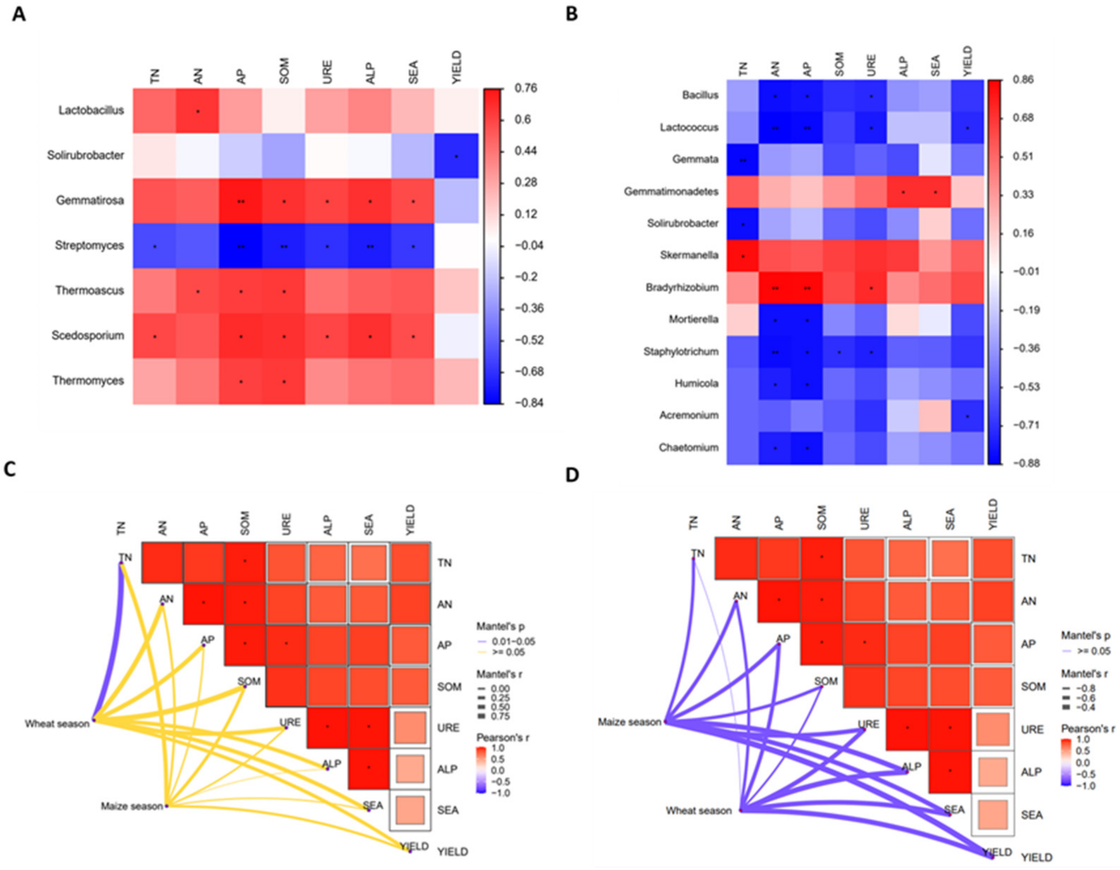

Figure 7. Correlation analysis between enzymes and microbes related to phyla and genera and soil nutrients in wheat–maize cropping seasons under different treatments. The relationship between the composition of the soil microbial community, differential microbes, and differentially expressed metabolites (DEMs) has been studied in relation to multiple environmental factors using Mantel. The (**A**) indicates the link between soil nutrients throughout the summer maize season, (**B**) shows the correlation during the winter wheat season, (**C**) assesses the correlation between bacteria in winter wheat–summer maize cropping seasons, and (**D**) explores the correlation between fungi in winter wheat–summer maize cropping seasons. The coloring of the edge shows the significance of statistical analysis, which was determined using 9999 permutations. The grid asterisk indicates the magnitude of significance, "*" represents $p < 0.05$, and "**" indicates $p < 0.01$.

4. Discussion

This study examined how different fertilizer treatments affected soil nitrogen levels during wheat–maize growing seasons. It highlighted the benefits of the integration of inorganic (NPK compound fertilizers) and bacterial fertilizers [44] and found that the total nitrogen (TN) content increased gradually from seeding to ripening under various fertilizer treatments [45], also discovered that the treatments with NPK compound fertilizers had more TN during the mature stage. In this study treatment C improved AN at the jointing stage in the development of nitrogen buildup. However, treatment A (60% NPK + 20% BF) significantly decreased the TN content throughout the mature stage, suggesting that it helped wheat absorb nitrogen in the later stages (Figure 1A). The results were in accordance with an earlier study [46], which revealed that the biofertilizers with NPK components could boost soil nitrogen.

Analysis of AN and AP levels showed substantial changes across treatments and growth phases. Treatment C had more AP throughout the jointing stage, whereas treatment A had a higher AP at the mature stage. These findings suggest that fertilizer application affects soil nutrient dynamics in different ways [32,47]. At the mature stage, P and SOM availability was significantly improved by treatment with NPK (Figure 1B,C). This emphasizes the necessity of gradually adding organic inputs to the soil [48]. In maize, during the jointing and mature stages, treatment C had a higher TN content and significant differences were identified at the mature stage. Treatment A (60% NPK + 20% BF) had higher levels of AK and AP than the other treatments at various growth stages (Figure 3A–C). This shows the efficiency of treatment in maize throughout its life cycle [49]. The study also emphasizes strategic fertilization, which partially replaces NPK compound fertilizers with biofertilizers. This substitution can boost soil nutrients and organic matter, thereby enabling sustainable agriculture. However, it emphasizes the importance of proper calibration and moderation while applying fertilizer, as exceeding optimal levels may not be beneficial. These findings provide valuable information for improving wheat and maize fertilization methods to increase agricultural productivity and soil fertility.

In wheat, crop development showed that substituting a portion of NPK compound fertilizers with biofertilizers affected the soil urease and (AP) activities. This study found that urease activity in wheat was steady throughout development. CK had much less urease activity than biofertilizer treatments. Compared to CK and treatment A (60% NPK + 20% BF), treatments B and C had higher urease activity, and jointing treatment C had greater urease activity than CK and B (Figure 2A). The integration of organic, bacterial, and NKP compound fertilizers may increase soil urease activity, promoting nitrogen mineralization and wheat plant nitrogen availability [50]. The biofertilizers also significantly increased AP activity compared with CK. Meanwhile, at the jointing stage, treatment C was much more active than CK. Treatments A and B resulted in much higher AP activity at the mature stage (Figure 2B). These results suggest that NPK fertilizers could be replaced with organic and bacterial alternatives to improve soil AP activity, phosphorus availability for plant absorption, and soil fertility [51].

The study also found that biofertilizers increased sucrase activity, indicating increased soil microbial activity and organic material decomposition. Organic inputs fuel soil bacteria and stimulate microbial metabolism, thereby boosting sucrase activity [52]. Biofertilizers increase soil enzyme activities, which are essential for soil health and fertility (Figure 2C). In maize, biofertilizers significantly increased urease and AP activities compared to CK, demonstrating the efficiency of organic inputs in the soil nitrogen cycle. Treatment A (60% NPK + 20% BF) increased urease and AP activities at the jointing and mature stages, suggesting that it increased nitrogen and phosphorus availabilities in maize soils. The observed increases in sucrase activity suggested that biofertilizers could boost soil microorganism activities and organic matter decomposition in maize soils [53]. This improved the soil health and fertility (Figure 4A–C). These findings demonstrated the benefits of adding biofertilizers to traditional NPK compound fertilizer schemes to boost soil enzyme activities, nitrogen cycling, and soil health and fertility in wheat and maize cropping rotation systems.

An analysis of the composition and abundance of bacterial and fungal communities in soils subjected to various fertilizer treatments during wheat–maize cultivation seasons revealed how fertilizer practices affected soil microbiota dynamics. In all treatments, at the phylum level, the bacterial and fungal populations changed significantly. *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Firmicutes*, and *Gemmatimonadetes* accounted for more than 25% of maize bacteria. Compared to CK, biofertilizer treatments had more *proteobacteria*, whereas the highest *Actinobacteria* was found in CK, and *Ascomycota* in treatment B, accounting for roughly 80% of the fungi. The end results proved that fertilization methods affected the relative abundance of several bacterial and fungal species [54]. Figure 5A,B indicated that fertilizer integration shaped the composition of soil microbial populations.

The bacterial and fungal populations in the soil from wheat changed across treatments. *Proteobacteria* and *Actinobacteria* were more prevalent in all the treatments, however, *Firmicutes* and *Acidobacteria* varied. Fungal *Ascomycota* abundance was higher in all treatments, especially treatments A and C. These results imply that biofertilizers may affect wheat crop soil bacterial and fungal communities. However, this affected soil health and plant-growth-related nutrient cycling (Figure 5C,D). The class-level investigation revealed significant differences in the bacterial and fungal populations, with *Alphaproteobacteria*, *Betaproteobacteria*, *Dothideomycetes*, *Saccharomycetes*, and *Agaricomycetes* showing these differences. Figure 5 shows that using biofertilizers instead of NPK changed the soil microbial community composition and diversity. This underlines the effect of fertilization on soil microbiome composition and variation [55]. In conclusion, the addition of biofertilizers to traditional fertilizer practices may alter soil bacterial and fungal systems. Changes in the soil composition and nutrient cycling affect soil health. To promote a diverse and resilient soil microbiota that supports sustained agricultural output, fertilizer management must consider microbial factors.

In the wheat–maize cropping seasons, different fertilization treatments changed the soil bacterial and fungal populations at the genus level. *Solirubrobacter*, *Sphingomonas*, *Nocardioides*, *Lactobacillus*, *Phycicoccus*, *Gematirosa*, *Haliangium*, *Rubrobacter*, *Streptomyces*, and *Bacillus* were the dominant soil bacteria found during the maize cropping season. The most dominant fungi were *Aspergillus*, followed by *Leucaogaricus*, *Mortierella*, *Themoascus*, *Candida*, *Humicola*, *Seedosporium*, *Chaetomium*, *Thermomyces*, and *Pseudogymnoascus*. The *Solirubrobacter* was more abundant in bio-organic fertilizer treatments than in CK. *Solirubrobacter* was highest in treatment B (27%) (Figure 6A,B). *Bacillus*, *Lactococcus*, *Burkholderia*, *Gemmatimonadetes*, *Gemmata*, *Solirubrobacter*, *Skermanella*, *Bradyrhizobium*, *Kaistobacter*, and *Lysobacter* were the dominant bacteria during the wheat cropping season. Each treatment increased the relative abundance of specific bacterial and fungal taxa compared to that in the CK, but the dominance varied. In wheat, all treatments had more *Bacillus* and *Lactococcus* than CK. *Candida* had the strongest reactivity in all treatments, particularly in treatment C, among wheat fungi. *Candida* and *Mortierella* increased by 60% and 68%, respectively, compared with CK (Figure 6C,D).

Fertilization treatments significantly affected the relative abundance of certain bacterial and fungal taxa in the wheat–maize cropping system. Changes in microbial community composition suggest that fertilization practices may affect soil microbiota, which can in turn affect soil health, nutrient cycling, and crop productivity [56,57]. The higher predominance of particular genera in the bio-fertilizer treatments suggests that these fertilizers may promote beneficial microbial taxa, which may improve soil fertility and plant growth. In conclusion, microbial elements should be considered in fertilizer management to improve soil health and agricultural sustainability.

In the wheat–maize cultivation system, the correlation analysis between enzymes and genes associated with phyla and genera and soil nutritional variables revealed links between microbial community makeup and soil enzymatic activities. The TN, AN, AP, SOM, URE, ALP, SEA, and yield were measured under different fertilization treatments. *Lactobacillus*, *Solirubrobacter*, *Gemmatirosa*, *Streptomyces*, *Thermoascus*, *Scedosporium*, and *Thermomyces* were examined during the maize harvest season for correlation analyses. *Solirubrobacter* and *Streptomyces* were positively correlated with TN, AN, AP, SOM, URE, ALP, SEA, and yield, however: the correlation was not significant at the early stage, but it was significant during the maturity stage (Figure 7A). These results suggest that microbial communities may affect soil nutrient levels and enzymatic processes during maize growth [58]. Similarly, this study examined the relationship between SOM and various bacterial groups (*Bacillus*, *Lactococcus*, *Gemmata*, *Gemmatimonadetes*, *Solirubrobacter*, *Skermanella*, and *Bradyrhizobium*) and fungal species during the wheat season. The study discovered strong relationships between SOM and URE (Figure 7A), suggesting plausible links between these specific bacteria, fungi, and soil enzymatic processes during wheat growth [59]. During the maize season, AP and URE activities were correlated, suggesting that soil nitrogen supply affected urease activity in maize soils. Figure 7C,D showed a strong correlation between AP and URE activity in wheat soils during both cropping seasons, demonstrating that phosphate availability was linked to urease activity. Correlations suggest that some microbial species regulate soil nutrient dynamics and enzymatic activities [60,61]. In agricultural environments, microorganisms are essential to soil health and fertility [11]. These microbial soil interactions require further study to improve fertilization and agricultural sustainability.

5. Conclusions

In summary, the study demonstrated that the partial replacement of NPK compound fertilizers with biofertilizers significantly improved soil nutrient contents and enzymatic activities. It improved soil health and nutrient availability in the winter wheat and summer maize (Figure 8). The use of bacterial fertilizer in treatment A showed the most promising results in increased maize yield. Further, the application of biofertilizers positively influenced the diversity and richness of soil microbial species, it notably increased *Acidobacteria* and *Verrucomicrobia* populations while decreasing *Actinobacteria*. This treatment also enhanced metabolic activities in the microbiota during the maize season including amino acid, carbohydrate, energy, lipid, and vitamin metabolisms during the maize season. The fungal community composition also changed, with an increase in *Ascomycota* and a decrease in *Basidiomycota* in treatments with substituted fertilizers. These findings highlight the advantages of incorporating biofertilizers into traditional fertilization practices for sustainable agriculture. This approach not only improves soil health but significantly boosts crop productivity.

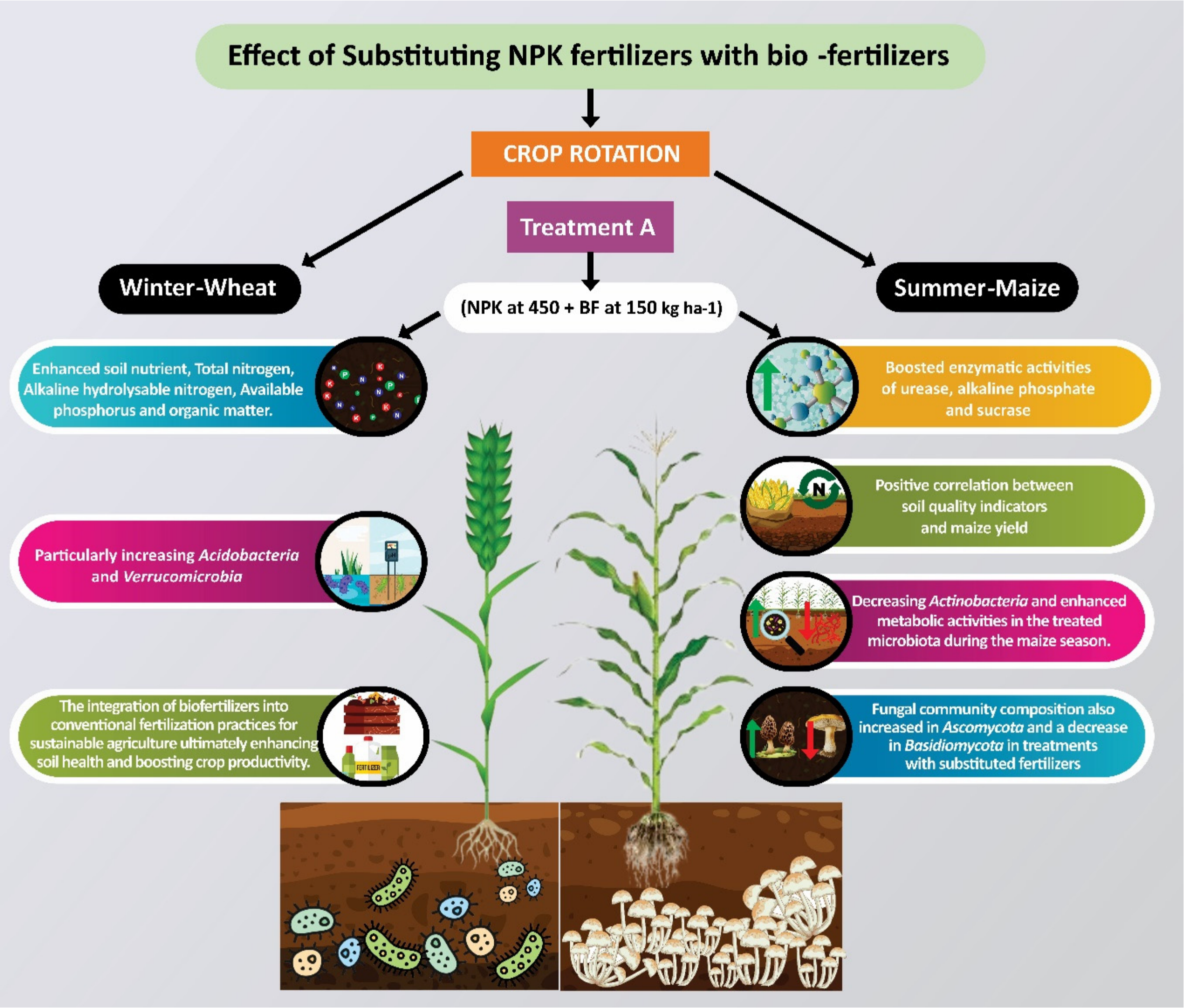

Figure 8. Proposed model of bio-fertilizers supplementation. Effects on enzymatic activities, soil properties, soil microbial community, changes in soil nutrients, and crop growth development and yield, in wheat–maize cropping seasons.

Author Contributions: Conceptualization, Z.Y. and Z.G.; field experiment and chemical analysis methods, J.C. and Y.Q.; investigation, X.L. and W.L.; statistical analysis, X.L.; sequencing data analysis, W.L.; writing—original draft preparation, A.A.; writing—review and editing, A.A. and W.Y.;

visualization, W.Y.; supervision, Z.Y.; funding acquisition, Z.Y. and Z.G. All authors have read and agreed to the published version of the manuscript.

Funding: The grants for this study were funded by the National Key Technology R&D Program of China (Ministry of Science and Technology of the People's Republic of China, Number: 2015BAD23B04-2), the particular funding for Scientific Research on Public Causes (Ministry of Agriculture and Rural Affairs of the People's Republic of China, Number: 201503120), the Shanxi Characteristic Wheat Industry Technology Innovative Strategic Alliance (Department of Science and Technology of Shanxi Province, Number: [2020]17), the Ministerial and Provincial Co-Innovation Centre for Endemic Crops Production with High-quality and Efficiency in Loess Plateau (Ministry of Education of the People's Republic of China and Shanxi Province, Number: SBGJXTZX-21), and the Modern Agriculture Industry Technology System Construction (Ministry of Agriculture and Rural Affairs of the People's Republic of China, Number: CARS-03-01-24).

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Cui, Z.; Dou, Z.; Chen, X.; Ju, X.; Zhang, F. Managing agricultural nutrients for food security in China: Past, present, and future. *Agron. J.* **2014**, *106*, 191–198. [CrossRef]
2. Chen, A.; Lei, B.; Lu, Y.; Mao, Y.; Zhang, D. Study on nitrogen application rate tolerance based on rice yield and ecological security in hilly areas of South China. *Trans. Chin. Soc. Agric. Eng.* **2013**, *29*, 131–139.
3. Zhao, J.; Luo, Q.; Deng, H.; Yan, Y. Opportunities and challenges of sustainable agricultural development in China. *Philos. Trans. R. Soc. B Biol. Sci.* **2008**, *363*, 893–904. [CrossRef]
4. Chao, G.; Bo, S.; Zhang, T.-L. Sustainable nutrient management in Chinese agriculture: Challenges and perspective. *Pedosphere* **2006**, *16*, 253–263.
5. Chen, F.; Xiao, T.; Zhu, Z.; Yang, X.; Ran, W.; Shen, Q. Effect of bio-organic fertilizers on root-knot nematode of muskmelon in field. *Plant Nutr. Fertil. Sci.* **2011**, *17*, 1262–1267.
6. Syed, S.; Wang, X.; Prasad, T.N.; Lian, B. Bio-organic mineral fertilizer for sustainable agriculture: Current trends and future perspectives. *Minerals* **2021**, *11*, 1336. [CrossRef]
7. Fan, T.; Stewart, B.; Yong, W.; Junjie, L.; Guangye, Z. Long-term fertilization effects on grain yield, water-use efficiency and soil fertility in the dryland of Loess Plateau in China. *Agric. Ecosyst. Environ.* **2005**, *106*, 313–329. [CrossRef]
8. Juan, L.; Zhao, B.-Q.; Li, X.-Y.; Jiang, R.-B.; Bing, S.H. Effects of long-term combined application of organic and mineral fertilizers on microbial biomass, soil enzyme activities and soil fertility. *Agric. Sci. China* **2008**, *7*, 336–343.
9. Mi, W.; Wu, L.; Ma, Q.; Zhang, X.; Liu, Y. Combined application of organic materials and inorganic fertilizers improving rice yield and soil fertility of yellow clayey paddy soil. *Trans. Chin. Soc. Agric. Eng.* **2016**, *32*, 103–108.
10. Arikan, Ş.; Pirlak, L. Effects of plant growth promoting rhizobacteria (PGPR) on growth, yield and fruit quality of sour cherry (*Prunus cerasus* L.). *Erwerbs-Obstbau* **2016**, *58*, 221–226. [CrossRef]
11. Han, Z.; Hou, H.; Yao, X.; Qian, X.; Zhou, M. Substituting Partial Chemical Fertilizers with Bio-Organic Fertilizers to Reduce Greenhouse Gas Emissions in Water-Saving Irrigated Rice Fields. *Agronomy* **2024**, *14*, 544. [CrossRef]
12. Zhai, L.; Wang, Z.; Zhai, Y.; Zhang, L.; Zheng, M.; Yao, H.; Lv, L.; Shen, H.; Zhang, J.; Yao, Y. Partial substitution of chemical fertilizer by organic fertilizer benefits grain yield, water use efficiency, and economic return of summer maize. *Soil Tillage Res.* **2022**, *217*, 105287. [CrossRef]
13. He, H.; Peng, M.; Ru, S.; Hou, Z.; Li, J. A suitable organic fertilizer substitution ratio could improve maize yield and soil fertility with low pollution risk. *Front. Plant Sci.* **2022**, *13*, 988663. [CrossRef]
14. Dhaliwal, S.S.; Sharma, V.; Shukla, A.K.; Verma, V.; Kaur, M.; Singh, P.; Gaber, A.; Hossain, A. Effect of addition of organic manures on basmati yield, nutrient content and soil fertility status in north-western India. *Heliyon* **2023**, *9*, e14514. [CrossRef]
15. Dhaliwal, S.S.; Sharma, S.; Sharma, V.; Shukla, A.K.; Walia, S.S.; Alhomrani, M.; Gaber, A.; Toor, A.S.; Verma, V.; Randhawa, M.K. Long-term integrated nutrient management in the maize–wheat cropping system in alluvial soils of North-Western India: Influence on soil organic carbon, microbial activity and nutrient status. *Agronomy* **2021**, *11*, 2258. [CrossRef]
16. Curtin, D.; Campbell, C.; Jalil, A. Effects of acidity on mineralization: pH-dependence of organic matter mineralization in weakly acidic soils. *Soil Biol. Biochem.* **1998**, *30*, 57–64. [CrossRef]
17. Murphy, B. Impact of soil organic matter on soil properties—A review with emphasis on Australian soils. *Soil Res.* **2015**, *53*, 605–635. [CrossRef]
18. Reeve, J.R.; Hoagland, L.A.; Villalba, J.J.; Carr, P.M.; Atucha, A.; Cambardella, C.; Davis, D.R.; Delate, K. Organic farming, soil health, and food quality: Considering possible links. *Adv. Agron.* **2016**, *137*, 319–367.
19. Yadav, K.K.; Sarkar, S. Biofertilizers, impact on soil fertility and crop productivity under sustainable agriculture. *Environ. Ecol.* **2019**, *37*, 89–93.

20. Kour, D.; Rana, K.L.; Yadav, A.N.; Yadav, N.; Kumar, M.; Kumar, V.; Vyas, P.; Dhaliwal, H.S.; Saxena, A.K. Microbial biofertilizers: Bioresources and eco-friendly technologies for agricultural and environmental sustainability. *Biocatal. Agric. Biotechnol.* **2020**, *23*, 101487. [CrossRef]
21. Kumar, D.; Dalal, R.P.S.; Arora, I. Bio-fertilizers a Future Prospect Towards Sustainable Agricultural Development. In *Innovative Approaches for Sustainable Development: Theories and Practices in Agriculture*; Springer: Berlin/Heidelberg, Germany, 2022; pp. 91–105.
22. Jing, W.; Sajnani, S.; Zhou, M.; Zhu, H.; Xu, Y. Evaluating the Ecological Impact of Wastewater Discharges on Microbial and Contaminant Dynamics in Rivers. *Water* **2024**, *16*, 377. [CrossRef]
23. Ojuederie, O.B.; Olanrewaju, O.S.; Babalola, O.O. Plant growth promoting rhizobacterial mitigation of drought stress in crop plants: Implications for sustainable agriculture. *Agronomy* **2019**, *9*, 712. [CrossRef]
24. Cheng, H.; Zhang, D.; Huang, B.; Song, Z.; Ren, L.; Hao, B.; Liu, J.; Zhu, J.; Fang, W.; Yan, D. Organic fertilizer improves soil fertility and restores the bacterial community after 1, 3-dichloropropene fumigation. *Sci. Total Environ.* **2020**, *738*, 140345. [CrossRef] [PubMed]
25. Chen, D.; Yuan, L.; Liu, Y.; Ji, J.; Hou, H. Long-term application of manures plus chemical fertilizers sustained high rice yield and improved soil chemical and bacterial properties. *Eur. J. Agron.* **2017**, *90*, 34–42. [CrossRef]
26. Zhang, L.-S.; Ma, Y.-A.; Li, B.-J.; Zhu, J.; Sun, S.-C. Effect of organic and inorganic fertilizers combined application on increase yield of winter wheat. *J. Plant Nutr. Fertil.* **2003**, *9*, 503–505.
27. Jiang, D.; Hengsdijk, H.; Ting-Bo, D.; Qi, J.; Wei-Xing, C. Long-term effects of manure and inorganic fertilizers on yield and soil fertility for a winter wheat-maize system in Jiangsu, China. *Pedosphere* **2006**, *16*, 25–32. [CrossRef]
28. Kennedy, A.C.; Stubbs, L. Soil microbial communities as indicators of soil health. *Ann. Arid Zone* **2006**, *45*.
29. Regmi, A.; Ladha, J.; Pathak, H.; Pasuquin, E.; Bueno, C.; Dawe, D.; Hobbs, P.; Joshy, D.; Maskey, S.; Pandey, S. Yield and soil fertility trends in a 20-year rice–rice–wheat experiment in Nepal. *Soil Sci. Soc. Am. J.* **2002**, *66*, 857–867. [CrossRef]
30. Cai, Z.; Qin, S. Dynamics of crop yields and soil organic carbon in a long-term fertilization experiment in the Huang-Huai-Hai Plain of China. *Geoderma* **2006**, *136*, 708–715. [CrossRef]
31. Manna, M.C.; Swarup, A.; Wanjari, R.; Mishra, B.; Shahi, D. Long-term fertilization, manure and liming effects on soil organic matter and crop yields. *Soil Tillage Res.* **2007**, *94*, 397–409. [CrossRef]
32. Ren, J.; Liu, X.; Yang, W.; Yang, X.; Li, W.; Xia, Q.; Li, J.; Gao, Z.; Yang, Z. Rhizosphere soil properties, microbial community, and enzyme activities: Short-term responses to partial substitution of chemical fertilizer with organic manure. *J. Environ. Manag.* **2021**, *299*, 113650. [CrossRef] [PubMed]
33. Dadenko, E.; Kazeev, K.S.; Kolesnikov, S.; Val'kov, V. Changes in the enzymatic activity of soil samples upon their storage. *Eurasian Soil Sci.* **2009**, *42*, 1380–1385. [CrossRef]
34. Guangming, L.; Xuechen, Z.; Xiuping, W.; Hongbo, S.; Jingsong, Y.; Xiangping, W. Soil enzymes as indicators of saline soil fertility under various soil amendments. *Agric. Ecosyst. Environ.* **2017**, *237*, 274–279. [CrossRef]
35. Li, P.; Lang, M.; Wang, X.X.; Zhang, T.L. Sorption and desorption of copper and cadmium in a contaminated soil affected by soil amendments. *CLEAN–Soil Air Water* **2016**, *44*, 1547–1556. [CrossRef]
36. Zhang, D.; Zhou, Z.; Zhang, B.; Du, S.; Liu, G. The effects of agricultural management on selected soil properties of the arable soils in Tibet, China. *Catena* **2012**, *93*, 1–8. [CrossRef]
37. Chen, J.; Arafat, Y.; Wu, L.; Xiao, Z.; Li, Q.; Khan, M.A.; Khan, M.U.; Lin, S.; Lin, W. Shifts in soil microbial community, soil enzymes and crop yield under peanut/maize intercropping with reduced nitrogen levels. *Appl. Soil Ecol.* **2018**, *124*, 327–334. [CrossRef]
38. Raiesi, F.; Salek-Gilani, S. The potential activity of soil extracellular enzymes as an indicator for ecological restoration of rangeland soils after agricultural abandonment. *Appl. Soil Ecol.* **2018**, *126*, 140–147. [CrossRef]
39. Stpniewska, Z.; Wolińska, A.; Ziomek, J. Response of soil catalase activity to chromium contamination. *J. Environ. Sci.* **2009**, *21*, 1142–1147. [CrossRef]
40. Langille, M.G.; Zaneveld, J.; Caporaso, J.G.; McDonald, D.; Knights, D.; Reyes, J.A.; Clemente, J.C.; Burkepile, D.E.; Vega Thurber, R.L.; Knight, R. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat. Biotechnol.* **2013**, *31*, 814–821. [CrossRef]
41. Edgar, R.C. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10*, 996–998. [CrossRef]
42. Cole, J.R.; Wang, Q.; Cardenas, E.; Fish, J.; Chai, B.; Farris, R.J.; Kulam-Syed-Mohideen, A.; McGarrell, D.M.; Marsh, T.; Garrity, G.M. The Ribosomal Database Project: Improved alignments and new tools for rRNA analysis. *Nucleic Acids Res.* **2009**, *37*, D141–D145. [CrossRef]
43. Kõljalg, U.; Nilsson, R.H.; Abarenkov, K.; Tedersoo, L.; Taylor, A.F.; Bahram, M.; Bates, S.T.; Bruns, T.D.; Bengtsson-Palme, J.; Callaghan, T.M. Towards a unified paradigm for sequence-based identification of fungi. *Mol. Ecol.* **2013**, *22*, 5271–5277. [CrossRef]
44. Liu, Y.; Wang, P.; Yu, T.; Zang, H.; Zeng, Z.; Yang, Y. Manure replacement of chemical fertilizers can improve soil quality in the wheat-maize system. *Appl. Soil Ecol.* **2024**, *200*, 105453. [CrossRef]
45. Mondal, S.; Kumar, R.; Mishra, J.S.; Dass, A.; Kumar, S.; Vijay, K.V.; Kumari, M.; Khan, S.R.; Singh, V.K. Grain nitrogen content and productivity of rice and maize under variable doses of fertilizer nitrogen. *Heliyon* **2023**, *9*, e17321. [CrossRef] [PubMed]
46. Ai, C.; Zhang, S.; Zhang, X.; Guo, D.; Zhou, W.; Huang, S. Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation. *Geoderma* **2018**, *319*, 156–166. [CrossRef]

47. Bonanomi, G.; De Filippis, F.; Zotti, M.; Idbella, M.; Cesarano, G.; Al-Rowaily, S.; Abd-ElGawad, A. Repeated applications of organic amendments promote beneficial microbiota, improve soil fertility and increase crop yield. *Appl. Soil Ecol.* **2020**, *156*, 103714. [CrossRef]
48. Kazem, B.M.; Hussein, H.J.; Hussein, A.A. The Effect of adding Fertilizer NPK-Chitosan is Growing and Productive (*Zea mays* L). *Univ. Thi-Qar J. Agric. Res.* **2024**, *13*, 146–152.
49. Dincă, L.C.; Grenni, P.; Onet, C.; Onet, A. Fertilization and soil microbial community: A review. *Appl. Sci.* **2022**, *12*, 1198. [CrossRef]
50. Futa, B.; Kraska, P.; Andruszczak, S.; Gierasimiuk, P.; Jaroszuk-Sierocińska, M. Impact of subsurface application of compound mineral fertilizer on soil enzymatic activity under reduced tillage. *Agronomy* **2021**, *11*, 2213. [CrossRef]
51. Novianti, F.; Syaiful, S.; Dachlan, A.; Fadhli, N. Efficiency of Fertilizing Maize Plants Through the Application of Slow Release NPK Tablet Fertilizer with Biofertilizer. *Indones. J. Agric. Res.* **2023**, *6*, 223–236. [CrossRef]
52. Mushalin, I.P.; Putri, H.F.N.; Fajri, H.A.M.; Yuniarda, S.; Khumairah, F.H.; Hibatullah, F.H.; Ambarita, D.; Nurbaity, A.; Herdiyantoro, D.; Kamaluddin, N.N. Current Status and the Role of Biofilm Biofertilizer for Improving the Soil Health and Agronomic Efficiency of Maize on Marginal Soil. *Int. J. Life Sci. Agric. Res.* **2024**, *3*, 596–602. [CrossRef]
53. Li, C.; Li, H.; Yao, T.; Su, M.; Li, J.; Liu, Z.; Xin, Y.; Wang, L.; Chen, J.; Gun, S. Effects of microbial inoculation on enzyme activity, available nitrogen content, and bacterial succession during pig manure composting. *Bioresour. Technol.* **2020**, *306*, 123167. [CrossRef]
54. Li, Y.; Wang, C.; Wang, T.; Liu, Y.; Jia, S.; Gao, Y.; Liu, S. Effects of different fertilizer treatments on rhizosphere soil microbiome composition and functions. *Land* **2020**, *9*, 329. [CrossRef]
55. Liu, J.; Shu, A.; Song, W.; Shi, W.; Li, M.; Zhang, W.; Li, Z.; Liu, G.; Yuan, F.; Zhang, S. Long-term organic fertilizer substitution increases rice yield by improving soil properties and regulating soil bacteria. *Geoderma* **2021**, *404*, 115287. [CrossRef]
56. Tahat, M.M.; Alananbeh, K.M.; Othman, Y.A.; Leskovar, D.I. Soil health and sustainable agriculture. *Sustainability* **2020**, *12*, 4859. [CrossRef]
57. Moorhead, D.L.; Rinkes, Z.L.; Sinsabaugh, R.L.; Weintraub, M.N. Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: Informing enzyme-based decomposition models. *Front. Microbiol.* **2013**, *4*, 56126. [CrossRef]
58. Qaswar, M.; Chai, R.; Ahmed, W.; Jing, H.; Han, T.; Liu, K.; Ye, X.; Xu, Y.; Anthonio, C.K.; Zhang, H. Partial substitution of chemical fertilizers with organic amendments increased rice yield by changing phosphorus fractions and improving phosphatase activities in fluvo-aquic soil. *J. Soils Sediments* **2020**, *20*, 1285–1296. [CrossRef]
59. Siczek, A.; Frąc, M.; Kalembasa, S.; Kalembasa, D. Soil microbial activity of faba bean (*Vicia faba* L.) and wheat (*Triticum aestivum* L.) rhizosphere during growing season. *Appl. Soil Ecol.* **2018**, *130*, 34–39. [CrossRef]
60. Wu, W.; Ma, B. Integrated nutrient management (INM) for sustaining crop productivity and reducing environmental impact: A review. *Sci. Total Environ.* **2015**, *512*, 415–427. [CrossRef]
61. Li, Y.; Wang, C.; Wu, J.; Zhang, Y.; Li, Q.; Liu, S.; Gao, Y. The effects of localized plant–soil–microbe interactions on soil nitrogen cycle in maize rhizosphere soil under long-term fertilizers. *Agronomy* **2023**, *13*, 2114. [CrossRef]