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Research Article

Different New Fertilizers Differentially Modulate Wheat Yield, Rhizosphere Microbiota and Soil Fertility

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Abstract

The use of alternative fertilizers offers a promising approach to improving soil health and crop productivity, yet their relative effects on rhizosphere microbial communities remain insufficiently understood. This two-year field study on the Huang-Huai Plain, China, assessed the impacts of controlled-release fertilizer (CRF), organic fertilizer (OF), and microbial fertilizer (MF), relative to a conventional synthetic fertilizer (CF), on soil properties, wheat yield, and microbial community composition. Soil chemical parameters, microbial diversity (via 16S rRNA and ITS sequencing), and wheat yield were analyzed. OF significantly enhanced soil organic matter (14.97%), available nitrogen (28.70%), phosphorus (20.59%), potassium (33.06%), and grain yield (17.58%) compared to CF, likely due to sustained nutrient release and stimulation of microbial activity. In contrast, CRF decreased soil organic matter (−19.2%) and phosphorus availability, with only modest yield improvement (3.50%). MF enriched plant-beneficial taxa, including Bacillus and Arthrobacter, and improved yield by 9.39%. Fungal communities showed greater responsiveness to fertilizer type than bacterial communities, with OF and CRF promoting notable increases in fungal diversity. LEfSe analysis revealed treatment-specific microbial biomarkers such as Saccharothrix (OF), Azotobacter (CRF), and Nitrospira (MF), while correlation analysis linked Cyphellophora (OF) and Epicoccum (CRF) to yield enhancement. These findings underscore the potential of organic amendments to simultaneously boost soil fertility, microbial diversity, and crop productivity, outperforming controlled-release and microbial fertilizers. MF demonstrated promise for microbiome-targeted interventions, whereas CRF may pose risks to long-term soil health. This study supports microbiome-informed, organic-inclusive fertilization strategies for sustainable agriculture.

Keywords

New Fertilizers, Rhizosphere Microbiome, Winter Wheat, Yield, Soil Fertility

1. Introduction

Wheat is the main staple food crop that sustains a large portion of the world's population, particularly in Asia. It holds immense importance in agronomy, economics, and food security. In agronomy, wheat cultivation is the cornerstone of global agriculture, with vast areas accounting for approximately 30.7% of the global cereal planting area, dedicated to

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production [1]. Economically speaking, wheat is the main commodity in global trade, with 25% of global wheat production used for export, affecting food prices and agricultural economy [2]. Moreover, wheat provides a significant portion of the calories and nutrients to billions of people around the globe [1], underscoring its critical role in food security. Given its multifaceted pivotal roles, optimizing wheat production through effective fertilization strategies is a priority for sustainable agriculture.

Global wheat production faces the escalating challenge of increasing yields to meet rising food demands while simultaneously minimizing the negative environmental consequences of intensive agriculture. Conventional fertilizers (CF) have been the mainstay of modern agriculture for decades, significantly contributing to the increase in wheat yield. Studies have shown that the application of CF can lead to substantial yield improvements by providing essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K) [3]. However, CF also has several known limitations. For instance, the rapid release of nutrients from CF can lead to nutrient imbalances and soil degradation over time [4]. Additionally, CF often result in significant nutrient losses through leaching and volatilization, contributing to water pollution and greenhouse gas emissions [5]. Moreover, the excessive use of CF can result in soil acidification, which negatively affects soil structure and microbial activity [6]. These issues highlight the need for alternative fertilization strategies that can maintain or enhance wheat yield while mitigating the negative impacts on soil health and the environment.

Alternative fertilizers (or advanced fertilizers) refer to a category of fertilizer products designed with innovative technologies or formulations—such as controlled-release fertilizers (CRF), microbial fertilizers (MF), and organic fertilizers (OF)—to enhance nutrient use efficiency, minimize environmental impact, improve crop performance, and serve as promising alternatives to conventional fertilizers. CRF achieve gradual release of nutrients over time through mechanisms such as polymer coating or encapsulation, aiming to match nutrient release with key growth stages of crops such as tillering and grain filling, thereby maximizing nutrient absorption efficiency [7]. Although CRF improves nitrogen utilization efficiency (nitrogen fixing bacteria enrichment) in the short term, they lead to a decrease in soil organic matter and phosphorus availability, which may pose a long-term threat to soil health. They need to be used in combination with organic fertilizers to balance nutrient release and soil sustainability [8]. Microbial fertilizers (MF) contain beneficial microorganisms that enhance the availability of soil nutrients through processes such as nitrogen fixation, phosphate solubilization, and the production of plant growth promoting substances [3]. MF enhances phosphorus dissolution and nitrification by enriching functional bacterial communities such as Bacillus and Nitrobacter, resulting in increased yield [9]. Organic fertilizers (OFs) are derived from plant or animal materials and can provide a slow-release source of nutrients [10]. They are of great significance in increasing soil organic matter (OM) content, which plays a key role in maintaining long-term soil health and fertility [11]. Meanwhile, organic fertilizers can enhance soil structure, improve water holding capacity, and activate microbial activity [4].

The rhizosphere, the narrow zone of soil directly influenced by plant roots, harbors a diverse and dynamic community of microorganisms that play a vital role in plant nutrient acquisition, disease suppression, and overall plant health [6]. Fertilizer application significantly impacts the composition and function of the rhizosphere microbiome [12]. For instance, chemical fertilizers (CF, CRF) can sometimes disrupt microbial communities and reduce diversity. Mechanistically, excessive nitrogen fertilizer inhibits the activity of nitrogen fixing bacteria and other microorganisms, reducing soil microbial community diversity [13]. Moreover, the abundance of readily available phosphorus and potassium forms reduce the available trace elements in the soil, inhibits the growth of microorganisms that rely on these elements, and excessive potassium indirectly affects related microbial communities [14]. OF and MF, however, can promote the colonization and functional expression of beneficial microbial communities in the rhizosphere [15]. In practice, OF provides carbon sources, optimizes ecological niches, regulates soil environment, improves nitrogen fixing efficiency, increases nitrogen fixing bacterial abundance and root nodule numbers [16]. For mycorrhizal fungi, OF provides carbon sources and signaling molecules, improves soil structure, promotes hyphal expansion, induces spore germination, and increases infection rate [17]. Nevertheless, MF is either directly inoculated with symbiotic nitrogen fixing bacteria to enhance its function, and/or auxiliary bacteria that work together to increase soil available nitrogen content and promote plant growth. Additionally, MF direct inoculation enhances symbiotic efficiency, promotes bacterial growth and inhibits pathogenic fungi, and protects the symbiotic environment [18]. The interplay between fertilizers and the rhizosphere microbiome also directly affects key soil properties, such as pH, organic matter (OM) content, total nitrogen (TN), available nitrogen (AN), available phosphorus (AP), and available potassium (AK) [19]. These properties, in turn, influence nutrient availability and plant growth [20]. Although studies have explored the potential benefits of controlled-release fertilizers (CRF), microbial fertilizers (MF), and organic fertilizers (OF) on soil health and crop productivity, there is still a significant knowledge gap regarding the underlying mechanisms through which these innovative fertilizers affect the nutrient balances and availability, soil quality, and rhizosphere microbial structure and function under wheat systems. In addition, there is currently a lack of systematic research on the effects of controlled-release fertilizers and microbial fertilizers on the rhizosphere microbiome of wheat under field conditions in Zhoukou Dancheng Agricultural High tech Zone, as well as how microorganisms such as Myxococcota and Gemmatimonadota in the micro-

biome are reflected in the improvement of wheat yield and soil fertility.

This study systematically addresses this gap through evaluating the comprehensive effects of controlled-release fertilizer (CRF), microbial fertilizer (MF), and organic fertilizer (OF) on the rhizosphere microbiome, soil properties (pH, OM, TN, AN, AP, AK), and yield of winter wheat compared to conventional fertilizer (CF) in a two-year field experiment in the Huang Huai Plain. We hypothesize that: (1) the MF and OF fertilizers would significantly improve soil chemical properties and diversify rhizosphere microbial community. (2) OF would result in a more diverse and beneficial rhizosphere microbial community compared to MF; and (3) OF would lead to comparable or superior wheat yields relative to CF, CRF, and MF. By elucidating the relationships between fertilizer type, rhizosphere microbiome composition, soil properties, and wheat yield, this study aims to provide insights into two significant implications. On the one hand, it helps to accurately grasp the characteristics of different fertilizers, optimize fertilization strategies, and use fertilizers reasonably, thereby ensuring wheat yield while maintaining soil health and promoting sustainable agricultural development. On the other hand, it can effectively reduce the environmental burden caused by traditional fertilization, minimize the pollution of water bodies by nutrient runoff, and mitigate the impact of greenhouse gas emissions on the climate.

2. Materials and Methods

2.1. Study Site Description

The field experiment was conducted at the Zhoukou Dancheng Agricultural High-Tech Zone in Henan Province, China (33.71 °N, 115.14 °E), located in the Huang-Huai Plain. The area experiences a temperate monsoon climate, with an average annual temperature of 14.5 ° C and a mean annual precipitation of 778.5 mm, based on 30-year meteorological data. The region is characterized by ample sunlight and moderate rainfall, providing favorable conditions for high-yield crop cultivation. The soil at the site is classified as a loam according to the USDA soil taxonomy.

2.2. Experimental Design

The experiment utilized a randomized complete block design (RCBD) with four fertilizer treatments and three replicates per treatment, resulting in 12 experimental plots. Each plot measured 15 m × 15 m (225 m^2). Winter wheat (Triticum aestivum L. cv. Yunong 908) was sown on October 18, 2022, and October 15, 2023, with a row spacing of 20 cm and a seeding density of 3.5 million plants per hectare, optimized for high-yield conditions in Henan Province. Harvesting occurred on June 2, 2023, and May 30, 2024, respectively.

The treatments were as follows: 1) CF (Conventional Fertilizer - Control): 750 kg ha^{-1}(N-P-K: 18-12-10) of synthetic compound fertilizer; 2) CRF (Controlled-Release Fertilizer): 750 kg ha^{-1}(N-P-K: 15-6-5), with polymer-coated urea for controlled nitrogen release; 3) OF (Organic Fertilizer): 600 kg ha^{-1}organic fertilizer containing 30% humic acid; and 4) MF (Microbial Fertilizer): 600 kg ha^{-1}microbial inoculant containing Bacillus subtilis (5×10^8 CFU g^{-1}). The amount of fertilizer used for the above treatment should be used according to the product instructions.

All fertilizers were manufactured by Xinlianxin Chemical Industry Group (Xinxiang County, Xinxiang City, Henan Province, China) and incorporated into the topsoil (0-10 cm) prior to sowing using a rotary tiller.

2.3. Soil Sample Collection

Rhizosphere soil samples were collected at wheat maturity (June 2023 and May 2024) to assess nutrient utilization, evaluate stable microbial communities following long-term fertilization, and minimize variation due to growth stages. Sampling was conducted using the five-point composite method within uniform crop growth zones, avoiding plot edges. Ten wheat plants per plot were carefully excavated to a depth of 20 cm. Loosely adhered soil was gently shaken off, and tightly bound rhizosphere soil (within 2 mm of root surface) was collected using sterile brushes and pooled per plot.

Collected samples were homogenized through a 2-mm sieve and divided into three portions: 1) Air drying (chemical analysis): Soil samples used for chemical analysis were air dried at 20-25 ℃ for 7 days. The dried samples are used to determine chemical properties such as soil organic matter (OM), total nitrogen (TN), available phosphorus (AP), and available potassium (AK).2) Cryopreservation (chemical analysis): Soil samples used for nutritional analysis are immediately stored at -20 ℃ after collection to maintain the stability of their nutritional composition until analysis is conducted. and 3) For microbiological analysis: Flash-frozen in liquid nitrogen and stored at −80°C for DNA extraction.

We collected initial soil samples from the study site prior to the experiment to assess its chemical quality. Baseline soil analyses revealed that OM = 30.98 mg/kg, pH = 7.65, TN = 1440.3 mg/kg, AN = 101.50 mg/kg, AP = 159.14 mg/kg, and AK = 0.31 mg/kg. The soil texture in this area is classified as loam soil based on the particle size, according to USDA taxonomy [15]. Regionally, the site is also reported as a calcareous lime concretion black soil, based on chemical and morphological characteristics [19]. Soil chemical properties were determined by wet oxidation method (Walkley Black method), 1:2.5 (w/v) soil water suspension measurement, micro Kjeldahl nitrogen determination method, alkali diffusion method, Olsen method, and 1 M ammonium acetate (pH 7) extraction combined with flame photometer, respectively.

Microbial community analysis was performed using bacterial 16S rRNA and fungal ITS sequencing protocols. DNA was extracted from 0.5 g of rhizosphere soil and its quality was verified. Bacterial and fungal communities were ampli-

fied by PCR using specific primers and reaction systems. After sequencing on the Illumina MiSeq platform, they were processed with QIIME2 and identified using the corresponding database. Record the grain weight and calculate the yield per unit area of wheat during harvest.

2.4. Indicator Measurements

2.4.1. Soil Chemical Properties

OM was assayed by wet oxidation (Walkley and Black) [21]. Soil total N content was determined by micro Kjeldahl digestion [22]. Available P was measured following Olsen extraction [23], while exchangeable K was analysed by a flame photometer after 1 M ammonium acetate extraction at pH 7. Soil pH was measured using a 1:2.5 (w/v) soil-to-water suspension. Alkali-hydrolyzable nitrogen (AN) was measured using the alkali diffusion method [24]. All measurements were performed in triplicate for technical accuracy.

2.4.2. DNA Extraction and Sequencing

Total microbial DNA was extracted from 0.5 g of rhizosphere soil using the FastDNA™ Spin Kit for Soil (MP Biomedicals, USA) following the manufacturer's protocol. The quality and integrity of DNA were verified by 2% agarose gel electrophoresis and spectrophotometry (NanoDrop 2000, Thermo Fisher Scientific), and the double stranded DNA was quantified by Qubit 4.0 fluorometer (Invitrogen). For bacterial community analysis, the V3-V4 region of the 16S rRNA gene was amplified using primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′). For fungal communities, the ITS1 region was amplified using primers ITS5F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′). PCR was performed in a 25 μL reaction system under the following conditions: pre-denaturation at 98 ℃ for 2 min; 30 cycles of 98 ℃ for 10 s, 55 ℃ for 30 s, and 72 ℃ for 30 s; with a final extension at 72 ℃ for 5 min. The quality and integrity of DNA were verified by 2% agarose gel electrophoresis and spectrophotometry (NanoDrop 2000, Thermo Fisher Scientific), and the double stranded DNA was quantified by Qubit 4.0 fluorometer (Invitrogen) [25]. The PCR amplification product was verified by 1.5% agarose gel electrophoresis for the size of the amplicon (about 460 bp for bacteria and 300 bp for fungi) to ensure the successful amplification of the target fragment. In order to optimize the separation and analysis of DNA fragments in different steps and ensure the accuracy of experimental results, agarose gel with different concentrations was selected for electrophoresis.

The sequencing was completed on the Illumina MiSeq platform (2 × 300 bp double ended sequencing). The raw data was processed by QIIME2 (version 2023.9), including quality filtering (Phred score ≥ 20, truncation length ≥ 150 bp), chimera removal (VSEARCH algorithm based on reference database), and OTU clustering with 97% similarity. The taxonomic annotations for bacteria and fungi are based on the SILVA (v138.1) and UNITE (v9.0) databases, respectively. The sequencing data was rarefied to the minimum sample depth (10000 reads) through sparse curves to ensure comparability. All microbiome data have been submitted to the NCBI sequence reading database (login number PRJNA1252672) and the original FASTQ file and metadata have been made public.

The quality control parameters include: removing sequences with single ended read length<150 bp and removing samples with chimerism ratio>5%. The integrity and transparency of the bioinformatics process are ensured through the standardized analysis module of the Majorbio cloud platform (www.majobio.com). The fungal data processing flow is consistent with that of bacteria, with the addition of ITSx truncation to remove non target sequences outside the conserved region.

2.5. Data Analysis

The alpha diversity index (Chao1 measures richness, Shannon measures evenness) is calculated using a sparse OTU table using the rarefied sequencing depth (10000 reads per sample), while beta diversity is evaluated using Bray-Curtis dissimilarity based on square root transformed OTU abundance data to reduce the influence of dominant taxonomic groups. Community differences were visualized using PCoA plots and the statistical significance of treatment on community composition was tested using PERMANOVA (999 permutations). Using LEfSe analysis to identify differentially enriched taxa (bacterial LDA score threshold>3.0, fungi>2.0, first subjected to Kruskal-Wallis rank sum test and paired Wilcoxon test). The reason for choosing these thresholds is mainly based on considerations of the significance and biological significance of the taxonomic groups among different treatment groups. The LDA score reflects the relative abundance of a taxonomic group in a specific treatment group compared to other groups, and a higher threshold ensures that the identified taxonomic group is statistically significant and biologically important. Pearson correlation analysis ($p<0.05$, FDR corrected multiple comparisons) was performed on the relative abundance of dominant microbial genera (top 20 abundances) and soil properties/yield data. Microbial data is processed and analyzed through standardized pipelines on the Majorbio Cloud Platform (www.mjorbio.com), which provides specific database classifications (with a confidence threshold of 70%) and analyzes community structure using the SparCC algorithm. For plant yield and soil chemical properties, SPSS version 26.0 (IBM, Armonk, New York, USA) was used for one-way ANOVA and Tukey's HSD test ($p<0.05$) after verifying normality and homogeneity of variance hypotheses using Shapiro-Wilk and Levene's tests, respectively. Sparse curves confirmed sequencing coverage>99% Good coverage. RDA quantified the contribution of soil variables to microbial community changes in CANOCO 5.0, and all visualizations were generated using OriginPro 2022 (OriginLab, Northampton, Massachusetts, USA). At the same time, the normalization steps, PERMANOVA related key points, LEfSe threshold and FDR

correction, platform pipeline details, sequencing depth, and validation of statistical hypotheses were clarified in terms of method transparency.

3. Results

3.1. Effects of Alternative Fertilizers on Soil Chemical Properties and Wheat Yield

Application of different fertilizer treatments significantly altered key soil chemical properties (Figure 1). In the CRF (Controlled-Release Fertilizer) treatment, soil organic matter (SOM) decreased by 19.2% compared to the control ($p < 0.001$), whereas organic fertilizer (OF) increased SOM by 14.97% ($p = 0.001$) (Figure 1A). Soil pH remained relatively stable across most treatments, with CRF and OF only showing significant increases relative to control (Figure 1B). Available nitrogen (AN) increased significantly by 28.74% under OF ($p = 0.003$), while CRF and microbial fertilizer (MF) treatments showed non-significant declines (Figure 1C). Similarly, available phosphorus (AP) rose by 20.59% in OF ($p = 0.045$), while CRF and MF treatments exhibited reductions of 26.17% ($p = 0.017$) and 12.01% ($p = 0.204$), respectively (Figure 1D). Available potassium (AK) increased significantly by 33.06% in OF ($p < 0.001$) (Figure 1E). In contrast, total nitrogen (TN) showed modest but non-significant increases across all fertilizer types (Figure 1F). Importantly, wheat yield improved under all treatments, with the largest increase observed in OF (17.58%, $p = 0.031$), followed by MF (9.39%, $p = 0.047$) and a marginal, non-significant increase in CRF (3.50%, $p = 0.071$) (Figure 1G). The statistical methods and degrees of freedom are detailed in Appendix Table A1.

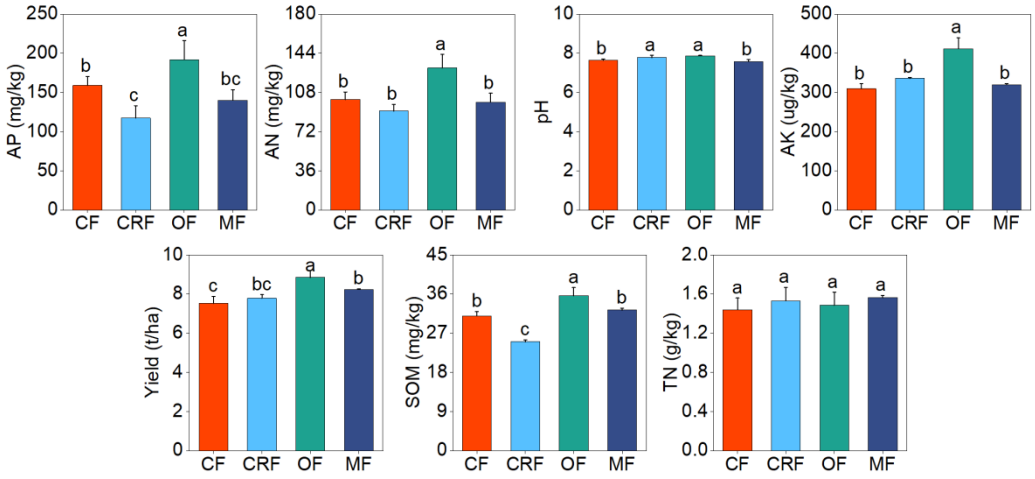


Figure 1. *Effects of different fertilizers on soil chemical properties and wheat grain yield. A; Soil organic matter (SOM), B; Soil pH, C; Available nitrogen (AN), D; Available phosphorus (AP), E; Available potassium (AK), F; Total Nitrogen (TN), and G; Wheat grain yield. Results are shown for conventional (CF), controlled-release (CRF), organic (OF), and microbial (MF) fertilizers. Data are presented as mean ±standard error (n = 4 treatments * 3 replicates = 12). Columns with different lowercase letters are significantly different (p < 0.05).*

3.2. Effects of Alternative Fertilizers on α and β Diversity of Wheat Rhizosphere Soil Microbial Communities

Alpha diversity, assessed using Chao1 and Shannon indices, did not differ significantly among treatments for either bacterial or fungal communities (Figure 2). However, slight, non-significant increases in fungal richness and diversity were observed under OF and CRF ($P>0.05$), indicating a potential trend toward improved fungal community complexity with these treatments.

Principal Coordinate Analysis (PCoA) based on Bray-Curtis distances revealed treatment-driven shifts in microbial community composition (Figure 3). At both phylum and genus levels, all treatments overlapped with each other, indicating no substantial distinction in bacterial communities structure under any treatment (PERMANOVA: phylum $p > 0.05$, $F=0.92687$, $R2=0.2257$; genus $p > 0.05$, $F=1.00284$, $R2=0.2485$). However, MF showed the least overlap, potentially exhibiting compositional alterations (Figure 3A, B).

In contrast, fungal communities exhibited clear separation among treatments at both phylum and genus levels (Figure 3C, D), suggesting a stronger sensitivity of fungal taxa to fertilizer type (PERMANOVA: phylum $p < 0.05$, $F=3.42218$, $R2=0.5911$; genus $p < 0.05$, $F=1.74057$, $R2=0.4519$).

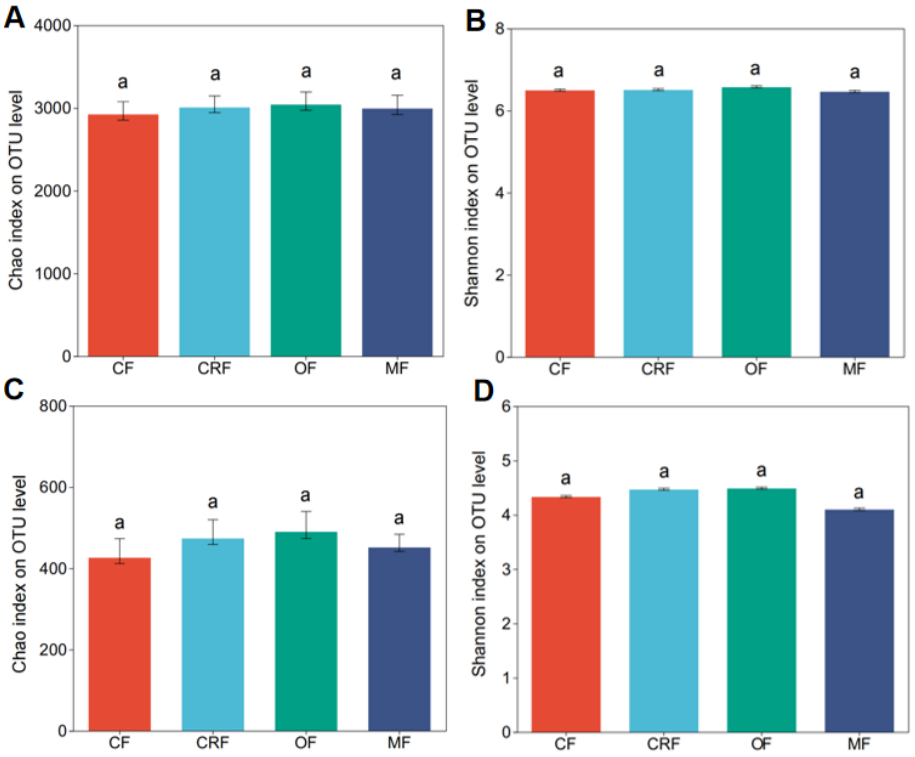


Figure 2. *Effects of fertilizers on bacterial and fungal alpha diversity. Chao1 estimates microbial richness of (A) bacteria and (C) fungi. Shannon diversity index accounts for abundance and evenness in (B) bacteria and (D) fungi. Treatments: conventional (CF), controlled-release (CRF), organic (OF), and microbial (MF) fertilizers.*

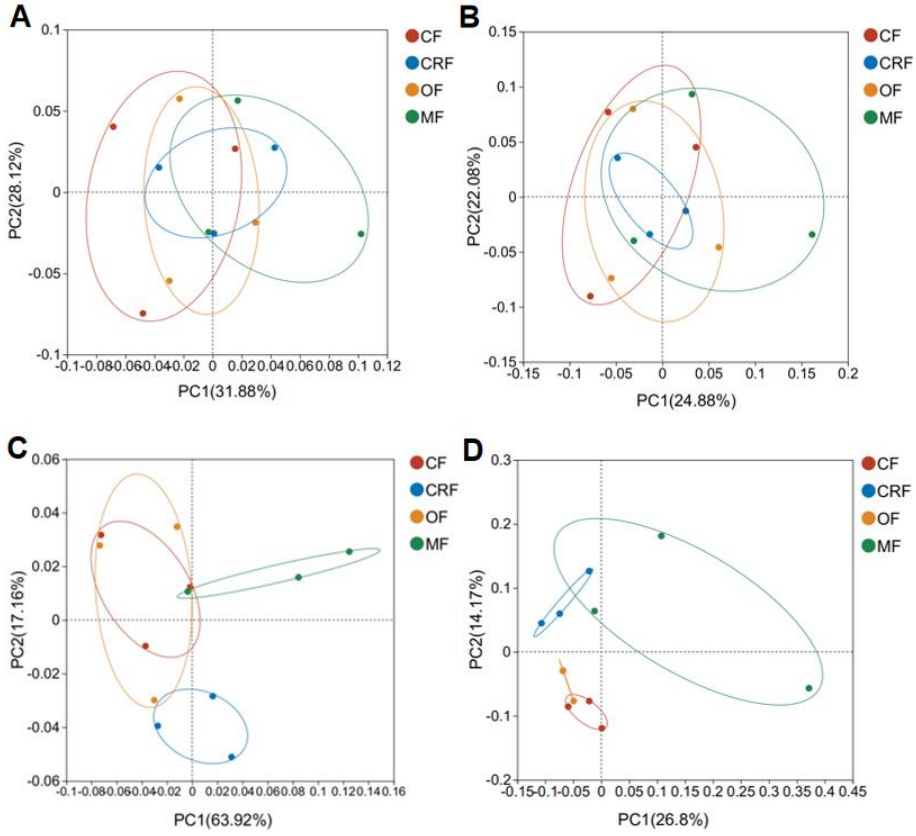


Figure 3. *Principal Coordinate Analysis (PCoA) of bacterial and fungal beta diversity based on Bray-Curtis distances. Results are shown at phyla (A: bacteria, C: fungi) and genera (B: bacteria, D: fungi) levels. Ellipses represent 95% confidence intervals for each treatment group: conventional (CF), controlled-release (CRF), organic (OF), and microbial (MF) fertilizers.*

3.3. Effects of Alternative Fertilizers on Microbial Community Composition

Bacterial communities across all treatments were dominated by phyla Myxococcota, Gemmatimonadota, Chloroflexi, Proteobacteria, and Actinobacteriota. Notably, OF treatment increased the relative abundance of Myxococcota (27% vs. 23% in CF) and Bacteroidota (27% vs. 26% in CF). MF treatment enriched Gemmatimonadota (28% vs. 25% in CF). At the genus level, fertilizer treatments induced notable shifts. In MF, Bacillus increased from 16% in CF to 32%, and Arthrobacter rose from 25% to 33% (Figure 4A, B).

Fungal communities were consistently dominated by the phylum Ascomycota (24-27%) across all treatments. However, genus-level changes were more pronounced. MF treatment led to a sharp increase in Oidiodendron (52% vs. 23% in CF). Conversely, Mortierella declined under CRF, OF, and MF compared to CF (Figure 4C, D).

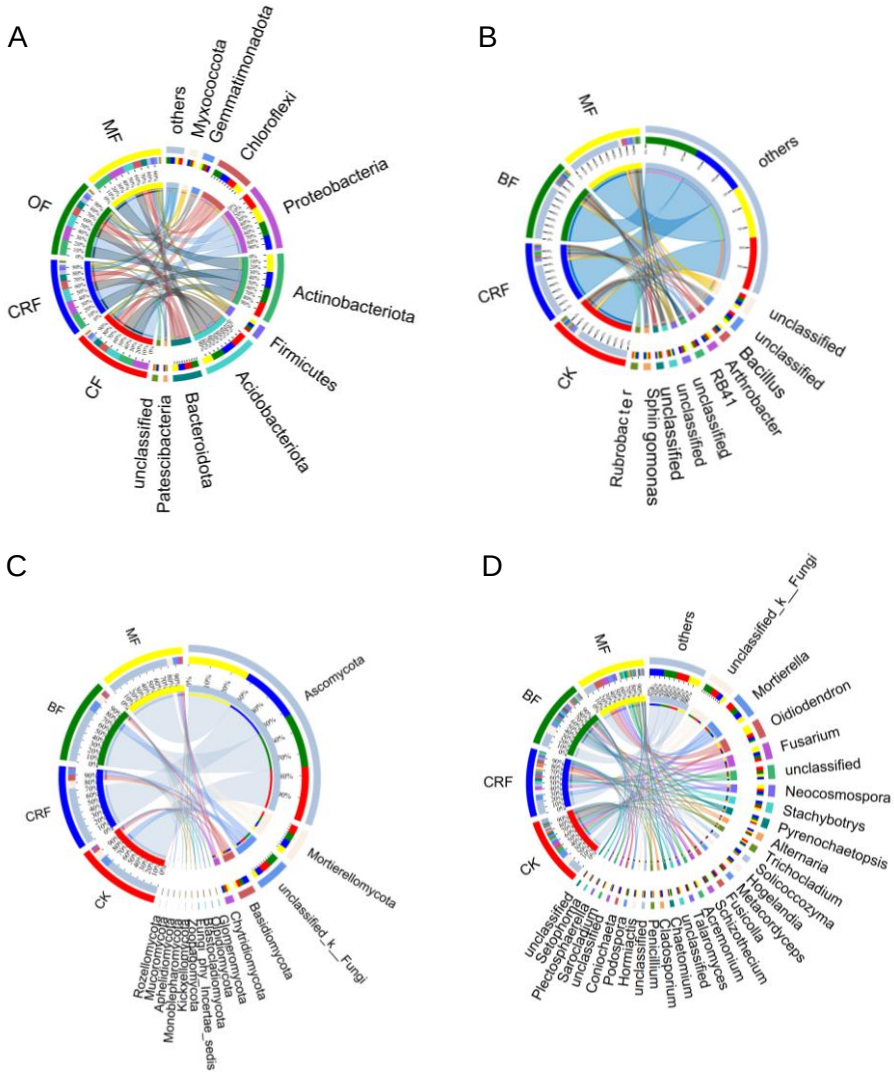


Figure 4. *Circos diagrams showing the relative abundance of microbial taxa across fertilizer treatments. Results are shown at phyla (A: bacteria, C: fungi) and genera (B: bacteria, D: fungi) levels. In each diagram, the left semicircle represents treatment groups—conventional (CF), controlled-release (CRF), organic (OF), and microbial (MF) fertilizers— while the right semicircle represents microbial taxa, with outer ribbons for taxonomic groups and inner ribbons for treatments. Ribbon widths correspond to proportional abundances within each treatment.*

3.4. Differentially Enriched Taxa Under Alternative Fertilizers

Linear Discriminant Analysis Effect Size (LEfSe) was used to identify key microbial biomarkers for each treatment (Figure 5).

For bacteria (LDA > 3), CRF significantly enriched Quadrisphaera, Azotobacter, Sporosarcina, and Chryseobacterium. OF enriched Saccharothrix. MF promoted Nitrospira, Fictibacillus, and Algoriphagus, associated with nitrification and organic matter degradation.

For fungi (LDA > 2), CRF enriched Solicoccozyma, Epicoccum, and Niesslia, while OF favored Schizothecium and Cyphellophora. MF enriched Arachnomyces, a genus linked to organic matter turnover.

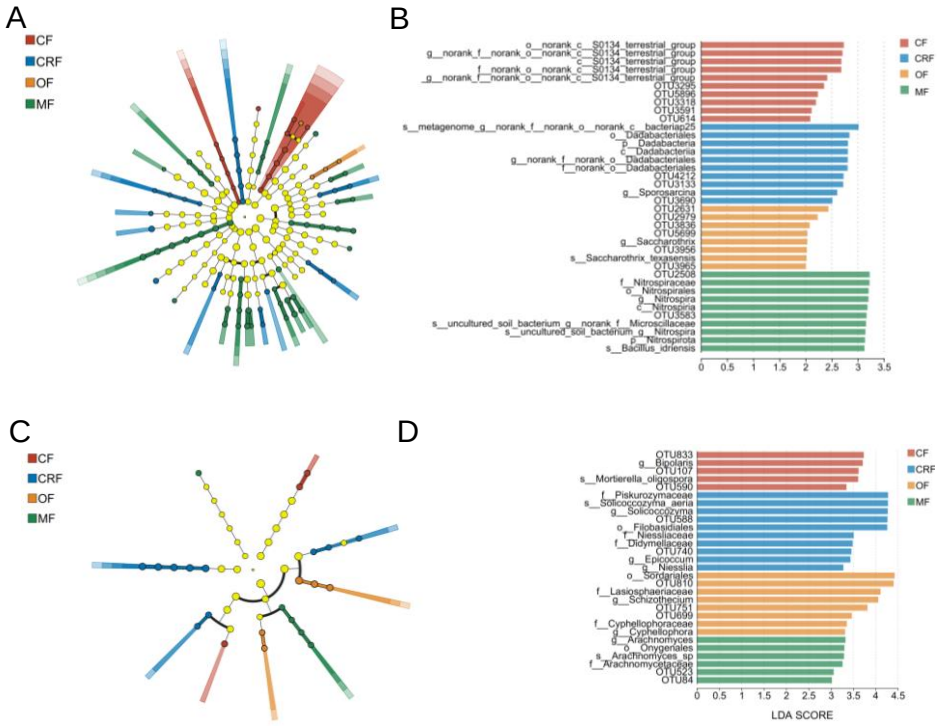
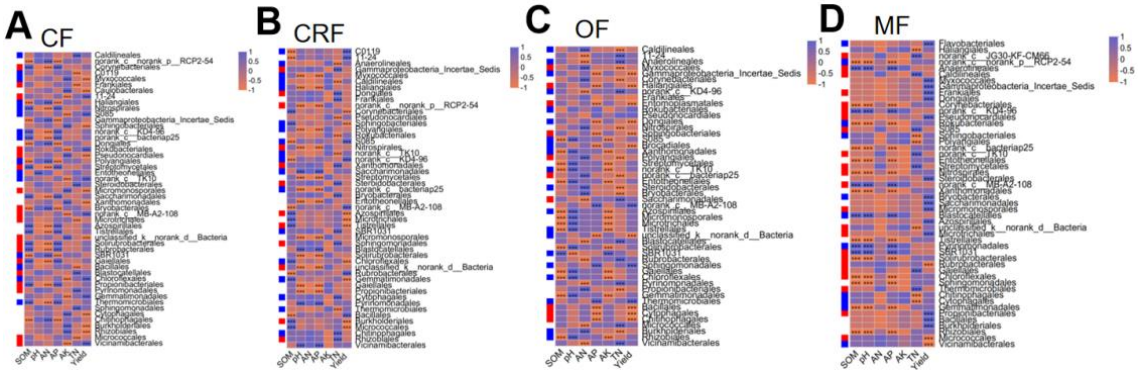


Figure 5. *Composition of the bacterial and fungal community in the soil at the genus level. A; bacterial, C; fungal cladograms: Nodes represent taxa significantly enriched in treatments (colored) or non-significant taxa (light yellow). LEfSe identifies taxa driving differences. B; bacterial (LDA > 3), D; fungal (LDA > 2) bar charts. Treatments: conventional (CF), controlled-release (CRF), organic (OF), and microbial (MF) fertilizers.*

3.5. The Correlation Among Dominant Microbial and Soil Properties and Yield Under Different Alternative Fertilizers

Pearson correlation analyses highlighted treatment-specific associations between microbial taxa, soil chemical properties, and wheat yield (Figure 5A-H).

Azotobacter, enriched in CRF, was negatively correlated with yield ($p < 0.001$), and showed a positive trend with SOM. In contrast, Bacillus, enriched in MF, exhibited positive correlations with both yield and nutrient availability.



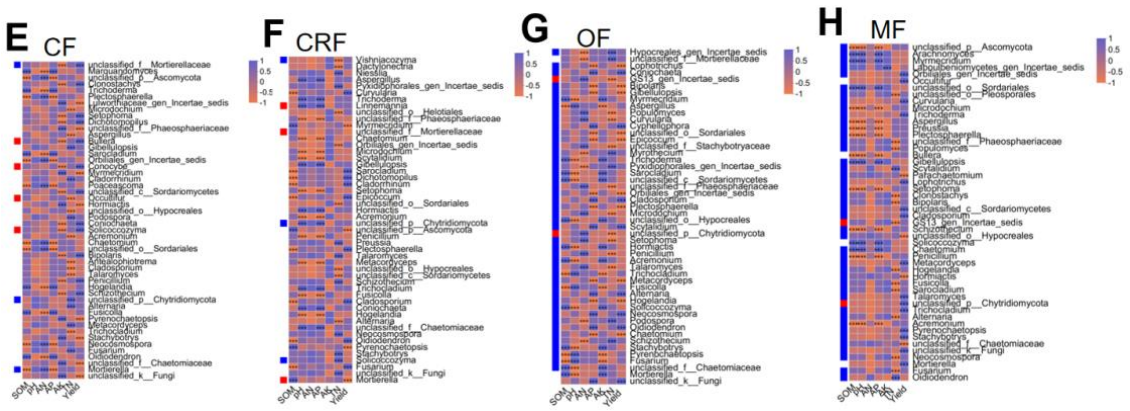


Figure 6. *Heatmap of correlations between microbial genera and soil properties or wheat yield under different fertilizer treatments. (A-D) bacterial genera correlations. (E-H) fungal genera correlations. Rows (Y-axis) represent microbial genera; columns (X-axis) represent soil properties and yield. Correlation coefficients (r-values) are color-coded, with significance levels indicated (*p < 0.05, **p < 0.01, ***p < 0.001).*

Several fungal taxa also demonstrated strong correlations with soil chemical properties and crop yield. Niesslia and Solicoccozyma (CRF) were positively correlated with TN ($p < 0.001$). Epicoccum (CRF) and Cyphellophora (OF) were positively correlated with wheat yield ($p < 0.001$). Cyphellophora also showed a strong positive correlation with AP ($p < 0.001$). Arachnomyces (MF) was positively correlated with SOM, pH, and AP ($p < 0.001$).

4. Discussion

4.1. Effects of Alternative Fertilizers on Soil Chemical Properties and Wheat Yield

4.1.1. Soil Organic Matter (SOM) Dynamics

Our findings demonstrate that while all fertilizer treatments tended to improve wheat yield, their effects on soil health indicators varied significantly, with OF exhibiting the most pronounced and beneficial overall impact. These changes in soil properties and yield were accompanied by significant shifts in the composition of the rhizosphere microbial communities, although alpha diversity was not significantly affected. The observed alterations in soil chemistry highlight the distinct nutrient release dynamics and interactions of each fertilizer type with the soil matrix. The significant increase in SOM under OF aligns with established literature demonstrating that organic amendments enhance SOM accumulation by providing carbon-rich residues [26]. This increase in SOM likely improved soil structure, water-holding capacity, and nutrient retention [27], contributing to the observed increases in available nitrogen, available phosphorus, and available potassium. In contrast, CRF led to a significant decrease in SOM. This may be attributed to a "priming effect," where the readily available nutrients in CRF stimulate microbial activity, leading to accelerated decomposition of existing SOM [28]. Additionally, unlike OF, the slow-release mechanism of CRF limit the input of readily decomposable organic carbon to the soil, potentially hindering the build-up of SOM over time [29]. While some studies have shown that increased plant yield from synthetic fertilizer application can indirectly contribute to SOM [30], our results suggest that this effect was not sufficient to offset the accelerated decomposition in the CRF treatment. Therefore, while CRF is designed to improve nutrient use efficiency, this decrease in SOM could have negative long-term consequences for soil health, suggesting that combining CRF with organic amendments might be a more sustainable approach. The MF treatment did not significantly alter SOM.

4.1.2. pH Modifications and Nutrient Availability

Soil pH showed relatively minor, albeit statistically significant, increases in both the CRF and OF treatments relative to CF. The mechanisms underlying these pH shifts likely differ. The increase with OF can be attributed to the release of alkaline compounds during organic matter decomposition and the potential presence of carbonates or bicarbonates in the organic material itself [31]. However, the pH increase with CRF may be related to the specific formulation of the fertilizer, potentially involving the coating material or the form of nitrogen used [32]. While the precise magnitude of the pH changes was minor, it is crucial to consider the initial soil pH and the optimal pH range for wheat when interpreting the ecological and agronomic significance of these shifts [33]. MF did not show significant changes. This pattern is likely due to the buffering capacity of components in the fertilizer formulations [34].

In terms of nutrient availability, CRF and MF showed less consistent effects on nutrient availability relative to OF. CRF exhibited non-significant declines in AN and a significant reduction in AP (26.17%, $p = 0.017$), raising concerns about potential nutrient imbalances or limitations. MF also showed a non-significant decline in AN, while AP exhibited a non-significant reduction (12.01%, $p=0.204$), suggesting a

limited impact of this treatment on nutrient availability within the timeframe of this study. The lack of a substantial positive response to MF, despite its potential for enhancing nutrient cycling [35], warrants further investigation, possibly focusing on the specific microbial strains used, application methods, and soil conditions. Available potassium (AK) increased only under OF, likely reflecting the potassium content of the fertilizer. Total nitrogen (TN) showed modest, non-significant increases across all fertilizer treatments, indicating that the overall nitrogen pool in the soil was not drastically altered. This highlights the critical distinction between total nutrient content and plant-available nutrient forms, with OF demonstrably enhancing the latter, suggesting that this fertilizer primarily affects the bioavailable nitrogen pools rather than the total nitrogen reserves [28].

4.1.3. Agronomic Performance and Yield Implications

The ultimate indicator of treatment effectiveness, wheat yield, showed improvements across all fertilizer treatments. However, the magnitude of the yield increase varied significantly. The superior yield performance of OF (+17.58%) likely results from the combined benefits of enhanced root growth and nutrient uptake [36]. The moderate yield increase under MF (+9.39%) may be attributed to the activity of plant growth-promoting rhizobacteria (PGPR) within the inoculant, such as Bacillus and Arthrobacter, which can enhance nutrient solubilization and phytohormone production [25]. CRF, despite its negative impacts on SOM and AP, resulted in a subtle, non-significant yield increase (3.50%, $p = 0.071$). This suggests that while CRF may provide readily available nutrients that support some initial growth, its long-term benefits are questionable, particularly in the context of soil health degradation. This may be attributed to that the nutrient release rate may not have been optimally synchronized with the crop's nutrient demands during critical growth stages.

4.2. Microbial Community Responses to Fertilizer Treatments

Our study investigated the impact of the different fertilizer treatments on soil microbial communities, focusing on both bacteria and fungi. The results reveal a contrasting response between these two major microbial groups, with fungal communities showing greater sensitivity to fertilizer type than bacterial communities. Fertilizer treatments substantially influenced microbial richness and diversity (Figure A1). Rarefaction analysis confirmed sufficient sequencing depth to representatively capture microbial diversity (Figure A2).

Analysis of alpha diversity, using the Chao1 (richness) and Shannon (diversity) indices, revealed no statistically significant differences among treatments for either bacterial or fungal communities (Figure 2). This suggests that, overall, the number of distinct microbial taxa (richness) and their relative abundance (diversity) were not drastically altered by the fertilizer applications. This lack of significant change in bacterial alpha diversity aligns with some previous research [26, 37], which suggests that bacterial communities can exhibit resilience to certain environmental perturbations, possibly due to functional redundancy within the community [38].

However, a subtle but potentially important trend was observed in the fungal communities. While not statistically significant, both OF and CRF treatments showed a slight increase in fungal richness and diversity compared to other treatments (Figure 2). This observation hints at a potential positive effect of these fertilizer types on fungal community complexity. This could be due to the slower, more sustained nutrient release provided by OF and CRF, which may favor a wider range of fungal species, including those with slower growth rates or specialized nutrient acquisition strategies [26]. The lack of a strong increase with MF is interesting. While microbial fertilizers aim to enhance microbial diversity [39], our results suggest that, at least in terms of overall richness and diversity, the effect was not as pronounced as with OF and CRF.

While alpha diversity indices (Chao1 and Shannon) did not differ significantly among treatments, Principal Coordinate Analysis (PCoA) based on Bray-Curtis distances revealed distinct patterns in microbial community composition (beta diversity) (Figure 3). This highlights that even if the overall richness and diversity remain similar, the types of microbes present can change significantly [40].

For bacterial communities, PCoA plots showed considerable overlap among all treatments at both the phylum and genus levels, indicating that the overall structure remained relatively similar. However, the MF treatment showed slightly less overlap, this could be due to direct interactions (competition, cooperation) between the introduced and resident bacteria [41], or indirect effects mediated by changes in the soil environment caused by the MF inoculum [42].

In contrast, fungal communities exhibited clear separation among treatments at both the phylum and genus levels, demonstrating that fungal community composition is much more sensitive to fertilizer type. This greater sensitivity of fungi to fertilizer treatments is consistent with other studies [43].

A closer examination of the taxonomic composition of bacterial and fungal communities showed specific shifts in response to different fertilizer treatments. At the phylum level, bacterial communities across all treatments were dominated by Myxococcota, Gemmatimonadota, Chloroflexi, Proteobacteria, and Actinobacteriota (Figure 4A). The OF treatment led to a slight increase in the relative abundance of Myxococcota (27% vs. 23% in CF) and Bacteroidota (27% vs. 26% in CF). Myxococcota are known for their predatory capabilities and production of secondary metabolites [44], suggesting that OF may have stimulated this group, potentially through increased substrate availability from organic matter decomposition. The increase in Bacteroidota, often associated with the breakdown of complex organic compounds [45], further supports the idea

that OF promoted the decomposition of organic matter. The MF treatment resulted in an enrichment of Gemmatimonadota (28% vs. 25% in CF). Gemmatimonadota are often found in drier, nutrient-poor environments [46], and their increase under MF is intriguing. It's possible that the introduced microbes in MF altered the microenvironment, creating niches favorable for Gemmatimonadota, or that they have synergistic interactions. More pronounced shifts were observed at the genus level (Figure 4B). Notably, the MF treatment significantly increased the relative abundance of Bacillus (from 16% in CF to 32%) and Arthrobacter (from 25% to 33%). This is a key finding, as Bacillus species are commonly used in microbial fertilizers due to their plant growth-promoting abilities, including phosphate solubilization, nitrogen fixation, and biocontrol of pathogens [47]. The increase in Bacillus strongly suggests that the MF treatment successfully introduced and established this genus in the soil. Arthrobacter is also known for its metabolic versatility and ability to degrade various organic compounds, including some pollutants [48]. Its increase could be a direct result of introduction via the MF or an indirect effect of altered conditions favoring its growth.

Fungal communities across all treatments were consistently dominated by the phylum Ascomycota (24-27%) (Figure 4C), which is typical for many soil environments [49]. The MF treatment caused a substantial increase in the relative abundance of Oidiodendron (52% vs. 23% in CF). Oidiodendron species are known to be ericoid mycorrhizal fungi, forming symbiotic relationships with plants in the Ericaceae family [50]. Conversely, Mortierella, a common soil saprotrophic fungus [51], decreased in relative abundance under CRF, OF, and MF treatments compared to the control (CF). This suggests that Mortierella may be outcompeted by other fungi under these altered nutrient conditions, or that it is sensitive to some component of the fertilizer treatments.

In conclusion, our findings demonstrate a differential response of soil bacterial and fungal communities to different fertilizer treatments. Different fertilizer treatments can have specific, targeted effects on the composition of soil microbial communities, even when overall diversity metrics remain relatively unchanged. The success of the MF treatment in increasing the abundance of certain genera suggests the potential for using microbial fertilizers to manipulate soil microbial communities for specific purposes. The OF treatment appears to promote subtle but potentially beneficial shifts in both bacterial and fungal composition, which may enhance key soil processes such as organic matter decomposition, nutrient cycling, and disease suppression.

4.3. Functional Implications of Microbial Shifts

Following our observations of limited treatment effects on bacterial diversity but significant shifts in fungal community composition, and the examination of the relative abundance of key taxa, we employed Linear Discriminant Analysis Effect Size (LEfSe) to identify specific microbial biomarkers associated with each treatment (Figure 5). This analysis provides further insight into the nuanced effects of fertilizer management on soil microbial community structure, as microbial composition suggest functional consequences for nutrient cycling and soil health.

4.3.1. Bacterial Biomarkers

While beta diversity analysis revealed considerable overlap in bacterial community structure, suggesting overall resilience, examination of relative abundances and LEfSe analysis revealed more subtle, yet potentially important, treatment-specific shifts. LEfSe analysis further identified Saccharothrix (LDA > 3) as a significant bacterial biomarker for the OF treatment. Saccharothrix is an actinomycete known for producing bioactive compounds, including antibiotics [52], which could further contribute to disease suppression and influence microbial community dynamics. The co-enrichment of Bacillus and Mortierella under OF further supports improved phosphorus solubilization and plant growth promotion through organic acid secretion and hormone production. The CRF treatment, while not showing large shifts in overall bacterial community structure, significantly enriched Quadrisphaera, Azotobacter, Sporosarcina, and Chryseobacterium (LDA > 3) according to LEfSe. Azotobacter is a well-known free-living nitrogen-fixing bacterium [53], suggesting a potential contribution of CRF to nitrogen availability. Sporosarcina is known for its ability to precipitate calcite and is involved in biomineralization processes [54]. The roles of Quadrisphaera and Chryseobacterium in soil environments are less well-defined, warranting further investigation. LEfSe analysis also identified Nitrospira, Fictibacillus, and Algoriphagus (LDA > 3) as biomarkers for the MF treatment. Nitrospira is a key player in nitrification, converting nitrite to nitrate [55], suggesting a potential enhancement of this crucial step in the nitrogen cycle. Fictibacillus and Algoriphagus have been associated with organic matter degradation [56, 57], further suggesting a role for the MF treatment in promoting decomposition processes.

4.3.2. Fungal Biomarkers

LEfSe analysis identified Arachnomyces (LDA > 2) as a biomarker for the MF treatment. Arachnomyces is a genus of keratinophilic fungi, often associated with the breakdown of complex organic matter [58]. Conversely, Mortierella declined under CRF, OF, and MF compared to CF. Mortierella species are common soil fungi, often involved in lipid metabolism and organic matter decomposition [59]. This suggests that Mortierella may be less competitive under these altered nutrient conditions, potentially due to increased competition from other fungi, or that it is sensitive to some component of the fertilizer treatments, or to the altered microbial community structure. The CRF treatment enriched Solicoccozyma, Epicoccum, and Niesslia (LDA > 2). Solicoccozyma has been found in various environments, including soil, and some species have shown potential for

biocontrol [58]. Epicoccum is a widespread fungal genus, often associated with plant material and known for producing bioactive compounds [60]. The ecological roles of Niesslia are less well-characterized. The OF treatment favored Schizothecium and Cyphellophora (LDA > 2). Schizothecium is a coprophilous fungus, typically found on dung [61], suggesting a potential role in the decomposition of organic matter derived from animal sources if present in the OF. Cyphellophora is a dematiaceous (darkly pigmented) fungus, often found in soil and decaying plant material [62].

Our findings, combining alpha and beta diversity analyses, relative abundance data, and LEfSe biomarker identification, demonstrate that different fertilizer treatments exert distinct influences on soil microbial community structure, particularly within the fungal community. While bacterial communities showed greater overall resilience, treatment-specific shifts in both bacterial and fungal taxa were observed, suggesting the potential for using fertilizer management to shape microbial communities for specific purposes, such as enhancing nutrient cycling, promoting plant health, or suppressing soilborne diseases.

The MF treatment, in particular, induced significant shifts in both bacterial and fungal composition, highlighting the potential, and the complexity, of using microbial inoculants to modify soil microbial communities. The enrichment of specific bacterial genera like Bacillus, Nitrospira, and Arthrobacter, and fungal genera like Oidiodendron and Arachnomyces, suggests a potential enhancement of functions related to plant growth promotion, nitrogen cycling, and organic matter decomposition. However, the mechanisms underlying these shifts, and their long-term consequences, require further investigation.

4.4. Linking Microbial Taxa to Soil Function and Crop Yield

Correlation analyses provided further insights into potential microbial contributions to soil health and yield (Figure A3, A4). The positive correlation of Cyphellophora (enriched in OF) with yield ($p < 0.001$) and AP ($p < 0.001$) suggests a potential association with improved plant performance, possibly through organic matter decomposition and nutrient release, as has been observed in other soil fungi [63]. Similarly, Epicoccum (CRF-enriched) showed a strong positive association with yield ($p < 0.001$), possibly due to its known biocontrol and stress-mitigating properties (e.g., disease resistance, abiotic stress). Conversely, the negative correlation between Azotobacter (enriched in CRF) and yield ($p < 0.001$) is intriguing. Azotobacter is typically considered a beneficial, free-living nitrogen fixing bacterium. This unexpected result suggests a potential competition with wheat for ammonium or nitrate under the specific conditions of the CRF treatment, or that high levels of readily available nitrogen from CRF reduce the need for nitrogen fixation, leading to a less beneficial interaction. It is also possible that, under these specific conditions, Azotobacter produces compounds that are not beneficial, or even inhibitory, to plant growth. Alternatively, this negative correlation may reflect indirect effects such as changes in microbial community dynamics or nutrient partitioning that warrant further investigation. The positive correlations of Niesslia and Solicoccozyma (both enriched in CRF) with TN may reflect their involvement in some aspects of nitrogen cycling, although the specific mechanisms require further investigation. These findings underscore the complexity of microbial interactions in fertilized soils and emphasize that microbial enrichment should be evaluated not just for presence, but for function and compatibility with plant needs.

Future research should aim to validate these correlations through functional assays and long-term field trials across diverse soil types and climatic conditions. In addition, integrating metagenomic, metatranscriptomic, or metaproteomic approaches would help link community composition with microbial activity and ecological function. It would also be beneficial to examine the interactions between the introduced microbial inoculants and the indigenous microbial community in more detail.

5. Conclusions

Our findings underscore the critical role of fertilizer type in shaping rhizosphere microbial communities, influencing soil nutrient dynamics, and ultimately affecting crop productivity. Among the treatments, organic fertilizer exhibited the most consistent positive effects, including increases in soil organic matter (SOM), microbial diversity, nutrient availability, and wheat yield. Microbial fertilizer led to moderate improvements, potentially through the activity of introduced plant growth-promoting microbes, though further validation of these mechanisms is needed. Controlled-release fertilizer, while beneficial for improving nitrogen use efficiency, may risk long-term soil degradation and microbial imbalance if not supplemented with organic inputs.

The strong correlations observed between specific microbial taxa and agronomic indicators suggest that microbiome-informed fertilization strategies could be developed to enhance both soil health and crop performance. However, establishing causal relationships between microbial presence and ecosystem function remains a key challenge. Future research should incorporate functional assays, long-term field trials, and multi-omics approaches to better understand microbial contributions and the sustainability of different fertilization regimes.

Abbreviations

CRF	Controlled-Release Fertilizer
OF	Organic Fertilizer
MF	Microbial Fertilizer

SOM	Soil Organic Matter
TN	Total Nitrogen
AN	Available Nitrogen
AP	Available Phosphorus
AK	Available Potassium
RCBD	Randomized Complete Block Design
Kg	Kilogram
Ha	Hectare
CFU	Colony Forming Unit
Cm	Centimeter
w/v	Weight to Volume
PCR	Polymerase Chain Reaction
OTU	Operational Taxonomic Unit
μL	Microliter
PCoA	Principal Coordinates Analysis
LEfSe	LDA Effect Size
LDA	Linear Discriminant Analysis

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Author Contributions

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Data Availability Statement

The data is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Appendix

Table A1. *The table shows statistical methods and degrees of freedom.*

ANOVA						
		Sum of squares	**freedom**	**mean square**	**F**	**significance**
SOM	Inter group	176.133	3.000	58.711	46.993	0.000
	within-subjects	9.995	8.000	1.249		
	total	186.127	11.000			
pH	Inter group	0.175	3.000	0.058	8.404	0.007
	within-subjects	0.056	8.000	0.007		
	total	0.231	11.000			
AN	Inter group	2699.083	3.000	899.694	11.910	0.003
	within-subjects	604.333	8.000	75.542		
	total	3303.417	11.000			
AP	Inter group	8933.801	3.000	2977.934	10.414	0.004
	within-subjects	2287.724	8.000	285.966		
	total	11221.526	11.000			
AK	Inter group	19267.254	3.000	6422.418	26.733	0.000

ANOVA

		Sum of squares	freedom	mean square	F	significance
	within-subjects	1921.925	8.000	240.241		
	total	21189.179	11.000			
	Inter group	0.026	3.000	0.009	0.682	0.587
TN	within-subjects	0.103	8.000	0.013		
	total	0.129	11.000			
	Inter group	3.015	3.000	1.005	13.376	0.002
Yield	within-subjects	0.601	8.000	0.075		
	total	3.616	11.000			

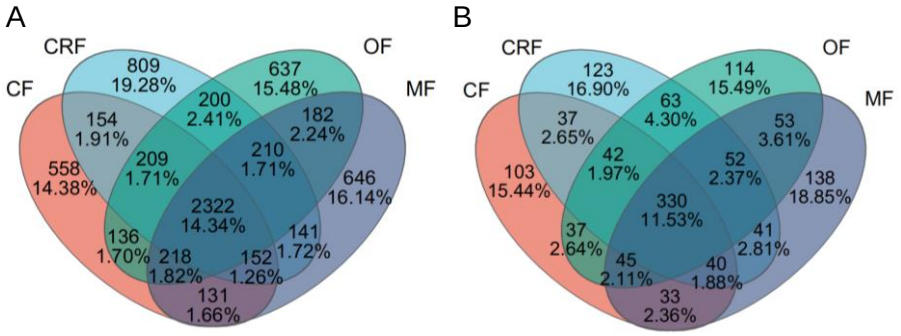

Figure A1. *Venn diagram represents unique bacteria and fungi species under different alternative fertilizers (CF, CRF, OF, MF).*

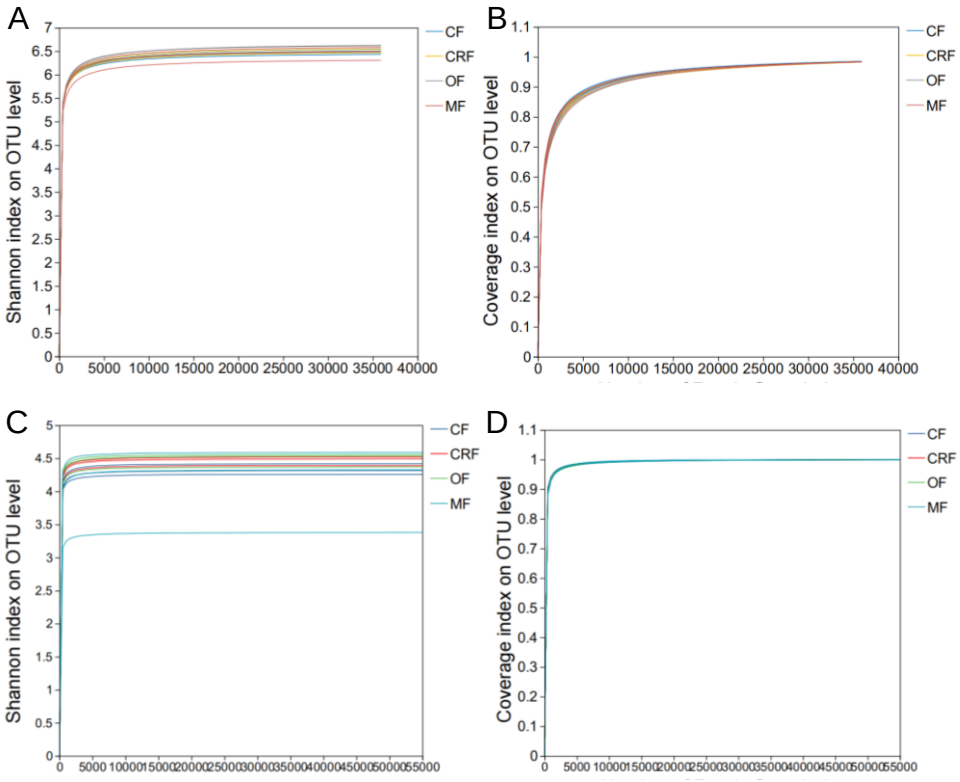

Figure A2. *Dilution curve of community coverage and diversity index of soil bacteria and fungi at OTU level.*

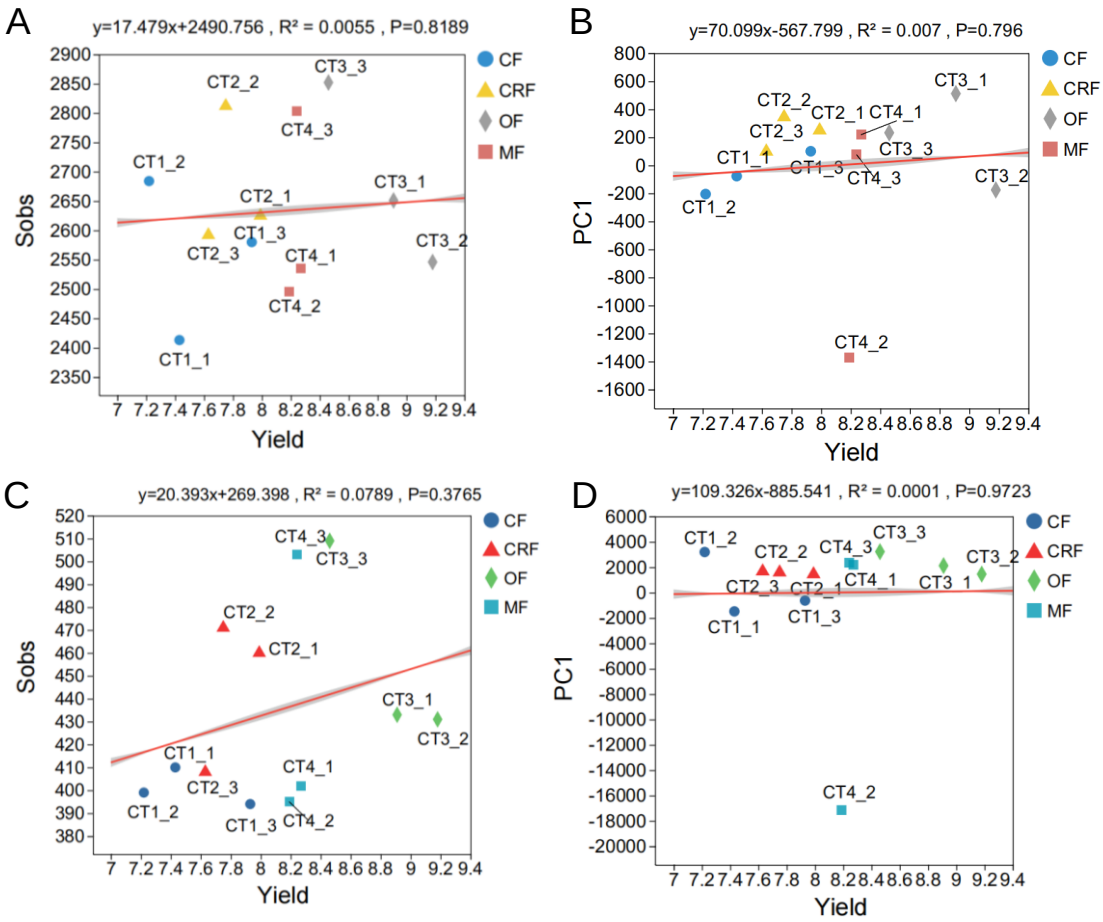

Figure A3. *This figure represents the correlation between yield and microbiota structure, assessing the size of the impact on differences in sample community composition.*

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Biography

Ran Wen is an undergraduate student enrolled in 2022 at the College of Agronomy, Henan Agricultural University, majoring in Agronomy (Shaoqui Innovation Experimental Class of New Agricultural Sciences), has achieved notable accomplishments as a core participant: a national silver award and a bronze award at the China International College Students Innovation Competition, a national bronze award at the Chuang Qing chun competition, and a first prize in the Central China Division of the National Agricultural Science Students Competition. Additionally, as the project leader, she presided over the 2023 Henan Agricultural University College Students Entrepreneurship Practice Program. Currently, mainly explores the fields of corn cultivation physiology and the regulation of crop stress resistance.

Yang Cao is an undergraduate student (Class of 2022) majoring in Seed Science and Engineering at the College of Agronomy, Henan Agricultural University, specializing in Crop Cultivation and Farming Systems. Currently engaged in research practice at the Henan Provincial Key Laboratory of Crop Physiology and Ecology, he is systematically studying the theories and technologies of high-yield and high-efficiency maize cultivation.

Sohaila Mohamed Ramadan Mohamed, B.Sc. Student | Department of Soil and Water Sciences, Faculty of Agriculture, Fayoum University, Egypt. Education: Expected B.Sc. in Land and Water Sciences (2025). Research Project: "Water Quality Assessment in Fayoum Governorate" Technical Skills: Field water sampling and laboratory analysis (spectrophotometry, titration), Water sampling techniques, Using laboratory equipment (pH meters, spectrophotometers), Data collection and report preparation Research Interests: Water resources, Soil management, Environmental sustainability.

Qihang Yu is an undergraduate student majoring in Agronomy at the College of Agronomy, Henan Agricultural University, enrolled in 2021. Currently, he focuses on maize stress cultivation research, assisting in the collection and statistical analysis of field phenotypic data. He is committed to integrating cutting-edge technologies with traditional agronomy to explore efficient and green development models for modern agriculture.

Wang Bo is a 2023-level undergraduate student majoring in Agronomy at the College of Agronomy of Henan Agricultural University. She has taken core courses such as Molecular Biology and Genetics as her main subjects. She has accumulated solid foundation in field data modeling and application of agricultural information technology. Currently, she is conducting research on the physiological mechanism of high-yield corn cultivation and is responsible for data collection and precise fertilization in field experiments.

Hecheng Liu, an undergraduate student majoring in Agronomy at the College of Agriculture of Henan Agricultural University since 2021, was awarded the title of "Innovation Star" of the Third Henan Province University Students in 2024. He is an outstanding representative of the university who has consecutively won this honor for three years. Focusing on agricultural science and technology innovation, he integrates classroom learning with the demands of rural areas through participating in scientific research, technology development, and the promotion of achievements, dedicated to serving the development of agriculture, rural areas, and farmers. Currently, he mainly explores the fields of corn cultivation physiology and the regulation of crop stress resistance.

Research Field

Ran Wen: Microorganism, Ecological environment, Maize cultivation, maize physiological stress and regulation, soil properties

Yang Cao: Microorganism, Ecological environment, Maize cultivation, maize physiological stress and regulation, soil properties

Sohaila Mohamed Ramadan Mohamed: Water resources-, Soil management,, Environmental sustainability

Qihang Yu: High temperature stress, drought stress, high temperature drought stress, soil properties, Stress Resistance Modulator Regulator Development

Wang Bo: High temperature stress, drought stress, high temperature drought stress, soil properties, Stress Resistance Modulator Regulator Development

Hecheng Liu: High temperature stress, drought stress, high temperature drought stress, soil properties, Stress Resistance Modulator Regulator Development