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Changes in the Physical, Chemical, and Bacterial Community Characteristics of Soil in Response to Short-Term Combined Organic–Inorganic Fertilizers in a Dry Direct-Seeded Paddy Field

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Abstract: Dry direct-seeded rice cultivation is a simple and labor-saving planting method wherein the combined application of organic and inorganic fertilizers can improve yield. However, the effects of combined fertilizers on soil properties and bacteria in dry direct-seeded rice paddy soil are unclear. Here, four treatments, conventional fertilization (NPK), seaweed bio-organic fertilizer + NPK, Jishiwang bio-organic fertilizer + NPK, and attapulgite organic fertilizer + NPK applied for three consecutive years were tested to explore their effects on soil physical, chemical, and bacterial community characteristics in a dry direct-seeded rice paddy field. The combined fertilizers increased alkaline hydrolysis-nitrogen and available potassium while decreasing the bulk density and pH; in addition, a marked increase in the relative abundance of soil macroaggregates (>5 mm) and clay particles and a decrease in that of sand was observed. Urease and neutral phosphatase activities were the highest with the Jishiwang organic fertilizer + NPK, whereas invertase and catalase activities were the highest with attapulgite organic fertilizer + NPK. All combined fertilizers considerably increased the bacterial richness index (ACE) and Chao index; Jishiwang bio-organic fertilizer + NPK also increased the Simpson index, whereas the seaweed bio-organic fertilizer + NPK reduced it. Proteobacteria and Acidobacteria accounted for 54.25–70.49% of the total bacterial relative abundance. The relative abundance of Verrucomicrobia, Chloroflexi, Firmicutes, and Nitrospirae increased with the combined fertilizers. The correlation network analysis showed predominant antagonistic relationships. A redundancy analysis demonstrated that total nitrogen, soil organic matter, urease, and invertase were the main environmental factors affecting bacterial composition. Combined fertilizers may improve soil physical and chemical properties, fertility, and bacterial richness.

Keywords: bacterial community; combined fertilizer; dry direct-seeded rice; microbial diversity; soil properties

1. Introduction

As a major food crop worldwide, rice can supply food to more than 50% of the global population [1]. In recent years, the shortage of water caused by climate change and environmental pollution has led to increased cultivation costs [2] and soil degradation, limiting the development of traditional rice planting. Direct-seeded rice has the advantages of simpler production schemes, high efficiency, labor savings [3], farmland water savings,

methane emission reduction [4], and economic benefits [5]. This system is preferred by rice farmers in many countries such as Cambodia, India, the United States, and China [6–9]. Dry direct-seeded rice cultivation is facing a series of challenges, such as variety selection, ensuring seedling emergence rate, weed control, fertilizer and water management, lodging prevention, and high and stable yield [10–13]. Compared with conventional transplanting cultivation, fewer scientific researchers are engaged in dry direct-seeded rice cultivation, especially in cold regions. Direct seeding rice cultivation is being used to a very small degree in production and is still in its infancy. Furthermore, dry direct-seeded rice cultivation technology has great potential for rice production.

Despite the potential benefits of direct-seeded rice cultivation, stable and high yields are difficult to achieve [14]. The application of large quantities of inorganic fertilizers leads to soil hardening, reduced soil quality, and an inability to keep soil fertility, resulting in environmental pollution, and stable high yields are often unattainable [15]. Direct-seeded rice production can hardly be improved using conventional fertilization. To face this challenge, a change in the pattern of fertilizer investment, focusing on a more reasonable, balanced fertilization scheme, with increased use of organic fertilizers, may be necessary. Rice cultivation, the domestic agricultural core of China, is decentralized; furthermore, industrialization is limited, soil testing of fertilizers has elevated costs, and central coordination is difficult. In this context, it is feasible to adopt strategies involving the greater use of organic fertilizers [16].

Organic fertilizers provide various nutrients and contain elements needed for crop growth, such as carbon and nitrogen, as well as many probiotics and required microelements. These fertilizers can trigger the release of fixed elements in the soil matrix, balance soil nutrients, and improve soil water dynamics while increasing soil fertility, thereby improving soil structure [17]. Wang et al. [17] reported that organic fertilizers can significantly increase total nitrogen, phosphorus, and potassium contents in the 0–60 cm soil layer. The total carbon content and pH of the soil were markedly increased by organic fertilizers [18]. Burger et al. [19] and Gatinger et al. [20] also showed that organic fertilizers could improve soil fertility by increasing the organic matter content. Furthermore, organic fertilizers or organic–inorganic combinations can reduce nutrient loss and mitigate N_2O emissions [21–23].

The microbial community is an important driving factor in the output and stability of global food and fiber production and constitutes a key point in the context of potential solutions for long-term fertilization needs. Organic fertilizers can provide a stable substrate for soil microbial metabolism, thus improving microbial biomass. Even over a short period, organic fertilizers can improve soil microbial activity [24]. Lori et al. [25] reported that organic fertilizer application remarkably increased soil bacteria abundance and enzyme activity.

Furthermore, one study reported that organic fertilizers increased the total number of soil bacteria, actinomycetes, and *Pseudomonas* [26]. The combined application of organic and inorganic fertilizers could result in immediate and slow effects, with beneficial effects on soil community growth and composition [27] as well as on soil nutrient cycling [28] and crop production [29].

In recent years, the combined application of organic and inorganic fertilizers has become one of the most active and fast-growing fields in fertilizer application research [30]. The current understanding of its effect on rice is mostly based on paddy fields under anaerobic conditions; research on dry direct-seeded rice under aerobic conditions is limited, and the effect of the combined application on soil properties and microbial diversity in these production settings remains nebulous. To the best of our knowledge, no other study has yet explored the effects of organic–inorganic fertilizers on dry direct-seeded rice paddy soil. A hypothesis has been proposed that the combination of different organic fertilizers and chemical fertilizers for three consecutive years would have an impact on the physical and chemical properties, water stable aggregates, and soil enzyme activities of paddy soil. These changes would induce changes in the composition and diversity of soil microbial

community structure, which could ultimately improve the physical and chemical properties of dry direct-seeded paddy fields, improve soil fertility, and increase soil bacterial richness.

2. Materials and Methods

2.1. Experimental Design

The field experiment was conducted based on a randomized complete block design located at Friendship Farm, Heilongjiang Province, China (46.45° N, 131.49° E) from May to September, 2018 to 2020. The soil, therein, is of black meadow type; the climate at the experimental site corresponds to the continental monsoon of the mid-temperate zone, with an average annual temperature of 3.1 °C and an annual precipitation of 501.2 mm. The meteorological conditions during the rice growth period from 2018 to 2020 and the physicochemical properties of the soil at a depth of 0–20 cm of this experimental site were consistent with a previous study [31]. Each treatment was repeated thrice; the treatments were NPK (conventional fertilization, control), OF1 + NPK (seaweed bio-organic fertilizer combined with NPK), OF2 + NPK (Jishiwang bio-organic fertilizer combined with NPK), and OF3 + NPK (attapulgite organic fertilizer combined with NPK). The plot area was 300 m^2 (20.8 × 14.4 m).

For the NPK treatment, the total nitrogen (urea, 46% N) quantity applied was 204.05 kg·N·ha^{-1}: distributed as basal fertilizer, 88.82 kg·N·ha^{-1}; tiller fertilizer, 69 kg·N·ha^{-1}; and panicle fertilizer, 46.23 kg·N·ha^{-1}. The total phosphorus (calcium triple superphosphate, 46% P_2O_5) quantity was 80.04 kg·P_2O_5·ha^{-1} and was applied once as basal fertilizer. The total potassium (potassium sulfate, 50% K_2O) applied was 115 kg·K_2O·ha^{-1}, divided into two applications (63.75 kg·K_2O·ha^{-1} as basal fertilizer and 51.25 kg·K_2O·ha^{-1} as panicle fertilizer).

For the OF1 + NPK treatment, based on conventional fertilization, 300 kg·ha^{-1} of the seaweed bio-organic fertilizer (commercial organic fertilizer, 1.10% N, 2.43% P_2O_5, 1.14% K_2O, 45.6% organic matter, and 2 × 10^7 g^{-1} of functional microorganisms) was applied as basal fertilizer in each year. The seaweed bio-organic fertilizer was purchased from Qingdao Pulebaiwo Biotechnology Co., Ltd., Qingdao, Shandong Province, China.

For the OF2 + NPK treatment, based on the conventional fertilization, 300 kg·ha^{-1} of the Jishiwang bio-organic fertilizer (commercial organic fertilizer, 1.32% N, 2.12% P_2O_5, 1.52% K_2O, 43.8% organic matter, and 5 × 10^7 g^{-1} of functional microorganisms) was applied as basal fertilizer in each year. The Jishiwang bio-organic fertilizer was purchased from Jiamusi Sanxing Agricultural Technology Service Co., Ltd., Jiamusi, Heilongjiang Province, China.

For the OF3 + NPK treatment, based on conventional fertilization, 750 kg·ha^{-1} of the attapulgite organic fertilizer (commercial organic fertilizer, 2.50% N, 2.05% P_2O_5, and 1.56% K_2O) was applied as basal fertilizer in each year. The attapulgite organic fertilizer was purchased from Dingfengyuan Aotu High Tech Development Co., Ltd., Zhangye, Gansu Province, China.

The rice variety tested was Longjing 31; this variety has 11 leaves on the main stem. The growth period was approximately 130 days, and the active accumulated temperature ≥10 °C was approximately 2350 °C. The seeds were coated with the Liangdun seed coating agent before sowing and mechanically sown on 19 April 2020. The sowing rate was 210 kg ha^{-1}, the sowing depth was 2–3 cm, and the row spacing was 20 cm. Simultaneously, the relevant base fertilizer was applied, and the soil was mechanically covered and pressed twice after sowing.

2.2. Soil Sampling

Four soil samples per treatment were collected after the rice harvest in 2020. The samples were separated into the 0–10 and 10–20 cm layers, and each layer sample was mixed. One portion of the soil was collected with a ring knife and dried to determine the bulk density (0–10 and 10–20 cm); another portion of fresh soil was used to determine soil aggregate distribution (0–10 and 10–20 cm), and a third portion of naturally air-dried soil

was crushed and sieved (1 mm) to determine chemical properties (0–10 and 10–20 cm) and enzyme activities (0–20 cm). Additionally, a portion of fresh soil was transported to the laboratory on ice and stored at −80 °C to determine soil microbial diversity.

2.3. Soil physical and Chemical Properties

Soil physical and chemical properties, including pH, organic matter (SOM), total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), and available potassium (AK), were determined according to the methods published by Lu et al. [32]. The soil pH was determined using a composite electrode with a water/soil ratio of 2.5:1; SOM was determined using a potassium dichromate volumetric method; TN was determined by the Kjeldahl method; TP was determined by $HClO_4$–H_2SO_4 digestion and molybdenum antimony anti-colorimetry; and AP was determined via $NaHCO_3$ extraction and molybdenum antimony anti-colorimetry.

2.4. Soil Enzymes

According to the method of Hou et al. [33], soil urease, invertase, neutral phosphatase, and catalase activities were obtained using the soil enzyme kit from Solarbio Science & Technology Co. (Beijing, China).

Soil urease activity was measured as follows: 5 g soil sample was mixed with reagents I, II, and III and then incubated for 24 h at 37 °C. Subsequently, the sample was centrifuged at 10,000× *g* at room temperature for 10 min, and the supernatant was obtained. The diluted supernatant was added to reagents IV and V, fully mixed, and placed at room temperature for 20 min. The supernatant was measured with a spectrophotometer at a wavelength of 630 nm. The activity of urease was expressed as 1 microgram of NH_3-N per gram of soil per day.

The soil invertase activity was measured as follows: 0.1g air dried soil was mixed with reagent I and then incubated for 15 min at 37 °C. Subsequently, the samples were added to reagent II and reagent III and incubated for 24 h at 37 °C, after which the samples were centrifuged at 10,000× *g* at 4 °C for 5 min, and the supernatant was obtained and ultimately diluted. Then, the supernatant were mixed with reagent IV and measured with a spectrophotometer at a wavelength of 450 nm. Invertase activity was expressed as 1 milligram reducing sugar per gram of soil per day, at 37 °C.

The soil neutral phosphatase activity was measured as follows: 0.1 g air dried soil was mixed with toluene and shaken gently for 15 min; then, the sample was added to reagent I, vigorously shaken, and placed in a 37 °C constant temperature incubator for 24 h. After 24 h, the sample was taken out, and reagent II was quickly added and well-mixed to stop the enzyme-catalyzed reaction. Subsequently, the sample was centrifuged at 10,000× *g* at 25 °C for 10 min, and the supernatant was obtained and placed on ice for testing. Neutral phosphatase activity was expressed as 1 nanomole of phenol per gram of soil per day.

The soil catalase activity was measured as follows: 0.1 g air dried soil was mixed with reagent I, shaken, and cultured at 25 °C for 20 min. Subsequently, reagent II was added, and the sample was centrifuged at 8000× *g* at 25 °C for 5 min. The supernatant was then obtained, and reagent III was added. Light absorption was measured at 240 nm. Catalase activity was expressed as 1 millimole of H_2O_2 degradation per gram of soil per day.

2.5. PCR Amplification and Sequencing

The total bacterial DNA was extracted from the soil samples using the Power Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA), according to the manufacturer's protocol. The DNA quality and quantity were assessed using the 260/280 and 260/230 nm absorbance ratios. Then, the DNA was stored at −80 °C until further processing. The V3–V4 region of the bacterial 16S rRNA gene was amplified with the common primer pair (forward primer, 5′-ACTCCTACGGGAGGCAGCA-3′; reverse primer, 5′-GGACTACHVGGGTWTCTAAT-3′) combined with adapter and barcode sequences. PCR amplification was performed in a total volume of 50 µL, which contained 10 µL of

buffer, 0.2 µL of Q5 High-Fidelity DNA Polymerase, 10 µL of High GC Enhancer, 1 µL of dNTP, 10 µM of each primer, and 60 ng of genome DNA. The thermal cycling conditions were as follows: an initial denaturation at 95 °C for 5 min, followed by 15 cycles at 95 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 7 min. The PCR products from the first PCR step were purified using VAHTS DNA Clean Beads. The second round of PCR was then performed in a 40 µL reaction, which contained 20 µL of 2× Phusion HF MM, 8 µL of double-distilled water, 10 µM of each primer, and 10 µL of the PCR products from the first step. The thermal cycling conditions were as follows: an initial denaturation at 98 °C for 30 s, followed by 30 cycles at 98 °C for 10 s, 65 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min. Finally, all PCR products were quantified using Quanti T dsDNA HS Reagent and pooled together. High-throughput sequencing analysis of bacterial rRNA genes was performed on the purified, pooled samples using the Illumina Hiseq 2500 platform (2 × 250 paired ends) from Biomarker Technologies Corporation, Beijing, China.

2.6. Data Analysis

The bioinformatic analysis in this study was completed using the Biomarker Biocloud Platform (www.biocloud.org, accessed on 20 May 2021). To obtain the raw tags, paired-end reads were merged using FLASH (v1.2.7, http://ccb.jhu.edu/software/FLASH/, accessed on 26 May 2021) [34]. Then, raw tags were filtered and clustered in the next steps. The merged tags were compared with the primers, and the tags with more than six mismatches were discarded by the FASTX-Toolkit, accessed on 27 May 2021. Tags with an average quality score <20 in a 50 bp sliding window were truncated using Trimmomatic (http://www.usadellab.org/cms/?page=trimmomatic, accessed on 30 May 2021) [35], and tags shorter than 350 bp were removed. We identified possible chimeras by employing UCHIME, a tool included in mothur (http://drive5.com/uchime, accessed on 30 May 2021). The denoised sequences were clustered using USEARCH (version 10.0), and tags with a similarity ≥97% were regarded as an operational taxonomic unit. Taxonomy was assigned to all units by searching against the Silva database (http://www.arb-silva.de, accessed on 4 June 2021) using UCLUST within QIIME. Data from the three years were pooled, and group means were assessed using one-way ANOVA followed by the least significant difference (LSD) test, performed using IBM SPSS v20.0 statistical software (SPSS, Chicago, IL, USA). Figures were generated using GraphPad Prism 7 software (San Diego, CA, USA).

3. Results

3.1. Soil Physical and Chemical Properties

Soil physical and chemical properties at different depths are shown in Table 1. Bulk density (BD) and pH showed a decreasing trend with the application of organic–inorganic fertilizers over three consecutive years, but BD reductions were notable only in the 0–10 cm soil layer. All organic–inorganic fertilizers considerably reduced soil pH compared with the NPK treatment, and soil pH was slightly higher in the 10–20 cm layer than in the 0–10 cm layer. In both cases, the trend was NPK > OF1 + NPK ≈ OF3 + NPK > OF2 + NPK. On the other hand, the organic–inorganic fertilizer OF2 + NPK considerably increased the content of alkaline hydrolyzable nitrogen (AHN) and available potassium (AK) in both soil layers. The combined fertilizer OF1 + NPK enhanced only the AK content in the upper layer, and OF3 + NPK also improved AHN in the deeper layer. The total potassium content (TK) increased considerably due to the incorporation of the combined fertilizers OF1 + NPK and OF3 + NPK only in the upper layer, and the total phosphorus (TP) increased markedly due to the incorporation of the combined fertilizer OF3 + NPK only in the 10–20 cm layer. Soil organic matter (SOM), total nitrogen (TN), and available phosphorus (AP) did not vary significantly due to the application of organic–inorganic fertilizers, but an increasing tendency for AP contents was verified, and the pattern was OF3 + NPK > OF2 + NPK >

OF1 + NPK > NPK. As a general trend, OF2 + NPK treatment led to greater improvements in AHN and AK.

Table 1. Effects of organic–inorganic fertilizers on soil physical and chemical properties in a dry direct-seeded rice paddy soil.

Soil Layer Depth	Treatment	BD g·cm^{-3}	pH	SOM g·kg^{-1}	TN g·kg^{-1}	TP g·kg^{-1}	TK g·kg^{-1}	AHN mg·g^{-1}	AP mg·g^{-1}	AK mg g^{-1}
0–10 cm	NPK	1.6 ± 0.05 ^{a,c}	6.6 ± 0.21 ^{a}	21.3 ± 0.64 ^{a}	1.1 ± 0.08 ^{a}	0.9 ± 0.02 ^{a}	19.2 ± 0.3 ^{b}	110.5 ± 8.3 ^{b}	37.7 ± 5.5 ^{a}	235.7 ± 20.6 ^{c}
	OF1 + NPK	1.4 ± 0.01 ^{b}	6.0 ± 0.10 ^{b}	21.8 ± 1.10 ^{a}	1.2 ± 0.03 ^{a}	0.9 ± 0.02 ^{a}	20.0 ± 0.5 ^{a}	116.8 ± 2.6 ^{a,b}	41.2 ± 4.9 ^{a}	269.0 ± 8.2 ^{b}
	OF2 + NPK	1.4 ± 0.06 ^{b}	5.9 ± 0.03 ^{b}	21.4 ± 0.29 ^{a}	1.1 ± 0.04 ^{a}	1.0 ± 0.03 ^{a}	19.6 ± 0.1 ^{a,b}	123.0 ± 1.5 ^{a}	43.7 ± 4.1 ^{a}	304.6 ± 18.8 ^{a}
	OF3 + NPK	1.4 ± 0.05 ^{b}	5.9 ± 0.21 ^{b}	22.4 ± 0.02 ^{a}	1.1 ± 0.02 ^{a}	1.0 ± 0.08 ^{a}	20.1 ± 0.3 ^{a}	118.3 ± 4.7 ^{a,b}	45.9 ± 2.5 ^{a}	295.6 ± 1.6 ^{a,b}
10–20 cm	NPK	1.6 ± 0.04 ^{a}	6.6 ± 0.03 ^{a}	19.4 ± 1.21 ^{a}	1.0 ± 0.06 ^{a}	0.8 ± 0.02 ^{b}	17.5 ± 0.9 ^{a}	100.8 ± 6.3 ^{b}	32.9 ± 3.3 ^{a}	208.1 ± 4.3 ^{b}
	OF1 + NPK	1.5 ± 0.1 ^{a}	6.4 ± 0.09 ^{b}	20.03 ± 1.47 ^{a}	1.1 ± 0.01 ^{a}	0.9 ± 0.01 ^{a,b}	18.0 ± 0.9 ^{a}	111.5 ± 5.7 ^{a,b}	34.4 ± 1.7 ^{a}	214.6 ± 29.6 ^{b}
	OF2 + NPK	1.5 ± 0.02 ^{a}	6.2 ± 0.09 ^{c}	19.9 ± 0.47 ^{a}	1.1 ± .03 ^{a}	0.9 ± 0.03 ^{a,b}	18.4 ± 0.7 ^{a}	115.8 ± 5.6 ^{a}	35.7 ± 1.6 ^{a}	262.2 ± 15.8 ^{a}
	OF3 + NPK	1.5 ± 0.04 ^{a}	6.4 ± 0.03 ^{b}	21.5 ± 2.18 ^{a}	1.1 ± 0.01 ^{a}	0.9 ± 0.06 ^{a}	18.4 ± 0.2 ^{a}	113.5 ± 1.7 ^{a}	37.6 ± 5.2 ^{a}	240.2 ± 5.1 ^{a,b}

NPK, conventional fertilization; OF1 + NPK, seaweed bio-organic fertilizer and NPK; OF2 + NPK, Jishiwang bio-organic fertilizer and NPK; OF3 + NPK, attapulgite organic fertilizer and NPK. BD, bulk density; SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AHN, alkaline hydrolizable-nitrogen; AP, available phosphorus; AK, available potassium. Different letters indicate significant differences ($p < 0.05$).

3.2. Relative Abundance of Water-Stable Aggregates

Organic–inorganic fertilizers applied for three consecutive years markedly altered the distribution of water-stable aggregates (Table 2). Macroaggregates (>5 mm) were dominant with the organic–inorganic fertilizer treatments, whereas microaggregates (<0.25 mm) were dominant with the NPK treatment. Compared with the NPK treatment, the OF2 + NPK and OF3 + NPK treatments extensively increased the fraction of water-stable aggregates >5 mm (by 87.01% and 90.79%, respectively), while the OF1 + NPK treatment markedly increased the fraction of 0.5–1 mm (by 78.14%). Furthermore, the OF3 + NPK treatment significantly increased the proportion of the 2–5 mm fraction (43.34%).

Table 2. Effects of organic–inorganic fertilizers on water-stable aggregate size fractions in a dry direct-seeded rice paddy soil.

Treatment	Proportion of Different Soil Water-Stable Aggregate Size Fraction (%)					
	>5 mm	2–5 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	<0.25 mm
NPK	27.80 ± 5.55 ^{b}	7.73 ± 0.06 ^{b}	7.34 ± 2.64 ^{a}	9.24 ± 0.75 ^{b}	7.26 ± 0.28 ^{a}	40.63 ± 6.18 ^{a}
OF1 + NPK	28.51 ± 2.97 ^{b}	7.91 ± 0.10 ^{b}	11.09 ± 2.37 ^{a}	16.46 ± 1.09 ^{a}	10.38 ± 2.18 ^{a}	25.65 ± 2.03 ^{b}
OF2 + NPK	54.85 ± 10.94 ^{a}	9.10 ± 0.45 ^{a,b}	11.76 ± 3.64 ^{a}	11.40 ± 4.40 ^{a,b}	10.020.76 ^{a}	2.86 ± 0.39 ^{c}
OF3 + NPK	53.04 ± 1.33 ^{a}	11.08 ± 2.50 ^{a}	10.91 ± 2.55 ^{a}	11.01 ± 3.93 ^{a,b}	9.87 ± 0.76 ^{a}	4.09 ± 1.60 ^{c}

NPK, conventional fertilization; OF1 + NPK, seaweed bio-organic fertilizer and NPK; OF2 + NPK, Jishiwang bio-organic fertilizer and NPK; OF3 + NPK, attapulgite organic fertilizer and NPK. Different letters indicate significant differences ($p < 0.05$).

3.3. Particle Size Distribution

As shown in Table 3, the paddy soil analyzed contained silt (2–50 μm) as the predominant particle size, followed by sand (50–200 μm) and clay (0–2 μm). Compared with the NPK treatment, all organic–inorganic fertilizers markedly increased the silt content

in both soil layers (in the range of 5.23% to 7.03%), but the differences among the three combined treatments were not significant. At the same time, organic–inorganic fertilizers considerably reduced the sand content in both soil layers, with reductions in the range of 19.91% to 25.93%, compared with the NPK treatment. The differences between the organic–inorganic treatments and NPK were notable, whereas the differences among organic–inorganic treatments were not.

Table 3. Effects of organic–inorganic fertilizers on clay, silt, and sand percentages in a dry direct-seeded paddy soil.

Treatment	Soil Layers	Clay Content (%)	Silt Content (%)	Sand Content (%)
NPK	0–10cm	6.98 ± 0.29 ^{a}	73.42 ± 1.47 ^{b}	19.59 ± 1.73 ^{a}
OF1 + NPK		7.12 ± 0.11 ^{a}	78.12 ± 0.19 ^{a}	14.76 ± 0.11 ^{b}
OF2 + NPK		7.05 ± 0.21 ^{a}	77.26 ± 0.47 ^{a}	15.69 ± 0.68 ^{b}
OF3 + NPK		7.43 ± 0.27 ^{a}	77.33 ± 0.40 ^{a}	15.24 ± 0.30 ^{b}
NPK	10–20cm	7.46 ± 0.39 ^{a}	72.45 ± 0.28 ^{b}	20.09 ± 0.37 ^{a}
OF1 + NPK		7.58 ± 0.18 ^{a}	77.54 ± 0.17 ^{a}	14.88 ± 0.23 ^{b}
OF2 + NPK		7.52 ± 0.16 ^{a}	76.47 ± 0.53 ^{a}	16.01 ± 0.46 ^{b}
OF3 + NPK		7.69 ± 0.09 ^{a}	76.74 ± 0.99 ^{a}	15.57 ± 1.02 ^{b}

NPK, conventional fertilization; OF1 + NPK, seaweed bio-organic fertilizer and NPK; OF2 + NPK, Jishiwang bio-organic fertilizer and NPK; OF3 + NPK, attapulgite organic fertilizer and NPK. Different letters indicate significant differences ($p < 0.05$).

3.4. Soil Enzymes

The application of organic–inorganic fertilizers considerably improved the activity of most soil enzymes (Table 4). The activity of soil urease and soil neutral phosphatase in the dry direct-seeded paddy soil increased with the use of combined fertilizers and showed no significant differences among treatments. Despite this, a defined trend in the magnitude of these increases could be detected, OF2 + NPK > OF1 + NPK > OF3 + NPK, and the relative change compared with the NPK treatment ranged from 6.28% (urease, OF3 + NPK) to 12.07% (OF2 + NPK, neutral phosphatase). In addition, the organic–inorganic fertilizers considerably improved invertase activity compared with the NPK treatment by 32.65% (OF3 + NPK), 25.58% (OF1 + NPK), and 16.82% (OF2 + NPK). Catalase activity was markedly enhanced with respect to NPK treatment only in the soils under OF3 + NPK treatment.

Table 4. Effects of organic–inorganic fertilizers on soil enzyme activity in a dry direct-seeded rice paddy soil.

Treatment	Urease $U \cdot g^{-1}$	Neutral Phosphatase $U \cdot g^{-1}$	Invertase $U \cdot g^{-1}$	Catalase $U \cdot g^{-1}$
NPK	270.73 ± 5.43 ^{b}	20,225.00 ± 925.68 ^{b}	25.45 ± 0.46 ^{c}	10.32 ± 0.14 ^{b}
OF1 + NPK	294.55 ± 7.73 ^{a}	22,041.67 ± 880.81 ^{a}	31.96 ± 2.70 ^{a,b}	10.79 ± 0.40 ^{b}
OF2 + NPK	297.54 ± 13.26 ^{a}	22,666.67 ± 118.15 ^{a}	29.73 ± 0.85 ^{b}	10.93 ± 0.28 ^{b}
OF3 + NPK	287.74 ± 2.58 ^{a}	21,908.33 ± 398.70 ^{a}	33.76 ± 0.74 ^{a}	12.06 ± 0.38 ^{a}

NPK, conventional fertilization; OF1 + NPK, seaweed bio-organic fertilizer and NPK; OF2 + NPK, Jishiwang bio-organic fertilizer and NPK; OF3 + NPK, attapulgite organic fertilizer and NPK. Different letters indicate significant differences ($p < 0.05$).

3.5. α-Diversity of Soil Bacteria

The application of all organic–inorganic fertilizers considerably improved the bacterial richness (ACE) and Chao1 indices; the magnitude of the increases compared with NPK fertilization was OF1 + NPK > OF2 + NPK > OF3 + NPK (Figure 1a,b), ranging from 4.14% to 5.18% increase for the former index and from 3.38% to 4.08% for the latter. The effect of the seaweed bio-organic fertilizer was the most notorious. Figure 1c,d demonstrates that the soil bacteria richness indices Simpson and Shannon had little variation under the four treatments: 0.9901–0.9946 and 8.65–8.97, respectively. Both indices showed the following

decreasing pattern, OF2 + NPK > OF3 + NPK > NPK > OF1 + NPK, with marked differences only in the case of the Simpson index for OF2 + NPK (marked increased) and OF1 + NPK (marked decrease), compared with the control NPK. Unlike the Simpson index, the Shannon index showed no significant differences when organic–inorganic fertilizers were compared with the NPK regime. To sum up, Jishiwang bio-organic and attapulgite organic fertilizers combined with NPK improved the diversity of the soil bacterial community, while the seaweed bio-organic fertilizer combined with NPK reduced it.

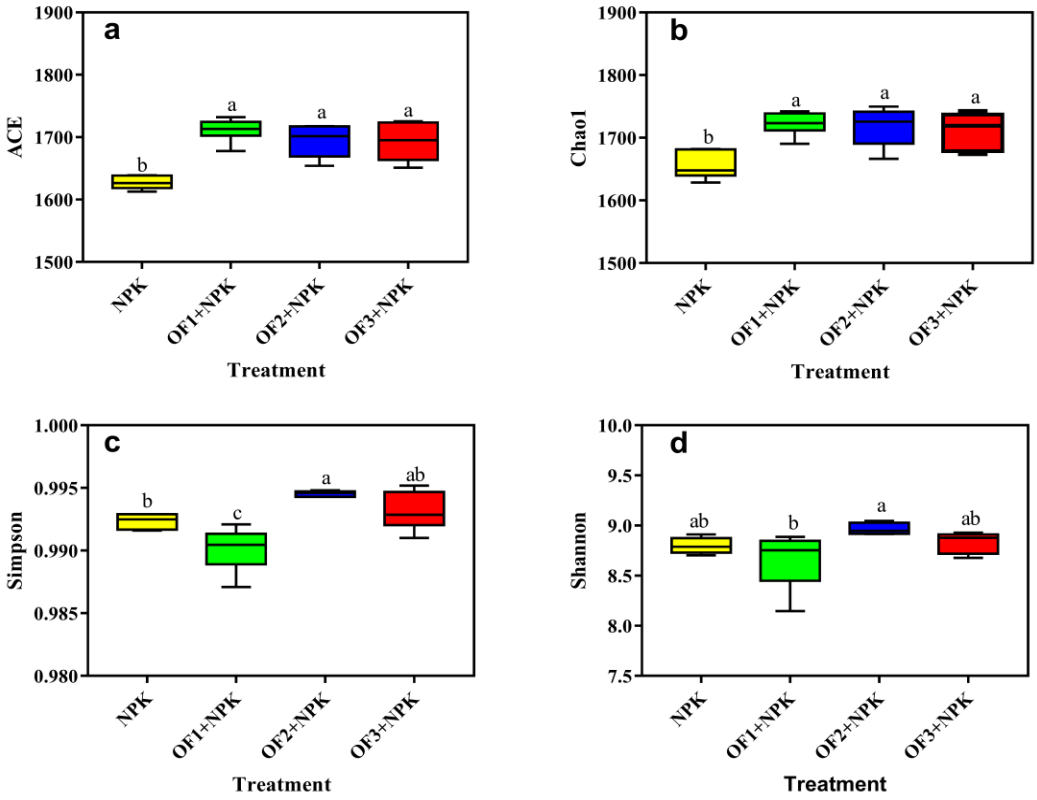


Figure 1. Effects of organic–inorganic fertilizers on soil bacterial diversity (**a**) ACE; (**b**) Chao1; (**c**) Simpson; (**d**) Shannon in a dry direct-seeded rice paddy soil. Different letters indicate significant differences ($p < 0.05$). NPK, conventional fertilization; OF1 + NPK, seaweed bio-organic fertilizer and NPK; OF2 + NPK, Jishiwang bio-organic fertilizer and NPK; OF3 + NPK, attapulgite organic fertilizer and NPK.

3.6. Soil Bacterial Community Composition

The relative abundance of different bacterial groups varied among treatments, but the overall composition of soil bacteria remained similar (Figure 2a). Proteobacteria (33.08–45.77%) and Acidobacteria (17.85–27.40%) were the dominant phyla, accounting for 54.25–70.49% of the total abundance, and the application of OF1 + NPK and OF3 + NPK treatments tended to reduce these populations. The relative abundance of Gemmatimonadetes decreased under the OF1 + NPK treatment by 58.60%, compared with NPK. The relative abundance of the less represented groups in the control treatment (NPK) such as Verrucomicrobia, Chloroflexi, Firmicutes, and Nitrospirae increased to different degrees in the plots under organic–inorganic fertilizers. The trend for the relative abundance of Verrucomicrobia, Chloroflexi, and Nitrospirae was OF1 + NPK > OF3 + NPK > OF2 + NPK > NPK, whereas the opposite pattern was detected for Proteobacteria and Gemmatimonadetes. Compared with the other treatments, OF1 + NPK increased the relative abundance of Verrucomicrobia, Chloroflexi, and Nitrospirae by 188.67–17,611.19%; this finding suggests that these groups might be antagonistic to Proteobacteria and Gemmatimonadetes.

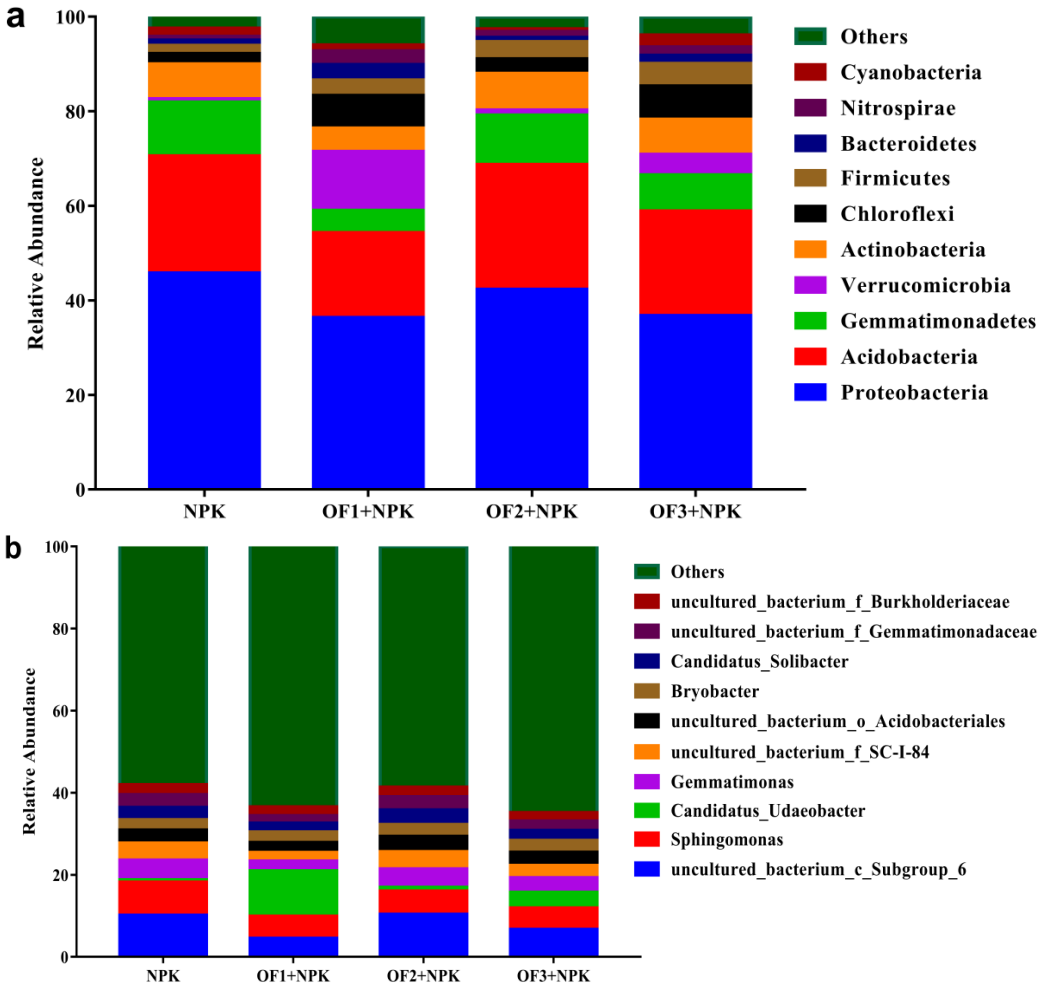


Figure 2. Composition of the bacterial community at the phylum (**a**) and genus (**b**) levels in a dry direct-seeded rice paddy soil under different fertilization treatments. NPK, conventional fertilization; OF1 + NPK, seaweed bio-organic fertilizer and NPK; OF2 + NPK, Jishiwang bio-organic fertilizer and NPK; OF3 + NPK, attapulgite organic fertilizer and NPK.

When analyzed at the genus level (Figure 2b), uncultured_bacterium_c_Subgroup_6 and *Sphingomonas* were the absolute dominant bacteria for the NPK, OF2 + NPK, and OF3 + NPK treatments, showing relative abundances of 4.49–10.15% and 5.19–8.06%, respectively. *Candidatus_Udaeobacter* and *Sphingomonas* were the dominant bacteria under the OF1 + NPK treatment, with relative abundances of 11.08% and 5.40%, respectively, indicating that seaweed bio-organic fertilizer combined with NPK can change the pattern of absolute dominant bacteria in dry direct-seeded rice paddy soil. However, compared with the NPK treatment, the OF1 + NPK treatment reduced the relative abundance of *Sphingomonas*, *Gemmatimonas*, uncultured_bacterium_f_SC-I-84, uncultured_bacterium_o_Acidobacteriales, *Candidatus_Solibacter*, uncultured_bacterium_f_Gemmatimonadaceae, and uncultured_bacterium_f_Burkholderiaceae. The OF2 + NPK treatment increased the relative abundance of *Candidatus_Udaeobacter*, uncultured_bacterium_o_Acidobacteriales, *Bryobacter*, and *Candidatus_Solibacter*, and the OF3 + NPK treatment increased the relative abundance of *Candidatus_Udaeobacter*, uncultured_bacterium_o_Acidobacteriales, and Bryobacter. In summary, the seaweed bio-organic fertilizer + NPK changed the absolute dominant bacterial population and inhibited some other relatively dominant bacteria. The Jishiwang bio-organic and attapulgite organic fertilizers combined with NPK had similar effects on the top ten bacterial species.

The contribution of PC1 to the difference in bacterial operational taxonomic units in the horizontal coordinate was 28.76%, whereas that of PC2 in the vertical coordinate was

13.28%, and the cumulative contribution rate of the first and second principal components was 42.04% (Figure 3). NPK was mainly distributed in the negative region for PC1 and PC2, OF1 + NPK was mainly distributed in the positive region for PC1 and the negative region for PC2, and OF2 + NPK and OF3 + NPK were mainly distributed around the origin. There was an obvious aggregation phenomenon with the OF2 + NPK and OF3 + NPK treatments. The distribution of NPK and OF1 + NPK indicates that the contributions of the bacterial groups in the OF2 + NPK and OF3 + NPK treatments were small; it also indicates that the application of organic–inorganic fertilizers altered the bacterial community structure.

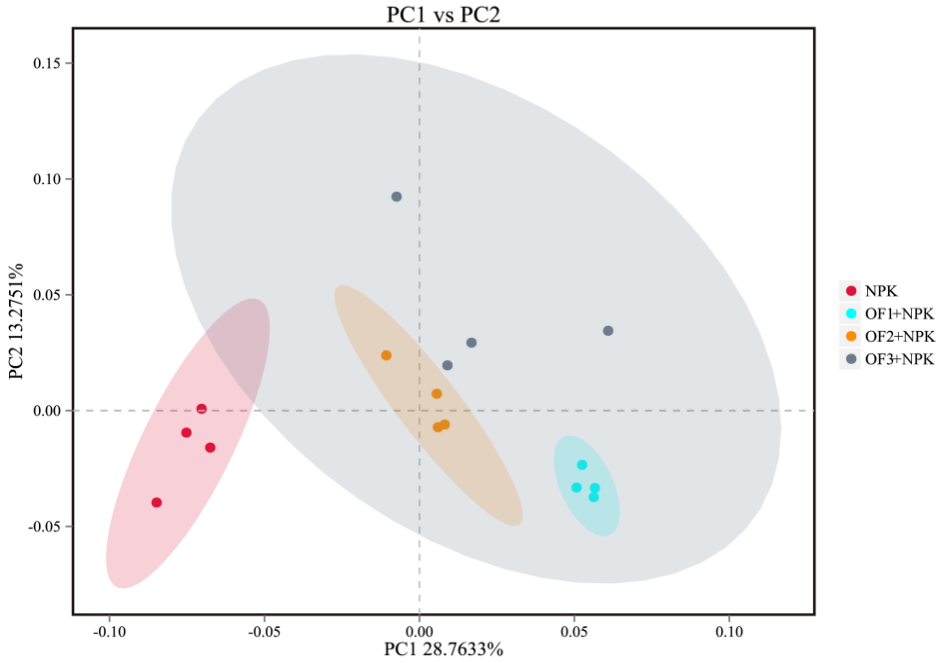
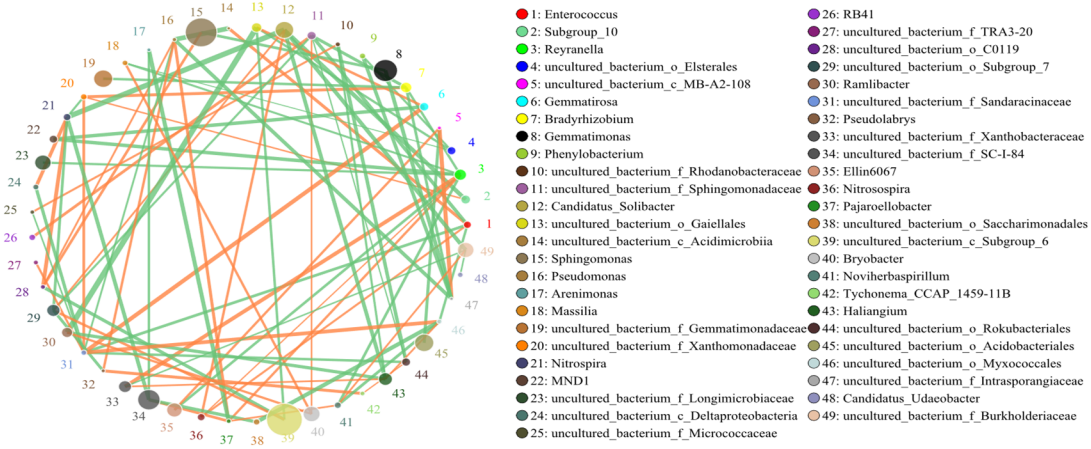


Figure 3. Principal component analysis of the soil bacterial community in a dry direct-seeded rice paddy soil under different fertilization treatments. NPK, conventional fertilization; OF1 + NPK, seaweed bio-organic fertilizer and NPK; OF2 + NPK, Jishiwang bio-organic fertilizer and NPK; OF3 + NPK, attapulgite organic fertilizer and NPK.

3.7. Relationship between Bacterial Community Structure and Soil Properties

The relationship among soil bacterial communities in the dry direct-seeded rice paddy soil was further explored. The degree of correlation among bacterial genera in each treatment was different. The number of pairwise-related species in soil treated with NPK was 84, of which 35 were positively correlated and 49 were negatively correlated; the number of pairwise-related species in soil treated with OF1 + NPK was 113, of which 49 were positively correlated and 64 were negatively correlated; and the number of pairwise-related species in soil treated with OF2 + NPK was 124, of which 51 were positively correlated and 73 were negatively correlated; the number of pairwise-related species in soil treated with OF3 + NPK was 98, of which 41 were positively correlated and 57 were negatively correlated (Figure 4a–d). Overall, the relationship between the main genera of soil bacteria under treatment with different organic and inorganic fertilizers is stronger than that of soil treated with NPK, and the number of positive correlations with OF1-3 + NPK treatment is considerably higher than that with NPK treatment.

a



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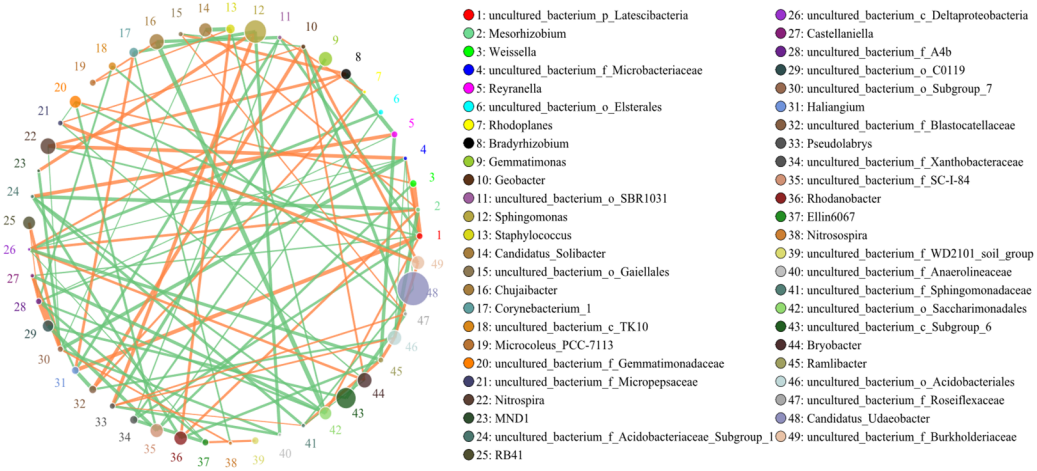

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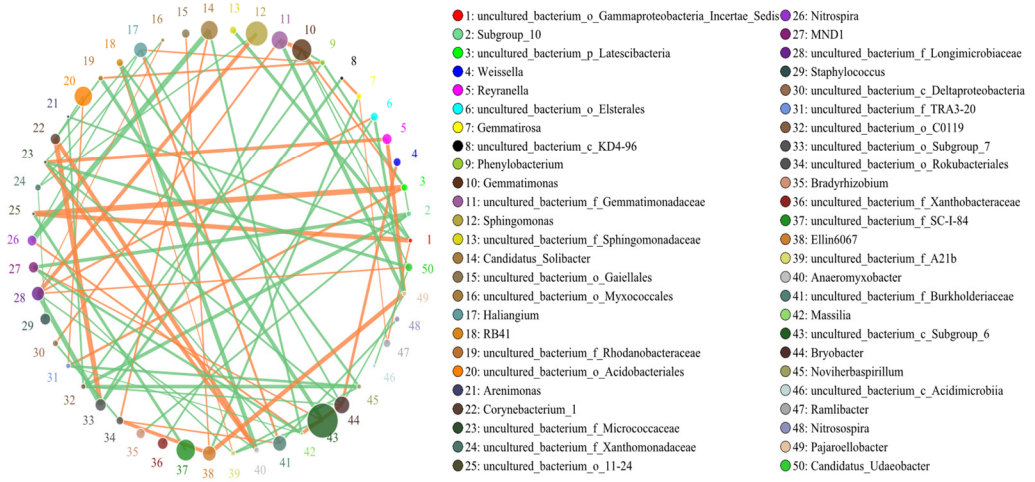

Figure 4. *Cont*.

d

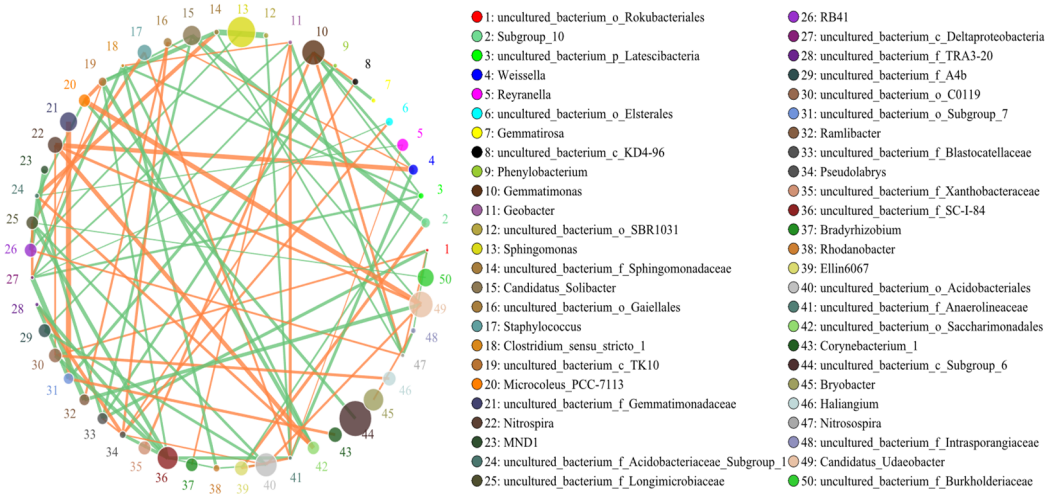

Figure 4. Correlation network analysis of soil bacteria phyla in a dry direct-seeded rice paddy soil under different fertilization treatments. NPK (**a**) conventional fertilization, OF1 + NPK (**b**) seaweed bio-organic fertilizer and NPK, OF2 + NPK (**c**) Jishiwang bio-organic fertilizer and NPK, and OF3 + NPK, (**d**) attapulgite organic fertilizer and NPK. Each node represents each dominant phylum and is identified by a different color. The edge connecting two nodes indicates the correlation between two phyla, with the orange line indicating a positive correlation and the green line indicating a negative correlation. The node size indicates the degree of association between the g phyla and other phyla of the community. The node size increases with the degree. Different colors represent different degrees of nodes.

Based on the redundancy analysis (RDA) linear model (Figure 5), the relationship between the soil bacterial community and soil properties was explored. Principal components 1 and 2 explained 47.41% and 15.20% of the bacterial community distribution, respectively, for a total of 62.61%. Total nitrogen, soil organic matter, urease, and invertase were the main environmental factors affecting the bacterial community composition. Proteobacteria were positively correlated with the pH but negatively correlated with the total potassium content and invertase activity. Acidobacteria were positively correlated with the pH and AHN but negatively correlated with the total potassium content, invertase activity, and soil organic matter. Verrucomicrobia and Nitrospirae were mainly positively correlated with the total nitrogen content; Verrucomicrobia were mainly negatively correlated with the total phosphorus content, and Nitrospirae were mainly negatively correlated with the soil bulk density. Chloroflexi were mainly positively correlated with soil organic matter, total potassium content, and invertase activity and negatively correlated with AHN and pH. Firmicutes were mainly positively correlated with urease activity and negatively correlated with soil bulk density. Urease activity and soil bulk density were negatively correlated.

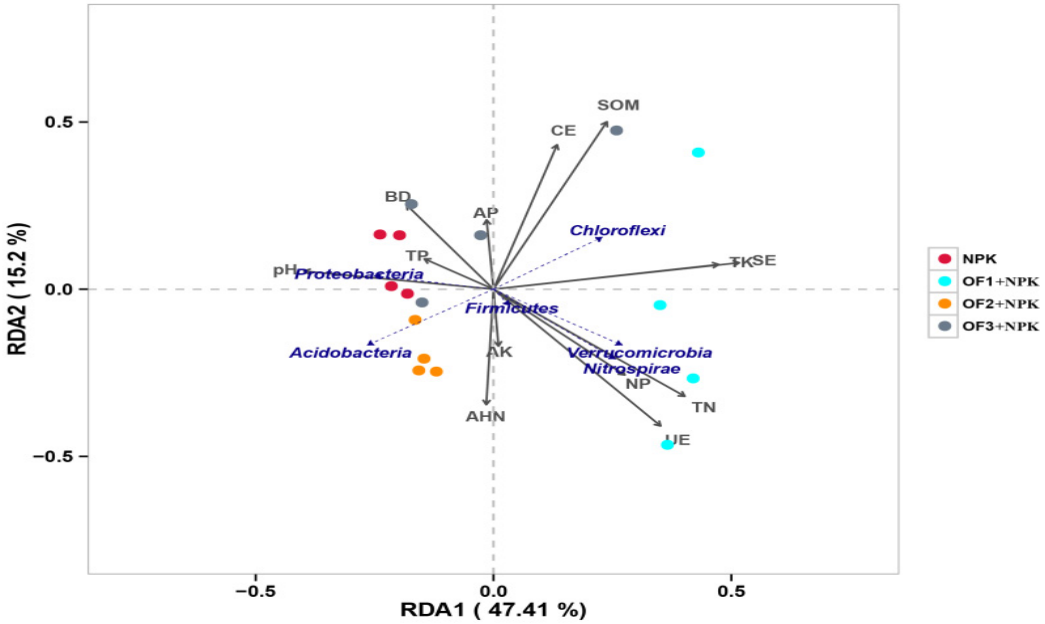

Figure 5. Redundancy analysis of the relationship between bacterial community distribution and soil properties under different fertilization treatments. NPK, conventional fertilization; OF1 + NPK, seaweed bio-organic fertilizer and NPK; OF2 + NPK, Jishiwang bio-organic fertilizer and NPK; OF3 + NPK, attapulgite organic fertilizer and NPK. AHN, alkaline hydrolyzable nitrogen; AK, available potassium; AP, available phosphorus; BD, bulk density; CE, catalase; NP, neutral phosphatase; SE, invertase; SOM, soil organic matter; TK, total potassium; TN, total nitrogen; TP, total phosphorus; UE, urease.

4. Discussion

Fertilization has an important effect on soil physical and chemical properties, while also supplying nutrients to crops. A single application of inorganic fertilizer increases soil bulk density and soil consolidation, which is not conducive to nitrogen accumulation [36]. Organic fertilizers increase soil organic matter content [19,20] and the sustainable utilization of soil nutrients, but nitrogen is easily leached. The combination of organic and inorganic fertilizers effectively improves soil fertility and reduces nitrogen leaching [37]. According to Gong et al. [38], organic fertilizers considerably affect soil nitrogen, phosphorus, and potassium levels. Zuo et al. [39] found that only the application of organic fertilizers in successive years could increase the nutrient content at deeper soil layers, even when the downward movement of superficial organic matter and nutrients with root stubble or root sediments may occur. In the present study, soil bulk density decreased, and soil nutrient levels increased with the application of organic–inorganic fertilizers compared with NPK. This is important, considering that available nitrogen, phosphorus, and potassium contents tend to decrease rapidly with rice maturity under the traditional NPK fertilization regime. Upon using organic–inorganic combinations, a slower release of nutrients through the action of soil microorganisms takes place, allowing for remarkable rises in available nitrogen and potassium contents [40]. Our findings suggest that accelerated mineralization of soil organic matter due to microbial activity occurs when organic–inorganic fertilizers are applied sustainedly in dry direct-seeded rice, and available nutrient contents tend to increase. However, due to structure and C:N ratio differences among the organic fertilizers tested, the magnitude of these increases varied. Furthermore, nutrients in the 0–10 cm soil layer could not be transferred to a deeper soil layer in the short term, altering the nutrient content of the 10–20 cm layer only to a slight extent.

Soil is usually composed of aggregates of various sizes [41], and the particle size distribution of these aggregates also has a significant impact on microorganisms [42]. Zhang et al. [43] showed that organic fertilizers considerably changed the particle size distribution

of soil aggregates. Compared with the NPK treatment, the application of organic–inorganic fertilizers mainly promoted the formation of >5 mm water-stable aggregates in the paddy soil, followed by 0.5–1 and 2–5 mm aggregates; however, there were no significant effects on the proportion of 1–2 and 0.25–0.5 mm aggregates. Our findings are consistent with those of Jiang et al. [44], who reported that the content of water-stable aggregates >1 mm in red soil in dry land was effectively improved by organic and inorganic compounds, whereas the total amount of water-stable >0.25 mm aggregates increased under NPK. To sum up, we found that organic–inorganic fertilizers promoted the transformation of small water-stable aggregates to larger water-stable aggregates. Soil particles are typically classified as sand (>50 µm), silt (2–50 µm), and clay (<2 µm) [45]. Organic carbon and total nitrogen associated with the sand fraction are considered the main active carbon and nitrogen components [46] and the most responsive to tillage and fertilization management changes [47]. Compared with those under NPK treatment, the organic–inorganic fertilizer treatments increased the soil clay and silt contents by 0.80–6.45% and 5.23–7.03%, respectively, and decreased the sand content by 19.91–25.93%. This finding demonstrates that continuous application of organic–inorganic fertilizers can gradually reverse desertification processes and improve soil conservation by promoting fine soil particle retention and intergranular cementation and improving the relative amount of more stable water-stable aggregates. Soil enzyme activity has attracted much attention in recent years because it is directly related to carbon and nutrient cycling in the soil [48,49]. Soil enzymes are mainly derived from microbial metabolism and plant root secretions [50]. It has been reported that fertilization can promote root metabolism, increase root exudates, and accelerate microbial reproduction, thus improving soil enzyme activity [51]. The application of combined organic–inorganic fertilizers extensively affected the soil enzyme activity, in which the soil urease, neutral phosphatase, and sucrase activities were notably improved compared with the conventional fertilization. This fully showed that the application of combined organic–inorganic fertilizers promoted the conversion and release of soil nitrogen and phosphorus nutrients, promoted the effective decomposition of organic substances, and improved the absorption efficiency of crop nutrients.

Various studies have shown that Proteobacteria are the most abundant phylum and can participate in the biological circulation of essential mineral nutrients in the soil [52–54]; a high proportion of amoebas in the rhizospheric soil (eventually supplied by an organic fertilizer) is beneficial for the conservation of soil fertility and plant growth [55]. Li et al. [51] showed that organic fertilizers increased the relative abundance of Proteobacteria in paddy soil, while pig manure decreased it. *Sphingomonas* have a relatively high abundance in nutrient-deficient environments [56]. Acidobacteria comprise oligotrophic bacteria [57], relatively abundant in acidic soils [58]. However, some studies have shown that Acidobacteria are closely associated with soil organic matter content in dry land soils [59]. Compared with NPK, the application of organic–inorganic fertilizers decreased the relative abundance of Proteobacteria. The redundancy analysis revealed that Proteobacteria abundance was positively correlated with soil pH; upon organic–inorganic fertilizers treatment, the soil pH was considerably lower than under NPK use, owing to the relative decrease in this phylum. Soil pH is an important regulating factor of soil microbial community composition, and acid bacteria show strong reverse response to soil pH, which also explains the positive correlation between Proteobacteria and pH. The application of Jishiwang bio-organic fertilizer markedly increased the relative abundance of Acidobacteria but decreased the soil pH, which is consistent with the results of Wang et al. [56], wherein the relative abundance of Acidobacteria was negatively correlated with soil pH. Some studies also reported that the relative abundance of Acidobacteria is positively correlated with soil pH, which might be due to the differences in soil and plant types. Compared with the NPK treatment, the organic–inorganic fertilizer treatments increased the relative abundance of the phyla Verrucomicrobia, Chloroflexi, Firmicutes, and Nitrospirae. Verrucomicrobia exist in various environments and are closely related to plant roots in soil [60]. Verrucomicrobia have a

high coding density for glycosidic hydrolase genes and generally play a key role in carbon cycling and polysaccharide degradation [61,62].

Through the redundant analysis linear model, we found that Verrucomicrobia were closely related to the soil total nitrogen content, neutral phosphatase activity, and urease activity, with considerably higher values for these two enzyme activities under the use of organic–inorganic fertilizers than NPK. The Chloroflexi phylum mostly contains anaerobes, which can ferment sugar and polysaccharides into organic acids and hydrogen and are associated with the accumulation of halogenated organic compounds produced by plant residue humification [63]. In this study, the relative abundance of Chloroflexi increased under the organic–inorganic fertilizer treatments; this might be related to pre-harvest rainfall, which could reduce the soil oxygen content and thus promote anaerobic conditions allowing for the preferential reproduction and biomass increase in Chloroflexi. Moreover, this study found that the phylum Chloroflexi was positively correlated with soil organic matter content, total potassium content, and invertase activity, indicating that Chloroflexi probably promoted invertase activity. Increasing Chloroflexi abundance was also beneficial for soil organic matter accumulation. Firmicutes members are oligotrophic bacteria, most of which can resist extreme conditions as spores [64]. Firmicutes were a major factor positively affecting urease activity, and since Nitrospirae members participate in the oxidation of soil nitrites, combined organic–inorganic fertilization can improve soil nitrogen levels through multiple actions.

5. Conclusions

At both soil depths analyzed, organic–inorganic fertilizers considerably increased AHN and available potassium and decreased soil pH, compared with the NPK treatment. Moreover, these combined fertilization treatments considerably increased the relative proportion of soil macroaggregates (>5 mm), with differences according to the type of organic fertilizer applied, and changed the relative proportion of sand (which decreased) to that of silt (which increased). Urease, neutral phosphatase, and invertase activities were considerably increased by organic–inorganic fertilizer regimes, but only the organic fertilizer attapulgite combined with NPK markedly increased catalase activity. The organic–inorganic fertilizers considerably modified soil bacterial community richness: the Jishiwang bio-organic and attapulgite organic fertilizers combined with NPK increased soil bacterial diversity, while the seaweed bio-organic fertilizer combined with NPK decreased it. Proteobacteria and Acidobacteria were the dominant phyla, and the application of the combined organic–inorganic fertilizers increased, to different degrees, the relative abundance of Verrucomicrobia, Chloroflexi, Firmicutes, and Nitrospirae. At the phylum level, the correlations among species were mainly negative, suggesting that antagonistic relationships may predominate among soil microbial functional communities.

The introduction of organic fertilizers in dry direct-seeded paddy soil may favor soil organic matter accumulation, accelerate polysaccharide decomposition, and regulate soil nitrogen levels. Total nitrogen content, soil organic matter, and soil urease and invertase activities are the main environmental factors affecting bacterial community composition. Therefore, the combination of organic–inorganic fertilizers is an effective strategy to improve soil fertility and reduce chemical fertilizer pollution.

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

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