RESEARCH ARTICLE

Effects of organic fertilizer on soil properties and soil microorganisms

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Organic fertilizers have been shown to positively impact soil microorganisms and the physical and chemical properties of soil. This study utilized high-throughput sequencing technology to analyze the composition and structure of the soil microbial community after organic fertilizer application. Enzyme activity, water content, and organic matter were also measured. The findings indicated that organic fertilizer application resulted in lower soil pH and reduced rates of miscellaneous bacteria, while increasing enzyme activity, water content, and organic matter. Moreover, the use of organic fertilizers improved alpha and beta diversity of soil microorganisms with *Firmicutes* experiencing a significant increase at the phylum level, and *Bacillus*, SBR1031, and *Sphingomonas* exhibiting higher relative abundance at the genus level. Conversely, levels of soil microorganisms like *Nocardioides*, *Solidubrobacter*, and *Microvirga* were lower in the organic fertilizer group compared to the control group. The application of PICRUST2 revealed significant up-regulation of PWY-6174 (mevalonate pathway II (*haloarchaea*)), ASPASN-PWY (super pathway of L-aspartate and L-asparagine biosynthesis), and PWY0-42 (2-methylcitrate cycle I) pathways in the organic fertilizer group, demonstrating the positive impact of organic fertilizers on microbial communities. Further analysis of the species composition of these pathways showed that in PWY-6174 pathway, Marine_ Group_ II in the control group displayed higher relative abundance than that in the organic fertilizer group ($P < 0.05$). In PWY0-42 pathways, the relative abundance of *Rhodanobacter*, *Chujaibacter*, *Dyella*, *Luteimonas*, and *Thermomonas* in organic fertilizer group was significantly higher than that in control group ($P < 0.05$). In ASPASN-PWY pathway, the relative abundance of *Bacillus*, *Fonticella*, *Romboutsia*, *Chloroplast*, and *Tepidimicrobium* in organic fertilizer group was significantly higher than that in control group ($P < 0.05$). In conclusion, this study indicated that organic fertilizers could effectively enhance the structure and composition of the soil microbial community, leading to improvements in the physical and chemical properties of soil.

Keywords: organic fertilizer; soil microorganism; enzyme activity; *Bacillus*; 16S rRNA.

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Introduction

Straw burning and overuse of chemical fertilizers harm soil microorganisms, reduce soil fertility, and cause soil degradation. These negative impacts threaten crop yields and food security, as well as the sustainable development of the environment and ecosystem. It is crucial to implement sustainable agricultural practices to protect and promote soil health and fertility [1-3]. In addition, the long-term use of chemical fertilizer has led to the continuous accumulation

of salt and nitrate in the soil, which forms the alkalization of raw salt on the soil surface, destructs soil structure, and deteriorates physical, chemical, and biological properties of soil [4-6], which, at least, will affect the germination and emergence of seeds, hinder nutrient absorption, and cause poor crop growth. In the worst case, it will cause plant damage, death, and even lose agricultural value [7, 8]. Reducing the use of chemical fertilizer, organic substitution, and increasing the application of biological fertilizer are the inevitable choices to solve the soil problem and realize the green and sustainable agricultural development [9, 10].

Organic fertilizer is a kind of fertilizer widely used in agricultural production. Numerous studies have demonstrated that the use of organic fertilizers plays a vital role in enhancing soil fertility, ensuring successful crop production, and improving the overall quality of crops [11, 12]. In order to reduce the harm of fertilizers to soil, organic fertilizers are used instead of fertilizers for crop cultivation. With the improvement of bioscience technology and the development of agricultural production, the massive application of organic fertilizer has played a positive role in slowing down agricultural pollution, maintaining the stable growth of agricultural production, and ensuring food security [13, 14]. Organic fertilizer can regulate soil microbial structure and soil physical and chemical properties, which has become a research hotspot. Organic fertilizers can promote the diversification of soil microorganisms, regulate the presence of heavy metals in soil, and consequently impact the absorption of nutrients by plants [15]. In soil, the movement and availability of heavy metals are related to the properties of soil, especially soil pH value, which has the most important impact on the bioavailability and form of heavy metals [16, 17]. In order to explore the biological regulation of biological fertilizer (organic fertilizer) on the microorganisms and the physical and chemical properties of soil, this study investigated the soil physical and chemical indexes with or without the application of organic fertilizer and detected the microbial community structure in the soil by using high-throughput sequencing technology for 16S rRNA gene sequencing. The results of this study would provide a basis for the rational application of organic fertilizer.

Materials and Methods

The fertilizer resource and experimental site management

The organic fertilizer used in the experiment was the biological bacterial fertilizer prepared by microbial fermentation of corn straw and pig manure, which was purchased from Hailan animal husbandry Co., Ltd, Xiping County, Zhumadian City, Henan province, China. The experiments were conducted in the biological organic fertilizer vegetable planting experimental site of Hailan animal husbandry Co., Ltd and were divided into control and organic fertilizer groups with an area of 5 × 20 m^2, respectively. Before vegetable planting, the soil was turned over and applied with organic fertilizer of 1.5 kg/m^2. For the control group, the general compound fertilizer was applied with 1,350 kg/hm^2 in soil, among them the contents of N, P_2O_5, and K_2O were all 202.5 kg/hm^2. The field management was carried out according to the high-yield field protocol with (1) scientific fertilization, (2) reasonable irrigation, (3) weed control and pest control, and (4) strengthening field management.

Soil collection and physical and chemical index detection

Soil samples were collected by the five-point sampling approximately one month after fertilization. The samples were then divided into two parts with one part for soil physical and chemical properties tests and the other part for soil microbial parameters tests. Activities of soil enzymes including catalase (S-CAT), sucrase (S-SC), alkaline phosphatase (S-AKP/ALP), and urease (UE) were measured by using commercial kits from Nanjing Jiancheng Biotechnology Research Institute, Nanjing, Jiangsu, China. The Thermo Multiskan FC ELISA reader (ThermoFisher, Bethesda, Maryland, USA) had been used for soil enzyme activity detection. The

soil contents of moisture, nitrogen, phosphorus, and potassium were measured by using direct drying method, element analyzer, alkali fusion method, and flame photometry, respectively, while the contents of organic matter, *Escherichia coli*, *Ascaris* egg mortality, effective viable bacteria number, and heavy metals were detected by using potassium dichromate oxidation external heating method, membrane filter, coating detection, dilution coating method, and atomic absorption spectroscopy (AAS) according to the Chinese national standard methods. The remaining soil was put in a 50 mL centrifuge tube containing sterilized 0.86% NaCl solution. After standing on ice for 30 mins, the soil sample was shaken well in every 5 mins to discard the impurities before centrifuged at 4,000 g for 30 mins at 4℃. After pouring out the supernatant, the soil sediment was stored in a clean centrifuge tube at -20℃ for standby.

Soil microorganism DNA extraction and sequencing library construction

The soil microbial DNA Extraction Kit (Tiangen Biochemical Technology Co., Ltd, Beijing, China) was used to extract the DNA of soil microorganisms according to manufacturer's instructions. The DNA was quantified by using Thermo Nanodrop 2000 (ThermoFisher, Bethesda, Maryland, USA) and the quality of DNA was checked by using 1.2% agarose gel electrophoresis. The V4-V5 region of 16S rDNA was amplified by using universal primers of 515F (5'-GTG CCA GCC GG-3') and 907R (5'-CCG TCA ATT CMT RAG TT-3') and Takara PCR kit (Takara Bio Inc, Shiga Prefecture, Japan). The amplification reaction system was 25 μL including 4 μL of 5× Taq FastFu Buffer, 2 μL of 2.5 mmol/L dNTPs, 0.4 μL of each 20 μmol/mL primer, 0.5 μL of DNA template (approximately 50 ng), and ddH_2O up to 20 μL. The amplification reaction procedure was pre-denaturation at 95℃ for 2 mins followed by 30 cycles of denaturation at 95℃ for 30 s, renaturation at 55℃ for 30 s, and extension at 72℃ for 1 min, and then a final extension at 72℃ for 5 mins by using ABI VeritiPro Thermal Cycler (Applied Biosystems, Leuven, Belgium). The amplified product was purified and recovered by using AMPureXP magnetic beads (Beckman, Bria, California, USA) and quantified by using fluorescence. The samples were then mixed according to the corresponding proportion to prepare the sequencing library by using TruSeq Nano DNA LT Library Prep Kit (Illumina, San Diego, California, USA).

High-throughput sequencing and sequencing data analysis

The quality of sequencing library was inspected by using both Agilent Bioanalyzer (Agilent, Santa Clara, California, USA) and Promega QuantiFluor (Promega, Madison, Wisconsin, USA). The qualified on-line sequencing libraries were then gradient diluted and mixed in proportion to the desired sequencing volume. NaOH was used prior to on-line sequencing to achieve the single-strand denaturation. Two-terminal sequencing of 2×300 was carried out with the MiSeq reagent kit V4-V5 (600 cycles) and the Illumina MiSeq sequencer (Illumina, San Diego, California, USA). To ensure the suitability of the initial sequencing data for analysis, quality filtering, denoising, splicing, and dechimerization were performed. The alpha diversity of soil microorganisms was then analyzed by using the Faith's PD, Observed Species, Simpson, Shannon, Chao1, and Pielou's Evenness indices in QIIME2 (https://qiime2.org), and rarefaction curves were drawn. Based on the flattened Amplicon Sequence Variant (ASV)/ Operational Taxonomic Unit (OTU) table, PCoA analysis was conducted by using the ape package in R software (https://www.r-project.org). Specific composition tables per sample for each classification level were generated by collapsing the ASV/OTU table. Phylum and genus level column diagrams were created by using QIIME2 software, while the ggtree package was used to show the position and evolutionary distance of each ASV/OTU in the form of histograms and heatmaps. Based on the ASV/OTU abundance table, the number of unique and common ASV/OTU was then counted by using the Venn Diagram package in R software to generate Wayne diagrams. To cluster each sample and taxon, the pheatmap package in R software was

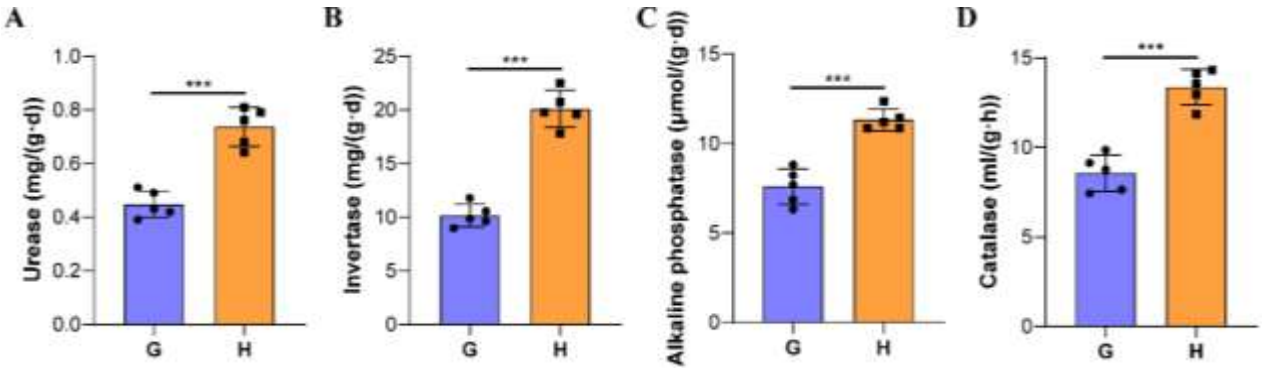

Figure 1. The physical and chemical properties of soil. **A.** The content of urease in soil. **B.** The content of invertase in soil. **C.** The content of alkaline phosphatase in soil. **D.** The content of catalase in soil.

used, and interactive graphs were created to show the top 20 bacteria with their relative abundance. The drawing installation package (Faith's PD, ggtree package, and Venn Diagram package, *et al*.) in R software was provided by Parsenor Biotechnology Co., Ltd., Shanghai, China. A comparison between the up-regulated and control groups was carried out by using the uncollapsed ASV/OTU table. The fitFeatureModel function of Metagnomeseq package in R software was used to fit the distribution of each ASV/OTU through a zero-inflated log-normal model. The significance of differences was assessed based on the fitting results of the model. This study used the Metacyc online analysis platform (https://metacyc.org) to analyze the metabolic pathways involved in two groups of microbiota. The correlation analysis was conducted between the top 10 bacterial species in soil abundance and soil enzymes and physical and chemical indicators by using the genescloud tools, a free online data analysis platform (https://www.genescloud.cn). Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUST2), a software that predicts the functional abundance of microbiota using marker gene sequences in samples [18], was employed for functional potential prediction of soil microorganisms. The essential function of PICRUST2 is to quantify metabolic pathways, analyze the differences in metabolic pathways, and examine species composition within metabolic pathways. The primary analytical approach followed the methodology outlined by Langille, *et al*. [19].

Statistical analysis

This study employed a completely randomized trial design. The analysis of all data was performed by using independent sample t-test in SPSS version 25.0 (IBM, Armonk, New York, USA) with statistically significant difference as $P < 0.05$ and very significant difference as $P < 0.01$.

Results

Soil physical and chemical indexes

Sucrase, urease, alkaline phosphatase, and catalase are closely related to the transformation of carbon, nitrogen, and phosphorus in soil. After applying organic fertilizer for one year, compared to the control group without applying organic fertilizer, the activities of urease, sucrase, alkaline phosphatase, and catalase in soil increased by 64.29%, 97.62%, 49.35%, and 56.04%, respectively (Figure 1). The use of organic fertilizer significantly enhanced the activity of soil enzymes. Additionally, the organic fertilizer group exhibited higher levels of soil organic matter content and living bacteria counts compared to the control group ($P < 0.01$). Meanwhile, ascaris egg mortality was nearly 100% and group content of *E. coli* was less than 3 most probable number (MPN)/g in the organic

Table 1. The detection of physical and chemical indexes of organic fertilizer.

Group	pH	Organic matter (%)	Living bacteria count (CFU/g)	Group content of *E. coli* (MPN/g)	Ascaris egg mortality (%)	As (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Cr (mg/kg)	Hg (mg/kg)
G1	8.02	2.1	1.1×10^5	9,200	98	6.90	0.10	15.5	30.1	0.01
G2	7.93	2.0	2.7×10^5	16,643	97	6.26	0.10	14.9	30.4	0.01
G3	8.03	2.1	8.9×10^5	9,490	98	5.72	0.11	15.2	27.6	0.01
G4	7.91	1.9	4.7×10^5	15,000	97	6.57	0.10	15.9	28.5	0.01
G5	8.08	2.2	3.8×10^5	15,000	98	6.02	0.11	14.5	30.2	0.01
H1	5.75	12.6	2.7×10^7	<3.0	99	6.57	0.14	17.7	28.2	0.00
H2	5.79	16.7	2.4×10^7	<3.0	100	6.68	0.18	23.5	27.1	0.00
H3	5.73	13.6	2.5×10^7	<3.0	100	6.43	0.16	24.2	29.4	0.00
H4	5.70	15.5	2.2×10^7	<3.0	100	6.79	0.22	37.1	30.1	0.00
H5	5.83	17.4	2.5×10^7	<3.0	100	6.30	0.15	16.7	26.5	0.00

Notes: G: the control group. **H:** the organic fertilizer group.

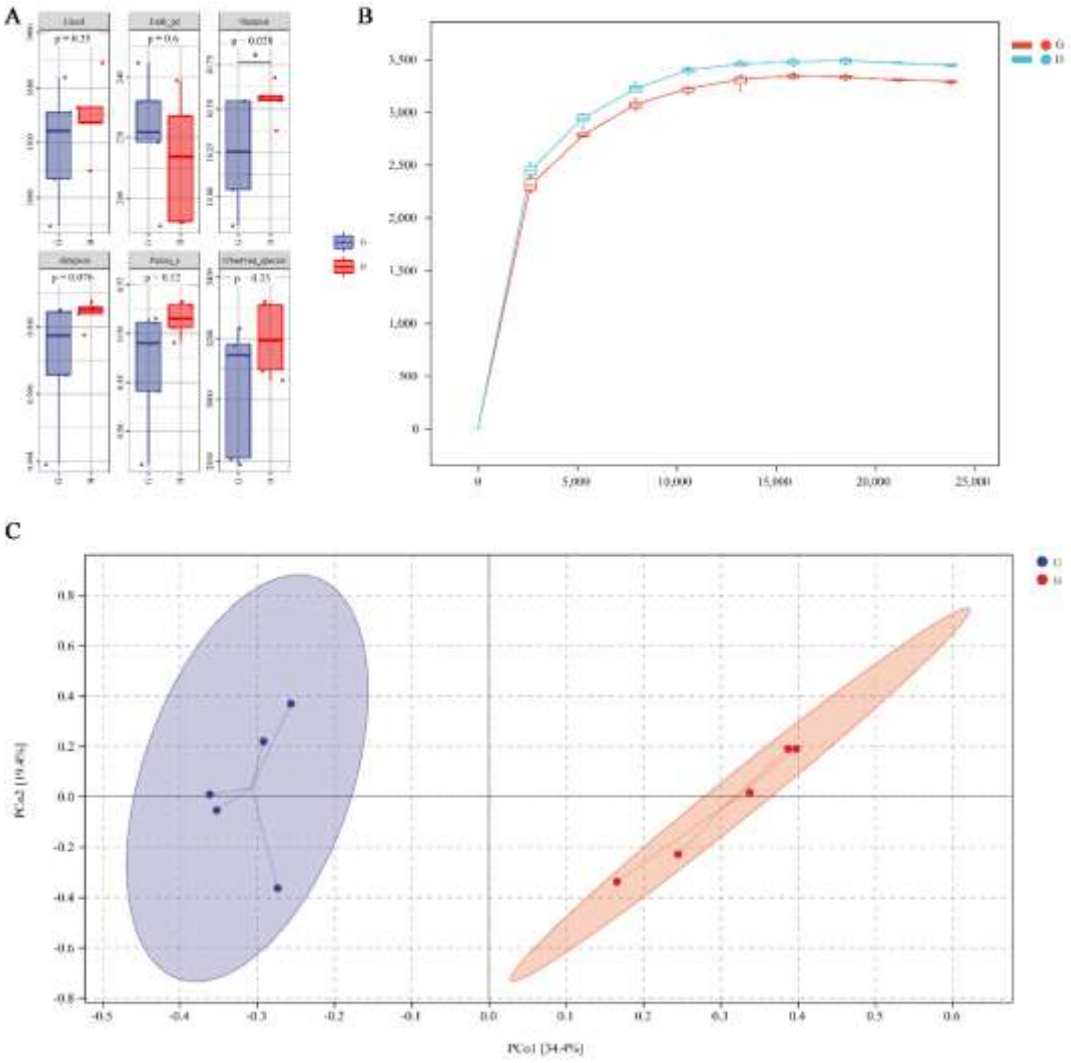

Figure 2. Alpha and beta diversities in control (G) and organic fertilizer (H) groups. **A.** alpha diversity index. **B.** rarefaction curve. **C.** PCoA analysis.

fertilizer group ($P < 0.05$). The organic fertilizer group also displayed very significant lower pH values in soil than that in the control group ($P < 0.01$). Notably, in both groups, the contents of heavy metals including As, Cd, Pb, Cr, and Hg in soil were far lower than the risk screening values stipulated in soil environmental quality, agricultural land risk management, and national control standard (GB15618-2018) in China ($P > 0.05$) (Table 1).

Alpha and beta diversity

Alpha diversity encompasses measures of diversity, species richness, and evenness within homogeneous habitats. In this study, the Pielou's index that represented evenness, Shannon and Simpson indexes that represented diversity, Chao1 and observed species indexes that represented richness, and Faith's PD index that represented evolution-based diversity of soil microorganisms were measured. The results demonstrated that all indexes of the organic fertilizer group were higher than that in the non-fertilizer group with Shannon index showing a statistically significant difference ($P < 0.05$) (Figure 2A). The rarefaction curve indicated that the sequencing data adequately reflected the diversity within the analyzed samples, and that additional sequencing was unlikely to reveal a substantial number of new ASV/OTUs (Figure 2B). In contrast, beta diversity describes the variation in species composition or the rate of species turnover across environmental gradients between different communities, referred to as between-habitat diversity. In this study, PCoA results were used to show the beta diversity of soil microorganisms (Figure 2C). There was a large difference between the soil group with organic fertilizer (group H) and the control group (group G). However, there was a small difference within each group, which indicated that the microbial community structure composition of each sample in the group was similar, while the microbial community structure composition of samples between groups was quite different.

Bacterial species composition

To visualize the abundance distribution trend of each sample and compare species composition differences between samples, heat maps were created by using abundance data at the genus and phylum levels for the top 10 and 20 average abundances. At the phylum level, *Actinobacteria* and *Proteobacteria* were the most dominant bacteria observed in both the organic fertilizer and control groups, but the control group displayed a significantly lower relative abundance of *Firmicutes* than that in the organic fertilizer group (Figure 3A). At the genus level, *Bacillus*, SBR1031, and *Sphingomonas* showed higher relative abundances in the organic fertilizer group than that in the control group, while in contrast, soil microorganisms such as *Nocardioides*, *Solirubrobacter*, and *Microvirga* displayed lower relative abundances in the organic fertilizer group than that in the control group (Figure 3B). The analysis of phylogenetic tree indicated that *Actinobacteria* and *Proteobacteria* were the main phyla of soil microorganisms, and further revealed that ASV_144 (*Solirubrobacter*) and ASV_102 (67-14) were closely related. Notably, their relative abundances in the organic fertilizer group were significantly lower than those in the control group ($P < 0.05$) (Figure 3C).

Bacterial species differences and marker analysis

ASV/OTU Venn diagram, Heatmap of species composition, and Metagenomicseq analysis were used to explore which species caused the difference of soil microorganisms between the control and the organic fertilizer groups. The results showed that there were 10,474 and 10,113 ASV/OTUs in soil microorganisms in control group and organic fertilizer group, respectively. The number of ASV/OTUs shared between the two groups was 1,334 (Figure 4A). The heatmap results of the top 20 bacteria in the average abundance showed that the relative abundance of *Bacillus*, SC-I-84, *Dechloromonas*, *Sphingomonas*, *Saccharimonadales*, and *Haliangium* in the organic fertilizer group were higher than that in the control group, while the relative abundance of soil microorganisms such

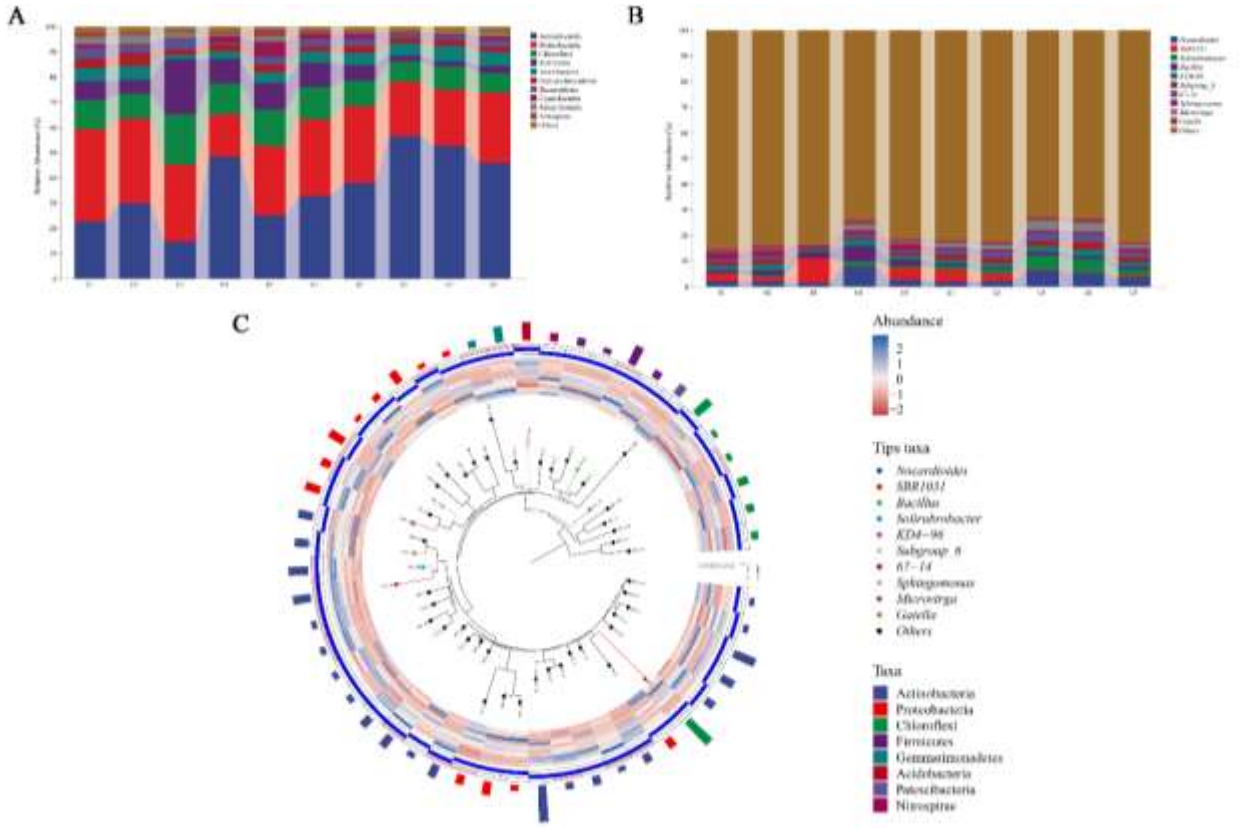

Figure 3. The species composition in control (G) group and organic fertilizer (H) group. **A.** species composition at the phylum level. **B.** species composition at the genus level. **C.** Phylotree evolutionary tree.

as *Mycobacterium*, *skermanella*, and *Streptomyces* in the organic fertilizer group was lower than that in the control group (Figure 4B). Metagenomeseq analysis displayed that, compared with the control group, the organic fertilizer significantly upregulated the bacteria phyla such as *Actinobacteria* and *Proteobacteria* ($P < 0.05$). Further analysis found that, compared with the control group, the organic fertilizer mainly significantly upregulated the bacteria phyla of *Actinobacteria* and *Proteobacteria* including *Blastococcus*, *Actinoplanes*, *Nocardioides*, *Gaiella*, 67-14, *Solidubrobacte*r, KD4-96, *Microvirga*, and *Ramlibacter* ($P < 0.05$) (Figure 4C).

Association analysis

The correlation analysis results showed that the soil microbial communities of *Solirubacter*, Subgroup_6, 67-14, and *Microvirga* were significantly negatively correlated with urease, sucrose, alkaline phase, catalase, organic matter, and phosphorus in soil, while *Bacillus* and *Sphingomonas* in soil were significantly positively correlated with urease, sucrose, alkaline phase, catalase, organic matter, and phosphorus in soil (Figure 5).

Functional potential prediction of soil microorganisms

Upon analyzing the diversity and species composition of soil microbiota, the functions and functional unit composition of the microbiota were investigated by using PICRUST2 to gain a general understanding of the functional potential of the microbiota present in the soil samples. The Metacyc was employed to analyze the metabolic pathways of biosynthesis, degradation/ utilization/estimation, detoxification, generation of precursors, metal and energy, glycan

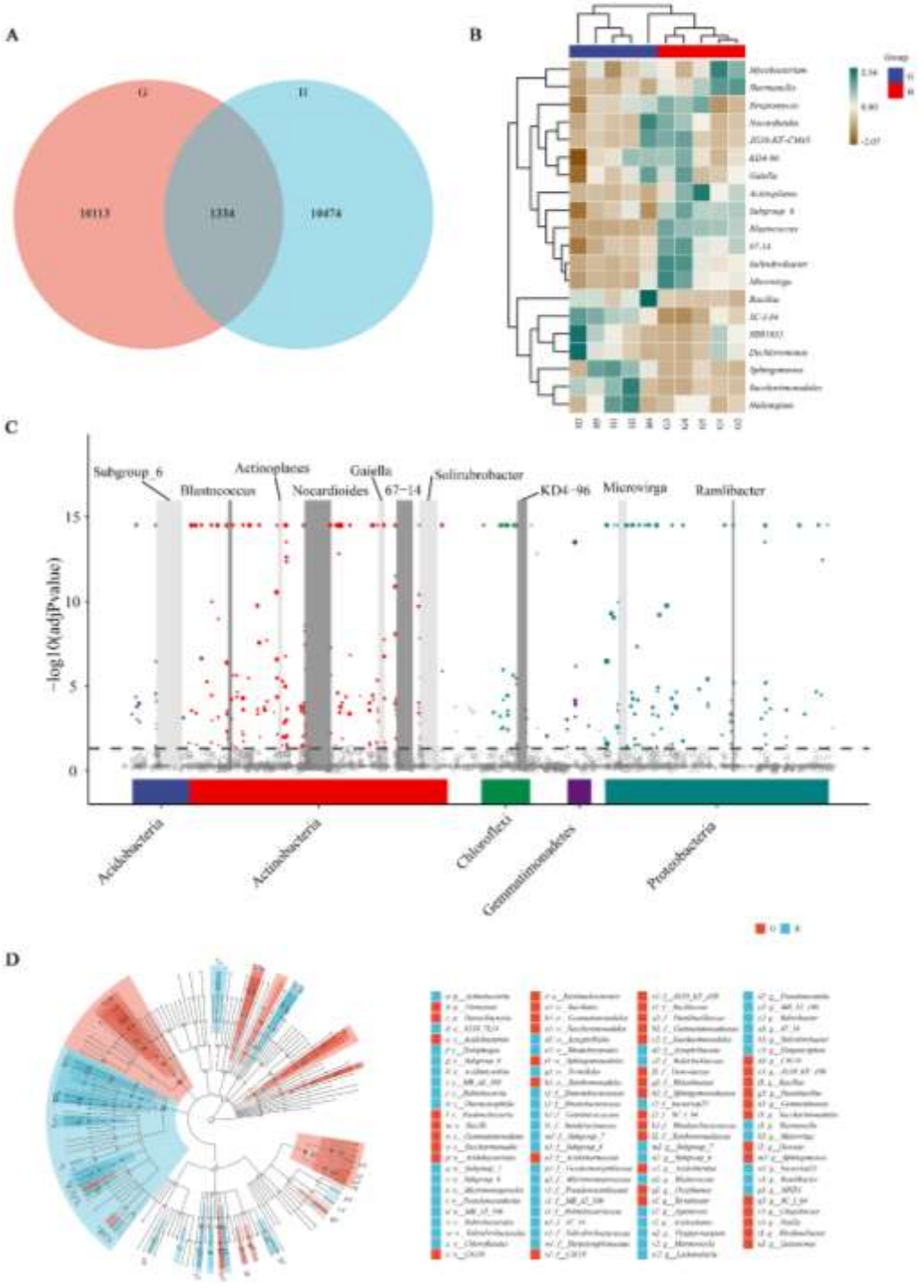

Figure 4. Analysis of species differences and marker species. **A.** ASV/OTU Venn diagram. **B.** genus level species composition heat map. **C.** Metagenomicseq analysis. **D.** Lefse analysis.

pathways, macromolecular modification, and metallic clusters to obtain insights into the metabolic processes of all life in soil microorganisms. The results showed that the biosynthetic pathways including prosthetic group, carbohydrate, vitamin biosynthesis,

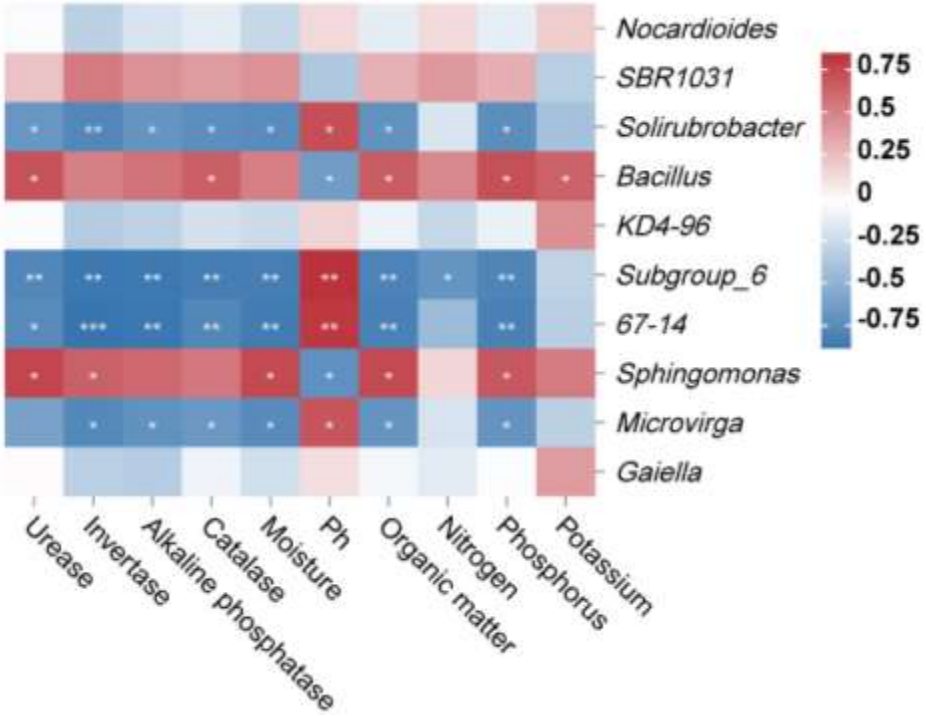

Figure 5. Association analysis between soil microbiota and soil physicochemical properties.

amino acid, cofactor, and electron carrier accounted for the majority of the relative abundance of soil microbiota (Figure 6A). By using Metagenomic-seq, the abundance data of metabolic pathways was further analyzed to identify any significant differences between groups. The results showed that the organic fertilizer significantly upregulated PWY-6174, ASPASN-PWY, and PWY0-42 pathways compared to the control group (Figure 6B). The pathway's species composition was analyzed by utilizing the metabolic pathway abundance table of the stratified samples (Figure 6C) (Supplementary Table S1). In the PWY-6174 pathway, the relative abundance of Marine_Group_II in the control group was observed to be higher than that in the organic fertilizer group ($P < 0.05$). Conversely, in the PWY0-42 pathway, the relative abundances of *Rhodanobacter*, *Chujaibacter*, *Luteimonas*, *Dyella*, and *Thermomonas* in the organic fertilizer group were significantly higher than that in the control group ($P < 0.05$). In the ASPASN-PWY pathway, the relative abundances of *Bacillus*, *Sphingomonas*, *Fonticella*, *Romboutsia*, *Chloroplast*, and *Tepidimicrobium* in the organic fertilizer group were significantly higher than that in the control group ($P < 0.05$).

Discussion

In this study, the soil microbiota applied with organic fertilizer was analyzed by using the 16S rRNA gene sequencing technology. The diversity index of soil microbial community reflects the complexity of soil microbial community structure. The higher the community alpha diversity indexes including Shannon index, Faith's PD index, Simpson index, and Chao1 index are, the more complex the microbial community structure is, the better the stability of the community is [20, 21]. There was significant difference in alpha diversity index between organic fertilizer group and control group ($P < 0.05$) in this study, indicating that the soil microbial community in organic fertilizer group had higher richness, diversity, and stability. The PCoA results further showed that the structural

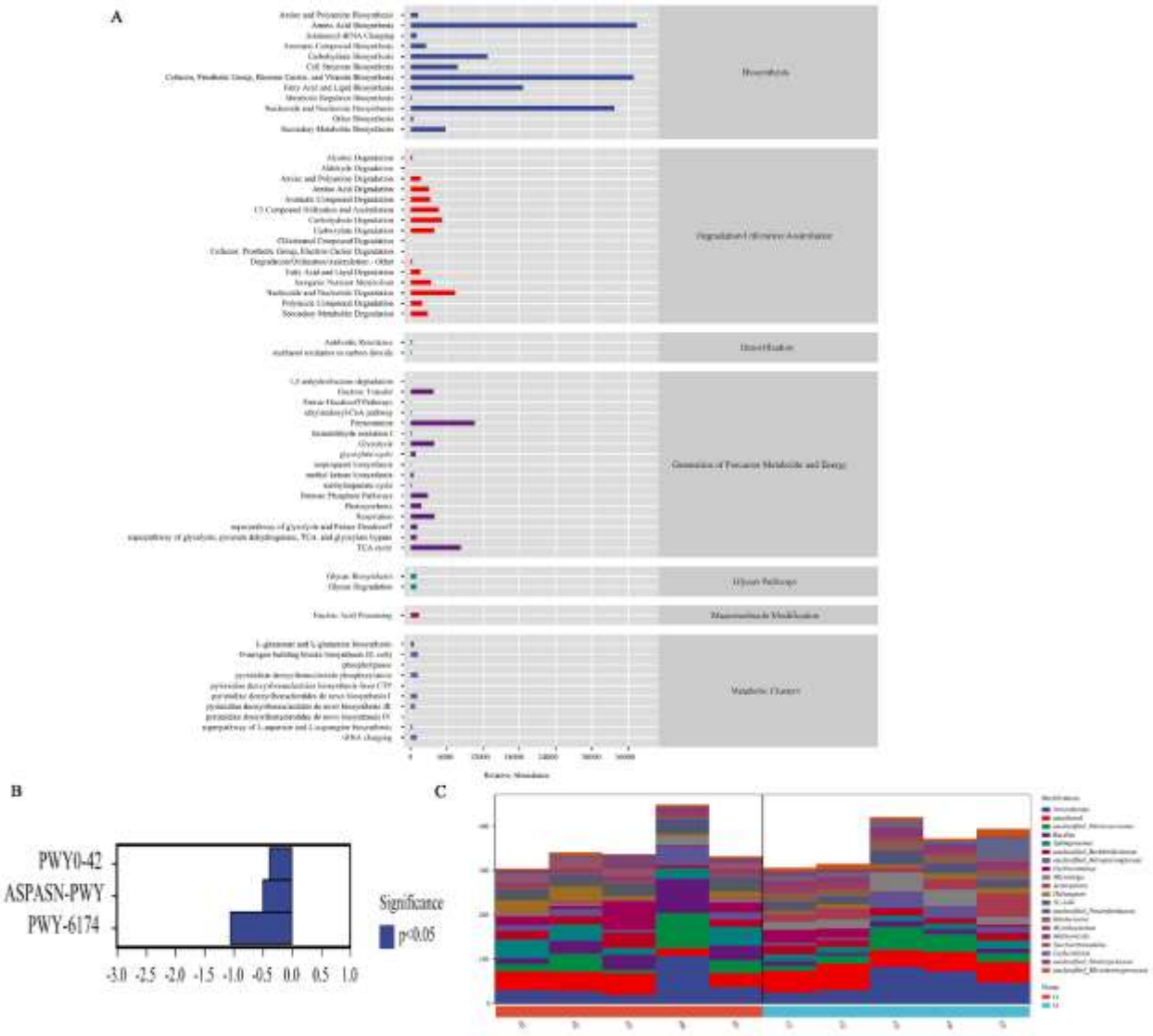

Figure 6. Difference analysis of metabolic pathway and its species composition. **A.** metabolic pathway statistics. **B.** differential analysis of metabolic pathways. **C.** species composition of metabolic pathways.

stability of soil microbial community in the organic fertilizer group might be better than that in the control group [22]. Furthermore, the smoothness of the sparse curve reflected the impact of sequencing depth on the observed sample diversity. The smoother the curve was, the more indicative it was of the sequencing results carrying enough information to reflect the diversity presented within the current samples. However, increasing sequencing depth beyond this point might result in the detection of a large number of undiscovered new ASV/OTUs [20]. At the phylum and genus levels, there were significant differences in species community compositions between the organic fertilizer and control groups. *Actinobacteria* and *Proteobacteria* were identified as the dominant bacteria in both groups with the relative abundance of *Firmicutes* being significantly higher in the organic fertilizer group than that in the control group. At the genus level, the relative abundances of *Bacillus*, SBR1031, and *Sphingomonas* in the organic fertilizer group were higher than that observed in the control

group. Conversely, the relative abundances of soil microorganisms including *Nocardioides*, *Solirubrobacter*, and *Microvirga* were lower in the organic fertilizer group than that in the control group. Yang, *et al*. reported that the application of rotten organic fertilizer significantly improved the physical and chemical properties of soil, which aligned with the results of this study [23]. Additionally, this study found that the relative abundance of *Firmicutes* was significantly higher in the organic fertilizer group than that in the control group. Moreover, at the genus level, the relative abundances of *Bacillus* and *Actinomycetes* were significantly higher in the organic fertilizer group than that in the control group, while the relative abundances of *Nocardioides*, *Solirubrobacter*, and *Microvirga* were lower in the organic fertilizer group. Differences in fecal microbial composition and available carbon source in organic matter could be important factors affecting soil bacterial diversity and community structure [24, 25]. The antibacterial substances produced by *Bacillus* could further enhance this effect by controlling various plant diseases [26]. *Bacillus thuringiensis* could produce a parasporal crystal during its formation and had become the largest microbial insecticide in the world [27]. In addition, *Bacillus* had biological activities such as phosphorus, potassium, and nitrogen fixation, which was conducive to improving crop yield [28]. *Bacillus* also had good stress resistance and had been widely used in the production of biological fertilizer and feed additives [29, 30]. *Actinoplanes* produced a variety of anti-bacterial antibiotics, which could produce creatmycin with special effects on *E. coli*. These microorganisms demonstrated strong prebiotic functions, which could not only inhibit plant pathogens, but also stimulate plant immunity, so as to enhance plant disease resistance [31-34]. Those results might explain why the physical and chemical properties of soil in organic fertilizer group were better than those in control group. There was an interactive relationship between soil microorganisms and plant roots. The growth and reproduction of soil microorganisms provided nutrient elements for plant growth. At the same time, plant roots also provided a living environment for microbial growth, indirectly selecting, and promoting the rhizosphere microorganisms of "survival of the fittest" [35]. The results of correlation analysis showed that *Bacillus* and *Sphingomonas* had a significant positive correlation with soil enzymes of urease, sucrose, alkaline phase, and catalase, while they had a significant negative correlation with pH, which indicated that *Bacillus* and *Sphingomonas* might play a crucial role in improving soil physical and chemical properties.

The microbial metabolic function prediction results revealed that the functional differences between the two groups of soil microorganisms were primarily associated with biosynthetic pathways including vitamin biosynthesis, carbohydrate biosynthesis, and amino acid biosynthesis among the others. Amino acids and trace elements in soil were crucial for crop growth and disease resistance [36]. For instance, glycine can enhance the absorption of phosphorus and potassium by crops, improve plant stress resistance [37, 38], and play a unique role in promoting plant growth, especially photosynthesis [39]. It increases chlorophyll content, improves enzyme activity, promotes carbon dioxide penetration, makes photosynthesis more vigorous, and contributes significantly to improving crop quality and increasing the content of VC and sugar. Further analysis indicated that PWY-6174, ASPASN-PWY, and PWY0-42 pathways were upregulated in the organic fertilizer group compared to the control group with the relative abundances of Marine_Group_II, *Bacillus*, and *Sphingomonas* being significantly higher than that in the control group. This finding indicated that the application of organic fertilizer might enhance soil physical and chemical properties through specific metabolic pathways by increasing the relative abundance of friendly microbiota in the soil. This study investigated the impacts of organic fertilizer on soil microorganisms and the physical and chemical properties of soil. The results demonstrated that organic fertilizer could significantly enhance the composition and structure of soil microbial communities and improve soil physical and chemical properties.

These findings suggested that organic fertilizer had the potential to alter soil properties, providing a basis for the substitution of chemical fertilizers and promoting sustainable agricultural development.

Availability of data and materials

The raw sequence files had been deposited in the National Center for Biotechnology Information Sequence Read Archive (Bethesda, Maryland, USA) and can be accessed by using the accession number PRJNA797718.

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