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Effects of organic fertilizers on plant growth and the rhizosphere microbiome

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ABSTRACT Application of organic fertilizers is an important strategy for sustainable agriculture. The biological source of organic fertilizers determines their specific functional characteristics, but few studies have systematically examined these functions or assessed their health risk to soil ecology. To fill this gap, we analyzed 16S rRNA gene amplicon sequencing data from 637 soil samples amended with plant- and animal-derived organic fertilizers (hereafter plant fertilizers and animal fertilizers). Results showed that animal fertilizers increased the diversity of soil microbiome, while plant fertilizers maintained the stability of soil microbial community. Microcosm experiments verified that plant fertilizers were beneficial to plant root development and increased carbon cycle pathways, while animal fertilizers enriched nitrogen cycle pathways. Compared with animal fertilizers, plant fertilizers harbored a lower abundance of risk factors such as antibiotic resistance genes and viruses. Consequently, plant fertilizers might be more suitable for long-term application in agriculture. This work provides a guide for organic fertilizer selection from the perspective of soil microecology and promotes sustainable development of organic agriculture.

IMPORTANCE This study provides valuable guidance for use of organic fertilizers in agricultural production from the perspective of the microbiome and ecological risk.

KEYWORDS metadata analysis, rhizosphere microbiome, metagenomics, antibiotic resistance genes, soil planet health

To address the challenge of the global food shortage crisis, chemical fertilizers have been extensively applied to crops (1). However, overuse of chemical fertilizers can result in soil acidification and compaction (2), cause declines in microbial function and diversity (3), and change microbial assembly processes (4). These changes can adversely affect soil ecology and food safety. Organic fertilizers are mainly derived or composted from plant- or animal-based materials, contain abundant nutrients, and carry various plant growth-beneficial microbes. They are a promising alternative to chemical fertilizers (1, 5, 6); however, the effects of organic fertilizers from different biological sources on plants and their risks to soil ecology remain poorly understood.

Organic fertilizers could avoid the adverse effects of synthetic fertilizers in traditional agricultural practices while improving soil nutrition and microbial communities (7, 8). In some cases, such as in apple orchards, organic fertilizer applied alone or with chemical fertilizer reduced damage from nitrate leaching and improved denitrification rate and efficiency (9). Long-term application of organic fertilizers also promoted soil functional traits and resilience (10). In addition, organic fertilizer could stimulate the interaction of protists and disease-suppressive bacteria, significantly reducing the abundance of pathogens and the dissemination of banana *Fusarium* wilt disease (8). However, organic fertilizers can also enrich risk factors in soil, such as heavy metals, microplastics,

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pathogens, viruses, and antibiotic resistance genes (ARGs) (11–14). A comprehensive evaluation of the impact of organic fertilizer on ecological health risks is needed.

Single case studies of organic fertilizer sometimes produce contradictory results due to the different experimental conditions such as soil properties, plant types, and fertilization strategies (15–17). For example, He et al. (15) and Han et al. (16) came to the opposite conclusion in their study of the effects of organic fertilizers on soil N-oxide emissions, possibly due to the difference in N content of fertilizers. He et al. reported that livestock manure fertilizer suppressed soil N-oxide emissions, while Han et al. observed a stimulation effect of livestock manure on soil N-oxide emissions. Single case studies and variability in organic fertilizer properties are thus not sufficient to reach universal conclusions. Metadata could provide a better understanding of the effect of organic fertilizers on the soil microbiome (18).

In this study, we used metadata to examine the effects of various types of organic fertilizers on soil microbial diversity and function. To verify this analysis, we constructed an experiment using pakchoi (*Brassica chinensis* L.), one of the most common vegetables in China. We determined effects on the soil microbiome and quantified the human health risk in soil after organic fertilizer application using metagenomic analysis. This study provides valuable guidance for use of organic fertilizers in agricultural production from the perspective of the microbiome and ecological risk.

MATERIALS AND METHODS

Data collection and preprocessing

We primarily collected articles related to organic fertilizer from Web of Science using "organic fertilizer," "16S," and organic fertilizer names such as "manure" and "slag" as the search terms. According to the accession number these articles provided, the 16S rRNA raw data were downloaded from the Sequence Read Archive database and the European Bioinformatics Institute database. We used the following selection criteria to minimize possible bias (19): (i) samples were not collected from treatments, such as pesticides, antibiotics, and heavy metals pollution; (ii) samples that had low quality sequence data and did not provide primer information were excluded; and (iii) accurate coordinate information was available for global analysis. After filtering, we used QIIME2 (v.2020.8) to classify and merge these raw data sets (20) and then removed adaptor and primer sequences from the reads using Cutadapt. The read quality of soil 16S rRNA gene data was assessed, and Dada2 (21) was used to quality control soil 16S rRNA gene data, denoise, and remove unnecessary sequences. Then, Dada2 was used to join paired-end reads (for pair-end reads) and to obtain amplicon sequence variants (ASVs).

Sequencing data consolidation

The merge-seqs and merge commands in QIIME2 were used to integrate the ASV representative sequences and ASV tables in different studies, respectively. The SILVA full-length gene sequence database was used to classify each 16S rRNA soil microbial representative sequence to eliminate PCR primer differences. Sequences in each sample were normalized to 5,000 reads, and rare reads (total sequence number <10) were removed. Sequences annotated to mitochondria and chloroplasts or that could not be classified at the kingdom level were also removed. The composition and abundance characteristic tables of soil microbes at different classification levels under different fertilization conditions were obtained and further used to calculate relative abundance.

Data classification and functional prediction

We obtained a total of 1,149 sets of 16S rRNA gene sequence data from 57 independent studies, including 14 types of organic fertilizer treatment (e.g., cow dung, goat manure, fish manure, horse manure, chicken manure, pig manure, earthworm manure, bamboo slag, bean fertilizer, seaweed fertilizer, weed fertilizer, city compost, wastewater sludge

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fertilizer, and unknown organic fertilizer). To further understand the effects of organic fertilizer on soil microbial function, we used PICRUSt (22) software to compare 16S rRNA gene sequencing data with the Greengene database and to predict functions associated with 16S ASVs. Then, the microbial community composition obtained by sequencing was mapped to the database and used to predict Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic functions of the microbial community.

Validation experimental design

Sterilized pakchoi seeds were cultivated in an artificial greenhouse using seedling boxes containing soil matrix (Shandong Mandeli Agricultural Technology Co., Ltd) for 2 weeks under 25°C ± 0.5°C, relative humidity of 80%, and 300-µmol photons/m^2/s cool-white fluorescent light (period of 12 h:12 h light:dark cycle). At the same time, we collected natural soil from United Village in Huzhou, China (30°34′21.2″N, 120°3′50.07″E). To provide a stable soil environment in the following cultivation test, soil samples were air-dried, homogenized, and sieved (<2 mm) beforehand, and then were mixed and incubated for a week in the same artificial greenhouse with soil matrix and reverse osmosis water in the ratio of 200 g:1,000 g:3,000 mL. After a week, four types of organic fertilizers were added to the cultured soil in the ratio of 200-g fertilizer:200-g natural soil, respectively.

These fertilizers were all composted, fermented commercial organic fertilizers provided by the Institute of Environmental Resources and Soil Fertilizer, Zhejiang Academy of Agricultural Sciences, China. Among them, the two animal-derived organic fertilizers (hereafter animal fertilizers) were fermented pig manure and chicken manure, while two plant-derived organic fertilizers (hereafter plant fertilizers) were fermented tree leaves and tea slag, respectively. We determined the initial content of important nutrients in the organic fertilizers and experimental soil beforehand, including phosphorus, potassium, ammonium nitrogen, and nitrate. The initial content of nutrients in four organic fertilizers are shown in Table S1. We added inorganic salts to a final ratio of N:K:P = 5:7:8 in the mixed soil and then continually incubated for 1 week. In addition, the control group added the same amount of soil matrix to ensure the total soil mass and elemental content were equal to the fertilizer treatment groups.

Two weeks later, seedlings were transferred into polycarbonate pots (200 mL) containing 150-g mixed soil mentioned above and cultivated in the same artificial greenhouse with the same environmental conditions. Four parallel groups were set up for each type of organic fertilizer treatment, and each group included five pakchoi seedlings. After 4 weeks of growth, we measured the aboveground and belowground weight of seedlings and collected 30 g of rhizosphere soil according to Lu et al. (23). We pooled rhizosphere soil samples from five plants in each parallel group as one sample.

DNA extraction and metagenomic sequencing

DNA extraction and metagenomic sequencing were performed using rhizosphere soil samples of different plants, as described previously (24). We weighed 0.5-g rhizosphere soil samples from each parallel group and extracted total DNA using a PowerSoil DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, USA). DNA quality and concentration were determined using an ND-1000 spectrophotometer (NanoDrop Technology, Wilmington, USA) and a Qubit (v.2.0) fluorometer (Thermo Fisher Scientific, Massachusetts, USA), respectively. DNA samples were stored at −20°C for further use.

A NEB Next Ultra DNA Library Prep Kit (New England Biolabs, Massachusetts, USA) was used according to the manufacturer's instructions to generate libraries for metagenomic sequencing. Qubit (v.3.0) fluorometry (Life Technologies, New York, USA) and electrophoresis (Agilent 4200; Agilent, California, USA) were used to assess the library quality. Subsequently, metagenomic sequencing was performed on an Illumina Novaseq platform (Illumina, California, USA).

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Assembly and analysis of metagenomic sequences

Raw reads were trimmed and filtered using Fastp (v.0.20.0). Data were further cleaned from contamination with host sequences using SoapAligner software for quality control. Then, the optimized sequence was assembled using Megahit (v.1.1.2), and the assembled scaffolds were interrupted at the N junction to obtain the sequence fragment without N, which is called scaftigs, with the shortest length of ≥500 bp. Next, open reading frames were predicted in each sample and assembled scaftigs using MetaGeneMark. The predicted gene sequences from all samples were clustered using CD-HIT (v.4.7) to obtain unique initial gene (unigene) clusters (95% identity with 90% coverage). The clean data were mapped to unigenes using BBMAP, and the gene abundance per sample was calculated as reads per kilobase per million mapped reads using SoapAligner based on the mapped reads number and gene length. The unigenes were mapped to the taxonomic information database of the NR database and Comprehensive Antibiotic Resistance Database using DIAMOND software (v.0.8.35) for species and ARG information, respectively. According to our previous study (25), we quantified the risk of ARGs to human health [risk index (RI)] based on three indicators, human accessibility, mobility, and human pathogenicity, and classified the ARGs as different ranks (Q1–Q4). DIAMOND software (v.0.8.35) was also used to compare unigenes with the KEGG database. We filtered the comparison results of each sequence and selected comparison results with HSP of >60 bits for further analysis.

Statistical analysis

For comparisons of microbial taxa and functional gene abundance between different groups, one-way analysis of variance (ANOVA) or analysis of similarities (Anosim) was used to calculate differences between groups. Differences were considered statistically significant if the *P* value is <0.05. Principal coordinate analysis (PCoA) of soil microbes and KEGG functional genes based on the Bray-Curtis distance and the alpha diversity (richness and Shannon index) of soil microbes and ARGs was performed using the vegan package (v.2.5–7) in RStudio and visualized in GraphPad Prism (v.8.0.2). The histograms and box plots were also generated using GraphPad Prism (v.8.0.2). Heatmaps of soil microbe abundance and biogeochemical cycle function gene abundance in different fertilizer groups were constructed using TBtools. All Venn diagrams were generated using EVenn (v.1.0, http://www.ehbio.com/test/venn). The network analysis at the genus level was performed based on a significant Spearman correlation matrix and visualized using the Gephi platform (v.0.9.7) based on the Fruchterman Reingold algorithm. To eliminate the influence of rare microbes in the networks, we focused on the microbes that were detected in at least 75% of the samples. The advanced volcano plots were performed using the OmicStudio tools at https://www.omicstudio.cn/tool. The world map and detailed map were constructed using the ggmap package (v.3.0.2) in RStudio.

RESULTS

Metadata analysis reveals the effect of organic fertilizers on soil microbial function

We collected and analyzed 152 samples treated with animal fertilizer, 238 samples treated with plant fertilizer, and 247 control samples (Fig. S1). PCoA revealed distinct patterns in the community of soil microbes after organic fertilizer treatments (Fig. 1A; $P = 0.001$, $R^2 = 0.3985$, Anosim). Richness (Fig. 1B; $P < 0.05$, one-way ANOVA) was significantly increased only in the animal fertilizer treatments. Co-occurrence networks and the topological attributes of networks (Fig. S2) indicated that average connectivity (average links per node, average *K*) of the network was much stronger in animal fertilizer-applied soil than others and so was closeness centrality (the shortest distance between a node and any other point) (Fig. 1C). Both animal and plant fertilizers decreased the betweenness centrality (the ability to control information transfer of other nodes) of microbial community networks compared to the control.

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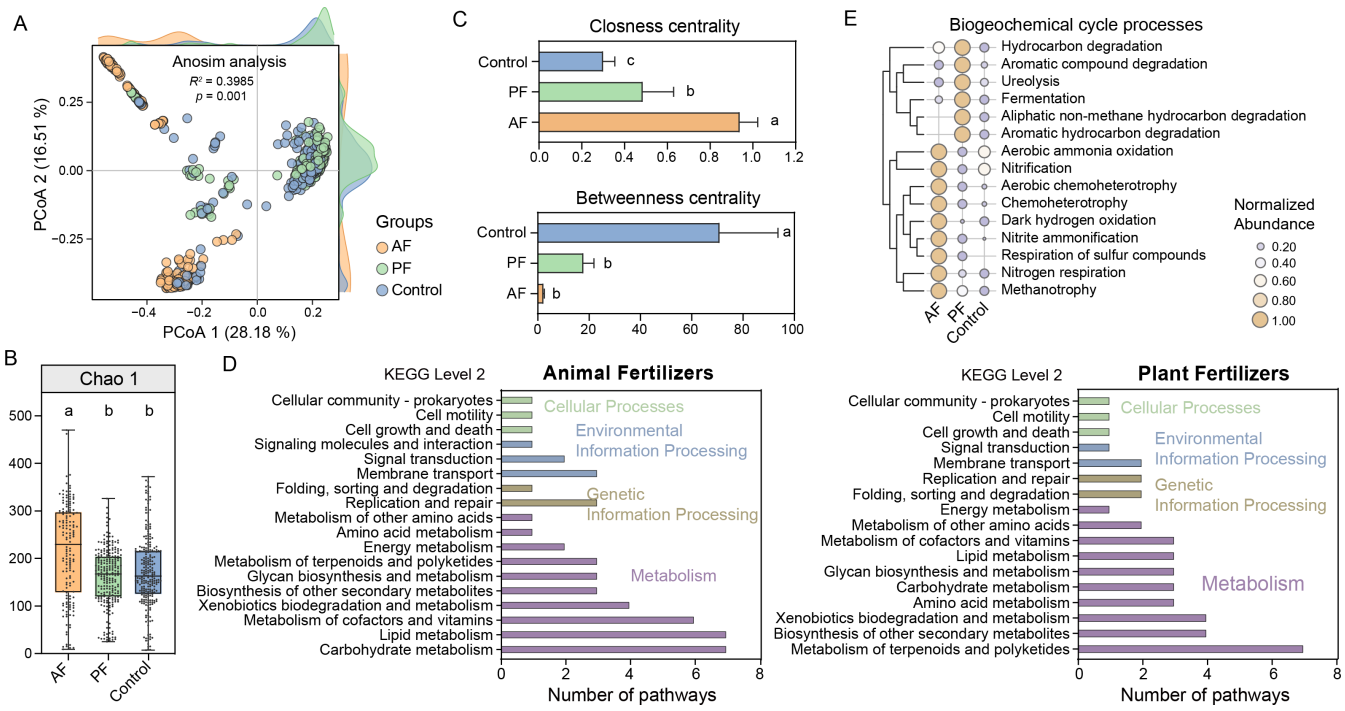


FIG 1 The effect on soil microbial communities and ecological functions of fertilization types by metadata analysis. "Control" represents no fertilizer application groups. (A) PCoA of Bray-Curtis dissimilarity with Anosim of the microbes in the three groups. The density plots indicate the difference in the structure of microbial communities along the PCoA 1 and PCoA 2 axes. (B) The richness of soil microbiome that applied three different fertilization managements. Different letters represent significant differences among the three groups. (C) The topological attributes (betweenness centrality and closeness centrality) of co-occurrence networks of microbes in animal fertilization, plant fertilization, and no fertilization soil. Different letters represent significant differences among the three groups. (D) Number of significantly upregulated KEGG functional pathways (second level) in the two groups. The different colors represent different classes of pathways at the first level. (E) The heatmap showed the 15 biogeochemical cycle processes that were significantly upregulated in soil treated with animal and plant fertilizers. Moreover, the size of the circle and the color scale represent the predicted functional gene abundance in three groups. AF, animal fertilizer; PCoA, principal coordinate analysis; PF, plant fertilizer.

We predicted microbial functions and found that animal and plant fertilizers could mainly upregulate the metabolism pathway in the microbial community functions (Fig. 1D). In detail, animal fertilizer mainly increased the abundance of carbohydrate metabolism (e.g., ko00030, ko00040, ko00051, ko00053, ko00500, and ko00520), lipid metabolism (e.g., ko00100 and ko00564), and metabolism of co-factors and vitamins (e.g., ko00670, ko00740, ko00750, ko00760, and ko00785) at the second level of metabolism pathway, while plant fertilizer mainly increased the abundance of metabolism of terpenoids and polyketides (ko00900), carbohydrate metabolism (ko00051 and ko00630), and glycan biosynthesis and metabolism (ko00550) at the second level of the metabolism pathway. Based on the FAPROTAX database, we performed functional annotation of the soil microbiome and found there were 15 biogeochemical cycle processes significantly increased in animal or plant fertilizer-applied soil than that in control (Fig. 1E). Nitrogen cycle processes such as aerobic ammonia oxidation, nitrification, nitrite ammonification, and nitrogen respiration were significantly promoted by animal fertilizer, and carbon cycle processes such as hydrocarbon degradation, aromatic compound degradation, and aromatic hydrocarbon degradation were significantly promoted by plant fertilizer.

Validation experiment verifying the effect of organic fertilizers on plant growth and soil microbial communities

To verify the effect of organic fertilizers on microbial communities, we performed a validation experiment using typical organic fertilizers and the common vegetable

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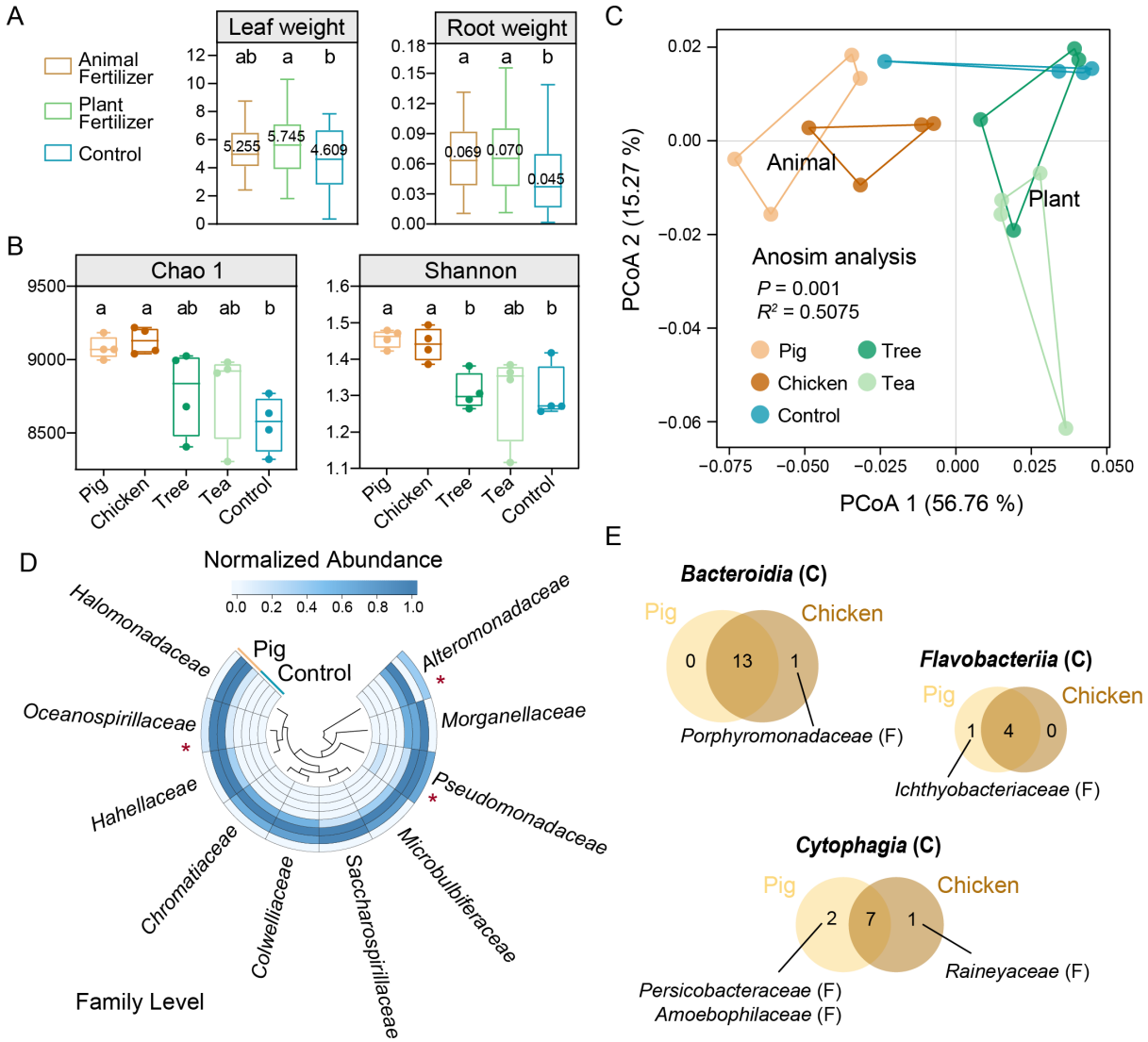


FIG 2 The effect of animal and plant fertilizers on the growth of plant and rhizosphere microbiome. The "pig," "chicken," "tree," and "tea" represent different derived organic fertilizers, pig manure, chicken manure, tree leaf, and tea slag, respectively. (A) The effect of two fertilizer treatments on the leaf and root weight of pakchoi. Different letters represent significant differences between the two groups. (B) The alpha diversity (Shannon and Chao 1 index) of the microbes in the five soil types. Different letters represent significant differences between the two groups. (C) PCoA of Bray-Curtis dissimilarity with Anosim of the microbes in the five soil types. (D) The circle heatmap shows changes in the abundance of Gammaproteobacteria (class) between the control group and the pig manure group. (E) The Venn plot indicates the special and shared families between the two fertilization types of soil from enriched classes. PCoA, principal coordinate analysis.

pakchoi (*Brassica chinensis* L.). Both animal and plant fertilizers significantly increased the weight of leaves and roots, and plant fertilizers showed a stronger positive effect (Fig. 2A). Animal fertilizer significantly ($P < 0.05$, two-tailed Student *t*-test) increased the alpha diversity (Shannon and Chao 1 index) of rhizosphere microbial communities, while plant fertilizer only increased this slightly (Fig. 2B). Meanwhile, according to the Bray-Curtis distance, animal fertilizers significantly ($P < 0.05$, two-tailed Student *t*-test) changed the structure of the microbial community (Fig. 2C; Fig. S3). Different derived organic fertilizers displayed different effects on microbial communities. For example, pig manure increased the abundance of Proteobacteria (Fig. S4A), such as Pseudomonadaceae, Alteromonadaceae, and Oceanospirillaceae (families) from Gammaproteobacteria (class) (Fig. S4B; Fig. 2D); chicken manure increased the abundance of Porphyromonadaceae and Raineyaceae (Fig. S4C; Fig. 2E). As for plant fertilizer treatments, the tea

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slag changed the composition of the microbial community by enriching Bacteroidetes (phylum), specially enriching the class Chitinophagia (Fig. S4C).

Organic fertilizers increase the stability of microbial interaction networks

For microbial interactions at the genus level (detected in at least 75% of samples), the complexity of the soil microbial network (L = 3191) and the average connectivity (average K = 2.78) in the plant fertilizer treatments were higher than in animal fertilizer treatments (Fig. 3A and B). Similarly, the average clustering coefficient and betweenness centrality were also higher in the plant fertilizer treatments (Fig. 3C and D). The ratios of negative edges, modularity, and closeness centrality in animal fertilizer-treated microbial networks were much higher. We calculated the robustness value (natural connectivity) of the microbial network after each random node was removed (at most 400 nodes), and the results demonstrated that the connectivity values of the soil microbial network were higher in the plant fertilizer treatment (Fig. 3E).

Organic fertilizers change microbial functional profiles

The two animal fertilizers significantly (P < 0.05, one-way ANOVA) changed some microbial functional gene compositions (Fig. 4A). Differential enrichment analysis

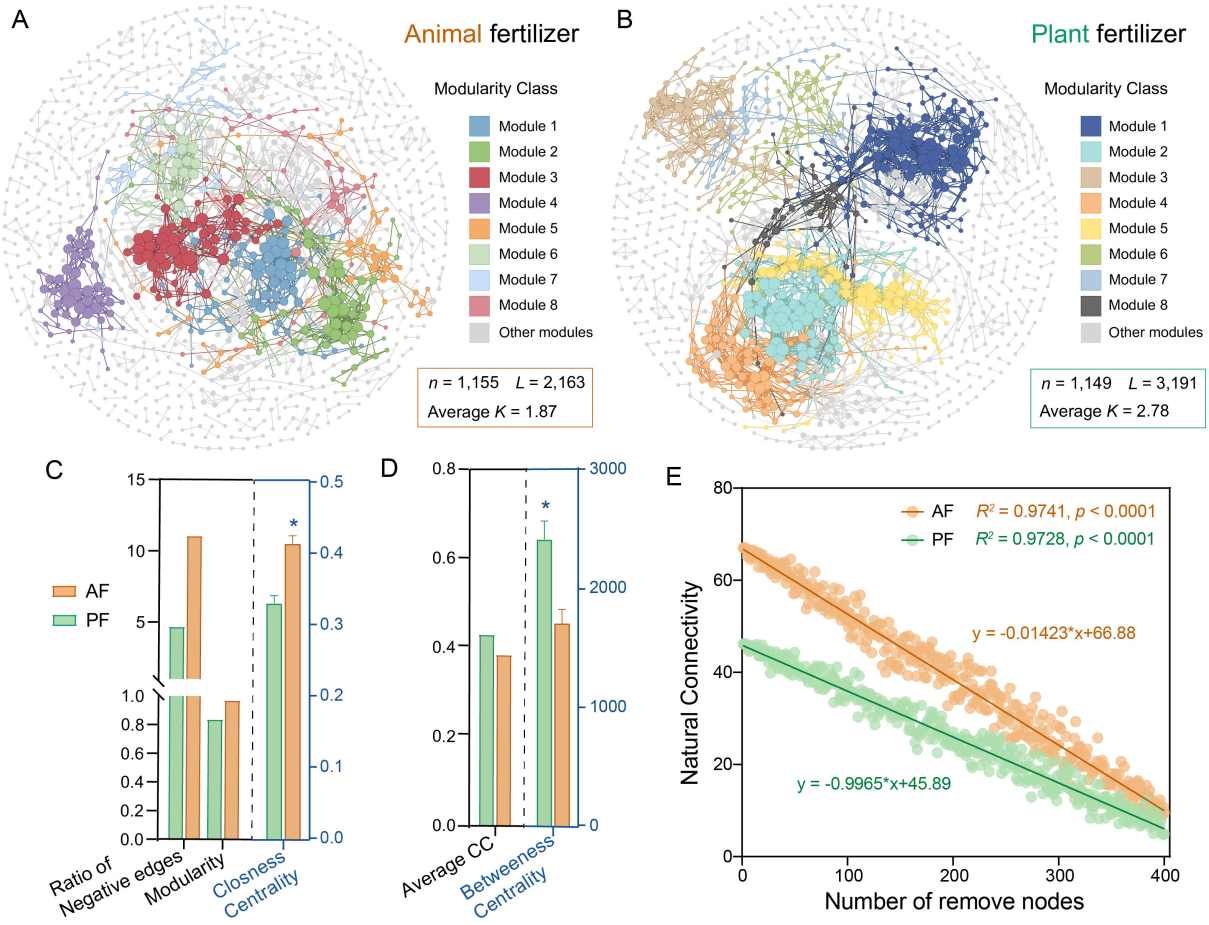


FIG 3 Co-occurrence network analysis and the topological attributes of networks. (A and B) The co-occurrence network is based on the spearman correlation matrix of animal and plant fertilizer-applied soil microbe genera (detected in at least 75% of samples). (C and D) The topological attributes of networks, including the ratio of negative edges, average *CC*, modularity, betweenness centrality, and closeness centrality. The asterisks represent significant differences between the two groups. (E) The robustness of two networks. The natural connectivity of the microbial network was calculated for every random node removed. "AF" and "PF" represent animal fertilizers and plant fertilizers, respectively. Average *K*, the average links per node; CC, clustering coefficient; *L*, the total number of links; *N*, the total number of nodes.

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indicated that biofilm formation genes (*K20963*) were at significantly higher abundance in both animal fertilizer treatments (Fig. S5). Pig manure mainly increased the abundance of carbohydrate metabolism pathway genes, while chicken manure increased abundance of genes involved in the metabolism of cofactors and vitamins (Fig. 4B). Genes related to metabolism of terpenoids and polyketides (*K14215*) and amino acid metabolism (*K19312*) significantly increased in tea slag treatments, while the genes involved in metabolism of cofactors and vitamins (*K00603*) and xenobiotic biodegradation and metabolism (*K20712*) significantly increased in the tree leaf treatment (Fig. S6). Meanwhile, the patterns of functional genes involved in carbon, nitrogen, phosphorus,

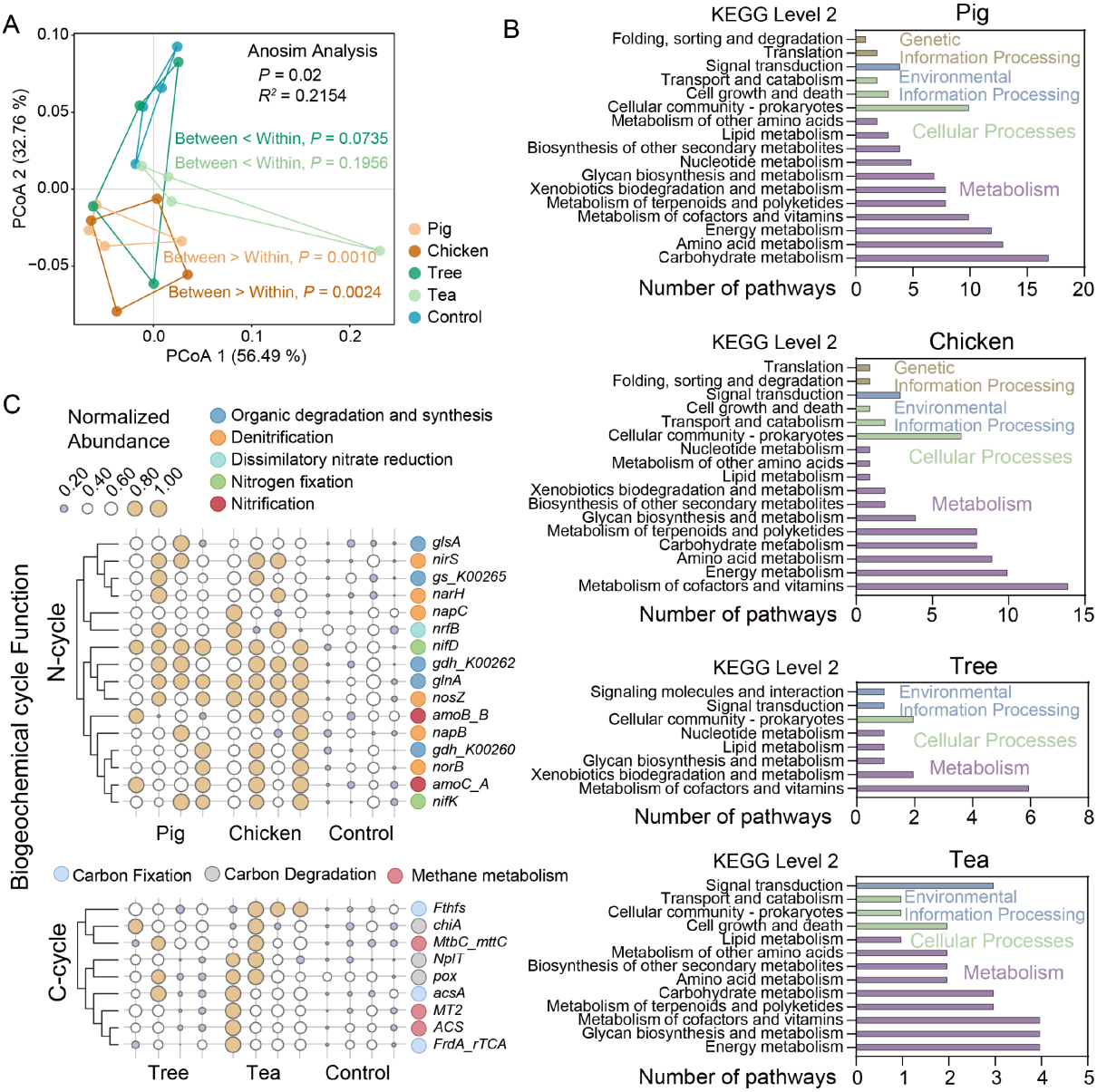


FIG 4 Functional variability in the microbiome between different fertilizations. "Pig," "chicken," "tree," and "tea" represent different derived organic fertilizers, pig manure, chicken manure, tree leaf, and tea slag, respectively. (A) PCoA of Bray-Curtis dissimilarity with Anosim of the microbial function in the five soil types. The "between" represents the distance between the fertilizer treatment and the control group, while the "within" represents the distance within each treatment group. The *P* value was calculated based on the Bray-Curtis distance with ordinary one-way ANOVA. (B) Number of significantly upregulated KEGG functional pathways (second level) in the four soil types. The different colors represent different classes of pathways at the first level. (C) The heatmaps show the abundance of the nitrogen cycling functional genes in animal fertilizers and the carbon cycling functional genes in plant fertilizers. Different color circles represent different functions genes belonged to. PCoA, principal coordinate analysis.

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and sulfur cycling among the three soil types were different (Fig. S7). Compared with the control, animal fertilizers significantly ($P < 0.05$, two-tailed Student *t*-test) enriched genes related to carbon and nitrogen cycling. Among them, animal fertilizers promoted the nitrogen cycle potential mainly through increasing the abundance of genes referred to organic degradation and synthesis of nitrogen, denitrification, and nitrification (Fig. 4C). Although plant fertilizers had a slight effect on the total carbon cycle, we still found that the genes related to carbon fixation, carbon degradation, and methane metabolism were significantly ($P < 0.05$, two-tailed Student *t*-test) enriched in plant fertilizer treatments.

Animal fertilizers have ecological health risks

The abundance and number of viruses related to human diseases and high-risk ARGs (Q1 level) were significantly increased in animal fertilizer treatments (Fig. 5; Fig. S8). Among them, we detected 56 viruses related to human disease, mostly related to bacterial infectious diseases and neurodegenerative diseases. It was worth noting that animal fertilizer significantly increased the abundance of viruses involved in neurodegenerative diseases and antimicrobial drug resistance (Fig. S9). At the same time, plant fertilizers showed a lower virus pollution risk. In addition, a total of 253 higher-risk ARGs (RI >0) and 289 low-risk ARGs (RI = 0) were detected in three groups. In detail, the diversity of risk ARGs (Fig. S10A) and among four fertilizers, pig manured soil significantly enriched macrolide antibiotic resistance genes (e.g., *mexA*, *mexB*, *mexK*, and *oprM*) and aminoglycoside antibiotic resistance genes [e.g., *aad(6)* and *AAC(6')-Ib7*] (Fig. S10B). The abundance of various ARGs, such as macrolide antibiotic resistance genes (e.g., *macB* and *ErmC*), sulfonamide antibiotic resistance genes (e.g., *sul1*), tetracycline resistance genes [e.g., *tet(L)*], and aminoglycoside antibiotic resistance genes [e.g., *ANT*

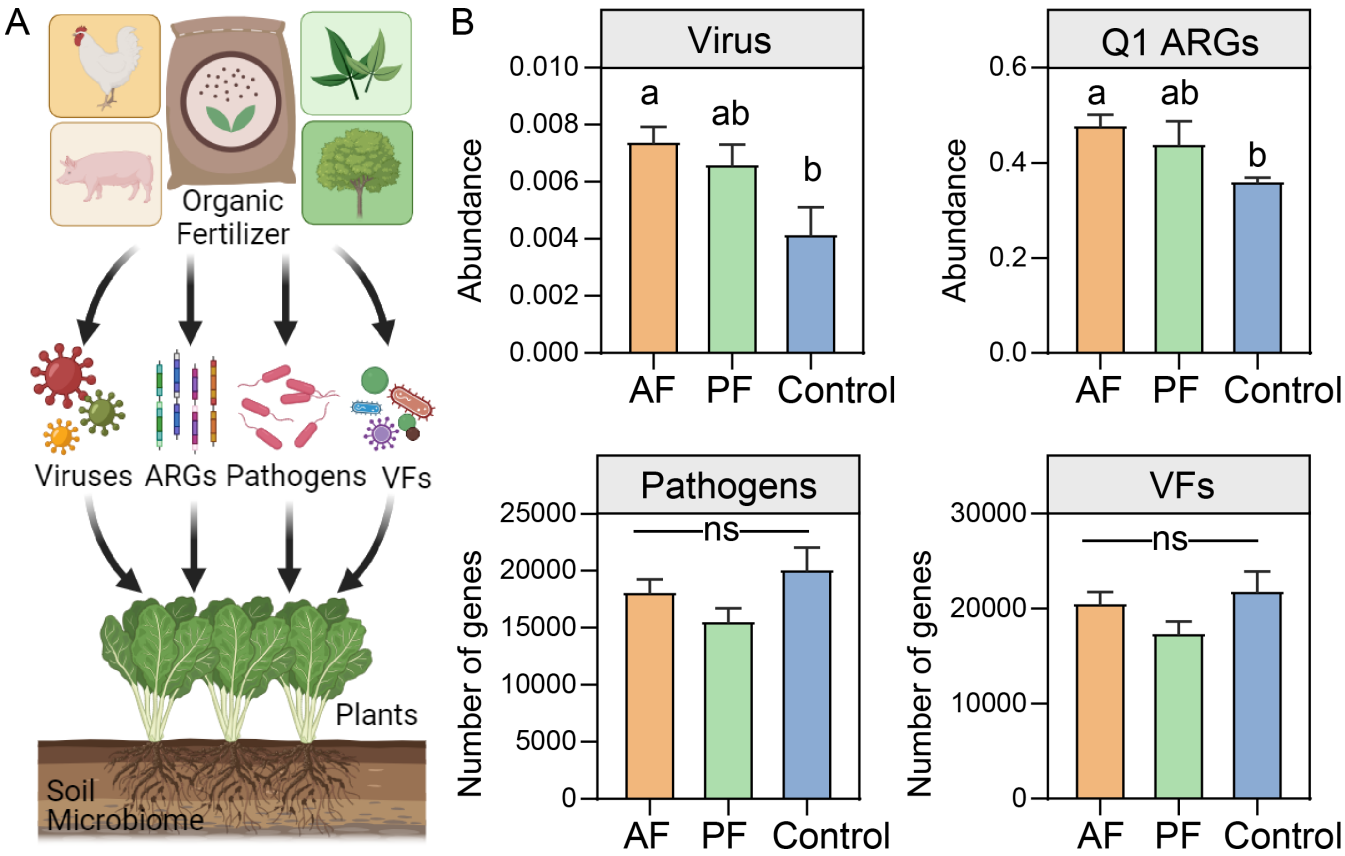


FIG 5 The effect of organic fertilization on human health risk in soil. (A) Schematic diagram of introducing human health risk factors into soil from organic fertilizers. (B) The abundance or number of genes of human health risk factors in soil, including virus, ARGs (Q1 level), pathogens, and VFs. AF, animal fertilizer; ns, not significant; PF, plant fertilizer; VF, virulence factor.

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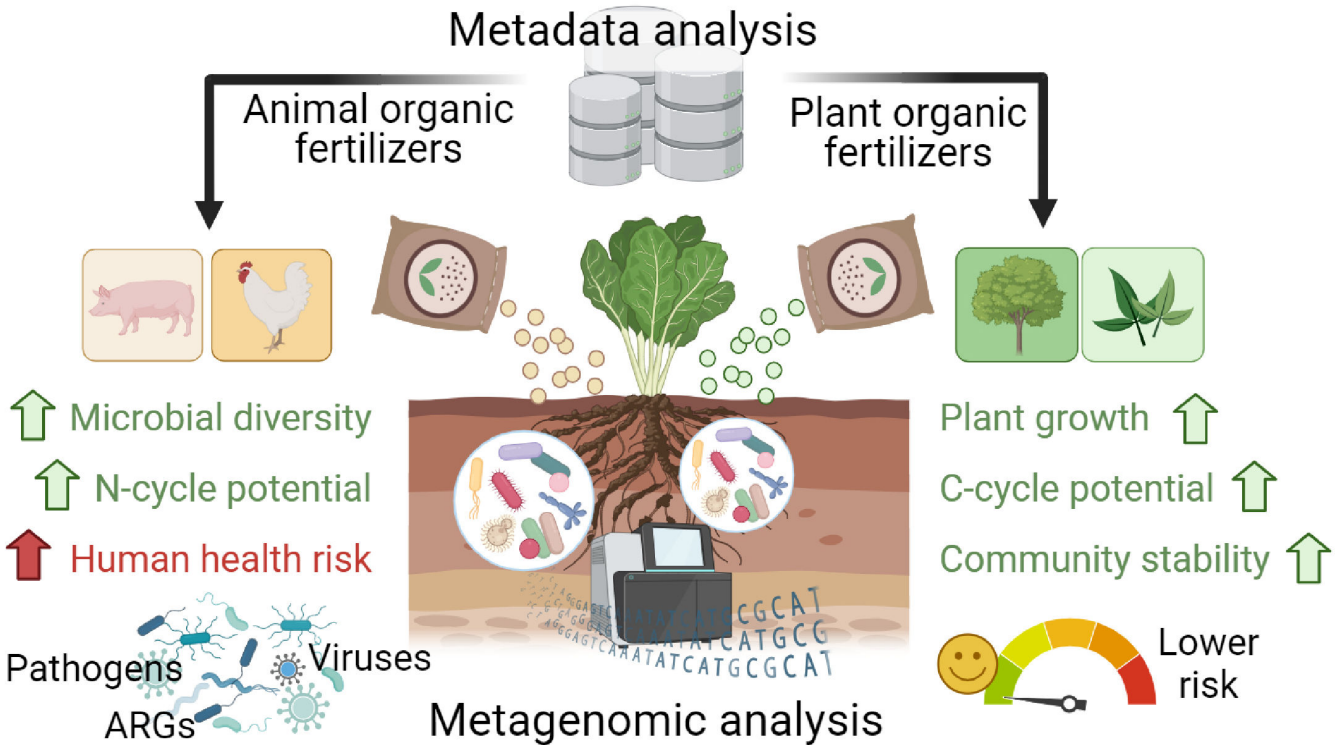


FIG 6 Schematic diagram of the methods of this study and comparison of advantages and disadvantages of animal- and plant-derived organic fertilizers.

(6)-la], was significantly increased by chicken manure application. The application of tree leaf fertilizer only enriched a macrolide antibiotic resistance gene, *mtrD*. The numbers of pathogens and virulence factor (VFs) genes were relatively lower in plant fertilizer treatments (Fig. 5B).

DISCUSSION

Composted and processed modern commercial organic fertilizers are widely applied in organic agriculture (26) and can effectively increase crop yields by activating soil-borne beneficial microorganisms (27, 28), stimulating rhizosphere microbial interactions (8), and upregulating biogeochemical cycles (29). Previous studies have shown that the main reason for the higher microbial diversity in soils treated with organic fertilizer is the activation of soil-borne microbial genera, which are mostly rare microbes (low abundance but present) in unfertilized soils (27).

Through metadata analysis, we found that there were differences in the modification of soil microbial community caused by plant fertilizer and animal fertilizer, and more details were subsequently acquired by our experimental verification and metagenomic sequencing. First, animal fertilizers greatly increased the diversity of rhizosphere microbial communities. Their input of organic matter is greater than plant fertilizers (30), which could drive larger shifts in microbial community structure and keystone taxa associated with crops (31, 32). Both animal and plant fertilizers increased the stability (e.g., closeness centrality) of rhizosphere microbial communities (Fig. 1C), especially evident in the plant fertilizer-applied microbial communities. Therefore, we speculated that the nutrient turnover and ecosystem processes in plant fertilizer treatments were likely more active (33, 34). We also demonstrated that both animal and plant fertilizers improve soil health by affecting important indicators of diversity and stability in the soil microbial community.

In addition, we showed that application of animal and plant fertilizers potentially strengthens microbial metabolic pathways in soil and rhizosphere, which could improve

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nutrient cycling or decomposition in the soil and rhizosphere (35, 36). Interestingly, animal and plant fertilizers might affect microbial metabolism in different pathways. For instance, animal fertilizer increased the metabolic potential of lipid and vitamin pathways, which could enhance microbial resistance to external pollution. The abundance of terpene, polyketide and glycan metabolism was enriched in plant fertilizer treatment soils, which could affect photosynthesis, seed germination, bolting, and flowering (37–40), thereby improving the yield of crops (41, 42). Moreover, the biogeochemical potential of soil microbes also differed between the animal and plant fertilizer treatments. The animal fertilization mainly enriched nitrogen cycle pathways, while plant fertilizer mainly enriched carbon cycle pathways. This may be related to the fact that animal and plant fertilizers effectively activated nitrogen fixing and carbon fixing bacteria in soil (43), respectively.

Organic fertilizer not only improved soil nutrient content and potentially activated beneficial bacteria but also enriched ecological risk factors in soil. Many studies have shown that application of organic manure from livestock and poultry enhanced ARG dissemination in soil ecosystems (13, 44, 45). As expected, our study found that two animal fertilizers significantly increased the health risk of ARGs in soil. Qian et al. (46) showed that ARG diversity and abundance were higher after poultry manure treatment, with most ARGs conferring resistance to aminoglycosides, macrolides and tetracycline. Similar results were obtained in the present study, where animal fertilizers mainly enriched high-risk ARGs (Q1 level) to macrolides and aminoglycosides. In comparison with plant fertilizers, animal fertilizer significantly increased the abundance of viruses related to human diseases and enriched pathogens and virulence factors in soil, causing serious soil ecological risk (47). Therefore, plant fertilizers are relatively safer in agricultural production.

Conclusion

The application of organic fertilizers represents a recent innovation that has been embraced in sustainable agriculture. This shift is in response to the increasing global food demand, the unpredictable nature of the environment, and the detrimental effects associated with the excessive use of chemical fertilizers, including environmental degradation, soil fertility reduction, and pollution. This study found that animal fertilizer not only increased microbial diversity but also resulted in higher human health risks (Fig. 6). On the contrary, plant fertilizer promoted the growth of plants and improved the stability of the soil microbial community with lower consequent health risks. Hence, plant fertilizers should perform better in agricultural production. Predictions about the ecological functions of soil microbes need to be validated by further genomics and transcriptomics analysis in the future.

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AUTHOR CONTRIBUTIONS

Yitian Yu, Conceptualization, Funding acquisition, Methodology, Resources, Writing – review and editing | Qi Zhang, Conceptualization, Conceptualization, Funding acquisition, Funding acquisition | Jian Kang, Conceptualization, Methodology, Visualization | Nuohan Xu, Conceptualization, Visualization | Zhenyan Zhang, Conceptualization, Funding acquisition | Yu Deng, Conceptualization, Writing – original draft | Michael Gillings, Writing – review and editing | Tao Lu, Funding acquisition, Investigation, Resources, Writing – review and editing | Haifeng Qian, Conceptualization, Funding acquisition, Methodology, Resources, Writing – review and editing

DATA AVAILABILITY

Metadata of 1,149 16S rRNA gene sequencing data of soil bacterial communities used in this study are listed in Table S2. Metagenomic sequencing data were deposited to the National Center for Biotechnology Information's Sequence Read Archive under the project accession number PRJNA1045731. All scripts and codes for machine learning, visualization, bioinformatics, and statistical analysis used in this study are available on GitHub.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental figures and table (AEM01719-23-S0001.docx). Fig. S1 to S10 and Table S1.
Table S2 (AEM01719-23-S0002.docx). The table of metadata information.

REFERENCES

1. Wang T, Cheng KK, Huo XJ, Meng PP, Cai ZH, Wang ZK, Zhou J. 2022. Bioorganic fertilizer promotes pakchoi growth and shapes the soil microbial structure. Front Plant Sci 13:1040437. https://doi.org/10.3389/fpls.2022.1040437
2. Wang C, Zheng MM, Song WF, Wen SL, Wang BR, Zhu CQ, Shen RF. 2017. Impact of 25 years of inorganic fertilization on diazotrophic abundance and community structure in an acidic soil in Southern China. Soil Boil Biochem 113:240–249. https://doi.org/10.1016/j.soilbio.2017.06.019
3. Liang YT, Ning DL, Lu ZM, Zhang N, Hale L, Wu LY, Clark IM, McGrath SP, Storkey J, Hirsch PR, Sun B, Zhou JZ. 2020. Century long fertilization reduces stochasticity controlling grassland microbial community succession. Soil Boil Biochem 151:108023. https://doi.org/10.1016/j.soilbio.2020.108023
4. Feng MM, Adams JM, Fan KK, Shi Y, Sun RB, Wang DZ, Guo XS, Chu HY. 2018. Long-term fertilization influences community assembly processes of soil diazotrophs. Soil Boil Biochem 126:151–158. https://doi.org/10.1016/j.soilbio.2018.08.021
5. Gouda S, Kerry RG, Das G, Paramithiotis S, Shin H-S, Patra JK. 2018. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol Res 206:131–140. https://doi.org/10.1016/j.micres.2017.08.016
6. Dincă LC, Grenni P, Onet C, Onet A. 2022. Fertilization and soil microbial community: a review. Applied Sciences 12:1198. https://doi.org/10.3390/app12031198

Downloaded from https://journals.asm.org/journal/aem on 17 June 2025 by 188.119.36.238.

7. Chowdhury SP, Babin D, Sandmann M, Jacquiod S, Sommermann L, Sørensen SJ, Fliessbach A, Mäder P, Geistlinger J, Smalla K, Rothballer M, Grosch R. 2019. Effect of long-term organic and mineral fertilization strategies on rhizosphere microbiota assemblage and performance of lettuce. Environ Microbiol 21:2426–2439. https://doi.org/10.1111/1462-2920.14631
8. Guo S, Tao C, Jousset A, Xiong W, Wang Z, Shen Z, Wang B, Xu Z, Gao Z, Liu S, Li R, Ruan Y, Shen Q, Kowalchuk GA, Geisen S. 2022. Trophic interactions between predatory protists and pathogen-suppressive bacteria impact plant health. ISME J 16:1932–1943. https://doi.org/10.1038/s41396-022-01244-5
9. Kramer SB, Reganold JP, Glover JD, Bohannan BJM, Mooney HA. 2006. Reduced nitrate leaching and enhanced denitrifier activity and efficiency in organically fertilized soils. Proc Natl Acad Sci USA 103:4522–4527. https://doi.org/10.1073/pnas.0600359103
10. Luo JP, Liao GC, Banerjee S, Gu SH, Liang JB, Guo XY, Zhao HP, Liang YC, Li TQ. 2023. Long-term organic fertilization promotes the resilience of soil multifunctionality driven by bacterial communities. Soil Biol Biochem 177:108922. https://doi.org/10.1016/j.soilbio.2022.108922
11. Anonymous. 2002. Peer reviewed: pathogen risks from applying sewage sludge to land. Environ Sci Technol 36:286A–293A. https://doi.org/10.1021/es0223426
12. Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV. 2006. Co-selection of antibiotic and metal resistance. Trends Microbiol 14:176–182. https://doi.org/10.1016/j.tim.2006.02.006
13. Zhu Y-G, Johnson TA, Su J-Q, Qiao M, Guo G-X, Stedtfeld RD, Hashsham SA, Tiedje JM. 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc Natl Acad Sci USA 110:3435–3440. https://doi.org/10.1073/pnas.1222743110
14. Weithmann N, Möller JN, Löder MGJ, Piehl S, Laforsch C, Freitag R. 2018. Organic fertilizer as a vehicle for the entry of microplastic into the environment. Sci Adv 4:eaap8060. https://doi.org/10.1126/sciadv.aap8060
15. He TH, Yuan JJ, Luo JF, Wang WJ, Fan JL, Liu DY, Ding WX. 2019. Organic fertilizers have divergent effects on soil N^2O emissions. Biol Fertil Soils 55:685–699. https://doi.org/10.1007/s00374-019-01385-4
16. Han ZQ, Wang JY, Xu PS, Li ZT, Liu SW, Zou JW. 2021. Differential responses of soil nitrogen-oxide emissions to organic substitution for synthetic fertilizer and biochar amendment in a subtropical tea plantation. GCB Bioenergy 13:1260–1274. https://doi.org/10.1111/gcbb.12842
17. Huttenhower C, Finn RD, McHardy AC. 2023. Challenges and opportunities in sharing microbiome data and analyses. Nat Microbiol 8:1960–1970. https://doi.org/10.1038/s41564-023-01484-x
18. Li H-Z, Yang K, Liao H, Lassen SB, Su J-Q, Zhang X, Cui L, Zhu Y-G. 2022. Active antibiotic resistome in soils unraveled by single-cell Isotope probing and targeted metagenomics. Proc Natl Acad Sci U S A 119:e2201473119. https://doi.org/10.1073/pnas.2201473119
19. Zhang Q, Xu NH, Lei CT, Chen BF, Wang TZ, Ma YT, Lu T, Penuelas J, Gillings M, Zhu YG, Fu ZW, Qian HF. 2023. Metagenomic insight into the global dissemination of the antibiotic resistome. Adv Sci 10:e2303925. https://doi.org/10.1002/advs.202303925
20. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:1091. https://doi.org/10.1038/s41587-019-0252-6
21. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869
22. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 31:814–821. https://doi.org/10.1038/nbt.2676
23. Lu T, Ke M, Lavoie M, Jin Y, Fan X, Zhang Z, Fu Z, Sun L, Gillings M, Peñuelas J, Qian H, Zhu Y-G. 2018. Rhizosphere microorganisms can influence the timing of plant flowering. Microbiome 6:231. https://doi.org/10.1186/s40168-018-0615-0
24. Lu T, Ke M, Peijnenburg W, Zhu Y, Zhang M, Sun L, Fu Z, Qian H. 2018. Investigation of rhizospheric microbial communities in wheat, barley, and two rice varieties at the seedling stage. J Agric Food Chem 66:2645–2653. https://doi.org/10.1021/acs.jafc.7b06155
25. Zhang ZY, Zhang Q, Wang TZ, Xu NH, Lu T, Hong WJ, Penuelas J, Gillings M, Wang MX, Gao WW, Qian HF. 2022. Assessment of global health risk of antibiotic resistance genes. Nat Commun 13:1553. https://doi.org/10.1038/s41467-022-29283-8
26. Tortosa G, Alburquerque JA, Ait-Baddi G, Cegarra J. 2012. The production of commercial organic amendments and fertilisers by composting of two-phase olive mill waste ("alperujo"). J Clean Prod 26:48–55. https://doi.org/10.1016/j.jclepro.2011.12.008
27. Semenov MV, Krasnov GS, Semenov VM, Ksenofontova N, Zinyakova NB, van Bruggen AHC. 2021. Does fresh farmyard manure introduce surviving microbes into soil or activate soil-borne microbiota J Environ Manage 294:113018. https://doi.org/10.1016/j.jenvman.2021.113018
28. Li QJ, Zhang DQ, Song ZX, Ren LR, Jin X, Fang WS, Yan DD, Li Y, Wang QX, Cao AC. 2022. Organic fertilizer activates soil beneficial microorganisms to promote strawberry growth and soil health after fumigation. Environ Pollut 295:118653. https://doi.org/10.1016/j.envpol.2021.118653
29. Luo GW, Li L, Friman VP, Guo JJ, Guo SW, Shen QR, Ling N. 2018. Organic amendments increase crop yields by improving microbe-mediated soil functioning of agroecosystems: a meta-analysis. Soil Biol Biochem 124:105–115. https://doi.org/10.1016/j.soilbio.2018.06.002
30. Kopittke PM, Menzies NW, Wang P, McKenna BA, Lombi E. 2019. Soil and the intensification of agriculture for global food security. Environ Int 132:105078. https://doi.org/10.1016/j.envint.2019.105078
31. Lin YX, Ye GP, Kuzyakov Y, Liu DY, Fan JB, Ding WX. 2019. Long-term manure application increases soil organic matter and aggregation, and alters microbial community structure and keystone taxa. Soil Boil Biochem 134:187–196. https://doi.org/10.1016/j.soilbio.2019.03.030
32. Wang XY, Bian Q, Jiang YJ, Zhu LY, Chen Y, Liang YT, Sun B. 2021. Organic amendments drive shifts in microbial community structure and keystone taxa which increase C mineralization across aggregate size classes. Soil Biology and Biochemistry 153:108062. https://doi.org/10.1016/j.soilbio.2020.108062
33. Faust K, Raes J. 2012. Microbial interactions: from networks to models. Nat Rev Microbiol 10:538–550. https://doi.org/10.1038/nrmicro2832
34. Coyte KZ, Schluter J, Foster KR. 2015. The ecology of the microbiome: networks, competition, and stability. Science 350:663–666. https://doi.org/10.1126/science.aad2602
35. Brown RW, Chadwick DR, Bending GD, Collins CD, Whelton HL, Daulton E, Covington JA, Bull ID, Jones DL. 2022. Nutrient (C, N and P) enrichment induces significant changes in the soil metabolite profile and microbial carbon partitioning. Soil Biology and Biochemistry 172:108779. https://doi.org/10.1016/j.soilbio.2022.108779
36. Li Q, Xing Y, Huang B, Chen X, Ji L, Fu X, Li T, Wang J, Chen G, Zhang Q. 2022. Rhizospheric mechanisms of bacillus subtilis bioaugmentation-assisted phytostabilization of cadmium-contaminated soil. Sci Total Environ 825:154136. https://doi.org/10.1016/j.scitotenv.2022.154136
37. Yamaguchi N, Winter CM, Wu M-F, Kanno Y, Yamaguchi A, Seo M, Wagner D. 2014. Gibberellin acts positively then negatively to control onset of flower formation in *Arabidopsis*. Science 344:638–641. https://doi.org/10.1126/science.1250498
38. Nisar N, Li L, Lu S, Khin Nay C, Pogson BJ. 2015. Carotenoid metabolism in plants. Mol Plant 8:68–82. https://doi.org/10.1016/j.molp.2014.12.007
39. Davière J-M, Achard P. 2016. A pivotal role of DELLAs in regulating multiple hormone signals. Mol Plant 9:10–20. https://doi.org/10.1016/j.molp.2015.09.011
40. Binenbaum J, Weinstain R, Shani E. 2018. Gibberellin localization and transport in plants. Trends Plant Sci 23:410–421. https://doi.org/10.1016/j.tplants.2018.02.005
41. Hantari D, Purnomo D, Triharyanto E. 2020. The effects of fertilizer composition and gibberellin on flowering and true shallot seed formation of three shallot varieties at the highlands. 4th International Conference on Climate594 Change 2019 (4th ICCC 2019) 423:012032. https://doi.org/10.1088/1755-1315/423/1/012032
42. Kasim N, Panggula NDP, Haring F, Ulfa F, Dachlan A, Widiayani N, Yulsan D. 2020. Growth and production of Katokkon (*Capsicum Chinense Jacq*) chili plants in lowland applied with gibberellins and liquid organic fertilizer 2nd International Conference on Food Security and Sustainable Agriculture in the Tropics, Vol. 486, p 012121. https://doi.org/10.1088/1755-1315/486/1/012121

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43. Xia L, Lam SK, Wolf B, Kiese R, Chen D, Butterbach-Bahl K. 2018. Trade-offs between soil carbon sequestration and reactive nitrogen losses under straw return in global agroecosystems. Glob Chang Biol 24:5919–5932. https://doi.org/10.1111/gcb.14466
44. Zhang N, Juneau P, Huang RL, He ZL, Sun B, Zhou JZ, Liang YT. 2021. Coexistence between antibiotic resistance genes and metal resistance genes in manure-fertilized soils. Geoderma 382:114760. https://doi.org/10.1016/j.geoderma.2020.114760
45. Zhu D, Ding J, Wang Y-F, Zhu Y-G. 2022. Effects of trophic level and land use on the variation of animal antibiotic resistome in the soil food web. Environ Sci Technol 56:14937–14947. https://doi.org/10.1021/acs.est.2c00710
46. Qian X, Gu J, Sun W, Wang X-J, Su J-Q, Stedfeld R. 2018. Diversity, abundance, and persistence of antibiotic resistance genes in various types of animal manure following industrial composting. J Hazard Mater 344:716–722. https://doi.org/10.1016/j.jhazmat.2017.11.020
47. Liang XL, Radosevich M, DeBruyn JM, Wilhelm SW, McDearis R, Zhuang J. 2023. Incorporating viruses into soil ecology: a new dimension to understand biogeochemical cycling. Crit Rev Env Sci Tec:1–21. https://doi.org/10.1080/10643389.2023.2223123

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