https://doi.org/10.17221/132/2023-PSE

Long-term application of pig manure fertiliser affects wheat yield and soil microorganism composition

*Yan Li[1,2,3], Zishuang Li[4], Lei Ma[1], Lingfei Zhang[1,5], Xiaolin Zhou[4], Hongjie Li[4], Jianlin Wei[1], Fuli Zheng[1], Deshui Tan[1,2]**

[1]*Institute of Agricultural Resources and Environment, Shandong Academy of Agricultural Sciences, Ji'nan, P.R. China*
[2]*State Key Laboratory of Nutrient Use and Management, Ji'nan, P.R. China*
[3]*Shandong Provincial Key Laboratory of Plant Nutrition and Fertilizer, Ji'nan, P.R. China*
[4]*Dezhou Academy of Agricultural Sciences, Dezhou, P.R. China*
[5]*College of Resources and Environment, Shandong Agricultural University, Taian, P.R. China*
**Corresponding author: tandeshui@163.com*

Citation: Li Y., Li Z.S., Ma L., Zhang L.F., Zhou X.L., Li H.J., Wei J.L., Zheng F.L., Tan D.S. (2023): Long-term application of pig manure fertiliser affects wheat yield and soil microorganism composition. Plant Soil Environ., 69: 374–386.

Abstract: To elucidate the mechanism of organic fertilizer promoting wheat yield increasing. In this work, we examined the impacts of the continuous application of pig manure fertiliser for 10 years on wheat yield in the calcaric fluvisol soil with a texture of sandy loam, and the relationship between soil microbial community composition and soil properties was also analysed. The wheat yield, yield components and wheat biomass were analysed by collected aboveground part. Soil nutrient, enzymatic activity and microorganism compositions were analysed by collected soil samples at the filling stage. The results showed that long-term application of pig manure fertiliser could remarkably increase wheat yield by improving soil nutrient availability, enzyme activities, and microbial composition. Moreover, soil pH, nitrogen content, dehydrogenase and urease were closely related to the soil microbial diversity. In conclusions, the long-term application of pig manure in combination with term mineral fertiliser could optimise microbial community composition by regulating the interaction between microbial species and enhancing soil enzyme activity and soil fertility, leading to increased wheat yield.

Keywords: soil fertility; degradation; soil bacteria; ecosystem; microorganism community

The Huang-Huai-Hai Plain is one of the main grain-producing areas in China. In order to obtain a high yield, farmers use mineral fertiliser for a long time, leading to soil acidification, compaction, nutrient loss, and fertility decline. A large amount of livestock and poultry manure has been produced with the rapid development of the livestock and poultry breeding industry in China, resulting in a massive waste of resources and severe environmental pollution (Lv et al. 2018). Therefore, the resource treatment of livestock and poultry manure has become an urgent matter to control environmental pollution and turn waste into treasure. Fertiliser utilisation is an essential way of resource treatment of livestock and poultry manure and also the essence of traditional agricultural technology. Compared with other animal manure, solid pig manure has a finer texture, containing 8.70–26.1% organic carbon (OC),

Supported by the National Natural Science Foundation of China, Project No. 41877100; by the National Key Research and Development Program of China, Project No. 2021YFD1901003; by the Agricultural Science and Technology Innovation Project of Shandong Academy of Agricultural Sciences, Project No. CXGC2023A14, and by the Earmarked Fund for China Agriculture Research System (CARS-03), the Smart Fertilisation Project.

https://doi.org/10.17221/132/2023-PSE

0.50–0.60% nitrogen (N), 0.20–0.22% phosphorus (P), and 0.29–0.37% potassium (K). It was including fats, proteins, cellulose, hemicellulose, organic acids, and inorganic salts, which are generally easy to be decomposed by microorganisms. Moreover, pig manure releases nutrients that can be absorbed and used by crops. Therefore, solid pig manure is a commonly used organic fertiliser, which can improve crop yield and soil fertility, especially the soil's organic carbon content. Besides improving soil fertility and enhancing crop yield, pig manure also contains some heavy metal residues. Current research on pig manure mostly focuses on the dynamic change of heavy metals and their impact on farmland (Liu et al. 2014), while only very few studies have been carried out on the interaction mechanism between yield and microorganisms after the application of pig manure.

As an important part of the soil environment, soil microorganisms are directly involved in the recycling and transformation of soil nutrients, maintenance of soil structure, and degradation of agricultural chemicals, and they play an essential role in maintaining soil health and sustainable agricultural production. Soil microorganisms are the most active and changeable part of the soil, often used to evaluate the biological characteristics of soil quality (Shi et al. 2020). In general, the higher the soil microbial diversity, the more complete the soil function and the more stable the ecosystem (Felipe-Lucia et al. 2020). Maintaining and restoring the ecosystem of microbial diversity has become a principal problem in the application as an important agricultural production measure in sustainable agriculture (Gupta et al. 2022) through regulating the input to the soil nutrients and organic carbon and stimulating the growth of microorganisms.

Organic fertiliser affects the soil microbial abundance, activity, and microbial community structure (Xiao et al. 2022). Many long-term location tests have shown that organic fertiliser or organic-inorganic compound fertiliser can significantly improve crop yield by replacing part of term mineral fertiliser (Sun et al. 2015). Long-term application of organic fertiliser or a combination of organic and inorganic fertiliser can maintain the diversity of soil bacteria, while long-term application of inorganic fertiliser can significantly reduce the diversity of soil bacteria. In addition, replacing part of term mineral fertiliser with solid pig manure organic fertiliser can increase soil microbial biomass and improve soil nutrient availability, leading to a significantly changed bacterial community structure (Liu et al. 2022). Both pig manure organic fertiliser and pig manure organic and inorganic compound fertiliser can increase soil microbial biomass, and the contents of carbon and nitrogen are increased with the increase of term mineral fertiliser replacement (Luan et al. 2020).

Therefore, to explore the impact of the long-term application of solid pig manure fertiliser on wheat yield and its underlying mechanism, we studied the effects of wheat yield, soil nutrient effect, enzyme activity, and soil microorganisms by different pig manure organic fertiliser treatments. In addition, the correlation between soil nutrient effect, enzyme activity, and soil microorganisms was also explored. Collectively, our findings provided valuable insights for improving wheat yield and soil quality in the wheat-corn rotation field.

MATERIAL AND METHODS

Experimental site. The experimental site was located in Dezhou, Shandong Province, China (117°5'E, 36°43'N), with a continental monsoon climate. The soil type was calcaric fluvisol soil, according to the FAO, with a texture of sandy loam. The annual average sunshine duration, temperature, and precipitation of the experimental site were 2 724.8 h, 12.9 °C, and 439.5–593.5 mm, respectively. The experiment was conducted from 2009 to 2018 using wheat as the experimental crop. The soil of the test field contains total nitrogen (TN) 1.24 g/kg, total phosphorus (TP) 0.98 g/kg, total potassium (TK) 21.63 g/kg, available nitrogen (AN) 58.12 mg/kg, available phosphorus (AP) 33.41 mg/kg, available potassium (AK) 234.25 mg/kg, organic carbon (OC) 11.83 g/kg, pH 8.74.

Experimental treatments. In this experiment, no fertiliser (NF); term mineral fertiliser (F); pig manure compost + term mineral fertiliser (POM), and pig manure organic-inorganic compound fertiliser (OIF) were set in wheat season. Each treatment was repeated four times. There are 16 plots, the plot area was 40 m^2, the protection line was about 5 m, and the interval was 1 m. Detailed fertiliser consumption was shown in Table 1. The mineral fertiliser was urea (N 46.0%), double superphosphate (P 19.7%), and potassium sulfate (K 41.5%). The nutrient content of pig manure compost was 1.63% N, 1.1% P, 0.84% K and 38.89% OC. The nutrient content of pig manure organic-inorganic compound fertiliser was 9.96% N, 1.07% P, 1.32% K and 29.38% OC. The total N, P and

https://doi.org/10.17221/132/2023-PSE

Table 1. Fertiliser dosage of each treatment in wheat-maize rotation system

Season	Treatment	Urea	Double superphosphate	Potassium sulfate	Pig manure compost	Pig manure compound fertiliser
		(kg/ha)				
Wheat season	NF	0	0	0	0	0
	F	652	261	200	0	0
	POM	456	183	140	3 600	0
	OIF	0	0	0	0	3 090
Maize season	NF	0	0	0	0	0
	F	544	90	90	0	0
	POM	380	63	63	3 600	0
	OIF	0	0	0	0	3 090

NF – no fertiliser; F – term mineral fertiliser; POM – pig manure compost + term mineral fertiliser; OIF – pig manure organic-inorganic compound fertiliser

K nutrient content of the F, POM and OIF treatments were consistent in the wheat-maize rotation period (300 kg N/ha; 120 kg P/ha; 100 kg K/ha). The irrigation time, irrigation frequency, and irrigation amount of each treatment remained the same. Field management measures complied with local agricultural habits. The experimental wheat cultivar was "JIMAI 22".

Investigation and sampling. The basic physical and chemical properties of the soil were determined before the test. The biomass and grain yield of wheat was measured at harvest, and the spike number, grain number per spike, and 1 000-grain weight were measured. The five-point compound method was used to randomly select soil samples at a depth of 0–25 cm in each plot. At the filling stage, soil nutrient characteristics at a depth of 0–25 cm were measured. Soil samples were stored at the Institute of Agricultural Resources and Environment, Shandong Academy of Agricultural Sciences, Jinan, China.

Soil TN was determined by Kjeldahl semi-micro-titration; TP was determined by sodium hydroxide melting, molybdenum blue colourimetric method; TK was determined by sodium hydroxide melting, flame photometry method; AN was determined by alkaline hydrolysis diffusion method; AP was determined by 0.5 mol/L $NaHCO_3$ extraction – molybdenum-antimony resistance colourimetric method determine; AK was extracted by 1 mol/L CH_3COONH_4 sub absorption spectrophotometer method; soil OM using $K_2Cr_2O_7$ oxidation (external heating oil bath) method, 0.2 mol/L $FeSO_4$ solution titration method; soil pH was measured by acidimeter method (soil to water ratio of 2.5:1).

The activities of soil urease (SUE) were determined by the colourimetric method of sodium phenol-sodium hypochlorite; soil phytase (SP) was determined by inorganic phospho-ammonium molybdate colour method; soil alkaline phosphatase (SA) was determined by the colourimetric method of disodium benzene phosphate; soil sucrase (SC) activity was determined by 3.5- dinitro salicylic acid method; soil laccase (SL) was determined by ABTS method; soil dehydrogenase (SD) was determined by 2,3,5-triphenyl tetrazolium chloride methods; soil luciferase diacetate hydrolase (SF) was determined by FDA colourimetric methods.

Rhizosphere soil was collected at the filling stage, and total genomic DNA was extracted using the Power Soil kit (MO BIO Laboratories, Carlsbad, USA) following the manufacturer's instructions. The specific primers of the bacterial 16S rRNA gene and the partial ITS region of fungi were used for the amplification. The PCR reactions, quality control, and purification processes followed the procedures of Jia et al. 2006. A library was constructed, and all sequences were generated with Illumina's MiSeq platform (2 × 250) using paired-end reads. All steps mentioned above were conducted at the Beijing Novogene Technology Co. LTD, Beijing, China (Li et al. 2019).

Statistical analysis. Microbial alpha diversity, principal coordinate analysis (PCoA) based on Bray-Curtis distance, redundancy analysis (RDA), and replacement multiple variance analysis (Adonis) were all calculated using the "vegan" package of R 4.0.5 (AT&T Bell Laboratories, USA). The level of different species in the Galaxy website (http://huttenhower.sph.harvard.edu/galaxy/) was calculated using linear

https://doi.org/10.17221/132/2023-PSE

Table 2. Wheat yield and its constituent factors

Treatment	Spike number (pieces/m^2)	Grain number per spike	Thousand-grain weight (kg)	Yield	Wheat biomass
				(kg/ha)	
NF	28.36 ± 2.28^c	28.73 ± 2.08^a	0.05 ± 0.002^a	4 060.2 ± 331.9^c	12 623.2 ± 786.5^c
F	49.73 ± 3.49^b	29.35 ± 1.33^a	0.05 ± 0.001^a	9 418.9 ± 433.9^b	15 595.4 ± 768.3^b
POM	48.13 ± 2.78^b	28.75 ± 0.98^a	0.05 ± 0.003^a	9 133.1 ± 377.0^b	16 543.2 ± 1240.0^b
OIF	64.60 ± 4.71^a	29.00 ± 0.68^a	0.05 ± 0.001^a	10 309.3 ± 673.4^a	19 783.5 ± 1155.7^a

Different lowercase letters after data within the same column indicate significant differences between the treatments ($P < 0.05$). NF – no fertiliser; F – term mineral fertiliser; POM – pig manure compost + term mineral fertiliser; OIF – pig manure organic-inorganic compound fertiliser

discriminant analysis (LEfSe), in which LDA greater than three species was defined as significant differences. SPSS 25.0 software (IBM, USA) was used for one-way ANOVA. The differences in the means were compared by the least significant difference (*LSD*) test, and $P < 0.05$ was considered statistically significant. Microsoft Excel 2019 (Microsoft, USA) was used for data processing.

RESULTS

Wheat yield and its constituent factors. After the long-term application of pig mature organic fertiliser, the yield of wheat remained relatively stable. The yield in OIF was significantly higher than F treatment by 9.45%. The yield in POM was slightly lower than F, and the difference was not significant. The wheat biomass in organic fertiliser treatment was higher than the term mineral fertiliser treatment alone, and the wheat biomass in OIF was the highest, which was 26.86% higher than F. The spike number in OIF was significantly higher than F, and the OIF was 14.87 pieces/m^2 higher than F. There were no significant differences in grain number per spike and 1 000-grain weight among the four treatments (Table 2). Therefore, the spike number was the main yield component of wheat increase in this study.

Soil property. After applying organic fertiliser, the contents of TN, TP, TK, AN, AP, AK, and OC in the soil were significantly increased. Compared with F, OIF treatment significantly increased the AN content by 12.48%; POM treatment significantly increased the contents of TN, TP, TK, AN, AP, AK, and OC in the soil by 3.40, 44.17, 2.05, 9.28, 47.03, 17.49, and 18.13%, respectively. Besides, the application of organic fertiliser significantly reduced the pH value of the soil (Table 3).

Microbial community composition. A total of 65 132 and 64 350 high-quality reads were analysed for bacteria and fungi in wheat season, respectively, which were then clustered into 10 235 and 1 881 OTUs (operational taxonomic units). The coverage of bacterial and fungal clone libraries was all above 97%. The α-diversity analysis showed the overall rich-

Table 3. Soil nutrient status in different treatments

Treatment	TN	TP	TK	AN	AP	AK	OC	pH
	(g/kg)			(mg/kg)			(g/kg)	
NF	1.05 ± 0.01^c	0.92 ± 0.06^c	16.62 ± 0.23^c	75.49 ± 5.75^c	12.44 ± 0.83^c	229.25 ± 20.87^c	5.31 ± 0.02^d	8.71 ± 0.01^a
F	1.43 ± 0.02^{ab}	1.20 ± 0.23^b	18.05 ± 0.13^{bc}	100.72 ± 5.08^b	35.38 ± 2.46^b	277.25 ± 11.21^b	6.53 ± 0.56^c	8.62 ± 0.03^b
POM	1.48 ± 0.06^a	1.73 ± 0.06^a	18.42 ± 0.30^a	110.07 ± 6.52^a	52.02 ± 3.91^a	325.75 ± 23.16^a	7.71 ± 0.54^a	8.52 ± 0.01^c
OIF	1.41 ± 0.04^b	1.05 ± 0.91^c	17.60 ± 0.23^b	113.29 ± 5.07^a	35.71 ± 3.21^b	271.55 ± 20.55^b	7.08 ± 0.52^b	8.35 ± 0.02^d

TN – total nitrogen; TP – total phosphorus; TK – total potassium; AN – available nitrogen; AP – available phosphorus; AK – available potassium; OC – organic matter; NF – no fertiliser; F – term mineral fertiliser; POM – pig manure compost + term mineral fertiliser; OIF – pig manure organic-inorganic compound fertiliser

https://doi.org/10.17221/132/2023-PSE

Table 4. Alpha diversity of bacteria

Treatment	Diversity		Richness	
	Shannon	Simpson	Chao 1	ACE
NF	10.04 ± 0.08[a]	0.997 ± 0.001[a]	4 329.67 ± 65.53[a]	4 382.27 ± 88.15[a]
F	10.05 ± 0.02[a]	0.997 ± 0.000[a]	4 324.81 ± 58.38[a]	4 381.11 ± 69.75[a]
POM	10.05 ± 0.04[a]	0.997 ± 0.001[a]	4 275.87 ± 34.71[a]	4 317.57 ± 44.30[a]
OIF	10.11 ± 0.07[a]	0.998 ± 0.001[a]	4 447.35 ± 51.50[a]	4 518.69 ± 56.49[a]

Different lowercase letters after data within the same column indicate significant differences between the treatments ($P < 0.05$; Duncan's test). NF – no fertiliser; F – term mineral fertiliser; POM – pig manure compost + term mineral fertiliser; OIF – pig manure organic-inorganic compound fertiliser

Table 5. Alpha diversity of fungi

Treatment	Diversity		Richness	
	Shannon	Simpson	Chao 1	ACE
NF	5.08 ± 0.19[a]	0.89 ± 0.02[a]	612.80 ± 16.75[a]	626.59 ± 15.70[a]
F	4.94 ± 0.13[a]	0.90 ± 0.01[a]	555.33 ± 4.71[b]	564.52 ± 11.06[b]
POM	5.13 ± 0.23[a]	0.91 ± 0.02[a]	604.61 ± 22.96[a]	618.34 ± 18.43[a]
OIF	4.44 ± 0.24[a]	0.90 ± 0.01[a]	495.61 ± 514.53[c]	507.12 ± 12.32[c]

Different lowercase letters after data within the same column indicate significant differences between the treatments ($P < 0.05$; Duncan's test). NF – no fertiliser; F – term mineral fertiliser; POM – pig manure compost + term mineral fertiliser; OIF – pig manure organic-inorganic compound fertiliser

ness and diversity of bacteria in the four treatments was not significantly different (Table 4). In contrast, the ACE and Chao1 indices in the four treatments revealed statistically significant (P-value < 0.05) alterations in the richness of fungi, and Shannon and Simpson indices were not significantly different.

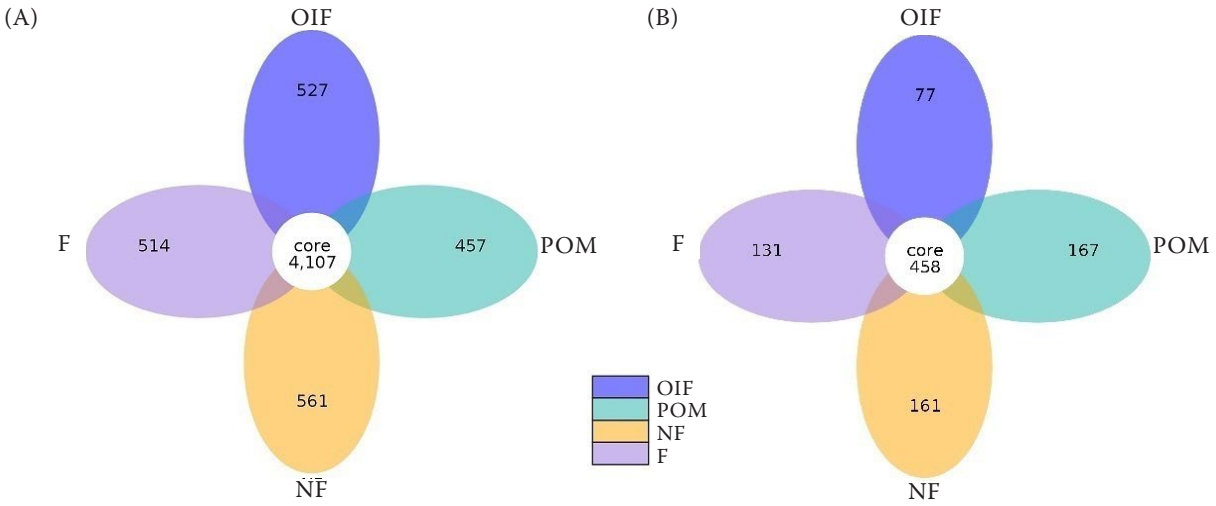


Figure 1. Venn diagrams of (A) shared bacteria and (B) fungi biomarkers different treatments. The white circle in the middle represents the common duplicate samples; the outside circle represents the samples in different treatments, and different colour represents a different sample. The numbers in the overlap region represent the number of OTU (operational taxonomic unit) shared by all samples. The numbers on the non-overlap region represent the number of OTU unique to the sample. NF – no fertiliser; F – term mineral fertiliser; POM – pig manure compost + term mineral fertiliser; OIF – pig manure organic-inorganic compound fertiliser

https://doi.org/10.17221/132/2023-PSE

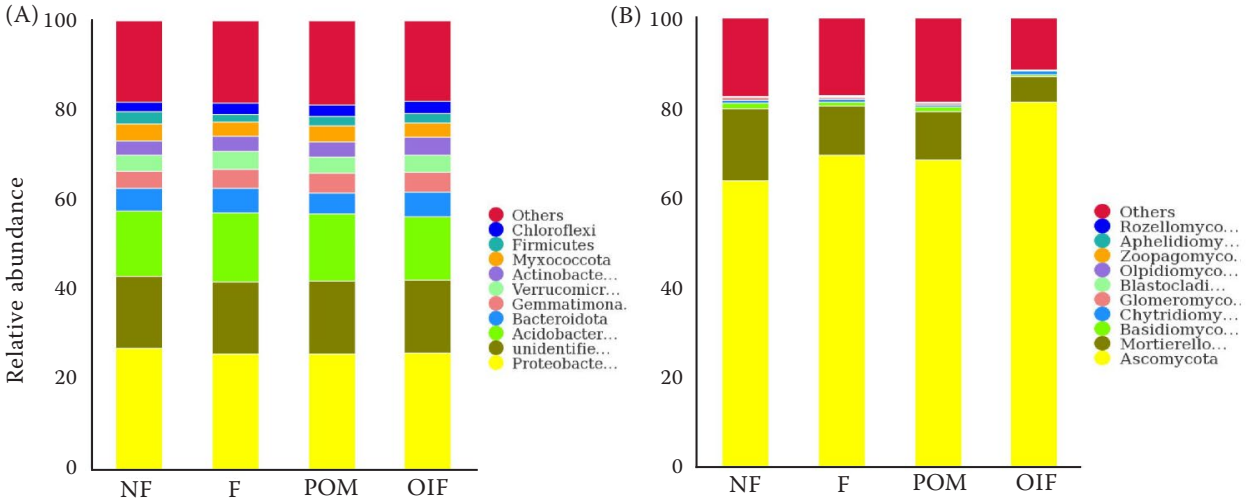


Figure 2. Abundance of (A) bacteria and (B) fungi phyla in soil sample in 2018. Only OTUs (operational taxonomic units) with an indicidence > 1% in at least one sample are shown

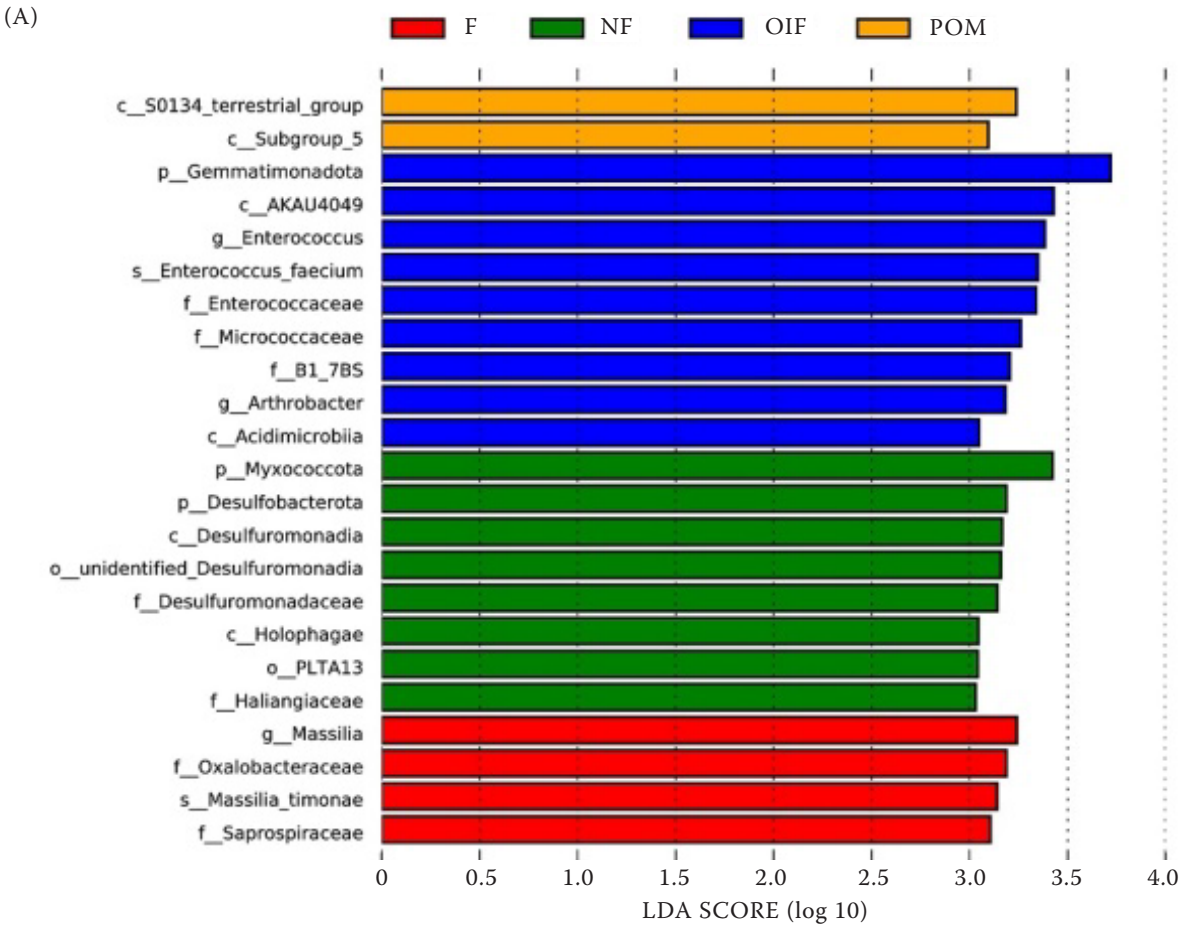
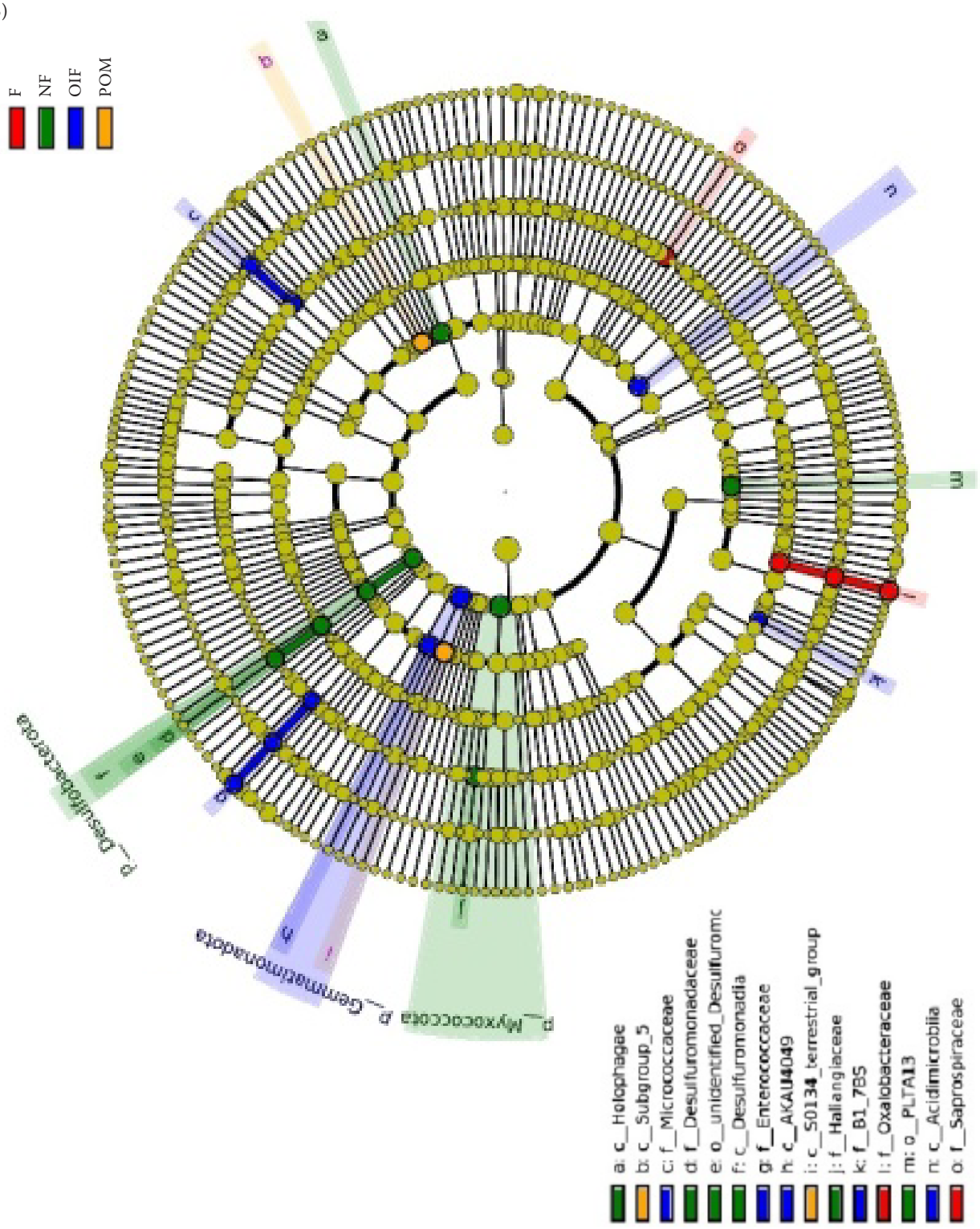


Figure 3. LEfSe cladograms showing taxa bacteria with different abundance values (LDA score > 3; $P < 0.05$). Histogram of LDA value distribution (A) and cladogram (B). The central point represents the root of the tree, and each ring represents the next taxonomic level (phylum, class, family, genus and species). NF – no fertiliser; F – term mineral fertiliser; POM – pig manure compost + term mineral fertiliser; OIF – pig manure organic-inorganic compound fertiliser

https://doi.org/10.17221/132/2023-PSE

(B)



Continued Figure 3. LEfSe cladograms showing taxa bacteria with different abundance values (LDA score > 3; $P < 0.05$). Histogram of LDA value distribution (A) and cladogram (B). The central point represents the root of the tree, and each ring represents the next taxonomic level (phylum, class, family, genus and species). NF – no fertiliser; F – term mineral fertiliser; POM – pig manure compost + term mineral fertiliser; OIF – pig manure organic-inorganic compound fertiliser

https://doi.org/10.17221/132/2023-PSE

The ACE and Chao1 in the POM treatment significant increased 8.87% and 9.53% compared with F, respectively (Table 5).

Structure and composition of soil microbial community. Figure 1 showed that the sum of total observed OTUs in bacteria was 6 166, including 4 107 OTUs common to all treatments, as well as 561, 514, 457, and 527 specific OTUs in the NF, F, POM, and OIF groups, respectively. The sum of the total observed OTUs in fungi was 994, including 458 OTUs common to all treatments, as well as 161, 131, 167, and 77 specific OTUs in the NF, F, POM, and OIF groups, respectively.

The species with the highest maximum abundance at different taxonomic levels between samples were analysed based on the species annotation results. The relative abundance of Proteobacteria was 25.80–27.08% in the measured soil bacteria. The remaining species with relative abundance greater than 1% were unidentified bacteria (16.13–16.40%), Acidobacteria (14.19–15.39%), Bacteroidetes (4.74–5.51%), Gemmatimonadota (3.76–4.61%), Verrucomicrobiota (3.50–4.12%), Actinobacteriata (3.28–3.92%), Myxococcota (3.11–3.65%), Firmicutes (1.58–2.72%), and Chloroflexi (2.31–2.74%) (Figure 2A). The analysis of fungi showed that the relative abundance of bacteria was significantly different among all treatments. Ascomycota (63.88–81.40%) was the dominant bacteria in the tested soil. Other species with relative abundance > 1% included Mortierellomycota (5.60–16.08%) and Basidiomycota (0.59–1.27%) (Figure 2B).

LEfSe at the genus level found that the bacterial species with significantly increased relative abundance in NF, F, POM, and OIF treatments were Haliangiaceae (Myxococcota), Desulfuromonadaceae (Desulfobacterota), and Holophagae (Acidobacteriota); Massilia, Oxalobacteraceae, and Saprospiraceae; Longimicrobiaceae (Gemmatimonadota) and Vicinamibacteraceae (Acidobacteriota); and Gemmatimonadota (Blastomonas), Enterococcus (Firmicutes), Micrococcaceae (Actinobacteriota), and Proteobacteria, respectively (Figure 3).

The relatively abundant fungi in NF, F, POM, and OIF treatments were Hypocreales (Ascomycota) and Glomeraceae (Glomeromycota); Fusicolla (Ascomycota); Dothideomycetes (Ascomycota), Mortierellales (Mortierellomycota), and Filobasidiales (Basidiomycota); and Chaetomiaceae and Sordariales (Ascomycota), respectively (Figure 4).

A PCoA based on weighted_unifrac distance found that the first two axes of the bacterial and fungal

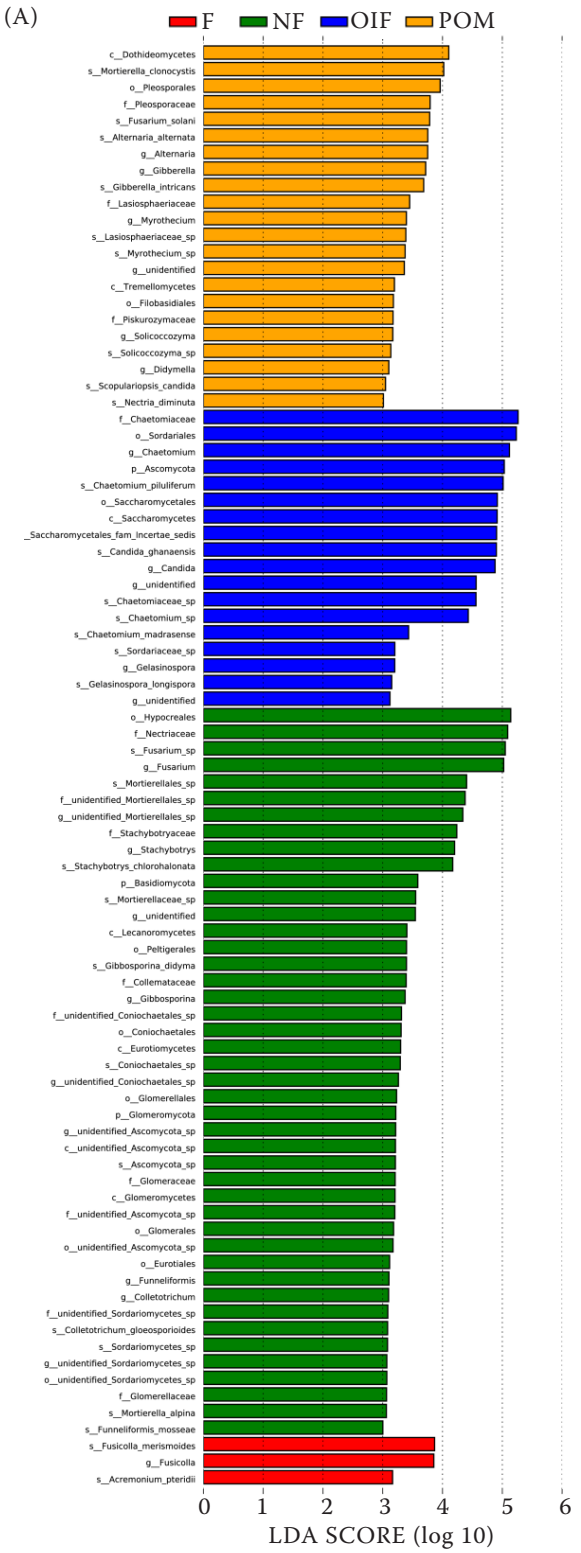
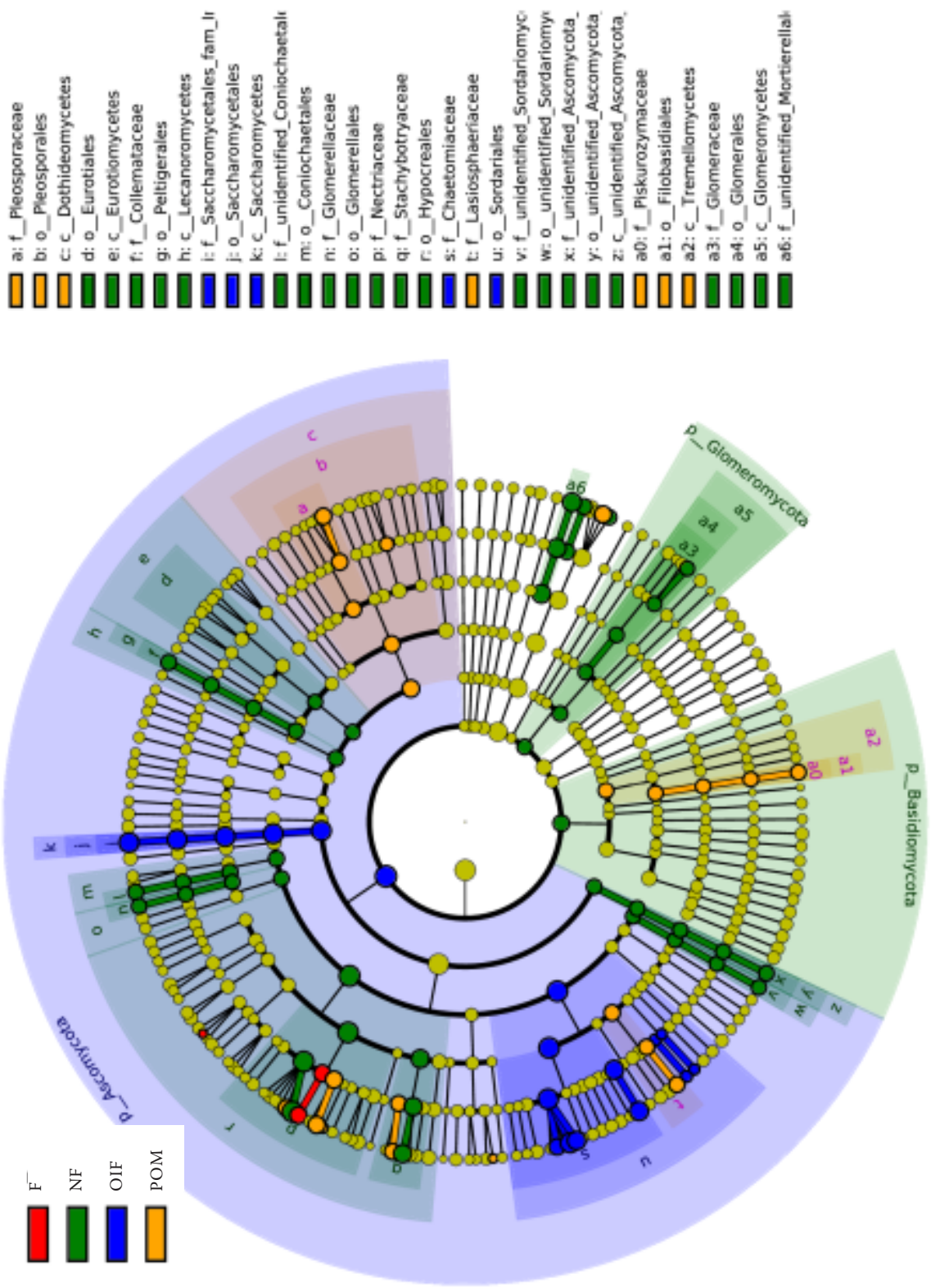


Figure 4. LEfSe cladograms showing fungi taxa with different abundance values (LDA score > 3; $P < 0.05$). Histogram of LDA value distribution (A) and cladogram (B). The central point represents the root of the tree, and each ring represents the next taxonomic level (phylum, class, family, genus and species)

https://doi.org/10.17221/132/2023-PSE

(B)



Continued Figure 4. LEfSe cladograms showing fungi taxa with different abundance values (LDA score > 3; $P < 0.05$). Histogram of LDA value distribution (A) and cladogram (B). The central point represents the root of the tree, and each ring represents the next taxonomic level (phylum, class, family, genus and species). NF – no fertiliser; F – term mineral fertiliser; POM – pig manure compost + term mineral fertiliser; OIF – pig manure organic-inorganic compound fertiliser

https://doi.org/10.17221/132/2023-PSE

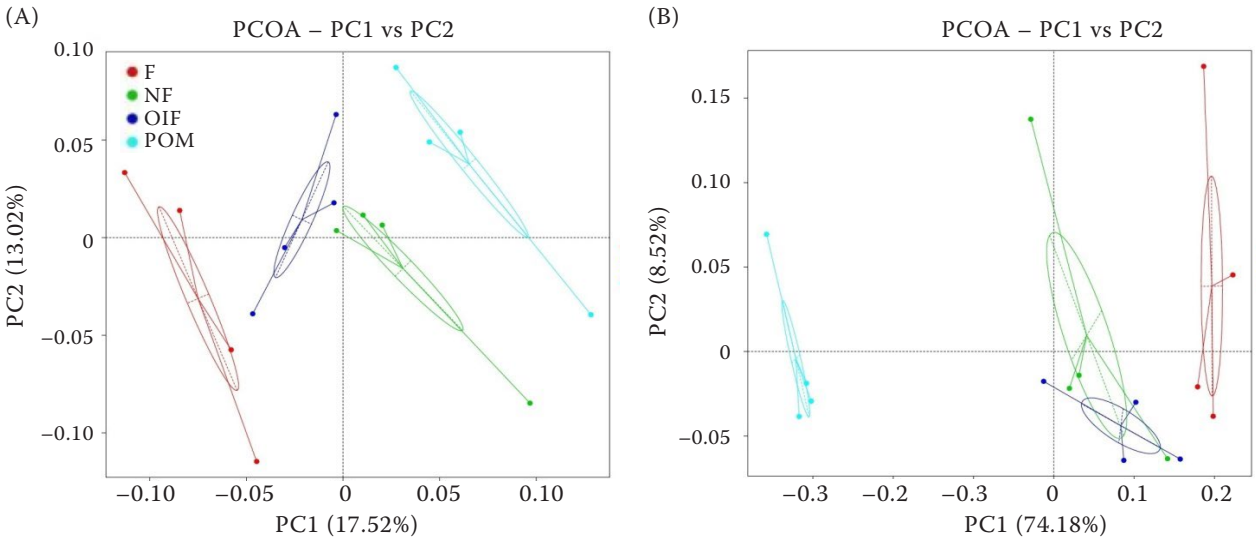


Figure 5. PCoA of (A) bacteria and (B) fungi community structure based on weighted unifrac distance. NF – no fertiliser; F – term mineral fertiliser; POM – pig manure compost + term mineral fertiliser; OIF – pig manure organic-inorganic compound fertiliser

principal coordinate together explained about 30.54% and 82.70% of the community differences (Figure 5). Adonis analysis found NF and POM have significant differences in bacteria; NF and OIF have significant differences in fungi (Table 6).

Soil enzyme activity. Organic fertilisers significantly changed soil enzyme activities. The enzyme activities of SUE, SP, SA, and SD in the POM treatment were increased by 130.30, 26.79, 39.77, and 24.17% compared with F, respectively. The enzyme activities of SUE, SP, SA, and SD in OIF were increased by 184.85, 28.57, 90.91, and 15.13% compared with F, respectively. The SL activity was significantly lower than F. There was no significant difference in SC and SF between POM, OIF, and F, while all were significantly higher compared with NF (Table 7).

Effects of environmental factors on soil microorganisms. The results showed that AN was the main factor leading to the variation of bacterial community among different treatments, and SD was the main enzyme factor leading to the variation of the bacterial community. Moreover, pH was the main factor leading to the variation of the fungal community among different treatments, TN was the main nutrient factor leading to the variation of the fungal community, SUE was the main enzyme factor leading to the variation of the fungal community (Table 8).

DISCUSSION

Soil property. Term mineral fertiliser combined with organic fertiliser can significantly improve the

Table 6. Adonis analysis of bacteria and fungi

Treatment	Bacteria		Fungi	
	r	*P*	*r*	*P*
NF-VS-F	0.197	0.038	0.365	0.024
NF-VS-POM	0.183	0.048	0.394	0.023
NF-VS-OIF	0.270	0.025	0.857	0.03
F-VS-POM	0.196	0.028	0.235	0.073
F-VS-OIF	0.170	0.239	0.766	0.034
POM-VS-OIF	0.225	0.025	0.839	0.026

r – correlation value; *P* – significance value. When $P < 0.05$, it indicates that there is a correlation between the two. The greater the absolute value of *r*, the better the correlation, with positive numbers indicating positive correlation and negative numbers indicating negative correlation. NF – no fertiliser; F – term mineral fertiliser; POM – pig manure compost + term mineral fertiliser; OIF – pig manure organic-inorganic compound fertiliser

https://doi.org/10.17221/132/2023-PSE

Table 7. Soil enzyme activity in different treatments

Treatment	SUE (mg/d/g)	SP (μmol/d/g)	SA (μmol/d/g)	SC (mg/d/g)	SL (nmol/min/g)	SD (μg/d/g)	SF (μmol/d/g)
NF	0.24 ± 0.01^{d}	0.36 ± 0.03^{c}	0.17 ± 0.04^{d}	31.52 ± 0.25^{b}	43.53 ± 0.23^{a}	60.42 ± 2.86^{c}	36.30 ± 2.60^{b}
F	0.33 ± 0.02^{c}	0.56 ± 0.05^{b}	0.88 ± 0.03^{c}	31.92 ± 0.36^{a}	43.73 ± 0.19^{a}	121.74 ± 6.97^{b}	40.08 ± 1.72^{a}
POM	0.76 ± 0.01^{b}	0.71 ± 0.04^{a}	1.23 ± 0.07^{b}	32.09 ± 0.09^{a}	42.72 ± 1.19^{ab}	151.17 ± 9.61^{a}	41.09 ± 0.38^{a}
OIF	0.94 ± 0.01^{a}	0.72 ± 0.06^{a}	1.68 ± 0.07^{a}	32.19 ± 0.22^{a}	42.28 ± 0.65^{b}	140.16 ± 10.73^{a}	40.27 ± 0.59^{a}

SUE – soil urease; SP – soil phytase; SA – soil alkaline phosphatase; SC – soil sucrase; SL – soil laccase; SD – soil dehydrogenase; SF – soil luciferase diacetate hydrolase; NF – no fertiliser; F – term mineral fertiliser; POM – pig manure compost + term mineral fertiliser; OIF – pig manure organic-inorganic compound fertiliser

soil OM and nutrient contents, enhance soil enzyme activity, ameliorate soil structure, and increase crop yield (Jiang et al. 2022). In the present study, POM treatment had the best effect on improving soil nutrient and OM contents (Table 2), fully indicating that pig manure compost + term mineral fertiliser treatment was more conducive to improving soil quality. Available nitrogen has the most direct relationship with wheat yield. The AN content of the OIF treatment was higher than that of POM, which was one possible reason why the yield and wheat biomass of the OIF treatment were significantly higher than those of other treatments. The in-depth analysis found that AN and TN also improved soil and increased wheat yield by affecting the microbial community structure.

Soil microbial community structure. Proteobacteria and Acidobacteria are the dominant bacteria in the measured soil bacteria, and some of them can degrade plant residues (Sui et al. 2019). It plays a very important role in soil material circulation and ecological environment construction. Further LEfSe at the genus level found that the species significantly increased in POM and OIF treatments were associated with soil nitrogen and organic carbon. Such as Longimicrobiaceae (Gemmatimonadota) and Vicinamibacteraceae (Acidobacteriota) in the POM treatment. Blastomonas, in addition to Enterococcus (Firmicutes) and Micrococcaceae (Actinobacteriota) in OIF. Studies have found that Actinomycetes have a strong ability to decompose complex macromolecular OM in soil. Many species in Firmicutes can secrete cellulase and participate in the degradation of cellulose in organic fertilisers (Chang et al. 2014).

Among fungi, Ascomycota and Mortierellomycota are the dominant strains of soil fungi. The species significantly increased in POM and OIF treatments were associated with soil nitrogen, phosphorus and organic carbon. Such as the soil organic carbon and phosphorus-related Mortierellales (Mortierellomycota) and Filobasidiales (Basidiomycota) in POM. And the species Chaetomiaceae and Sordariales (Ascomycota)

Table 8. Correlation analysis between the soil microorganism and soil environmental factors

Variable	Bacteria		Fungi	
	r	*P*	*r*	*P*
AN	**0.428**	0.008	0.2024	0.042
AP	0.425	0.008	0.1569	0.074
AK	0.4131	0.005	0.0311	0.524
OM	0.3559	0.004	0.1073	0.135
pH	0.0752	0.226	**0.7914**	0.001
TN	0.3739	0.002	**0.2029**	0.034
TP	0.0846	0.755	0.0179	0.476
TK	0.4227	0.014	0.1796	0.087
SUE	0.0903	0.139	**0.6468**	0.001
SP	0.2777	0.022	0.2475	0.033
SC	0.2506	0.063	0.1064	0.172
SA	0.2786	0.02	0.6011	0.002
SL	0.0471	0.571	0.3496	0.012
SD	**0.3659**	0.004	0.2217	0.037
SF	0.2751	0.064	0.1364	0.128

r – correlation value; *P* – significance value. When $P < 0.05$, it indicates that there is a correlation between the two. The greater the absolute value of *r*, the better the correlation, with positive numbers indicating positive correlation and negative numbers indicating negative correlation. AN – available nitrogen; AP – available phosphorus; AK – available potassium; OM – organic matter; TN – total nitrogen; TP – total phosphorus; TK – total potassium; SUE – soil urease; SP – soil phytase; SA – soil alkaline phosphatase; SC – soil sucrase; SL – soil laccase; SD – soil dehydrogenase; SF – soil luciferase diacetate hydrolase

https://doi.org/10.17221/132/2023-PSE

in OIF. Some strains of Morpita can significantly increase the contents of AP, AK, available calcium, available magnesium, and available boron, which can promote plant growth (Li et al. 2018, Ning et al. 2022) and degrade hemicellulose, cellulose, and lignin (Koechli et al. 2019). To sum up, there were differences in microbial community structure among different treatments, indicating that different types of pig manure could improve soil and enhance soil fertility by increasing the contents of bacteria and fungi.

Soil enzyme activity. The activities of soil enzymes can represent the exuberance of soil material metabolism and, to a certain extent, also reflect the absorption, utilisation, and growth of crop nutrients, which is an essential indicator of soil fertility, soil quality, and soil health (Lv et al. 2018). In this study, the activities of enzymes SUE related to nitrogen, SA related to phosphorus decomposition and SD related to OC decomposition and utilisation were also significantly increased after the application of organic fertilisers (Table 3), indicating that the application of pig manure organic fertiliser was beneficial to the improvement of soil enzyme activity (Yang et al. 2022).

Environmental factors and soil microbial community structure. The pH was the main factor leading to the variation of fungal community between treatments in this study. A large number of studies have shown that soil microbial diversity is mainly regulated by soil pH. Soil pH can disturb the structure and diversity of the soil microbial community by affecting soil composition and chemical properties. Soil bacteria and fungi predominate in more alkaline and more acidic soils, respectively. The soil pH was decreased after organic fertiliser treatment. Therefore, it had a greater impact on soil fungal diversity than bacterial diversity (Cai et al. 2017). The nitrogen cycle is one of the cores of the element cycle in the soil ecosystem, and its four main processes, namely biological nitrogen fixation, ammonification, nitrification, and denitrification, are all driven by microorganisms (Ramond et al. 2022). Studies have found that pig manure can increase soil microbial biomass by accumulating soil AN (Knoblauch et al. 2017). In the present study, nitrogen content was the main nutrient factor contributing to the structural diversity of bacterial and fungal communities, which also confirmed the importance of nitrogen to microbial community diversity.

SD was the main enzyme factor leading to the variation of the bacterial community. Its activity can reflect the number of active microorganisms in the soil system and its degradation activity to OM, which can be used as the degradation performance index of soil microorganisms. In the present study, there was a close relationship between SD and bacterial community change, indicating that the addition of organic fertiliser changed the content of soil OM and then affected soil microbial diversity.

REFERENCES

Cai F., Pang G., Li R.X., Li R., Gu X.L., Shen Q.R., Chen W. (2017): Bioorganic fertilizer maintains a more stable soil microbiome than chemical fertilizer for monocropping. Biology Fertility of Soils, 53: 861–872.

Chang Y.C., Choi D.B., Takamizawa K., Kikuchi S. (2014): Isolation of *Bacillus* sp. strains capable of decomposing alkali lignin and their application in combination with lactic acid bacteria for enhancing cellulase performance. Bioresource. Technology, 152: 429–436.

Felipe-Lucia M.R., Soliveres S., Penone C., Fischer M., Ammer C., Boch S., Boeddinghaus R.S., Bonkowski M., Buscot F., Fiore-Donno A.M., Frank K., Goldmann K., Gossner M.M., Hölzel N., Jochum M., Kandeler E., Klaus V.H., Kleinebecker T., Leimer S., Manning P., Oelmann Y., Saiz H., Schall P., Schloter M., Schöning I., Schrumpf M., Solly E.F., Stempfhuber B., Weisser W.W., Wilcke W., Wubet T., Allan E. (2020): Land-use intensity alters networks between biodiversity, ecosystem functions, and services. Proceedings of the National Academy of Sciences, 117: 28140–28149.

Gupta A., Singh U.B., Sahu P.K., Paul S., Kumar A., Malviya D., Singh S., Kuppusamy P., Singh P., Paul D., Rai J.P., Singh H.V., Manna M.C., Crusberg T.C., Kumar A., Saxena A.K. (2022): Linking soil microbial diversity to modern agriculture practices: a review. International Journal of Environmental Research and Public Health, 19: 3141.

Jia X., Han S.J., Zhao Y.J., Zhou Y.M. (2006): Comparisons of extraction and purification metheds of soil microorganism DNA from rhizosphere soil. Journal of Forestry Research, 17: 31–34.

Jiang Y., Zhang R., Zhang C., Su J., Cong W.F., Deng X. (2022): Long-term organic fertilizer additions elevate soil extracellular enzyme activities and tobacco quality in a tobacco-maize rotation. Frontier in Plant Science, 13: 973639.

Knoblauch C., Watson C., Becker R., Berendonk C., Wichern F. (2017): Change of ergosterol content after inorganic N fertilizer application does not affect short-term C and N mineralization patterns in a grassland soil. Applied Soil Ecology, 111: 57–64.

Koechli C., Campbell A.N., Pepe-Ranney C., Buckley D.H. (2019): Assessing fungal contributions to cellulose degradation in soil by using high-throughput stable isotope probing. Soil Biology and Biochemistry, 130: 150–158.

https://doi.org/10.17221/132/2023-PSE

Li F., Chen L., Redmile-Gordon M., Zhang J.B., Zhang C.Z., Ni Q., Li W. (2018): Mortierella elongata's roles in organic agriculture and crop growth promotion in a mineral soil. Land Degradation and Development, 29: 1642–1651.

Li Y., Fang F., Wei J.L., Wu X.B., Cui R.Z., Li G.S., Zheng F.L., Tan D.S. (2019): Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut: a three-year experiment. Scientific Reports, 9: 201065023.

Liu K.L., Li D.M., Huang Q.H., Yu X.C., Ye H.C., Xu X.L., Hu H.W., Wang S.L. (2014): Ecological benefits and environmental carrying capacities of red paddy field subjected to long-term pig manure amendments. Scientia. Agricultura Sinica, 47: 303–313.

Liu M., Linna C., Ma S., Ma Q., Song W., Shen M., Song L., Cui K., Zhou Y., Wang L. (2022): Biochar combined with organic and inorganic fertilizers promoted the rapeseed nutrient uptake and improved the purple soil quality. Frontiers in Nutrition, 9: 997151.

Luan H., Gao W., Huang S., Tang J., Li M., Zhang H., Chen X., Masiliūnas D. (2020): Substitution of manure for chemical fertilizer affects soil microbial community diversity, structure and function in greenhouse vegetable production systems. PLoS One, 15: e0214041.

Lv F.L., Hou M.M., Zhang H.T., Qiang J.C.R., Zhou Y.T., Lu G.Y., Zhao B.Q., Yang X.Y., Zhang S.L. (2018): Replacement ratio of chemical fertilizer nitrogen with manure under the winter wheat-summer maize rotation system in Lou soil. Journal of Plant Nutrition and Fertilizer, 24: 22–32.

Ning Q., Chen L., Li F., Zhang C.Z., Ma D.H., Cai Z.J., Zhang J.B. (2022): Effects of mortierella on nutrient availability and straw decomposition in soil. Acta Petrologica Sinica, 59: 206–217.

Ramond J.B., Jordaan K., Díez B., Heinzelmann S.M., Cowan D.A. (2022): Microbial biogeochemical cycling of nitrogen in arid ecosystems. Microbiol and Molecular Biology Reviews, 86: e0010921.

Shi Y.C., Ziadi N., Hamel C., Belanger G., Abdi D., Lajeunesse J., Lafond J., Lalande R., Shang J.L. (2020): Soil microbial biomass, activity and community structure as affected by mineral phosphorus fertilization in grassland. Applied Soil Ecology, 146: 103391.

Sui X., Zhang R., Frey B., Yang L., Li M.H., Ni H. (2019): Land use change effects on diversity of soil bacterial, Acidobacterial and fungal communities in wetlands of the Sanjiang Plain, northeastern China. Science Report, 9: 18535.

Sun R.B., Zhang X.X., Guo X.S., Wang D.Z., Chu H.Y. (2015): Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biology and Biochemistry, 88: 9–18.

Xiao X., Li J., Lyu J., Feng Z., Zhang G., Yang H., Gao C., Jin L., Yu J. (2022): Chemical fertilizer reduction combined with bio-organic fertilizers increases cauliflower yield *via* regulation of soil biochemical properties and bacterial communities in Northwest China. Frontiers in Microbiology, 13: 922149.

Yang Y., Li M., Wu J., Pan X., Gao C., Tang D.W.S. (2022): Impact of combining long-term subsoiling and organic fertilizer on soil microbial biomass carbon and nitrogen, soil enzyme activity, and water use of winter wheat. Frontier in Plant Science, 12: 788651.

Received: March 27, 2023
Accepted: August 7, 2023
Published online: August 18, 2023