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Fertilizer management methods affect bacterial community structure and diversity in the maize rhizosphere soil of a coal mine reclamation area

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Abstract

Background The filling or mixed stack mode is a frequently used coal mine reclamation engineering technique that results in changes in soil microbial community structure and nutrient content, which lead to considerable deviations from the characteristics of restored coal mine reclamation areas that can be used for farming. Fertilization is an effective strategy for improving soil fertility in such areas; however, the response of soil bacterial communities, especially in the crop rhizosphere soil, to different fertilization techniques in such soils remains unclear. Therefore, we investigated the effects of different fertilization management methods, including no fertilizer, farmers' practice, inorganic fertilizer, organic fertilizer, and organic–inorganic fertilizer, on maize yield, rhizosphere soil bacterial community and diversity, soil physicochemical properties, and nitrogen cycle-related gene abundance (*nifH*, *AOB*, and *nirS*).

Results The results showed that organic–inorganic fertilizer treatment significantly improved maize yield. The relative abundance of the dominant phyla did not significantly differ between the treatment groups. However, the Chao 1 and Shannon indices of the bacterial community significantly changed between the no fertilizer and organic–inorganic fertilizer treatments. Notably, organic–inorganic fertilizer application significantly increased the copy numbers of *nifH* and *nirS*. Further, moisture, bulk density, and available phosphorus content were identified as the major driving factors responsible for the changes in bacterial community structure, diversity, and copy numbers of *nifH*, *nirS*, and *AOB*.

Conclusions The results of this study revealed that organic–inorganic fertilizer application improved soil bacterial diversity and the copy numbers of *nifH* and *nirS* in maize rhizosphere soil. Therefore, we concluded that organic–inorganic fertilizer is an effective strategy for the restoration of maize rhizosphere soil properties and bacterial communities in coal mine reclamation areas.

Keywords Coal gangue, Coal mine reclamation, Crop management, Fertilization management, Rhizosphere, Sustainability

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Background

Coal gangue, generated during the coal mining process, accounts for approximately 10–15% of coal production (Li and Wang 2019; Zhang and Ling 2020). This low coal yield results in resource wastage and the degradation of the original geological landform (Chen and Lu 2021). The filling or mixed stack mode is often used for

soil reconstruction in coal mine reclamation areas. This filling mode involves the use of fly ash and coal gangue as filling materials followed by covering with natural soil. However, the soil thus reclaimed is associated with issues, such as a low nutrient content (Zhang et al. 2014) and poor physical fertility (Chen et al. 2018). Studies have shown that 8–12 years after reclamation with fly ash, the soil nutrient content is still lower than that of normal agricultural soils (Hu et al. 2002), with low biological activity (Xu et al. 2012). The mixed stack mode has been widely used to reverse mining-related subsidence owing to the ease with which it is applied and its simple equipment requirements. However, the upper and lower layers of the reclaimed soil become mixed, resulting in poor nutrient content and weak microbial activity (Hu et al. 1994). Reportedly, it takes at least 13 years for the physical and chemical properties of farmland soils reclaimed using this method to return to normal; thus, improving the nutrient content and microbial activity in coal mine reclamation areas within a shorter time frame is crucial (Hu et al. 2021; Zhang et al. 2020).

Previous studies have shown that fertilizer application (Li et al. 2021) and crop cultivation (Xu et al. 2018) can improve the nutrient content of reclaimed soil, but the response of bacterial and microbial communities in such reclaimed soil to fertilizer treatment is still unclear. As the interface between soil and crops, the rhizosphere is an important factor affecting crop nutrient absorption and fertilizer utilization efficiency. Plant roots, including those of maize, predominantly modify the rhizosphere. Therefore, the rhizosphere hosts numerous active microbial communities that are affected by mineral nutrients supplied through fertilizer (Ali et al. 2009; Philippot et al. 2013). Studies have also revealed that the microorganisms in the rhizosphere are strongly affected by environmental heterogeneities, such as plant species and crop management techniques (Chen et al. 2019; Zhu et al. 2016). Zhao et al. (2018) found that fertilizers can reduce rhizosphere bacterial diversity and abundance. In contrast, organic manure improves rhizosphere soil microbial communities in a long-term fertilized field (Enebe and Babalola 2020; Wu et al. 2020b). Unlike chemical fertilizers, organic fertilizers when applied continuously strengthen the relationship between soil microbial function and crop yield (Li et al. 2022a, b, c, d). The effect of the combined application of organic and inorganic fertilizers on the soil microbial community, especially in the rhizosphere, needs further clarification.

Nitrogen plays a key role in soil health and the growth of crops, and is among the nutrients commonly supplied in fertilizers. Inorganic fertilizers have become the leading option due to their high efficiency and association with significantly increased yields (Li et al. 2022c). Further, the application of organic fertilizers has been repeatedly proven to significantly improve soil structure. Additionally, organic fertilizers are rich in nutrients beneficial to the material balance of agricultural ecosystems (Cao et al. 2020). Therefore, we hypothesized that different fertilization treatments with equal nitrogen contents will result in soils with different physical and chemical properties as well as microbial activities and abundances. As a result, nitrogen cycling in the soil may also be affected. Nitrogen fixation, nitrification, and denitrification, which are driven by microbes, are vital nitrogen cycle processes that mediate soil nitrogen availability (Li et al. 2022b; Putz et al. 2018; Song and Niu 2022). Enhanced nitrogen rates stimulate the relative abundance of *nirA* (which encodes nitrate reductase), *nirK* (which encodes nitrite reductase containing copper ions), and *nosZ* (which encodes nitrous oxide reductase; Linton et al. 2020). Furthermore, in the rhizosphere microbial communities in maize, the relative abundance of bacteria related to the nitrogen cycle is significantly increased, indicating the promotion of nitrogen fixation and ammonization (Henneron et al. 2020; Yu et al. 2019). However, whether different fertilization treatments containing equal amounts of nitrogen would differently affect the abundance of *nifH* (which encodes nitrogenase ferritin), *nirS* (which encodes nitrous reductase containing cytochrome cd1), and *AOB* (which encodes ammoxidation) gene copies of rhizosphere soil bacteria remains unclear.

In this study, we investigated the rhizosphere soil of maize in a coal mine reclamation area fertilized starting in July, 2014. High-throughput 16S rRNA Illumina MiSeq technology and quantitative polymerase chain reaction (qPCR) methods were employed to understand the composition and structure of the bacterial community and to evaluate *AOB*, *nifH,* and *nirS* gene copies in maize rhizosphere soil undergoing different fertilization treatments for 8 years. The study was performed based on the hypothesis that fertilization could influence rhizosphere soil bacterial community structure and diversity and *AOB*, *nifH,* and *nirS* gene copies by altering the physicochemical properties of the soil. The specific goals were to: (1) determine whether the continuous application of different fertilization treatments affects the maize rhizosphere soil bacterial community; (2) characterize the bacterial community and evaluate *AOB*, *nifH*, and *nirS* copies according to fertilization treatment; and (3) confirm whether fertilization treatment choice changes the soil bacterial community by changing the physicochemical properties of the soil. Our study will provide valuable insights into optimal fertilization management practices for sustainable coal mining area reclamation.

Results

Organic–inorganic fertilizer treatment increased maize yield and height

The addition of fertilizer significantly increased maize height and yield compared with the no fertilizer treatment (Fig. 1). Under organic–inorganic fertilizer treatment, maize height and yield were 61.4 ± 7.81 cm and 32.70 ± 3.82 kg/ha greater, respectively, than those observed after the no fertilizer treatment, presenting a significant difference ($p < 0.05$; Supplementary Table 1, Additional File 1). Furthermore, Tukey analysis showed that the organic–inorganic fertilizer treatment significantly increased maize height compared with the organic fertilizer, inorganic fertilizer, farmer's practice, and the no fertilizer treatments. The maize yield obtained under the organic–inorganic fertilizer treatment was also significantly higher than those obtained under the other treatments.

Physical and chemical properties of the rhizosphere soil of maize

The physical and chemical properties of the rhizosphere soil of maize are presented in (Supplementary Table 2, Additional File 1). The pH range of the maize rhizosphere soil was 8.18–8.41, and the pH corresponding to the organic fertilizer treatment was significantly lower than that corresponding to the other treatments (Table 1). The bulk density (BD) observed for the no fertilizer group was significantly higher than that observed for the other groups. The moisture content, which was the highest for the organic–inorganic fertilizer treatment group, was significantly higher than that observed for the inorganic and organic fertilizer groups. The lowest moisture content was observed for the no fertilizer group. Fertilizer application significantly affected the NH_4^+-N, NO_3^--N, and total nitrogen (TN) contents. Meanwhile, the NH_4^+-N content for the organic–inorganic fertilizer group was significantly lower than that for the other treatment groups. The NO_3^--N and TN contents in the no fertilizer group were the lowest, at 1.64 mg/kg and 0.024%, respectively. The order of available phosphorus (AP) and available potassium (AK) contents following the five treatment groups was: organic–inorganic fertilizer > organic fertilizer > inorganic fertilizer > farmers' practice > no fertilizer, with the organic–inorganic fertilizer group showing significantly higher AP and AK contents than the other treatment groups. In conclusion, the long-term application of fertilizers significantly affected the pH, BD, moisture, and NH_4^+-N, NO_3^--N, TN, AP, and AK contents of maize rhizosphere soil.

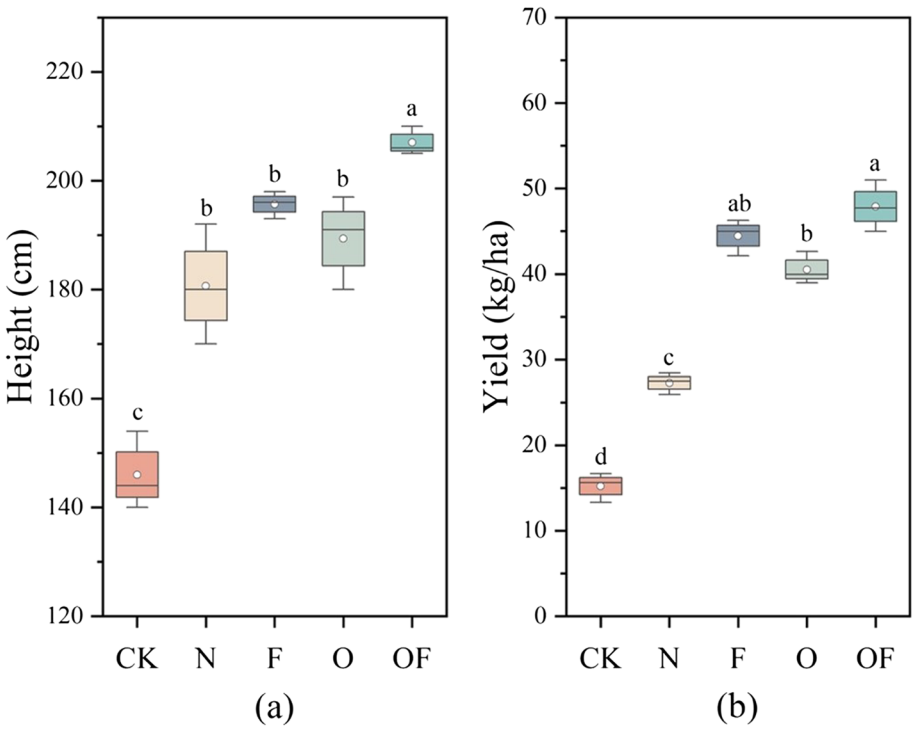

Fig. 1 Effect of fertilizer treatment on maize height and yield. (**a** height; **b** yield) Note: CK, no fertilizer; N, farmers' practice; F, inorganic fertilizer; O, organic fertilizer; OF, combined application of organic and inorganic fertilizers. Five numerical points of box plot: maximum observed value (upper edge); 75% quantile; median; 25% quantile; minimum observed value (lower edge). The points outside the box diagram are abnormal points. Different letters indicate significant differences between treatments according to Tukey test ($p < 0.05$)

Table 1 Chemical and physical properties of maize rhizosphere soil under different fertilizer treatments

Treatment	pH	BD (g/cm^3)	Moisture (%)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	TN (g/kg)	AP (mg/kg)	AK (mg/kg)
CK	8.49 ± 0.06a	1.34 ± 0.004a	6.16 ± 0.01e	3.54 ± 0.15b	1.64 ± 0.18b	0.54 ± 0.01c	5.35 ± 0.01e	127 ± 1.00e
N	8.41 ± 0.02ab	1.27 ± 0.001b	6.33 ± 0.02d	4.57 ± 0.12a	2.62 ± 0.65ab	0.56 ± 0.02bc	5.92 ± 0.02d	135 ± 0.58d
F	8.32 ± 0.05b	1.25 ± 0.005c	6.55 ± 0.02c	4.12 ± 0.25ab	3.10 ± 0.51a	0.58 ± 0.01bc	6.68 ± 0.03c	141 ± 0.58c
O	8.18 ± 0.01c	1.24 ± 0.008 cd	7.36 ± 0.01b	2.67 ± 0.29c	2.02 ± 0.48ab	0.62 ± 0.04ab	6.89 ± 0.01b	157 ± 1.00b
OF	8.35 ± 0.08b	1.23 ± 0.002d	7.56 ± 0.02a	0.79 ± 0.31d	1.81 ± 0.10b	0.65 ± 0.03a	8.46 ± 0.01a	176 ± 1.53a

CK No fertilizer, *N* Farmers' practice, *F* Inorganic fertilizer, *O* Organic fertilizer, *OF* Combined application of organic and inorganic fertilizers, *BD* Bulk density, *TN* Total nitrogen, *AP* Available phosphorus, *AK* Available potassium. Different letters indicate significant differences between treatment groups according to Tukey test ($p < 0.05$)

Bacterial community structure and diversity under different fertilization conditions

In total, 5988 operational taxonomic units (OTUs) were identified in the soil bacterial communities. These OTUs belonged to 41 phyla, 149 classes, 161 orders, 830 families, and 3050 genera (Supplementary Table 3, Additional File 1). In this experiment, the highly variable V3–V4 region of the bacterial 16S rRNA gene with a length of approximately 468 bp was selected for double-end sequencing, and the number of reads was 60,000.

The soil samples were dominated by Proteobacteria, Actinobacteria, and Chloroflexi. The total relative abundance of these three bacterial phyla accounted for 65.79–89.95% of the bacterial sequence (Fig. 2a). The presence of these dominant phyla was no different among the treatment groups (Supplementary Table 4, Additional File 1). However, the relative abundance of Gemmatimonadetes, Nitrospirae, and Verrucomicrobia in the organic–inorganic fertilizer treatment group was significantly higher than that in the no fertilizer group ($p < 0.05$). Furthermore, the organic–inorganic fertilizer treatment group exhibited a significantly reduced relative abundance of Bacteroides and Firmicutes. At the genus level, fertilization significantly affected the relative abundance of *subgroups_6*, *KD4-96*, *Nocardioides*, *Sphingomonas*, and *Solidubrobacter*. The proportions of *subgroups_6*, *Sphingomonas*, and *Solidubrobacter* in the fertilization treatment groups were higher than those in the no fertilizer group. In contrast, the abundances of *KD4-96* and *Nocardioides* in the no fertilizer group were significantly higher than those in the other treatment groups (Supplementary Table 5, Additional File 1).

The α-diversity analysis revealed that the bacterial community diversity index markedly varied between

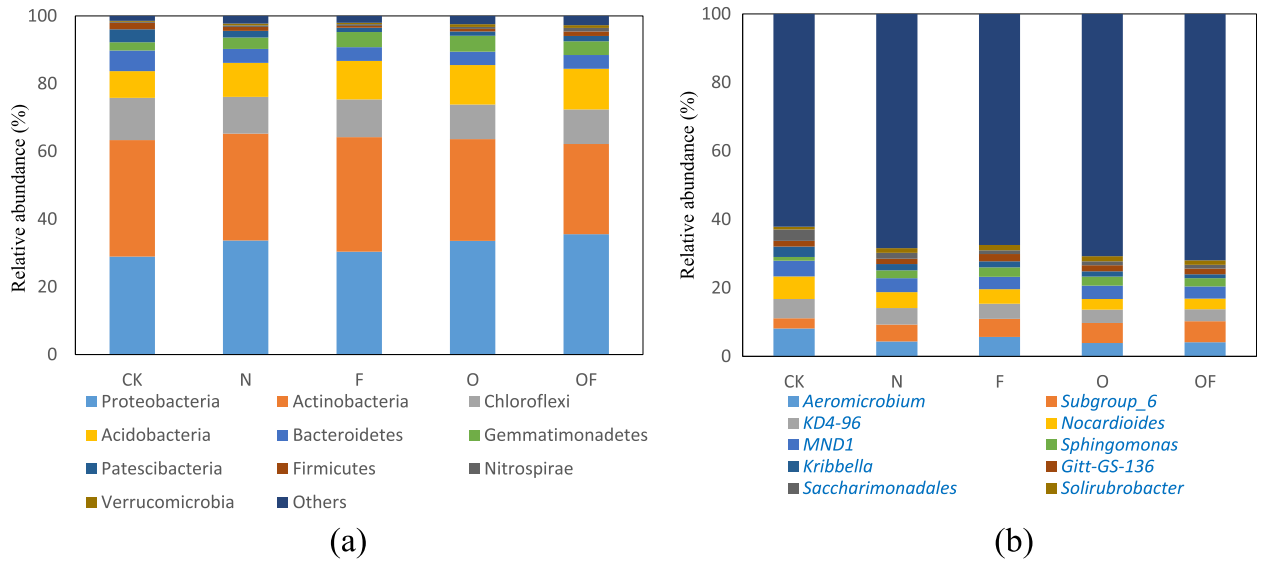

Fig. 2 Bacterial community composition of the maize rhizosphere. (**a** phylum level; **b** genus level) Note: CK, no fertilizer; N, farmers' practice; F, inorganic fertilizer; O, organic fertilizer; OF, combined application of organic and inorganic fertilizers. Phyla and genera with abundances less than 1% are merged into "Others". Phylum and genus abundance in each treatment was the average from all samples

the no fertilizer and organic–inorganic fertilizer groups. Organic–inorganic fertilizer application increased the Chao 1 and Shannon indices over those of the no fertilizer treatment (Supplementary Table 6, Additional File 1). Collectively, the bacterial α-diversity was significantly influenced by the organic–inorganic fertilizer treatment (Fig. 3).

Copy numbers of nifH, AOB, and nirS genes

Subsequently, we attempted to further clarify the effects of long-term fertilization on the copy numbers of genes related to the nitrogen cycle (Supplementary Table 7, Additional File 1). Thus, we found that the copy number of *nirS* in the maize rhizosphere soil was higher than those of *nifH* and *AOB* (Fig. 4). Specifically, the copy number of *nirS* ranged as $6.62–43.28 \times 10^4$/g, and the variance results showed that fertilization significantly affected its abundance. In addition, organic–inorganic fertilizer application was associated with a significantly higher copy number (a 6.54-fold increase) of *nirS* than that for the other treatment groups, particularly the no fertilizer treatment. Further, only the organic–inorganic fertilizer treatment significantly increased the copy number of *nifH*. The differences among the other treatment groups were not significant. The copy number of *AOB* was the highest for the inorganic fertilizer treatment group.

Redundancy analysis between physicochemical properties, bacterial diversity, and the copy numbers of nifH, nirS, and AOB

Redundancy analysis (RDA) showed that 63.56% and 24.53% of the total variation could be explained by axis 1 and axis 2, respectively (Fig. 5). The soil's TN, AP, and AK contents positively influenced the Chao1 and Shannon indices. The copy numbers of *nifH* and *nirS* were negatively related to NO_3^--N, NH_4^+-N, and BD contents. Correlation analysis also identified moisture, BD, and AP as the major factors driving the bacterial diversity and the copy numbers of *nifH*, *nirS*, and *AOB* (Table 2).

Adding fertilizers significantly increased the copy number of *nifH*, especially under organic–inorganic fertilizer treatment, which showed a significantly higher copy number of *nifH* than the other treatments, and a higher corresponding TN content. Meanwhile, the copy number of *nirS* under organic–inorganic fertilizer treatment was also significantly higher than those corresponding to the other treatments. The NO_3^--N content was also significantly lower than that observed under the other treatments, further confirming this point. However, it was

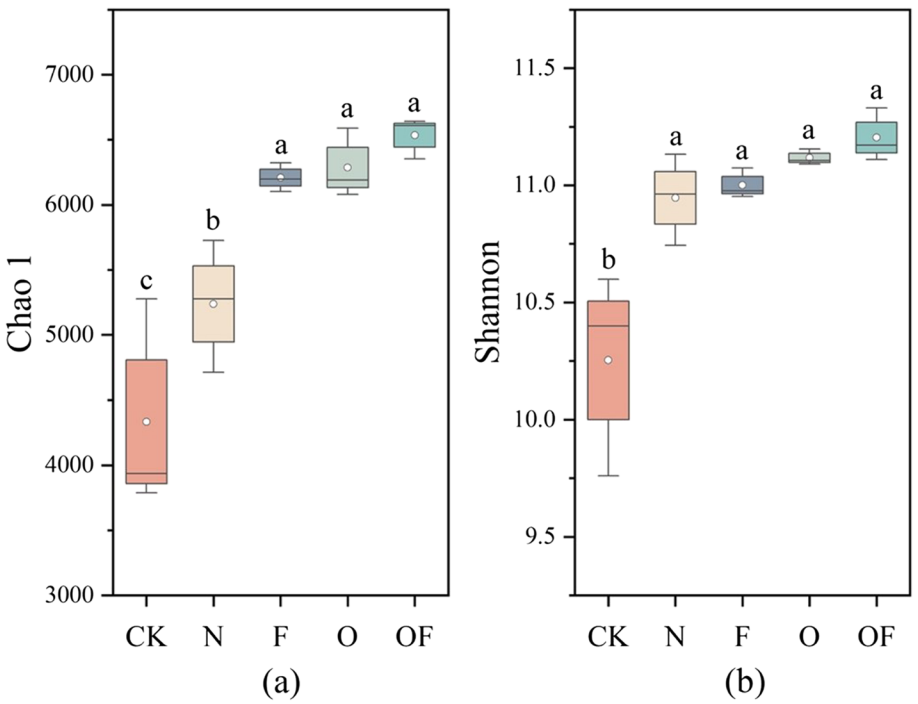

Fig. 3 Chao1 and Shannon indices of rhizosphere bacterial communities. (**a** Chao 1 indices; **b** Shannon indices) Note: CK, no fertilizer; N, farmers' practice; F, inorganic fertilizer; O, organic fertilizer; OF, combined application of organic and inorganic fertilizers. Five numerical points of box plot: maximum observed value (upper edge); 75% quantile; median; 25% quantile; minimum observed value (lower edge). The points outside the box diagram are abnormal points. Different letters indicate significant differences between treatments according to Tukey test ($p < 0.05$)

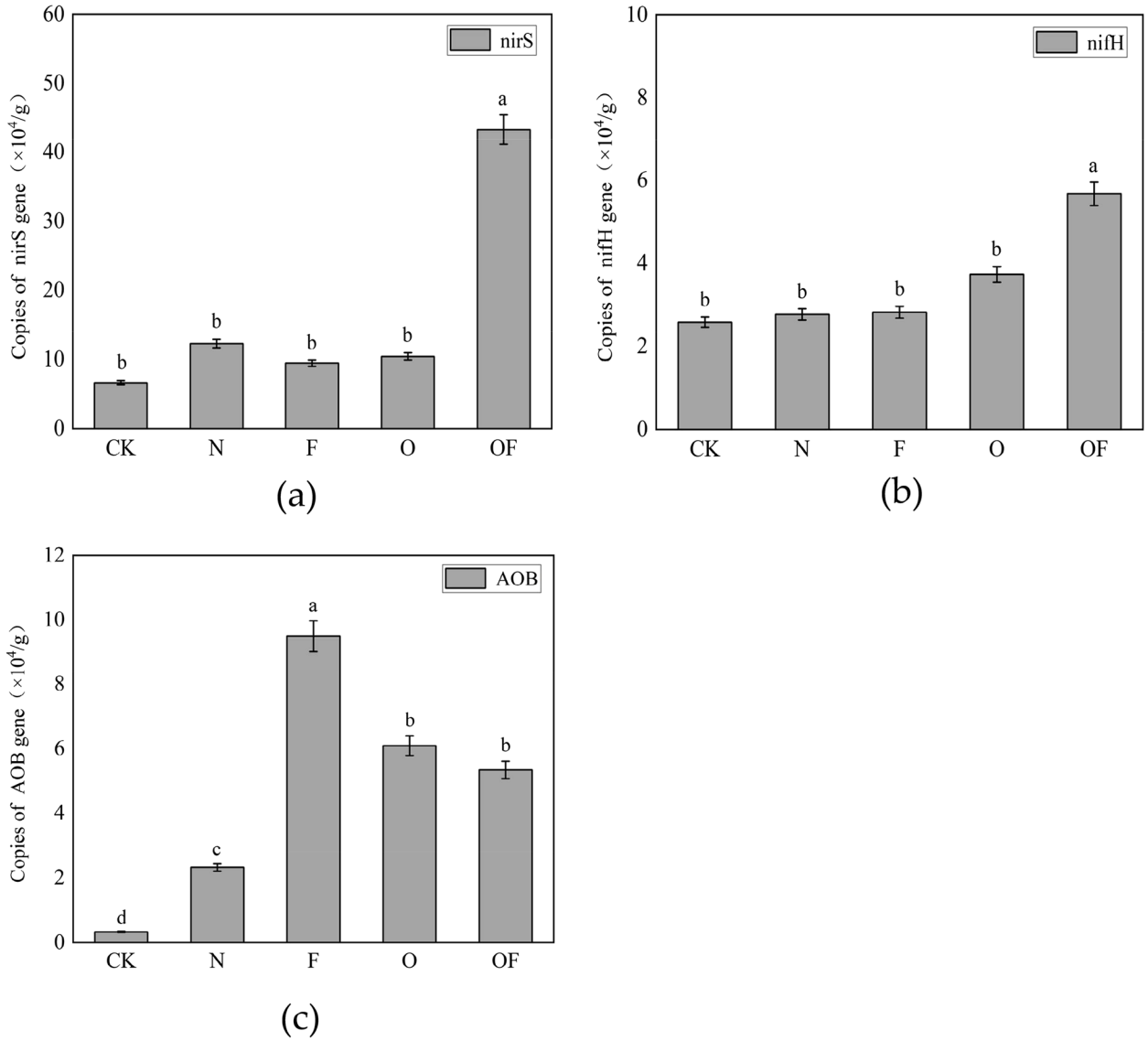

Fig. 4 Effect of fertilizer treatments on copy numbers of *nirS, nifH*, and *AOB* in maize rhizosphere soil (**a** *nirS*; **b** *nifH*; **c** *AOB*). Note: CK, no fertilizer; N, farmers' practice; F, inorganic fertilizer; O, organic fertilizer; OF, combined application of organic and inorganic fertilizers. Different letters indicate significant differences between treatments according to Tukey test ($p < 0.05$)

interesting to note that the copy number of *nirS* was not significantly different among the no fertilizer, farmers' practice, inorganic fertilizer, and organic fertilizer treatments, while the NO_3^--N content was significantly different among these four treatments, possibly due to the fact that the NO_3^--N content was not solely determined by the copy number of *nirS*. Similarly, at the phylum level, we found that Nitrospirae was significantly more abundant under organic–inorganic fertilizer treatment than under the other treatments, and the lower NO_3^--N content under this treatment might have been due to the large amount of NO_3^--N absorption by the maize during the sampling period. The NH_4^+-N content and copy number of *AOB* under inorganic fertilizer treatment were significantly higher than those under organic fertilizer treatment, possibly because NH_4^+-N content does not depend only on the copy number of *AOB*.

Discussion

The obvious advantage of the long-term application of fertilizer is an increase in maize yield and the improvement of soil physical and chemical properties. In this study, we observed that maize height and yield increased after 8 years of fertilizer application compared with

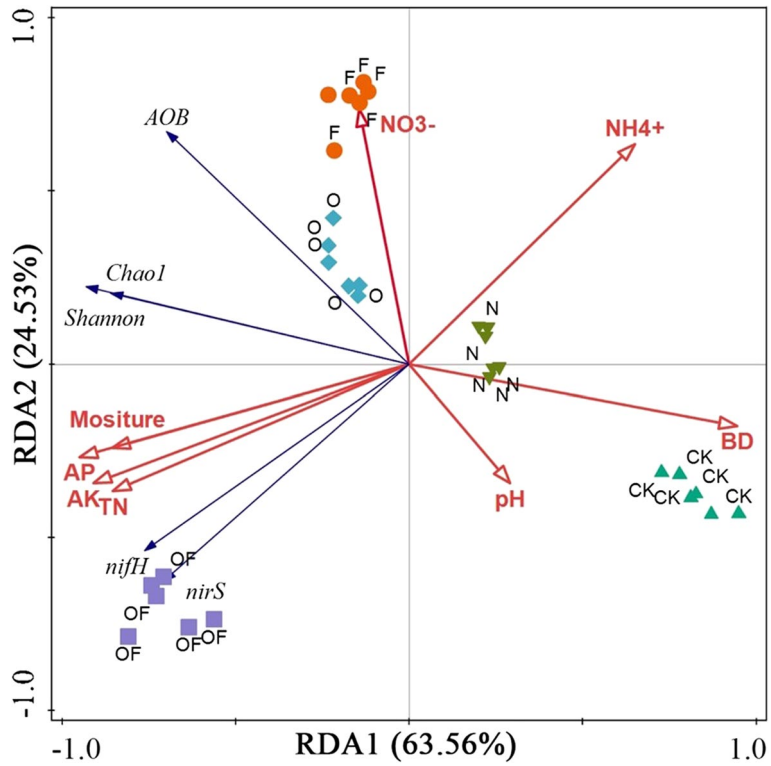

Fig. 5 Summary of redundancy analysis (RDA) of soil physicochemical properties and bacterial diversity.

Table 2 Correlation between copy numbers of nitrate epitope genes and physicochemical properties of maize rhizosphere soil

Index	pH	Moisture (%)	BD (g/cm^3)	NO_3^--N (mg/kg)	NH_4^+-N (mg/kg)	TN (g/kg)	AP (mg/kg)	AK (mg/kg)
Chao1	-0.279	0.778*	-0.905*	0.248	-0.478	0.728*	0.794*	0.768*
Shannon	-0.081	0.683*	-0.910*	0.304	-0.353	0.633*	0.700*	0.691*
nifH	-0.353	0.840*	-0.611*	-0.443	-0.091	0.371	0.530*	0.425
AOB	-0.145	0.398	-0.749*	0.328	-0.750*	0.865*	0.845*	0.869*
nirS	0.130	0.682*	-0.548*	-0.276	-0.672*	0.751*	0.809*	0.775*

BD Bulk density, *TN* Total nitrogen, *AP* Available phosphorus, *AK* Available potassium

* Indicates significant differences between treatments based on Pearson correlation double tailed test ($p < 0.05$)

the maize yield obtained under the no fertilizer treatment, and the highest yield was observed in response to organic–inorganic fertilizer treatment. These results are consistent with those of previous reports, indicating that organic–inorganic fertilizer application can increase maize height and yield (Laura et al. 2016). We also observed that moisture, TN, AP, and AK contents in maize rhizosphere soil were higher under the organic–inorganic fertilizer treatment than under the other treatments, consistent with the results reported by Cai et al. (2019), who suggested that the increased nutrient concentrations observed under organic–inorganic fertilizer treatment can be primarily attributed to the combination of the rich nutrient content of organic fertilizers and the fast release of nutrients from inorganic fertilizers. However, the concentrations of other nutrients (e.g., NH_4^+-N and NO_3^--N) were lower after the organic–inorganic fertilizer treatment than after the other treatments. This is strongly associated with plant absorption behavior as plant roots directly take up NO_3^--N; notably, the copy number of *nirS* was the highest under the organic–inorganic fertilizer treatment. These characteristics may explain why the levels of NH_4^+-N and NO_3^--N were lower in the rhizosphere of maize treated with organic–inorganic fertilizer than in those of maize under other fertilization treatments.

Rhizosphere microbial communities, known as the "extended genome" or "second genome" of plants, play vital roles in plant growth and development (Cui et al. 2018; Zhao et al. 2021). Long-term fertilization is the main factor controlling soil microbial composition and structure (Hartmann et al. 2015). Bacterial communities are essential for maintaining soil fertility and ecosystem function and are typically sensitive to plant species and crop management techniques implemented in the rhizosphere (Aira et al. 2010; Di Salvo et al. 2018; Ramirez et al. 2010). Analysis of the taxonomic components of microorganisms revealed that the phyla Proteobacteria, Actinobacteria, and Chloroflexi are sensitive to fertilizer application (Dai et al. 2018; Soman et al. 2017). Cao et al. (2020) found that the bacterial community structure at the phylum level in reclaimed soil in a coal mining area is significantly altered when inorganic fertilizer and organic manure are applied. However, in this study, the fertilizer management method did not affect the relative abundances of the dominant phyla, which contradicts previously reported results, possibly owing to the differences in the amounts of nitrogen fertilizer. Cao et al. (2020) applied 195 kg/hm^2 nitrogen fertilizer, which is more than the amount applied in this study. Nitrogen fertilizer can stimulate the growth and proliferation of microorganisms, thereby affecting microbial abundance (Zheng et al. 2019; Li et al. 2022a). However, the soil nutrient content in coal mine reclamation areas is relatively low. Further, the addition of exogenous nitrogen (150 kg/hm^2) may not affect the soil environment sufficiently to change the relative abundance of the dominant phyla.

Previous studies have revealed that organic–inorganic fertilizer treatment results in the highest bacterial levels and community diversity (Daquiado et al. 2016; Sun et al. 2015a, b; Yu et al. 2015). Based on a long-term fertilization experiment, Fan et al. (2021) analyzed the effects of different fertilizer treatments on bacterial diversity. They found no significant difference between NPK treatment and the unfertilized control, while organic fertilizers significantly increased bacterial diversity compared with the control. Liu et al. (2021) reported similar results, noting that organic–inorganic fertilizer treatment improved the α-diversity of the rhizosphere soil bacterial community. The effect of fertilizer application on bacterial diversity varies with soil texture and water management but has no association with nitrogen application (Li et al. 2018). With the significant increase in mining activities, soil microbial diversity in reclaimed areas is crucial for subsequent agricultural production. The results of the present study demonstrated that fertilizers increased maize rhizosphere soil bacterial diversity (Chao1 and Shannon indices), especially after organic–inorganic fertilizer treatment, consistent with a previous report (Shi et al. 2020). The bacterial Chao1 index in organic–inorganic fertilizer, organic fertilizer, and inorganic fertilizer treatments was significantly higher than that observed following the farmers' practice and no fertilizer treatments, indicating that organic and inorganic fertilizer treatments are more conducive for bacterial diversity. This may be because the treatment continues to slowly release effective nutrients, making an important contribution to the continuous enrichment of rare microbial species from the soil to the rhizosphere of crops, resulting in a significant increase in rhizosphere microbial species and a significant increase in bacterial diversity.

In this study, genes involved in N fixation (*nifH*) were widely distributed in the soil that received the organic–inorganic fertilizer treatment (Fig. 4). Coelho et al. (2008) showed that inorganic–organic fertilizer affected the rhizosphere copy number of *nifH*. This result suggested that organic–inorganic fertilizer treatment provides sufficient nitrogen to stimulate microbial activity, so that the nitrogen in the soil is fixed by microorganisms and becomes microbial nitrogen, which is more easily mineralized than original nitrogen. Thus, provides more available nitrogen, resulting in an increase in the yield of maize (Fig. 1). Ammonia-oxidizing bacteria drive soil nitrification and play an important role in nitrogen cycling in soil ecosystems (Wang et al. 2015). This study showed that adding fertilizer significantly increases the copy number of *AOB* compared to that observed under no fertilizer treatment; this is consistent with previously reported findings (Cui et al. 2016). However, there were significant differences in the impact of the different fertilization treatments on *AOB* under equal nitrogen levels. The copy number of *AOB* was highest under the inorganic fertilizer treatment and was significantly higher than those obtained under the other fertilization treatments, indicating that inorganic fertilizer application improves the copy number of *AOB*. This may be related to the fact that ammonia-oxidizing bacteria possessing urease are able to grow using urea, and undergo ammonia oxidation via urea hydrolysis in the natural environment (Lu et al. 2012). The copy numbers of *nirS*, which are involved in denitrification, were higher after the organic–inorganic fertilizer treatment than after the other treatments. The addition of the organic–inorganic fertilizer provided a large amount of carbon to the soil as well as rich electron donors, thereby promoting denitrification by microorganisms. At the same time, the combination of organic and inorganic fertilizers is also beneficial to the growth of other microorganisms, increasing CO_2 emissions and accelerating oxygen consumption, which provide a good low-oxygen microenvironment for denitrification (Song and Niu 2022). The organic–inorganic

fertilizer treatment significantly affected the microbial communities involved in the nitrogen cycle (Sun et al. 2015a, b; Ouyang et al. 2018). According to qPCR results, the copy numbers of *nifH* and *nirS* were highest for the organic–inorganic group. We also found that the number of *Azotobacter* sp., *Azotobacter*, *Azomonas*, *Beijerinckia*, *Derxia*, and *Pseudomonas*/*Alcaligenes* Castellani & Chalmers OTUs differed among the different fertilization schemes. The genera with denitrification functions were significantly different among the treatments, with a maximum of 120 OTUs under organic–inorganic treatment, as verified by qPCR.

Environmental factors have important effects on the structure and composition of bacterial communities in rhizosphere soil (Liu et al. 2021; Wu et al. 2020a). When soil nutrient content is high, competition among bacterial populations decreases and diversity increases. Further, the diversity of bacterial communities is related to soil physical and chemical properties, and changes in soil conditions can in turn affect the various types of microorganisms within the soil (Zhang et al. 2015). Previous studies have revealed that fertilizer application changes microbial community diversity by affecting various soil physicochemical properties (Bulluck et al. 2002; Du et al. 2019; Guo et al. 2019). In this study, the different fertilization management schemes significantly increased soil BD as well as its moisture, TN, AP, and AK contents. Notably, organic–inorganic fertilizer treatment positively affected the bacterial community structure and diversity but had no effect on BD, consistent with previously reported results (Chen et al. 2016; Zhang et al. 2015). Thus, organic–inorganic fertilizer may increase the abundance and community diversity of soil bacteria by providing rich nutrients and a suitable environment for growth.

Conclusions

Our study revealed that organic–inorganic fertilizer application improved bacterial diversity and the copy numbers of *nifH* and *nirS* in maize rhizosphere soil. Additionally, adding organic–inorganic fertilizer increased the contents of moisture, TN, AP, and AK, nutrients, which are required by maize for growth. Furthermore, the changes in microbial diversity and copy numbers of *nifH* and *nirS* were driven by soil moisture, BD, and AP. Overall, these results provide a basis for the broader application of organic–inorganic fertilizer in sustainable coal mining area reclamation. In addition, we would further study the relationship between different proportions of organic and inorganic fertilizers and bacterial communities to deepen our understanding of the bacterial community in maize rhizosphere soil and improve the application of organic–inorganic fertilizer treatment.

Methods

Site description

The study area, located in the Gujiao Tunlan coal mine reclamation area (112° 06' E, 37°53' N) in Taiyuan, Shanxi Province, China, is part of the Loess hilly area. The annual average temperature and precipitation in this area are 9.5 °C and 460 mm, respectively, reflecting a temperate continental monsoon climate (Bai et al. 2022).

In 2002, the Tunlan Mine of Shanxi Coal Power Group began to discharge coal gangue in natural gullies. The gangue discharge activity ended in 2013, and the surface of the gangue landfill area was then covered with the surrounding natural soil. The average thickness of the initial covering soil was 70 cm. The experiment was conducted in 2014, a year after covering with natural soil.

Experimental design

The experiment involved the use of a single-factor completely randomized design. The factor was fertilizer treatment, with five levels: no fertilizer, farmers' practice, inorganic fertilizer, organic fertilizer, and organic–inorganic combined application; each fertilizer treatment was performed in triplicate. Thus, the study involved a total of 15 plots. The area of each plot was 10 m × 10 m, the width of each aisle 2 m, and the total area of the whole site 1972 m^2 (Fig. 6). The fertilizer amount in the no fertilizer treatment was 0, while the other treatments involved equal amounts of nitrogen (150 kg/hm^2; An et al. 2020). For the inorganic fertilizer treatment, a compound fertilizer with $N:P_2O_5:K_2O = 18:12:10$ was used. For the organic fertilizer treatment, organic matter, nitrogen, phosphorus, and potassium contents of 53.48%, 2.16%, 1.68%, and 2.2%, respectively, were provided by the Pingyao Guoqing Tongying Poultry Co., Ltd. (Jinzhong, China). The organic–inorganic fertilizer treatment included equal ratios of organic and inorganic fertilizers. All the

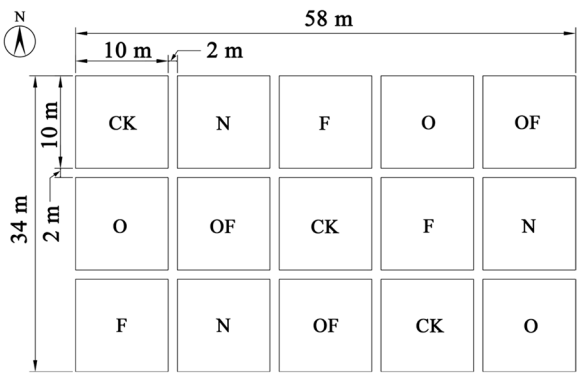

Fig. 6 Test plot size and distribution map. Note: CK, no fertilizer; N, farmers' practice; F, inorganic fertilizer; O, organic fertilizer; OF, combined application of organic and inorganic fertilizers

fertilizers were applied as a base fertilizer before spring sowing. The planting crop was maize (Xianyu 335), and the planting density was 0.6 × 0.3 m (line × row).

After the completion of the remediation project in 2014, the test site was leveled and uniformly treated to ensure consistent properties in the topsoil. The pH was 8.61, and the organic carbon, TN, alkali hydrolyzed nitrogen, AP, and AK contents were 3.05 g/kg, 0.42 g/kg, 13.33 mg/kg, 1.52 mg/kg, and 81.10 mg/kg, respectively.

Rhizosphere soil sample collection

After examining the distribution of maize roots, we found that the roots were predominantly distributed within the 0–30 cm depth range. Next, we selected six plants from each plot in July 2021. We used a shovel to remove a 40 × 20 cm soil block (sampling at each corn spacing and distance direction) from each selected maize plant, and thereafter, separated the block into 0–30 cm segments. The maize spacing and distance samples were then mixed into a single sample. Thus, a total of six independent soil samples were obtained for each plot; overall, 90 samples were included.

The maize rhizosphere soil was collected using the "shaking off method" as follows: the loosely attached soil on the root was gently shaken off, and any soil tightly attached to the root was dusted off with a sterile brush, put into a sterile self-sealing bag, temporarily stored in an ice box, and quickly transported to the laboratory. The collected rhizosphere soil samples were divided into three parts: the first sample was transferred to a sterile tube and stored at -70 ℃ for high-throughput sequencing; the second sample was stored in a refrigerator at -4 ℃ to determine soil NH_4^+-N and NO_3^--N concentrations; the third sample was used to determine other soil chemical and physical properties.

Determination of soil chemical and physical properties

The maize rhizosphere soil samples collected were divided into two parts. The first part was used to measure moisture content and BD, while the second part was milled and passed through a 20-mm sieve to determine the soil pH and nutrient content. The moisture content of the samples was determined using the gravimetric method by baking it to a constant weight at 105 ℃ (Jiao et al. 2023). Further, the ring knife method was used to measure the BD of soil samples and calculate the mass of the dry soil per unit volume (Suzuki et al. 2022). Soil pH was determined using a glass electrode with 2.5:1 water-soil ratio (v/w) (Guangzhou, China), and TN was determined using the Kjeldahl method (Hangzhou, China). AP was determined via $NaHCO_3$ molybdenum blue colorimetry and ultraviolet spectrophotometry, and available potassium (AK) was determined via NH_4Ac extraction and flame photometry (Beijing, China; Long et al. 2022). NH_4^+-N and NO_3^--N content were determined via spectrophotometry (Shanghai, China; Bao 2007).

DNA extraction

Genomic DNA was extracted from a 0.25-g sample of rhizosphere soil using the OMEGA Soil DNA Kit (m56350-02; Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions (Li et al. 2020). The quantity of the extracted DNA was measured using the NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), while the quality of extracted DNA was determined via agarose gel electrophoresis. For the PCR amplification of the V3–V4 region of bacterial 16S rRNA gene, we used the forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and the reverse primer 806R (5′-GGACTACHVGGG TWTCTAAT-3′) (Yang et al. 2022). Further, we used the Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) for PCR amplicon purification and the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) for PCR amplicon quantification. After the individual quantification step, the amplicons were pooled in equal amounts. Then, we used the Illumina MiSeq platform with NovaSeq 6000 SP Reagent Kit (500 cycles) for paired-end 2 × 250 bp sequencing at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

Illumina MiSeq analysis

Illumina MiSeq sequencing was used to generate double-ended raw sequence data. Briefly, using the demux plugin, we demultiplexed the raw sequence data followed by cutting the primers with the cutadapt plugin. First, we used cutadapt (v2.3) to cut the primer fragment of the sequence, and next, discarded the sequences of unmatched primers. The fastq_mergepairs module of Vsearch (v2.13.1_linux_x86_64) was used for sequence splicing, the fastq_ filter was used for quality control, and the derep_fulllength module was used to remove duplicate sequences. We also used the cluster_size module to cluster the sequences after the removal of duplicate sequences at a 98% similarity level and used the uchime_denovo module to remove chimeras. Thereafter, we used perl script (https://github.com/torognes/vsearch/wiki/VSEARCH-pipeline) to filter the chimeras in the sequence set after quality control to obtain high-quality sequences. We again used the cluster_size module to cluster the high-quality sequences at a 97% similarity level to form OTUs. Sequence data analyses were mainly performed using QIIME2 and R packages (v3.2.0).

Table 3 Primer information used in the experiment

ID	Primer name	Sequence (5′–3′)
AOB	amoB-1F	GGGGTTTCTACTGGTGGT
	amoB-2R	CCCCTCKGSAAAGCCTTCTTC
nifH	nifH-PolyF	TGCGAYCCSAARGCBGACTC
	nifH-PolyR	ATSGCCATCATYTCRCCGGA
nirS	cd3aF	GTCAACGTGAAGGAAACCGG
	R3cd	GAGTTCGGATGGGTCTTGA

Quantitative real-time PCR analysis

Quantitative real-time PCR was performed to determine the copy numbers of *nifH*, *AOB*, and *nirS* (Table 3) in soil samples according to the methods described by Walker et al. (2001). The reaction was conducted using an ABI Real-Time 7500 system (Applied Biosystems, Waltham, MA, USA) as follows: 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, with a final extension of 30 s at 60 °C. Then, the samples were sent to Magigene Biotechnology (Shanghai, China) for 2 × 250 bp sequencing using the Illumina NovaSeq platform.

Statistical analysis

The mean and standard deviation of variables per plot were calculated using Excel 2010. One-way analysis of variance (ANOVA in SPSS 20.0; IBM, Armonk, NY, USA) was used to analyze the differences between treatments ($p<0.05$). When the main effect of the ANOVA was significant, Tukey test was employed to separate the means. Multiple relationships between soil physicochemical properties and Chao1 and Shannon indices and the copy numbers *of nifH*, *AOB*, *nirS* and soil chemical and physical properties were evaluated via RDA.

Abbreviations

qPCR	Quantitative polymerase chain reaction
AP	Available phosphorus
AK	Available potassium
RDA	Redundancy analysis
BD	Bulk density

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-023-01729-4.

Additional file 1: The Supplementary Tables contain additional information regarding fertilizer treatments, maize rhizosphere soil chemical and physical properties, and bacterial communities. **Supplementary Table 1.** Effect of fertilizers on maize height and yield. **Supplementary Table 2.** Chemical and physical properties of maize rhizosphere soil under different fertilizer treatments. **Supplementary Table 3.** Bacterial community OTUs in maize rhizosphere soil under different fertilization treatments. **Supplementary Table 4.** Bacterial community composition of the maize rhizosphere (phylum level). **Supplementary Table 5.** Bacterial community composition of the maize rhizosphere (gene level). **Supplementary Table 6.** Chao 1 and Shannon indices of rhizosphere bacterial communities.

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Authors' contributions

D.S.J., Q.Z., and H.J.B. conceived and designed the experiments; H.J.B., Z.J.L., and D.S.J. performed the experiments; H.J.B., Z.J.L., and M.G.X. collected samples and analyzed the data; H.J.B. wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The data generated and analyzed during this study are available in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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