Fertilization treatments affect soil CO₂ emission through regulating soil bacterial community composition in the semiarid Loess Plateau

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A growing body of literature have emphasized the effects of fertilization regimes on soil respiration and microbial community in the semiarid region, however, fertilization treatment effects on the soil CO₂ emission, soil bacterial community, and their relationships from long-term experiments is lacking. In the present study, we investigated the effects of long-term fertilization regimes on soil bacterial community and thereafter on soil CO₂ emission. A 9-year field experiment was conducted with five treatments, including no fertilizer (NA) and four fertilization treatments (inorganic fertilizer (CF), inorganic plus organic fertilizer (SC), organic fertilizer (SM), and maize straw (MS)) with equal N input as N 200 kg hm⁻². The results indicated that CO₂ emission was significantly increased under fertilization treatments compared to NA treatment. The bacterial abundance was higher under MS treatment than under NA treatment, while the Chao1 richness showed opposite trend. MS treatment significantly change soil bacterial community composition compared to NA treatment, the phyla (Alphaproteobacteria and Gammaproteobacteria) and potential keystone taxa (Nitrosomonadaceae and Beijerinckiaceae) were higher, while the Acidobacteriota was lower under MS treatment than under NA treatment. CO₂ emission was positively correlated with the abundance of Alphaproteobacteria, Gammaproteobacteria, and keystone taxa, negatively correlated with these of Acidobacteriota. Random forest modeling and structural equation modeling determined soil organic carbon, total nitrogen, and the composition and network module III of the bacterial community are the main factors contribute to CO₂ emission. In conclusion, our results suggest that the increased CO₂ emission was affected by the varied of soil bacterial community composition derived from fertilization treatments, which was related to Alphaproteobacteria, Gammaproteobacteria, Acidobacteriota, and potential keystone taxa (Nitrosomonadaceae and Beijerinckiaceae), and highlight that the ecological importance of the bacterial community in mediating carbon cycling in the semiarid Loess Plateau.

Soil releases large amounts of CO₂ to the atmosphere through respiration, which leads to an increase in atmospheric CO₂ concentration, thus threatens ecological sustainability¹. Agricultural system is one of the most active components of the terrestrial ecosystem and plays crucial roles in global C cycling². Thus, even small changes in the agricultural system may significantly alter soil respiration, and profoundly affect the atmosphere CO₂ concentration³ and global C cycling⁴. Soil microbial and plant roots respiration are the key components of soil respiration⁵; meanwhile, these two pathways are simultaneously influenced by biological and abiotic properties⁶. To date, the information is limited to the biological mechanisms of these factors regulating soil respiration.

Evidence has demonstrated that soil temperature³, soil moisture⁴, plants diversity⁷, and soil physicochemical properties⁸ are the important factors for predicting soil respiration. However, uncertainties are particularly
high in farming ecosystems, in which small changes in climate and fertilization practices can strongly impact microbial C metabolism and cycling dynamics. Soil microorganisms play significant roles in predicting CO2 emission through microbial processes. Especially, microbial diversity plays important ecological roles in mediating multiple ecosystem functions, including soil CO2 emission and climate regulation. In addition to the diversity, the microbial community structures and co-occurrence network may be responsible for soil CO2 emission. Studies have shown that Proteobacteria and Actinobacteria are positively correlated with CO2 emission, suggesting that the compositions of these microbial community have potential roles in driving CO2 emission. So far, how the composition and network of the microbial community changed CO2 emission has not been fully assessed under field studies in dryland farming system.

Maize (Zea mays L.) is the dominant crop for food security in the semiarid Loess Plateau of China. Fertilization is often considered to provide the limiting element for maize growth in this region. The long-term dependence on inorganic fertilizer results in a decline in soil indicators and N use efficiency, while organic plus inorganic fertilizer is helpful to improve soil fertility and maize yield. The fertilizer application also has profound impacts on soil C pools and C fluxes. Although a large number of experiments have been conducted to explore the effects of fertilization on soil CO2 emission, it is still controversial showed positive, negative, or neutral effects on CO2 emission due to the complexity of farmland system. Therefore, understanding the effects of fertilization on CO2 emission is vital for predicting regional and global C cycling.

Here, we aimed to explore how the bacterial community regulate the response of CO2 emission to fertilization treatments. In the present study, a 9-year field experiment with five treatments was performed in the semiarid Loess Plateau. Specifically, we addressed the following questions: (i) how does soil CO2 emission change in response to fertilization treatments? (ii) how do fertilization treatments affect the diversity, composition, and co-occurrence network of soil bacterial community? and (iii) what is the ecological mechanism of soil bacterial community in driving soil CO2 emission across fertilization treatments? We hypothesized that fertilization treatments significantly changed the composition and co-occurrence network of soil bacterial community through improving soil chemical properties. We expected that the bacterial community composition mediated soil CO2 emission under fertilization treatments.

Results
Soil chemical properties and CO2 emission. Soil pH, SOC, TN, AP, C/N, and total CO2 emission obey normality, while NO3-N do not obey normality. Soil pH was significantly lower under CF, SC, and SM treatments than under NA treatment (Fig. 1). SOC was significantly higher under MS treatment than under CF and SC treatments, while NO3-N exhibited the inverse trend. The C/N ratio under MS treatment was significantly
improved compared to CF treatment. No significant difference in TN and NH₄–N was observed among fertilization treatments. Soil respiration exhibited similar variations during the growing season of maize (Fig. 2a,b). Compared to NA treatment, the mean Rs and total CO₂ emission (TCE) increased by 28.8–74.5% and 27.1–72.7% under fertilization treatments in both years, respectively (Fig. 2c,d). Furthermore, the mean values of Rs and TCE were significantly enhanced under MS treatment than under CF, SC, and SM treatments.

Soil bacterial community. Both the abundance, Chao1 richness, and Shannon index of soil bacterial community obey normality. The abundance of soil bacteria represented by the copy number of 16S rRNA gene ranged from 0.57 × 10⁷ to 1.14 × 10⁷ copies g⁻¹ soil (Fig. S1). The bacterial abundance was significantly higher under MS treatment than under NA and CF treatments. The Chao1 richness was significantly increased under SM treatment, but decreased under MS treatment compared to NA treatment. No significant differences were observed in Shannon index among five treatments.

The bacterial community compositions were mainly consisted of Alphaproteobacteria (11.3%), Gammaproteobacteria (12.0%), Acidobacteriota (17.7%), Actinobacteriota (13.6%), (Fig. 3a). The abundance of Alphaproteobacteria and Gammaproteobacteria were significantly increased, but that of Acidobacteriota was decreased under MS treatment compared with the other treatments. The dominant families in the bacterial community were Chitinophagaceae (4.3%), Nitrosomonadaceae (4.2%), Vicinamibacteraceae (4.1%), and Gemmatimonadaceae (3.6%) (Fig. 3b). The abundance of Chitinophagaceae was significantly decreased under CF, SC, SM, and MS treatments compared to NA treatment. The abundance of Nitrosomonadaceae and Beijerinckiacaeae were significantly higher under MS treatment than under NA, CF, SC, SM treatments, while Gemmatimonadaceae exhibited opposite trend (Fig. 3b). Principal coordinate analysis indicated that the bacterial community composition was significantly affected by fertilization treatments (Fig. S2). The bacterial abundance was positively correlated with SOC, C/N ratio, and TCE, while the bacterial community composition was negatively correlated with pH, SOC, and TCE (Fig. 4). TCE was positively correlated with the abundance of Alphaproteobacteria, Gammaproteobacteria, but negatively correlated with these of Acidobacteriota (Table S1).

Co-occurrence networks of soil bacterial community. To reveal the co-occurrence pattern of soil bacterial community in five treatments, a network was constructed based on 15 samples (Fig. 5). There were 140 nodes, 798 edges, and 4 modules in the networks. The bacterial network had more positive edges (750) than negative edges (48) (Table S2). In particular, the statistical keystone taxa belonged to Nitrosomonadaceae, Vicinamibacteraceae, and Beijerinckiacaeae (Table S3), and the edges associated with potential keystone taxa were mostly positive in module III. Modules I and IV were significantly correlated with pH and NO₃–N, Module II was negatively correlated with pH and SOC. Module II and IV had negatively correlations with TCE, Module
III had positive correlation with TCE (Fig. 4). In addition, TCE was positively correlated with the relative abundance of \textit{Nitrosomonadaceae} and \textit{Beijerinckiaceae} (Fig. S3).

**Soil properties and bacterial community regulated CO\textsubscript{2} emission.** Random forest modeling revealed that SOC and TN were the main soil properties for predicting CO\textsubscript{2} emission (Fig. 6a). As for the bacterial community, the bacterial compositions, and modules III and VI in the bacterial network significantly affected soil CO\textsubscript{2} emission. Structural equation modeling (SEM) further suggested that SOC and TN had significant correlations with the composition and network (modules III and IV) of the bacterial community (Fig. 6b). Importantly, CO\textsubscript{2} emission was negatively associated with the bacterial community composition ($p<0.001$), but positively correlated with the module III of bacterial network ($p<0.05$).
Discussion

Fertilization treatments affected soil CO2 emission and the bacterial community. In the semi-arid Loess Plateau, nitrogen fertilization is often regarded as the common practice to improve soil quality and crop yield due to where there is a low N level. However, fertilization regimes also significantly influence soil CO2 emission. The effect of fertilization treatments on CO2 emission was related to the autotrophic (Ra) and heterotrophic respiration (Rh), which was significantly affected by crop growth and soil substrate. In the present study, soil CO2 emission significantly increased by 27.1–72.7% under fertilization treatments compared to the NA treatment. This result was consistent with the results from previous studies that reported increased CO2 emission after fertilization in farmland ecosystems. The CF treatment promoted CO2 emission could attributed to the synchrony between nutrient supply and crop nutrient demand, and enhance maize growth, leaf area index, biomass, promoted the allocation of carbohydrates belowground and maize roots activity, thus resulting in the increase CO2 emission through Ra. The increased CO2 emission under SC, SM, and MS treatments mainly because the high level of SOC, which supply higher concentrations of nutrient substrates for the microbial community, thus stimulated soil heterotrophic respiration and CO2 emission. Moreover, CO2 production from SOC mineralization also significantly contributes to total CO2 emission. CO2 emission was significantly higher under MS treatment than under other treatments may because the high SOC mineralization in early stage of maize, which would increase soil respiration.
In this study, organic treatments (SC, SM, and MS) increased the bacterial abundance compared to the NA and CF treatments. The result was in accordance with previous researches. The large bacterial abundance was probably explained by high SOC and appropriate C/N under organic treatments. Soil C/N ratio has also been considered to play crucial roles in enhancing soil bacterial abundance. The appropriate C/N supply a favorable habitat for the bacterial community and the readily available C substrates can serve as food resources for the bacterial growth. In addition, the bacterial abundance under MS treatment was significantly higher than under NA treatment may attribute to more maize straw could help some soil-dominant bacteria proliferation, like Proteobacteria. We found that the Chao1 richness was significantly decreased under MS treatment, but increased under SM treatment compared to the NA treatment. Previous studies have reported that the application of maize straw shows stimulatory effects on bacterial phyla Proteobacteria and Firmicutes, and fungal ascomycetes, and reduces species richness due to the disappearance of locally rare species. In contrast, soil bacteria can take full advantage of nutrients in organic fertilizer and thus improve the bacterial Chao1 richness.

Soil bacterial community composition was significantly varied by fertilization treatments, and significantly correlated with soil pH and SOC, which was consistent with the findings of previous studies. Soil pH plays a key role in regulating soil bacterial community, and that high SOC dominated by the fresh labile organic C has significant effects on the composition of the bacterial community. Therefore, the difference in soil bacterial community composition could be explained by significant changes in pH and SOC across the five treatments. The bacterial communities were mainly assigned to Alphaproteobacteria, Gammaproteobacteria, Acidobacteriota, Actinobacteriota. All these bacteria are previously reported that have specific roles in utilizing SOC for respiration. The MS treatment exhibited the highest relative abundance of Alphaproteobacteria and Gammaproteobacteria, suggesting that a high SOC level is beneficial to growth and colonization of these two classes. The phylum Proteobacteria has generally been categorized as copiotrophs, preferring nutrients-rich environments. The phylum Acidobacteriota is significantly correlated with soil pH and N availability. Thus, the high abundance of the phylum Acidobacteriota under CF and SC treatments may be explained by the fact that the pH was closer to neutral and increased NO₃-N.

Co-occurrence network is a crucial tool to assess the function of soil microbial community, which reveals the potential connections and ecological niches structure among bacterial taxa in soil. In this study, the high edges between nodes in the bacterial network suggested the close relationships between bacterial taxa. The underlying connections among the taxa in bacterial network indicate the exchanging nutrients and metabolic products.
The high positive correlations in the bacterial network represented potential commensalism relationships existing between the bacterial taxa. In addition, each module group represents the clustering of species that may share the same ecological niches, and have similar responses to environmental changes. Moreover, the modules in networks have been determined to originate from interaction specificity, habitat heterogeneity, resource partition, and ecological niche overlaps, and optimize ecological functions of nutrients cycling and resources availability in the farmland system. Furthermore, we discovered that Nitrosomonadaceae, Verrucomicrobacteria, and Beijerinckiaceae were the presumed keystone taxa. The keystone taxa play strong roles in maintaining potential biochemical potential functions of soil microbial community.

Soil bacterial community regulated CO2 emission. Soil bacterial community have been reported as new insights in regulating soil respiration and CO2 emission. Soil microbial respiration is a key component of soil respiration, and is positively correlated with soil microbial biomass. The present study showed that soil CO2 emission was positively correlated with soil bacterial abundance, suggesting that increasing microbial populations may significantly contribute to soil CO2 emission, this result was consistent with previous study. Although soil bacterial diversity plays important roles in regulating soil basic respiration, our study found no relationship between soil CO2 emission and bacterial diversity. It has been documented that soil respiration dynamics is determined by a certain taxa in the bacterial composition, rather than over diversity. Therefore, a better understanding of soil respiration is required to incorporate associations in the bacterial community. We found that soil bacterial community composition had a negative correlation with soil CO2 emission, which was in agreement with Liu et al. and Chen et al., who reported that the bacterial community composition may play crucial roles in regulating soil CO2 emission. It has been broadly reported that copiotrophs and oligotrophs have the ability of utilizing C for respiration. Generally, oligotrophs are proposed to exhibit lower respiration rates than copiotrophs. The Alphaproteobacteria and Gammaproteobacteria are considered as potential copiotrophs, whereas Actinobacteria are classified as oligotrophs. In the present study, the relative abundance of Alphaproteobacteria and Gammaproteobacteria was significantly increased under MS treatment than under other treatments, while that of Actinobacteria followed a decreasing pattern. Consequently, the activity of bacterial populations with the changes in oligotroph-to-copiotroph ratio within the community may be accounted for the negative relationship between the bacterial community composition and CO2 emission across fertilization treatments. Furthermore, we found there were remarkable differences among the four distinct modules in the network, and the module III in network exerted a strong positive relationship with soil CO2 emission. Meanwhile, the keystone taxa were all in module III of the network. The potential keystone taxa in the soil microbial community are identified to exert significant contributions to SOC sequestration and carbon cycling dynamics irrespective of their abundance. In the present study, the keystone taxa Nitrosomonadaceae and Beijerinckiaceae had positive correlations with soil CO2 emission. Studies have revealed that these two dominant taxa occupy specific ability soil C for respiration. The family Beijerinckiaceae is positively correlated with decomposition rates of organic matter, Nitrosomonadaceae promote soil C mineralization, and eventually increased soil CO2 emission. Thus, soil bacterial community shift with high abundance of Nitrosomonadaceae and Beijerinckiaceae may considerably improve soil respiration and CO2 emission.

Taken together, our results highlight that the increased CO2 emission was affected by the varied of soil bacterial community composition derived from fertilization treatments, which was related to Alphaproteobacteria, Gammaproteobacteria, Acidobacteriota in the semiarid Loess Plateau. We further provide new insights into the potential underlying mechanisms of keystone taxa (Nitrosomonadaceae and Beijerinckiaceae) mediating CO2 emission in farmland systems through farmland management practices. However, it is worth noting that the associations between soil bacterial community composition and CO2 emission based on statistical correlations do not necessarily represent causal relationships. Future research using stable isotope tracking is warranted to verify the causal links between soil bacterial community and CO2 emission.

Conclusion

Our results indicated that fertilization treatments significantly increased CO2 emission compared to the NA treatment. The bacterial abundance was higher, while the Chao1 richness was lower under MS than under NA treatment. Fertilization treatments significantly affected the Alphaproteobacteria, Gammaproteobacteria, Acidobacteriota, thus changed soil bacterial community composition. Soil organic carbon, total nitrogen, and the community composition and network module III of soil bacterial community contributed to CO2 emission. We further identified CO2 emission was positively correlated with the potential keystone taxa (Nitrosomonadaceae and Beijerinckiaceae). Overall, this study provided the increased CO2 emission was affected by the varied of soil bacterial community composition derived from fertilization treatments, which was related to Alphaproteobacteria, Gammaproteobacteria, Acidobacteriota, and potential keystone taxa (Nitrosomonadaceae and Beijerinckiaceae), and may highlight that the ecological importance of the bacterial community in mediating carbon cycling in the semiarid Loess Plateau.

Materials and methods

Site description and experimental design. The filed study was located at the Rainfed Agricultural Experimental Station (35°28’N, 104°44’E) of Gansu Agricultural University in Gansu province, China. Long-term mean annual temperature and precipitation of the area were 6.4 °C and 390 mm, respectively. The soil at the experimental site is described as Calcaric Cambisol according to the Food and Agriculture Organization classification system. The field experiment with five treatments was started in 2012, including no fertilizer (NA), inorganic fertilizer (CF), inorganic plus organic fertilizer (SC), organic fertilizer (SM), and maize straw (MS). Each treatment had three replicates and 15 plots (13 m length and 3.3 m width) were formed based on a rand-
Sampling and soil properties assays. In August 2021, nine soil samples were collected from topsoil depth (0–20 cm) in each plot following an S-shaped sampling strategy, and then mixed to form a composite sample. Visible residues and roots were removed using a 2-mm soil sieve. The samples were then separated into two parts. One part was immediately stored at −80 °C for soil bacterial community analysis, and the other part was air-dried for chemical analysis. Six soil properties were determined, including soil pH, soil organic C (SOC), total N (TN), nitrate N (NO3-N), ammonium N (NH4-N), and the ratio of SOC to TN (C/N ratio). Detailed soil chemical analysis was done as described by Bao.

Soil respiration rate and CO2 emission. After crop seeding, three PVC collars were randomly pressed into each plot to measure soil respiration (Rs). The Rs rate was determined using a Li-8100A automated soil CO2 flux system from 09:00 am to 11:00 am with about 15-day intervals throughout the growing season from May 6 to October 16 in 2020, and April 29 to September 15 in 2021, respectively. Total CO2 emission (TCE) across the maize growing season was calculated according to the following equation:

\[
TCE = \sum \left[ \frac{Rs(i+1) + Rs(i)}{2} \times \left[ t(i + 1) - t(i) \right] \times 0.1584 \times 24 \right] \times 0.2727 \times 10
\]

where \( i+1 \) is current measuring date, \( i \) is previous measuring date, and \( t \) is days after seeding.

Soil bacterial community. Soil DNA was extracted from 0.5 g fresh samples using the EZNA® Soil DNA Kit. The extracted DNA concentration and purity were measured using a NanoDrop-2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The abundance of bacteria was evaluated by quantifying the copy number of 16S rRNA gene using the primers 515F and 907R. Standard curves were obtained using tenfold serial dilutions of plasmids DNA containing 16S rRNA gene. The amplification efficiency and correlation coefficients for the standard curve were 97.0% and 0.99, respectively. The bacterial abundance was calculated based on the standard curves.

High-throughput sequences of PCR products were obtained from the Illumina MiSeq platform. Raw sequences were trimmed and quality-filtered using the QIIME software (version 1.2.11) to obtain clean paired-end reads. FLASH (version 1.2.11) software was used to merge the clean paired-end reads and used MOTHUR software (V1.35.1) to remove the barcode and primers. Operational taxonomic units (OTUs) were clustered at a similarity level of 97% by the UPARSE (v7.1) software package. Bacterial sequences were taxonomically identified based on SILVA database. The bacterial diversity (Shannon index and Chao1 richness) was calculated using MOTHUR software. The bacterial sequences have been deposited in the NCBI database (Accession number: PRJNA821401).

Statistical analysis. All data were checked for normality by the Shapiro–Wilks test using SPSS 22.0 (IBM SPSS, USA), and for homoscedasticity using Levene's test with the general linear model. Either square root or natural log transformation was used to transform data to achieve normality when assumptions could not be met. One-way ANOVA with LSD testing (\( p < 0.05 \)) was used to evaluate the significant differences in soil properties, CO2 emission, and the bacterial community among the five treatments. The relationships between soil properties, soil bacterial community, and CO2 emission were assessed by Pearson’s correlation coefficients analysis. Principal coordinate analysis (PCoA) was performed to explore the Bray–Curtis distances of soil bacterial community under the five treatments. The permutational multivariate analysis of variance (PERMANOVA) was used to test the significance of the principal coordinate analysis.

Co-occurrence networks were constructed to visualize the relationships among bacterial community. 15 samples were mixed together and OTUs with a number of more than 10 were selected for network analysis. Correlations between the bacterial taxa were calculated by Spearman's correlation. A true co-occurrence was considered a statistically robust correlation between species when the Spearman's correlation coefficient was > 0.6 or < −0.6 and the \( P < 0.05 \). To reduce the chances of obtaining false-positive results, Benjamini–Hochberg method was used to test the correlations. Gephi software was used to visualize the co-occurrence network and soil taxa were categorized into the most associated modules. OTUs with high degree, high eigenvector centrality, and high closeness/betweenness centrality were considered as the keystone taxa.

The main predictors of CO2 emission were estimated using random forest analysis. The ‘randomForest’ package was used to investigate random forest modeling. The significance of model predictors was determined using the package ‘rIPermute’. The significant predictors from random forest analysis were further selected for structural equation modeling (SEM) analysis. SEM analysis was applied to determine the direct and indirect contributions of soil properties and the bacterial community to CO2 emission. SEM analysis was performed using AMOS 22.0 software (SPSS, Chicago, IL, USA). The model fitness was evaluated by \( \chi^2 (p > 0.05) \), goodness-of-fit, and root mean square error of approximation.
Data availability
The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA821401.

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Author contributions
L. L., J. X. and Z. L. designed the experiment. J. W., L. X., and Y. Z. collected data. J. W. Wrote the main manuscript. L. L., Y. J., and Z. E. revised the manuscript. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

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