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ORIGINAL PAPER

Effects of Short-Term Application of Chemical and Organic Fertilizers on Bacterial Diversity of Cornfield Soil in a Karst Area

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Abstract

Among karst mountain agricultural fertilization measures, the partial replacement of chemical fertilizers with organic fertilizers is important for protecting its vulnerable mountain environment and developing ecologically-friendly agriculture. In this study, we investigated the effects of short-term organic fertilizer application on the bacterial diversity of maize soil in karst areas and the potential of using organic fertilizer as a partial substitute for chemical fertilizers. Two maize fields with different parent materials in a karst region were selected for a short-term field control experiment using chemical fertilizer and organic fertilizer treatment, combined with the high-throughput sequencing method of 16S rDNA gene amplicons. (i) The soil physicochemical properties and bacterial diversity of different parent material soil are different, but the main dominant bacterial types are similar. (ii) Short-term organic fertilizer treatment, rather than chemical fertilizer treatment, increased the bacterial richness significantly, especially for some functional bacteria (such as *Nitrospira*, *Gemmatimonas*). (iii) Analysis of the correlation between environmental factors and bacterial diversity indicated that soil pH and total P had the most significant effects on bacterial community structure ($r = 0.91$, $p = 0.001$; $r = 0.33$, $p = 0.001$). This study showed that it is an effective method to maintain a richer bacterial community and increasing the abundance of some functional bacteria by increasing organic fertilizers and reducing chemical fertilizers in the farm soil in karst regions, which could also be applied to other fragile agricultural ecosystems in the world.

Keywords Karst · Soil parent material type · Short-term fertilization · Soil physicochemical properties · Bacterial diversity

1 Introduction

The community characteristics of bacteria are sensitive to changes in soil nutrients, pH, and other external conditions, reflecting changes in soil quality in a timely manner (Chu et al. 2010; Shen et al. 2010). In addition, bacteria also play a critical role in the agricultural ecosystem when evaluating soil quality, maintaining soil fertility and crop productivity (Nacke et al. 2011). The appropriate community structure, rich diversity, and a high microbial activity are significant factors in maintaining soil ecosystems and productivity (Cardinale et al. 2006; Zhao et al. 2014a). Previous studies have shown that moderate fertilization is an important agricultural measure to improve plant nutrition, increase soil organic matter, and achieve high crop yield, it can also affect the abundance, activity, and community structure of soil microorganisms (Chu et al. 2007; Islam et al. 2011). Soil microbial biomass and diversity tended to differ due to different fertilization treatments, with the increase of microbial biomass and diversity being more pronounced with manure and compost than with mineral fertilizer (Geisseler et al. 2017; Wang et al. 2018).

Long-term application of organic fertilizers will improve the physicochemical properties and structure of soil (Bronick and Lal 2005; Diacono and Montemurro 2011) and increase soil microbial biomass and diversity (Gu et al. 2019).

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However, long-term application of chemical fertilizers can also cause the soil fertility and microbial diversity to decrease, as well as increasing groundwater pollution, soil acidification, and the air pollution represented by the greenhouse effect (Geisseler and Scow 2014; Pan et al. 2014). A study indicates that bioorganic-mineral fertilizers can restore soil polluted by excessive application of chemical fertilizers and improve soil quality (Yang et al. 2020). Few people have compared the effects of short-term application of chemical and organic fertilizers on bacterial community structure in karst areas. As the soil ecological environment is complicated, the rapid dissolution and diffusion of fertilizers will lead to change in local soil microorganisms because of fertilization. This short-term stimulation effect may exert an important effect on the microbial community (Dai et al. 2017).

Karst is a special landform developed on carbonate rocks such as limestone or dolomite, which is widely found in southwestern China (Tang et al. 2019; Zhang et al. 2017). The karst ecosystem is one of the three most fragile ecosystems in the world, and land in karst area is highly susceptible to environmental disturbances (Calò and Parise 2006). Natural conditions and long-term human planting methods (e.g., over-cultivation, excessive application of fertilizers and pesticides) led to the low productivity of arable land in karst areas. The application of chemical fertilizers has been the main way of increasing agricultural production in karst areas. With the rapid increase in population imposing demand on agricultural production, the use of chemical fertilizers has also increased rapidly. However, the over and unchecked application of chemical fertilizers, results in degradation of the physicochemical properties of soils and significant environmental pollution problems (Singh et al. 2014; Guo et al. 2010). In view of the widely recognized beneficial effects of organic fertilizer application on crop growth, we hypothesized that organic fertilizers could not only increase soil organic matter content but also change soil bacterial community structure, thus creating a soil environment more conducive to crop growth. The study aimed at investigating the effects of a short-term application of organic fertilizer and chemical fertilizer on the soil bacterial community structure in karst area. A combined method involving field experiments and high-throughput sequencing of the 16S rDNA gene was used in our study. The results obtained here could provide a theoretical basis for increasing the application of organic fertilizer and decreasing the application of chemical fertilizer in karst farm soil while maintaining the fragile karst ecological environment.

2 Materials and Methods

2.1 Site Description

The experimental sites were selected from natural farmland near Longga Village and Shuangtang Village, Puding County, Anshun City, Guizhou Province, China (Fig. 1). Among them, the limestone parent material sample plot (105° 45′ 0.74″, 26° 22′ 0.62″, 1175 m) is located near Longga village with an area of 298 m^2, and the dolomite parent material sample plot (105° 45′ 53.13″, 26° 20′ 42.8″, 1108 m) is located near Shuangtang village with an area of 281 m^2. Puding County is a typical karst mountainous area with strong karst development. The exposed area of carbonate rocks is 863.7 km^2, accounting for 84.42% of the County's surface area (Qin et al. 2014). The study area experienced a typical subtropical monsoon climate, with a mean annual temperature of 15.1 °C and a mean annual precipitation of 1367 mm (Liu et al. 2016). The main soil types are limestone soil according to the Chinese General Soil Classification, which are similar to Mollic Inceptisols according to the USDA Soil Taxonomy (Soil Survey Staff. 2010). The soil pH is between 4.6 and 8.4.

2.2 Experimental Design and Treatments

Given that the default experimental plot is homogeneous, two fertilization treatments were established in each sample plot, and three parallel groups were established in each fertilization treatment. So, a sample plot was divided into six experimental groups (on average) by deep ditching (each trench is 400 mm deep and 1000 mm wide and can prevent the exchange of nutrients and water between different treatment groups and eliminate boundary effects: online resource, Fig. S1).

After the first batch of soil samples were collected, the land was plowed and sown on 20 April 2018, and the sowing rate was 21,240 plants ha^{-1}. Subsequently, fertilizer treatments for each test field were applied (Table 1). We fertilized once at the earing stage of corn, and urea was applied at 15 g m^{-2}. The maize yield was measured at harvest time on 30 August 2018. The specific ingredients of fertilizers and their manufacturers are described in the online supplementary material.

2.3 Soil Sampling

The first batch of soil samples were taken before fertilization and sowing in April 2018, and the second batch of soil samples were collected during the corn harvest in August 2018. When collecting two batches of soil samples (bulk soil), we used the same location for sample collection. For each replicate site, five replicate samples were collected in an X-shaped pattern (we removed about 2 mm of cover from the surface layer and collected the 150-mm-thick topsoil) and then homogenized this to one composite sample, then divided the bulk soil mass into two sub-samples and stored them on ice. One sub-sample was stored at − 80 °C for microbial sequencing analysis, and the other sub-samples were air-dried at room temperature, ground, and then sieved for analysis of soil physicochemical properties.

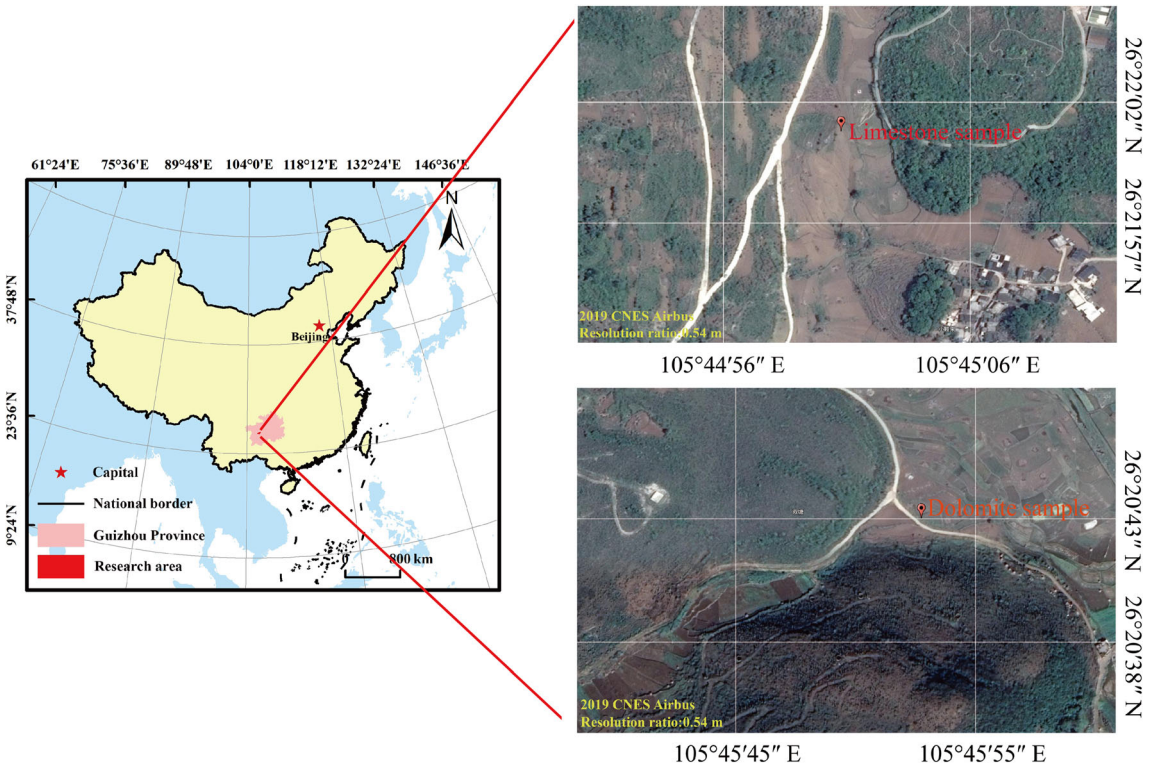

Fig. 1 Location of the experiment sites

2.4 Soil Physicochemical Properties Analysis

The physicochemical properties of soil samples from the areas with different parent materials before, and after, fertilization were tested and analyzed. The pH value of the soil was measured by the water extraction potential method (water-soil ratio 2.5:1) (Marcos et al. 2019). The total organic carbon (TOC), total carbon (TC), and total nitrogen (TN) of the samples were determined using an elemental analyzer (Vario EL type III, Elementar, Germany) (Liu et al. 2014). Total phosphorus (TP) and total potassium (TK) were determined by sodium hydroxide melting–flame spectrophotometry (Ren et al. 2016). Alkali-hydrolyzed nitrogen (AN) was determined by the alkaline hydrolysis diffusion method, available phosphorus (AP) was measured by $NaHCO_3$ method, and available potassium (AK) was determined by using ammonium acetate extraction-flame spectrophotometry (Lu 1999). Ammonia nitrogen (NH_4^+–N) was measured by sodium reagent colorimetry and nitrate nitrogen (NO_3^-–N) was measured by UV spectrophotometric determination (Lu 1999).

2.5 DNA Extraction in Samples and High-Throughput Sequencing

In this study, total soil bacterial DNA was extracted using the E.Z.N.A® Soil DNA Kit (OMEGA, USA) (Han et al. 2019).

Table 1 Fertilizer combinations of different treatment groups in different soil parent material experimental fields

Sample field ID	Treatment	Based fertilizers (fertilization date 20 April 2018)					Topdressing (fertilization date 2 July 2018)
		Organic fertilizer (g m^{-2})	Chemical compound fertilizer (g m^{-2})	Urea (g m^{-2})	Calcium superphosphate (g m^{-2})	The ratio of N, P_2O_5, K_2O	Urea (g m^{-2})
Dolomite parent material plot	DCF	0.00	22.50	0.00	0.00	14%:16%:15%	15.00
	DOF	99.33	0.00	4.86	5.94	14%:16%:15%	15.00
Limestone parent material plot	LCF	0.00	22.50	0.00	0.00	14%:16%:15%	15.00
	LOF	99.33	0.00	4.86	5.94	14%:16%:15%	15.00

Treatment: *DCF* the chemical fertilizer treatment groups of dolomite parent material soil plot, *DOF* the organic fertilizer treatment groups of dolomite parent material soil plot, *LCF* the chemical fertilizer treatment groups of the limestone parent material plot, *LOF* the organic fertilizer treatment groups of the limestone parent material plot. This is imposed according to the quantity. There is no standard error

DNA quality and purity were checked using 1% agarose gel electrophoresis and spectrophotometry (Liu et al. 2020). Using the sample total DNA as a template, the V3–V4 region of the bacterial 16S rDNA was amplified using primers 338F 5′-ACTCCTACGGGAGGCAGCAG-3′ and primer 806R 5′-GGACTACHVGGGTWTCTAAT-3′ (Fadrosh et al. 2014). PCR reactions, containing 25 μl 2× Premix Taq (Takara Biotechnology, Dalian Co. Ltd., China), 1 μl of each primer (10 mM), and 3 μl DNA (20 ng μl^{-1}) template in a volume of 50 μl, were amplified by thermocycling: 5 min at 94 °C for initialization; 30 cycles of 30 s denaturation at 94 °C, 30 s annealing at 52 °C, and 30 s extension at 72 °C; followed by 10 min final elongation at 72 °C. The PCR instrument was a BioRad S1000 (Bio-Rad Laboratory, CA, USA) (Gong et al. 2019). The specificity test for the PCR amplification product was undertaken on 1.5% agarose gel and purified using the EZNA Gel Extraction Kit (Omega, USA) (Han et al. 2019).

2.6 Illumina Sequencing and Data Processing

The specific sequencing process was completed by Guangzhou Magigene Technology Co., Ltd. PE250 sequencing was performed on the constructed amplicon library using the Illumina Hiseq2500 platform and 250 bp paired-end DNA sequencing reads were generated. Unprocessed FASTQ files were obtained for the analysis. The Trimmomatic software (V0.33, http://www.usadellab.org/cms/?page=trimmomatic) was used for double-ended data filtering (Bolger et al. 2014). We used the FLASH (V1.2.11, https://ccb.jhu.edu/software/FLASH/) software for sequence stitching (Magoc and Salzberg 2011). The mothur software (V1.35.1, http://www.mothur.org) was used for sequence quality control (Schloss et al. 2009). The Usearch software (V10, http://www.drive5.com/usearch/) was used for OTU clustering and chimera and singleton removal (Edgar 2013). The assign_taxonomy.py script in Qiime (http://qiime.org/scripts/assign_taxonomy.html) was adopted to compare the representative sequence of each OTU after removing chimera (Caporaso et al. 2010). All representative sequences were classified with the Silva (V132) database with 75% confidence threshold (Liu et al. 2019). The methodological information on bioinformatic analysis and calculation procedures applied here were aligned with protocols described on the online Majorbio Cloud Platform constructed by Shanghai Majorbio (http://www.majorbio.com/) (Liu et al. 2020).

The 16S rDNA V3–V4 region amplification sequences in this experiment were deposited in the NCBI Sequence Read Archive (SRA) database, under accession number SRP239277 (these sequences data can be retrieved from NCBI after June 2021).

2.7 Statistical Analysis

Statistical analysis in this study was carried out by one-way ANOVA (Tukey HSD test) to test significant differences between treatments using the SPSS 22 (IBM, USA) software. Bacterial α-diversity was calculated using the mothur software (1.30.2, https://www.mothur.org/wiki/Download_mothur) and the β-diversity test was performed using the Qiime software (1.9.1, http://qiime.org/install/index.html) (Liu et al. 2019). ANOSIM (analysis of similarities) test was applied to determine whether, or not, statistical differences in bacterial community structures were significant among treatments (Liu et al. 2020). By means of an algorithm for Bray–Curtis dissimilarity, non-metric multi-dimensional scale analysis (NMDS) was used to analyze the shifts in bacterial community structures (Zhu et al. 2019). Canonical correspondence analysis (CCA) was conducted to determine the relationships between bacterial community structures and environmental variables. In addition, the heatmap was drawn in the R software (Version 3.6.3).

3 Results

3.1 Effects of Fertilization on Maize Yield and Soil Physicochemical Properties

The corn production was measured during the maturity period. In the dolomite parent material plot, the corn production of the organic fertilizer treatment group (7081.19 kg ha^{-1}) was higher than that of the chemical fertilizer treatment group (6452.69 kg ha^{-1}). The same phenomenon was observed in the limestone parent material plot, where the corn yield of the organic fertilizer group (7122.69 kg ha^{-1}) was higher than that of chemical fertilizer treatment group (4651.19 kg ha^{-1}). According to the analysis of soil physicochemical properties (Table 2), short-term fertilization could slightly increase soil pH. Before fertilization, except for total potassium (TK) and ammonium nitrogen (NH_4^+-N), most soil nutrients in DPS were higher than those in LPS. Regardless of the dolomite parent sample or the limestone parent sample, there was no significant difference in soil nutrient indices between the chemical fertilizer treatment group and the organic fertilizer treatment group (DCF and DOF, LCF, and LOF) after short-term fertilization. Therefore, short-term organic fertilizer treatment can increase crop yield to a certain extent, but it has no obvious effect on change in soil physicochemical properties.

Table 2 Physicochemical properties of soils with different parent material before and after fertilization

Soils properties	DPS	DCF	DOF	LPS	LCF	LOF
pH	7.92 ± 0.07a	8.16 ± 0.05a	8.04 ± 0.10a	5.58 ± 0.60c	5.51 ± 0.54c	6.20 ± 0.42b
TOC	42.75 ± 2.73a	43.10 ± 7.25a	43.42 ± 2.54a	17.90 ± 0.92b	18.06 ± 1.79b	19.52 ± 1.61b
TC	67.76 ± 4.25a	66.43 ± 8.71a	64.57 ± 5.92a	19.31 ± 0.66b	20.24 ± 0.89b	21.46 ± 0.71b
TN	2.90 ± 0.09a	2.81 ± 0.06a	2.87 ± 0.03a	1.97 ± 0.07c	2.04 ± 0.05bc	2.11 ± 0.10b
C/N	23.30 ± 1.23a	23.67 ± 3.55a	22.54 ± 2.28a	9.83 ± 0.20b	9.94 ± 0.21b	10.19 ± 0.30b
TP	1.00 ± 0.12a	0.75 ± 0.10bc	0.87 ± 0.06ab	0.65 ± 0.06c	0.62 ± 0.05c	0.69 ± 0.01c
TK	7.45 ± 0.58d	7.54 ± 1.17d	7.13 ± 0.88d	39.42 ± 0.75a	36.04 ± 1.00b	34.61 ± 0.67c
AN	214.27 ± 15.47a	175.00 ± 16.52bc	187.33 ± 10.41b	194.33 ± 17.98b	159.00 ± 5.20c	156.33 ± 5.13c
AP	16.77 ± 3.61a	10.14 ± 3.69bc	12.77 ± 1.11ab	6.96 ± 2.24c	10.52 ± 0.32bc	13.31 ± 3.31ab
AK	160.00 ± 41.46ab	123.67 ± 9.81b	110.33 ± 10.50b	146.67 ± 47.59ab	152.00 ± 51.39ab	196.67 ± 10.41a
NO_3^-–N	5.38 ± 1.73 cd	17.69 ± 7.18a	15.10 ± 1.84ab	4.90 ± 3.18d	9.85 ± 7.27bcd	11.19 ± 1.03abc
NH_4^+–N	9.72 ± 4.73b	14.83 ± 0.44ab	13.09 ± 0.79ab	11.43 ± 6.46ab	16.21 ± 1.23a	14.47 ± 0.39ab

The data in the table are mean ± standard deviations; in the same row, different letters represent statistically significant differences (one-way ANOVA, $p < 0.05$)

Treatment: *DPS* the dolomite parent material plot before fertilization, *DCF* the chemical fertilizer treatment groups of dolomite parent material plot, *DOF* the organic fertilizer treatment groups of dolomite parent material plot, *LPS* the limestone parent material plot before fertilization, *LCF* the chemical fertilizer treatment groups of the limestone parent material plot, *LOF* the organic fertilizer treatment groups of the limestone parent material plot, *pH* soil pH value, *TOC* the total organic carbon (g kg^{-1}), *TC* total carbon (g kg^{-1}), *TN* total nitrogen (g kg^{-1}), *C/N* the C/N ratio (%), *TP* total phosphorus (g kg^{-1}), *TK* total potassium (g kg^{-1}), *AN* alkaline hydrolysis nitrogen (mg kg^{-1}), *AP* available phosphorus (mg kg^{-1}), *AK* available potassium (mg kg^{-1}), *NO_3^-–N* nitrate nitrogen (mg kg^{-1}), *NH_4^+–N* ammonia nitrogen (mg kg^{-1})

3.2 Effect of Fertilization on Soil Bacterial Diversity

In this study, a total of 2,020,347 high-quality effective sequences of bacterial 16S rDNA were obtained. The average sequence length of the samples is 467 bp. At the 97% sequence similarity threshold level, 693,491 OTUs were obtained. The dilution curve of bacteria tended to flatten with the increase of sequencing depth, indicating that the number of sequences obtained could better represent the bacterial community (online resource, Fig. S2). Before fertilization, the soil bacterial abundance and diversity of DPS were higher than those of LPS (Table 3). Fertilization treatment increased the bacterial richness in the soil, and the improvement imparted by organic fertilizer treatment was more significant compared to that of chemical fertilizer treatment ($p < 0.05$). Meanwhile, the effect of organic fertilization treatment in the limestone parent plot was more significant than that in the dolomite parent plot (Table 3).

3.3 Effects of Fertilization on Bacterial Community Structure and Composition

To compare the bacterial community composition of samples from different treatment groups before and after fertilization, a non-metric multi-dimensional scaling analysis (NMDS) was performed (Fig. 2). The MDS1 axis can distinguish the bacterial community structure of dolomite parent soil samples from that of limestone parent soils. The fertilization treatment affected the bacterial community of both limestone and dolomite parent soil samples. The bacterial community of these soils differed between different fertilization treatments, especially in the dolomite parent soil samples, which indicated that there were significant differences (ANOSIM test, $R = 0.7037$, $p = 0.001$) in bacterial community structures of different parent soils among different fertilization treatments.

All OTUs were classified into 36 phyla and 775 genera. Before fertilization, the dominant phyla (relative sequence abundance > 1%) types of DPS and LPS were similar, but the relative abundances of Actinobacteria (8.70 ± 1.48 and 3.33 ± 0.67, respectively), Gemmatimonadetes (3.92 ± 0.62 and 5.95 ± 1.53, respectively), Planctomycetes (4.17 ± 0.74 and 1.48 ± 0.97, respectively), Proteobacteria (27.73 ± 2.21 and 36.59 ± 8.12, respectively), and Verrucomicrobia (6.54 ± 0.61 and 4.46 ± 1.34, respectively) were significantly different (Fig. 3; Table S1; $p < 0.01$). After fertilization, the relative abundances of Planctomycetes, Chloroflexi, Gemmatimonadetes, Latescibacteria, and Nitrospirae in the dolomite parent soils increased, however, the relative abundances of Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, and Proteobacteria decreased, but the difference between DPS, DCF, and DOF was insignificant (Fig. 3; Table S1). After fertilization, the relative abundances of Planctomycetes, Gemmatimonadetes, Chloroflexi, Nitrospirae, and Actinobacteria increased in limestone parent soils. Among them, Planctomycetes increased significantly in LOF, Gemmatimonadetes increased significantly in LOF and

Table 3 α-Diversity indices of soil bacteria in different treatments

Sample	Observed OTUs	Chao1	Shannon	Simpson	Coverage
DPS	2168 ± 63.06a	2709.98 ± 67.07c	6.66 ± 0.33a	0.01 ± 0.01a	0.96 ± 0.00b
DCF	2287 ± 81.74a	3016.75 ± 153.24abc	6.63 ± 0.14a	0.00 ± 0.00a	0.96 ± 0.00b
DOF	2289 ± 72.03a	3097.68 ± 194.99ab	6.72 ± 0.07a	0.00 ± 0.00a	0.96 ± 0.00b
LPS	1733 ± 318.08b	2231.48 ± 410.63d	6.18 ± 0.31b	0.01 ± 0.00a	0.97 ± 0.01a
LCF	1889 ± 180.34b	2753.26 ± 111.20bc	6.06 ± 0.32b	0.01 ± 0.01a	0.96 ± 0.00b
LOF	2419 ± 91.06a	3261.35 ± 117.01a	6.85 ± 0.06a	0.00 ± 0.00a	0.95 ± 0.00c

In the same column, different letters represent statistically significant differences (one-way ANOVA, $p < 0.05$)

Treatment: *DPS* the dolomite parent material plot before fertilization, *DCF* the chemical fertilizer treatment groups of dolomite parent material plot, *DOF* the organic fertilizer treatment groups of dolomite parent material plot, *LPS* the limestone parent material plot before fertilization, *LCF* the chemical fertilizer treatment groups of the limestone parent material plot, *LOF* the organic fertilizer treatment groups of the limestone parent material plot

LCF, and Patescibacteria increased significantly in LCF (Fig. 3; Table S1). The relative abundances of Acidobacteria and Proteobacteria decreased in the limestone parent soil after fertilization, but the difference was insignificant.

At the genus level (Fig. 4), the effects of different fertilization treatments on bacterial abundance also showed different results in the plots of the two soil parent materials. For instance, the relative abundance of bacterial genus in DCF treatment group and DOF treatment group was not significantly different, while the relative abundance of bacterial genus in LCF treatment group and LOF treatment group was significantly different (Fig. 4). Some genera were more abundant in soils with OF (DOF and LOF) treatments as compared with CF (DCF and LCF) treatments, namely *Ramlibacter*, *Gemmatimonas*, *Nitrospira*, *MND1* (belonging to Gammaproteobacteria), *Haliangium*, *SM1A02*, *Sphingomonas*, *Steroidobacter*, *Nocardioides*, *Terrimonas*, *Chryseolinea*, *Flavobacterium*, and *Solirubrobacter* and many of these bacteria are considered to play important roles in soil diversity and nutrient cycling. Compared with the chemical fertilizer treatment group, the relative abundance of dominant bacteria genera in the organic fertilizer treatment group was increased, especially in the limestone parent soil (Fig. 4).

3.4 Correlation Between Soil Physicochemical Properties and Bacterial Diversity

After the soil physicochemical factors were analyzed by VIF variance expansion factor, a combination of physicochemical factors (pH + TP + AP + AN + AK + NO_3^-–N + NH_4^+–N) related to the influence of bacterial community composition

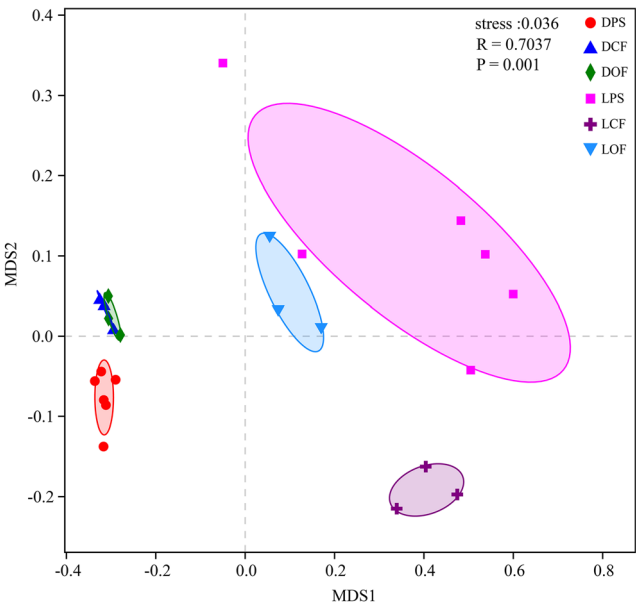

Fig. 2 Non-metric multi-dimensional scaling (NMDS) analysis of bacterial community. The distance between the points indicates the differentiation in the community

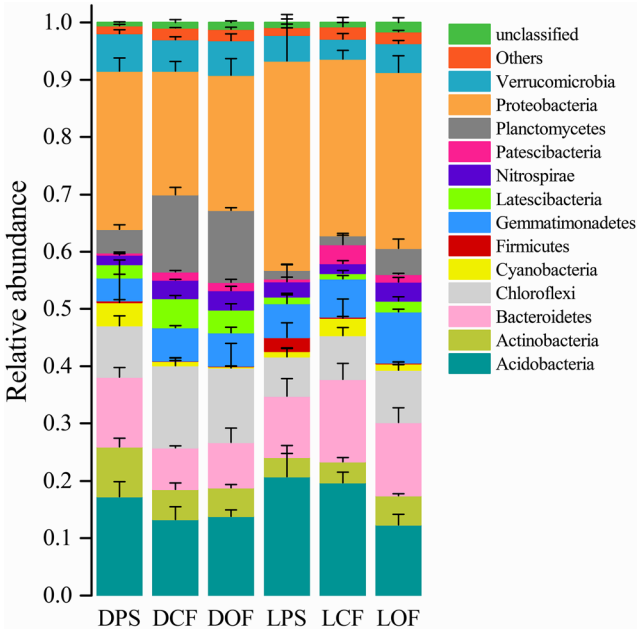

Fig. 3 Relative abundance of the dominant bacterial phyla (abundance greater than 1%) under different fertilization treatments

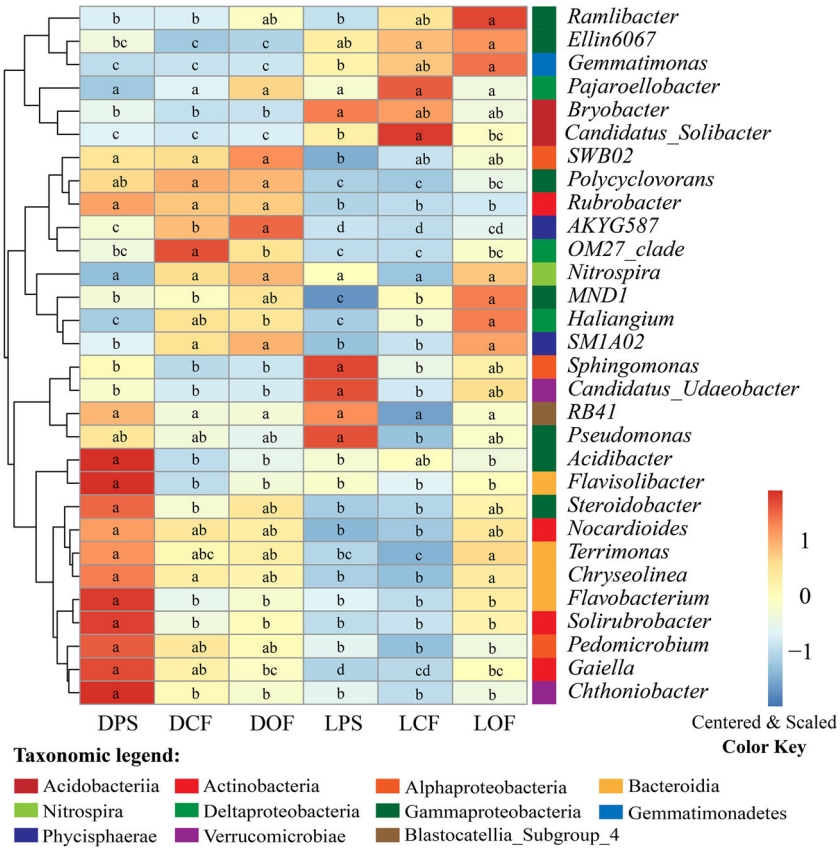

Fig. 4 A heatmap diagram of the relative abundance of the dominant 30 genera before and after fertilization. The redder the rectangle, the higher the abundance of the genus in the sample. Different letters in the same row indicate significant differences among different treatment groups at $p < 0.05$ according to Tukey's test. The right colored vertical bar in the heatmap corresponds to the class level classification of each genera

was selected for environmental factor correlation analysis. Through DCA analysis, the non-linear model CCA was employed to analyze the association between bacterial community and environmental factors (Fig. 5). The first two axes could explain a total of 24.9% of the sample-environment

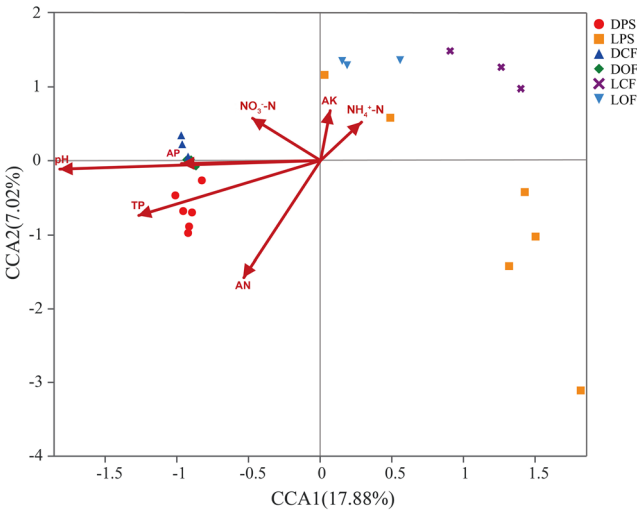

Fig. 5 CCA analysis of the effects of environmental parameters on bacterial community structure. TP, total phosphorus; TK, total potassium; AN, alkaline hydrolysis nitrogen; AP, available phosphorus; AK, available potassium; NO_3^-–N, nitrate nitrogen; NH_4^+–N, ammonia nitrogen

relationship. Mantel testing showed a high correlation between environmental variables (pH, TP) and bacterial communities ($r = 0.91$, $p = 0.001$; $r = 0.33$, $p = 0.001$), indicating that pH and TP had greater effects on the distribution of bacterial communities. When the applied fertilizer had effects on soil pH and phosphorus content, it significantly affected the soil bacterial community structure. Spearman correlation analysis was conducted on all soil physicochemical properties and the top 15 species of bacteria (online resource, Fig. S3). There is a significantly positive or negative correlation between the physicochemical properties of most soils and bacterial species, and the correlation between different bacterial species and physicochemical properties varies.

4 Discussion

4.1 Response of Soil Properties and Maize Yield to Short-Term Different Fertilization Treatments

Soil physicochemical properties, which are commonly considered to be indicators of soil quality, have been defined as the capacity to sustain plant productivity (Karlen et al. 1997). In our study, the soil pH changed upon application of different fertilizer treatments, which is consistent with the results of Zhao et al. (2014b) and Zhao et al. (2016). Especially in

limestone parent material plots, the pH value of the LOF treatment group was significantly higher than that of the LCF group (Table 2). We also observed an increase, albeit not significant, in soil TC and TOC in response to fertilization, and which in the organic fertilizer treatment groups were generally higher than in the chemical fertilizer treatment groups. That was the same result as long-term fertilization (Blanchet et al. 2016). After short-term fertilization treatment, there was no significant difference between N elements (TN, AN) and P elements (TP, AP) in the organic fertilizer treatment groups and the fertilizer treatment groups, but in terms of numerical values, the organic fertilizer treatment groups were slightly higher than the fertilizer treatment groups (Table 2). This indicates that short-term fertilization cannot significantly distinguish the effects of organic fertilizer treatment from chemical fertilizer treatment on soil physicochemical properties, but it can also be seen that organic fertilizer treatment can improve some soil fertility indices. Long-term fertilization experiments have proved that organic fertilizer treatment or the combination of organic fertilizer and inorganic fertilizer treatments can increase the contents of soil C, N, P, K, and other elements, which is conducive to the improvement of soil fertility (Chen et al. 2018; Gu et al. 2019).

According to the research of Li et al. (2017), NPK fertilizers could be at least partially replaced by manure to sustain a high maize yield in the North-East China Plain and we highly recommend the combined application of chemical fertilizers and manure. Over the 9 years of the investigation, Li et al. (2018) proved that the average wheat yield increased by 9.9 to 17.4% for organic fertilization treatments, compared to the initial yield for each treatment, whereas the average yield of chemical fertilization treatment over the same period was reduced by 6.5%. In this study, the maize yields of the organic fertilizer treatment groups were numerically higher than that of the chemical fertilizer treatment groups in both limestone and dolomite parent material plots, although no statistical analysis was conducted. The reason for these results was mainly because chemical fertilizer-only application may result in deficiencies in the amounts of micro-elements such as Mg, S, and Zn, while organic fertilizer supplied these micro-elements directly in addition to the regulation of nutrient release intensity and rate (Li et al. 2016).

4.2 Response of Bacterial Diversity and Community Structure to Short-Term Different Fertilization Treatments

Fertilizers significantly influenced bacterial abundance and biodiversity (Table 3). In our study, the abundance and biodiversity of bacteria in the organic fertilizer treatments were higher than that in the chemical fertilizer treatments, and the differences in the limestone parent material plots were significant ($p < 0.05$). Organic fertilizers were considered as an important component to increase microbial biomass as compared to chemical or other inorganic fertilizers (Okur et al. 2009; Lentendu et al. 2014; Wang et al. 2018) and were evinced to be the complete nutrients combination required for bacterial growth (De Angelis et al. 2010). Long- or short-term application of chemical fertilizers caused a lower level of soil microbial biomass than organic fertilizers (Geisseler and Scow. 2014; Khalil et al. 2016).

The NMDS analysis result showed that the bacterial community of soil was different given different fertilization treatments (Fig. 2). In our study, organic fertilization increased the abundance of some functional bacteria, e.g., *Gemmatimonas*, *Nitrospira*, *Ramlibacter* (belonging to Gammaproteobacteria), *MND1* (belonging to Gammaproteobacteria), *Haliangium* (belonging to Deltaproteobacteria), *Steroidobacter* (belonging to Gammaproteobacteria), *Nocardioides* (belonging to Actinobacteria), and *Solirubrobacter* (Fig. 4). *Gemmatimonas* and *Nitrospira* are related to soil N content, mainly contributing to conversion of nitrites to nitrates (Cesarano et al. 2017). Both *Ramlibacter*, *Steroidobacter*, and *MND1* belong to Gammaproteobacteria and participate in nitrification (Baker et al. 2013). Actinobacteria is a type of bacterium involved in the organic matter cycle and can produce many antibacterial and exogenous compounds (Jones et al. 2009). *Haliangium* is related to element cycling in soil (Chen et al. 2018). *Solirubrobacter* and *Steroidobacter* can use different substances (such as polysaccharides, amino acids, agar, and steroids) as carbon sources (Sakai et al. 2014). *Solirubrobacter* is one of the bacteria that can promote plant growth changed (Franke-Whittle et al. 2015).

Many organic colloids contained in organic fertilizers provide a material basis for the formation of soil organic and inorganic composite aggregates, which is conducive to the generation of reactive calcium ions and the increase of humus content. Organic fertilizers also contain a wealth of beneficial functional groups, which add new carbon sources and energy to the soil, facilitate the formation of soluble carbon and nitrogen, and thus affect the diversity and community structure of bacteria. The supply of organic materials increases the availability and the number of C sources for soil microorganisms, which increases the energy available thereto and promotes their metabolism and reproduction (Li et al. 2018; Ma et al. 2012). Bacterial diversity levels were directly influenced by soil pH because most bacterial taxa exhibit relatively narrow growth tolerances (Rousk et al. 2010; Zhou et al. 2015). The application of organic fertilizer regulated the balance of soil pH (Table 2), and our study indicated that pH exerted a greater effect on the distribution of bacterial communities (Fig. 5). Therefore, the bacterial diversity and community composition of the organic fertilizer treatments were better than that when applying chemical fertilizer treatments.

4.3 Relationship Among Soil Parent Matter, Physicochemical Properties, and Bacterial Community

Previous studies have shown that soil parent material is the key to determining the differences in soil properties and mineral element composition (Cardelli et al. 2017) and is related to nutrient cycling in the soil-ecosystem and its fertility (Anda et al. 2015). Therefore, the soil parent material indirectly affects soil bacterial community through its physicochemical properties and mineral element composition. In our study, both soil physicochemical properties and bacterial community in dolomite parent material plots are obviously different to these in limestone parent material plots (Table 2; Figs. 2 and 3).

Changes in pH value affect soil fertility, structure, and growth of vegetation communities, thereby indirectly changing soil bacterial community structure (Lauber et al. 2009). In our study, pH and TP were proven to be the main factors affecting soil bacterial communities, as found in previous research results (Feng et al. 2018; Huang et al. 2019). Many biological factors directly affect carbon mineralization in soils, and carbon sources are one of the most important factors affecting microbial communities (Liu et al. 2018); the same results were obtained in our study (online resource, Fig. S3). Generally, microorganisms degrade organic nitrogen into mineral nitrogen to maintain their growth (Parfitt et al. 2005). This study also proves that there is a close relationship between the element N and the bacterial community.

5 Conclusions

Short-term organic fertilizer treatment can significantly increase the number of bacterial communities and species abundance of the soil in the karst region, and organic fertilizer treatment can increase the abundance of some functional bacteria in the soil. The environmental factor analysis of microbial diversity showed that the physicochemical properties of soil, such as pH and total phosphorus, had a significant effect on the distribution of bacterial community, and the physicochemical properties of soil exerted varying degrees of influence on the various bacterial species. It was confirmed that organic fertilizer can replace chemical fertilizers to some extent, providing a basis for increasing organic fertilizer and reducing chemical fertilizer in karst areas, thus protecting the karst ecological environment. At the same time, this study also provides biological theoretical support for the management of agricultural fertilization and ecological maintenance in other similar fragile ecosystems in the world.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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