

The Residue of Tetracycline Antibiotics in Soil and Vegetable and Its Effect On The Diversity of Soil Bacterial Community

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Research

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Abstract

[Background] Tetracycline antibiotics (TCs) are a broad-spectrum antibiotic, widely used in livestock and poultry breeding. The residue of tetracycline antibiotics in animal manure may cause changes of soil microbial community.

[Methods] On the basis of the investigation and analysis of TCs pollution in the soil of main vegetable bases and the livestock manure of major large-scale farms in Chongqing, China, the effects of tetracycline antibiotics on the structure and diversity of soil microbial community were investigated by high-throughput sequencing.

[Results] The TCs content in soil was increased by applying livestock manure. The contents of TC, OTC and CTC in the soil under pig manure treatment were 171.07-660.20 $\mu\text{g}\cdot\text{kg}^{-1}$, 25.38-345.78 $\mu\text{g}\cdot\text{kg}^{-1}$ and 170.77-707.47 $\mu\text{g}\cdot\text{kg}^{-1}$, respectively. The contents of TC, OTC and CTC in the soil under the treatment of chicken manure were 166.62-353.61 $\mu\text{g}\cdot\text{kg}^{-1}$, 122.25-251.23 $\mu\text{g}\cdot\text{kg}^{-1}$ and 15.12-80.91 $\mu\text{g}\cdot\text{kg}^{-1}$ respectively. The content of tetracycline antibiotics (TCs) in edible parts of *Brassica juncea var. gemmifera* was increased after livestock manure treatment, among which pig manure had the greatest influence on the increase of TCs content, and the larger the dosage of pig manure was, the more significant the increase of TCs content was. Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi and bacteroidetes under livestock manure treatment were the dominant phyla, accounting for 85.2%-92.4% of the total abundance of soil bacteria.

[Conclusions] The soil OTUs under the treatment of pig manure was higher than that under the treatment of chicken manure. Biogas residue after fermentation treatment can effectively reduce the environmental and ecological risks caused by antibiotic residues.

Background

Farmland is the main place for the release of antibiotics, and the application of livestock manure into farmland as organic fertilizer is considered to be one of the most important ways for antibiotics to enter the soil (Quaik et al. 2020). China consumes 60%-65% of the world's total antibiotics (Tasho and Cho 2016). Among them, Tetracycline antibiotics (TCs) are one of the most widely used antibiotics in the world including Tetracycline (TC), Oxytetracycline (OTC) and Chlortetracycline (CTC). Residual concentration of antibiotics detected in the livestock manure from livestock and poultry farms in eastern China was up to 1420 $\text{mg}\cdot\text{kg}^{-1}$ (Chen et al. 2012). Tetracycline antibiotics have a strong persistence in soil and are easy to accumulate, posing a potential threat to the ecosystem and human health (Pan and Chu 2017a).

Soil microorganisms play a key role in maintaining ecosystem functions (Lladó et al. 2018). The application of livestock manure will affect the microbial community in the soil ecosystem of vegetable fields (Sun et al. 2015; Francioli et al. 2016) by on one hand providing nutrients such as C, N, P and K to

the soil to promote the increase of microbial population (Ninh et al. 2015), and on the other hand by bringing harmful substances such as heavy metals and antibiotics into the soil to inhibit microbial growth (Pan and Chu 2017a). Many researchers have linked soil microbial communities to soil nutrient composition, but few have linked them to soil antibiotic content. The content of antibiotics in soil depends on the type and application dosage of livestock manures (Quaik et al. 2020), so different fertilization treatments may have different effects on the diversity of soil microbial community. In recent years, high-throughput sequencing technology has provided a good platform for the assessment of soil microbial diversity and its functional analysis, among which 16S rRNA amplification sequencing technology has become an important means to study the microbial community composition in environmental samples (Hess et al. 2011).

It is of great significance for current agricultural production to study the effects of tetracycline antibiotics on crops and the environment with manure type and application dosage as variables (Xiong et al. 2018). On the basis of the investigation and analysis of TCs pollution in the soil of main vegetable bases and the livestock manure of major large-scale farms in Chongqing, the field experiment was conducted to study the residues of tetracycline antibiotics in *brassica juncea var. gemmifera* and soil under application of different kinds of livestock manures at different dosages. The effects of tetracycline antibiotics on the structure and diversity of soil microbial community were investigated by high-throughput sequencing.

Materials And Methods

The experimental field was located in Yuxi vegetable research and development center in Xiguan Village, Qitang town, Bishan District, Chongqing. The basic physical and chemical properties of the soil were 1.04 g·kg⁻¹ total nitrogen, 143.93 mg·kg⁻¹ alkali-hydrolyzed nitrogen, 1.12 g·kg⁻¹ total phosphorus, 131.53 mg·kg⁻¹ available phosphorus, 18.76 g·kg⁻¹ total potassium, 237.02 mg·kg⁻¹ available potassium, 36.10 g·kg⁻¹ organic matter, with pH of 5.19 and cation exchange capacity (CEC) of 21.73cmol·kg⁻¹. The pig manure, chicken manure and biogas residue used in the experiment were purchased from a large farm nearby. The biogas residue was fermented from the same pig manure sampling site. The contents of tetracycline antibiotics are shown in table 1. The vegetable planted was *Brassica juncea var. gemmifera* (Lee et Lin).

Table 1 Contents of tetracycline antibiotics in three organic fertilizers

Types	TC/mg·kg ⁻¹	OTC/mg·kg ⁻¹	CTC/ mg·kg ⁻¹
Pig manure	81.08	23.94	80.92
Chicken manure	28.06	527.16	0.89
Biogas residue	2.90	41.37	0.16

Experimental design

A total of 9 treatments were set up in the experiment, including low amount of pig manure (1350 kg·667m⁻²), LPM; medium amount of pig manure is (2700 kg·667m⁻²), MPM; high amount of pig manure (4050 kg·667m⁻²), HPM; low amount of chicken manure (450 kg·667m⁻²), LCM; medium amount of chicken manure (900 kg·667m⁻²), MCM; high amount of chicken manure (1350 kg·667m⁻²), HCM; inorganic NPK fertilizer (50 kg·667m⁻²), CM; biogas residue (2700 kg·667m⁻²), BR; No fertilizer, CK. The plot area of the test field was 9 m², each treatment was repeated for three times, with random block group arrangement. Irrigation, fertilizer, pests and diseases in the test area shall be routinely managed. When harvesting *brassica juncea var. gemmifera*, soil samples with depth of 0-20cm were collected using "S" sampling method for determination of soil TCs. The content of TCs in edible part of *brassica juncea var. gemmifera* was determined.

Determination of tetracycline antibiotics in soil and chicken manure

The samples were frozen at -20°C for 3 days, freeze-dried by a freeze-dryer, ground and screened via a 1mm sieve, and stored at -20°C for preparation. Soil sample extraction: 2.0000g soil sample was accurately weighed and added into a 50ml centrifuge tube, followed by addition of 10ml of extract, mixed by swirling and shaking for 1min, and then underwent ultrasonic treatment for 10min. The supernatant was collected after centrifugation at 4000r·min⁻¹ for 10min. After that, the supernatant was extracted twice with 8ml and 6ml of extract, respectively. The supernatant extracted three times was combined, and then evaporated to a volume of about 12ml at 40°C. Solid phase extraction: SPE column was activated with 5ml of methanol and 5ml of ultra-pure water in turn, and the extraction solution passed through the column at a flow rate of 1ml·min⁻¹. Then, the columns were washed with 5ml of ultrapure water and 5ml of 5% methanol solution, vacuumed for 20min, and eluted with 5ml of 0.01mol·L⁻¹ methanol oxalate solution to collect eluent. Subsequently, nitrogen was blown to near dry at 40°C, the volume was fixed to 1ml with methanol, and the eluent was stored in the automatic sampler after passing a 0.22µm filter membrane. Sample extraction of chicken manure: 1.0000 g of pig manure/chicken manure sample were weighed and added in a 50ml centrifugal tube, followed by addition of the extraction solution and extract (the process is the same as the soil sample). After that, the extracts were combined, with addition of 5ml of n-hexane for lipid removal, and then evaporated to a volume of about 12ml at 40°C. The SPE column and SAX column were activated with 5ml of methanol and 5ml of ultra-pure water in turn, and the extracted solution passed through the column at a flow rate of 1ml·min⁻¹. The treatment process of other samples was the same as that of soil samples.

The pretreatment method was mainly referred to the method proposed by Jacobsen et al. (2004), with some optimization. Samples of chicken manure were determined by high performance liquid chromatography (HPLC) and soil samples were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Liquid phase conditions: 40 column temperatures, flow rate of 0.4 ml·min⁻¹, injection volume of 2 samples, gradient elution procedure: 1-3 min, 15-40% B phase.3-4 min, 40%-95% B phase;4-5 min, 95% B

phase;5-5.1 min, 15%B phase;5.1-8 min, 15% B phase.

Mass spectrometry conditions: ion source: electrospray ion source (ESI); Scanning method: positive ion scanning; Monitoring mode: multi-reflection monitoring mode (MRM). Dry gas: N₂; Dry temperature: 400°C; Dry air velocity: 15 L·min⁻¹; Atomizer pressure: 30 psi; Capillary electrical pulse pressure: 4500 V. The LLOQ of tetracycline, oxytetracycline and aureomycin was 0.007 µg·mL⁻¹–0.014 µg·mL⁻¹–0.021 µg·mL⁻¹, respectively. The recoveries of three antibiotics were determined by standard additions method. The average recovery rate of OTC, TC and CTC was 58.3%, 57.1% and 87.4%, respectively, with all variation coefficients less than 11.04%.

Determination of TCs content in plants

Plant samples were analyzed by HPLC-MS/MS. The specific methods are as follows:

Liquid phase: the column temperature was 40°C, the flow rate was 0.4 ml·min⁻¹, the injection amount was 2µL, the gradient elution procedure: 1-3 min, 15-40% B phase; 3-4 min, 40%-95% B phase; 4-5 min, 95% B phase; 5-5.1 min, 15%B phase; 5.1-8 min, 15% B phase.

Mass spectrometry conditions: ion source: electrospray ion source (ESI); Scanning method: positive ion scanning; Monitoring mode is multi-reflection monitoring mode (MRM). Dry gas: N₂; Dry temperature: 400°C; Dry air velocity: 15 L·min⁻¹; Atomizer pressure: 30 psi; Capillary electrical pulse pressure: 4500 V. The LLOQ of tetracycline, oxytetracycline and aureomycin were 7 µg·kg⁻¹–14 µg·kg⁻¹–21 µg·kg⁻¹, respectively. The recoveries of three antibiotics were determined by standard additions method. The average recovery rate of OTC, TC and CTC was 76.3%, 77.0% and 87.4%, respectively, which met the analysis requirements.

16S rRNA gene sequencing method

Based on Illumina sequencing technology platform (Genepioneer Biotechnologies, Nanjing, China), 16S rRNA gene sequencing was carried out by PE250 sequencing method. The bacterial 16S rRNA gene V4/V5 region was selected for amplification and sequencing, and 420bp amplification fragments were obtained using primers 515F (5'- TGCCAGCMGCCGCGG- 3') and 907R9 (5'- CCGTCAATTCMTTTRAGTTT- 3'). The coupling was added, and 2×250bp paired-end data was obtained by sequencing based on NovaSeq platform. By splicing, a longer sequence could be obtained, so as to conduct 16S analysis. Raw Data obtained by sequencing was spliced and filtered to obtain Clean Data. Operations such as chimera removal, combining and clustering of data were carried out using Usearch software. In Usearch clustering, Reads were sorted from large to small in terms of abundance, and OTU clustering was conducted according to the similarity level of 97%, then chimeric was removed, and Operational Taxonomic Units (OTUs) were obtained. Next, Reads of each sample were randomly flattened, with corresponding OTU sequences being extracted. The RDP classifier bayesian algorithm was used to plot the dilution curve of Alpha diversity index on the Qiime platform, and the reasonable flattening parameters were selected according to the dilution curve. Qiime was used to analyze the flattened OTU. First, a Read was extracted

from each OTU as a representative sequence, which was then compared with the RDB (Relational Database) Database to annotate each OTU. According to the number of sequences in each OTU, the OTU abundance table was obtained, based on which subsequent analysis was carried out.

Data analysis

Duncan's multiple comparison was used to analyze the significant difference between different treatments ($P < 0.05$). Based on the OTU abundance obtained by 16S rRNA sequencing, principal component analysis was used to compare the difference of bacterial community under different treatments. Pearson correlation analysis was carried out to calculate the correlation coefficient between the relative abundance of TCs and that of major phylum level bacteria. CANOCO 4.5 was used to make the graph. PCA, correlation analysis and variance analysis were performed by SPSS Statistics 21.0. Heatmap was plotted by R3.1.0.

Results And Discussion

Soil TCs content

It can be seen from figure 1 that, compared with the control group, the application of livestock manure increased the TCs content in the soil, and the pig manure had the greatest impact on the increase of TCs. Moreover, with the increase of the application dosage of pig manure, the contents of TC, OTC and CTC in soil treated with low, medium and high contents of pig manure were 171.07, 25.38, 170.77 $\mu\text{g}\cdot\text{kg}^{-1}$, 256.32, 266.30, 356.49 $\mu\text{g}\cdot\text{kg}^{-1}$, and 660.20, 345.78, 707.47 $\mu\text{g}\cdot\text{kg}^{-1}$, respectively. Compared with pig manure treatment, the TCs residue in the soil treated with chicken manure was lower, but the TC, OTC and CTC contents in the soil treated with low, medium and high contents of chicken manure were 166.62, 122.25, 15.12 $\mu\text{g}\cdot\text{kg}^{-1}$, 334.82, 126.49, 18.39 $\mu\text{g}\cdot\text{kg}^{-1}$ and 353.61, 251.23, 80.91 $\mu\text{g}\cdot\text{kg}^{-1}$, respectively. The contents of TC, OTC and CTC in soil treated with biogas residue were 70.42, 134.25 and 33.24 $\mu\text{g}\cdot\text{kg}^{-1}$ respectively. The soil treated with pig manure has higher contents of tetracycline and aureomycin, the soil treated with chicken manure had higher contents of tetracycline and oxytetracycline, and the soil treated with biogas residue had higher contents of oxytetracycline and oxytetracycline.

Plant Tcs content

It can be seen from figure 2 that, compared with the control group, the contents of tetracycline antibiotics (TCs) in edible parts of *brassica juncea* var. *gemmifera* were increased by the treatment of different livestock manures, among which pig manure had the greatest influence on the increase of TCs. Moreover, the content of TCs increased with the increase of dosage of pig manure. The contents of tetracycline (TC), oxytetracycline (OTC) and aureomycin (CTC) in *brassica juncea* var. *gemmifera* treated with low, medium and high contents of pig manure reached 15.22, 28.73, 24.05, 43.73, 62.16, 48.05, 199.68, 151.49 and 101.96 $\mu\text{g}\cdot\text{kg}^{-1}$, respectively. Compared with pig manure treatment, the TCs residue in *brassica juncea* var. *gemmifera* was lower, but the contents of TC, OTC and CTC in *brassica juncea* var.

gemmifera treated with low, medium and high contents of chicken manure reached 31.07, 25.57, 22.35, 29.89, 22.49, 17.25, 34.99, 30.41 and 22.45 $\mu\text{g}\cdot\text{kg}^{-1}$, respectively. The concentrations of TC and OTC were 28.81 and 22.54 $\mu\text{g}\cdot\text{kg}^{-1}$, respectively, and the CTC content was lower than the detection line.

Correlation between TCs content in plants and soil

As can be seen from table 2, there was a significant positive correlation between pig manure dosage and CTC content in soil, OTC and CTC contents in plants ($P < 0.05$), while there was a significant positive correlation between chicken manure dosage and OTC content in plants, indicating that the application of pig manure and chicken manure was an important factor affecting the contents of antibiotics in soil and plants. There was a significant positive correlation between OTC, TC and CTC contents in soil and OTC, TC and CTC contents in plants, indicating that TCs absorbed by plants was significantly correlated with the TCs content in soil.

Table 2 Pearson correlation analysis of TCs content in *brassica juncea var. gemmifera* and TCs content in soil

	Pig manure dosage	Chicken manure dosage	Plant OTC	Plant TC	Plant CTC	Soil OTC	Soil TC	Soil CTC
Pig manure dosage	1							
Chicken manure dosage		1						
Plant OTC	0.952*	0.954*	1					
Plant TC	0.948	0.961	0.975**	1				
Plant CTC	0.976*	0.873	0.977**	0.930**	1			
Soil OTC	0.942	0.862	0.797*	0.761*	0.804**	1		
Soil TC	0.865	0.846	0.864**	0.868*	0.895*	0.847**	1	
Soil CTC	0.976*	0.757	0.976**	0.909**	0.966**	0.751*	0.801**	1

Characteristics of bacterial community in soil

By using high-throughput sequencing technology, through sequence assembly, quality control and removal of chimeric sequences, more than 30,000 reads were obtained for each soil sample. Each sample was flattened under 29,555 sequences and clustered at 97% clustering level. A total of 1688 OTUs were generated from 9 samples.

The Venn diagram (figure 3) shows the amount and intersection of OTUs detected in five treatments: HPM, HCM, BR, CM and CK. A total of 675 identical OTUs were detected in the soil under the five treatments. Soil-specific OTUs (30) of pig manure treatment and soil-specific OTUs (38) of chicken manure treatment were less than CK (57), BR (58) and CM (61). Table 3 shows the OTU number, richness and diversity of soil bacteria. The number of OTUs under BR treatment was the highest. The number of OTUs under all dosages of pig manure treatment was higher than that under all dosages of chicken manure treatment. There was no significant difference in bacterial richness (Chao1) and diversity (Shannon and Simpson) among the treatments.

Table 3 OTUs, richness (Chao1) and diversity (Shannon and Simpson) of bacterial communities treated with different livestock manures

Treatment	OTUs	Shannon	Simpson	Chao1
LPM	1110	7.52	0.98	1312.57
MPM	1219	7.89	0.98	1326.14
HPM	1146	7.71	0.98	1304.43
LCM	1035	7.40	0.98	1213.49
MCM	1045	7.43	0.98	1184.22
HCM	1089	6.96	0.97	1308.75
BR	1225	7.89	0.98	1380.00
CM	1157	7.52	0.97	1317.36
CK	1149	7.57	0.98	1302.96

Fig. 4 shows the bacterial community composition at the top20 gate level in soil treated with different livestock manures. Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi and bacteroidetes are the dominant phyla, accounting for 85.2%-92.4% of the total bacterial abundance in the treated soil. Proteobacteria is the most important category of soil bacteria, with a relative abundance of 40.9%-50.2%. Proteobacteria is the largest category of bacteria, including many human pathogens, such as *Escherichia coli*, *Salmonella*, *Vibrio cholerae*, *Helicobacter pylori* and so on. The relative abundance of proteobacteria was the highest under LPM treatment while lower under MPM, MCM and BR treatments. Actinomycetes are a class of antibiotic-producing phylum, with the highest relative abundance under CM treatment and the lowest relative abundance under HCM and HPM treatments. The relative abundance of Chloroflexi was the highest under MCM treatment, but lower under HPM and LCM treatments. The relative abundance of bacteroidetes was the highest under LCM treatment but lower under HPM and HCM treatments. Different types and application dosages of fertilizer caused differences in the relative abundance of bacteria in each phylum, which indicated that the application dosage and type of fertilizer could affect the structure of soil microbial community.

Correlation analysis between bacterial community and TCs contents in soil

To investigate the effect of tetracycline antibiotics in livestock manure on the structure of bacterial communities (10 dominant bacteria were selected as example), this study carried out Redundancy analysis (RDA) for all sample with the residual amounts of TC, OTC and CTC in soil as the environmental factors. As shown in Fig. 5, contents of TC, OTC, CTC in soil were correlated with *Granulicella*, but showed no correlation with other 9 bacteria, indicating that TCs residue in soil had a weak effect on microbial community.

Discussion

When livestock manure is applied into farmland as organic fertilizer, antibiotics will also be transferred to the soil (Miller et al. 2016; Prosser and Sibley 2015; Quaik et al. 2020). In this study, compared with the CK treatment, the application of livestock manure increased the TCs residue in the soil, with the application dosage ranking as pig manure > chicken manure > biogas residue, which is consistent with the content of TCs in pig manure and chicken manure. The content of single antibiotics in the soil treated with pig manure reached 171.07-707.47 $\mu\text{g}\cdot\text{kg}^{-1}$, that treated with chicken manure was 15.12-334.82 $\mu\text{g}\cdot\text{kg}^{-1}$, and that treated with biogas residue was 33.24-134.25 $\mu\text{g}\cdot\text{kg}^{-1}$. According to the standards stipulated by the European Union (EMA 2006), the antibiotic content in the soil causing the environmental risk is 100 $\mu\text{g}\cdot\text{kg}^{-1}$, which is exceeded by most of the soil TCs in this field experiment. Excessive application of livestock manure will lead to severe antibiotic residues in soil (Zhang 2013), and the ecological risks brought by this cannot be ignored.

Antibiotics in soil can be absorbed and enriched by vegetables and enter the human body through the food chain, thus posing a threat to human health (Chung et al. 2017; Zhang et al. 2019). In this study, the contents of tetracycline antibiotics (TCs) in edible parts of *brassica juncea* var. *gemmifera* were increased by the treatment of livestock manure, in which the increase of TCs contents in *brassica juncea* var. *gemmifera* treated by pig manure was much higher than that under other treatments. Moreover, the TCs contents increased with the increase of the application dosage of livestock manure. The TCs residue in *brassica juncea* var. *gemmifera* was lower under chicken manure treatment than that under pig manure treatment. The biogas residue is fermented from the same pig manure sampling point. When the application dosage of biogas residue was equal to and the medium amount of pig manure, the TCs content in *brassica juncea* var. *gemmifera* under biogas residue treatment was far lower than that under the treatment of medium amount of pig manure, indicating that manure fermentation can reduce the TCs-induced risk to vegetables.

The correlation analysis showed that the contents of TCs in edible part of *brassica juncea* var. *gemmifera* was significantly correlated with the residual TCs in soil ($P < 0.05$). Some studies believe that the absorption process of antibiotics by plants is an active absorption (Kong et al. 2007). The absorption of antibiotics in plant roots is related to the ion trap and electrostatic interaction in cell walls (Miller et al. 2016). After the antibiotics are absorbed by the root system, they enter the above-ground part of the plant

through xylem under transpiration action, and most of them are accumulated in the cell fluid and the comoplast (Goldstein et al. 2014). The transport factors and enrichment factors of antibiotics are related to lipophilicity. Compounds with high lipophilicity are easy to be absorbed by the intracellular fat, resulting in reduced transport capacity, so tetracycline antibiotics with high water solubility are more likely to be transported and accumulated by plants (Wu et al. 2015). Hu et al.(2010) found that the contents of antibiotics in some vegetable tissues exceeded that in the corresponding soil, which had a enrichment effect on antibiotics. However, in this study, the TCs contents in edible parts of *brassica juncea* var. *gemmifera* were generally lower than that in soil, which is consistent with the report by Chi et al.(2018). This may be due to that *brassica juncea* var. *gemmifera* has a low enrichment capacity of tetracycline antibiotics.

Soil microbial communities, including bacteria, archaea and fungi, play an important role in maintaining the function and sustainability of soil ecosystems. When applying organic fertilizers to increase crop yield, soil microbial processes can be stimulated, which is mainly due to that the long-term application of organic fertilizers can increase soil organic matter content and improve soil fertility (Diacono and Montemurro 2010). In this study, the specific bacteria OTUs in soil under HPM and HCM treatments was lower than that under CK and BR treatments. Similar studies have shown that the application of cow manure can reduce the OTUs of soil bacteria (Hartmann et al. 2015; Bonanomi et al. 2016). Calleja-cervantes et al.(2015) found that Shannon diversity index of soil bacteria did not change significantly after 15 days of application of organic fertilizer. Long-term fertilization experiments showed that the application of organic fertilizer significantly improved the diversity of soil bacteria^l(Francioli et al. 2016; Hartmann et al. 2015). The effect of organic fertilizer application on soil bacterial community structure needs to be further studied.

Some studies have found that due to the selection pressure of antibiotics in soil, bacteria with antibiotic resistance proliferate, while those without antibiotic resistance will degenerate (Tamminen et al. 2011). However, the redundancy analysis in this study showed that soil TCs had no significant effect on other 9 dominant bacteria in soil except *Granuliclla*, which may be related to the low TCs contents and short affect time (Calleja-Cervantes et al. 2015). Nevertheless, the long-term effect of antibiotics on soil microbial community should not be ignored.

Conclusions

The application of livestock manure increased the residual contents of tetracycline antibiotics in soil and in *brassica juncea* var. *gemmifera*, where the increment order can be ranked as pig manure > chicken manure > biogas residue. Moreover, with the increase of application dosage of pig manure and chicken manure, the residual content of tetracycline antibiotics in soil and in *brassica juncea* var. *gemmifera* both increased. The residual amount of tetracycline and aureomycin was higher in the soil treated by pig manure, the residual of tetracycline and oxytetracycline was higher in the soil treated by chicken manure, and the residual amount of oxytetracycline and oxytetracycline was higher in the soil treated by biogas residue. Soil Proteobacteria, Acidobacteria, Actinobacteria, Chioroflexi and bacteroidetes under livestock

manure treatment are the dominant phyla. The application of livestock manure changed the diversity and abundance of soil bacterial community. The number of OUTs, richness and diversity of soil bacteria were the highest under biogas residue treatment. The number of OTUs under pig manure treatment was higher than that under chicken manure treatment. Biogas residue after fermentation treatment can effectively reduce the environmental and ecological risks caused by antibiotic residues.

Abbreviations

TCs: Tetracycline antibiotics; TC: Tetracycline; OTC: Oxytetracycline; CTC: Chlortetracycline; CEC: cation exchange capacity; LPM: low amount of pig manure; MPM: medium amount of pig manure; HPM: high amount of pig manure; LCM: low amount of chicken manure; MCM: medium amount of chicken manure; HCM: high amount of chicken manure; CM: chemical NPK fertilizer; BR: biogas residue; CK: No fertilizer; SPE: Solid phase extraction; HPLC: high performance liquid chromatography; LC-MS/MS: liquid chromatography tandem mass spectrometry; ESI: ion source: electrospray ion source; MRM: Monitoring mode: multi-reflection monitoring mode; OTUs: Operational Taxonomic Units.

Declarations

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Authors' contributions

LSX and WHX conceived the study. WZW collected the data presented in the manuscript. LSX and WZW performed statistical analyses. All authors contributed to the writing and revision of the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The sampling of soil and vegetable did not require permission and the species are not classified as endangered, and are not under any protection in any of the sampled areas.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

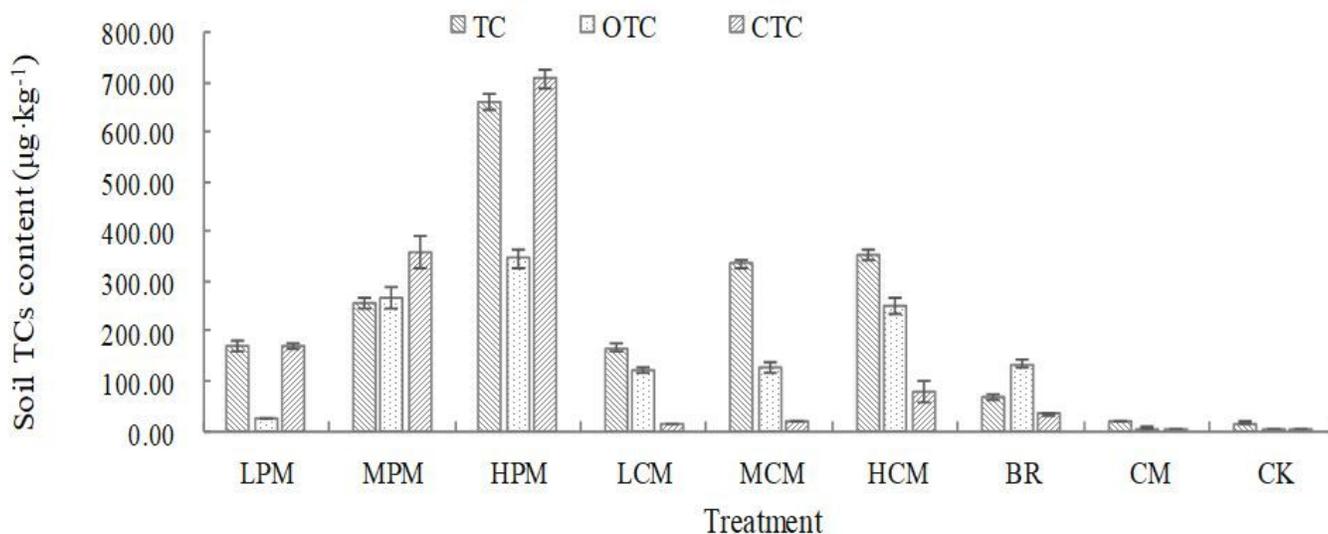


Figure 1

residual content of TCs in soil under treatment of different livestock manures

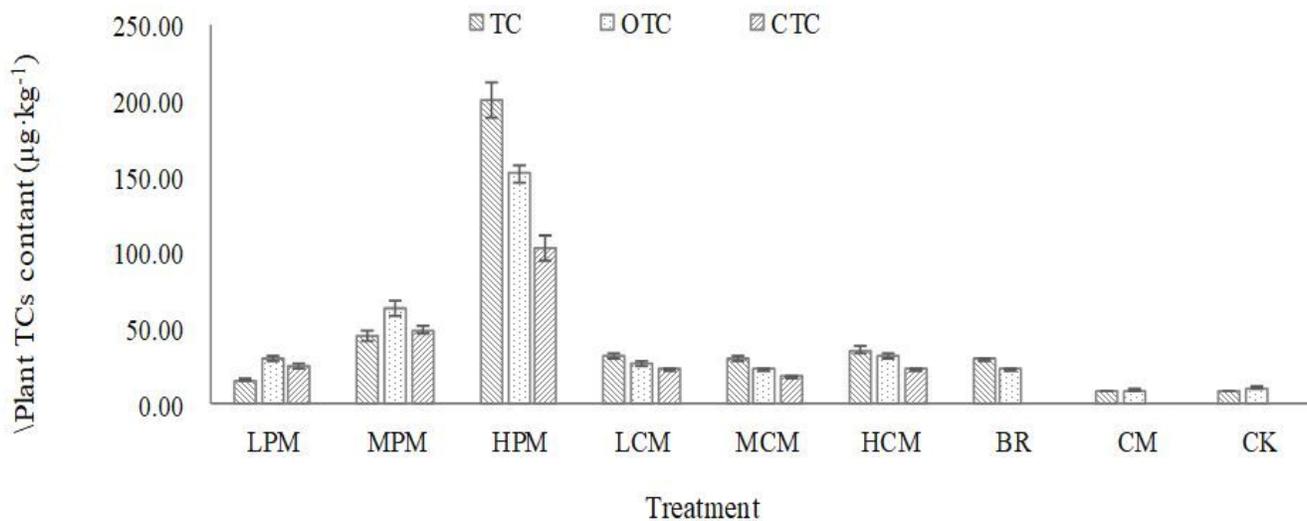


Figure 2

TCs content in edible parts of brassica juncea var. gemmifera treated with different livestock manures

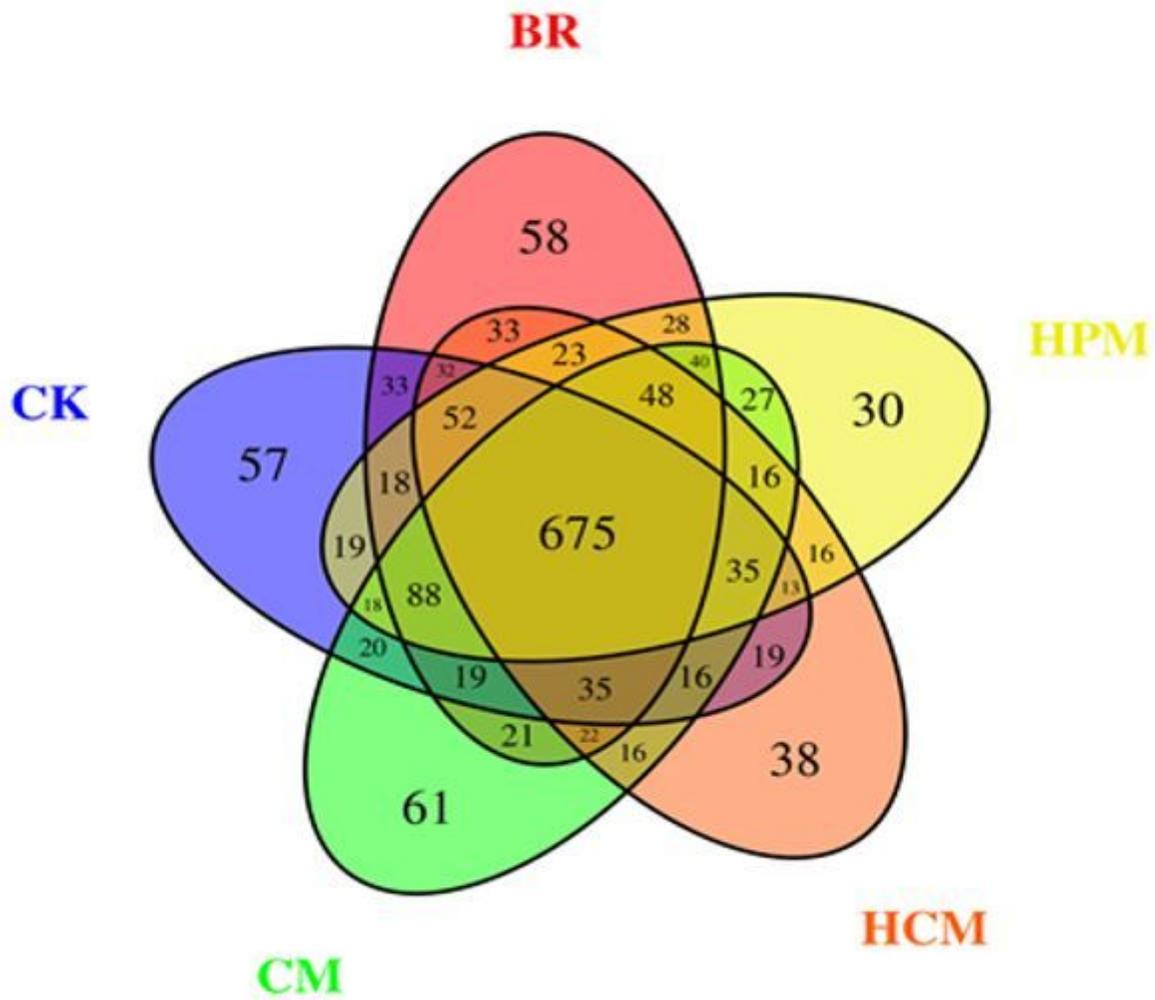


Figure 3

Venn diagram of the number of bacteria OTUs in soil treated with different livestock manures

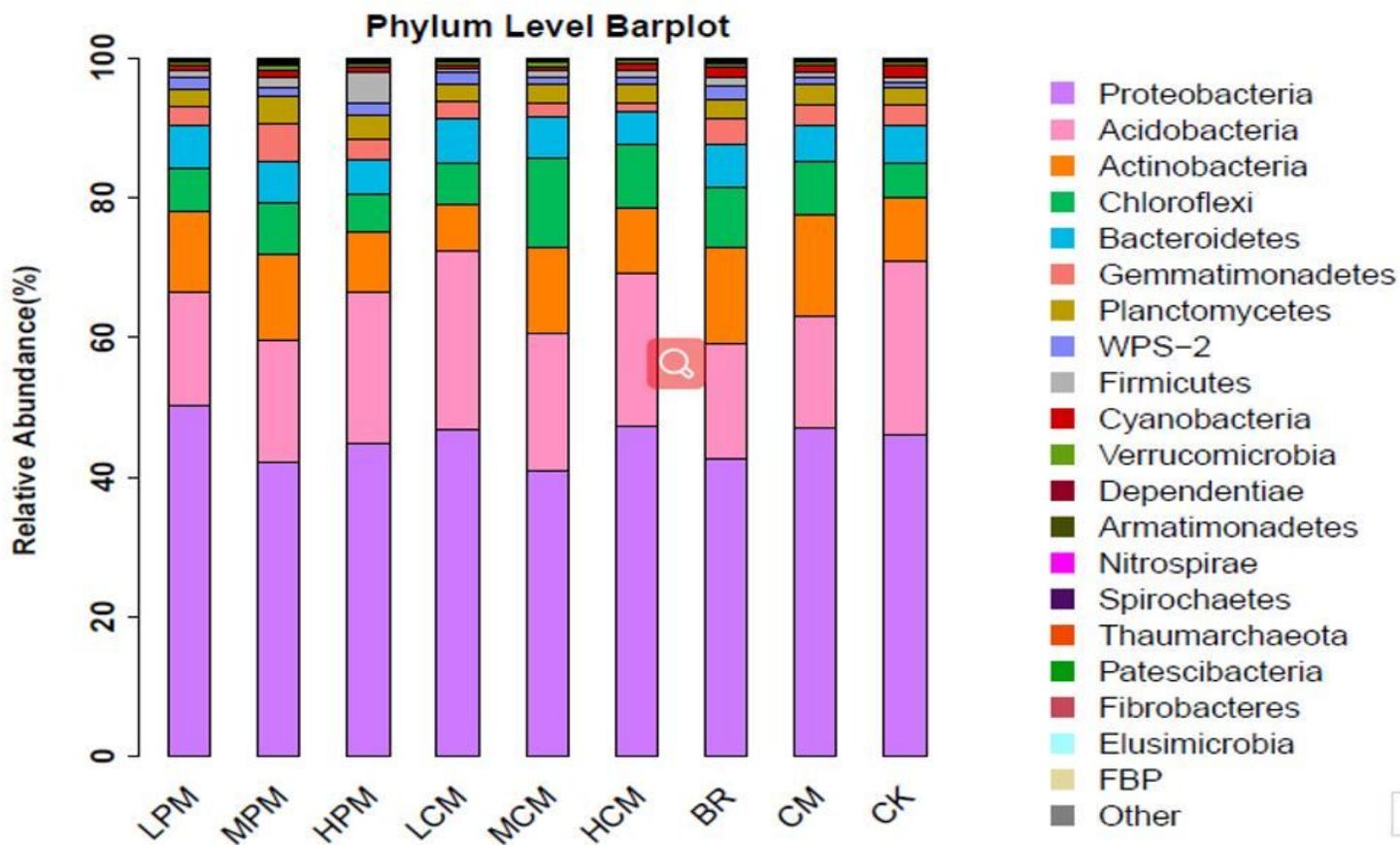


Figure 4

characteristics of bacteria with relative abundance ranking top 20 in soil under treatment of different livestock manures

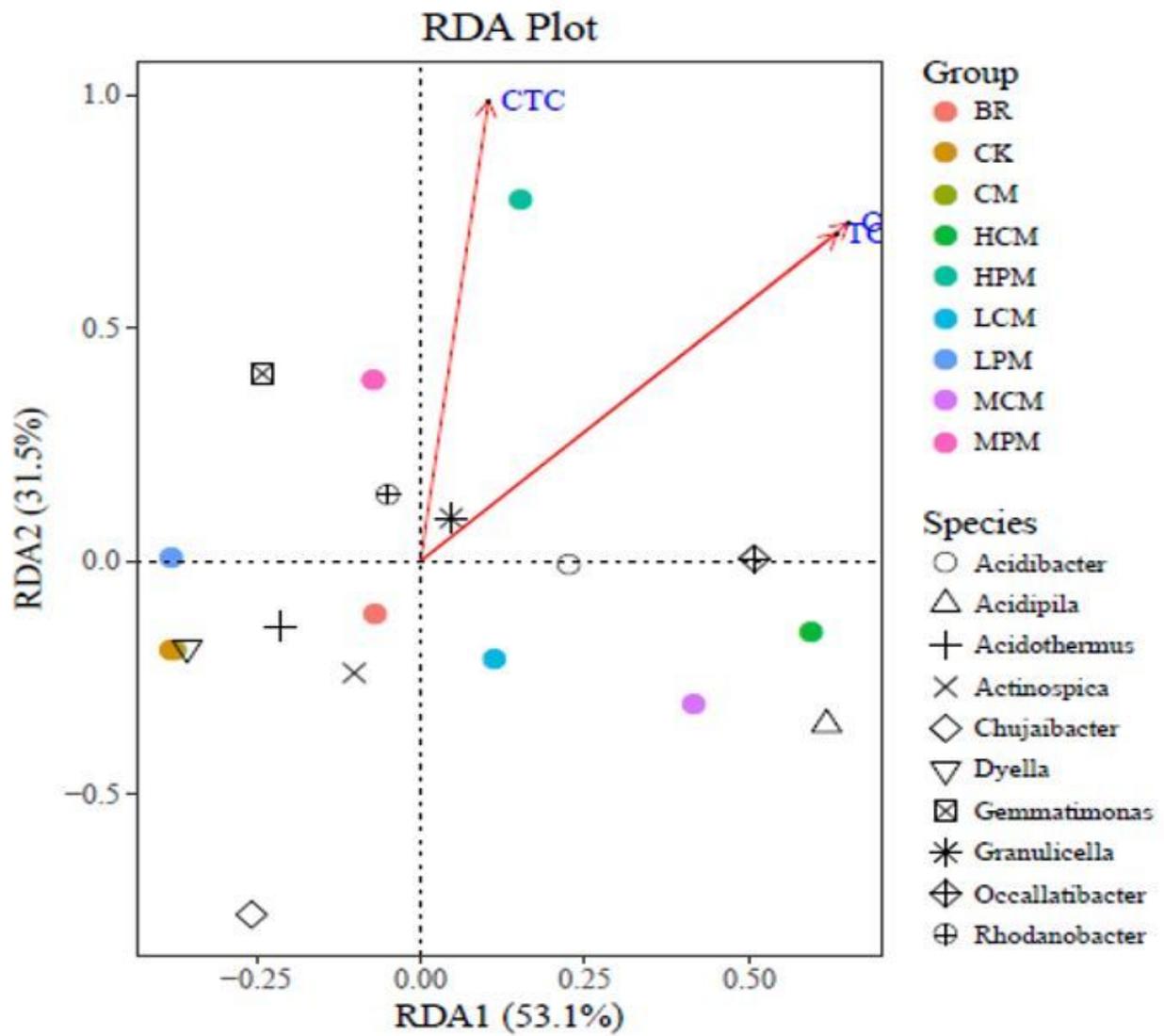


Figure 5

Redundancy analysis of major bacterial communities and TCs residue in soil