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SOIL RESEARCH

# Occurrence of veterinary antibiotics in arable soil with different fertilisation modes: a field study

Xiaona Xie[A,B], Yutong Li[C], Jun Wang[A], Ye Zhou[A], Zhengzhou Yang[A] and Zhengjie Zhu[A,B,*]

For full list of author affiliations and declarations see end of paper

*Correspondence to:
Zhengjie Zhu
Baise University, Baise 533000, China
Email: zhuzhjie@163.com

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## ABSTRACT

**Context.** Antibiotics contained in animal manure can contaminate arable soils and even groundwater. Systematic studies on the presence of veterinary antibiotics (VAs) in soils under different fertilisation modes are urgently needed to provide ample evidence for preventing and controlling VAs pollution. **Aims.** This study tried to illustrate the effect of different fertilisation modes on the occurrence of soil VAs in a long-term field experiment. **Methods.** Soil samples were collected from a 30-year fertilisation experiment with four fertiliser application modes: (1) no fertiliser (CK); (2) chemical fertiliser (TR1); (3) cattle manure (TR2); and (4) combination of chemical fertiliser and cattle manure (TR3). **Key results.** Results showed that tetracyclines and sulfonamides were ubiquitously detected in soil samples with the concentration from not detectable (ND) to 6.95 μg $kg^{-1}$ and ND to 3.85 μg $kg^{-1}$, respectively, which were significantly lower than those of cattle manure (5.13–1628 μg $kg^{-1}$). In addition, the long-term fertilisation generally improved soil properties and increased levels of VAs. The combined application of chemical fertiliser and cattle manure could significantly improve contents of soil nutrients. The correlation coefficient showed that the concentration of antibiotics and soil properties, such as soil organic matter, total nitrogen and available phosphorus, had positive correlation ($P < 0.05$). **Conclusions.** This study indicated that different fertilisation methods had significant effects on the occurrence of antibiotics in arable soil. **Implications.** The combination of inorganic and organic fertiliser application was a reasonable fertilisation mode to improve the soil fertility and control antibiotics contamination.

**Keywords:** agricultural soil, arable soil, cattle-manure, long-term fertilisation, soil nutrients, sulfonamides, tetracyclines, veterinary antibiotics.

## Introduction

Antibiotics are a type of natural or synthetic chemical capable of inhibiting or killing microorganisms, that have existed and substantially benefited public health for decades (Tasho and Cho 2016). Improper or excessive use of antibiotics could cause large amounts of undigested antibiotic residues in urine and faeces of humans and animals as maternal drugs or active pharmaceuticals (Sarmah *et al.* 2006). In addition, antibiotics and their metabolites have been excreted into various environments along with sewage, sludge or manure of hospitals and farms (Kumar *et al.* 2012). Numerous studies reported that antibiotics had been detected in different environmental mediums, such as animal manure (Martínez-Carballo *et al.* 2007; Hu *et al.* 2010; Hou *et al.* 2015; Guo *et al.* 2016; Pan and Chu 2018; Zhou *et al.* 2020), sewage treatment plants (Wu *et al.* 2016), water (Zhang *et al.* 2015; He *et al.* 2018; Liu *et al.* 2018), soil (Hu *et al.* 2010; Li *et al.* 2015; Guo *et al.* 2016; Tasho and Cho 2016; Sun *et al.* 2017), and even in groundwater (Hu *et al.* 2010; Ma *et al.* 2015). China is one of the largest producers and consumers of antibiotics. For example, in 2013, approximately 92 700 tons of antibiotics were used in China. However, an estimated 54 000 tons of them were excreted and finally released into environment (Zhang *et al.* 2015).

Veterinary antibiotics (VAs) are widely used for treating bacterial diseases and promoting growth in animals (Sarmah *et al.* 2006; Kumar *et al.* 2012; Zhou *et al.* 2013).

However, large amounts of agricultural soils in China had the problem of accumulation of residual VAs, caused by the application of animal manure as fertiliser because of farmers' limited knowledge of VAs treatment and regulation (Chen *et al.* 2018). Recently, the accumulation of VAs in agricultural soils became an increasing concern over the world. Previous work suggested that livestock and poultry manure was one of the main sources of VAs in agricultural soil (Ho *et al.* 2014), with residual VAs being absorbed by vegetables and subsequently polluting the food chain (Hu *et al.* 2010). VA residues could also cause the enrichment of harmful bacteria and increasing antimicrobial resistance (Martínez 2008; WHO 2014), posing serious threats to global public health. In addition, VA residues also induced toxic effects on vegetable crops; e.g. wheat, Chinese cabbage and tomato (Jin *et al.* 2009). It is noticed that such toxic effects could occur at very low levels because of long-term exposure (Kuppusamy *et al.* 2018). For food safety and health, it is mandatory to monitor and solve the antibiotics contamination problems.

The occurrence, fate and risks of VAs in agricultural soils had been a hot issue in recent years. VAs contamination was a common phenomenon in agricultural soils (Hu *et al.* 2010; Chen *et al.* 2014; Ho *et al.* 2014; Tang *et al.* 2015; Guo *et al.* 2016; Wei *et al.* 2019; Cai *et al.* 2020). Antibiotics commonly used in livestock husbandry include macrolides, β-lactams, cephalosporins, sulfonamides, tetracyclines and fluoroquinolones. These can be detected in agricultural soils ranging from not detectable (ND) to mg $kg^{-1}$ levels (Kuppusamy *et al.* 2018), reflecting the diversification and abuse of antibiotics. The detected residual concentrations of individual antibiotics were up to 2.68 mg $kg^{-1}$ in Tianjin (Hu *et al.* 2010), 8.40 mg $kg^{-1}$ in Xuzhou (Zhang *et al.* 2016), 2.01 mg $kg^{-1}$ in Tianjin (Wei *et al.* 2016), and 4.72 mg $kg^{-1}$ in Yangtze River Delta (Sun *et al.* 2017). Moreover, Gros *et al.* (2019) demonstrated that VAs were detected in manure-amended soils with average concentrations ranging from 0.078 to 150 μg $kg^{-1}$. Hu *et al.* (2010) reported that the concentration ranges of all the selected antibiotics in the manure and manure-amended soil were 0.1–184 mg $kg^{-1}$ and 0.1–2683 μg $kg^{-1}$ in northern China, respectively. Concentrations of tetracyclines and quinolones antibiotics in organic farm soils in Guangzhou were 2.32–305 μg $kg^{-1}$ (Xiang *et al.* 2016) and 0.46–55.2 μg $kg^{-1}$ (Wu *et al.* 2014), respectively. Hence, the tetracyclines antibiotics were usually measured as predominant antibiotics with high levels of both residual concentrations and detected frequencies in agricultural soils throughout the world, resulting in a potential threat to both plants and soil organisms (Kuppusamy *et al.* 2018).

To date, many researchers have focused on the fate and sources of VAs in agricultural soils worldwide (Kumar *et al.* 2012; Tasho and Cho 2016; Kuppusamy *et al.* 2018; Qiao *et al.* 2018). In consideration of the extension of resource utilisation of animal manure in China, here we present the occurrence of typical VAs in arable soils under different fertilisation modes (no fertiliser, chemical fertiliser, cattle manure, and combined fertiliser) from a 30-year fertilisation experiment, and reveal the relationships between the VAs and soil properties under exclusive application of cattle-manure. We also provide a reference for the control of antibiotics.

## Materials and methods

### Chemicals and standards

Four types of tetracyclines (TCs) including: (1) tetracycline (TC); (2) oxytetracycline (OTC); (3) chlortetracycline (CTC); and (4) doxycycline (DC), as well as four types of sulfonamides (SAs) including: (1) sulfameter (SM); (2) sulfamethazine (SMZ); (3) sulfathiazole (STZ); and (4) sulfadiazine (SDZ) were used in this study (Dr. Ehrenstorfer GmbH, Germany). To quantify the concentrations of antibiotics, the deuterated antibiotics such as tetracycline-D6 (Toronto Research Chemicals Inc., Canada), sulfamethazine-D4 and sulfadimethoxine-D6 (Dr. Ehrenstorfer) were used as the internal standards. Sulfamethoxazole-D4 and demeclocycline (Dr. Ehrenstorfer), another two deuterated antibiotics, were performed as the surrogate standards to assay the recovery of each analysis. Other analytical grade chemicals were from Sinopharm Chemical Reagent Co. Ltd, China, except methanol, formic acid, oxalic acid and acetonitrile (HPLC grade) were from Tedia Company Inc., USA, and ammonium acetate from Sigma-Aldrich, USA. Ultrapure water was produced by a Milli-Q apparatus (Millipore, Bedford, MA).

The stock solution of each standard (1.0 mg $L^{-1}$) was dissolved in methanol, and stored at −20°C in dark. Working solutions were prepared freshly by methanol dilution, and stored at 4°C in dark. The extraction solvent was a mixture of EDTA-sodium phosphate buffer (SPB, pH = 4) prepared based on Huang *et al.* (2013). Briefly, SPB was prepared by mixing $NaH_2PO_4$ (10.56 g) and $H_3PO_4$ (0.82 mL) in 1 L water. EDTA-SPB (pH = 4) was obtained by dissolving $Na_2EDTA$ (80 g) in 1 L SPB.

### Experimental design and sampling

Soil samples were collected at October 2015, from a 30-year fertilisation experiment at the Soil Fertility and Fertilisation Efficiency Monitoring Base, located in the Agricultural Science Institute of Xuzhou, Jiangsu Province (34°16′N, 117°17′E). Four fertiliser application modes were designed: (1) no fertiliser (CK); (2) chemical fertiliser (combination of nitrogen, phosphorus and potassium, TR1); (3) exclusive application of cattle manure (TR2); and (4) combination of chemical fertiliser and cattle manure (TR3). Each treatment contained four replicated districts. Chemical fertiliser contained pure N (8 g $kg^{-1}$), $P_2O_5$ (4 g $kg^{-1}$) and $K_2O$ (6 g $kg^{-1}$). Cattle manure contained pure N (6.3 g $kg^{-1}$), $P_2O_5$ (5.1 g $kg^{-1}$) and $K_2O$ (7.4 g $kg^{-1}$). The annual amount of fertiliser in TR1, TR2 and TR3 was 37 500 kg $hm^{-2}$ from 1984 until now.

A total of 16 surface soil (0–20 cm) samples were collected by five-point sampling method from the area of each district (33 $m^2$) using a pre-cleaned stainless soil auger and transferred into cloth bags. Stones and residual roots were removed. All samples were immediately transferred to the laboratory, and stored at −20°C in dark. Soil samples were sieved through a stainless steel sieve (0.25 mm), freeze-dried, and then sealed in brown glass bottles for the antibiotic extraction. Physico-chemical properties of soil are in Table 1.

### Extraction and analytical procedures

Antibiotics in manure and soil samples were analysed as described by Wei *et al.* (2019). Briefly, each 0.5 g manure or 5 g soil sample was mixed with 2.0 mL 150 mg $L^{-1}$ $Na_2EDTA$ solution, and then mixed with 5 mL acetonitrile/methanol (65/35, v/v), and finally mixed with 5 g anhydrous $Na_2SO4$ and 0.5 g NaCl. The supernatant was pipetted and centrifuged (3000g, 10 min, room temperature) using a d-SPE sorbent consisting of 12.5 mg $C_{18}$, 12.5 mg primary secondary amine and 225 mg $Na_2SO4$.

Purified samples were analysed using a liquid chromatography-tandem mass spectrometry system equipped with a $C_{18}$ column (3 μm × 150 mm × 4.6 mm) at 40°C, and the injection volume was 20 mL. The mobile phase was methanol-acetonitrile (1:1, v/v) and 0.3% formic acid solution with 0.1% ammonium formate (v/v). Mobile phase A and mobile phase B were 0.1% formic acid (v/v), and methanol-acetonitrile (1:1, v/v).

## Results

### Variations of soil physico-chemical characteristics

With more than 30 years of different fertilisation modes, soil physico-chemical characteristics in the current study are listed in Table 2. Soil nutrients under different fertilisation modes are significantly diverse. The pH of the top soil samples in different treatments are weakly alkaline. The fertilisation significantly decreases soil pH, but increases the total organic matter (OM) and cation exchange capacity (CEC). The cattle manure could increase soil total N (TN) and active phosphate (AP), but decrease active potassium (AK). Compared with the control treatment, fertilisation raises soil TN, AK, AP, OM about 34.6–121%, 55.4–145%, 95.7–1184% and 29.8–97.8%, respectively.

### Distributions of selected antibiotics in soils and cattle manure

Eight typical VAs were detected ubiquitously in soils of the long-term experiment with different fertilisation modes. Detection frequencies for all tested antibiotics reached 100% in fertilisation experiments (TR1, TR2, TR3), and only 62.5% in the control treatment (CK). It is noticed that the residue of TCs in the soil was significantly higher than that of SAs ($P < 0.05$) as shown in Table 3.

Concentrations of antibiotics in current arable soils were at a fairly low level ranging from ND to 6.95 μg $kg^{-1}$. Average concentrations for SAs in order were SDZ > SM > STZ > SMZ, and for TCs were CTC > TC > DC > OTC. Thus, SDZ (2.38 ± 1.44 μg $kg^{-1}$) or CTC (2.72 ± 2.50 μg $kg^{-1}$) were the dominant SAs or TCs under different treatments.

Distributions of SAs and TCs in cattle manure are shown in Fig. 1. Concentrations of TCs are 2-fold higher than that of SAs. CTC is the dominant antibiotic (1628 μg $kg^{-1}$), followed by TC (296.8 μg $kg^{-1}$), DC (152.5 μg $kg^{-1}$) and OTC (36.7 μg $kg^{-1}$). For SAs antibiotics in cattle manure, the concentration of SM is the highest with a mean value of 18.5 μg $kg^{-1}$, while SMZ (5.13 μg $kg^{-1}$) is the lowest.

### Occurrence of selected antibiotics in different treatments

Different long-term fertilisation treatments affected soil antibiotics residues. Residues of four types of TCs and SAs

**Table 1.** The physico-chemical properties of soil with the mean values.

| | pH | Total N (g $kg^{-1}$) | Total P (g $kg^{-1}$) | Organic matter (g $kg^{-1}$) | Physical clay content (mg $kg^{-1}$) | Clay content (mg $kg^{-1}$) | Cation exchange capacity (cmol $kg^{-1}$) |
|---|---|---|---|---|---|---|---|
| Soil | 8.01 | 0.66 | 0.74 | 10.8 | 0.01 | 59.8 | 20.4 |

**Table 2.** Soil properties with different fertilisation treatments.

| Treatment | pH | TN (g $kg^{-1}$) | AK (g $kg^{-1}$) | AP (g $kg^{-1}$) | Soil OM (g $kg^{-1}$) | CEC (cmol $kg^{-1}$) | C/N |
|---|---|---|---|---|---|---|---|
| CK | 8.08 ± 0.02a | 0.81 ± 0.03b | 27.67 ± 2.08d | 9.41 ± 0.14d | 14.57 ± 1.10d | 14.80 ± 0.44b | 10.47 ± 0.59a |
| TR1 | 7.74 ± 0.06b | 1.09 ± 0.05b | 52.00 ± 2.00b | 18.42 ± 1.41c | 18.91 ± 1.21c | 15.72 ± 0.53b | 10.04 ± 0.77a |
| TR2 | 7.98 ± 0.05a | 1.51 ± 0.03a | 43.00 ± 1.00c | 120.86 ± 7.49a | 24.68 ± 1.31b | 18.69 ± 0.96ab | 9.48 ± 0.37ab |
| TR3 | 7.79 ± 0.03b | 1.79 ± 0.05a | 67.67 ± 0.58a | 108.54 ± 5.70b | 28.82 ± 2.06a | 20.65 ± 0.93a | 9.16 ± 0.43b |

The values of soil samples with different lowercase letters were significantly different from each other ($P < 0.05$).

**Table 3.** The concentrations of veterinary antibiotics in the soil samples (μg $kg^{-1}$).

| Antibiotics | | Minimum | Maximum | Mean ± s.d. | Median |
|---|---|---|---|---|---|
| Sulfonamides | SM | ND | 2.92 | 1.64 ± 1.00 | 2.04 |
| | SMZ | 0.32 | 1.73 | 0.90 ± 0.53 | 0.78 |
| | STZ | 0.48 | 2.98 | 1.31 ± 0.90 | 0.99 |
| | SDZ | 0.49 | 3.85 | 2.38 ± 1.44 | 2.93 |
| Tetracyclines | TC | 0.22 | 4.14 | 1.54 ± 1.53 | 0.91 |
| | OTC | 0.19 | 3.58 | 1.51 ± 1.10 | 1.40 |
| | CTC | ND | 6.95 | 2.72 ± 2.50 | 1.59 |
| | DC | ND | 3.90 | 2.05 ± 1.28 | 1.56 |

ND, not detected.

in different treatments are shown in Figs 2, 3, respectively. Residual VA concentrations of TR1 treatment are below 0.5 μg $kg^{-1}$, and similar to that of CK. In TR2, concentrations of TCs (2.2–6.95 μg $kg^{-1}$) and SAs (1.4–3.85 μg $kg^{-1}$), are higher than those of the other three treatments, indicating that the pure cattle manure could enhance significantly soil antibiotics level. Hence, pure manure might be the primary polluted source of antibiotics in soils. These results suggest that the combined application of organic–inorganic fertiliser could reduce concentrations of TCs in soils, especially for CTC. However, it is not the case for SAs because there was no significant difference among all concentrations. As shown in Fig. 4, the occurrence of total concentrations of TCs and SAs under different treatments is similar to that of the individual antibiotics. Compared with TR2, the concentration of TCs and SAs in TR3 is reduced about 58.9% and 28.8%, respectively. Typically, as the major compounds in soil, the concentration of CTC and SDZ in TR3 decreased about 73.6% and 20.6%, respectively. However, the total concentration of SAs is higher than that of TCs in TR3, suggesting that a higher decreasing tendency for TCs antibiotics might be owing to the compound fertiliser.

## Correlations between antibiotics and soil properties

Table 4 lists correlation coefficients between antibiotics and soil properties in different fertilisation treatments. Strong positive correlations among different classes of antibiotics are observed. Concentrations of TC, OTC, CTC, DC are significantly ($P < 0.05$) correlated with those of SM, SMZ, SDZ and STZ. Generally, the chemicals in the branch of the same group have a close correlation, and show their positive correlations in soils. It indicates that the residues of soil antibiotics would increase along with the application dosages. Additionally, AP has an extremely strong positive correlation with TCs and SAs; CEC, TN and OM are positively correlated with antibiotics. Notably, OTC, SM and SDZ have extremely strong correlations with soil properties except AK, whereas TC and CTC are weakly correlated with soil properties. Therefore, soil properties, such as AP, TN, SOM and CEC, played a vital role in the residual of antibiotics.

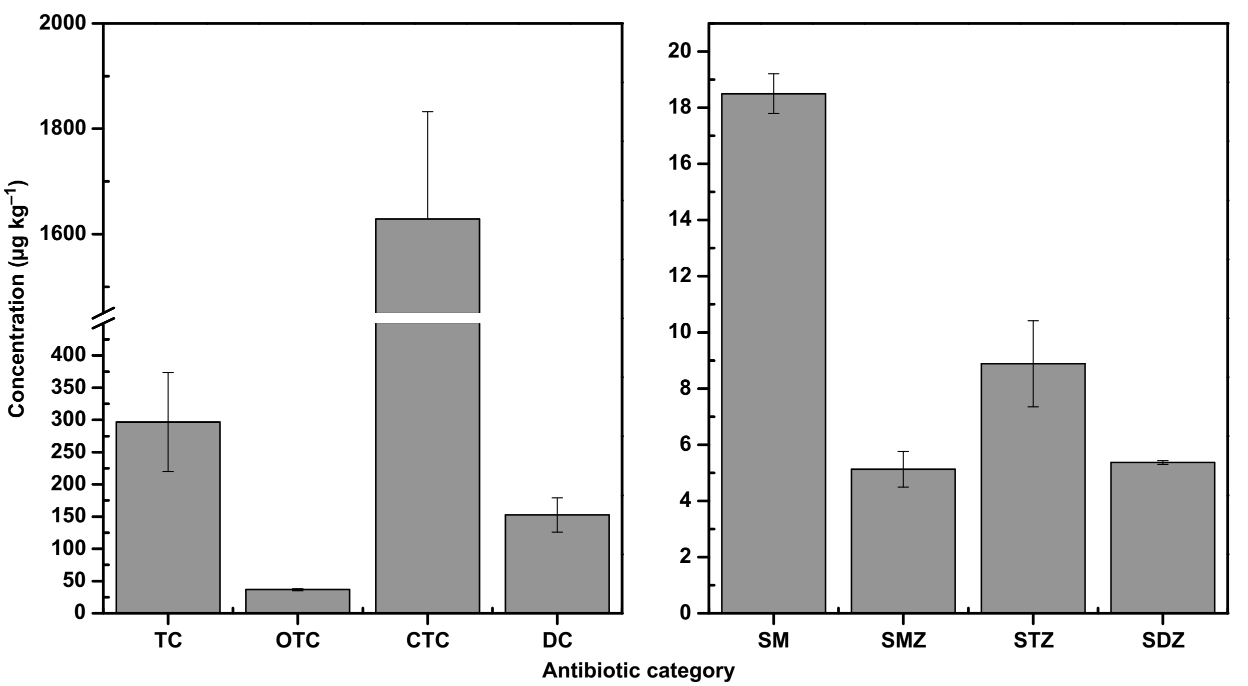


**Fig. 1.** Antibiotics in manure.

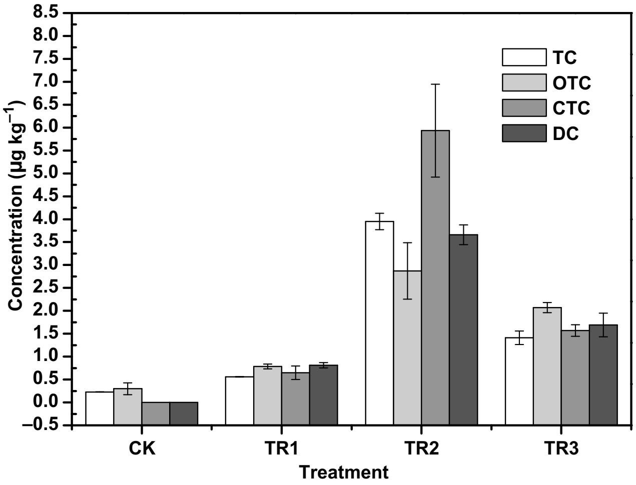


**Fig. 2.** TCs in soil with different fertiliser treatments.

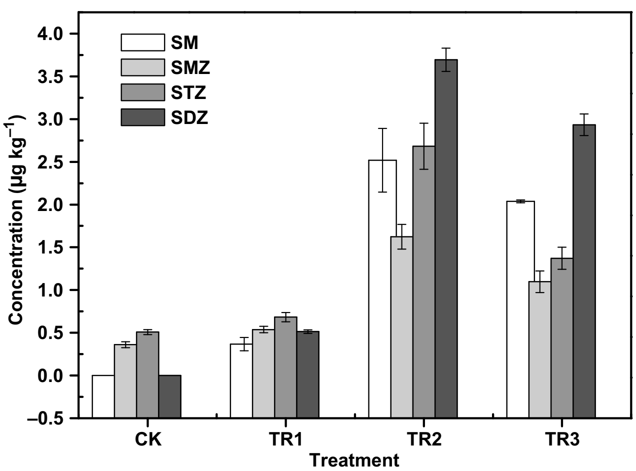


**Fig. 3.** SAs in soil with different fertiliser treatments.

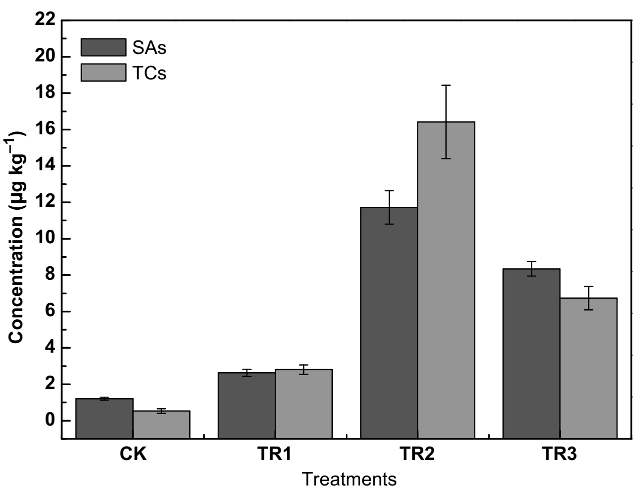


**Fig. 4.** Total antibiotics in soil with different fertiliser treatments.

## Discussion

### Occurrence of typical VAs in soil–manure system

As shown in Fig. 3, both fertilisation modes and antibiotics residues of manure could affect directly the accumulation of the antibiotics in soil. Both detection frequencies and residual concentrations of SAs were lower than those of TCs. CTC was the major individual antibiotic in the present field with the maximum concentration of 1628 μg $kg^{-1}$ and 6.95 μg $kg^{-1}$ in cattle manure and arable soils, respectively. According to standards of veterinary medicines from the Food and Drug Administration, if residual levels of antibiotics are lower than the eco-toxic effect trigger value of 100 μg $kg^{-1}$ in soil, this would pose a low environmental risk.

The levels of eight typical antibiotics in the applied cattle manure and manure-amended soil of the present work were much lower than those of the previous results from various agricultural soils around the world, even for soils continuously manured over 30 years (Hamscher *et al.* 2002; Martínez-Carballo *et al.* 2007; Karcı and Balcıoğlu 2009; Ho *et al.* 2014; Hou *et al.* 2015; Guo *et al.* 2016). A large number of studies had shown that the residual VAs that came from agricultural soil fertilised with manure was a common phenomenon. An investigation undertaken in Austria revealed that 46 mg $kg^{-1}$ of CTC, 29 mg $kg^{-1}$ of OTC, 23 mg $kg^{-1}$ of TC and 20 mg $kg^{-1}$ of sulfadimidine were detected in pig manure (Martínez-Carballo *et al.* 2007). Zhao *et al.* (2010) also found that OTC (0.15–59.6 mg $kg^{-1}$), CTC (0.16–27.6 mg $kg^{-1}$), SMZ (0.1–0.66 mg $kg^{-1}$) or SDZ (0–3.12 mg $kg^{-1}$) were detected in chicken, pig and cattle manure samples. In manure samples, a higher range of concentrations of TCs (54.1 ± 5776 μg $kg^{-1}$) was detected than that of SAs (3.2 ± 5.2 μg $kg^{-1}$) in Jiangsu Province (Guo *et al.* 2016), similar to those reported in other cities of China (Hou *et al.* 2015; Qian *et al.* 2016; Xie *et al.* 2016; Zhou *et al.* 2020). However, the concentrations of SAs (0.1–35.5 mg $kg^{-1}$) were higher than those of TCs (ND–0.5 mg $kg^{-1}$) in manure collected from Turkey (Karcı and Balcıoğlu 2009).

The concentration of antibiotics varies greatly across soils from different origins. Our result is consistent with previous conclusions; i.e. TCs were commonly examined as a class of predominant antibiotic in the livestock and poultry manure with a broad concentration range from ND to hundreds of ppm. After the application of these polluted manures to the field, the soil would act as an antibiotics pool. Residual TCs and SAs in agricultural soils have been detected worldwide. Hu *et al.* (2010) reported that the OTC was the predominant antibiotic in amended soil with concentrations of 124–2683 μg $kg^{-1}$, followed by CTC (1–1029 μg $kg^{-1}$), TC (0.1–105 μg $kg^{-1}$), and SMZ (0.03–0.9 μg $kg^{-1}$). An investigation in the vegetable bases in Beijing revealed that TCs and SAs in soils were detected with concentrations of 6.1–430 μg $kg^{-1}$ and ND–13 μg $kg^{-1}$, respectively

**Table 4.** The relationships between antibiotics and soil properties.

| | OTC | CTC | DC | SM | SMZ | STZ | SDZ | CEC | AK | AP | SOM | TN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TC | 0.93* | 1.00* | 0.98* | 0.88* | 0.96* | 0.99* | 0.88* | 0.57 | 0.10 | 0.84* | 0.58 | 0.59 |
| OTC | | 0.90 | 0.97* | 0.99* | 0.99* | 0.95* | 0.99* | 0.83* | 0.44 | 0.98* | 0.84* | 0.85* |
| CTC | | | 0.98* | 0.84* | 0.94* | 0.98* | 0.85* | 0.52 | 0.05 | 0.80* | 0.53 | 0.54 |
| DC | | | | 0.92* | 0.98* | 0.99* | 0.93* | 0.67 | 0.26 | 0.89* | 0.69 | 0.70 |
| SM | | | | | 0.98* | 0.92* | 1.00* | 0.90* | 0.50 | 0.99* | 0.89* | 0.90* |
| SMZ | | | | | | 0.98* | 0.98* | 0.78 | 0.34 | 0.95* | 0.78* | 0.79 |
| STZ | | | | | | | 0.92* | 0.64 | 0.16 | 0.88* | 0.65 | 0.65 |
| SDZ | | | | | | | | 0.89* | 0.49 | 0.99* | 0.89* | 0.89* |
| CEC | | | | | | | | | 0.77* | 0.92* | 0.99* | 0.99* |
| AK | | | | | | | | | | 0.52 | 0.82* | 0.81* |
| AP | | | | | | | | | | | 0.91* | 0.92* |
| SOM | | | | | | | | | | | | 1.00* |

*Significant at $P < 0.05$.

(Li *et al.* 2015). In addition, the occurrence of several typical antibiotics was described, including OTC (ND–1620 μg $kg^{-1}$), TC (ND–275 μg $kg^{-1}$), DC (ND–814 μg $kg^{-1}$), SMZ (ND–0.98 μg $kg^{-1}$), SDZ (ND–1.21 μg $kg^{-1}$), and SDM (ND–35.4 μg $kg^{-1}$) from soils fertilised by manure on a national scale (Zeng *et al.* 2019). Apparently, the concentration of TCs tended to be higher than that of SAs. The same tendency was confirmed by other researchers (Wu *et al.* 2014; Zhang *et al.* 2015; Huang *et al.* 2016; Qian *et al.* 2016; Xie *et al.* 2016; Sun *et al.* 2017; Wei *et al.* 2019). In contrast, similar concentrations were observed in vegetable soils of the Pearl River Delta, including OTC (ND–80 μg $kg^{-1}$), TC (ND–74 μg $kg^{-1}$), CTC (ND–105 μg $kg^{-1}$), SMZ (ND–74 μg $kg^{-1}$), SDZ (ND–86 μg $kg^{-1}$), SM (ND–120 μg $kg^{-1}$), SMX (ND–55 μg $kg^{-1}$), and SDM (ND–40 μg $kg^{-1}$) (Li *et al.* 2011) in the wastewater irrigation fields in Beijing and Tianjin, including SDZ (ND–97 μg $kg^{-1}$), SMX (ND–90 μg $kg^{-1}$), OTC (ND–112 μg $kg^{-1}$), and CTC (ND–5.2 μg $kg^{-1}$) (Chen *et al.* 2014). However, in a paddy soil adjacent to a composting facility in Korea, the concentration of TCs (4.07–7.02 μg $kg^{-1}$) was lower than that of SAs (24.4–38 μg $kg^{-1}$) (Ok *et al.* 2011). Ji *et al.* (2012) also reported that the highest level of antibiotic existed in SAs (5.85–33.4 μg $kg^{-1}$) rather than TCs (4.54–24.7 μg $kg^{-1}$) in soils near a poultry farm in Shanghai. Moreover, the residual concentrations of eight selected antibiotics in the study area above were as low as 0.1% of those in cattle manure, which could result from leaching, photodegradation, biodegradation and uptake by vegetables (Blackwell *et al.* 2009; Hu *et al.* 2010).

The occurrence and fate of antibiotics in soil varied with compounds. There was a variation between concentrations of TAs and SAs in present field experiments, which depended on types and dosages of the antibiotics, as well as their molecular structures and physico-chemical properties, such as water solubility, volatility, and adsorption capacity (Hu *et al.* 2010; Albero *et al.* 2018). Although eight antibiotics were at low levels, they were still likely to persist for several months to years in soils (Jechalke *et al.* 2014). Thus the potential risk of the emergence and spread of the bacterial resistance via vegetables must be taken into consideration.

## Occurrence of typical VAs in different treatments

In this study, compared with CK treatment, concentrations of soil antibiotics and nutrients were slightly increased in TR1, suggesting that no significant accumulation of antibiotics occurred in 30 years of chemical fertiliser application. Moreover, compared to the combined application in TR3, AP significantly increased under the application of exclusive manure fertiliser in TR2; in contrast, SOM and TN decreased. Our results indicate that the effect of combined application on soil nutrients was better than that of pure manure additive. Although the application amount of manure in TR3 was half of TR2, the content of antibiotics was more than half that of TR2. This might be explained by the change of soil properties under different fertilisation patterns, which might further affect the adsorption and degradation of antibiotics. Soil properties have been reported to play an essential role in the occurrence and metabolic fate of antibiotics (Tasho and Cho 2016). In this study, AP was the primary property that affected residues of VAs in all soil samples. OTC, SM, and SDZ showed significant positive correlations with AP, SOM, CEC, and TN (Table 4) suggesting that these properties might be the key factors, which caused an excess of accumulation of OTC, SM and SDZ in TR3. Previous studies also reported that pH had a negative influence on the adsorption of antibiotics, whereas SOM and CEC were positively associated with the adsorption of antibiotics (Thiele-Bruhn 2003; Jones *et al.* 2005; Zhang *et al.* 2010; Leal *et al.* 2013), which was consistent with our results. Further, residues of VAs might be affected by their physico-chemical properties and management system (Ho *et al.* 2014; Tasho and Cho 2016). Yin *et al.* (2012) suggested that the content of soil antibiotics was sensitive to environmental and anthropogenic factors. Sorption has been considered as a major process determining the metabolic fate of soil antibiotics. Previous studies had revealed the adsorption of SAs (ter Laak *et al.* 2006; Leal *et al.* 2013; Wang *et al.* 2015) and TCs in soils (Sassman and Lee 2005; Zhang *et al.* 2010; Fernández-Calviño *et al.* 2015), while the adsorption capacity of TCs was stronger than that of SAs in manure and soil (Kong *et al.* 2012; Pan and Chu 2016; Tasho and Cho 2016). SAs had the lower $K_d$ values than TCs, indicating that SAs had stronger water solubility, and thus had easier migration from manure or soil into groundwater and surface water (Doretto *et al.* 2014). Hence, numerous studies suggested that SAs occurred in soil–manure system at low concentrations. It also confirmed that TCs had much stronger adsorption to the soil by exploring the leaching behaviour of antibiotics (Blackwell *et al.* 2009). Besides, TCs had multiple polar and ionisable functional groups, which might involve strong absorption of soil particles through cation exchange and complexation (Pan and Chu 2016). The other explanation was that SAs were more easily hydrolysed and degradable by soil microorganisms than TCs (Thiele-Bruhn 2003). Thus, TCs could be more easily accumulated in soils and should be paid more attention.

Water management of fields, such as drying–rewetting cycles, has shown a great effect on the persistence of antibiotics (Jechalke *et al.* 2014; Albero *et al.* 2018). Previous studies suggested that the antibiotic dissipation rate under soil with frequent drying–rewetting cycles was lower than that under continuous irrigation (Jechalke *et al.* 2014). Frequent drying–rewetting cycles might cause a higher accessibility of polar sorption sites of SOM. Drying–rewetting cycles could increase AP as well, particularly in manure-fertilised soil (Sun *et al.* 2018). According to our results, it might further disturb the occurrence of antibiotics. For decades, many studies claimed that the combined, but not exclusive, fertiliser application could significantly promote soil nutrients (Hartmann *et al.* 2015; Francioli *et al.* 2016). Our results showed that the combined application of organic–inorganic fertiliser could dramatically improve soil properties. Long-term usage of organic manure became non-point source pollution and lead to the accumulation of antibiotics in soil. Such pollution also enriched bacterial resistance and accelerated greenhouse effect. The accumulation of antibiotics in arable soils would make irreversible changes to soil physico-chemical properties and micro-ecological function. As a non-renewable resource, the potential risk of leaching, absorbtion by vegetables, and horizontal resistant gene transfer in arable soil should be paid much more attention. The findings in this study shed light on a better understanding of the occurrence of VAs in arable soil, and would help to establish more regulations and strategies to reduce the improper use of antibiotics from different sources.

## Conclusions

In this study, the occurrence of eight typical VAs was detected in manure and soil samples from a 30-year field experiment with four types of fertilisation modes. TCs and SAs were ubiquitously detected in soil samples with concentrations of ND–6.95 μg $kg^{-1}$ and ND–3.85 μg $kg^{-1}$, respectively, which were even 2-fold to 3-fold less than those of other agricultural soils. Total concentrations of antibiotics in agricultural soils were significantly lower than those in cattle manure (5.13–1628 μg $kg^{-1}$). In addition, the long-term fertilisation generally increased soil properties and levels of VAs. The combined application of organic–inorganic fertiliser could significantly improve concentrations of soil nutrients compared with the exclusive application, whereas it reduced the residual VAs from manure fertiliser into soil. Furthermore, concentrations of antibiotics had a positive correlation with soil properties, including available phosphorus, CEC, SOM and total N. This study suggested that fertilisation modes had significant effects on the occurrence of antibiotics in arable soils. Hence, the combined application of organic and inorganic fertiliser was an effective fertilisation mode to improve the soil fertility and control antibiotics contamination.

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**Data availability.** The data sets supporting the results of this article are included within the article.

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**Author affiliations**

[A]Baise University, Baise 533000, China.

[B]Guangxi Key Laboratory of Biology for Mango, Baise 533000, China.

[C]Chongqing Research Academy of Eco-Environmental Sciences, Chongqing 401147, China.