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Fate of antibiotic resistance genes in abandoned swine feedlots in China: seasonal variation

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Abstract

Background: Environmental hygiene concerns are needed to be settled before the reuse of abandoned swine feedlot sites. However, few researchers have focused on the fate of antibiotic resistance genes (ARGs) in soil microbiota around abandoned swine feedlots. In this study, we examined the seasonal alterations of ARGs and bacterial community composition in soil using quantitative PCR and high-throughput sequencing of 16S rRNA gene.

Results: The seasonal variation patterns were different for different ARG subtypes and soil sampling sites. The bacterial community composition at the genus level generally showed no significant alteration from winter to summer. Moreover, the co-occurrence network suggested that the bacterial genera host range of ARGs was broader in the summer than in the winter.

Conclusion: This study offers further data on ARG transfer risk in soil, emphasizing the necessity of continuous concern before land-reuse of abandoned feedlots.

Highlights

- Seasonal change of ARGs in soil varied between ARG subtypes and sampling sites.
- Variation trend of ARGs in abandoned feedlots was similar to that in working ones.
- Composition of bacterial community changed little between seasons.
- Co-occurrence of ARGs and bacteria was more intensive in summer than in winter.
- ARG transfer risk was still not reduced after abandonment for 1.5 years.

Keywords: ARGs, Abandoned swine feedlot, ARG distributing pattern, Bacterial community composition

Background

Swine feedlots have been widely known as an important hotspot for antibiotic resistance genes (ARGs) [1, 2]. This issue remains unsettled, because ARGs can persist for extended periods in environmental matrices including soil and water [3, 4]. Moreover, in addition to vertical gene transfer, ARGs derived from microorganisms in animal manure may be transmitted to indigenous soil microorganisms through horizontal gene transfer (HGT), and this process fosters the proliferation of antibiotic-resistant bacteria [5].

In recent years, the intensive swine farming industry has greatly downsized due to the African swine fever outbreak and increasingly strict environmental policies in

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China, leaving a large number of abandoned swine farms. The soil at abandoned sites will need to be restored and reused. However, the fate of ARGs in soil microbiota near abandoned swine feedlots is unclear and has seldom attracted necessary attention. In our previous work [6], the ARG composition and soil bacterial community structure in soil near abandoned and working swine feedlots were examined after swine feedlots were demolished for 1 year. Considering that time and meteorological conditions may influence bacterial community composition and the dynamic fate of ARGs, further studies are needed to explore the fate of ARGs as time goes by and seasons change. Thus, ARG profiles in soil microbiota need to be investigated before the reuse of these sites to assess the potential environmental hygiene conditions.

In this study, ARG distributing patterns using quantitative polymerase chain reaction (qPCR) and bacterial community composition with 16S rRNA gene high-throughput sequencing were investigated. The aim of this study is to investigate the distributing patterns of ARGs in soil adjacent to abandoned and working swine feedlots and demonstrate whether these can be significantly altered by seasons (winter and summer). The latter has been a main point of difference to our previous work [6]. To our knowledge, this is the first study to explore ARG seasonal variations around abandoned swine feedlots. Since there are no efficient methods to eliminate ARG pollution in soil currently, the ARG transmission risks need to be continually investigated. This work will offer a basic reference for environmental risks of ARGs transferring to soil and help understand the potential transmission risk of ARGs in the land reuse of abandoned swine feedlots.

Methods and materials

Background information of chosen feedlots

One typical working and one abandoned swine feedlot were investigated in each of two cities in China. Namely, Shunyi District in Beijing and Hefei in Anhui Province, as the representative regions of animal husbandry in northern China and southern China, respectively. The working and abandoned feedlots in Beijing and Hefei were indicated as BW, BA and HW, HA, respectively (Additional file 1: Table S1). The abandoned feedlot and the working one in Beijing were ~2 km away from each other. In Hefei, they were ~3 km away from each other. The two chosen abandoned feedlots, namely, BA and HA had been demolished 1 (winter) and 1.5 years (summer) at the start of the sampling. Weather information for June and December was collected from the local meteorological stations (Table 1). There were no manure or wastewater treatment facilities in our experimental feedlots. The wastewater that flushed out swine manure was directly discharged into a ditch outside the feedlot enclosures and were designated as BWW, BAW, HWW and HAW, respectively. In addition, six soil cores taken from soils without a history of animal breeding and manure fertilization were collected upstream of the two chosen feedlots in Beijing as a blank control (CK soil).

Sampling

Soils were sampled from fields adjacent to the investigated swine feedlots. A rectangular area of nearly one thousand square meters was divided along one side of the wastewater discharging ditch. The two sides of the rectangle parallel to the ditch were one and ten meters away from the ditch, respectively, and the other two sides perpendicular to the ditch were just outside the discharging outlet and 100 m downstream along the ditch (Additional file 1: Fig. S1). For each point, three points were sampled with a soil probe, and the samples were thoroughly homogenized and treated as one sample. Soil cores from 0 to 20 and 20 to 50 cm were taken as the topsoil and subsoil samples, respectively. Soil samples were immediately transported back to the laboratory, about 50 g soil from each sample was frozen at − 20 °C and the remaining was aired for 1 week before analysis. In addition, six soil cores in the region of 10 km upstream of the two Beijing feedlots were sampled as the control (CK) soil for comparison with soil samples adjacent to the Beijing feedlots. Meanwhile, no control samples were collected from Hefei to keep the homogeneity of soil properties for calculating the relative abundance (fold change) of ARGs. Sampling was done twice, once in December 2017 and the other in June 2018. A part of these data have been previously

Table 1 Information of the two sampling cities in June and December

City	Location	Soil type	Month	ADMIT (°C)	ADMAT (°C)	AAT (°C)	Rainfall (mm)
Beijing	40° N, 116° E	Meadow cinnamon	June	19	30	11.5	74
			December	− 6	3		2
Hefei	31° N, 117° E	Yellow–brown	June	21	29	15.7	132
			December	1	9		29

ADMIT average daily minimum temperature, *ADMAT* average daily maximum temperature, *AAT* average annual temperature

reported [6]. The approximate locations of soil cores taken at two depths and wastewater sites are indicated in Additional file 1: Fig. S1.

Pollution source sampling involved acquiring wastewater samples from the discharging ditches. As for the abandoned feedlots, sediment at the bottom of wastewater ditches was collected in triplicate, then mixed thoroughly and treated as one sample. Samples were stored at − 20 °C before analysis.

Soil characteristic analysis

Determination of soil pH: soil samples (5 g) were added to 50 mL plastic centrifuge tubes with 25 mL deionized water and shaken for 30 min. The pH was measured in the liquid after settling of the soil using an electrode (Mettler Toledo, Shanghai, China). Total nitrogen (TN) was determined from 0.6 g soil in a digestion tube to which was added 8 mL H_2SO_4 conc. and 10 drops of $HClO_4$. The mixture was maintained at 180 °C for 45 min, 240 °C for 90 min and 320 °C for 120 min for digestion. TN was determined using a Kjeldahl nitrogen analyzer (Shanghai Qianjian Instrument Co., Ltd, Shanghai, China) that was titrated with 0.01 M HCl.

Total phosphorus (TP) was determined from 1.0 g soil in a 100 mL digestion tube and digested as per TN above. TP was then determined by spectrophotometry at 700 nm using a UV-4802 instrument (Unico, Dayton NJ, USA). Soil nitrate (NO_3^-–N) and nitrite (NH_4^+–N) were extracted using 6 g fresh soil in 100 mL 1 M KCl that was then oscillated for 1 h. Measurements were carried out in a continuous flowing analyzer (Skalar San++, the Netherlands). Soil organic carbon (SOC) was determined by the $K_2Cr_2O_7$ oxidation method with ferrous sulfate (0.2 M) titration. Cu, Zn and Cd were extracted using 0.6 g soil in 12 mL aqua regia (HCl: HNO_3, 3/1 v/v) and 3 mL $HClO_4$. Cu and Zn were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin Elmer Optima 5300 DV, USA). Cd was determined with an inductively coupled plasma mass spectrometer (ICP-MS) (Perkin Elmer Sciex, USA).

DNA extraction and quantitative PCR

Before DNA extraction, 0.3 g soil for each soil sample was prepared for DNA extraction. For wastewater samples, 3 mL of wastewater was transferred to a centrifuge tube and was centrifuged at 10,000×*g* for 5 min, then the supernatant was removed and the precipitation was retained for DNA extraction. Sample DNA was extracted using a PowerSoil DNA Isolation Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. 20 target genes were quantified, including 6 tetracycline (*tet*) resistance genes (*tetA*, *tetG*, *tetM*, *tetO*, *tetW* and *tetX*), 3 sulfonamide (*sul*) resistance genes (*sul1*, *sul2*, *sul3*), 3 quinolone (*qnr*) resistance genes (*qnrA*, *qnrB* and *qnrS*), 2 chloramphenicol (*cml*) resistance genes (*cfr* and *floR*), 3 macrolide (*mac*) resistance genes (*ereA*, *ermB* and *mefA/E*), and the class 1 and class 2 integron genes (*intI1* and *intI2*) as indicators of potential HGT. The 16S rRNA gene was used to determine soil bacterial population. Real time qPCR reaction volume of 20 μL was made by mixing 10 μL qPCR 2× SYBR Green premix (Tiangen FastFire, Beijing, China), 0.2 μL of 10 mM forward and reverse primer, 1 μL template DNA and 8.6 μL ultrapure water in a 2-step amplification procedure with two replicates (Additional file 1: Text S2). Real time qPCR was carried out by a Biorad CFX 96 well Thermocycler (Biorad, Hercules, CA, USA) using gene-specific primers (Additional file 1: Table S2).

Gene abundance was calculated using normalized abundance (ARG copies/16S rRNA gene copy) and a relative quantitative method [7]. The relative abundance presented as the fold change value ($2^{-\Delta\Delta Ct}$) of each ARG and integron genes, which was used to calculate the difference of ARG composition between the polluted soil (soil adjacent to swine feedlots) and the CK soil. The corresponding functions of fold change have been listed below. The average normalized ARG abundance of the CK soil was used as the reference (ΔCt (CK soil)) when calculating fold change. Values were considered as significantly different when the $2^{-(\Delta\Delta Ct+2SD)} > 1$ or $2^{-(\Delta\Delta Ct-2SD)} < 1$.

$$\text{Fold change } (2^{-\Delta\Delta Ct}) = 2^{-[\Delta Ct(\text{polluted soil}) - \Delta Ct(\text{CK soil})]} \quad (1)$$

$$\Delta Ct = \log(\text{ARG copy number}) - \log(\text{16S rRNA gene copy number}) \quad (2)$$

where the polluted soil refers to soil adjacent to swine feedlot, CK soil refers to control soil without a history of animal farming or manure application.

Bacterial 16S rRNA gene amplicon sequencing and analysis

High-throughput sequencing of bacterial V3–V4 hypervariable regions of 16S rRNA gene was carried out by Beijing Allwegene (Beijing, China) on the Illumina Miseq PE300 platform (Illumina, Sam Diego, CA, USA). The primer sets used were 338F (5′-barcode-ACTCCT ACGGGAGGCAGCAG-3′) and 806R (5′-GGACTA CHVGGGTWTCTAAT-3′) [8]. The detailed process of PCR and subsequent bioinformatics analysis are presented in section Additional file 1: Text S1. The raw data were deposited in the NCBI Sequence Read Archive (SRA) under the BioProject PRJNA723211.

Statistical analysis

Statistical analyses and graphs were mainly carried out and generated using Rstudio based on R 3.5.2 [9]. Non-metric multidimensional scaling analysis (NMDS) using the Bray–Curtis dissimilarity and redundancy analysis (RDA) were carried out using 'vegan' package in R [10]. Network graphs were generated using 'igraph' and 'mixOmics' packages in R [11, 12]. Networks were further illustrated and visualized by Cytoscape 3.6.1 [13]. ANOVA or *t* tests were considered significantly different when $p < 0.05$. Details for the above methods can be seen in our previous publication [6].

Results

ARG distributing patterns in samples

The normalized and relative abundance (fold change) of ARGs were calculated to compare the variation of ARG composition among different sites and seasons. The results showed that the seasonal variation patterns were different for different ARG subtypes and soil sampling sites (Fig. 1 and Additional file 1: Table S3). While most of

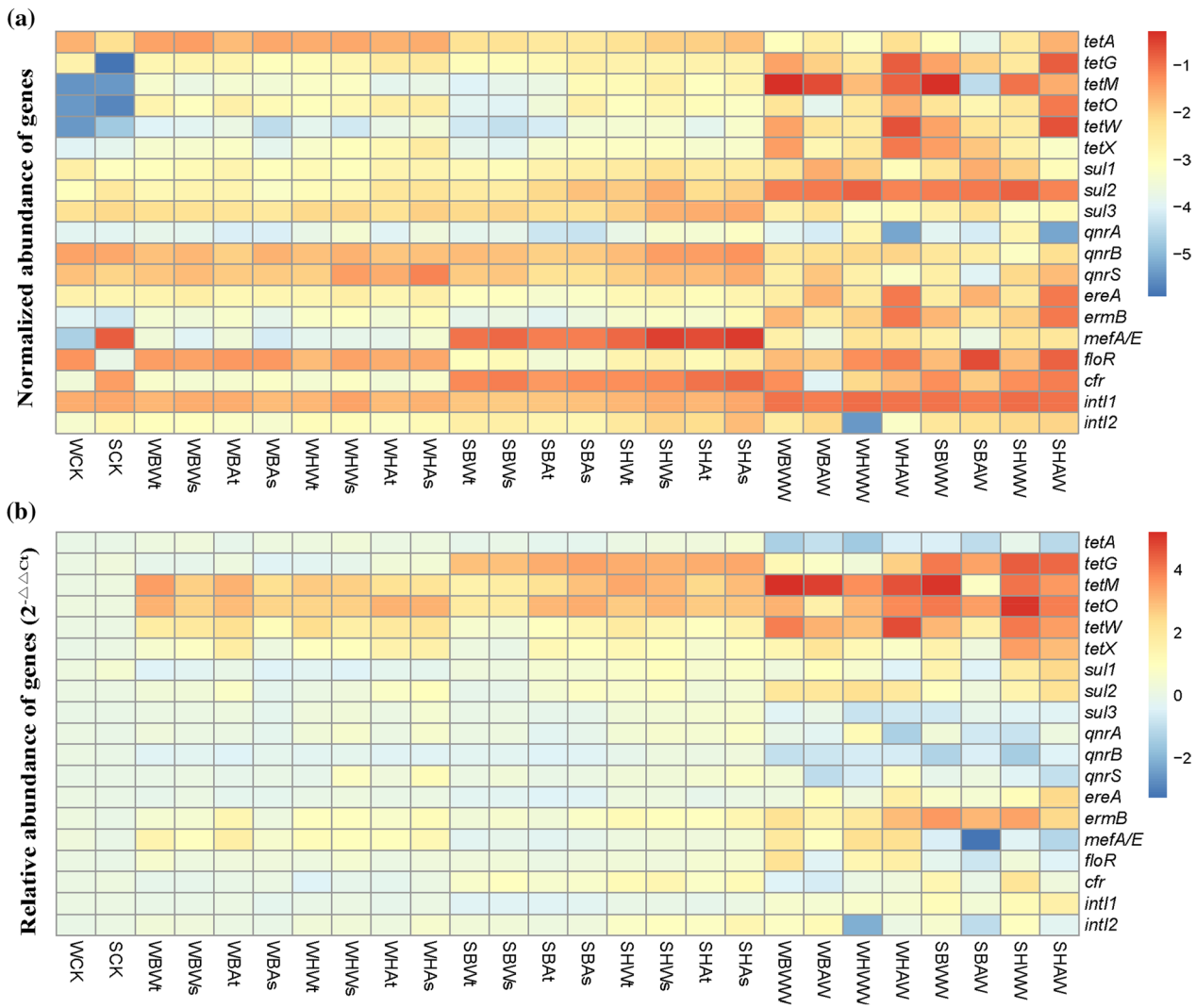

Fig. 1 ARGs grouped by season in soils and wastewater adjacent to swine feedlots. **a** Normalized ARG abundance relative to 16S rRNA gene; **b** ARG abundance relative to the CK soil (fold change). The initial letters in the abbreviations W and S represent winter and summer, respectively. The latter two capital letters, 'CK' represent control soil without a history of livestock farming or manure application; 'BW', working feedlot in Beijing; BA, abandoned feedlot in Beijing; 'HW', working feedlot in Hefei; 'HA', abandoned feedlot in Hefei. Lower case letters 't' and 's' indicate topsoil and subsurface soil, respectively. The number involved represents sampling sites indicated in Additional file 1: Fig. S1. Samples with a last capital 'W' represents wastewater (the pollution source) or sediment collected from the wastewater discharging ditches

the target genes sampled from the majority sites showed no significant difference between two seasons, five genes (*tetA*, *mefA/E*, *floR*, *cfr*, *intI2*) derived from most sites showed a significant difference in normalized abundance between winter and summer (Additional file 1: Table S3). For example, *mefA/E* and *cfr* significantly increased from winter to summer, while *floR* significantly decreased from winter to summer (*t* test, $p < 0.01$). The normalized abundance of *tetA* was significantly decreased in most soil sites from winter to summer except for HWs, HAt and HAs (*t* test, $p < 0.05$). *tetG* also showed a similar trend, although it was only significant for CK, BWs and BAs (*t* test, $p < 0.05$) (Additional file 1: Table S3).

In addition, wastewater samples generally carried a higher ARG abundance than soil samples (Fig. 1), highlighting the heaviness of ARG load in the pollution source. Especially that the relative abundance of *tetM*, *tetO* and *tetW* was significantly higher than that in CK soil samples. Moreover, significant higher *tetM*, *tetO* and *tetW* relative abundance in soil adjacent to pig farms than that in CK soil was also found, this was most likely due to the transmission of ARGs from wastewater to the adjacent soil, as these three genes were frequently found in abundance in pig manure [14].

The normalized abundance of *floR* significantly decreased, while that of *mefA/E* and *cfr* significantly increased in summer ($p < 0.05$). These differences were more obvious when presented as a bar chart which demonstrated that SHW and SHA contained more abundant normalized ARGs than other soil groups (Additional file 1: Fig. S2). Generally speaking, a significant increase of normalized ARG abundance was observed from winter to summer in HW and HA ($p < 0.05$).

Correlation of ARGs and physiochemical characteristics of soil

A correlation heatmap was generated to explore the Pearson correlations between specific physiochemical characteristics and normalized ARG abundance (Fig. 2). The result indicated that the majority of ARGs displayed weak and non-significant correlations with the other physiochemical characteristics, while exceptions did exist. For example, *tetM* had a weak positive correlation with TN, *sul2* showed a weak positive correlation with TN and NO_3^-–N. *mefA/E* and *cfr* showed medium ($r = 0.52$) and weak ($r = 0.40$) positive correlations with NH_4^+–N, respectively. TP, TN, NH_4^+–N, Cu and Zn also showed weak positive correlations with *sul1* and *mefA/E* ($0.3 < r < 0.5$).

Bacterial community structure in winter and summer

The α diversity of bacterial communities in our samples was calculated. There was no significant difference found between winter and summer both in soil and wastewater or sediment samples, except for BA, where the α diversity was significantly decreased from winter to summer (Additional file 1: Fig. S4). NMDS analysis was then carried out to examine β-diversity, and the result showed that there was no significant difference in the bacterial community structure according to season at the genus level (NMDS stress = 0.166) (Fig. 3), even though the weather characteristics were quite different (Table 1).

The relative abundance of Actinobacteria and Acidobacteria showed decreasing and increasing trends, respectively, from winter to summer in Beijing but not in Hefei. Table 2 lists any phylum that showed a significant difference in relative abundance (the percentage of the single phylum to the whole soil microbiota at the phylum level) between winter and summer. The result indicated that Firmicutes, Actinobacteria, Gemmatimonadetes, Nitrospirae, and Planctomycetes showed no significant differences in relative abundance between winter and summer for the majority of our soil sites (Table 2). Firmicutes were consistently more abundant in soils adjacent to the Beijing swine feedlots than for the Beijing CK soils.

Co-occurrence network of bacterial genera and ARGs

In our present study, the co-occurrence networks for ARGs and soil bacteria in winter and summer displayed some common features (Figs. 4 and 5). For example, there were more potential genera hosts on the network of the working swine feedlots in each season than those of the abandoned swine feedlots.

A co-occurrence network for summer wastewater and sediment samples was also generated (Fig. 6), and compared this to our previous winter network (Fig. 5 in [6]). A comparison of these networks indicated that the potential ARG hosts at the phylum level for wastewater and sediment were different from those in the soil samples. Many genera members in Firmicutes that were present on this summer network were also present on the corresponding winter network and included *Tissierella*, *Lactobacillus* and *Fastidiosipila* (Fig. 6). On the other hand, the frequency of phyla presented on the above networks was not proportional to their relative abundance in our soil, wastewater, or sediment showed in Additional file 1: Fig. S4.

Discussion

Spatial and temporal variation of ARG abundance

Our results of ARG abundance emphasized that different gene elements responded differently to the same environmental variations. For example, the normalized abundance of efflux pump gene *tetA* showed a decreasing trend from winter to the contiguous summer. This gene was found flanked by MGEs (mobile genetic elements, MGEs); therefore, its transmission was associated

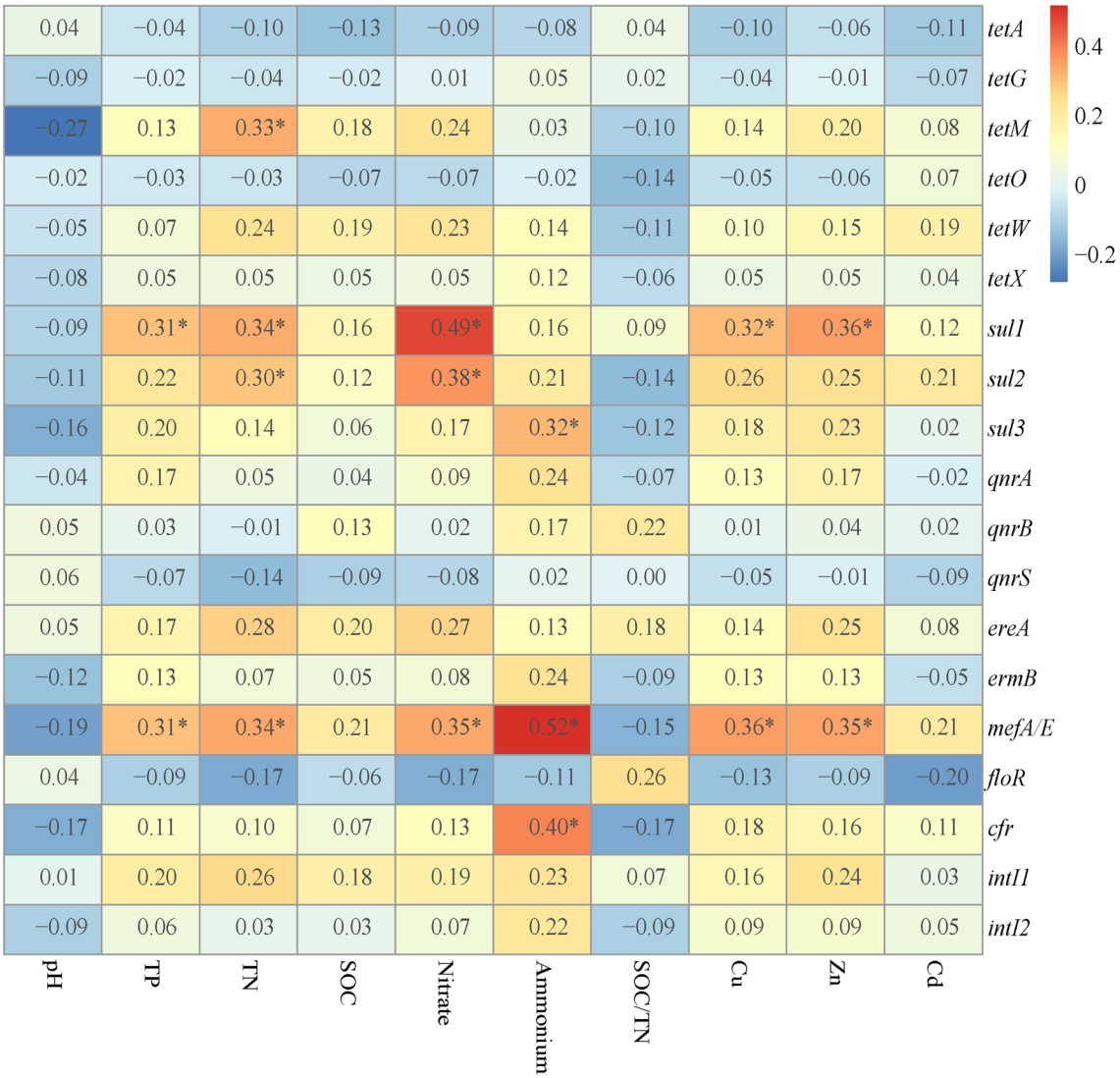

Fig. 2 Heatmap of correlation between ARGs and environmental factors. *, significant correlation with Pearson coefficient of $p < 0.05$

with the HGT of the MGEs that carried them [15]. The normalized abundance of *floR* significantly decreased, while that of *mefA/E* and *cfr* significantly increased in summer ($p < 0.05$). The *floR* and *mefA/E* genes encode efflux pumps, while *cfr* catalyzes ribosomal methylation [16, 17]. In previous studies, these three genes were also frequently found associated with MGEs, such as plasmids and transposons, suggesting MGEs were most likely involved in their variation [16, 18, 19]. In addition, *intI2* showed an increasing trend from winter to the contiguous summer, this could facilitate the transmission of the linked ARGs, for integrons permit tandem integration and expression of mobile cassettes coding for ARGs [20].

In addition, three RPP genes—*tetM*, *tetO* and *tetW* were found significantly increased in soil adjacent to swine feedlots in Beijing, compared with the nearby CK soil. Previously, these three *tet* genes were found to be prevalent in swine intestinal microbiota and swine manure, indicating their close linkage with animal manure [21–23]. It was found that the distributing patterns of the three *tet* genes were independent of seasons, and there were also consistent distributing patterns of these genes between the adjacent soil and wastewater, implicating wastewater as the primary polluting source. These genes are commonly carried on MGEs that facilitate transfers between different bacterial taxa in an ecosystem [24]. For example, *tetM*, *tetO* and *tetW* are

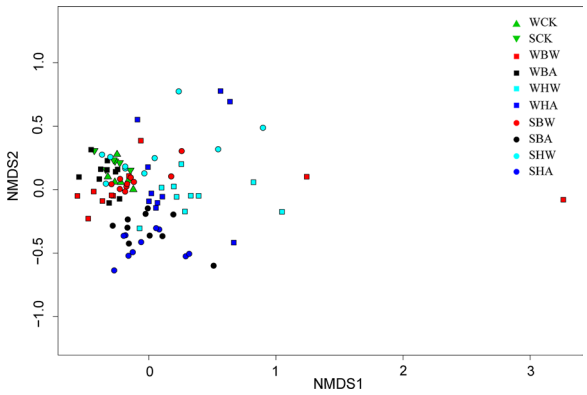

Fig. 3 Non-metric multidimensional scaling (NMDS) analysis (Bray–Curtis dissimilarity) of bacterial community in winter and summer at the genus level. The initial letters in the abbreviations 'W' and 'S' represent winter and summer, respectively. The latter two capital letters, 'CK' represent control soil without a history of livestock farming or manure application; 'BW', working feedlot in Beijing; 'BA', abandoned feedlot in Beijing; 'HW', working feedlot in Hefei; 'HA', abandoned feedlot in Hefei

present in numerous genera due to HGT [14]. Especially, *tetM* is commonly carried on Tn*916*-like transposons which makes it possess the broadest host range among all tetracycline resistance genes [18, 25]. Moreover, RPP *tet* genes have a wider spectrum of resistance to tetracyclines than most of *tet* efflux pump genes [26]. These facts highlighted the environmental risks of ARG pollution induced by swine manure into soil, since RPP *tet* genes are prevalent in swine manure [21, 27].

Overall, there were no significant differences in the ARG composition between topsoil and subsoil (Fig. 1 and Additional file 1: Table S3). Samples taken at greater depths might be needed to distinguish ARG composition in soils subjected to long term ARG pollution by swine wastewaters. Moreover, abandonment for 1.5 years did not alleviate ARG transfer risk compared with being abandoned for 1 year, and to the contrary, total ARG normalized abundance increased from winter to summer in this present study. These data indicated that a time span of 1.5 years was far from enough to recover a background ARG abundance. Therefore, a long-term survey is essential to study the recovery situation in soil near these abandoned feedlots.

The positive correlations between ARGs (e.g., *mefA/E*, *cfr*, *sul1*) and environmental factors (e.g., NH_4^+–N, TP, TN, NH_4^+–N, Cu and Zn) in Fig. 2 indicated that these factors should be concerned, especially since swine manure usually contained these components in abundance.

Bacterial community variation in winter and summer

Combining the results of NMDS analysis in Fig. 3 at the genus level and comparison of relative abundance of bacterial community between two seasons at the phylum level, our present study indicated that seasonal change did not substantially alter the bacterial community structure from winter to summer, even though the weather characteristics were quite different (Table 1). The reason might be that the complex soil colloid offered a relative stable environment for bacterial community. However, further studies are needed in future to verify this speculation as previous studies seldom focus on the bacterial community alteration over season. Among the seven phyla that did show a significant alteration in relative abundance between winter and summer (Table 2), Actinobacteria decreased, while Acidobacteria increased for Beijing but not for Hefei. This discrepancy might be the result of climatic differences based on geography. Beijing has four distinct seasons with larger temperature fluctuations between winter and summer and is much drier.

Table 2 Relative abundance of dominant phyla in winter and summer soil samples

Group	Actinobacteria	Acidobacteria	Firmicutes	Gemmatimonadetes	Nitrospirae	Planctomycetes	Saccharibacteria
WCK	25.6 ± 4.1%*	15.7 ± 1.7%*	0.5 ± 0.1%	3.4 ± 0.5%	1.3 ± 0.3%*	2.5 ± 0.5%	0.4 ± 0.1%
SCK	9.9 ± 1.1%*	31.1 ± 2.5%*	0.4 ± 0.1%	3.2 ± 0.4%	2.5 ± 0.3%*	3.3 ± 0.3%	0.7 ± 0.2%
WBW	27.8 ± 3.8%*	7.6 ± 1.8%*	8.4 ± 2.3%*	3.4 ± 0.5%	0.8 ± 0.2%*	0.8 ± 0.2%*	0.9 ± 0.3%
SBW	16.7 ± 3.0%*	34.1 ± 4.3%*	1.5 ± 0.2%*	4.8 ± 0.5%	2.5 ± 0.3%*	1.5 ± 0.1%*	0.7 ± 0.1%
WBA	21.3 ± 2.5%*	13.9 ± 1.6%	3.3 ± 1.0%	5.0 ± 0.4%	1.7 ± 0.2%	1.4 ± 0.1%	0.5 ± 0.1%*
SBA	14.7 ± 1.5%*	21.1 ± 3.4%	6.9 ± 2.8%	3.5 ± 0.5%	1.4 ± 0.4%	1.4 ± 0.2%	1.6 ± 0.3%*
WHW	13.3 ± 1.3%	8.8 ± 1.4%	3.6 ± 0.7%	5.9 ± 0.5%	2.0 ± 0.6%	0.4 ± 0.1%*	1.1 ± 0.2%*
SHW	14.6 ± 2.6%	11.6 ± 2.1%	3.6 ± 0.9%	5.4 ± 0.9%	2.2 ± 0.8%	0.8 ± 0.2%*	2.3 ± 0.4%*
WHA	17.2 ± 1.7%	13.8 ± 1.6%	1.7 ± 0.4%	5.5 ± 0.4%*	2.6 ± 0.4%	0.8 ± 0.1%	0.6 ± 0.1%*
SHA	24.1 ± 2.8%	13.5 ± 3.0%	3.4 ± 0.7%	4.0 ± 0.2%*	3.6 ± 0.8%	1.0 ± 0.1%	1.3 ± 0.2%*

Numbers are shown as mean ± standard error

*Indicates a significance difference in abundance between winter and summer ($p < 0.05$)

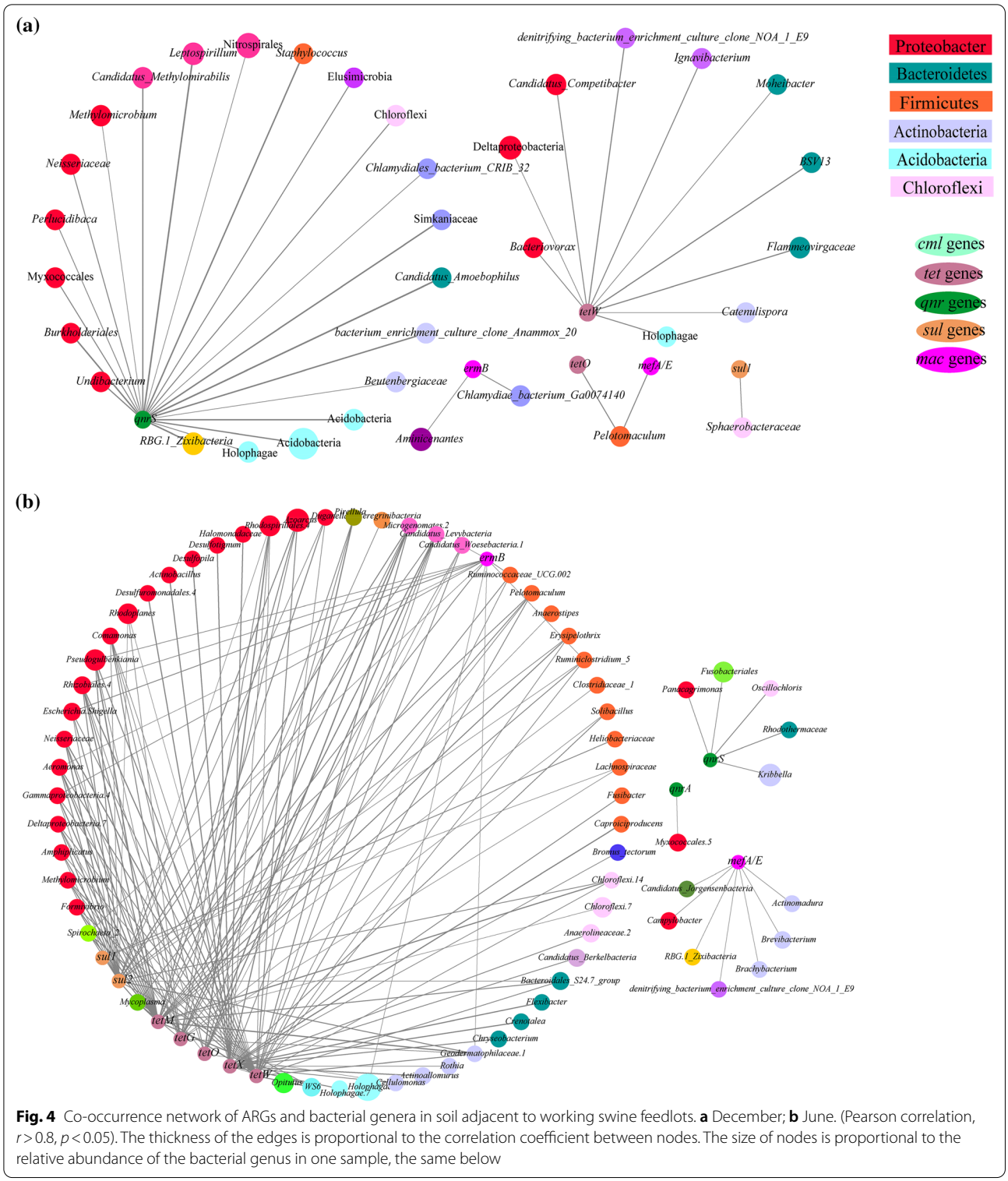

Fig. 4 Co-occurrence network of ARGs and bacterial genera in soil adjacent to working swine feedlots. **a** December; **b** June. (Pearson correlation, $r > 0.8$, $p < 0.05$). The thickness of the edges is proportional to the correlation coefficient between nodes. The size of nodes is proportional to the relative abundance of the bacterial genus in one sample, the same below

These factors would exert stress on the soil bacterial community resulting in a competitive advantage for Actinobacteria in winter, since they possessed high GC content and could robustly resist these stressors. However, Hefei in southern China experienced fewer temperature extremes between winter and summer and received more rainfall than Beijing. For example, the average daily temperatures in December and June in Beijing are −6–3

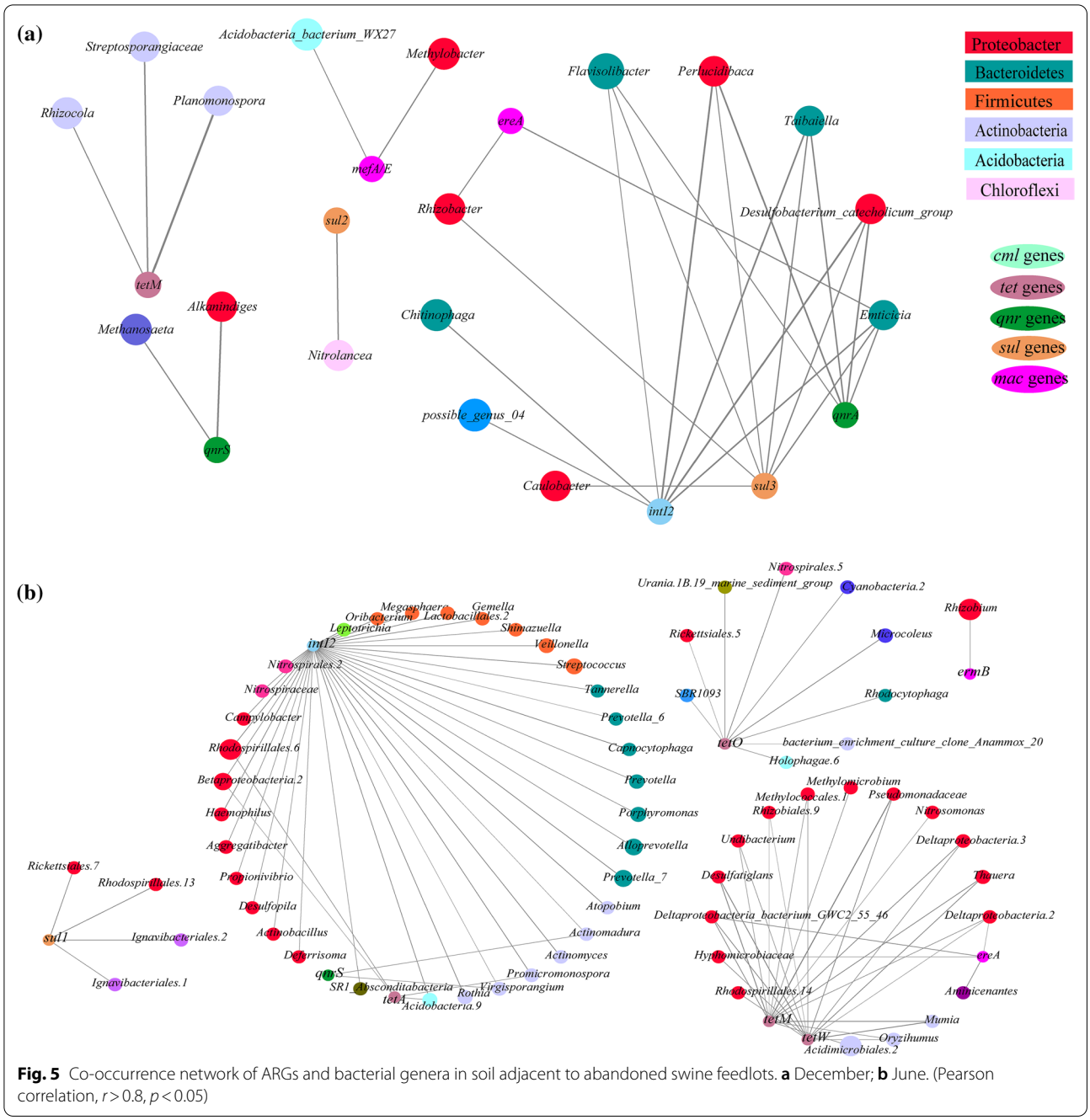

Fig. 5 Co-occurrence network of ARGs and bacterial genera in soil adjacent to abandoned swine feedlots. **a** December; **b** June. (Pearson correlation, $r > 0.8$, $p < 0.05$)

and 19–30 °C, while in Hefei, they are 1–9 and 21–29 °C, respectively (Table 1). The average rainfall from December to May in Beijing was only 74.1 mm, while in Hefei, it was 362 mm. In Beijing, the relative humidity varied from 44 to 77% compared with Hefei at 73–80% (China Meteorological Bureau, available at https://www.weather-atlas.com/). The soil types also differed, and together these components were reflected in the combined Actinobacteria population trend. Moreover, unlike other bacterial phyla in soil, Actinobacteria relative abundance was negatively correlated with soil moisture. In contrast, Acidobacteria populations were positively correlated to soil moisture content [28]. Actinobacteria were drought resistant and could proliferate in arid soil conditions [29]. These variables were probably major contributors to the seasonal variation of Actinobacteria and Acidobacteria in Beijing, because the rainfall is extremely concentrated in summer and winter is a dry season there (China

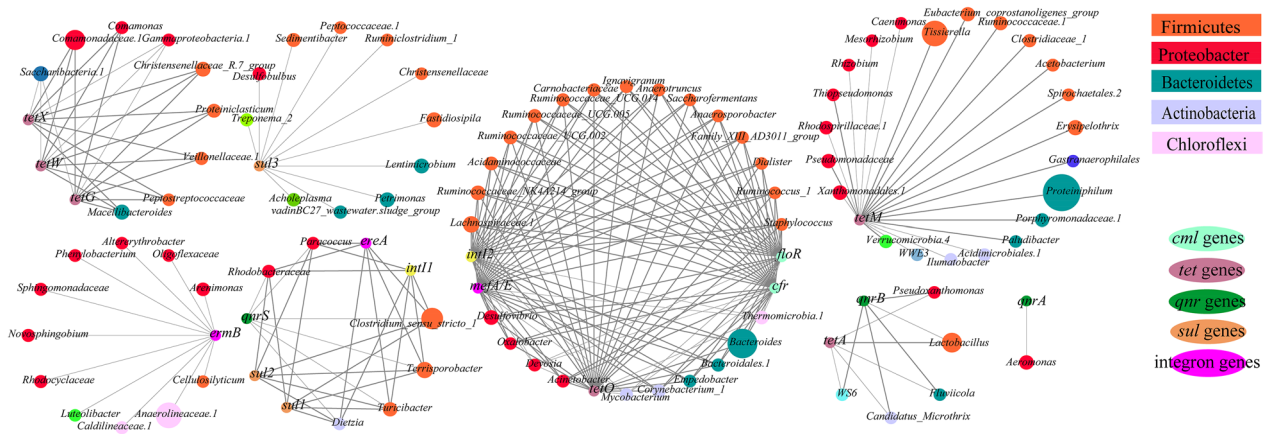

Fig. 6 Co-occurrence network of ARGs and bacterial genera in wastewater and sediment of swine feedlots. (Spearman correlation, $\rho > 0.8$, $p < 0.05$)

Meteorological Bureau). However, the lack of a gradient and continuous alteration of soil moisture data as a variable in the present study made it impossible to determine whether soil moisture directly altered bacterial phyla. Therefore, further experiments are needed to address this question.

Moreover, ref. [30] concluded that Firmicutes were the predominant phylum in swine intestinal microflora, and the long-term exposure of swine manure to soil is linked to Firmicutes abundance. In our study, the persistence of Firmicutes in soils adjacent to swine feedlots in Beijing suggested that this is a result of long-term manure pollution from the adjacent wastewater. The above characteristics of Actinobacteria and Firmicutes were also most likely to contribute to ARG profiles in the corresponding soil sites, because Actinobacteria and Firmicutes were among the major phyla that host ARGs and caused their transmission [31, 32].

Co-occurrence patterns of bacterial taxa with ARGs

In this present study, there were more potential genera hosts on the network of the working swine feedlots than those of the abandoned swine feedlots in the same season. The possible reason for this might be that the HGT level was higher in summer than in winter both in abandoned and working feedlots, as several studies have shown that HGT was highly temperature-dependent [33–36]. There were also studies showed that higher ambient temperatures favor HGT between bacterial taxa, and transfer frequencies have been linked to specific temperatures [35, 37–39]. For example, the transformation level of *Pseudomonas stutzeri* at 12 °C was 0.7% of that at 30 °C [37]. An increase in temperature across different regions was conceived to facilitate the HGT of antibiotic resistance level for common pathogens [38]. However, this assumption could not serve as a conclusion for sure in the current scenario, because HGT level could not be speculated merely based on co-occurrence network analysis. More studies are needed to infer the correlation between HGT level and season in soil microbiota.

Overall, the potential ARG hosts at the phylum level in the soil adjacent to swine feedlots in both seasons were quite similar (Additional file 1: Fig. S5). They were primarily composed of Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria and Acidobacteria, which are in accordance with previous studies [4, 32]. In addition, the frequency of phyla presented on the above networks was not proportional to their relative abundance in our soil, wastewater, or sediment showed in Additional file 1: Fig. S4. More specifically, Firmicutes possessed the most nodes in summer, underlining the significant input of antimicrobial-resistant bacterial from swine manure to the wastewater. Because Firmicutes were proved to be the most dominant phylum in the swine feces (> 90% in adult swine) [40]. The above result was consistent with the results of wastewater and sediment samples in winter from our previous study [6]. The above results suggested that the host taxa were constrained to a group of particular phyla that included Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, Acidobacteria and Cloroflexi. Proteobacteria, Firmicutes and Actinobacteria had also been implicated as main ARG carriers and played important roles in disseminating ARGs in previous studies, [41–43]. This implied some barriers exist in horizontally transferring ARGs to other phyla within soil microbiota. The mechanisms that might act as these barriers included host restriction–modification systems, plasmid incompatibly, and the lack of integrative conjugative element target sites [18].

Conclusions

Generally, no significant differences in ARG and bacterial taxa variations were found based on soil depth within 50 cm. The abundance of ARGs was not significantly reduced after 1.5 years compared with 1 year of abandonment. Overall, our results indicated that the response of ARGs to season when normalized to 16S rRNA gene in soil microbiota was subtype and site specific. The co-occurrence network analysis suggested that the HGT level was likely increased from winter to summer. Potential ARG host taxa in adjacent soil differed from those of wastewater samples; Proteobacteria were the most frequent ARG hosts in the former and Firmicutes in the latter. A long-term study is still necessary to understand the transmission risk of ARGs around the abandoned swine feedlots.

Supplementary Information

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Additional file 1. Text 1. PCR and bioinformatics analysis for bacterial community composition. **Text 2**. Amplification procedure for qPCR. **Table S1**. Operational history of the investigated swine feedlots. **Table S2**. Primers used in this study. **Table S3**. Normalized abundance of ARGs in soil samples. **Fig. S1**. The sampling sites. **Fig. S2**. Bar chart of total normalized ARG abundance at different sites, seasons, as well as different soil depths. **Fig. S3**. α diversity index of winter and summer samples. **Fig. S4**. Bar chart of bacterial relative abundance at the phylum level in winter and summer.

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Authors' contributions

NL: investigation, formal analysis, data curation, writing—original draft. JC: Conceptualization, Writing - review & editing. CL: data curation, preparation. BL: investigation, methodology. CZ: conceptualization, methodology, supervision. HL: writing—reviewing and editing, validation, formal analysis, data curation, funding acquisition. All authors read and approved the final manuscript.

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Availability of data and materials

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

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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