Silicon dioxide nanoparticles have contrasting effects on the temporal dynamics of sulfonamide and $\beta$-lactam resistance genes in soils amended with antibiotics

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Abstract

Nanoparticles (NPs) and antibiotic resistant genes (ARGs), as emerging environmental contaminants, have been reported to be accumulated in the soil environment. The use of NPs have raised increasing concerns about their environmental impacts, but the combined effect of NPs and antibiotics on ARGs remains less understood. Here, we established laboratory microcosms to explore the impacts of different concentrations of SiO₂ NPs on $\beta$-lactam and sulfonamide resistance genes in soils amended with $\beta$-lactam or sulfonamide. Illumina sequencing and quantitative PCR revealed that the addition of NPs increased the bacterial community diversity but had no significant effects on the bacterial abundance. Moreover, NPs and sulfonamide jointly increased the abundances of sulfonamide resistance genes, while the exposure of NPs and $\beta$-lactam decreased $\beta$-lactam resistance genes. The detected ARGs were associated closely with two mobile genetic elements (MGEs, the tnpA and intI1 genes), indicating that MGEs may contribute to the dissemination of ARGs. Correlation analysis indicated the shifts in potential bacterial hosts and the frequency of horizontal gene transfer were important factors explaining the patterns of ARGs. Furthermore, structural equation models indicated that NPs exposure decreased the abundances of $\beta$-lactam resistance genes by driving changes in bacterial community and MGEs, whereas the increased abundances of sulfonamide resistance genes were mainly associated with the bacterial community, diversity and MGEs mediated by NPs and antibiotics. These results suggested that the combined effects of NPs and antibiotics on soil bacterial resistance were different due to the types of antibiotics.

1. Introduction

The presence of nanoparticles (NPs), an emerging environmental contaminant, has achieved a pivotal status in human life, due to their extensive applications in commodities, cosmetics, textiles, etc (Lowry et al 2012). The global nanotechnology market is predicted to increase from $39.2 billion in 2016 to $90.5 billion in 2021, indicating a considerable demand for NPs (Qi et al 2018). A wide variety of NPs, such as magnetic NPs, metal NPs, and plastic NPs, are becoming a steadily increasing environmental threat due to their widespread use. Previous studies have shown that NPs, used as greenhouse materials, soil conditioners and fertilizers, have accumulated in agricultural soils (Tomasz et al 2011, Lowry et al 2012, Song et al 2018). NPs are now prevalent in considerable concentrations in the aquatic and soil systems, and because of their durability, slow degradation, and ability to release and absorb organic pollutants, NPs pollution has become an environmental issue of global concern (Rios et al 2007, Lusher et al 2015).

As pharmaceutical compounds, antibiotics have been used widely in hospitals, agriculture, livestock and aquaculture for a long time (Vera and Lúcia, 2011, Le et al 2018, Tran et al 2018). It is believed that...
continuous, improper and high usage of antibiotics have contributed to the evolution and development of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in various receiving environments (Brown et al. 2006, Laxminarayan et al. 2013, Zhang et al. 2017). Antibiotics, different from other classes of drugs, are poorly absorbed by animals, with around 30%–90% of doses excreted to the environment through manure in the form of the original antibiotics (Thames et al. 2012, Zhou et al. 2013, Zhan and Xiao 2017, Berendsen et al. 2018). The long-term selection pressure imposed by antibiotics can contribute to the enrichment and dispersal of ARGs in the environment (Hu et al. 2016). ARGs in the environment are subjected to high mobility between different microorganisms catalyzed by mobile genetic elements (MGEs), such as plasmids, integrons, and transposons (von Wintersdorff et al. 2016). Therefore, environmental ARGs may be transferred to human pathogens and bacteria, which might have a detrimental effect on human health (Nikolina et al. 2014). Soil has been recognized as an important reservoir of ARGs, which poses a great threat to the environment and public health (Pruden et al. 2006, Forsberg and Dantas 2012). Therefore, the fates of ARGs in soils have attracted substantial attention recently.

NPs typically show distinct chemical and physical properties, and due to their inherent reactivity with other contaminants, NPs may serve as a carrier and co-exist with other contaminants resulting in long-term environmental and health risks (Azizi et al. 2016, Jean-net et al. 2016, Wang et al. 2016). Various metal oxide NPs were found to accumulate in sludge and co-exist with ARGs, indicating that NPs may be a stimulant to microbe, affecting the propagation of ARGs (Tou et al. 2017). TiO₂ and ZnO NPs have been observed to enhance the prevalence of ARGs in soil microbial communities through the mechanisms of co-selection and horizontal gene transfer. Some NPs may also affect the spread of ARGs by affecting bacteriophage associated with horizontal gene transfer. For example, Ag and ZnO NPs enhance the internalization of bacteriophage MS2, which is responsible for horizontal gene transfer (Hajipour et al. 2012). CuO and ZnO NPs can lead to the spread of ARB in activated sludge and promote the horizontal transfer of ARGs (Haining et al. 2019). With the increasing usage of antibiotics and NPs, their concentrations and co-existence in soil as well as potential for environmental impact will gradually rise. It is widely known that the presence of antibiotics in soil will enhance the propagation of ARB and increase the transport of ARGs, but whether novel contaminant NPs affect the influence of antibiotics on ARGs remains known. We have little understanding of whether the exposure of soil microbial communities to NPs-antibiotics addition can increase the occurrence and dissemination of ARGs. Evidences from previous studies demonstrated the heavy metals promoted the emergence of metal resistance, and participated in the co-selection processes for ARGs (Zhou et al. 2016, Zhang et al. 2018). To avoid the influence of metal on bacterial community and ARGs, the non-metallic nanomaterials were chosen in this study. SiO₂ NPs have been widely used in various fields of human life. For example, in agriculture, SiO₂ NPs are used to make agricultural seed treatment agents, and also be used in herbicides and pesticides. Therefore, SiO₂ NPs may accumulate in agricultural soils, and have a joint effect with the antibiotics on soil microorganisms.

This study was designed to investigate the influence of SiO₂ NPs and two types of antibiotics (β-lactam and sulfonamide) on the bacterial community, ARGs and MGEs. Soils containing β-lactam or sulfonamide were exposed to different doses of nano-SiO₂ in laboratory microcosms for 14 d. The effects of NPs-antibiotics on the abundance of bacteria, ARGs and MGEs were assessed through quantitative PCR (qPCR). The effects of NPs-antibiotics on the bacterial community composition and diversity were evaluated using Illumina MiSeq sequencing. Finally, the direct and indirect relationships among the NPs concentrations, the bacterial community, MGEs, and ARGs were assessed by structural equation models (SEMs). We tested the following hypotheses: (1) The NPs-antibiotics exposure will affect the soil bacterial community compositions; and (2) The existence of NPs-antibiotics will promote the occurrence and transportation of ARGs in soils.

2. Materials and methods

2.1. Preparation of antibiotics, NPs and soil

NPs (99.5%) used in this study were purchased from Alfa Aesar (Shanghai, China), and the detailed information is provided in table S1, available online at stacks.iop.org/ERSL/15/034001/mmedia. The concentrations of 1.25, 2.5 and 5 g L⁻¹ stock solution of NPs were prepared by adding 0.125, 0.25 and 0.5 g of NPs to 0.1 L of distilled water (pH 7.0), respectively. The NPs were sonicated for 1 h (25 °C, 250 W, 40 kHz) each time before dilution. The soil for the experiment was collected from Pangguangou Nature Reserve in Shanxi, China, which was known to have minimal anthropogenic disturbance. Six soil sampling plots (20 m × 30 m) were established at Pangguangou Nature Reserve in Liyang Mountain. After removing surface vegetation and coverings, soils at 0–10 cm were collected and mixed for each plot. Soils were sieved (<2 mm) to remove soil impurities, hand-mixed and stored in plastic bags. Part of each soil sample was stored at 4 °C until analysis, and the other part was air-dried and sieved to establish a laboratory microcosm, the physicochemical characterization of the soil is shown in table S2. β-lactam (ampicillin sodium, ≥99.5%) and sulfonamide (≥99.5%) were purchased from Kelong (Sichuan, China), because these
antibiotics are widely used in agriculture and animal husbandry, and β-lactam and sulfonamide resistance genes have been frequently detected in global and Chinese farmlands (Sarmah et al. 2006, Younghoe et al. 2008, Satoru and Phuong 2012). The concentrations of NPs used in this study were comparable to those used in previous studies (Qi et al 2018, Haining et al 2019).

2.2. Laboratory microcosm incubation

We established a laboratory microcosm to explore the combined effect of β-lactam (30 mg kg⁻¹) or sulfonamide (50 mg kg⁻¹) with SiO₂ NPs on the changes of ARGs. Each microcosm consisted of 250 g sieved soil (dry weight equivalent) in a 1 l sterile plastic bottle. Soil water content was maintained at 60% of the soil water-holding capacity by adding sterilized water to each microcosm. NPs-β-lactam and NPs-sulfonamide were added to each microcosm and thoroughly mixed in the soil matrix. For each antibiotic type, the SiO₂ NPs exposure doses were 25, 50, 100 mg kg⁻¹ soil. Soil without SiO₂ NPs was used as the control. The treatments with different concentrations of NPs for β-lactam or sulfonamide amended soils were denoted as B0, B25, B50 and B100 and H0, H25, H50 and H100, respectively. Microcosms were placed in a constant incubator at 25 °C. Three replicate microcosms of each NPs treatment were destructively harvested on day 14, as β-lactams are rapidly degraded in soil (Tran et al. 2016, Tran et al. 2019). All soil samples were stored at −80 °C for further analysis.

2.3. DNA extraction and qPCR analysis of ARGs

Genomic DNA was extracted from 0.25 g soil using TIANNAMP Soil DNA Kit (TIANGEN, China) according to the manufacturer’s protocol. The concentration and quality of the extracted DNA were determined using Infinite 200 PRO (TECAN, Sweden). The extracted DNA was stored at −20 °C for qPCR analysis and high-through sequencing.

We used the qPCR technique to quantify the abundance of 12 ARGs, including 10 β-lactam resistance genes (ampC3, ampC2, blaOXA-10, blaPER, mecA, osa-2, blaZ, fox-5, blaPSE, and blaTEM) and 2 sulfonamide resistance genes (sul I and sul II). Additionally, the transposon-transposase gene (tnpA) and class 1 integrase-integrase gene (intI1) were quantified to investigate the horizontal gene transfer potential of ARGs. The PCR reactions were as follows: 3 min of denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min In order to normalize the abundance of ARGs and MGEs in each sample, the bacterial 16 S rRNA gene was also quantified. All the qPCR analysis was performed on ABI StepOnePlus™ (Thermo, USA). The 10 μl reaction mixture consisted of 5 μl of TB Green Premix Ex Taq II (Tli RNaseH Plus, Takara), 0.4 μl of each primer, 0.2 μl ROX Reference Dye, 1 μl template DNA (around 10 ng μl⁻¹), and 3 μl H₂O. Melting curve analysis was performed at the end of each qPCR run to check the specificity of the amplicons (table S3).

2.4. Illumina MiSeq sequencing

The V3–V4 hypervariable regions of the bacterial 16 S rRNA gene were amplified with primers 338 F and 806 R using a thermocycler PCR system (GeneAmp 9700, ABI, USA). PCR reactions were carried out using the following reaction mixture: 4 μl of 5× FastPfu Buffer, 2 μl of 2.5 mM dNTPs, 0.8 μl of each primer (5 μM), 0.4 μl of FastPfu Polymerase and 10 ng of template DNA. The PCR conditions used were 3 min at 95 °C, 27 cycles of 30 s at 95 °C, 30 s at 55 °C, and 45 s at 72 °C, and at 72 °C for 10 min PCR products were separated through 1% agarose gel electrophoresis and purified using Wizard™ SV Gel and PCR Clean-Up System (Promega, USA). Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio BioPharm Technology Co. Ltd (Shanghai, China).

2.5. Processing of sequencing data

Raw fastq files were quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (i) Reads were truncated at any site with an average quality score <20 over a 50 bp sliding window; (ii) Sequences with >10 bp overlap were merged with mismatch no more than 2 bp; (iii) Sequences of each sample were separated according to barcodes (exactly matching) and primers (<2 nucleotide mismatching), and reads containing ambiguous bases were removed. Operational taxonomic units (OTUs) were clustered at 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) with a novel ‘greedy’ algorithm that performed chimera filtering and OTU clustering simultaneously. The taxonomy of each 16 S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16 S rRNA database using the confidence threshold of 70%.

2.6. Statistical analysis

The bacterial diversity was characterized for each treatment by calculating the Shannon index, Simpson index, and richness estimator indices Chao1 and abundance-based coverage estimation (ACE) using the ‘Vegan’ package in the R software. One-way analysis of variance (ANOVA) and the Tukey’s HSD test were used to examine the significant effects of the different treatments on microbial diversity indices and abundance of ARGs, MGEs and bacterial 16 S rRNA gene. Pearson’s correlation analysis was used to explore the relationships among β-lactam and sulfonamide resistance genes, MGEs, as well as the bacterial community. SEMs were constructed to explore the
3. Results

3.1. Effect of NPs exposure treatments on the bacterial community

Different concentrations of NPs exposure treatments all induced significant ($P < 0.05$) increases in the Shannon, Simpson, ACE and Chao1 indexes of the bacterial community in the β-lactam and sulfonamide amended soils (figure 1). The diversity indexes of the bacterial community all increased significantly in soils of the NPs exposure treatments ($P < 0.05$). The Shannon and Simpson indexes were significantly different ($P < 0.05$) in the B25, B50 and B100 treatments, and the H25, H50 and H100 treatments (figures 1(a), (b), (e), (f)). However, no significant difference ($P > 0.05$) was observed in the Chao1 index across the treatments in the B25, B50 and B100 treatments, or the H25, H50 and H100 treatments (figures 1(c), (g)).

The β-diversity of bacterial communities indicated dramatic variations among the four treatments (figure 2(a)). The significance of this clustering was tested through ANOSIM with 999 permutations, which revealed that the bacterial community structures among the four treatments differed significantly in soils with antibiotics (ANOSIM, β-lactam: $r = 0.9815$, $P = 0.001$; sulfonamide: $r = 0.9045$, $P = 0.001$). The bacterial community compositions were profiled according to their relative abundance at the phylum level (figure 2(b)). In β-lactam-amended soils, approximately 99.84% of the bacterial sequences were classified into 25 different phyla across all samples. Proteobacteria, Actinobacterial, Chloroflexi, Firmicutes, and Bacteroidetes were the dominant phyla in the four treatments and together accounted for >80% of the total bacterial sequences. The phyla Acidobacteria, Cyanobacteria, Gemmatimonadetes, Verrucomicrobia, Planctomycetes, and Armamatimonadetes were also present in the samples with lower relative abundances (<5%). In sulfonamide-amended soils, Proteobacteria was the most dominant phylum, and Bacteroidacteria was another dominant phylum. The phyla Chloroflexi, Bacteroidetes, Firmicutes, Gemmatimonadetes, Acidobacteria, Cyanobacteria, Planctomycetes, Verrucomicrobia, and Armamatimonadetes were also widely distributed in all the soil samples.

Among these bacterial phyla in β-lactam-amended soils, the relative abundances of Firmicutes, Actinobacteria, Acidobacteria and Gemmatimonadetes increased with the concentrations of NPs, while Bacteroidetes, Cyanobacteria, Verrucomicrobia, Planctomycetes, and Armamitimonadetes decreased in their relative abundances (figure 2(a); table S4). Similar with the β-lactam-amended soils, the abundances of Actinobacteria, Acidobacteria, Chloroflexi and Gemmatimonadetes increased in the NPs exposure soils amended with sulfonamide, while Bacteroidetes decreased (figure 2(b)).

Contrary to the several bacterial phyla in β-lactam amended soils, Armamitimonadetes, Planctomycetes, Cyanobacteria and Verrucomicrobia increased in NPs exposure soils amended with sulfonamide (figure 2(b)). The changes of bacterial communities were analyzed at the genus level (figure 3). A total of 55 genera were identified, and the relative abundances of 29 and 43 genera were increased by NPs exposure in β-lactam- and sulfonamide-amended soils, respectively.

3.2. Effects of NPs exposure on the abundance of bacteria, ARGs and MGEs

One-way ANOVA and Tukey’s HSD test were used to compare the abundance of 16S rRNA gene, ARGs and MGEs across the four treatments (figure 4). The bacterial abundance had no significant difference across the four treatments in β-lactam and sulfonamide amended soils (figures 4(a), (d)). In β-lactam amended soils, the tnpA gene showed significant differences in NPs exposure soils, and its relative abundance decreased with the increasing concentration of NPs ($P < 0.05$; figure 4(b)). The intI1 gene significantly increased in sulfonamide amended soils, while decreased in NPs exposure soils amended with β-lactam ($P < 0.05$; figures 4(c), (f)).

The changes of ARGs in both β-lactam and sulfonamide amended soils are shown in figure 5. In β-lactam-amended soils, the relative abundance of total ARGs was about 0.0012 without NPs addition, while decreased to 0.00095, 0.00065 and 0.00063 in the B25, B50 and B100 treatments, respectively (figure 5(a)). As for the β-lactam resistance genes, the blaTEM and blaPSE genes were dominant among the four treatments (figure 5(a); table S5). The different concentrations of NPs exposure resulted in varied changes in different ARGs. The relative abundance of the blaTEM gene was the lowest in the B50 treatment, whereas the blaPSE gene was the lowest in the B100 treatment. Furthermore, the oxa-2 and ampC3 genes were not detected in the B50 and B100 treatments, respectively (figure 5(a)). The total relative abundance of sulfonamide resistance genes increased under NPs exposure, and the sulII gene increased significantly in the B25, B50 and B100 treatments (figure 5(b); table S5).

3.3. Correlations among the bacterial community, ARGs and MGEs

Majority of the bacterial phyla mentioned above exhibited a significant correlation with β-lactam resistance genes ($P < 0.05$; table 1). Proteobacteria
was significantly and positively correlated with the \textit{ampC2}, \textit{blaOXA-10}, \textit{blaPER}, \textit{blaZ}, and \textit{oxa-2} genes ($P < 0.05$) and had no significant correlation with other ARGs (table 1). Acidobacteria was positively correlated with the \textit{ampC2}, \textit{blaPER}, \textit{blaPSE} and \textit{blaTEM} genes ($P < 0.05$; table 1). Interestingly, the \textit{blaPSE} and \textit{blaTEM} genes had significantly positive correlations with the bacterial phyla that decreased with increasing concentrations of NPs, while showed negative correlations with the bacterial phyla that increased with increasing concentrations of NPs ($P < 0.05$; table 1). With regard to the correlation of sulfonamide resistance genes and bacteria, both \textit{sulI} and \textit{sulII} genes had significantly positive correlations with Actinobacteria, Chloroflexi and Gemmatimonadetes, while were negatively correlated with Proteobacteria ($P < 0.01$; table 1). The \textit{sulI} and \textit{sulII} genes were negatively correlated with the \textit{tnpA} gene ($P < 0.01$), while showed positive correlation with the \textit{intI1} gene ($P < 0.01$; table 2). Meanwhile, the \textit{blaPSE} and \textit{blaTEM} genes were significantly and positively correlated with the \textit{tnpA} and \textit{intI1} genes ($P < 0.01$; table 2).

SEM were constructed to explore the direct and indirect effects of NPs and antibiotics on the ARGs in both soils (figure 6). In the $\beta$-lactam-amended soils (figures 6(a), (c)), NPs were found to have significantly influences on bacterial diversity and MGEs ($P < 0.001$).
Furthermore, a significant positive correlation between MGEs and ARGs was observed ($P < 0.05$). Similarly, in the sulfonamide-amended soils (figures 6(b), (d)), NPs were found to have significant effects on bacterial community ($P < 0.001$), bacterial diversity ($P < 0.05$) and MGEs ($P < 0.05$). Positive correlation of MGEs and ARGs was also observed in the sulfonamide amended soil ($P < 0.05$). Overall, NPs, through modifying the abundances of MGEs, were the important drivers of ARGs in this study.

### 3.4. Co-occurrence patterns between ARGs, MGEs and the bacterial community

We conducted the network analysis to explore the relationships among ARGs, MGEs and the bacterial community, and to identify the possible hosts of ARGs in complex environmental scenarios (Li et al 2015).

ARGs/MGEs and the bacterial genera in the same module may co-occur under the same environmental pressure. We assumed that the non-random co-occurrence patterns between ARGs and microbial taxa could indicate the possible host information of ARGs if the ARGs and co-existing microbial taxa possessed a strong and significant positive correlation (Spearman’s $\rho > 0.6$, $P < 0.05$). It was found that various ARGs closely correlated with diverse species, and the relations were clearly classified into two groups: ARGs with the decreased genus and ARGs with the enriched genus (figure 7). Based on the results of the network analysis in $\beta$-lactam amended soils, various ARGs were clearly separated into four modules, and positively or negatively ($P < 0.05$) correlated with various bacterial taxa (figure 7(c)). Three groups of bacterial genera were possible hosts of ARGs based on the co-occurrence patterns.
occurrence results in the β-lactam amended soils (figures 7(a), (b)). For instance, the decreased bacterial genera of *Bradyrhizobium*, *Arthrobacter*, *Mesorhizobium*, *Ensifer*, *Arenimonas* and *Phycicoccus* were suggested to be hosts of genes including *bla*PER, *bla*TEM, *bla*PSE oxa-2, *ampC2*, *trp*A and *irr*I (figures 7(a), (b)). *Shinella* were the potential hosts of *fox*-5 and *mec*A, and 15 enriched genera were the potential host of *bla*Z, *amp*C3, *bla*OXA-10 (figures 7(a), (b)). The resultant network in the sulfonamide amended soils could be separated clearly into three modules (figure 7(c)). *Bacillus*, *Nocardoides*, *Arenimonas*, *Sphingomonas*, *Mesorhizobium*, *Promicromonaspora*, *Blastococcus*, *Streptomyces*, *Bradyrhizobium* and *Methylphaga* were the potential hosts of *sulI*, *sulIII* and *intI*. Interestingly, all these potential hosts increased in NPs-sulfonamide exposure treatments (figures 7(c), (d)).

4. Discussion

4.1. Effect of NPs on the bacterial abundance, diversity and composition

Bacteria can communicate with each other using diffusible molecules known as autoinducers to coordinate population activities, and some environmental stimuli can regulate quorum sensing, affect bacterial communication and change the bacterial community composition (Wagner et al 2006). It was interesting that no significant difference in the total abundance of bacteria exposed to four concentrations of NPs was observed in both β-lactam sulfonamide amended soils in this study (figures 4(a), (d)). The result was in agreement with previous studies, which indicated that both metal oxide NPs and non-metal NPs had no significant effect on bacterial abundance (He et al...
In order to further explore the effects of NPs on the soil bacteria, we assessed the changes in the bacterial diversity and bacterial community composition in the four NPs exposure treatments. The NPs exposure treatments changed the alpha-diversity of soil bacteria and the bacterial diversity indexes increased in NPs exposure soils amended with β-lactam or sulfonamide (figure 1). Some previous studies reported opposite results from our study (Ge et al. 2013). However, some other studies indicated that NPs could potentially stimulate the growth of some bacterial taxa and increase the soil bacterial community diversity, which was similar to our findings (He et al. 2011, Shah et al. 2014, Pallavi et al. 2016). The apparent contradictory results could be partially caused by the inherent toxicity differences among NPs and also possibly by differences in environmental parameters (Heinlaan et al. 2008, Ju-Nam and Lead, 2008, Nowack 2009, Hadduck et al. 2010, Shouls-Wilson et al. 2011). Moreover, owing to their tiny size and stabilization, NPs can be transported easily into the soil and then undergo agglomeration, sorption, desorption, dissolution, and migration differently in different soils because of their core and surface chemistry (He et al. 2007, Klaine et al. 2012). NPs may thereby indirectly affect soil bacteria by changing nutrient availability or the bioavailability of co-occurring contaminants (such as antibiotics) and by changing physical properties of the soil due to their large surface area and high reactivity (Bernhardt et al. 2010). NPs can also affect surface sites that can bind to natural organic compounds and change their bioavailability to soil bacteria affecting the growth of some microbes in the soil (Liu et al. 2008).

In line with previous studies, the bacterial community compositions underwent some changes after an NPs exposure in this study. At the phylum level, during the short exposure period, the relative abundance of Bacteroidetes decreased in both β-lactam and sulfonamide amended soils, whereas the relative abundances of Actinobacteria, Firmicutes and Gemmatimonadetes increased with increasing NPs concentrations (figure 2; table S4). At the genus level, the relative abundances of Sphingobacterium decreased in both β-lactam and sulfonamide amended soils with the increasing concentrations of NPs, whereas the relative abundances of Flavobacterium and Streptomyces increased (figure 3). Meanwhile, the relative abundances of Sphingomonas decreased in NPs-β-lactam amended soils but increased in NPs-sulfonamide soils (figure 3). Among these bacterial genera, Sphingobacterium, Sphingomonas and Streptomyces are known as decomposer of recalcitrant organic pollutants and metabolize biopolymers including protein, cellulose,
chitin and lignocellulose (White et al 1996, Chater et al 2010). The genera Bradyrhizobium and Rizobium, which contain symbiotic N₂-fixing bacteria, declined in NPs-β-lactam amended soils but increased in NPs-sulfonamide soils (figure 3). Thus, both NPs-antibiotics may interfere with symbiotic N₂ fixation in legume crop (Priester et al 2012). The genus Methyloversatilis, which are vital for methane oxidation to CO₂, decreased in both β-lactam and sulfonamide amended soils with the increasing concentrations of NPs (figure 3). Aerobic denitrifying bacteria, Bacillus and Pseudomonas, which can convert nitrate and nitrite into gaseous nitrogen, also changed in both NPs-antibiotics exposure soils (Robertson and Kuenen 1984, Kim et al 2005, 2008). Collectively, it is possible that the differential sensitivity of different bacteria to NPs could be attributed to different mechanism of action for NPs and others co-occurring contaminants. Since various NPs are chronically released into the environment and hard to degrade, they are likely to accumulate in soil and thus cause long-term effects on soil bacteria.

4.2. Effects of SiO₂ NPs exposure on the fate of ARGs

β-lactam and sulfonamide resistance genes showed opposite responses to the exposure of NPs (figure 5). As for the β-lactam resistance genes, significant decreases ($P < 0.05$) in the total abundance of ARGs were observed in NPs exposure soils. However, the treatment of NPs led to a significant increase ($P < 0.05$) in the total abundance of sulfonamide resistance genes in this study. We further analyzed the changes of dominant ARGs to verify the observed effects of NPs with a full insight into the changes of detected ARGs. Different types of ARGs encode resistance to β-lactam and among them are the commonly occurring and clinically important blaTEM gene (Livermore 1998, Jacoby 2005), which was
| Phylum            | ARGs       | MGEs       | Sulfonamide | ARGs       | MGEs       |
|-------------------|------------|------------|-------------|------------|------------|
|                   | $\beta$-lactam |           |             | $\beta$-lactam |           |             |
|                   |            | $\text{ampC3}$ | $\text{ampC2}$ | $\text{blaOXA-10}$ | $\text{blaPER}$ | $\text{blaPSE}$ | $\text{blaTEM}$ | $\text{blaZ}$ | $\text{fox-5}$ | $\text{mecA}$ | $\text{oxa-2}$ | $\text{tnpA}$ | $\text{intI1}$ | $\text{suII}$ | $\text{suII}$ | $\text{intI1}$ | $\text{tnpA}$ |
| Firmicutes        | $-0.25$    | $0.34$     | $0.78^{**}$ | $0.07$     | $-0.87^{**}$ | $-0.35$     | $0.86^{**}$ | $0.3$      | $-0.82^{**}$ | $0.5$    | $-0.79^{**}$ | $-0.72^{**}$ | $-0.13$     | $0.01$     | $-0.34$     | $0.28$     |
| Actinobacteria    | $0.73^{**}$ | $0.15$     | $0.28$     | $-0.13$    | $-0.47$    | $-0.80^{**}$ | $-0.06$    | $-0.69^{*}$ | $-0.81^{**}$ | $-0.34$  | $-0.71^{**}$ | $-0.5$      | $0.68^{**}$ | $0.70^{**}$ | $0.59^{*}$  | $-0.32$    |
| Acidobacteria     | $-0.52$    | $-0.71^{**}$ | $-0.25$ | $-0.85^{*}$ | $-0.76^{**}$ | $-0.56^{*}$ | $0.13$     | $0.56$     | $-0.47$    | $-0.35$  | $-0.66^{*}$ | $-0.82^{**}$ | $0.3$       | $0.31$     | $0.37$      | $0.13$     |
| Gemmatimonadetes  | $-0.45$    | $0.38$     | $0.79^{**}$ | $0.18$     | $-0.76^{*}$ | $-0.1$      | $0.96^{**}$ | $0.49$     | $-0.63^{*}$ | $0.67^{*}$ | $-0.62^{*}$ | $-0.59^{*}$ | $0.79^{**}$ | $0.76^{**}$ | $0.83^{**}$ | $-0.58^{*}$ |
| Bacteroidetes     | $-0.76^{**}$ | $-0.71^{**}$ | $-0.65^{*}$ | $-0.51$    | $-0.49$    | $0.59^{*}$ | $-0.2$     | $0.73^{**}$ | $0.56$    | $-0.19$  | $0.36$      | $0.05$      | $-0.29$     | $-0.2$     | $-0.54$     | $0.036$    |
| Cyanobacteria     | $-0.22$    | $-0.17$    | $-0.55$    | $0.17$     | $0.87^{*}$ | $0.75^{*}$ | $-0.45$    | $0.16$     | $0.99^{*}$ | $-0.02$  | $0.95^{**}$ | $0.82^{**}$ | $0.18$     | $0.17$     | $0.30$      | $0.25$     |
| Verrucomicrobia   | $0.55$     | $-0.36$    | $-0.76^{*}$ | $-0.19$    | $0.71^{**}$ | $0$        | $-0.97^{**}$ | $-0.59^{*}$ | $0.53$    | $-0.72^{*}$ | $0.53$      | $0.37$     | $0.39$     | $0.40$      | $0.053$    |
| Planctomycetes    | $0.28$     | $-0.47$    | $-0.86^{**}$ | $-0.24$    | $0.77^{*}$ | $0.18$     | $-0.94^{**}$ | $-0.33$    | $0.71^{**}$ | $-0.65^{*}$ | $0.66^{*}$ | $0.59^{*}$ | $0.13$     | $0.12$     | $0.28$      | $0.30$     |
| Armatimonadetes   | $0.62^{*}$ | $-0.38$    | $-0.73^{*}$ | $-0.27$    | $0.59^{*}$ | $-0.16$    | $-0.97^{**}$ | $-0.65^{*}$ | $0.38$    | $-0.79^{**}$ | $0.39$      | $0.41$     | $0.46$     | $0.37$      | $0.66^{*}$ | $-0.42$    |
| Proteobacteria    | $-0.04$    | $0.94^{*}$ | $0.89^{**}$ | $0.91^{**}$ | $0.04$     | $0.5$      | $0.745^{**}$ | $0.03$     | $-0.057$  | $0.94^{**}$ | $0.11$      | $0.25$     | $-0.63^{*}$ | $-0.66^{*}$ | $-0.58$    | $0.27$     |
| Chloroflexi       | $0.37$     | $-0.59^{*}$ | $-0.37$    | $-0.79^{*}$ | $-0.49$    | $-0.92^{**}$ | $-0.43$    | $-0.33$    | $-0.59^{*}$ | $-0.82^{**}$ | $-0.67^{*}$ | $-0.64^{*}$ | $0.73^{**}$ | $0.70^{**}$ | $0.79^{**}$ | $-0.49$    |

Note. Values in bold indicate statistical significance. Significance levels are shown at $^* P < 0.05$ and $^{**} P < 0.01$. 

Table 1. The results of Pearson’s correlation analysis of the abundances of bacteria, MGEs and ARGs in $\beta$-lactam amended soils and sulfonamide amended soils, respectively.
Table 2. The results of Pearson's correlation analysis of the abundances of MGEs and ARGs.

|                | ampC3 | ampC2 | BlaOXA-10 | blaPER | blaPSE | blaTEM | blaZ | fox-5 | mecA | oxa-2 | sulI  | sul II |
|----------------|-------|-------|-----------|--------|--------|--------|------|-------|------|-------|-------|-------|
| ampC3          | -0.05 | 0.099 | -0.357    | 0.418  | 0.94   | 0.80   | -0.38| -0.015| 0.97 | 0.127 |       |       |
| ampC2          | 0.2   | 0.324 | -0.19     | 0.59   | 0.96   | 0.78   | -0.349| -0.257| 0.83 | 0.177 | 0.73  | 0.69  |
| BlaOXA-10      | -0.05 | 0.099 | -0.357    | 0.418  | 0.94   | 0.80   | -0.38| -0.015| 0.97 | 0.127 |       |       |
| blaPER         | -0.38 | -0.015| 0.94      | 0.80   | 0.97   | 0.127  |       |       |      |       |       |       |
| blaPSE         | 0.418 | 0.94  | 0.80      | 0.97   | 0.127  |       |       |       |      |       |       |       |
| blaTEM         | 0.94  | 0.80  | 0.97      | 0.127  |       |       |       |       |      |       |       |       |
| blaZ           | -0.38 | -0.015| 0.94      | 0.80   | 0.97   | 0.127  |       |       |      |       |       |       |
| fox-5          | -0.38 | -0.015| 0.94      | 0.80   | 0.97   | 0.127  |       |       |      |       |       |       |
| mecA           | 0.97  | 0.127 | 0.97      | 0.127  |       |       |       |       |      |       |       |       |
| oxa-2          | 0.127 |       | 0.127     |       |       |       |       |       |      |       |       |       |
| sulI           |       |       |           |       |       |       |       |       |      |       | -0.76 | -0.84 |
| sul II         |       |       |           |       |       |       |       |       |      |       | -0.76 | -0.84 |

Note. Values in bold indicate statistical significance. Significance levels are shown at *P < 0.05 and **P < 0.01.
previously shown to be the dominant ampicillin resistance gene (Bailey et al. 2010). The relative abundance of the blaTEM gene was about 0.0001 without NPs treatment in this study. However, when soils were exposed to NPs, the relative abundance of the blaTEM gene decreased (figure 5(a)). The influences of different NPs on the blaTEM gene were not consistent in previous studies, in which the relative abundance of the blaTEM gene decreased in Nd₂O₃ and Gd₂O₃, NPs but increased in La₂O₃ and wastewater samples (Wu et al. 2010, Hsu et al. 2010). Furthermore, the blaOXA-10 gene significantly enriched in NPs exposure soils, while the mecA gene decreased (P < 0.05). Among sulfonamide resistance genes, the sulI and sulII genes were the most dominant ones in all the samples (figure 5(b)). Their abundances increased to 173%–226% and 128%–150% with the exposure of NPs, respectively, which was consistent with the results reported in a previous study (Haining et al. 2019). Collectively, we provide evidence that the NPs exposure contributed to the propagation of ARGS of sulfonamide resistance genes (sulI and sulII genes) but had adverse effects on the most β-lactam resistance genes.

4.3. Effects of SiO₂ NPs on the horizontal gene transfer potential of ARGs

The dissemination and propagation of ARGs could occur via horizontal transfer (HGT) from resistant bacteria to susceptible strains either between different species or across genera, and HGT could potentially enhance the accumulation and persistence of ARGs in soil via MGEs (Gaze et al. 2011, Qing et al. 2015, Hu et al. 2017). Bacteria can use MGEs, especially integrons, to stockpile and express different exogenous resistance genes, which play a crucial role in the global problem of ARGs (Michael et al. 2008, Partridge et al. 2010). Therefore, the abundances of the transposon–transposase gene (tnpA) and class 1 integron–integrase gene (intI1) were determined in this study. We found that the relative abundance of tnpA decreased significantly with the NPs treatments in β-lactam amended soils (P < 0.05; figures 4(b), (e)). Meanwhile, the tnpA gene in sulfonamide amended soils decreased slightly in the B50 and B100 treatments (figures 4(b), (e)). The adverse effects of NPs exposure on the tnpA gene in β-lactam amended soils were higher than that on the tnpA gene in sulfonamide amended soils. The intI1 gene is the one of most prevalent integrons in both soil and wastewater samples (Wu et al. 2010, Hsu et al. 2014). The relative abundance of intI1 in NPs exposure treatments decreased significantly in β-lactam
amended soils while increased in sulfonamide amended soils ($P < 0.05$; figures 4(c), (f)).

Pearson’s correlation analysis results indicated that the trpA gene was correlated positively with blaPSE, blaTEM and mecA, while correlated negatively with sulI and sulII ($P < 0.05$). Notably, blaPER, blaPSE, blaTEM, mecA, sulI and sulII were all correlated positively with intI1 ($P < 0.05$). Some studies have presented evidence for the close relationship between ARGs and MGEs. For example, by analyzing the intI1 and gene cassette of Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa, Sumita and Fukasawa (1996) found that intI1 was closely related to β-lactam antibiotics, and indicated that integrons can be horizontally transmitted in these species. Meanwhile, the transfer of sulfonamide resistance genes via high mobility MGEs, such as intI1, was reported in previous studies. For instance, Di Cesare et al (2016) have reported the co-occurrence between intI1 and sulI, indicating the possibility of transfer of sulfonamide resistance genes mediated by intI1 in the environment. Hence, the increased relative abundance of intI1 by the NPs exposure in this study, indicated that more sulfonamide resistance genes might be transferred among bacteria. The SEM analysis shown that NPs indirectly affected the abundance of ARGs via directly affecting MGEs, in both soils (figure 6). Thus, we suggested that MGEs effected by NPs might be responsible for the changes of ARGs and the hypothesis was supported by previous findings that ZnO NPs indirectly facilitated the increase in ARG via directly affecting MGEs (Shi et al 2019). Moreover, it was reported that NPs could penetrate into the cells, disturb intracellular metabolism and cause DNA damage. Hence, we supposed that the specific properties of NPs might contribute to the changed abundance of MGEs and influence the transportation of ARGs. (Yang et al 2013). Collectively, we assumed that the presence of NPs may contribute to the HGT of sulfonamide resistance genes in the soil environment. The decreases of β-lactam resistance genes (especially blaPSE and blaTEM) in this study, might be attributed to the intI1 gene abundance decreased by the NPs exposure.

4.4. Linkages of the bacterial community, ARGs, and MGEs

Pearson’s correlation analysis revealed the correlations between the bacterial phylum and ARGs/MGEs in this study (tables 1, 2). Bacteroidetes and Cyanobacteria were positively correlated with blaTEM and similar results were also observed in a recent study, in which the Bacteroides was significantly correlated with

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**Figure 7.** Network analysis of the correlations among the bacterial community, ARGs and MGEs. The left figures are the co-occurrence analysis among ARGs, MGEs and the bacterial community (at the genus level) which in soils containing β-lactam (a) and sulfonamide (c). Different colors represent ARGs, MGEs and the bacterial phyla in different modules. The right figures are the network analysis on the correlations among two types of the bacterial phyla, ARGs and MGEs in soils containing β-lactam (b) and sulfonamide (d). A connection stands for a strong (Spearman’s $r > 0.6$) and significant ($P < 0.05$) correlation. The size of each node is proportional to the number of connections (i.e. degree), and a connection represents a significant positive correlation (navy blue) or significant negative correlation (blue, $P < 0.05$).
The significant correlation between Bacteroides, Cyanobacteria and blaTEM (table 1; Zhang et al 2016). The significant correlation between Bacteroides, Cyanobacteria and blaTEM may be associated with characteristics of blaTEM, which often associated with conjugative transposons and also have a very wide host range (Liu and Pop 2009). Meanwhile, the blaPE gene had significant and positive correlations with Verrucomicrobia, Planctomycetes and Armatimonadetes (table 1). As for the linkages of the bacterial phylum and sulfonamide resistance genes, the sulI and sulII genes had significant and positive correlations with Chloroflexi and Gemmatimonadetes, but negatively correlated with Proteobacteria (table 1). Similar correlation was also observed in previous studies (Zhang et al 2016, Haining et al 2019). Based on the network analysis, Bradrhizobium, Arthrobacter, Mesorhizobium, Ensifer, Arenimonas and Phycicoccus could explain the abundance of the decreased β-lactam resistance genes including blaPER, blaTEM, blaPSE oxa-2, ampC2, ttpA and intI1 (figures 7(a), (b)). As for the increased sulfonamide resistance genes, the genus Arenimonas was the potential host bacteria of sulI and sulII (figures 7(c), (d)), which has been demonstrated in a previous study (Liu and Pop 2009). Network analysis further elucidated the primary effects of bacterial community on ARGs by determining their potential host bacteria. Furthermore, SEMs confirmed the important role of MGEs in shaping the patterns of ARGs (figure 6). The SEMs by compiling all the data together showed that bacterial community and bacterial diversity had indirect impacts on the β-lactam ARGs, via directly affecting MGEs (figures 6(a), (c)). Similar results were observed in sulfonamide-amended soils, in which bacterial community and bacterial diversity indirectly impacted ARGs, via directly affecting MGEs (figures 6(b), (d)). Collectively, changes in bacterial community, followed by effected MGE abundances, were the dominant factor stimulating ARGs transportation in the presence of NPs. We supposed that the pattern of ARGs was mainly associated with the changes of MGEs mediated by NPs exposure.

5. Conclusions

We provide evidence that the diversity of the soil bacterial community increased after certain concentrations of NPs exposure (<100 mg kg⁻¹), but the existence of NPs had no significant effect on the abundance of bacteria. Meanwhile, the exposure of NPs and sulfonamide was found to promote the propagation of sulfonamide resistance genes, while NPs and β-lactam jointly decrease the β-lactam resistance genes in soils. NPs-antibiotics can indirectly affect ARGs by influencing their potential bacterial hosts and MGEs. This study provides a new insight into the understanding of the fate of ARGs with NPs and antibiotics joint stimuli.

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Data availability statement

The data that support the findings of this study are available upon request from the authors.

References

Azizi S, Mohammad M, Abdul Rahim R, Moghaddam A B, Moniri M, Ariff A, Saad W Z and Namvab F 2016 ZnO-Ag core–shell nanocomposite formed by green method using essential oil of wild ginger and their bactericidal and cytotoxic effects Appl. Surf. Sci. 384 517–24
Bailey J K, Pinjyn J L, Anantham S and Hall R M 2010 Commensal Escherichia coli of healthy humans: a reservoir for antibiotic resistance determinants J. Med. Microbiol. 59 L331–9
Ben M T, Frenk S, Drot I, Minz D and Berkowitz B 2013 Effects of metal oxide nanoparticles on soil properties Chemosphere 90 640–6
Berendsen B J A, Lahr J, Nibbeling C, Jansen L J M, Bongers I E A, Wipfli E L and Schans M G M V 2018 The persistence of a broad range of antibiotics during calves, pig and broiler manure management Chemosphere 204 267–76
Bernhardt E S, Colman B F, Hochella M F, Cardinale B J, Niibert R M, Richardson C J and Yin L Y 2010 An ecological perspective on nanomaterials in the environment J. Environ. Qual. 39 1954–65
Besaury L, Pavlak B and Quillet L 2014 Expression of copper-resistance genes in microbial communities under copper stress and oxic/anoxic conditions Environ. Sci. Pollut. Res. Int. 23 4013–23
Brown K D, Kulis J, Thomson B, Chapman T H and Mawhinney D B 2006 Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico Sci. Total Environ. 366 772–83
Chater K F, Bíró S, Lee K J, Palmer T and Schrempp H 2010 The complex extracellular biology of Streptomyces FEMS Microbiol. Rev. 34 171–98
Collins D, Luxton T, Kumar N, Shah S, Walker V K and Shah V 2012 Assessing the impact of copper and zinc oxide nanoparticles on soil: a field study PLoS One 7 e42663
Colman B P et al 2013 Low concentrations of silver nanoparticles in sewage sludge cause adverse ecosystem responses under realistic field scenario PLoS One 8 e57189
Di Cesare E M, Eckert S, D’Urso R, Bertoni D C, Gillan R and Wattiez G 2016 Corno, Co-occurrence of integrase 1, antibiotic and heavy metal resistance genes in municipal wastewater treatment plants Water Res. 94 208–14
Forsberg K J and Dantas G 2012 The shared antibiotic resistome of soil bacteria and human pathogens Science 337 1107–11
Gaze W H, Li Hong Z, Abdouslam N A, Hawkey P M, Leo C B, Jeremy R, Helen B, Susan D, Paul K and Boxall A B A 2011 Impacts of anthropogenic activity on the ecology of class I integrons and integron-associated genes in the environment ISME J. 5 1253–61
Ge Y G, Priester J H, Van De Werfhorst L C, Schimmel J P and Holden P A 2013 Potential mechanisms and environmental controls of TiO₂ nanoparticle effects on soil bacterial communities Environ. Sci. Technol. 47 14411–7
nanoparticles on carbon mineralization and bacterial abundance J. Hazard. Mater. 283 529–35
Song Y K, Hong S H, Jang M, Han G M, Jung S W and Shim W J 2018 Combined effects of UV exposure duration and mechanical abrasion on microplastic fragmentation by polymer type Environ. Sci. Technol. 51 4368–76
Sumita Y and Fukasawa M 1996 Meropenem resistance in Pseudomonas aeruginosa Chemotherapy 42 47–56
Thames C H, Pruden A, James R E, Ray P P and Knowlton K F 2012 Excretion of antibiotic resistance genes by dairy calves fed milk replacers with varying doses of antibiotics Front. Microbiol. 3 139
Tomaz P, Bakhtiyor R, Agnieszka G, Xiaoke H, Dasari T P, Andrea M, Huey-Min H, Andrey T, Danuta L and Jerzy L 2011 Using nano-QSAR to predict the cytotoxicity of metal oxide nanoparticles Nat. Nanotechnol. 6 175–8
Tou F, Yang Y, Feng J, Niu Z, Pan H, Qin Y, Guo X, Meng X, Liu M and Michael F H 2017 Environmental risk implications of metals in sludges from waste water treatment plants: the discovery of vast stores of metal-containing nanoparticles Environ. Sci. Technol. 51 4831–40
Tran N H, Chen H, Do T V, Reinhard M, Ngo H H, He Y and Gin K Y 2018 Simultaneous analysis of multiple classes of antimicrobials in environmental water samples using SPE coupled with UHPLC–ESI-MS/MS and isotope dilution Talanta 159 163–73
Tran N H et al. 2019 Occurrence and risk assessment of multiple classes of antibiotics in urban canals and lakes in Hanoi, Vietnam Sci. Total Environ. 692 157–74
Tran N H, Reinhard M and Gin K Y 2018 Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions—a review Water Res. 133 182–207
Vera H and Lúcia S 2011 Degradation and removal methods of antibiotics from aqueous matrices—a review J. Environ. Manage. 92 2304–47
Vikesland P J, Pruden A, Pjä A, Aga D S, Buergermann H, Li X, Manaia C M, Namb M, Wigginton K R and Zhang T 2017 Towards a comprehensive strategy to mitigate dissemination of environmental sources of antibiotic resistance Sci. Technol. 51 13061–9
von Wintersdorff C J, Penders J, van Niekerk J M, Mills N D, Majumder S, van Alphen L B, Savelkoul P H and Wolffs P F 2016 Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer Front. Microbiol. 7 173
Wagner V E, Frelinger J G, Barth R K and Iglewski B H 2006 Quorum sensing: dynamic response of Pseudomonas aeruginosa to external signals Trends Microbiol. 14 35–8
Wang Y, Zhu X, Lao Y, Lv X, Tao Y, Huang B, Wang J, Zhou J and Cai Z 2016 TiO2 nanoparticles in the marine environment: physical effects responsible for the toxicity on algae Phaeodactylum tricornutum Sci. Total Environ. 565 818–26
White D C, Sutton S D and Ringelberg D B 1996 The genus Sphingomonas: physiology and ecology Curr. Opin. Biotechnol. 7 301–6
Wu S, Dalsgaard A, Hammerum A M, Porsbo L J and Jensen L B 2010 Prevalence and characterization of plasmids carrying sulfonamide resistance genes among Escherichia coli from pigs, pig carcasses and human Acta Veterinaria Scand. 52 1–7
Yadav R C, Patra A K, Purakayastha T J, Singh R and Kumar C 2014 Effect of engineered nanoparticles of fe and zn oxides on enzyme activity and bacterial abundance in soil at ambient and elevated atmospheric CO2 Proc. Natl Acad. Sci. India 84 649–56
Yang Y, Zhang C and Hu Z 2013 Impact of metallic and metal oxide nanoparticles on wastewater treatment and anaerobic digestion Environ. Sci.: Process. Impacts 15 39–48
Younghee K, Jung J, Kim M, Park J, Boxall A B A and Choia K 2008 Prioritizing veterinary pharmaceuticals for aquatic environment in Korea Environ. Toxicol. Pharmacol. 26 167–76
Zhan X and Xiao L. 2017 livestockwaste 2016 — International conference on recent advances in pollution control and resource recovery for the livestock sector Front. Environ. Sci. Eng. 11 16
Zhang B, Wang M M, Wang B, Xin Y, Gao J and Liu H 2017 The effects of bio-available copper on macrolide antibiotic resistance genes and mobile elements during tylosin fermentation dregs co-composting Bioresour. Technol. 251 230–7
Zhang F, Zhao X, Li Q, Liu J and Zhu J 2018 Bacterial community structure and abundances of antibiotic resistance genes in heavy metals contaminated agricultural soil Sci. Environ. Pollut. Res. Int. 25 9547–55
Zhang J, Chen M, Sui Q, Wang R, Tong J and Wei Y S 2016 Fate of antibiotic resistance genes and its drivers during anaerobic co-digestion of food waste and sewage sludge based on microwave pretreatment Bioresour. Technol. 217 28–36
Zhang Y J, Hu H W, Gou M, Wang J T, Chen D and He J Z 2017 Temporal succession of soil antibiotic resistance genes following application of swine, cattle and poultry manures spiked with or without antibiotics Environ. Pollut. 233 1621–32
Zhou B, Wang C, Zhao Q, Wang Y, Hua M, Wang J and Wang S 2016 Prevalence and dissemination of antibiotic resistance genes and co-selection of heavy metals in Chinese dairy farms J. Hazard. Mater. 320 10–7
Zhou S J, Yang G G, Liu S, Zhang B Q, Lai H J, Chen Z F and Pan C G 2013 Excretion masses and environmental occurrence of antibiotics in typical swine and dairy cattle farms in China Sci. Total Environ. 444 183–95