Co-occurrence of genes for antibiotic resistance and arsenic biotransformation in paddy soils

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abstract

Paddy soils are potential hotspots of combined contamination with arsenic (As) and antibiotics, which may induce co-selection of antibiotic resistance genes (ARGs) and As biotransformation genes (ABGs), resulting in dissemination of antimicrobial resistance and modification in As biogeochemical cycling. So far, little information is available for these co-selection processes and specific patterns between ABGs and ARGs in paddy soils. Here, the 16S rRNA amplicon sequencing and high-throughput quantitative PCR and network analysis were employed to investigate the dynamic response of ABGs and ARGs to As stress and manure application. The results showed that As stress increased the abundance of ARGs and mobile genetic elements (MGEs), resulting in dissemination risk of antimicrobial resistance. Manure amendment increased the abundance of ABGs, enhanced As mobilization and methylation in paddy soil, posing risk to food safety. The frequency of the co-occurrence between ABGs and ARGs, the host bacteria carrying both ABGs and ARGs were increased by As or manure treatment, and remarkably boosted in soils amended with both As and manure. Multidrug resistance genes were found to have the preference to be co-selected with ABGs, which was one of the dominant co-occurring ARGs in all treatments, and manure amendment increased the frequency of Macrolide-Lincosamide-Streptogramin B resistance (MLSB) to co-occur with ABGs. Bacillus and Clostridium of Firmicutes are the dominant host bacteria carrying both ABGs and ARGs in paddy soils. This study would extend our understanding on the co-selection between genes for antibiotics and metals, also unveil the hidden environmental effects of combined pollution.

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Introduction

Recently, antibiotic resistance genes (ARGs) have been recognized as one of the six emerging contaminants identified by UNEP (UNEP, 2016) and widely distributed in different habitats such as humans (Pal et al., 2016), water (Su et al.,
The high frequency of antibiotic resistance in environments is a global public health concern, which is challenging the therapy efficacy of life-saving antibiotics (UNEP, 2016). In soil environment, there are abundant ARGs and antibiotic resistant bacteria (ARBs), which can be transferred to humans through food chains, dust, and so on (Winkworth-Lawrence and Lange, 2016). In recent decades, ARGs and ARBs in soil environment attracted great concern due to their significant contribution to ARGs dissemination.

The use of livestock and poultry manure as fertilizers is one of the main routes of ARGs accumulated in soils (Binh et al., 2008; Zhao et al., 2018a; Zhu et al., 2013). Although manure application can introduce mineral nutrients and organic matter for crop growth and production (Hao et al., 2008), it has also been recognized as an environmental reservoir of ARGs and mobile genetic elements (MGEs), thus manure carrying these genes has been regarded as emerging environmental pollutants (Binh et al., 2008; Gillings et al., 2008; He et al., 2020; Pruden et al., 2006; Qiao et al., 2018; Zhu et al., 2013). Therefore, the potential risks of manure applications as organic fertilizer should not be ignored owing to its contribution to ARGs dissemination.

In addition to antibiotics, soils impacted by agricultural activities also have high metal(loid) burden (Jarup, 2003; Poole, 2017; Seiler and Berendonk, 2012), which can not only alter the distribution, composition, and abundance of ARGs, but also induce co-selection of ARGs with metal resistance genes (MRGs) (Li et al., 2017; Zhang et al., 2021; Zhu et al., 2013). Co-selection between ARGs and MRGs have been extensively revealed, including co-resistance (different resistance determinants present on the same genetic element), cross-resistance (the same genetic determinant responsible for resistance to both antibiotics and metals), and co-regulatory resistance (shared regulatory system to antibiotic and metal resistance) (Baker-Austin et al., 2006; Seiler and Berendonk, 2012). Recently, MRGs of various metals have been shown could be co-selected with ARGs, such as arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), and zinc (Zn) (Pal et al., 2015, 2017; Zhao et al., 2019). Additionally, those genes referred to antibiotic and metal resistances are usually associated with MGEs, such as integrons, plasmids, and transposons, which may be horizontally transferred among microbes (Andersson and Hughes, 2014; Suzuki et al., 2012). However, although the phenomenon of co-selection is widely recognized, the relevant mechanisms are not clear, the questions such as what kinds of ARGs are preferably associated with MRGs, and the dynamic variation of the co-selections, also what bacteria might carry both ARGs and MRGs are not well answered.

Arsenic (As) is ubiquitous in soil environment due to both natural and anthropogenic activities (Koljonen et al., 1988; Nordstrom, 1996; Zhao et al., 2010). Arsenic contamination in soils poses a serious health risk to human through various exposure pathways, such as food chain and groundwater (Barringer and Reilly, 2013; Smith et al., 2000). In order to adapt to the stress of As pollution, soil microbes have evolved a series of As resistance genes to detoxify and biotransform As, including arsenite (As(III)) oxidation, arsenate (As(V)) reduction, arsenic (de)methylation, and arsenic transport (Rosen, 2002; Zhao et al., 2018b). Biotransformation of As facilitated by As biotransformation genes (ABGs) not only endows microorganisms with the ability to detoxify As, but also profoundly affects As toxicity and bioavailability to plants (Rahman and Hassler, 2014). Therefore, ABGs could be crucial biomarkers for understanding As biogeochemical processes and environmental risk (Wang et al., 2021).

Due to the promotion of organic fertilizer and widespread As contamination, antibiotic-contaminated manure may be applied to soils contaminated with As and result in combined pollution by As and ARGs in the soil (Duan et al., 2012; Manyi-Loh et al., 2018). Therefore, it is of great significance to investigate the interaction between As and ARGs, also assess the environmental risk of this combined contamination. We hypothesized that swine manure application at arsenic contaminant fields may promote the co-occurrence of ABGs and ARGs, and thus might induce soil bacteria to carry both ABGs and ARGs, which would pose increasing environmental risk. Hence, by using Illumina sequencing and high throughput quantitative PCR (HT-qPCR) based on ABG and ARG chips, the objectives of this study were to (1) explore the co-occurrence patterns between ABGs and ARGs in soil amended with As and/or swine manure; (2) identify the putative host bacteria carrying both ABGs and ARGs in paddy soils. The results of this study would shed light on the mechanisms of interaction between ABGs and ARGs, advance our understanding of the hidden health risk associated with the co-occurrence of ARGs and MRGs.

1. Materials and methods

1.1. Soil collection and swine manure preparation

Topsoil (0-20 cm) without manure-applied over at least ten years was collected from a paddy field in Jiaxing, Zhejiang Province, China (30°50’7.7”N, 120°43’5.7”E). Swine manure was obtained from the local private pig farm that uses veterinary antibiotics. Then, the collected soil and swine manure were respectively air-dried, sieved (< 2 mm), and then stored in dark at room temperature until use. Detailed properties of soil and swine manure are provided in Appendix A Table S1, including basic physicochemical properties, metal(loid) concentrations, abundances of 16S rRNA gene, ARGs, MGEs, and ABGs.

1.2. Experimental design and sample collection

The 28-day incubation experiments were carried out using modified 250-mL conical flasks with facilities for volatile As and soil solution collection at 25°C. Four treatments were initially performed with 100 g dry soil and different amendments: (1) no amendment (CK), (2) 37.5 mg As(V)/kg (added as Na2AsO4•12H2O (AR, Sinopharm Group Co., Ltd., China)) (As), (3) 5 g dry swine manure (M), and (4) 37.5 mg As(V)/kg and 5 g dry swine manure (As+M). Each treatment was replicated 4 times, and the incubations were started by mixing with 120 mL Milli-Q water. During the incubation time, Milli-Q water was supplemented every two days to maintain water contents, and the air was continuously pumped into the flasks at the speed of 0.5 L/min to refresh the air and the volatile As can be preserved by 2% AgNO3 (AR, Sinopharm Group Co.,
Zhao et al., 2018b). A total of 296 primer sets were used to analyze the antibiotic resistome, including all major classes of antibiotics (283 primer sets), transposase genes (8 primer sets), integron-integrate genes (4 primer sets), and the 16S rRNA gene (Appendix A Table S2). Meanwhile, a total of 80 primer sets were used to analyze the ABGs, consisting of four types (79 primer sets) and one 16S rRNA gene (Appendix A Table S3). All the detailed instruction of the experimental procedure was followed by our previous study (Chen et al., 2017b). Briefly, each DNA sample was diluted to around 20 ng/µL using sterile water and amplified by qPCR in technical triplicates at a threshold cycle (CT) of 31 as the detection limit with a Wafergen SmartChip Real-Time PCR system in 100-nL reaction volumes (Su et al., 2015). Negative controls without templates were included. Relative copy number (N) was calculated using the following Eq. (1):

\[ N = 10^{(31-\text{CT})/10}/3 \]  

where, CT refers to quantitative PCR results (Chen et al., 2016). Only when the copy numbers of three technical triplicates for each DNA sample were all above the detection limit, that targeted gene was considered to be detected.

1.6. Statistical analysis

One-way analysis of variance (ANOVA) with Tukey’s Studentized Range (HSD) test was used to analyze the effects of As and swine manure amendment on the relative abundance of bacteria and targeted genes, with significance at \( P < 0.05 \). ANOVA was performed with SPSS software (version 23). As for bacterial communities, the metrics including observed OTUs, Chao1, Shannon index, and Phylogenetic diversity (PD) were used to describe bacterial alpha diversity for each sample. To evaluate the profiles of bacterial communities, Bray-Curtis distance between samples was employed for principal coordinate analysis (PCoA, cmdscale function from “stats” R package) with a PERMANOVA test (adonis function from “vegan” R package) (Dixon, 2003). To investigate the co-occurrence patterns between ABGs and ARGs, as well as putative bacteria carrying both ABGs and ARGs, the “WGCNA” R package was performed by calculating a correlation matrix containing all pairwise Spearman rank correlations among all ABGs, ARGs, and OTUs across all samples under different treatments (Gauthier, 2001; Langfelder and Horvath, 2008; Zhang and Horvath, 2005). P-values were adjusted by Benjamini-Hochberg false discovery rate (FDR) test (Benjamini and Hochberg, 1995), then the Spearman rank correlations were restricted to those having correlation coefficient \( > 0.8 \) and \( < -0.8 \) (adjusted \( P < 0.001 \)). The topological properties of these networks were characterized by various indices, including nodes, edges, modularity (MD), average path length (APL), network diameter (ND), average degree (AD), and graph density (GD). Network visualizations were performed in Gephi (version 0.9.2, http://gephi.org) (Bastian et al., 2009). Chord and bubble diagrams were generated with the “circlize” and “ggplot2” R packages, respectively (Gu et al., 2014). The Venn diagram showing the unique and shared putative bacteria carrying both ABGs and ARGs across different treatments was performed with the “ggplot” R package. The other figures and tables were generated by Microsoft Office Excel 2016.
Fig. 1 – The relative abundance of ABGs (a) and ARGs (b) under different treatments. The data are presented at the mean ± standard deviation (SD) (n = 4). ABGs: arsenic biotransformation genes; ARGs: antibiotic resistance genes; MLSB: Macrolide-Lincosamide-Streptogramin B resistance; CK: controlled soil samples; As: As-amended treatment; M: swine manure treatment; As+M: As-amended and swine manure treatment.

2. Results

2.1. Dynamic variation of arsenic biotransformation genes (ABGs)

Totally 61 unique ABGs were detected in all treatments, ranging from 6 to 41 in different samples (Appendix A Fig. S1a). The relative abundance of ABGs ranged from $2.15 \times 10^{-4}$ to $1.07 \times 10^{-2}$ copies per 16S rRNA gene, averaging $3.09 \times 10^{-3}$ copies per 16S rRNA gene (Fig. 1a). Generally, the detected numbers and relative abundances of ABGs were increased with extended incubation times ($P < 0.05$), which increased by 6-29 times after 28-day incubation. Additionally, swine manure amendment significantly enriched ABGs. For example, comparing with the relative abundances of ABGs in CK at Day-14 incubation, which increased by 34-fold in M and 114-fold As+M treatment (Fig. 1a). However, the relative abundances of ABGs were not significantly affected by As amendment only. In addition, swine manure amendment significantly increased the proportion of arsenic (de)methylation-associated genes. For example, at Day-28, the proportion of arsenic (de)methylation-associated genes in treatments with manure (M and As+M) was about 50%, while it was 13% in CK (Fig. 1a).

2.2. Dynamic variation of ARGs and MGEs

A total of 185 unique ARGs were detected in all samples, ranging from 26 to 122 in different samples (Fig. 1b). The relative abundance of ARGs was ranged from $1.23 \times 10^{-3}$ to $1.31$ copies per 16S rRNA gene (Fig. 1b). The amendment of swine manure significantly increased the number and relative abundances of ARGs during the whole incubation stages ($P < 0.05$). After the 28-day incubation, the relative abundances of ARGs in M and As+M treatment were 21 and 37-fold higher than that of CK, respectively. In addition, after the 28-day
incubation, As treatment also significantly enriched ARGs, the relative abundances increased by 3-fold compared to CK. Arsenic and manure treatment significantly changed the compositions of ARGs ($P < 0.05$) (Fig. 1b). Genes conferring resistance to beta lactamase and multidrug were dominant in the CK and As treatments, while those to aminoglycoside, Macrolide-Lincosamide-Streptogramin B resistance (MLSB), and tetracycline were dominant in the M and As+M treatments. In addition, compared to CK, As treatment significantly enriched the genes conferring resistance to multidrug ($P < 0.05$). Totally 11 unique MGEs were detected in all samples, including 8 transposase genes and 3 integron-integrase genes (Appendix A Fig. S2a). The relative abundance of MGEs ranged from $8.21 \times 10^{-5}$ to $9.30 \times 10^{-2}$ copies per 16S rRNA gene (Appendix A Fig. S2a). The abundance of MGEs was not affected by As treatment, whereas significantly increased by swine manure amendment ($P < 0.05$) (Appendix A Fig. S2a). There were significant positive correlations between the relative abundance of MGEs and ARGs (Pearson’s $r = 0.84$, $P < 0.0001$), but no between MGEs and ABGs (Appendix A Fig. S2b-c).

2.3. Patterns of co-occurrence between ABGs and ARGs

Mantel test showed that the dynamic variation of ABGs was significantly correlated with that of ARGs (Spearman’s $r = 0.27$, $P = 0.001$). Therefore, the co-occurrence patterns between ABGs and ARGs were investigated through network analysis based on strong ($r > 0.8$) and significant ($P < 0.001$) correlations (Fig. 2a, Appendix A Table S4). The results showed that As+M treatment had the highest complexity (average degree) of networks, followed by treatments of M, As, and CK, containing 164, 126, 77, and 74 nodes, 390, 240, 149, and 130 edges, re-
spectively. In all treatments, positive correlations were much more than negative ones (Appendix A Table S4). Additionally, the co-occurring genes involved in the networks were also varied among different treatments (Fig. 2b, Appendix A Table S4). For ARGs, in treatments without manure, genes for multidrug had the highest frequency of co-occurrence with ABGs, accounting for >30% of the genes involved in co-occurrence. In the treatments with manure, genes for MLSB became the highest co-occurring ARG with ABGs, accounting for about 30% (Fig. 2c). Manure amendment also increased the co-occurrence of ARGs for aminoglycoside with ABGs, but decreased the co-occurrence of genes for multidrug (Fig. 2c). Regarding to ABGs, As(V) reduction genes were the dominant ABGs co-occurring with ARGs, accounting for 28%-46%. Swine manure amendment increased the co-occurrence between arsenic (de)methylation genes with ARGs. The amendment of manure also increased the co-occurrence frequency of ABGs with MGEs (Fig. 2).

2.4. Bacterial community compositions and structures

A total of 2144 unique OTUs were obtained from all the treatments. At the phylum level, Firmicutes and Proteobacteria were the dominant bacteria in all samples, with Firmicutes accounting for 45%-83% (Fig. 3a). Extending incubation increased the abundance of Proteobacteria, but decreased that of Firmicutes. The amendment of swine manure significantly increased the abundance of Firmicutes, but decreased those of Actinobacteria and Planctomycetes (Fig. 3a). The bacterial beta diversity was further analyzed based on Bray-Curtis distances. The results showed that the first two principal-coordinate analysis (PCoA) axes explained a total of 69% variance of bacterial structure (Fig. 3b). Permutational multi-variate analysis of variance (PERMANOVA test) showed that both incubation duration and swine manure treatments significantly affected the bacterial compositions (incubation duration, 27.64%, P = 0.001; treatments, 24.50%, P = 0.001), whereas As treatment did not significantly change the bacterial community structure (PERMANOVA test 0.09%, P = 0.835). The indexes of observed OTUs, Chao1 index, and phylogenetic diversity (PD), which represent bacterial alpha diversity, were significantly increased by swine manure amendment at early stages of incubation but decreased over time. In soils without manure amendment, these indexes increased over time (Appendix A Table S5).

2.5. Characterization of putative bacteria carrying both ABGs and ARGs

The results of the Mantel test showed that there were strong associations between bacterial communities, ABGs, and ARGs (Spearman’s $r = 0.27-0.46$, $P = 0.001$). The bacteria which were significantly related to both ARGs and ABGs were identified as bacteria carrying both ABGs and ARGs via network analysis (Fig. 4). Results showed that As+M treatment had the most OTUs carrying both ARGs and ABGs, followed by treatments of M, As, and CK (Fig. 4). The complexity of networks among different treatments followed the order of As+M > M > As > CK (Appendix A Table S6). There was an interaction between As and swine manure, e.g., As increased the edges between ARGs and bacteria, swine manure increased those between ABGs and bacteria. In the network of CK, the nodes were randomly dispersed, with the highest modularity of 0.58, while in As and manure treatments, the nodes were assembled according to some hub nodes, with significantly decreased modularity (Fig. 4, Appendix A Table S6).

We further identified the co-occurring genes among different treatments. In treatments of CK and As, genes for As(V) reduction, beta lactamase, and multidrug resistance were highly co-occurring, while in treatments with swine manure, genes for arsenic transport, aminoglycoside, beta lactamase, MLSB, and multidrug resistance were highly co-selected (Fig. 4b). The bacteria preferred to carry both ARGs and ABGs also varied among treatments. Generally, the dominant bacteria in these networks at the phyla level were Firmicutes and Proteobacteria (Appendix A Table S7). At the OTU level, in all treatments, OTU1300 and OTU1878 belonged to Bacillus of Firmicutes were the putative hosts. OTU2395 belonged to Clostridium of Firmicutes was induced to have more edges with genes for As(V) reduction, beta lactamase, and MLSB resistance by As stress, but not affected by swine manure amendment.

2.6. Co-occurrence of ABGs and ARGs and soil indigenous bacteria

Since there are abundant and diverse bacteria in swine manure, to eliminate the bias caused by the bacteria intro-
duced with swine manure, shared OTUs among all treatments (including CK), representing soil indigenous bacteria, were picked up by Venn analysis. Among all the four treatments, there were 172 shared OTUs (Appendix A Fig. S3a). These shared OTUs belonged to 16 phyla, and were dominated by Firmicutes, Chloroflexi, Proteobacteria, Acidobacteria, and Actinobacteria (Appendix A Fig. S3b-c). Additionally, the co-occurrence profiles between shared OTUs and genes were constructed using network analysis to investigate the co-selection between ARGs and ABGs in soil indigenous bacteria (Appendix A Fig. S3d-g). The results further confirmed that the amendment of As or/and swine manure remarkably increased the co-occurrence between ABGs/ARGs, and shared OTUs in As + M treatment, compared to CK treatment (Appendix A Table S8). The co-occurred frequency between putative host bacteria and MGEs, which might increase the risk of horizontal gene transfer (HGT), also increased in M and As + M treatments (Appendix A Fig. S3d-g, Appendix A Table S8).

To further identify the putative bacteria carrying both ABGs and ARGs, the phylogenetic classification of shared OTUs was employed. Results showed that most shared OTUs carrying both ABGs and ARGs were classified in the phylum of Firmicutes (Appendix A Fig. S3c). In addition, As or/and swine manure treatment increased the co-occurrence between ABGs and ARGs in Firmicutes (Appendix A Table S9). The predominant subtypes of co-occurring ARGs were beta lactamase and multidrug, and the co-occurrence between MGEs and ABGs in Firmicutes was also enhanced in swine manure amended soils (i.e., M, and As + M treatments) (Appendix A Fig. S4). Further analysis at genera levels within Firmicutes demonstrated that Bacillus frequently co-occurred with ABGs and ARGs, which also had the highest relative abundances in all treatments at genus level, accounting for 22.08%, 21.30%, 7.81%, and 7.92% in CK, As, M, and As + M, respectively. Clostridium also showed high abundance and co-occurrence with ABGs and ARGs, with relative abundances of 5.16%, 4.32%, 1.97%, and 2.42% in CK, As, M, and As + M, respectively. In addition, Desulfitobacterium, Gracilibacter, Tuberculibacter, and Tumoribacillus were also putative host bacteria carrying both ABGs and ARGs in Firmicutes. Within Proteobacteria, Achromobacter, Geobacter, Rhodanobacter, Sideroxydans, and Thiobacillus were the putative host bacteria carrying both ABGs and ARGs.

3. Discussion

Considering the combined contamination of As and ARGs, their interactions are of significance to be investigated. In this study, the dynamic variation of ARGs and ABGs, as well as their co-occurrence were characterized under As or/and manure treatments. Results demonstrated that there are distinct interactions between ARGs and ABGs, applying ARGs carrying swine manure to As contaminated paddy soil significantly increased the abundance of ARGs and MGEs, increased As mobility, and enhanced As methylation and volatilization,
posing mutually reinforcing risks to environment and food safety.

3.1. Swine manure could be ARGs reservoir and As stress enriches ARGs in paddy soil

Our results demonstrated that swine manure amendment significantly increased the relative abundance and number of ARGs in paddy soils (Fig. 1b, Appendix A Fig. S1b), suggesting that swine manure might be the primary reservoirs of ARGs as previous studies described (Binh et al., 2008; Heuer et al., 2011; Munir and Xagoraraki, 2011; Zhao et al., 2018a; Zhu et al., 2013). For instance, Su et al. (2014) suggested that the amendment of manure increased the diversity and abundance of ARGs in soil. Arsenic treatments did not change the relative abundance of ARGs after 14 days incubation, while it was dramatically increased after 28 days incubation, and the relative abundance of multidrug was remarkably boosted (Fig. 1b). These results suggested that As stress can induce soil bacteria to carry ARGs, and this enrichment has been found in gut and water environments (Guo et al., 2014; Wang et al., 2019). In addition, in the soils amended with both As and manure, the relative abundance of MGEs was dramatically increased, representing the potential risk of horizontal gene transfer (HGT). These results clearly demonstrate that swine manure could be reservoir ARGs and MGEs, applying to metal(loid)-contaminated soils would enhance its dissemination risk of antimicrobial resistance.

3.2. Swine manure induces ABGs abundance and enhances As mobilization and methylation

Swine manure amending significantly increased the relative abundance of arsenic (de)methylation genes in soils (Fig. 1a). Consequently, the amounts of methylated arsenical species in soil solution were significantly increased, and As volatilization was also enhanced by manure amending (Appendix A Fig. S5). These results demonstrated that swine manure amending could modify the abundance and composition of ARGs in soils, and further affect As biogeochemical cycles in paddy soil. Our previous studies have elaborated that amending several other organic matters, such as rice straw, in paddy soils could also enhance the microbial methylation of As (Huang et al., 2012; Jia et al., 2013; Yang et al., 2018). It has been reported that Methanogens and sulfate-reducing bacteria (SRB) boost in flooded paddy soil when the organic substance was amended, and these microbes, the dominating hosts of arsM gene, facilitate As (de)methylation processes (Yang et al., 2018, 2020). Between As and CK treatments, although no significant difference was found in the relative abundances, the composition of ABGs was modified, with a higher abundance of genes for arsenic (de)methylation and transport in As treatment (Fig. 1a), performing As detoxification function response to As stress.

3.3. Combined contamination boosts co-occurrence between ARGs and ABGs

The frequency of co-occurrence between ARGs and ABGs was increased under As stress or manure application, and dramatically boosted in soils amended with both As and manure (Fig. 2, Appendix A Table S4). Among the co-occurred ARGs, multidrug resistance genes were found to have preference to be co-selected with ABGs, which was one of the dominant co-occurring ARGs in all treatments (Fig. 2). Multidrug resistance, resistant to three or more antibiotics, is of great risk to human health by reducing the efficacy of antibiotics used for therapies (Ouyang et al., 2015; Zhu et al., 2013). During recent decades, multidrug resistance genes are undergoing especially rapid geographical spread (McCann et al., 2019), which might be due to its preference of co-selection with other genes response to environmental stress, such as heavy metal contamination. Swine manure amendment increased the frequency of MLSB with ABGs, and the co-occurrence frequency of beta lactamase resistance genes with ABGs also increased (Fig. 2). This result is consistent with the study of Li et al. (2017), which has revealed that As-associated genes are more likely to co-select with beta lactam, bacitracin, and fosfomycin-related resistance genes. Meanwhile, the co-occurrences between arsenic (de)methylation genes and ARGs, and between ABGs and MGEs also increased in soils amended with As or/and swine manure, especially in the treatments of the compound amendment. These results clearly demonstrated that applying ARGs carrying swine manure to As or other metal(loid) contaminated paddy soil would significantly increase the frequency of co-occurrence between ARGs (or MGEs) and ABGs (or other MRGs), which might magnify their environmental risk.

3.4. Firmicutes are the dominant host bacteria carrying both ABGs and ARGs

In the present study, we explored the putative host bacteria carrying both ABGs and ARGs by using network analysis (Fig. 4). Results showed that both As stress and manure application can induce bacteria to carry both ABGs and ARGs, and especially in combined treatments with both As and manure (Fig. 4). This result indicates that As stress could induce ARGs selection, and manure amendment could also induce ABGs selection, so that combined exogenous pollution could induce bacteria to have abilities to resist both As and antibiotics. It has been found that some ARGs, including aminoglycosides, beta lactamase, MLSB, and tetracycline, could be co-selected with ABGs in the same bacteria (Ding et al., 2019; Pal et al., 2015). Furthermore, to eliminate the bias caused by the abundant bacteria introduced with swine manure, the co-selection of ARGs and ABGs by indigenous bacteria were analyzed (Appendix A Fig. S3a). Results suggested that exogenous pollution, especially exogenous combined contamination, could induce indigenous bacteria to carry both ABGs and ARGs. In addition, combined As and swine manure amendment increased the connections between MGEs and putative host bacteria (Appendix A Fig. S3c), suggesting more bacteria carry MGEs. It has been reported that the risk of HGT increased in soil environments with combined pollutants (Chen et al., 2017a; Han et al., 2018; Johnson et al., 2016).

Firmicutes are reported to be dominant hosts of As-associated genes in soils, and which are also the phylum probably carrying and disseminating ARGs (Dunivin et al., 2019; Huerta et al., 2013). Intriguingly, our results also demonstrated that Firmicutes is the absolute dominant bacteria in
soils (Fig. 3, Appendix A Fig. S3c, Appendix A Fig. S4), and the dominant hosts carrying both ARGs and ABGs (Appendix A Fig. S3d-g). Previous studies revealed that gut microbiota of Firmicutes could carry both MRGs and ARGs, such as As- associated genes, beta lactam, bacitracin, MLSB (Li et al., 2017; Wang et al., 2019b). In Firmicutes, Bacillus and Clostridium are two dominant genera in our soils (Appendix A Fig. S3b), and which showed a high frequency of co-occurrences with both ARGs and ABGs (Appendix A Fig. S3d-g). Bacillus, which has strong resistance to external harmful stress, has been reported to harbor multiple ARGs (Gueimonde et al., 2013; Guérout-Fleury et al., 1995), and also carry ABGs for As detoxification (Sato and Kobayashi, 1998; Yang et al., 2020; Yang et al., 2018). Clostridium, which is the dominant bacteria in paddy soil, participates in iron and arsenate reduction (Stolz et al., 2007; Yang et al., 2018, 2020), and also harbor multiple ARGs (Diarra et al., 2007; Frey and Falquet, 2015).

4. Conclusions

This study characterized the dynamic co-occurrence patterns between ABGs and ARGs in paddy soils amended with As and/or swine manure, and identified the putative host bacteria carrying both ABGs and ARGs. Results revealed that not only the co-occurrence between ABGs and ARGs, but also the putative host bacteria can be significantly increased by As or manure amendment, especially boosted under compound As and manure amendment. These results provide solid evidence for the co-selection between ABGs and ARGs, also between bacteria and genes, which may shed light on the complex association of ARGs and metals. This study would advance our understanding of the hidden health risk from the combined pollution of ARGs and metals.

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Appendix A Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jes.2022.02.027.

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