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LAND POLLUTION (G HETTIARACHCHI, SECTION EDITOR)

Fate of Land Applied Emerging Organic Contaminants in Waste Materials

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Abstract
Purpose of review In this review, we describe recent research on: (1) the occurrence of antibiotic resistant genes (ARGs) in waste materials and in soils receiving land application of ARG-contaminated waste materials; (2) the environmental behavior of ARGs in soils; (3) approaches to waste material management in terms of the elimination and reduction of ARGs. Land application of organic waste materials provides valuable nutrients to plant growth, but it can also introduce emerging organic contaminants, for example, antibiotics, into the environment. The widespread use of antibiotics and their entry into the environment are believed to result in the development and proliferation of antibiotic-resistant bacteria and ARGs in humans and animals, as well as in the environment. For this reason, ARGs are classified as emerging organic contaminants.
Recent findings Land application of manure, biosolids from wastewater treatment plants, and wastewater can potentially load elevated levels of antibiotics, antibiotic-resistant bacteria, and ARGs into soils. A host of environmental and biological factors may influence the environmental behavior of ARGs, including persistence and mobility, as well as their transmission among microorganisms in soil matrixes. Traditional on-site waste material management approaches may not be able to completely remove ARGs from the waste material before it is applied to the soils. However, recent research has suggested certain waste material land application management approaches may be able to further reduce the input of ARGs and mobility in the environment.
Summary Knowledge of the environmental behavior of ARGs is essential for better understanding and predicting the development and spread of antibiotic resistance in the environment. Information on the ecological consequences of ARG contamination and the possible entry of ARGs into the human food chain is also needed for better risk assessment of land application of ARG-contaminated waste materials. There is an urgent need to develop effective management and land application approaches to minimize/eliminate the input and mobility of ARGs in the soil environment.

Keywords Manure, biosolids, wastewater · Soils, environmental behaviors · Horizontal gene transfer · On-site waste material treatment · Environmental risk assessment

Introduction

Land application of organic waste materials, such as manure and wastewater treatment plant (WWTP) biosolids, is a common practice in agriculture to provide additional nutrients to plants [1]. Wastewater irrigation is also a common practice to enrich soil nutrients and overcome water shortages in many countries. However, these land application practices can introduce undesirable pollutants into the environment, of which those commonly addressed are heavy metals, organic compounds, and salts [2]. These practices could also introduce a broad spectrum of emerging organic contaminants into the environment [3]. Emerging organic contaminants, also or better termed as contaminants of emerging concern, are defined by the U.S. Environmental Protection Agency as "chemicals

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and other substances that have no regulatory standard, have been recently "discovered" in natural streams, often because of improved analytical chemistry detection levels, and potentially cause deleterious effects in aquatic life at environmentally relevant concentrations" [4].

One environmentally significant class of emerging organic contaminants is antibiotics. Antibiotics are widely used for treating human illness and animal diseases and promoting animal growth, as well as for a multitude of agricultural purposes [5]. For example, about 80% of the more than 17 million kilograms of antibiotics sold yearly in the USA is used in 60–80% of food-producing animal production for therapeutic and sub-therapeutic purposes [6–8]. Up to 95% of the antibiotics administered to animals and humans can eventually be excreted in urine or feces as parent compounds or metabolites [9–11], resulting in their frequent detection at significant levels in manure from a variety of farm animals [12–19] and in wastewater and biosolids [20–24]. Research has demonstrated that the overuse and/or misuse of antibiotics can facilitate the development and proliferation of antibiotic resistance in the microbiome of animal and human digestive systems [25, 26], resulting in increased levels of antibiotic-resistant bacterial populations and elevated levels of antibiotic resistance genes (ARGs) in animal manure [27–31] and human waste [32]. Therefore, land application of animal manure, wastewater, and biosolids can be sources of antibiotics, antibiotic-resistant bacteria (ARB), and ARGs in the environment.

The concentrations of antibiotics in biosolids and biosolids-amended soils have been reported to reach levels of up to 58 mg kg^{-1} [dry weight (d.w.)] and 900 μg kg^{-1} (d.w.), respectively [3], and the concentrations of antibiotics in wastewater treatment plant influents and effluents to reach levels of up to 122 μg L^{-1} and 6 μg L^{-1}, respectively [33]. In soils irrigated with wastewater, antibiotics at concentrations ranging from 1.4 to 66.7 μg kg^{-1} have been detected [34]. In manure and wastewater collected from a swine feedlot in China, the highest total tetracycline and total sulfonamide concentrations were 167 mg kg^{-1} and 65 μg kg^{-1}, respectively, in the manure and 389 and 7.6 μg L^{-1}, respectively, in the wastewater [35]. In another study, 17 antibiotics were detected in approximately 39% of the 219 dried manure-based fertilizer samples tested, with the mean concentrations ranging from 20 μg kg^{-1} to 2 mg kg^{-1}; one sample contained the highest concentration of antibiotic, namely, 73 mg kg^{-1} doxycycline [23]. The highest concentrations of tetracycline and chlortetracycline reported in pig manure-amended soils were 199 μg kg^{-1} and 7.3 μg kg^{-1}, respectively [36].

Research has shown that elevated levels of antibiotics in the soil environment can potentially further enhance the populations of ARB and levels of ARGs in soils in addition to those that are already associated with the land-applied waste materials [5, 37–39]. Elevated ARGs in the environment have been linked to increasing occurrence of antibiotic resistance in human populations and reduced/compromised effectiveness of antibiotic therapy for humans [40, 41]. In the European Union alone, ARB are estimated to cause 25,000 deaths and cost more than U.S. $1.5 billion in healthcare expenses and productivity losses every year [42]. Antibiotic resistance has become a global concern and was recently identified as the "major health security challenge of the 21st century" by the World Health Organization [43, 44]. It should be noted that while most ARB-induced infections result from the overuse of antibiotics by humans in hospitals [45], studies have revealed that ARB have been isolated from humans and a wide range of animals not subject to significant antibiotic exposure [46], implying a role of environmental microbiomes in the rise of antibiotic resistance [47].

The development of antibiotic resistance among bacteria arises from ARG expression. ARGs are DNA sequences encoding proteins responsible for the resistance [48]. Because of their essential role in the development of antibiotic resistance, ARGs were recently included as an important class of emerging organic contaminants that can greatly impact environmental and human health [49]. Extensive reviews on the occurrence, environmental behaviors, and ecological and human health impacts of antibiotics and ARB in soils subject to land applications with waste materials have been published in recent years [5, 50]. However, similar systematic information on ARGs in the soil environment is lacking. Therefore, the main objective of this review is to synthesize the most recent literature on: (1) the occurrence and distribution of ARGs in land applications of waste materials; (2) the environmental behaviors of ARGs in soils receiving land applications of waste materials; (3) the effectiveness of current on-site management approaches in reducing ARGs in waste materials; (4)) current knowledge gaps on the factors contributing to the enrichment of ARGs in soils, the potential risk to ecological and human health, and the best approaches to land application management with waste materials.

Occurrence of ARGs in Land-Applied Waste Materials and the Affected Soils

Land-Applied Waste Materials

The reported occurrence and distribution of a diverse ARGs in a wide range of waste materials is summarized in Table 1. Culture-independent methods, such as quantitative PCR assays and metagenomics sequence analysis, have been commonly used to identify, detect, and quantify ARGs [72–74]. As shown in Table 1, the detected ARGs conferred resistance to all major classes of antibiotics and encompassed all of the main resistant mechanisms.

Table 1 Abundance of antibiotic resistance genes detected in wastewater, manure, and biosolids from animal production sites and wastewater treatment plants

ARGs	Resistance mechanism	Animal production				Wastewater treatment plant				References
		WW/MS	FM	CM	LS	IF	EF	B	RB	
Tetracycline										
*tet*A	Efflux	−3 to 0[a], 8[b]	−3 to -4[a], 10[b]		10[b]	3-9[b]	4-7[b]	−3[a], 9[b], 3[c]	5-8[b]	[51–62]
*tet*B	Efflux	−3 to -1[a], 4[b]	−3 to -4[a]			3-7[b]	4-6[b]	−4[a], 7[b]	8[b]	[52, 53, 55, 58, 60–62]
*tet*C	Efflux	−4 to 0[a]	−5[a], 8[b]	−5[a], 6[b]		5-9[b]	4-8[b]	9[b], 3[c]	8[b]	[51–55, 61]
*tet*D	Efflux					4[b]	4[b]	9[b]	8[b]	[52, 55]
*tet*E	Efflux	−1[a]	−3[a]			3[b]	4[b]	−3[a], 9[b]	8[b]	[52, 54, 55, 58, 62]
*tet*G	Efflux	−2[a], 7-10[b]	−3[a], 10[b]	−2[a], 9[b]		3-9[b]	4-8[b]	10[b], 7[c]	8[b]	[51–55, 59, 61, 63]
*tet*H	Efflux	−1[a], 7[b]	9[b]			2[b]	4[b]	9[b]	8[b]	[52, 55, 59]
*tet*J	Efflux		−2[a], 10[b]	−2[a], 9[b]						[52, 55]
*tet*K	Efflux									[52, 55]
*tet*L	Efflux	−3[a],	−3 to -2[a], 10[b]	−2[a], 9[b]		5-7[b]	4-6[b]	8[b], 2[c]	8[b]	[51–53, 55, 61]
*tet*M	Protection	−2 to 1[a], 7-11[b]	−4 to 2[a]	−3[a]		4-8[b]	4-7[b]	9[b], 2[c]	8[b]	[51–55, 59, 61, 63–65]
*tet*O	Protection	−2 to 0[a], 7[b]	−3 to -2[a], 10-11[b]	−5 to -4[a], 6[b]		5-9[b]	2-8[b]	−1[a], 10[b], 3[c]	8[b]	[51–56, 58, 61, 62]
*tet*Q	Protection	−2 to -1[a], 4-7[b]	−3 to -2[a], 10[b]	−3[a]	10[b]	4-9[b]	4-7[b]	9[b]	8[b]	[52–55, 59, 61, 65]
*tet*S	Protection	−1[a]	−3[a], 10[b]	−5[a], 6[b]		3[b]	4[b]	9[b], 4[c]	8[b]	[51, 52, 55, 59]
*tet*T	Protection	−1[a], 7[b]				2[b]	4[b]	9[b]	8[b]	[52, 54, 55, 59]
*tet*W	Protection	−2 to 0[a],7[b]	−3 to -2[a], 11[b]	−5 to -3[a], 6[b]	10[b]	5-9[b]	3-8[b]	9[b]	6-8[b]	[52–55, 57, 59, 61, 65, 66]
*tet*X	Inactivation	−2[a],7-11[b]	−2[a]		10[b]	3-9[b]	4-8[b]	9[b], 3[c]	5-8[b]	[51–53, 55, 57, 59, 61, 63]
*tet*Z	Efflux		−3[a], 9[b]	−2[a], 9[b]						[52]
*tet*B/P	Protection		−3[a], 10[b]	−4[a], 7[b]						[52, 55, 59]
Sulfonamides										
*Sul*1	Protection	−2 to 2[a], 8-10[b]	−5 to -2[a], 9[b]	−2[a]	10[b]	4-10[b]	3-9[b]	−1[a], 11[b]	6-10[b]	[52–54, 56–59, 62–66]
*Sul*2	Protection	−2 to 1[a], 8[b]–11[b]	−3 to 2[a], 10[b]	−2[a]	10[b]	3-9[b]	5-8[b]	−1[a], 11[b]	10[b]	[52–54, 56, 58, 59, 62, 63, 65]
*Sul*3	Protection	−2[a]	−3[a], 9[b]			5[b]	4[b]	−1[a], 11[b]	10[b]	[52, 54, 58, 59, 62]
*Sul*A	Protection		−4[a]							[52, 62]
FCA										
*oqx*B	Efflux	−3[a]								[54]
*qnr*A	Protection		−6 to −4[a]	−6[a]						[62, 65]
*qnr*B	Protection		−8[a]	−8[a]		2[b]	3[b]	9[b]	7[b]	[52, 65]
*qnr*D	Protection	−3[a]				4[b]	3[b]	9[b]	7[b]	[52, 54]
*qnr*S	Protection	−1[a]	−5 to -4[a]	−5[a]		3-5[b]	3[b]	9[b]	7[b]	[52, 54, 62, 65, 66]
cfr	Deactivate	−2[a], 6[b]	9[b]							[59, 67]
*cml*A	Efflux	−3 to -1[a], 5[b]	9[b]							[59, 67]
*fex*A	Unknown	−3[a], 5[b]	11[b]		11[b]					[59, 67]
*fex*B	Unknown	−3[a], 5[b]	10[b]							[59, 67]
*flo*R	Efflux	−3[a], 5[b]								[59, 67]
*acr*A	Efflux	−3[a]								[54]
*acr*B	Efflux	−3[a]								[54]
MLSB										
*vga*A	Efflux									[68]
*vga*C	Efflux									[68]
*vga*D	Efflux									[68]
*vga*E	Efflux									[68]

Table 1 (continued)

ARGs	Resistance mechanism	Animal production				Wastewater treatment plant				References
		WW/MS	FM	CM	LS	IF	EF	B	RB	
*erm*A	Protection	−2 to 0[a]					5[b]			[60, 68]
*erm*B	Protection	0[a], 7-11[b]	10-11[b]		10[b]	4-6[b], 3[c]	3-4[b], 1[c]	10[b]	9[b]	[52, 54, 59, 63, 66, 68–70]
*erm*C	Protection	−2 to -1[a]	7[b]			3[b]	4[b]	10[b]	9[b]	[52, 54, 59, 70]
*erm*E	Protection	−1[a], 7[b]								[59]
*erm*F	Protection	11[b]				2[b]	3-5[b]			[56, 63, 70]
*ere*A	Deactivate	9[b]								[63, 71]
*mef*A	Efflux	11[b]								[63]
*Lnu*A	Deactivate	−1[a]								[68]
Aminoglycoside										
aph	Deactivate	−1[a]								[54]
*aad*A	Deactivate									
*aad*D	Deactivate	−1[a]								[54]
aac	Deactivate	−1[a]								[54]
*str*A	Deactivate							−3[a]		[58]
*str*B	Deactivate							−3[a]		[58]
Beta Lactamase										
*mec*A	Protection						2[b]			[60]
*bla*vim	Deactivate					1[c]	2[c]			[69]
*bla*TEM	Deactivate					4[b]	2[b]	−2[a]		[58, 66]
*bla*SHV	Deactivate							−2[a]		[58]
*bla*CT-X-M	Deactivate							−1[a]		[58]
blaOX-A58	Deactivate		−6[a]							[64]
ampC	Deactivate						−9[a]			[71]
Vancomycin										
*van*A	Protection					4[b], 1[c]	−10[a], 4[b], 2[c]			[56, 69, 71]

The data are expressed as log values (gene copies/16 s rRNA copies, gene copies/g dry solid, gene copies/ml water, or gene copies/ng DNA).

WW Wastewater/manure slurry, *FM* fresh animal manure, *CM* composted manure, *LS* lagoon biosolids, *IF* influent, *EF* effluent, *B*, wastewater treatment plant (WWTP) biosolids, *RB* recycled biosolids, *FCA* fluoroquinolone, quinolone, florfenicol, chloramphenicol, and amphenicol resistance genes. *MLSB* macrolide–lincosamide–streptogramin B resistance

[a] Gene copies/16 s rRNA copies (relative abundance)

[b] Gene copies/g dry solid or copies/mL water (absolute abundance)

[c] Gene copies/ng DNA extracted

Tetracycline resistance (*tet*) genes have been found to have the highest diversity in a variety of waste materials, with a total of 19 reported *tet* genes that include 11 genes encoding efflux pumps, seven genes encoding ribosome protection, and one gene encoding enzymatic inactivation (Table 1). The most frequently reported types of ARGs are those which encode macrolide (e.g., *erm*B), sulfonamide (e.g., *sul*1), and tetracycline (e.g., *tet*A) resistance genes (Table 1). While absolute abundance (gene copies/unit matrix) has been used in many studies, total extracted DNA or copies of 16 s rRNA copies from sample matrices have been used in some studies to normalize the total number of ARG copies to be expressed as relative abundance [51, 69].

The absolute abundance of ARGs has been reported to range from 10^5 to 10^{11} gene copies g^{-1} (d.w.) and from 10^2 to 10^{11} copies mL^{-1} in solid waste materials and wastewater samples, respectively (Table 1). The absolute abundance of species–specific-ARG also varies largely case by case. For example, the sulfonamide resistance (*sul*) gene *sul*1 was the most abundant ARG gene detected in WWTP effluents in

northern China, with an absolute abundance of 10^5 copies mL^{-1} [52]; however, its absolute abundance was four orders of magnitude lower than that detected in the WWTP effluents in eastern China (10^9 copies mL^{-1}) [53]. The relative abundances of four *tet* genes in activated biosolids from a Hong Kong WWTP ranged from 10^0 to 10^4 gene copies ng^{-1} DNA [51]. The relative abundances of chloramphenicol resistance genes and two *sul* genes (*sul*1 and *sul*2) in wastewater from a number of pig, cattle, and chicken farms in China ranged from 10^{-3} to 10^{-2} and from 10^1 to 10^2 copies/16 s rRNA copies, respectively [54, 67]. Clearly, comparing studies with ARG results expressed as absolute abundance with those expressed as relative abundance is difficult without knowing the levels of total DNA or 16sRNA in a sample. Therefore, a standardized culture-independent ARG analysis is essential for meaningful comparisons of ARG abundance from different investigations.

The observed statistically significant positive correlations between antibiotics and resistance genes in different waste materials suggest that antibiotics exert a selection effect on resistant bacteria and the corresponding ARGs [35, 67, 68]. For example, the relative abundances (10^{-6} to 10^0 copies/16 s rRNA copies) of 14 *tet* and three *sul* genes in manure and wastewater samples from a swine facility were found to be positively correlated with the concentrations of the antibiotic [35].

Soils Amended With Waste Materials

Among all of the environmental compartments, soil is the major receiver and reservoir of ARB and ARGs. First, soil is a major reservoir of antibiotics naturally produced by the indigenous microorganisms [75]. Furthermore, extensive use of antibiotics can result in their eventual release into the soil environment due to land application of waste materials that often have elevated antibiotic concentrations [75]. Elevated antibiotic concentrations in soils can potentially create a selective pressure on both ARB and ARGs in those soils [75]. Land application of waste materials with elevated levels of ARB and ARGs may further enhance their absolute abundance in soils.

Recent research has provided ample evidence that land application of waste material can significantly elevate the abundance of ARGs in soils. For example, the relative abundances (10^{-6} to 10^{-3} copies/16 s rRNA copies) of six *tet* genes increased by one to two orders of magnitude in paddy surface soils (0–5 cm) with long-term (9–33 years) manure application compared to soil without manure application (10^{-7} to 10^{-3} copies/16 s rRNA copies) [76]. The high relative abundance of ARGs (10^{-3} to 10^{-1} copies/16 s rRNA copies) in the manure led to significantly higher relative abundance of ARGs in manure-amended soils compared to the control soils. The authors of this study also reported that the relative abundances of four *tet* genes (*tet*A, *tet*M, *tet*Q, *tet*W) positively correlated with the concentrations of tetracyclines in soils (R^2 ranged from 0.45 to 0.52, $p < 0.01$) , as well as with soil pH (R^2 ranged from 0.54 to 0.62, $p < 0.05$) and soil organic matter contents (R^2 ranged from 0.47 to 0.58, $p < 0.01$). The results of this study indicate that both antibiotics and soil properties play an important role in the selection of ARGs in soils [76]. As such they are consistent with those of another study in which the relative abundances of three *tet* genes, *tet*G, *tet*L, and *tet*B(P), were found to be one to two orders of magnitude higher in soils with 6-year-long land applications of fresh or composted swine manure than in the control soils without manure application (10^{-5} to 10^{-4} copies/16 s rRNA copies) [55]. In another study a total of 149 unique ARGs were detected in swine manure-amended soils, and the diversities of the ARGs were threefold higher in the manure-amended soils than in the control soils [77]. The abundances of the top 63 unique ARGs were significantly enriched in the manure-amended soils, at an overall median enrichment of 192-fold, compared to control soils [77]. In Finland, which has the fourth lowest use of veterinary antibiotics per production animal among the 25 European Union countries, only two- to sevenfold increases in the relative abundances of *tet*M, *sul*1, and *bla*OXA-58 were detected in soils receiving manure from animals treated with lower levels of antibiotics compared to those in the investigations discussed in the preceding text [64]. Antibiotics are only allowed in Finland for therapeutic purposes, so antibiotic concentrations in manure are low. Nevertheless, the results of this study demonstrate that although the enrichment of ARGs is much lower in soils amended with manure from animals which only received antibiotics for therapeutic purposes, reducing the use of antibiotics in animal production does not appear to completely prevent the ARG enrichment in the manure-amended soils [64].

Wastewater irrigation and the application of biosolids may also elevate the abundance of ARGs in soils. For example, the relative abundances of three *sul* and ten *tet* genes in long-term (>30years) wastewater-irrigated soils in China were one to two orders of magnitude higher than those in the non-irrigated soils (not determined to 10^{-3} copies/16 s rRNA copies) [78]. The authors of this study also found that the relative abundance of ARGs were positively correlated with the concentrations of oxytetracyclines, sulfadiazine, and sulfamethoxazole in the soils. These findings are consistent with those of another study which showed that the relative abundances of *sul*1 and *sul*2 in soils irrigated for 100 years with wastewater were one to two orders of magnitude higher than those in the non-irrigated soils (10^{-5} copies/16 s rRNA copies) [79]. In a laboratory study, the absolute abundance of *sul*2 increased by fivefold in soils following repeated irrigation (60 days) with secondary wastewater effluent compared to the control soils [56]. To the contrary, in a study examining soils with high absolute abundance of ARGs (10^8 to 10^9 gene copies g^{-1} soil) in the natural soil microbiome, irrigation with wastewater for

6–18 years did not result in a significant enrichment of relative abundance of ARGs compared to the soils irrigated with fresh water [80]. The authors of this latter study suggested that the ARB in wastewater were not able to compete with the abundant native soil microbiome or survive in the soil environment and, therefore, did not significantly contribute ARGs to the native soil bacteria.

A total of 108 unique ARGs were found to be significantly enriched in soils receiving applications of biosolids for >10 years compared to those which did not [81]. Among the 108 ARGs detected in this study, *mex*F [encoding FCA (fluoroquinolone, quinolone, florfenicol, chloramphenicol, and amphenicol) resistance] showed the highest enrichment (up to 3845-fold), while some unique ARGs, such as *flo*R, *aad*E (encoding aminoglycoside resistance), *pikR*2 [encoding MSLB (macrolide–lincosamide–streptogramin B resistance) resistance], *erm*B, and *erm*D were enriched by more than 100-fold. In a different field study, the relative abundances of ten ARGs were found to be significantly elevated up to levels of 10^{-1} copies/16 s rRNA copies in the biosolids-treated plots compared to the control plots, in many of which the ARGs were below the detection limit (absolute abundance = 5 copies g^{-1} soil) [82]. Even 530 days after application of the biosolids, the relative abundance of one macrolide resistance gene (*mph*A) in the biosolids-applied soil was still detectable—at a level sixfold higher than that in the control plot. Because the relative abundance of gene targets was highly variable from year to year, the authors concluded that it was difficult to distinguish whether the enriched gene originated from the contents of the applied biosolids or from native soil bacteria [82].

Environmental Behaviors of ARGs in Soils Amended With Waste Materials

Antibiotic resistance genes, carried by either the bacterial chromosome or mobile genetic elements (e.g., plasmids), can be present either in the host cells of resistant bacteria or in the environmental media [83]. Free forms of ARGs can be released into environmental media from the host cells, either by the active secretion of resistant bacteria or by the rupture of dead cells [84]. Following their release into the soils, multiple factors, including soil physicochemical properties and biological conditions, could affect and/or determine the multitude of potential environmental behaviors of the ARGs, such as sorption/desorption, transformation, transport, and gene transmission. The possible fate of ARGs in soils receiving land application of waste materials is illustrated in Fig. 1.

Sorption and Persistence of ARGs Introduced to Soils Receiving Land Applications of Waste Materials

The sorption of DNA to soil can be significantly influenced by many factors, such as soil organic matter content, soil solution chemistry, and surface properties of the organic and inorganic components of the soil [85–87]. A batch experiment showed that the sorption of DNA (solid mass 10 mg, solution volume 1 mL, DNA initial concentration 100 μg mL^{-1}) in a natural soil (andosols) gradually decreased from 100 to 25% when the pH of the soil slurry increased from 2 to 5 and that it was relatively constant at 20–25% between pH 5 and 9 [88]. Because the isoelectric point of DNA is around pH 5, the relative amount of negative charges on the DNA molecule would increase at higher pH, resulting in decreased sorption of the DNA onto the andosols. The authors of the same study also showed that DNA sorption to soil particles was 75% when the ionic strength (for $MgCl_2$) was 640 mM at pH 6.7–7.1 [88]. One explanation for this effect is that the high levels of Mg^{2+} in the soil solution may have reduced the repulsive force between the negatively charged DNA molecule and the negative charges on soil particles by acting as bridging cations [85]. These results indicate that environmental relevant conditions, such as normal soil solution pH range (6–8) and soil solution ionic strength (<10 mM), are unfavorable for DNA sorption onto soil particles.

ARGs can persistent in soils for extended periods. A field study has shown that the relative abundances of *sul*1 and *sul*2 decreased one order of magnitude (from 10^{-6} to 10^{-5} and from 10^{-5} to 10^{-4} copies/16 s rRNA copies, respectively) in a soil during the first 10 days after swine manure application [89]. However, after 10 days, the relative abundance of *sul*1 (10^{-6} copies/16 s rRNA copies) remained at a stable level until 175 days, while that of *sul*2 further declined by another one order of magnitude (from 10^{-6} to 10^{-5} copies/16 s rRNA copies). These results are consistent with those in another field study in which the relative abundance increased by 40 and 58% for *sul*1 and by 67% and 107% for *sul*2, respectively, in loamy sand and silt loam that had been repeatedly amended with pig manure [90]. Similarly, the half-lives of five ARGs, namely, *erm*B, *sul*1, *tet*A, *tet*W, and *tet*X, ranged from 13 to 150 days and from 31 to 440 days in a biosolids-applied sandy loam and a silty loam, respectively [57]. Therefore, separate from the long-term application of ARG-containing waste materials that are able to continuously introduce ARGs into soils, the persistence of ARGs may also contribute to the accumulation of ARGs in soils.

Transmission of ARGs Among Microorganisms in Soils Receiving Waste Material Applications

There are two pathways of genetic transmission of ARGs: vertical inheritance and horizontal gene transfer

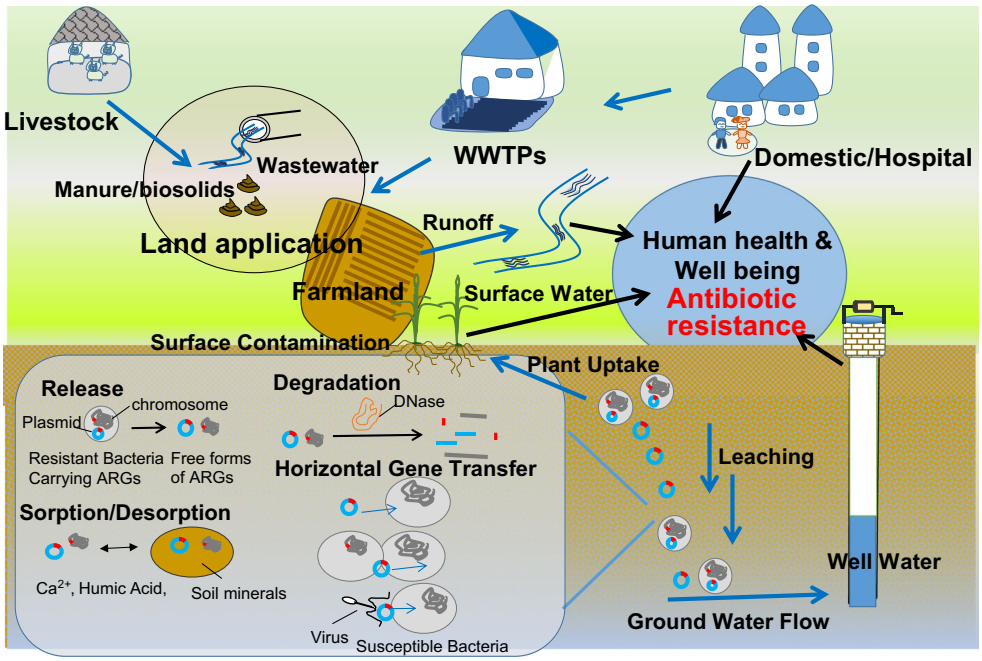

Fig. 1 Land application of waste materials and fate of antibiotic resistance genes (*ARGs*) in soils. *WWTPs* Wastewater treatment plants

[91]. In vertical inheritance (as known as vertical gene transfer), ARGs can be transmitted during the growth of resistant bacteria when they are passed on from parental resistant bacteria to its own progeny (offspring). In horizontal gene transfer (known as lateral gene transfer), susceptible bacteria may acquire ARGs via the intake and recombination of ARGs from other microorganisms that are already resistant or from the environmental media through three major pathways: conjugation, transformation and transduction [92]. The ARGs of parental bacteria may be the result of gene mutation (e.g., following exposure to antibiotics), horizontal gene transfer (from an unrelated resistant microorganism), or vertical inheritance (from its own parent).

Compared to the number of studies on gene transfer by vertical inheritance among microorganisms in waste materials-applied soils, relatively more studies have examined the possibility of horizontal gene transfer by monitoring the abundance of mobile genetic elements (e.g., transposases and introns) in soils [77, 81]. Since these mobile genetic elements play an important role in bacterial genetic exchange, their enrichment in soils may increase the likelihood of horizontal ARG transfer in soils. Land application of waste materials may elevate the abundance of mobile genetic elements in soils. For example, nine mobile genetic elements were enriched by as much as 1000-fold in ARG-polluted soils from farmland adjacent to a swine feedlot in China, and there was a positive correlation ($R^2 = 0.97$) between the relative abundance of *tet* genes and transposase genes [77]. These results suggest that manure application may accelerate the dissemination of ARGs in soils through horizontal gene transfer. Similarly, a positive correlation ($R^2 = 0.47$, $p < 0.01$) between the relative abundance of mobile genetic elements and ARGs was observed in soils to which biosolids were applied for >10 years [81].

Extracellular DNA can be adsorbed by surface-reactive particles in soils and sediments while still retaining protection against degradation by nucleases and maintaining the capacity to transform competent bacterial cells [93]. Consequently, the extended persistence of ARGs via sorption can increase the incidence of potential horizontal gene transfer. The concentration of DNase required to inhibit transformation by bound DNA has been shown to be higher than that required to inhibit transformation by comparable amounts of free DNA [94]. In that same study, considerably more bound DNAase than free DNase was required to inhibit transformation by the same amount of free DNA [94].

Under certain selection pressures (e.g., treatment with antibiotics), ARB can survive while other bacteria are killed, resulting in the proportion of the population of resistant bacteria increasing in a microbial community [91, 95, 96]. As the resistant bacteria in this scenario would be able to pass their ARGs to subsequent generations via vertical inheritance, the selection pressure would lead to an elevated level of relative abundance of ARGs. Importantly, antibiotic exposure is not the only factor that can exert a selective pressure; selective pressure can also be exerted by other factors, such as heavy metals (e.g., As, Cu, Zn, Mn, Co, Ag, Hg, and Ni) and biocides. Co-selection of antibiotic and metal resistance has been widely reported [97–100]. The authors of one study reported

that 17% of bacterial genomes (a total of 2522 genomes located on chromosomes or plasmids) carried both ARGs and biocide/metal resistance genes [101] and also suggested that heavy metal or biocides exposure has the potential to select ARB through chromosomal biocide/metal resistance genes. Some studies have reported higher concentrations of heavy metals and ARGs in land to which waste materials have been applied compared to those in control soils [77, 102]. For example, a positive correlation between Cu, Zn, and As and the relative abundance of total ARGs (R^2 ranged from 0.38 to 0.52, p <0.05) was observed in the manure-amended soils in one study [77]. A long-term (4–5 years) field experiment showed that Cu contamination could significantly affect the abundance, diversity, and mobility potential of a broad spectrum of ARGs in two contrasting agricultural soils (red and fluvo-aquic soil) [103].

The relative abundances of a β-lactamase resistance gene (blaCEP04) encoding *Amp*C increased in soils to which manure from animals not treated with antibiotics was applied [104]. The authors of this study observed that nutrients in the manure induced a bloom of *Pseudomonas* spp. which may be an important biological reservoir of β-lactamase resistance genes in the soil, leading them to highlight the role(s) of unexpected selection pressures (due to manure application) that could increase the abundance of resident ARB in soils and ARGs. On the other hand, the nutrients in manure may also promote the growth and production of β-lactam producers (e.g., a certain group of fungi, actinomycetes, unicellular bacteria) in soils, resulting in a selection pressure [104]. Unfortunately, this study did not examine the antibiotic concentrations in the manure and the manure-amended soil [104]. Therefore, the possibility that trace levels of antibiotics can be generated from the native microbiome of manure or manure-amended soils cannot be completely ruled out, even when the manure is from animals not treated with antibiotics.

Transport of ARGs Into the Aquatic Environment

Numerous studies have examined surface runoff losses and the leaching of contaminants, such as nitrate [105], phosphate [106], pesticides [107], and antibiotics [108], from agricultural soils amended with waste materials. For example, in one study the relative surface runoff losses of chlortetracycline, monensin, and tylosin were <5% of the total amount applied to the soil in manure [108], suggesting that the antibiotics were retained in the soil profile with the potential to exhibit continued pressure on the soil microbiome. Likewise, ARGs applied to agricultural fields through land application of waste materials can move into groundwater through leaching or into surface water via surface runoff. The transport behavior of ARGs can be mitigated by sorption, but under unfavorable sorption conditions, ARGs can be transported over a considerably long distance [109, 110]. For example, the transport of antibiotic resistance plasmids in quartz sand-packed columns was found to be significantly inhibited at high ionic strength (0.05 M for $CaCl_2$), a condition which enhances ARG sorption [110]. The same study also showed that following a reduction in ionic strength, the resistance plasmids were desorbed, demonstrating that ARG sorption by soils is a reversible process. These results suggest that water irrigation and rainfall, which can reduce the ionic strength of the soil solution, can potentially enhance the transport of ARGs. In a rainfall simulation study [111], the absolute abundances of *tet*Q and *erm*B were one to two orders of magnitude higher in surface runoff from manure-amended plots than in runoff from the control plots. Thus, surface runoff can act as a non-point source of ARGs pollution in the environment.

The absolute abundances of *tet*O, *tet*X, and *tet*M in soil samples collected from farmlands adjacent to swine feedlots were detected at up to 10^7 copies g^{-1} soil at a depth of 60–80 cm [58]. In this study, the abundance of ARGs at this soil depth were one to two orders of magnitude lower than those in top soil (0–20 cm). The presence of ARGs in deep soil layers may result from vertical transport, but this study did not report the abundance of ARGs in the same layer of a soil without manure application, making it difficult to draw a definite conclusion on the potential for downward ARG movement along soil profiles [58]. To the contrary, another study showed that the absolute abundance of ARGs in both soils at a depth of 5–20 cm and in the receiving water of the stream immediately down gradient of a field amended with manure, sawdust, and straw mixtures did not increase compared to those before land application [112]. The authors suggested that ARG transport to the aquatic systems may depend on characteristics of the waste materials applied and the soils, as well as the application approach [112].

If ARGs reach the aquifer, the groundwater flow may further carry them to a larger area. One important consequence of elevated levels of ARGs in groundwater could be their impact on well water, which is a very important drinking water source in many countries. Up to 1.5×10^4 copies ng^{-1} DNA of *tet*Z and *tet*Q were detected in groundwater wells adjacent to a manure lagoon in a pig production farm, while no ARGs were detected in a background monitoring well [113]. Similarly, many communities also rely on surface water as drinking water sources. The authors of one study reported a positive correlation between the relative abundance of riverine *sul*1 and both the size of animal feeding operations upstream ($R^2 = 0.35$, $p < 0.001$) and the treatment capacity of wastewater treatment ($R^2 = 0.34$, $p < 0.001$); these results suggest that surface water may represent a key dissemination pathway of ARGs [114]. The levels of ARGs in South Platte River samples collected from sites impacted by animal feeding operations or/and waste water treatment plants were two to three orders of magnitude higher than those in samples from pristine sites [114].

Association of ARGs With Plants Grown in Soils Amended With Waste Materials

Plant surfaces provide a natural habitat for a variety of bacteria, yeast, and fungi as phyllosphere microorganisms [115]. Plants are also hosts to a variety of endophytic microorganisms [116]. Microorganisms associated with plants can be potential hosts of ARGs and through the consumption of ARB-contaminated plant products, humans can ultimately be exposed to these ARGs. Reports of elevated levels of ARGs in soils amended with waste materials have led to concern about the association between ARGs and the edible portions of plants and that this association will result in a potential negative impact on human health [117, 118]. One possible pathway leading to an association between plants and ARGs is through plant surface contamination. As plants are close to the ground, contamination may occur by soil resuspension, rain splash, and animal disturbance [119]. The other possible pathway is through plant root uptake. Research has shown that DNA fragments of 120–3000 nucleotides in length could be actively absorbed by *Arabidopsis thaliana* root cells [120]. If an ARG were to be carried by DNA fragments within this length range, it could potentially be taken up by plants. However, this pathway is only speculation and has to be further tested by research on plant uptake of ARGs. The passive uptake of antibiotics with water by plants grown in manure-amended soils and soils irrigated with wastewater has also been reported [121–123]. It is therefore possible that antibiotics taken up by plants may introduce a selective pressure on antibiotic-resistant endophytic bacteria and result in a yet further increased abundance of ARGs associated with plants. More studies are needed to investigate this notion.

The limited number of reports published to date suggests the possibility of an elevated association of ARGs with plants grown in soils with elevated levels of antibiotics and ARGs. In one comparative study, the relative abundances of *sul*1, *tet*G, *tet*C, *tet*A, and *tet*M detected in leaves of lettuce grown in soils with the higher levels of antibiotics (100–200% higher) were three to four levels of magnitude higher than those with the relatively lower levels of antibiotics; the abundance of ARGs in the former was also three to four orders of magnitude higher than that in the latter [124]. A separate study also detected three or four orders of magnitude higher relative abundances of ten ARGs (*tet*W, *tet*M, *tet*B, *tet*Q, *sul*1, *sul*2, *erm*A, *erm*B, *cat*I, and *cat*II) in the stem, leaf, fruit, and seed of bell peppers grown in cow manure-applied soils compared to soils with reduced levels of ARGs [125]. More investigation is needed to enable more definitive conclusions to be drawn on the impacts of the land application of waste materials on the association of ARGs with plants, the extent of ARGs entering the food chains, and the subsequent effects on human health.

ARGs in the Air Near Soils Amended with Waste Materials

Antibiotic resistance genes can also reach humans through the inhalation of aerated solids (aerosols) containing ARB or ARGs originating from contaminated soils after land application. For example, the absolute abundances of *sul* and *bla*SHV were higher (up to 750 and 870 copies m^{-3}, respectively, in air samples) in air samples collected near three conventional farms than in those collected at an organic beef cattle farm [126]. However, the authors of this study did not compare the ARG abundance in air samples collected upwind and downwind. The same study reported that the bacteria isolated from the air samples near the three conventional farms showed more resistance to certain antibiotics compared to those collected at organic locations. A number of microorganisms can survive by forming resistant spores under harsh environmental conditions [127]. In addition, non-spore-forming species may enter into a viable but not culturable state. One study showed that antibiotic pressure can induce a viable but not culturable state in *Staphylococcus aureus* growing in biofilms [128]. These strategies allow the bacteria to survive for a much longer time, possibly enabling these ARB to be transported for long distances in a windblown environment. Thus, more studies are needed to gain a better understanding of this subpopulation of ARB and the fate of their corresponding ARGs.

On-site Waste Material Treatment for the Elimination/Reduction of ARGs

Before a waste material is applied to land, it is common practice to pre-treat it to destroy pathogens and remove other organic contaminants [129]. Since antibiotics can create a selective pressure on resistant bacteria and ARGs, management approaches that can reduce the release and spread of antibiotics in the environment may not be efficacious in terms of reducing the abundance of ARGs in the waste materials because the environmental behaviors of ARGs could be very different from that of antibiotics due to DNA being a macromolecule (biopolymers) and antibiotics being small molecules. In host cells, ARGs can amplify via gene replication that accompanies the proliferation of resistant bacteria, which might occur even in the absence of antibiotics, resulting in the hereditary and horizontal transfer of the ARGs to a greater population of bacteria. Efficient waste material management approaches for the removal of ARGs have yet to be identified.

Pretreatment of manure

Some manure management approaches can remove resistant bacteria. For example, multidrug-resistant bacteria were reduced by >90% by co-digestion of dairy manure and waste

milk under anaerobic conditions [130]. According to the U.S. Food and Drug Administration's (FDA) Food Safety Modernization Act, manure management is acceptable for land application if the numbers of pathogens (e.g., *Salmonella sp.* and *Listeria sp.*) are reduced to below standardized levels. Of all the manure management approaches, composting is a common practice to prevent and minimize pathogens in manure and may also reduce the levels of antibiotics [131]. However, since ARGs can be released from the cells upon rupturing, any reduction in the abundance of resistant bacteria during composting does not necessarily indicate a reduced abundance of ARGs in manure. The change in ARG abundance during composting has also been studied directly [65, 132]. In one of these studies [65], the relative abundances of *tet*M, *tet*O, *tet*Q, and *tet*W in swine manure decreased by 38.5 to 93.5% after 6 days of composting, and these decreases correlated with a reduced concentration of tetracycline; in contrast,, the relative abundances of *sul*1 and *sul*2 increased by approximately 100 and 350%, respectively, during the same period. These results indicate that proliferations of unique ARGs could occur during composting [65]. A separate study found that the relative abundances of five *tet* genes (*tet*A, *tet*C, *tet*G, *tet*L, and *tet*X) and two *sul* genes (*sul*1 and *sul*2) in swine manure significantly increased by one to two orders of magnitude from 10^{-5} to 10^{-3} copies/16 s rRNA copies to 10^{-3} to 10^{-1} copies/16 s rRNA copies after 32 days of composting [133]. Another study showed that of the 158 ARGs investigated, the abundances of 94 ARGs significantly reduced while 23 were significantly enriched during composting of a swine manure for 6 days [134]. The contradictions in outcomes regarding the changes in the abundance of ARGs during manure composting suggest that the efficiency of composting in the removal of ARGs prior to land application depends on the species of ARGs. Composting conditions such as pH and temperature in both the thermophilic and mesophilic stages affect the levels that antibiotics are reduced, and perhaps ARG levels also [135]. According to U.S. federal guidelines, two composting approaches, namely, static and tuned, are recommended. Therefore, assessments of U.S. FDA-approved manure management approaches are warranted.

Pretreatment of WWTP wastewater and biosolids

Wastewater or biosolids from WWTP are hotspots for ARGs [72], and the treatment of these hotspots is another opportunity to control ARGs before they are introduced into the environment [136]. Traditional treatments in WWTP are effective for removing resistant bacteria during the disinfection process using UV and chlorination treatments. However, the efficiency of the disinfection process to remove ARGs is variable. In one study, the absolute abundances of four erythromycin resistance genes (*erm*A, *erm*B, *ere*A, and *ere*B) and two *tet* genes (*tet*A and *tet*O) was reduced by three orders of magnitude (initial concentrations ranged from 10^0 to 10^2 gene copies mL^{-1}) during UV treatment of wastewater, indicating the effectiveness of the UV treatment in reducing ARG abundance in municipal wastewater; in contrast, chlorination could not eliminate or reduce the levels of ARGs in the wastewater [137]. In another study, the absolute abundances of *tet*A, *ere*A, and *ermB* fell by 24, 87, and 40%, respectively, with chlorination treatment while there was no significant change in the absolute abundances of *tet*B, *tet*O, *ere*B, and *erm*A; in fact, even an increased dose of chlorination did not improve the reduction rate of ARGs [137]. A recent study at a drinking water treatment plant demonstrated that chlorination can even cause an enrichment of the total abundance of ARGs [139]. There are also some studies suggesting that disinfection processes (UV and chlorination) do not contribute to the reduction of ARGs given that no significant difference in ARGs abundance between pre- and post-disinfected wastewater samples was found, indicating that ARG removal from wastewater is system-specific [140, 141].

The effect of anaerobic digestion of wastewater is ARG-specific. For example, although anaerobic digestion reduced the absolute abundances of *tet*G, *tet*M, *tet*O, *tet*W, *tet*X, *sul*1, *sul*2, *ere*A, *erm*B, *erm*F, and *mef*A in swine wastewater by 37–95% [63], when ARGs were normalized to 16 s rRNA gene, the relative abundances of *tet*G, *tet*M, *sul*1, *sul*2, *ere*A were found to be enriched by 2–530%. The reason why the relative abundance of unique ARGs increased is yet to be identified. It may be that the ARB carrying these genes proliferated under selective pressure during anaerobic digestion or that these ARGs horizontally transferred to other species. Regarding anaerobic digestion of biosolids, the absolute abundances of *tet*A, *tet*W, *sul*1, *sul*2, and *bla*TEM decreased by 30–98% during anaerobic digestion [142]. In another study, Metagenomics analysis revealed that 21 of the ARGs in biosolids were removed at a >90% efficiency during anaerobic digestion. However, *aad*A, *mac*B, and *sul*1 were found to be enriched by 26–33% during the thermophilic anaerobic digestion process, while *tet*M, *sul*1 and genes encoding *ere*A were enriched by 40–60% during the mesophilic anaerobic digestion process [143]. Whether anaerobic digestion could reduce the abundance of ARGs in wastewater and biosolids is still inconclusive, but the process appears to be species-specific. Further research is needed on this subject.

Knowledge gap

Environmental risk assessment of ARGs associated with waste material

To date, there have been few documented approaches to assess the risk of ARGs. A previous review summarized the research

needed to enable human health risk assessment for environmental development and the transfer of antibiotic resistance [144]. ARGs are ubiquitous in soils, however, the long-term and repeated land application of waste material contaminated with ARGs can significantly increase the levels of ARGs, as described in preceding sections of the current review. Pertinent to any assessment of the environmental risk of ARGs associated with waste material is the key question of whether the elevated levels of ARGs in the environment can increase the likelihood of those soil bacteria acquiring resistance and, therefore, contributing to the development and spread of ARB that could become potential human pathogens.

There are natural barriers which may prevent the spread of ARGs. With respect to bacteria, potential human pathogens may acquire ARGs from soils containing ARB [145]. However, ARB introduced by land application of waste material may not able to compete with indigenous organisms [146]. One study showed that a 15-month period of land application of biosolids did not increase the percentage of antibiotic-resistant culturable bacteria above the background levels [147]. Another study showed that 20 years of applying Class B biosolids to soil did not increase either the overall microbial population in the soil or bacterial or viral pathogens [148]. With regards to DNA, soil through sorption can mitigate ARG transport, but as the process is reversible subsequent mobilization would be a concern. Soil also is abundant in DNase enzymes, which could degrade desorbed DNA, thus limiting its persistence in soils and its long-range transport.

The frequency of horizontal gene transfer in soils receiving applications of waste materials needs to be further evaluated and validated. In one study, the transferability of *sul*1 was analyzed in soil amended with manure from sulfadiazine-treated pigs, by capturing resistance plasmids from soil communities into *Escherichia coli* [149]. Manure from pigs not receiving sulfadiazine treatment was used as a control treatment. During a 6-month experimental period, the transfer frequency of resistant plasmids was up to two orders of magnitude higher in the soil amended with sulfadiazine-treated manure than in the control soil, indicating that the application of waste materials may potentially encourage horizontal gene transfer in soils through the excessive loading of resistant bacteria and ARGs [149]. On the contrary, some studies have shown a lack of evidence of horizontal gene transfer in soils [150, 151]. For example, in a greenhouse study, horizontal gene transfer from a model strain (*Bacillus subtili*) to soil bacteria was not observed during 6 months of cultivation [151]. Competent cells are essential for DNA to across their cell membranes, however, it is still questionable if there is an adequate number of natural competent cells in soils [152, 153]. Without a selective pressure, resistant bacteria are at a competitive disadvantage to sensitive bacteria [154]. One potential benefit of natural competence is that starving bacteria can take up exogenous DNA as a nutrient for competitive survival in environments containing limited nutrients [155, 156]. Conjugative plasmid transfer between bacteria was observed under an oligotrophy environment [157]. However, if an oligotrophy condition is important, the incidence of horizontal gene transfer would be low in the rhizosphere where vast amount of nutrients is excreted by plants. In addition, spatial separation of donor and recipient cells in the soil may also limit the possibility of horizontal gene transfer [158]. Further research is needed to identify the factors influencing the competency of indigenous soil bacteria, and improved quantitative models for horizontal gene transfer are needed.

On the other hand, some studies have suggested that it is the shift in the bacteria community—rather than horizontal gene transfer—that plays the dominant role in the enrichment of ARGs in soils. In one study, mobile genetic elements only explained 4.1% variation of ARGs in soils after the application of biosolids [81] and, similarly, in another study mobile genetic elements only contributed to 2.6% of the variation in ARGs during biosolids composting [159]. Five bacterial phyla, including *Chloroflexi*, *Planctomycetes*, *Firmicutes*, *Gemmatimonadetes*, and *Bacteroidetes*, were found to be significantly correlated with the relative abundance of ARGs in soil. In another study, six genera, including *Flavobacterium*, *Poriferibacter*, *Bacteroides*, *Acinetobacter*, *Actinobaculu*, and *Streptococcus*, were found to be correlated with ARGs in a WWTP, indicating that these genera played important roles in shaping the ARG profiles [160]. More data are required to understand the biological reservoir of ARGs in both waste material and waste material-applied soils.

Best Land Application Management Approaches for Waste Materials

Since common waste material pretreatment approaches are not efficient in reducing ARGs in waste materials, improved practices in waste materials land application strategies might be another option to control the dissemination of ARGs in soils. The transport of ARGs in soils can largely depend on a farmer's approach(es) to the land application of waste material. For example, a recent study has shown that the injection of animal manure into the soil subsurface, a best land application management approach originally designed for reducing surface runoff of N and P [161], can reduce the surface runoff of antibiotics applied with dairy manure [162]. A comparison of the abundance of ARGs in runoff and manure land applied via broadcast, incorporation and injection methods after rainfall simulation revealed that the broadcast method resulted in significantly higher *erm* genes in runoff than did the incorporation and injection methods. However, the authors found no clear trend in ARG levels in the soil, likely because different host cells may respond differently to the soil environments created by various land application methods [111]. More information is needed to evaluate whether the manure

subsurface injection approach is also an effective approach for minimizing and preventing the spread of ARB and ARGs in a wide range of soils amended with different types of waste materials.

It has also been shown that narrow grass hedges as buffer strips along waste material-applied fields are effective in reducing the levels of tylosin and *erm*(B) in runoff, suggesting that the implementation of buffer strips could be another management practice to control the transport of antibiotics, ARB, and ARGs in surface runoff of waste material-amended soils [163]. More investigation is needed to test whether this practice would be universally effective for different ecosystems. Also Adding other amendments when waste materials are applied to soils may also help reduce ARG abundance in waste material-applied soils. For example, when calcined eggshell waste was added to soils, the relative abundance of ten ARGs (*tet*W, *tet*M, *tet*B, *tet*Q, *sul*I, *sul*II, *erm*A, *erm*B, *cat*I, and *cat*II) fell by two to four orders of magnitude compared to the untreated soils [125]. Other materials that can be used as co-amendments with waste materials for controlling enrichment of ARGs in the waste material-affected soils need to be further investigated.

Conclusions

Waste materials contain high levels of antibiotics, ARB, and ARGs. Soil application of waste materials may introduce these emerging organic contaminants into the environment. Although the majority of antibiotic resistant bacterial infection is due to the overuse of antibiotics in the clinical environment, the contribution of elevated levels of antibiotics, ARB, and ARGs in the environment to the development of antibiotic resistance in potential pathogens should not be ignored. A better understanding of the environmental behaviors of ARGs in soils is critical to the control of ARG pollution. There is the potential risk that human health-related pathogens are becoming more resistant due to elevated levels of ARGs in the environment. Current strategies incorporating traditional waste material management approaches are not promising in reducing ARGs in waste materials. More importantly, agricultural activities may introduce selective pressure on natural soil-borne ARGs. It is unrealistic to expect to be able to eliminate completely ARGs in waste material-amended soils since ARGs could also be naturally produced. The question to be asked is at which levels should ARGs in soils be considered as safe for the ecosystem and humans. At the present time there are no environmental regulation standards for ARGs in the environmental matrixes. More studies are needed for environmental risk assessment. Information on the biological reservoirs of ARGs, the transferability of ARGs, and the possibility of ARGs entering food chain are also essential. Effective land application management approaches for waste materials are needed to prevent and minimize the accumulation and dispersion of ARGs in waste material-amended soils.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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