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Review

The effects of biochar on antibiotic resistance genes (ARGs) removal during different environmental governance processes: A review

Binbin Shao [a], Zhifeng Liu [a,*], Lin Tang [a,*], Yang Liu [b], Qinghua Liang [a], Ting Wu [a], Yuan Pan [a], Xiansheng Zhang [a], Xiaofei Tan [a], Jiangfang Yu [a]

[a] College of Environmental Science and Engineering, Hunan University and Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, PR China
[b] School of Minerals Processing and Bioengineering, Key Laboratory of Biometallurgy of Ministry of Education, Central South University, Changsha 410083, PR China

HIGHLIGHTS

- The applications of biochar in different environmental systems for controlling ARGs were summarized.
- The process and mechanism of ARGs removal promoted by biochar were interpreted and discussed.
- Effects of biochar properties on ARGs removal were highlighted.
- Future challenges of using biochar to control ARGs dissemination were proposed.

GRAPHICAL ABSTRACT

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ABSTRACT

Antibiotic resistance genes (ARGs) pollution has been considered as one of the most significant emerging environmental and health challenges in the 21st century, many efforts have been paid to control the proliferation and dissemination of ARGs in the environment. Among them, the biochar performs a positive effect in reducing the abundance of ARGs during different environmental governance processes and has shown great application prospects in controlling the ARGs. Although there are increasing studies on employing biochar to control ARGs, there is still a lack of review paper on this hotspot. In this review, firstly, the applications of biochar to control ARGs in different environmental governance processes were summarized. Secondly, the processes and mechanisms of ARGs removal promoted by biochar were proposed and discussed. Then, the effects of biochar properties on ARGs removal were highlighted. Finally, the future prospects and challenges of using biochar to control ARGs were proposed. It is hoped that this review could provide some new guidance for the further research of this field.

1. Introduction

The excessive use and abuse of antibiotics in clinic, livestock, and aquaculture have stimulated the rapid emergence and spread of antibiotic resistance genes (ARGs), which has been considered as one of the most significant emerging environmental and health challenges in the 21st century (Fang et al., 2022; Fu et al., 2021a; Shao et al., 2022; Wu et al., 2022). Statistics indicated that the number of deaths from antibiotic-resistant infections was estimated at 7 × 10^5 per year worldwide, and the World Health Organization announced that we are

* Corresponding authors.
E-mail addresses: zhifengliu@hnu.edu.cn (Z. Liu), tanglin@hnu.edu.cn (L. Tang).

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fast running out of treatment options, which could even cause more than 10×10^6 deaths if no effective action is taken (Barancheshme and Munir, 2017; Zheng et al., 2021; Liang et al., 2021). Metagenomic studies revealed that diverse ARGs are widely distributed in various environmental media, including river, lake, ocean, effluent, tap water, soil, sludge, vegetable, and even the air (Lian et al., 2020; Peng et al., 2021; Shao et al., 2021; Zhang et al., 2022c), and they can continuous proliferation and spread via vertical gene transfer (VGT, the resistance genes are inherited from parental cells) and horizontal gene transfer (HGT, unresistant bacteria obtain resistance genes from mobile genetic elements (MGEs) by conjugation, transduction, and transformation), which is increasing at an unprecedented rate (Fang et al., 2022; Guo et al., 2017; Wu et al., 2021; Xie et al., 2022; Zheng et al., 2021). Consequently, the ARGs are considered to be a type of emerging contaminant, and the risk of global dissemination of ARGs has been aggravated via globalization and environmental factors, it is urgent to control and reduce the burden of ARGs in environmental system as well as their impact on human health and ecological environment.

So far, many treatment technologies, including the activated sludge process, disinfection process, composting process, advanced oxidation process, etc., have been introduced to control the proliferation of ARGs in different environmental systems (Ahmed et al., 2020, 2021; Chen et al., 2019; Feng et al., 2019, 2022, Shao et al., 2020a,b; Yan et al., 2019; Yu et al., 2021; Yue et al., 2022). Although these treatment technologies can inhibit the abundance of ARGs to a certain extent by adsorption, degradation, and other mechanisms, the removal efficiency of ARGs is not ideal in general, and sometimes they can promote the spread of ARGs. For example, although the traditional disinfection technology (such as chlorine and ultraviolet) in the wastewater treatment plant can effectively inactivate the resistant bacteria, however, the lysis of the resistant bacteria containing ARGs will release intracellular ARGs, resulting in a significant increase in the concentration of extracellular ARGs in wastewater (Liu et al., 2021c; X. Liu et al., 2021a; Z. Liu et al., 2021a; Shao et al., 2019; Wu et al., 2021; Yang et al., 2022; Zhang et al., 2022a,b,b,c; Zhao et al., 2021). Therefore, how to effectively control the proliferation and dissemination of ARGs in the environment, to restrain the harm of ARGs to the ecology and human health, has become a hot and challenging issue for environmental scientists, which has received many efforts in the past decades.

In the last decade, the biochar, which is a stable carbon-rich product produced by pyrolysis of biomass under anoxic conditions, has been widely studied in different environmental governance processes (including soil remediation, water treatment, aerobic composting, anaerobic digestion, etc.) due to the cost-effective and environmentally-friendly characteristics (Feng et al., 2021a; Tan et al., 2015; Wang et al., 2020a; Zhao et al., 2021). Firstly, the porous structure, large specific surface area (SSA), and abundant active sites endow biochar as an excellent adsorbent, and it can be used to adsorb organic and inorganic pollutants from different environmental media (Tan et al., 2015; Xiao et al., 2018). Moreover, the biochar also can serve as an efficient catalyst by generating persistent free radicals (PFRs) on itself or activating oxidants to produce various active species, thus can degrade organic pollutants, reduce heavy metals, and inactivate pathogenic bacteria (Ruan et al., 2019; Zhang et al., 2021c; Zhu et al., 2020). Besides, the biochar often is used as the carrier or amendment to alter the properties of different environmental systems, thus promoting the removal of various pollutants (Ajeng et al., 2020; Cheng et al., 2022). The multiple roles and impacts of biochar on pollutants endow them great application prospects in different environmental governance processes. Recently, the effects of biochar on the fate of ARGs in different environmental governance processes also has been studied, and these studies indicate that the biochar can affect the environmental properties or/and interact with ARGs to control the proliferation and dissemination of ARGs in the environmental systems (Fang et al., 2022; Ngigi et al., 2020; Wu et al., 2020; Zhang et al., 2021a). For instance, Qiu et al. (2021a) found that the rice stalk biochar and wheat stalk biochar could efficiently reduce the emergence of extracellular and intracellular *sul2* ARGs in soil and hinder its VGT. Zhou et al. (2021) reported that the maize straw biochar could alleviate the proliferation of ARGs in chicken manure composting, and the abundance of ARGs reduced by 98.7%. Xu et al. (2021) reported that the pig manure biochar could restrain the HGT of ARGs and reduce the dissemination of ARGs in the liquid phase by 41.4% during anaerobic co-digestion of pig manure and sewage sludge. These studies manifested that biochar could play a positive role in controlling ARGs in different environmental governance processes. Meanwhile, various mechanisms for the suppression and reduction of ARGs by biochar have been proposed, including (1) biochar could interact with ARGs by adsorption, aggregation, and/or decomposition to prevent the proliferation and dissemination of ARGs; (2) biochar could affect the bacterial community and MGEs, thus restraining the HGT of ARGs; (3) biochar could reduce the co-selective pressure and improve the environmental factors in the environmental system, thus decreasing the abundance of ARGs (Duan et al., 2017; Fang et al., 2022; Fu et al., 2021d; Ngigi et al., 2020; Qian et al., 2019). However, previous studies also have shown that the biochar properties (such as, pore diameter, SSA, heavy metal content, pH, etc.) will change markedly with different raw materials and pyrolysis temperatures, thus impacting the interaction between biochar and ARGs/environmental factors, and changing the fate of ARGs in the environmental systems (Ding et al., 2019; Fang et al., 2022; Fu et al., 2021a; Xiao et al., 2018). These existing results are insufficient to establish the relationship between biochar properties and their impacts on ARGs variation during different environmental systems, which requires more detailed study in the future.

Up to now, there are many review papers that have summarized and discussed the study of biochar in the environmental field. For instance, Tan et al. (2015) reviewed the application of biochar in removing contaminants from water. Wang et al. (2020a) reviewed the stabilization of heavy metal-contaminated soil by biochar. Godlewska et al. (2017) reviewed the application of biochar in composting for contaminants reduction. Xiao et al. (2018) reviewed the multiple and multilevel structures of biochar and their potential environmental applications. These reviews have made great strides to summarize and characterize biochar applications, which significantly promoting the related research. Although many reviews on biochar have been published, none of a critical review has been paid to the effects of biochar on the fate of ARGs during different environmental governance processes and the relevant mechanisms, which is urgently needed. In this review, the applications of biochar to control ARGs in different environmental governance processes were summarized firstly. Then, the related processes, mechanisms, and influence factors of biochar promoting ARGs removal in these environmental systems have been summarized and interpreted. Finally, the prospects and challenges of using biochar to control ARGs were proposed (Fig. 1). This review would inspire some new ideas for the control of ARGs pollutants in the environment by using biochar.

2. Control of ARGs in different environmental systems by using biochar

Based on previous studies, biochar is often used to control ARGs in soil systems, aqueous environments, and solid waste treatment processes (including aerobic composting and anaerobic digestion processes) (Table 1).

2.1. In soil systems

Soil is a vital place for animals, plants, and microorganisms to survive, and the quality of soil plays a significant influence on human health and the ecological environment. However, with the development of society, a large number of pollutants have been discharged into the soil, thus causing a considerable impact on the soil ecological environment (Shi et al., 2022; Yin et al., 2022; Zhang et al., 2022b; Zhi et al.,

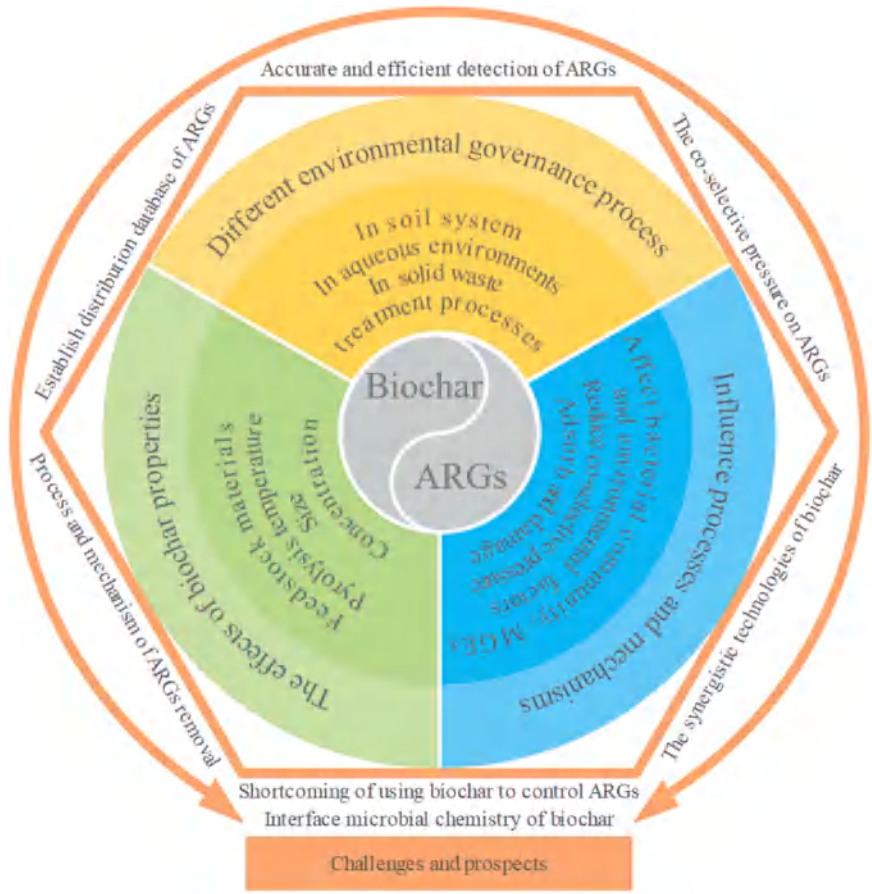

Fig. 1. An overview of the effects of biochar on antibiotic resistance genes (ARGs) removal during different environmental governance process.

2020). Typically, the ARGs are an emerging contaminant and readily get into the soil by manure application, reclaimed wastewater irrigation, and wastewater discharge. The enriched ARGs in soil could rapid expansion, and cause harm to plants and microorganisms (Fu et al., 2021a; He et al., 2021; Kavitha et al., 2018; Zhang et al., 2021a; Zhou et al., 2019). Therefore, the remediation of ARGs contamination in soil is of great significance to food safety and human health. Recently, the application of biochar in soil systems has attracted a good deal of attention, it has been widely recommended that biochar as a soil amendment can enhance carbon sequestration, improve soil quality and nutrient availability, increase the soil aggregate stability and water holding capacity, reduce organic and inorganic pollutants, and change bacterial community structure, thus alleviating the contamination of ARGs in soil and inhibiting ARGs transfer (An et al., 2018; Hu et al., 2022; Kavitha et al., 2018; Li et al., 2019; Ye et al., 2018; Zheng et al., 2021). As Duan et al. (2017) reported that the relative abundances (RAs) of ARGs decreased by 51.8%, 43.4%, and 44.1% in lettuce leaves, roots, and soil after the bamboo biochar was added into the soil-lettuce system, which mainly attributes to the fact that biochar can reduce the abundance of ARGs host bacteria and pathogenic bacteria in the soil. In general, the total ARGs abundance in the biochar-treated soils was significantly lower than those in the compost-amended soils during cultivation (Fig. 2a) (Zhou et al., 2019). In addition to plants, the effects of ARGs dissemination on soil fauna also should not be ignored. Ding et al. (2019) confirmed that biochar (including plant-derived biochar (soybean, bark, rice, and bamboo) and feces-derived biochar (sludge and manure)) could significantly change the ARGs in the collembolan gut by influencing the soil characteristics and the bacterial community of gut. Meanwhile, the higher heavy metal content in biochar, the more conducive to the proliferation of ARGs in the collembolan gut (Fig. 2b). Although most studies have shown that biochar could effectively mitigate ARGs contamination in soil systems to some extent, not all biochar consistently performed the positive impacts. For instance, Chen et al. (2018) found that the rice stalk biochar could decrease the abundance of ARGs and MGEs in non-planted soil but was insufficient to mitigate ARGs and MGEs levels in planted soil and crops (Fig. 2c). This is because planting could influence the effect of biochar on soil antibiotic resistome by altering microbial community compositions. The above studies demonstrate the uncertainties about the effectiveness of using biochar to mitigate ARGs contamination in soil. Hence, more efforts are still needed to further study the impacts of biochar on ARGs behavior in soil, and even exploit modification methods to boost the performance of biochar as a versatile amendment to remediate ARGs contaminated soil (Zheng et al., 2021).

2.2. *In aqueous environments*

Metagenomic studies suggested that diverse ARGs are widely distributed in water environments, including river, lake, ocean, effluent, tap water, and even groundwater. However, the traditional water treatment technologies are not ideal for removing ARGs prior to discharge, reuse, or other applications (Feng et al., 2021b; Lian et al., 2020; Wang et al., 2021b). Recent studies indicated that the biochar could effectively suppress the proliferation and spread of ARGs in aqueous environments by adsorption, decomposition, and other mechanisms (Barancheshme and Munir, 2017; Fang et al., 2022; Fu et al., 2021b; Li et al., 2020; Lian et al., 2020; Liu et al., 2021, 2021b). As reported by Lian et al. (2020), the rice straw-derived bulk-biochar could remove ARGs (*ampC* and *ermB*) from aqueous environments by adsorption, while the nano-biochar not only could adsorb ARGs but also could damage ARGs by oxidation, thus significantly inhibiting the proliferation and transfer of ARGs. Fang et al. (2022) reported that the rice

Table 1
Recent typical studies of using biochar to control ARGs during different environmental governance processes.

Different environmental governance processes		Biochar properties	Removal types and efficiency of ARGs and MGEs	Action mechanisms	Ref.
In soil systems	Contaminated soil-hyperaccumulator system	Maize straw biochar (600 ℃; 5%)	**Types:** 64 ARGs (including aminoglycoside, β-lactam, chloramphenicol, macrolide, fluoroquinolone, multidrug, tetracycline, trimethoprim, vancomycin, and others) and 14 MGEs (including integrons, transposons and insertion sequence common regions) were detected. **Efficiency:** Biochar amendment increased the detected number and absolute abundance of ARGs and MGEs in the soil while decreasing the corresponding number and absolute abundance in plant tissues.	Biochar can improve the environmental factors (carbon sequestration, nutrient/water retention, soil porosity structure) of soil and reduce the selective pressure of Cd and oxytetracycline on the endophytes of the plant, thus mitigating the prevalence of ARGs and MGEs in the soil-plant system.	(Fu et al., 2021a)
	Contaminated farmland soil-potato system	Maize straw biochar (500 ℃; 0.5%)	**Types:** 6 ARGs (namely *sul1, sul2, cat1, cat2, ermA, ermB*) were detected. **Efficiency:** Biochar amendment could effectively decrease the classes and the accumulative abundance of ARGs in the edible parts of potato. The lowest abundance of ARGs was detected in the biochar application treatment, with the accumulative ARGs level of 8.9×10^2 and 7.2×10^2 copies mL^{-1} in potato peel (*sul1* + *cat1* + *ermA*) and tuberous root (*sul1*), respectively.	Biochar played a positive role in relieving the poisonous effect of antibiotics in the root tissue of potatoes. Meanwhile, biochar application could clearly reduce the endophytic antibiotic resistant bacteria in potato peel and tuberous root, thus alleviating the accumulation risk of ARGs in vegetable.	(Jiao et al., 2018)
	Manured soil system	/ (500–600 °C; 1%)	**Types:** 18 ARGs (namely *sul1, sul2, tetG, tetB/P, tetH, tetO, tetW, tetC, cmlA, floR, fexA, cfr, ermA, ereA, aadA, qnrD, qnrS,* bla_{TEM}) and 3 MGEs (namely *intI1, intI2, Tn916*) were detected. **Efficiency:** ARGs dissipated more slowly in the biochar-amended soil than in soil without biochar-amendment.	Biochar amendment contributed to the maintenance of bacterial diversity. Succession of microbial community may have sustained the transfer and resilience of ARGs.	(He et al., 2021)
	Manured soil- ryegrass system	Wheat stalk biochar (650 ℃; 2%)	**Types:** 11 ARGs (namely *tetW, tetM, tetO, tetQ, tetH, sul1, sul2, gyrA, qnrA, ermF*) were detected. **Efficiency:** ARGs abundance was significantly lower under the biochar−ryegrass treatments, in which the ryegrass dissipates ARGs in soil, and biochar helps accelerate this process. Dissipation rate of these ARGs (ARGs/16 S rRNA): *tetW* (0.0083 ± 0.0008), *tetM* (0.0662 ± 0.009), *tetO* (0.0025 ± 0.0008), *tetQ* (0.0001 ± 0.00002), *tetH* (0.0003 ± 0.0001), *sul1* (0.05186 ± 0.003), *sul2* (0.0706 ± 0.031), *gyrA* (0.0125 ± 0.006), *qnrA* (0.00003 ± 0.00001), *ermF* (0.0112 ± 0.004).	Biochar can change the environmental factors (the specific surface area of soil, total root length, and average surface area of roots), which explained 95.46% of variation in the dissipation of ARGs. Soil pH and trace elements exerted weaker effects on ARGs after the application of biochar.	(Liang et al., 2017)
	Manured soil-pakchoi system	Crop straw biochar (500 °C; 2%)	**Types:** 283 ARGs (including β-lactam, chloramphenicol, macrolide, multidrug, sulfonamide, tetracycline, vancomycin and others) and 12 MGEs (including 8 transposases and 4 integrases) were detected. **Efficiency:** The fresh biochar significantly elevated the number and abundance of ARGs in the manured soil, but did not show such effect under pakchoi cultivation. The presence of aged biochar caused a marked reduction of ARGs in the planted soil.	It was through altering microbial composition that aging process affects the profile of ARGs in biochar-amended soil.	(Cheng et al., 2021)
	Manured soil- lettuce system	Bamboo biochar (600 °C; 2%)	**Types:** 8 ARGs (namely *tetG, tetC, tetW, tetX, sul, sul2, ermF, ermX*) and 1 MGEs (namely *intI1*) were detected. **Efficiency:** The RAs of ARGs were reduced by 51.8%, 43.4%, and 44.1% in lettuce leaves, roots, and soil, respectively with the addition of biochar. However, the application of	The reduction of Firmicutes due to biochar treatment was the main factor responsible for the removal of *tet* ARGs in leaves. Biochar led to the disappearance of human pathogenic bacteria, which was significantly correlated with the RAs of *erm* ARGs.	(Duan et al., 2017)

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Table 1 (*continued*)

Different environmental governance processes	Biochar properties	Removal types and efficiency of ARGs and MGEs	Action mechanisms	Ref.
		biochar had no significant effects on *intI1*.		
Organic fertilizer amended soil- pakchoi system	Rice straw biochar (500 °C; 0.5%)	**Types:** 285 ARGs (including β-lactam, chloramphenicol, macrolide, multidrug, sulfonamide, tetracycline, vancomycin and others) and 10 MGEs (including 8 transposases and 2 integrases) were detected. **Efficiency:** After biochar amendment, the abundance of ARGs was significantly decreased in non-planted soil, but no significant decrease of ARGs was found in rhizosphere and phyllosphere.	Biochar maintained or increased soil microbial diversity is potentially more useful in mitigating ARGs spread and accumulation.	(Chen et al., 2018)
Organic fertilizer amended soil- pakchoi system	Composted pig manure biochar (400–450 °C; 1.2%)	**Types:** 285 ARGs (including β-lactam, chloramphenicol, macrolide, multidrug, sulfonamide, tetracycline, vancomycin and others) and 10 MGEs (including 8 transposases and 2 integrases) were detected. **Efficiency:** The total ARGs and MGEs abundance in the biochar-treated soils were significantly lower than those in the compost-amended soils during cultivation.	Biochar application can enhance the adsorption of antibiotics and immobilization of heavy metals, thus providing a co-selective pressure to facilitate the dissipation of to ARGs. Meanwhile, biochar could change the microbial community composition thus reducing the HGT of ARGs.	(Zhou et al., 2019)
Unconventional water resources (reclaimed water or piggery wastewater) irrigated soil-rhizobox system	Wheat straw biochar (1.0%)	**Types:** 37 ARGs (including 35 *tet* genes and 2 *sul* genes) and 10 MGEs (including 8 transposases and 2 integrases) were detected.**Efficiency:** With piggery wastewater irrigation, biochar addition dramatically reduced the RAs of ARGs in rhizosphere soil and bulk soil samples after 30 days. However, after 60 days, biochar addition dramatically increased the RAs of ARGs in samples. Biochar addition resulted in the abundance was not notably decreased or increased in rhizosphere soil samples irrigated with distilled water. And for the RAs of ARGs in soil samples collected at different sampling time under reclaimed water irrigation, biochar addition had no statistically significant change on it. The behavior of MGEs was similar to ARGs behavior in soils but dissimilar to ARGs in root endophytes.	Biochar addition reduced the concentrations of antibiotics and then decreased abundance of ARGs. Furthermore, biochar addition increased the abundance of MGEs showing a strong relationship with ARGs in soil samples. Apart from this, the higher abundance of non-dominant phyla due to biochar addition (Firmicutes, Bacteroidetes and Proteobacteria) contributed to the higher abundance of ARGs.	(Cui et al., 2018)
Farmland soil-lettuce system	Maize straw biochar (300–700 ℃; 0.5%)	**Types:** 2 ARGs (namely *sul1*, *sul2*) were detected. **Efficiency:** With biochar amendment, the relative abundances (RAs) of *sul* genes in soil significantly decreased to the level of 10^{-7} (ARG copies per 16 S rRNA copy), which was three orders of magnitude lower than that of control soil. The RAs of *sul* genes in lettuce reduced to a level of 10^{-9} and 10^{-10} in the roots and old leaves, respectively, and no detection of *sul* genes in the new leaves.	Biochar application can prompt soil sulfonamides dissipation, change soil properties, impede the transfer and colonization of resistant bacteria, thus decreasing the RAs of *sul* genes in soil and plant.	(Ye et al., 2016)
Farmland soil- fauna system	soybean, bark, rice, bamboo, sludge, manure biochar (500 °C; 0.5%)	**Types:** 285 AGRs (including β-lactam, chloramphenicol, macrolide, multidrug, sulfonamide, tetracycline, vancomycin and others) and 10 MGEs (including 8 transposases, 2 integrases) were detected. **Efficiency:** Compared with the controls, most biochar amendments did not affect the RAs of ARGs in the collembolan guts and soil, except for the manure-derived biochar amendment, which significantly enriched the ARGs abundance in the collembolan guts. Meanwhile, the plant-derived and feces-derived biochars had different effects on the compositions of gut and soil associated ARGs.	Biochar amendment significantly altered the ARGs compositions of the collembolan guts and soils. Biochar containing elevated doses of heavy metals contributed to proliferation of ARGs in the collembolan guts. Biochar Changed the gut microbial community, MGEs and soil properties explained 84% of the total ARGs variations in the collembolan guts.	(Ding et al., 2019)
				(Qiu et al., 2021a)

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Table 1 (*continued*)

Different environmental governance processes		Biochar properties	Removal types and efficiency of ARGs and MGEs	Action mechanisms	Ref.
	Unmanured agricultural soil-pakchoi column system	Rice stalk Wheat stalk biochar (400 ℃; 1%, 3%)	**Types:** 2 ARGs (namely *sulI*, *sulII*), 1 MGEs (namely *intI1*) were detected. **Efficiency:** Two biochar amendment could effectively inhibit the occurrence of *sul2* gene in soil, however, it did not work out with the *sul1* gene and *intI1*.	Biochar addition can enhance dissolved organic matter export from soil, change its composition and impede the vertical transport of sulfamethazine, thus affecting the vertical transfer of ARGs.	
	Undisturbed soil column system	Maize straw biochar (300–700 ℃; 0.5%)	**Types:** 5 ARGs (namely *tetW, tetM, tetQ, catI, cmlA*) were detected. **Efficiency:** Biochar significantly stimulated the ARGs vertical dissipation in the soil column, the ARGs level in the 0 ∼ − 0.2 m top soil in the biochar application declined to 1.8E6 ± 1.4E5, 1.1E6 ± 6.1E5, 5.5E5 ± 3.1E4, 3.4E5 ± 1.6E4, and 1.7E5 ± 1.1E4 copies g^{-1} for *tetM, tetQ, tetW, catI, and cmlA*, respectively, and the five corresponding ARGs level in the bottom soil layer (−4 ∼ −5 m) was 4.2E4 ± 5.1E3, 2.3E4 ± 9.2E3, 1.6E4 ± 3.1E3, 2.1E4 ± 3.4E2, and 1.1E4 ± 4.2E3 copies g^{-1}, respectively.	Biochar contributed to the improvement of the physical structure and biochemical properties of the soil, creating favorable conditions for the colonization and reproduction of the indigenous bacteria, thus mitigating the vertical transfer risk of ARGs in the soil system.	(Sun et al., 2019)
In aqueous systems	Deionized water	Rice straw biochar (400, 700 °C)	**Types:** A model extracellular DNA (eDNA, encoding ARGs) and 2 ARGs (namely *ampC, ermB*) were detected. **Efficiency:** About 60.0% and 31.3% of the adsorption were completed for eDNA by nano700 and nano400, respectively. However, the eDNA adsorption on bulk-BCs was much slower and plateaued at nearly 12 h. Meanwhile, only adsorption was observed on bulk-BCs, while not only adsorption but also fragmentation of these eDNA molecules was found to occur on nano-BCs. Further, nano-BCs exhibited high adsorption for both *ampC* and *ermB* than the bulk ones. More strikingly, the amplification of *ampC* and *ermB* was greatly impeded by nano-BCs.	Bulk-biochar can remove ARGs by only adsorption, however, nano-biochar can remove ARGs by both adsorption and fragmentation (t hydroxyl radicals produced from persistent free radicals (PFRs) on nano-BCs played a major role in the damage of ARGs).	(Lian et al., 2020)
	Distilled water	Pine sawdust biochar (300, 500, 700 °C; 50 mg/mL)	**Types:** The conjugative transfer frequency of plasmid RP4 (conjugative transfer is the dominant pathway for HGT of ARGs between bacteria). **Efficiency:** The conjugative transfer of ARGs between bacteria changed significantly after treatment with dissolved biochar. When the pyrolysis temperature was 300 °C, the transfer frequency was 2.96-fold higher than the control; when it was 500 °C, there was no significant difference in the transfer frequency compared to the control. When the pyrolysis temperature was 700 °C, the transfer frequency decreased significantly.	The dissolved biochar could significantly reduce the Cu(II) concentration in water by the humic acid-like components which may be an effective mean to eliminate the heavy metal facilitation of the conjugative transfer of ARGs between bacteria. Meanwhile, the dissolved biochar also could cause the death of harmful bacteria in water thus decreasing the conjugative transfer efficiency of ARGs.	(Liu et al., 2021c)
	Ultrapure water	Ce modified excess sludge biochar (400 °C; 3.3 g/L)	**Types:** Ampicillin resistance gene (ARG_{Amp}) was detected. **Efficiency:** When the initial concentrations of ARG_{Amp} were 8.25, 16.60, 32.85 and 41.43 mg/L, the removal rates by adsorption (Ce-biochar) were 62.29%, 41.25%, 30.38% and 28.37%, respectively. The removal rates including the adsorption and the persistent free radicals (PFRs) reaction (Ce-biochar (PFRs)) were 84.49%, 55.32%, 48.07%, and 36.63%, respectively. After adding H_2O_2, the removal rates up to 100%, 99.53%, 78.94% and 55.93%, respectively.	ARG_{Amp} can be removed by biochar adsorption, meanwhile the PFRs in biochar and the generated·OH can directly destroy the ARG_{Amp} structure through oxidation.	(Wu et al., 2021)
	Ultrapure water	Rice straw, peanut shell biochar (300, 700 °C; 2, 4, 8 mg/mL)	**Types:** The horizontal transfer efficiency of eARGs. **Efficiency:** The transformation efficiency decreased markedly with increasing biochar concentration from 2	For the low temperature biochar, the transformation inhibition was mainly attributed to biochar dissolutions, while it was mainly caused by biochar solid particles for the high temperature	(Fang et al., 2022)

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Table 1 (*continued*)

Different environmental governance processes		Biochar properties	Removal types and efficiency of ARGs and MGEs	Action mechanisms	Ref.
			to 8 mg/mL. Compared with the biochar-free treatment, the transformation efficiency was reduced by 44.1%, 79.4%, 15.5%, and 30.7% in the presence of 2 mg/mL RS300, RS700, PS300, and PS700, respectively. When the biochar concentration increased to 8 mg/mL, the transformation efficiency was reduced by more than 80%, even to 90% with the 700 ℃ biochar.	biochar. In biochar dissolutions, the condensation and aggregation of plasmids, the decrease in cell membrane permeability, and the reduction in bacteria survival were responsible for the inhibitory effect on the ARGs HGT. For biochar solid phase, the adsorption of plasmids on biochar played an important role in inhibiting the transformation process of ARGs.	
	heavy metals and dye co-contaminated wastewater	β-cyclodextrin modified rice straw biochar (500 °C; 10 g/L)	**Types:** 8 ARGs (namely tetW, tetM, sul1, sul2, blaTEM, oxa1, qnrS, ermB) and 1 MGEs (*intI1*) were detected. **Efficiency:** When only under the stress of HMs, β-BC performed 78.99%, 82.99%, 31.189%, 48.381%, 19.753%, 52.031%, 33.834%, and 43.84% removal efficiency of total RAs for *tetW, tetM, sul1, sul2, bla_{TEM}, oxa1*, qnrS, and *intI1*, respectively. Under the co-stresses of HMs and dye with β-BC amendment, the removal efficiency of total RAs reached to 88.168%, of which, *tetW, tetM, sul1, sul2, bla_{TEM}, oxa1, qnrS*, and *intI1*, got up to 68.524%, 55.075%, 64.563%, 90.860%, 87.490%, 72.790%, 77.437% and 49.219%, respectively.	Biochar simultaneously promoted the removal of heavy metals and dye, which leads to the decline of ARGs abundance. Second, the *intI1* was greatly deposited to about 50% under biochar treatment, which contributed to the less transfer of ARGs between bacteria then higher removal of ARGs. Third, the biochar may alter the community structure that reduce the generation of ARGs.	(Wu et al., 2020)
	Unfiltered effluent	Sewage sludge biochar (600 °C)	**Types:** 5 ARGs (namely *ermB, sul1, sul2, qnrS, blaC_{TX-M}*) and 1 MGEs (*intI1*) were detected. **Efficiency:** Selected ARGs and MGEs present on the free-floating extracellular DNA fraction and on the total environmental DNA (i.e., both extra/intracellular) were removed at 85% and 97% by sewage-sludge biochar, respectively.	Sewage-sludge biochar displayed the highest adsorption efficiencies for ARGs and MGEs removal from treated effluents.	(Calderon-Franco et al., 2021)
In solid waste treatment systems	Manure composting: pig manure (PM) and duck manure (DM)	Rice straw (RSB), mushroom (MB) biochar (500 ℃; 5%)	**Types:** 12 ARGs (namely *tetB, tetL, tetM, tetW, tetQ, tetX, sul1, sul2, cfr, cmlA, fexA, floR*) were detected. **Efficiency:** The effect of biochar addition on the average removal value of ARGs depended on the type of biochar and manure. In pig manure compost, MB addition increased the average removal value of ARGs (18.79%~131.62%), while RSB addition decreased. And both biochar additions had a negative influence on the average removal value of ARGs in duck manure compost.	Biochar addition reduced the percentage of bioavailable arsenic in composting, and some ARGs had positive correlations with bioavailable arsenic level.	(Cui et al., 2017)
	Swine manure composting	Sawdust, corn stover, peanut hull biochar (600 ℃; 6%, 12%, 24%)	**Types:** 12 ARGs (namely *tetC, tetG, tetX, tetM, tetO, sul1, sul2, gyrA, qnrD, oqxB, aac(6′)-Ib, ermB, ermF*) were detected. **Efficiency:** *tetM, tetO, ermB* were reduced in all tested groups; *tetC, tetG, tetX, sul1, sul2, ermF, qnrD, aac(6′)-Ib* were mostly reduced under low level biochar addition but increased under higher level biochar addition; *gyrA* increased under medium biochar addition and reduced in other groups; *oqxB* remained comparatively stable throughout the composting process. The average removal rates of ARGs in the control, low, medium and high levels biochar addition groups were 0.24 logs, 0.52–0.72 logs, –0.52–0.18 logs and –0.19–0.21 logs, respectively.	The addition levels of biochar are more important than types of biochar on the removal of ARGs in the composting. Low level biochar addition could enhance the removal of ARGs, while medium level biochar addition would foster the propagation of ARGs.	(Wang et al., 2018)
	Chicken manure composting	Rice straw (RSB), mushroom (MB) biochar (500 ℃; 5%)	**Types:** 12 ARGs (namely *tetA, tetB, tetL, tetM, tetW, tetQ, tetO, tetX, sul1, sul2, fexA, floR, cmlA, cfr, fexB*) and 1 MGEs (*intI1*) were detected. **Efficiency:** MB addition resulted in a higher removal rate than that in the control composting experiment.	Biochar addition affected the bacterial community in composting, in the RSB group, the higher abundance of host and pathogenic could enhance the dissemination and abundance ARGs. Meanwhile, Biochar could change the concentration of bioavailable heavy	(Cui et al., 2016)

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Table 1 (*continued*)

Different environmental governance processes		Biochar properties	Removal types and efficiency of ARGs and MGEs	Action mechanisms	Ref.
			However, RSB addition yielded opposite results. The average removal rate was 0.86, 0.61 and 1.49 log units in control group, RSB group and MB group, respectively.	metals (Cu, Zn and As), which has high correlation with ARGs.	
	Vermicomposting of dewatered sludge	Corncob, rice husk biochar (1.25%, 5%)	**Types:** 12 ARGs (namely *ermF, tetM, tetX, sul1, sul2*) and 1 MGEs (*intI1*) were detected. **Efficiency:** Compared to the control group, *ermF* and *tetX* gnens significantly decreased by 0.32–0.45 times and with corncob biochar treatment. Rice husk biochar (5%) could effectively decrease *sul1* and *sul2* genes. However, the abundance of the i*ntI1* gene in all treatment groups with biochar addition increased, with significant increases of 0.47–1.35-fold in corncob biochar treatments. In addition, the abundance of the i*ntI1* gene increased with biochar concentration.	Biochar could enhance the abundances of bacterial and eukaryotic, meanwhile, biochar addition may directly affect microbial community structures, moreover, biochar could change the environmental factors (electrical conductivity, organic matter, ammonia, etc.) of composting, thus affecting the behavior of ARGs.	(Kui et al., 2020)
	Chicken manure composting	Bamboo biochar (600 °C; 5%, 10%, 20%)	**Types:** 12 ARGs (namely *tetC, tetG, tetW, tetX, sul1, sul2, dfrA1, dfrA7, ermB, ermF, ermQ, ermX*) and 1 MGEs (*intI1*) were detected. **Efficiency:** The RAs of most ARGs (*tetC, tetG, tetW, tetX, sul2, drfA1, drfA7, ermB, ermF, ermQ, ermX*) and *intI1* declined by 21.6–99.5%, whereas *sul1* increased by 7.5–17.7 times. The average RAs reductions with 0%, 5%, 10%, and 20% biochar were 0.85, 1.05, 1.08, and 1.15 logs, respectively.	Biochar significantly decreased the level of bioavailable heavy metals (Cu, Zn), thereby reducing the co-selecting ARGs. Meanwhile, biochar changed the environmental factors (especially temperature) of composting, thus decreasing the RAs of ARGs.	(Li et al., 2017)
	Chicken manure composting	Maize straw biochar (400 °C; 5%)	**Types:** 8 ARGs (namely *tetW, tetO, tetC, tetG, sul1, sul2, ermB, ermC*) and 1 MGEs (*intI1*) were detected. **Efficiency:** The average abundance of total ARGs distinctly reduced to 0.05 and 0.02 copies/bacterial cell, the removal rates of total ARGs was 92.5% and 98.7%, for control and biochar groups, respectively. The abundances of *intI1* in biochar treatment almost completely removed after composting.	Biochar addition could reduce the potential host bacteria (such as *Lactobacillus* and *Fastidiosipila*), suppress the abundance of MGEs, thus mitigating the accumulation and spread of ARGs during composting.	(Zhou et al., 2021)
	Cattle manure wastewater anaerobic digestion	Bamboo biochar (600 ℃; 5, 20, 50 g/L)	**Types:** 23 ARGs (namely *tetA, tetB/P, tetC, tetE, tetG, tetM, tetO, tetQ, tetT, tetW, tetX, sul1, sul2, dfrA7, aac(6′)-Ib-cr, qnrA, parC, qnrC, qnrS, ermB, ermF, ermQ, ermX*) and 4 MGEs (namely *intI1, intI2, ISCR1, Tn916/1545*) were detected. **Efficiency:** The addition of 50 g/L biochar increased the absolute abundances of ARGs by 1.88 times compared with control. The addition of 5 g/L and 20 g/L biochar decreased the RAs of five (*tetX, dfrA7, qnrA, aac(6′)-Ib-cr, qnrS*) and seven (*tetX, sul2, dfrA7, qnrA, aac(6′)-Ib-cr, qnrS, ermX*) ARGs, respectively. 5 g/L biochar only reduced the RA of *ISCR1*, whereas it had no significant effects on the RAs of *intI1*, *intI2*, and *Tn916/1545*. The addition of 20 g/L biochar made the RAs of *ISCR1* and *Tn916/1545* at a low level, the RAs of ISCR1 was 0.89 log lower than that in control.	Biochar mainly affected the distribution of ARGs by influencing the RAs of Firmicutes and Proteobacteria, and the influence of 20 g/L biochar was greater than that of 5 g/L. Meanwhile, biochar decreased the abundance of MGEs, thereby reducing the risk of ARGs spreading.	(Sun et al., 2018)
	Swine manure anaerobic digestion	Sawdust biochar (500 °C; 15 g/L)	**Types:** 8 ARGs (namely *tetA, tetC, tetG, tetM, tetO, tetT, tetX, tet34*) and 1 MGEs (i*ntI1*) were detected. **Efficiency:** Biochar addition generally mitigated ARGs enrichment. under high tetracycline (50 mg/L) pressure, the reduction efficiency of antibiotic efflux pump-associated ARGs (*tetA, tetC, tetG*) was 45.6–59.8%, the reduction	Biochar not only beneficial for depressing the tightly-bound extracellular polymeric substances secretion behaviors by microbes, but also alleviated ARGs accumulation via microbial community modulation by reducing the potential host of *Firmicutes* enrichment under antibiotics exposure.	(Wang et al., 2021a)

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Table 1 (*continued*)

Different environmental governance processes	Biochar properties	Removal types and efficiency of ARGs and MGEs	Action mechanisms	Ref.
		efficiency of antibiotic target protection (*tetM, tetO, tetT*) and inactivation-associated ARGs (*tetX*) was 62.5–91.6%. The content of *intI1* also decreased significantly.		
Anaerobic co-digestion of pig manure and sewage sludge	Dehydrate pig manure biochar (235 °C; 4 g/L)	**Types:** 23 ARGs (namely *tetA(58), otr (A), tetB(P), tetT, tetA(46), tetM, sul1, sul2, sul3, sul4, evgS, msbA, rpoB2, mtrA, macB, oleC, lmrD, parY, novA, kdpE, mupA, mupB, ileS*) were detected. **Efficiency:** The total abundance of ARGs in the biochar group was 34.9% less than control group in the liquid phase. Especially at 14% total solids, the total abundance of ARGs decreased from 2.14×10^3 to 1.95×10^3 reads with the addition of biochar, among which the reduction rates of *etB(P), sul1, rpoB2, macA, mupA* and *mupB* were more prominent.	Biochar with large specific surface area and pore structure can adsorb ARGs and also expand the living space of microorganisms. In addition, biochar dissolved molecular phenols and organic acids produced by dissolution may inhibit conjugative transfer to a certain extent. Thus. the decreased bacterial mobility might further hinder the contact of microorganisms, limit the exchange of genetic elements and reduce HGT of ARGs.	(Xu et al., 2021)
Anaerobic digestion of swine wastewater	Swine manure biochar (300, 500, 700 °C; 20 g/L)	**Types:** 15 ARGs (namely *tetM, tetG, tetX, tetA, tetC, sul1, sul2, ermF, ermX, ermB, qnrA, qnrS, ampC,* $bla_{CTX\text{-}M}$, bla_{TEM}) and 2 MGEs (namely *intI1, intI2*) were detected. **Efficiency:** After biochar addition, the total RAs of ARGs decreased from 0.17×10^{-4} to 0.13×10^{-5}. For *sul1* and *sul2*, the RAs were 21.4% and 24.0% lower than that in control, the RAs of *tetM/ermB* decreased by 23.6–27.7%. Biochar mitigated 74.8% of the MGEs abundance.	Biochar addition could reshape microbial community, reduce the abundance of ARGs host bacteria, thus restraining the HGT and abundance of ARGs.	(Wang et al., 2022)
Anaerobic digestion of swine manure	Rice husk biochar (300 °C; 5%, 10%, 15%, 20%)	**Types:** 13 ARGs (namely *sul1, sul2, parC, aac(6′)-Ib-cr, ermB, ermF, tetW, tetG, tetX,* $bla_{CTX\text{-}M}$, bla_{TEM}, *aac(6′)-II, aadA1*) and 1 MGEs (*intI1*) were detected. **Efficiency:** The abundance of most detected ARGs including *parC, tetX,* $bla_{CTX\text{-}M}$, bla_{TEM}, *ermF* were reduced more than 85%, *aac(6′)-Ib-cr, ermB* and *tetW* exhibited a removal efficiency of about 20–40%, in the treatments with various dose of biochar. The total RAs of these detected ARGs were reduced by 36.6%, 51.4%, 51.7%, 45.8% and 40.6% in B-C, B-5%, B-10%, B-15% and B-20%.	The *intI1* had direct effects on ARGs, while biochar indirectly affected ARGs by changing *intI1* and microbial structure. Meanwhile, ARGs abundance were significantly negative correlated with methane production, thus biochar addition could increase methane yield and reduce ARGs dissemination.	(Yang et al., 2021)

Notes:

1. In some studies, the specific values of the removal efficiency of ARGs did not be exhibited;
2. If the types of detected ARGs is too big, the specific names of ARGs were not listed, only the category names were listed;
3. The abbreviations of different ARGs: tetracycline ARGs—*tet, otr,*; sulfonamide ARGs—*sul, dfr*; quinolone ARGs—*gyr, qnr, oqx, par, aac(6′), nov*; macrolide ARGs—*erm, ere, mac, ole, lmr*; aminoglycoside ARGs—*aac, aph, aad, kdp*; β-lactam ARGs—*bla*; vancomycin ARGs—*van*; trimethoprim ARGs—*dfr*; chloramphenicol ARGs—*cat, cml, fos, flo, fex, cfr*; ampicillin ARGs—*amp*; mupirocin—*mup, ile*; multidrug—*evg, msb, rpo, mtr*; integron—*intI*; transposon—*Tn*; insertion sequence common region—*ISCR*.

straw biochar showed a stronger inhibitory effect on the transformation of ARGs-carrying plasmids in the aqueous environment. The biochar dissolutions produced under low pyrolysis temperature could induce intramolecular condensation and even agglomeration of plasmids, while the biochar solids formed under high pyrolysis temperature could adsorb plasmids, thus effectively hindering their transformation into competent bacteria (Fig. 3a). During the wastewater treatment system, Wu et al. (2020) found that the β-cyclodextrin functionalized biochar could remarkably decrease the RAs of all detectable ARGs (including *tetW*, *tetM*, *sul1*, *sul2*, *blaTEM*, *oxa1*, *qnrS*, *ermB*, and *intI1*) during the wastewater anaerobic reaction system under the co-stresses of heavy metals and dye. The biochar could promote the removal of heavy metals and dye and reduce the co-selective pressure effect on ARGs, which is one of the reasons. Besides, the biochar might alter the bacterial community structure that reducing the antibiotic-resistant bacteria proportion, which was another driver of ARGs removal. Although the relevant research is limited, biochar has shown great potential in removing ARGs pollutants from aqueous environments.

2.3. In solid waste treatment processes

Aerobic composting is an efficient technology of solid waste treatment, which is often used in the treatment and resource recovery of manure, sludge, and so on. During the aerobic composting, the microorganism can rapidly decompose organic matter and transform them into humic substances under aerobic conditions. Meanwhile, the contained contaminants are degraded, inactivated, and stabilized (Godlewska et al., 2017; Yue et al., 2022). Recent studies indicated that aerobic composting is also an efficient technology for reducing the content of ARGs in manure and sludge, and the addition of biochar could perform a significant impact on the reduction of ARGs abundance (Fu et al., 2021d; He et al., 2022; Qian et al., 2018). This is because the

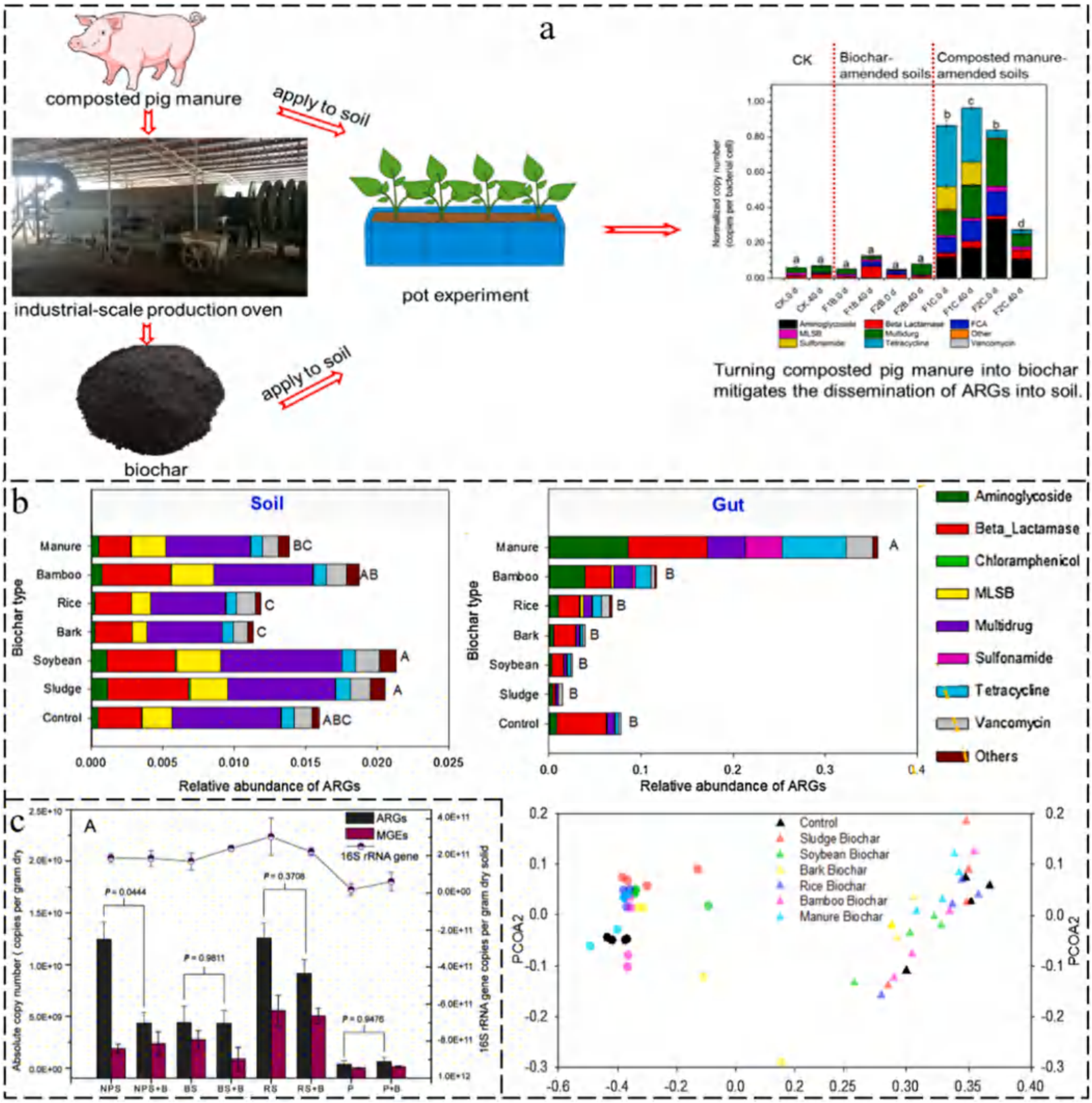

Fig. 2. (a) Normalized copy numbers of ARGs in soils after the addition of pig manure-based composts and pig manure biochar (Zhou et al., 2019). (b) The RAs of ARGs in the collembolan gut microbiota and soil samples from the different biochar treatments, the effects of different biochar types on the bacterial community compositions of the gut microbiota and soil microbiota (Ding et al., 2019). (c) Absolute abundances (AAs) of ARGs and MGEs in soil-crop system after the addition of biochar (Chen et al., 2018).

abundant functional groups and porous structure of biochar could improve the environmental conditions of composting (such as temperature, pH, moisture, etc.), accelerate the stabilization and removal of contaminants, promote microbial activity and the humification process, thus effectively decreasing the abundance of ARGs and controlling the proliferation and dissemination of ARGs (Chen et al., 2022; Cui et al., 2016, 2017; Guo et al., 2019; Li et al., 2017; Qian et al., 2019; Zhou et al., 2021). As reported by Li et al. (2017), the bamboo biochar performed a remarkable effect on ARGs removal in chicken manure composting. The results showed that the RAs of most ARGs (*tetC*, *tetG*, *tetW*, *tetX*, *sul2*, *drfA1*, *drfA7*, *ermB*, *ermF*, *ermQ*, and *ermX*) and *intI1* decreased by 21.6–99.5%. The average RAs reduction with 0%, 5%, 10%, and 20% biochar addition were 0.85, 1.05, 1.08, and 1.15 logs, respectively (Fig. 3b). The change of composting conditions (mainly the temperature, C/N ratio, and pH) and the decrease of bio-available heavy metals by biochar were responsible for the reduction of ARGs abundance. Qiu et al. (2021b) reported that the total abundances of ARGs (*ermB, ermC, sul1, sul2, tetC, tetG,* and *tetO*) declined by 17.6% after the addition of maize straw biochar compared to control during sewage sludge composting. The analysis revealed that the biochar could decline the abundance of

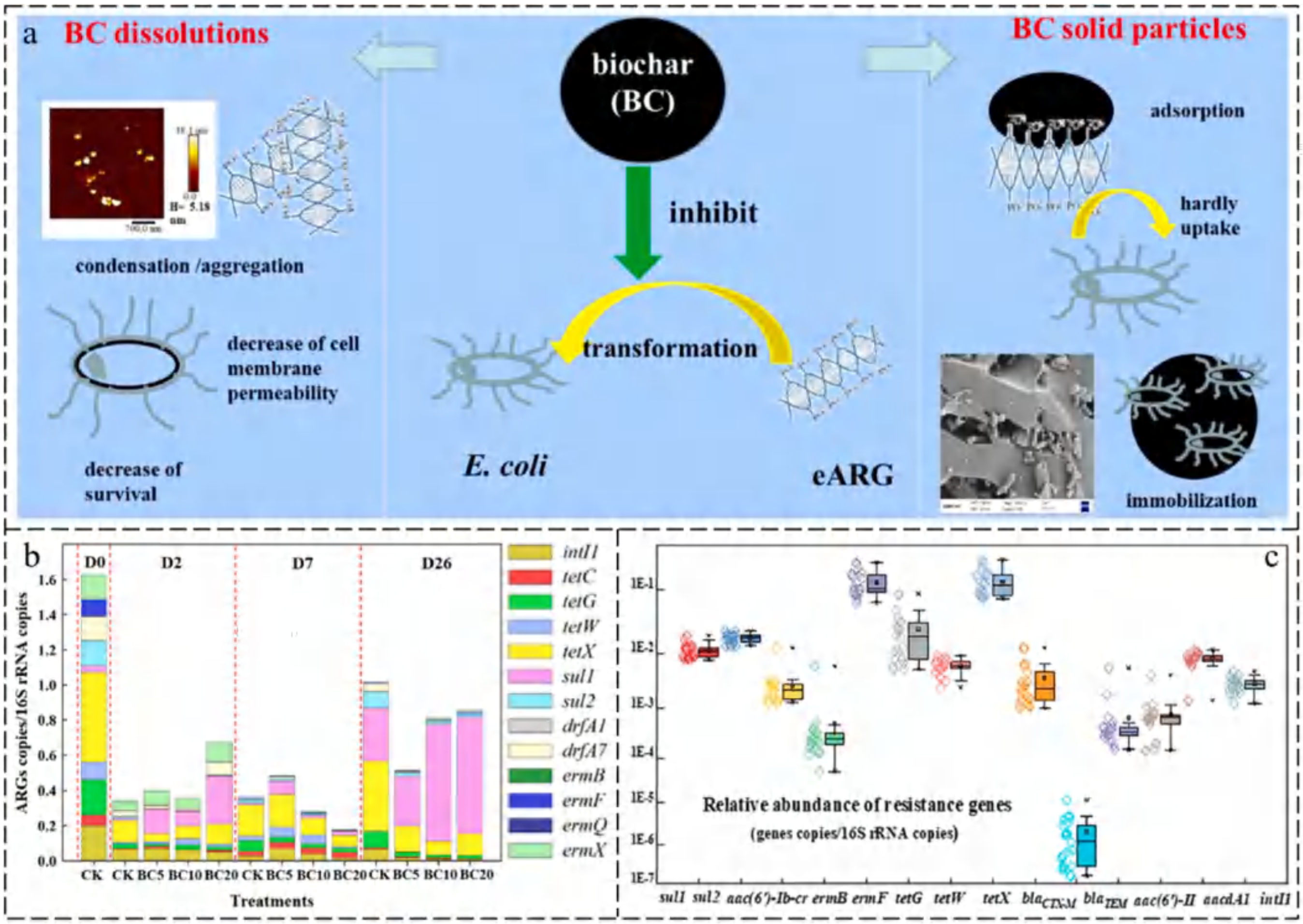

Fig. 3. (a) The effects of biochar on ARGs at different pyrolysis temperatures (Fang et al., 2022). (b) Effects of bamboo charcoal on ARGs during chicken manure composting (Li et al., 2017). (c) The RAs of ARGs during anaerobic digestion of swine manure (Yang et al., 2021).

bacterial pathogens. Besides, the co-selective pressure of Cu, Zn, and Pb on ARGs was less in biochar treatments, thus reducing the abundance of ARGs during composting. These studies indicated that the different biochar and parameters have different effects on ARGs behavior in different composting systems, but the introduction of biochar contributes to the reduction of most ARGs and restrains the ARGs transfer during composting.

Similar to aerobic composting, anaerobic digestion is also an efficient solid waste treatment method and is frequently studied in resource recovery and pollution control of organic waste (Xu et al., 2021; Yang et al., 2021). The microbial community during anaerobic digestion can produce biogas by using the organic matter. Meanwhile, the contaminants (including organic pollutants, heavy metals, pathogenic bacteria, etc.) in the matrix will be degraded, stabilized, and inactivated, and the residue can be applied as an organic fertilizer (Wang et al., 2021a, 2022; Xu et al., 2021). Previous studies have shown that biochar can improve the efficiency of biogas production, accelerate organic pollutant degradation and heavy metal stabilization, immobilize microbial cells, and facilitate electron transfer between interspecies during the anaerobic digestion (Wang et al., 2022). Recently, the related researchers also reported that the addition of biochar to anaerobic digestion system has significant effects on controlling the proliferation and dissemination of ARGs. Just as Yang et al. (2021) reported, the abundances of *parC*, *tetX*, $bla_{CTX\text{-}M}$, bla_{TEM}, *aac(6')-Ib-cr*, *ermB*, *tetW* reduced effectively during anaerobic digestion of swine manure with the assistance of rice husk biochar. Compared to the control group, the removal efficiency of total RAs of these detected ARGs increased by 14.8%, 15.1%, 9.2%, and 4.0% in 5%, 10%, 15%, and 20% biochar addition, respectively (Fig. 3c). The increased removal efficiency of ARGs should attribute to the effects of biochar on *intI1* removal and the change of microbial community. Sun et al. (2018) also reported the impacts of bamboo biochar on ARGs (including tetracycline, sulfonamide, fluoroquinolone, and macrolide ARGs) and MGEs (i.e., *intI1, intI2, ISCR1* and *Tn916/1545*) during anaerobic digestion of cattle farm wastewater. The results displayed that the RAs of 5/13 ARGs decreased at 5 g/L biochar, while the total RAs of ARGs decreased dramatically at 20 g/L biochar. Biochar mainly affected the ARGs profiles by affecting the host bacteria abundance (i.e., *Firmicutes* and *Proteobacteria*) and MGEs abundance (particularly *intI2* and *ISCR1*), and the effects of high concentration biochar (20 g/L) were more than that of low concentration biochar (5 g/L). The above results demonstrate that biochar can decrease the abundance of ARGs in the anaerobic digestion, thus reducing the environmental risk of ARGs spread.

3. Influence processes and mechanisms of biochar on ARGs removal

The versatility of biochar will cause multiple effects on the environmental system when it is applied to different environmental governance processes, thus affecting the behavior of ARGs in the environmental system (Fu et al., 2021a; Xiao et al., 2017) (Fig. 4). According to previous studies, the addition of biochar will have a great impact on the bacterial community, MGEs, and other environmental factors, thus changing the fate of ARGs (Cheng et al., 2021; Sun et al.,

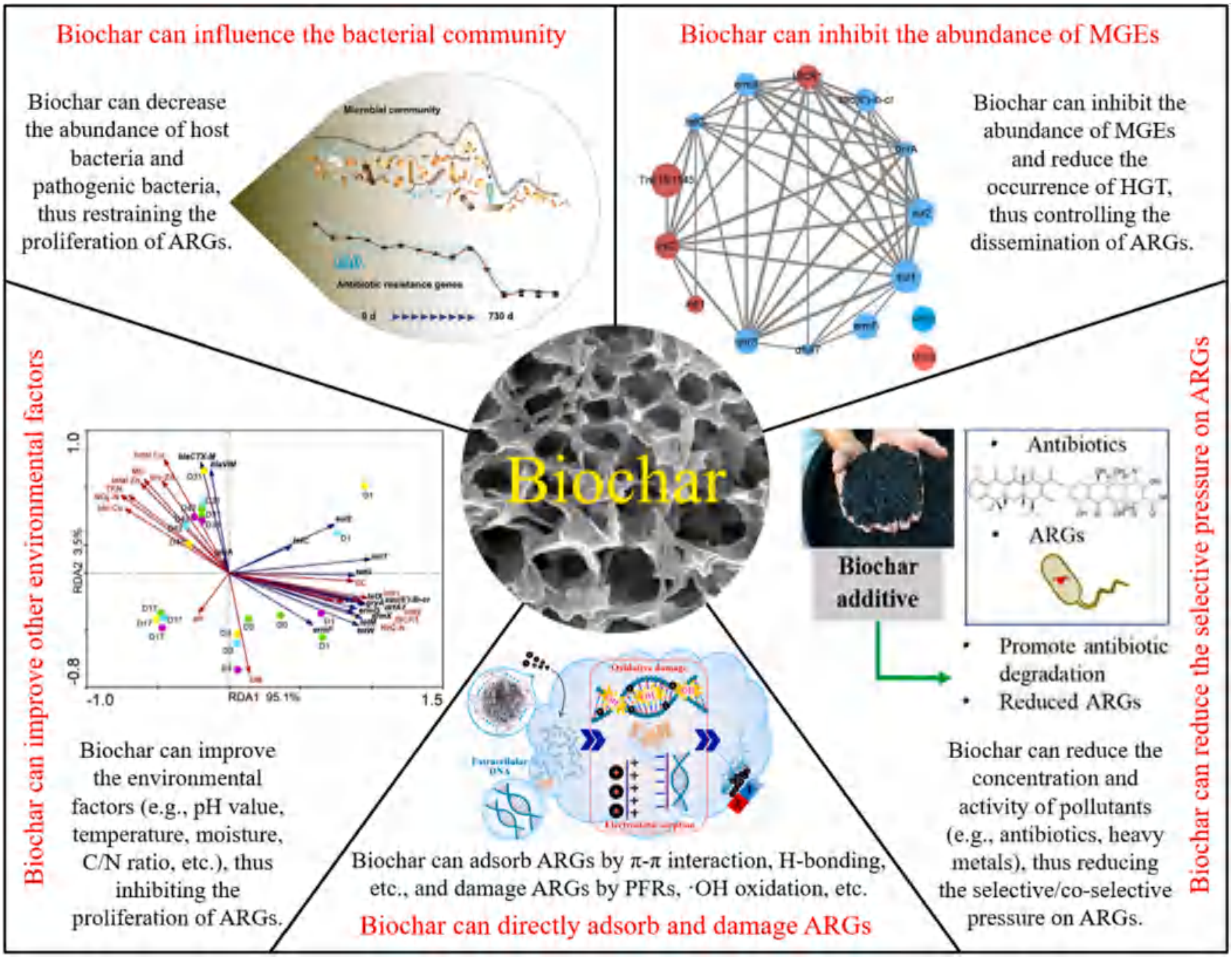

Fig. 4. The influence processes and mechanisms of using biochar to control ARGs during environmental governance.

2018; Yang et al., 2021; Zheng et al., 2021). Moreover, biochar can also directly decrease the abundance of ARGs through the adsorption and/or oxidation processes (Calderon-Franco et al., 2021; Lian et al., 2020). Although a number of studies have indicated that biochar plays a crucial role in alleviating ARGs pollution in different environmental systems, the related processes and mechanisms are still vague and need to be further studied.

3.1. Biochar can influence the bacterial community

The change of bacterial community has an essential effect on the variation of ARGs, and many studies indicated that the addition of biochar in different environmental systems (such as in soil (Duan et al., 2017), in aerobic composting (Cui et al., 2016; Guo et al., 2019; Qian et al., 2019), in anaerobic digestion (Sun et al., 2018; Wang et al., 2021a; Xu et al., 2021), in wastewater (Fu et al., 2021c; Wu et al., 2020) could change the bacterial community structure, maintenance bacterial diversity, decrease the host bacteria and pathogenic bacteria abundance (He et al., 2021; Shi et al., 2022; Zhou et al., 2021). Meanwhile, the biochar could serve as a new habitat for microbes, which might affect the abundance, community structure, and activities of bacteria by protecting them from desiccation and serving as a source of nutrients (Akdeniz, 2019; Duan et al., 2017). Consequently, the species and abundances of ARGs would be significantly influenced by the bacterial community succession (Cui et al., 2016; Duan et al., 2019; Fang et al., 2022; Zhang et al., 2021a). Just as previously reported, the addition of bamboo biochar could change the bacterial community structure and decrease the host bacteria abundance in the soil-lettuce system, which was the main reason for the variation of ARGs abundance (Duan et al., 2017). In detail, the decrease of host bacteria *Firmicutes* in soil and lettuce caused by biochar was the major reason for the decline of tetracycline ARGs in leaves. Meanwhile, network analysis was also performed to further elucidate the effects of the bacterial communities on ARGs by identifying their potential host bacteria. Obviously, *sul1* and *intI1* had the greatest diversity in terms of possible host bacteria. *ermF* and *ermX* had significant positive correlations with some pathogenic bacteria, such as *Bacteroides fragilis* and *Pseudomonas aeruginosa*. In addition, *tetW* and *sul2* had significant correlations with the same potential host bacteria (*Lysinibacillus, Streptococcus*, *Ruminiclostridium_1*, *Escherichia coli*, and *unidentified_Xanthomonadales*) (Fig. 5a). During the anaerobic digestion process, Sun et al. (2018) found that the bamboo biochar mainly affected the ARGs profiles by affecting *Firmicutes* and *Proteobacteria*. Network analysis showed that forty-eight relationships were formed in the network and each ARGs possesses four possible host bacteria. Meanwhile, among six phyla were distributed, where *Firmicutes* explained 61.5% of the total, *Aminivibrio* has the most ARGs with nine, and *Advenella* and *Hydrogenispora* also possess quite a few ARGs with eight and seven, respectively. Redundancy analysis further indicated that *Synergistales* and *Burkholderiales* were positively associated with most ARGs, and the bacterial community is closely related to the

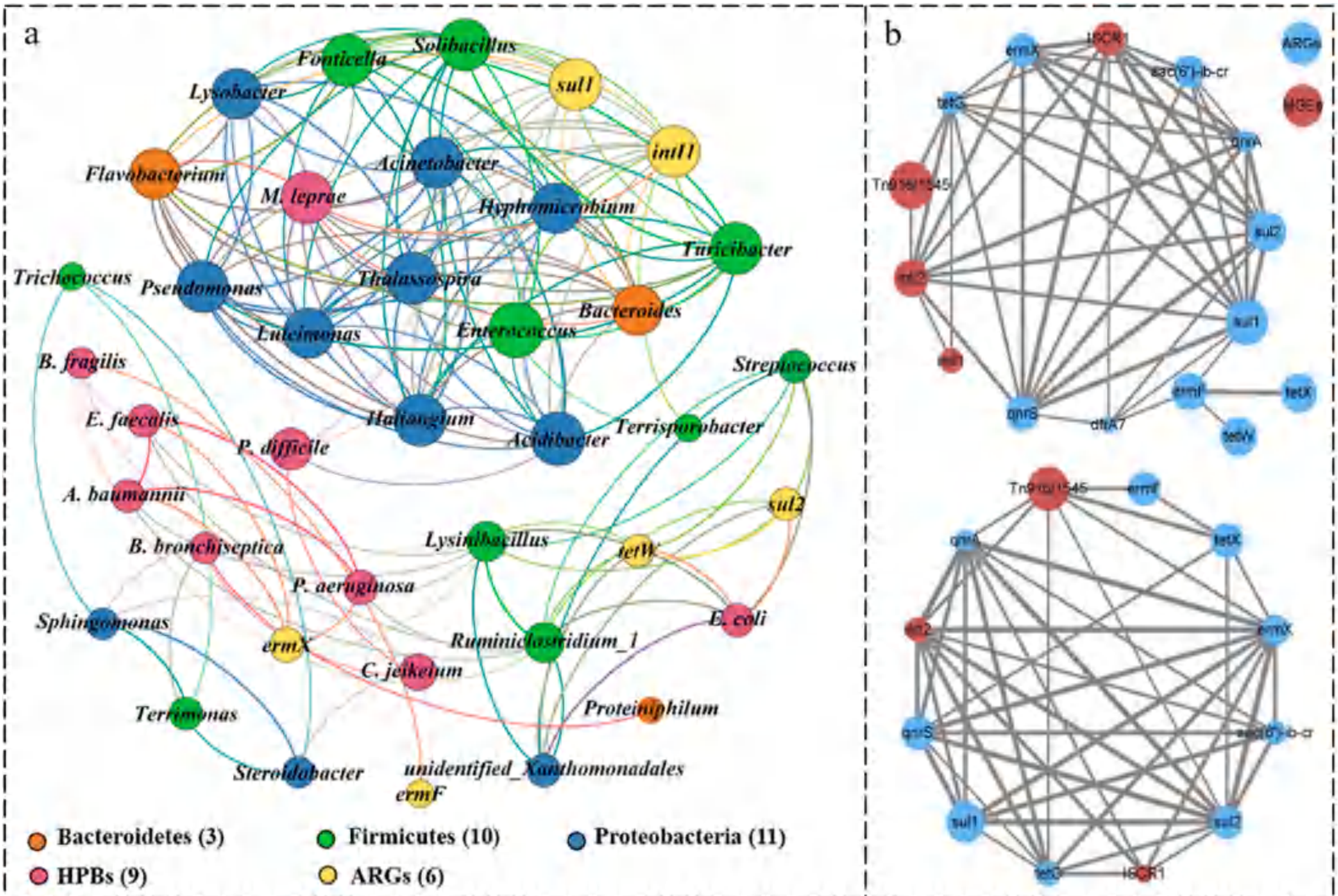

Fig. 5. (a) Network analysis of co-occurring ARGs (relative abundances), human bacterial pathogens, and potential host bacteria (top 30 genera) (Duan et al., 2017). (b) Network analysis of ARGs and MGEs in control and in the treatments with added biochar (Sun et al., 2018).

change of ARGs abundance. As reported previously, pathogenic bacteria are an important source of ARGs, and the death of pathogenic bacteria could have a great influence on the fate of ARGs. Li et al. (2017) verified that the bamboo biochar could elevate temperature during chicken manure composting, thereby effectively killing pathogenic bacteria, which significantly reduced the abundance of ARGs. In conclusion, the variation of ARGs abundance is closely related to the bacterial community, and biochar could change ARGs abundance by affecting the distribution of the bacterial community.

3.2. Biochar can inhibit the abundance of MGEs

The MGEs can significantly promote the HGT of ARGs in the environment, and they mainly comprise integrons (*intI*), transposons (*Tn*), and insertion sequence common regions (*ISCR*). Integrons are generally located on plasmids and chromosomes, they can capture many different types of ARGs, thus increasing the HGT of ARGs. Transposons can transfer between chromosomes and plasmids, and possibly even between Gram-positive and Gram-negative bacteria, thus increasing the proliferation of ARGs. Insertion sequence common regions are complex genetic element which could integrate non-genetic cassette ARGs (Chen et al., 2018; Cheng et al., 2021; Zhou et al., 2019). ARGs can be carried by MGEs to spread among different bacteria, hence, controlling the abundance of MGEs is an effective means to inhibit the propagation of ARGs and decrease the environmental risk of ARGs (Sun et al., 2018). The above discussion confirmed that the biochar has important effects on microbial community structure. Meanwhile, the porous structure of biochar can enlarge the spaces between microbes and may reduce the likelihood of connectivity among microbes. It is known that the connectivity among microbes is the first bottleneck for HGT. Therefore, the biochar can effectively control the proliferation and dissemination of ARGs by inhibiting the abundance of MGEs and reducing the occurrence of HGT (Chen et al., 2018; Cheng et al., 2021; Guo et al., 2019; Li et al., 2017; Zheng et al., 2021; Zhou et al., 2019). As reported by Sun et al. (2018), the bamboo biochar performed an inhibiting action on the total abundance of MGEs (*intI1*, *intI2*, *ISCR1*, and *Tn916/1545*) in the anaerobic digestion system, and they were the dominant elements that influenced the distribution of ARGs, especially *intI2* and *ISCR1* (Fig. 5b). Guo et al. (2019) also reported that the bamboo biochar performed appreciable effects on MGEs during aerobic composting. The results showed that the total MGEs (namely *ISCR1*, *intl1,* and *intl2*) decreased significantly. Compared with day 0, the abundance of MGEs in each treatment reduced observably by 94.75–98.40% after 42 days. Compared to other factors, the dynamic change of MGEs performed a more strongly influence on ARGs fate, which accounted for 36.41% of the variations in ARGs. Meanwhile, significant positive correlations between 10/16 ARGs and MGEs were found. These conclusions support the important roles of MGEs in the transmission of ARGs via HGT. Therefore, the removal of ARGs may be achieved by controlling MGEs. During the soil remediation process, a high positive correlation between ARGs and MGEs also was found, and the prevalence of ARGs may be dominantly mediated by MGEs. The biochar addition could perform effects on MGEs by both direct and indirect (influencing the concentration of pollutants in the soil) pathways (Fu et al., 2021a). Based on these previous studies, using biochar to control the abundance of MGEs in the environment is an important measure to restrain the propagation and spread of ARGs, in which the biochar can affect the MGEs by direct and indirect pathways, thereby effectively controlling the HGT of ARGs.

3.3. Biochar can reduce the selective/co-selective pressure on ARGs

The above discussion indicated that the bacterial community and MGEs are essentially determine the proliferation and dissemination of ARGs. In truth however, the prevalence of ARGs is mainly attributed to the misuse and overuse of antibiotics through continuous selection pressure. Therefore, reducing the concentration of antibiotic pollutants in the environmental system is a necessary means to control the ARGs. Similarly, except for antibiotic pollutants, the heavy metals also can pose a co-selective pressure on ARGs, thereby increasing the abundance of ARGs in the environment. Consequently, the decrease of concentration or activity of heavy metals has an important to ARGs proliferation (Lu et al., 2020; Wang et al., 2020b, 2021c). Based on previous studies, biochar is found to decrease the concentration of antibiotics directly by adsorption and indirectly by increased microbial/enzymatic degradation. Meanwhile, biochar also can reduce the concentration, the bioavailability, and mobility of heavy metals by adsorption. As a result, the selective stress of antibiotics and co-selective pressure of heavy metals could be significantly reduced, thus alleviating the proliferation and dissemination of ARGs (Duan et al., 2017; Fu et al., 2021a; Li et al., 2017; Shi et al., 2022; Zhang et al., 2022b). As Duan et al. (2017) reported that bamboo biochar application in a soil-lettuce system could accelerate the degradation of oxytetracycline (OTC) in the soil. Meanwhile, the biochar amendment significantly reduced the OTC concentration in lettuce leaves and roots. The redundancy analysis indicated that the OTC accounted for 6.3% of the total variation of ARGs abundance, and it was significantly correlated with *tetW*, *ermF*, *sul1*, and *intI1*. It can be seen that biochar can reduce the bio-accessible fractions of antibiotics and mitigate the uptake of antibiotics into plant tissues from contaminated soil, thus restraining the proliferation and dissemination of ARGs. Qiu et al. (2021b) reported that the significant positive correlations between ARGs abundance and heavy metals (Cu, Zn, and Pb) were found during sewage sludge composting, which exhibited 10.8%

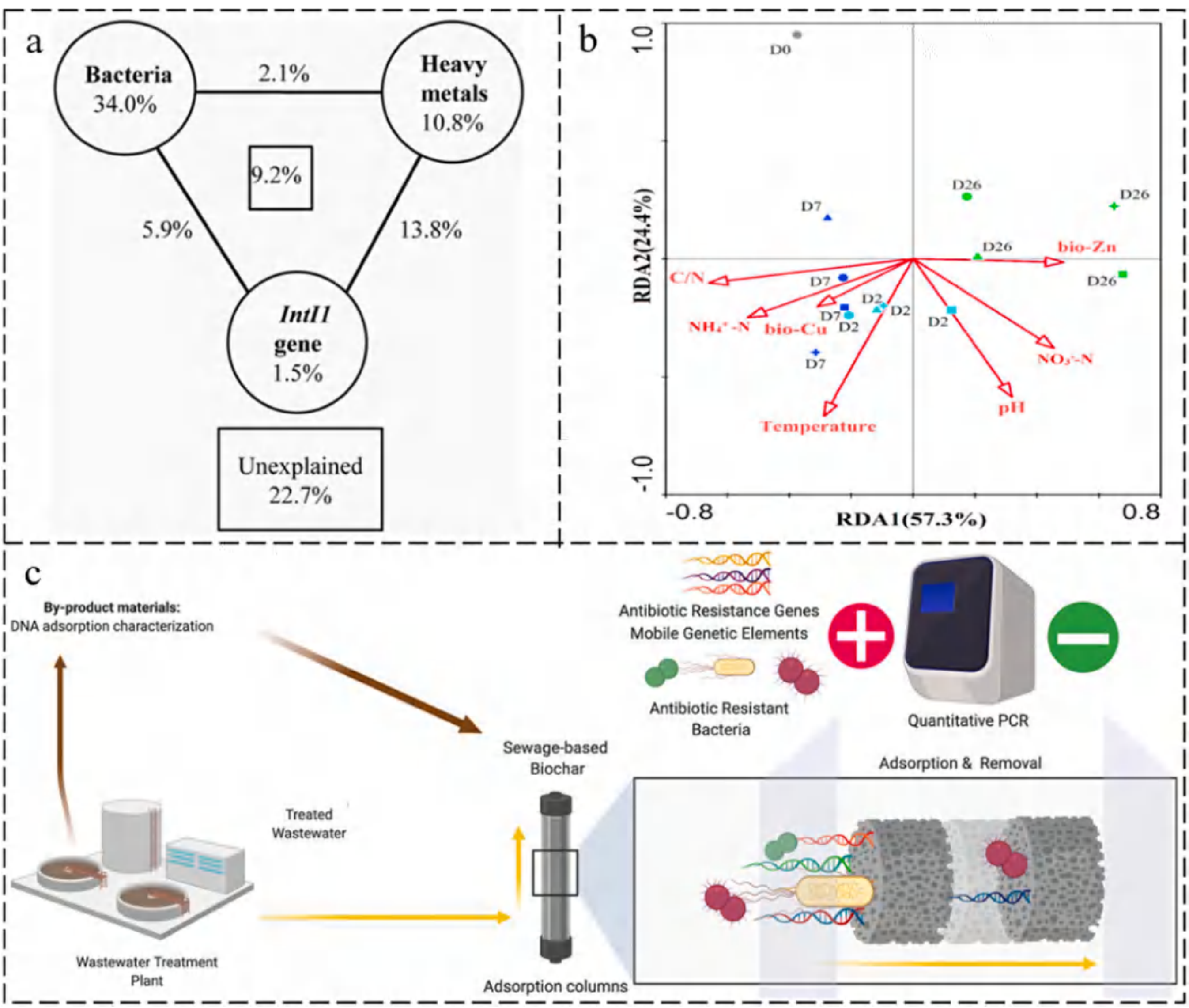

Fig. 6. (a) The relative contribution of the bacterial community, heavy metals and *intI1* gene on the ARGs profiles based on variation partitioning analysis (Qiu et al., 2021b). (b) Redundancy analysis of the relationships between environmental factors and the ARGs profiles during chicken manure composting (Li et al., 2017). (c) The adsorption removal of ARGs from treated effluents by Sewage-sludge biochar (Calderon-Franco et al., 2021).

contribution to the ARGs patterns, especially for *sul1*, *tetO,* and *intI1* (Fig. 6a). The addition of maize straw biochar could decrease the concentrations of these heavy metals, which significantly reduced the co-selective pressure for ARGs and further aid in attenuation of ARGs. Although the selective pressure of antibiotics and the co-selective pressure of heavy metals on ARGs have been confirmed, the co-selective pressure of other xenobiotic pollutants on ARGs is still unclear, and more attention should be paid.

3.4. Biochar can improve other environmental factors

Previous studies manifested that adding the rich functional groups and higher pore structure of biochar can affect many environmental factors (such as pH, temperature, moisture, C/N ratio, etc.) in the environmental governance processes (Akdeniz, 2019; Chen et al., 2018; He et al., 2021; Liang et al., 2017; Qiu et al., 2021a). For example, the biochar can enhance temperature, reduce the pH, increase micro-aeration, prevent leachate formation, and retain nutrients in the composting system. While the biochar can availably change the pH, improve the cation exchange capacity, mineral nitrogen content, air flux, and maintain water during the soil remediation. Meanwhile, the change of these environmental factors will have a great impact on microbial community structure, microbial activity, pollutant behavior, and so on (Fu et al., 2021a; Guo et al., 2019; Kavitha et al., 2018; Kui et al., 2020; Qian et al., 2018). Therefore, these environmental factors also have an important influence on the fate of ARGs, and the biochar could effectively inhibit the proliferation of ARGs by affecting these environmental factors. Just as Li et al. (2017) reported that the bamboo biochar addition could increase the temperature, C/N ratio, NH_4^+-N, and NO_3^--N, but reducing pH value during chicken manure composting, and the change of these environmental factors performed a significant influence on the ARGs profiles. In detail, the temperature, C/N ratio, and pH accounted for 26.1%, 19.4%, and 17.5% of the total variation in the ARGs profiles (Fig. 6b). In addition, the variation of these environmental factors could further affect the microbial community structure and reduce pathogenic bacteria, thus restraining the HGT and abundance of ARGs. Fu et al. (2021d) also studied the relationships between the environmental factors (including temperature, pH, moisture content (MC), electrical conductivity (EC), organic carbon, NH_4^+-N) and ARGs variation in composting after the addition of tree leave biochar. The results showed that the addition of biochar could increase the temperature and EC of composting, but decrease the pH, MC, organic carbon, and NH_4^+-N. Compared to other factors, the pH and organic carbon were mainly responsible for the change of ARGs. The above results verified that the environmental factors play a significant role in the proliferation of ARGs, the introduction of biochar could change the environmental factors and thus changing the fate of ARGs in the environmental system. However, the influence processes and mechanisms of environmental factors on ARGs are still unclear, so further research is urgently needed.

3.5. Biochar can adsorb and damage ARGs

Biochar is an excellent adsorbent due to its rich oxygen-containing groups, huge SSA and complex pore structure. It has been widely used in the adsorption removal of organic pollutants and heavy metals in the environment. ARGs are typical biological macromolecules, recently, some studies have indicated that the biochar can effectively adsorb ARGs in wastewater by hydrogen bonding, π-π interaction, ligand exchange, hydrophobic interactions, and pore confinement. In addition, biochar can generate the PFRs, which can participate in the degradation of organic matter by mediating the production of reactive oxygen species (ROS) and directly transferring electrons (Bimová et al., 2021; Calderon-Franco et al., 2021; Fang et al., 2021; Fu et al., 2021b; Lian et al., 2020; Wu et al., 2021). Therefore, biochar can not only reduce ARGs by adsorption, but also can directly damage ARGs by PFRs and ROS oxidation and some other processes. For example, Calderón-Franco et al. Calderon-Franco et al. (2021) found that the detected ARGs (*ermB*, *sul1*, *sul2*, *qnrS*, $bla_{CTX\text{-}M}$, *intI1*) present on the free-floating extracellular DNA fraction and the total environmental DNA (namely both extra/-intracellular DNA) were removed at 85% and 97% by sewage-sludge biochar adsorption from wastewater treatment plants effluent in up-flow column system (Fig. 6c). Wu et al. (2021) investigated the removal process and mechanism of ampicillin ARGs by Ce modified excess activated sludge biochar, the results shown that the removal ratios of ampicillin ARGs by adsorption, PFRs oxidation and·OH oxidation were 28.37%, 8.26%, and 27.56%, respectively. The mechanism analysis indicated that the adsorption was achieved by hydrogen bonding and π-π stacking of nucleobases. The large groove space structure in the closed-loop double helix structure of ampicillin ARGs has a large steric hindrance, which may be the primary adsorption site. Meanwhile, the CeO_2 exists in Ce modified biochar, which could also adsorb ampicillin ARGs by binding it to the DNA phosphate backbone. The oxidation process by PFRs and·OH could directly destroy the ampicillin ARGs structure, in which the phosphor diester bond in the base stacking structure and the phosphate bond in the nucleotide were the possible action sites of PFRs. Treated ampicillin ARGs products were in the form of base-pair residues or short-chain double helix structures. The·OH could be integrated into the bases of nucleotide molecules to produce greatly reactive free radical adducts. Therefore, they could initiate molecular dehydrogenation and intermolecular proton transfer, resulting in oxidation of the base to the scission of the phosphate sugar backbone of ARGs (Fig. 7). In addition to PFRs and·OH oxidation, the damaging effect on ARGs caused by the dissolutions of biochar also should not be ignored. The dissolutions of metal cations and their organic complex could interact with the DNA of ARGs through electrostatic attraction, π-π interaction, and hydrogen bond, and covalently bind to DNA, which could push the double helices together and enhance base stacking, finally resulting in the condensation and subsequent aggregation of DNA of ARGs, thus hindering their transformation into host bacteria and restraining the proliferation and dissemination of ARGs (Fang et al., 2022). The adsorption and damage of ARGs by biochar are important for controlling the amplification of ARGs, more related studies should be done, especially in soil remediation and solid waste treatment.

4. Effects of biochar properties on ARGs removal

The physicochemical properties of biochar have important influence on its application. In general, the physicochemical properties of biochar mainly depend on the feedstock materials and pyrolysis temperature (Cheng et al., 2021; Das et al., 2021; Tomczyk et al., 2020; Xiao et al., 2018). Generally, the feedstock materials with high organic carbon and low ash can promote the development of the pore structure of biochar, and the feedstock materials with high lignin content are beneficial to obtain biochar with high yield. The C content, pH, SSA and porosity, aromatics and thermal stability of biochar increased with the increase of pyrolysis temperature. However, the yield, polarity, and the acid oxygen-containing functional groups decrease with the enhancement of pyrolysis temperature (Das et al., 2021; Hassan et al., 2020; Ji et al., 2022; Wang et al., 2018). In addition, biochar with different sizes also has distinct physicochemical properties, such as the nano-biochar possess a larger SSA and more abundant PFRs than that of bulk-biochar (He et al., 2018; Kazemi Shariat Panahi et al., 2020; Lian et al., 2020; Luo et al., 2021). Furthermore, the concentration of biochar also has a direct effect on its application process. Therefore, it is essential to study the influence of different properties of biochar on the ARGs removal process, which has practical guiding significance for its application.

4.1. Feedstock materials of biochar

An extensive range of biomass materials (e.g., rice straw, shell,

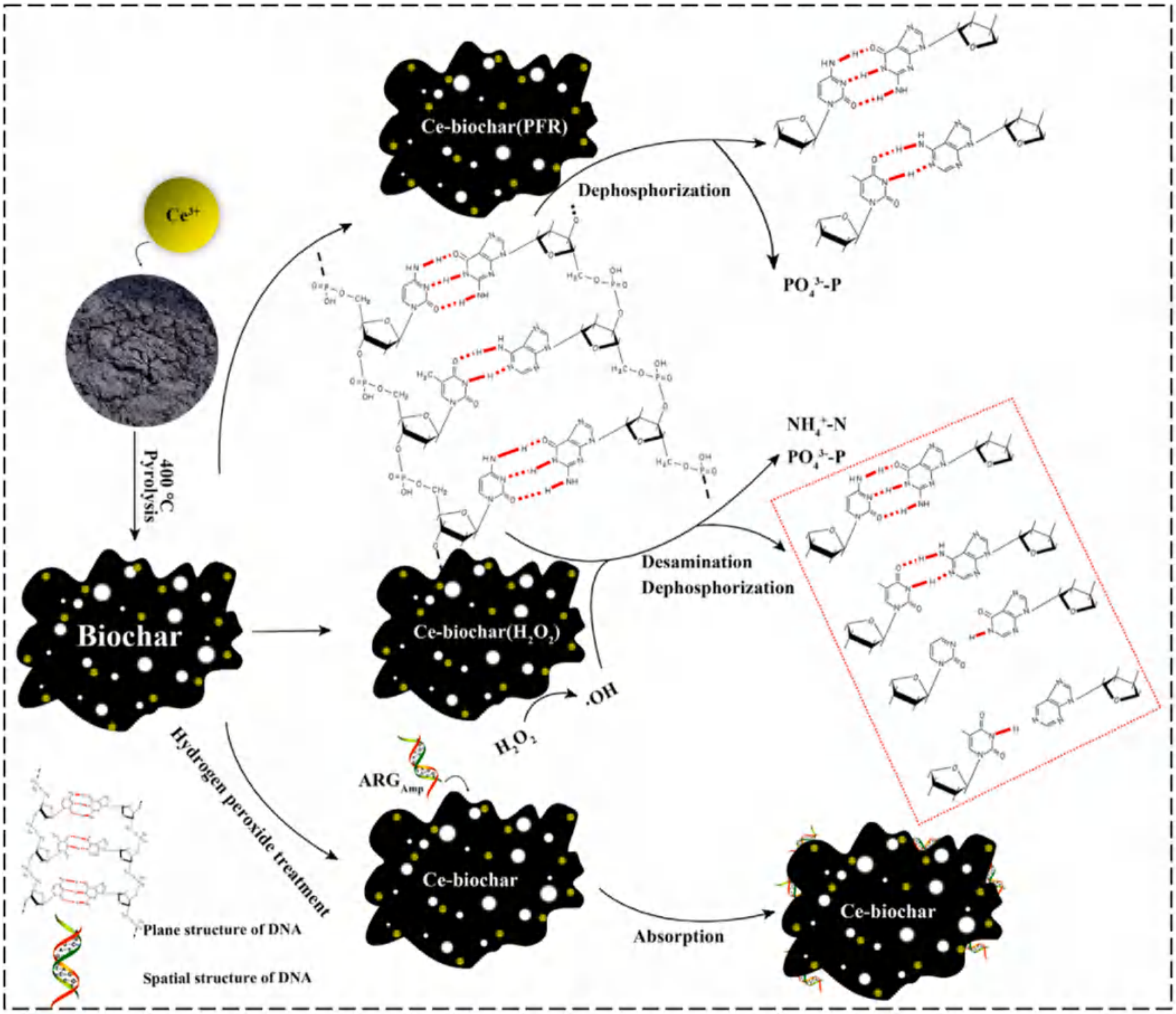

Fig. 7. The interaction mechanism between biochar and ARGs in wastewater (Wu et al., 2021).
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sludge, manure, etc.) has been used as feedstock materials to produce biochar for environmental governance. Many studies have shown that the biochar properties, including surface morphology, functional groups, hydrophobicity, stability, and zeta potential etc., are influenced by the variation of structural cellulose, hemicellulose, lignin, and inorganic elements contents of different feedstocks. Generally, the feedstock materials with high content of cellulose and hemicellulose will contribute to the formation of rich oxygen-containing groups on biochar. In contrast, a high content of lignin in feedstocks could cause the high degree of aromatic structure in biochar (Das et al., 2021; Hassan et al., 2020; Kazemi Shariat Panahi et al., 2020; Ortiz et al., 2020; Tomczyk et al., 2020). The distinctive physicochemical characteristics of biochar originating from different feedstock materials would significantly affect the removal of ARGs from the different environmental systems (Fig. 8a) (Hassan et al., 2020; Wang et al., 2018). For instance, Cui et al. (2016, 2017) reported that the mushroom biochar (high organic carbon content) addition contributed to the removal of ARGs, while the rice stalk biochar (high lignin content) addition caused opposite results, during chicken manure and pig manure composting. However, during duck manure composting, both mushroom biochar and rice stalk biochar addition had a negative effect on the elimination of ARGs. Ding et al. (2019) studied the impacts of different biochar (plants (soybean, bark, rice, and bamboo)-derived biochar and feces (sludge and manure)-derived biochar) amendments on ARGs compositions and abundance in the soil and collembolan gut. The results showed that the distribution of detected ARGs in collembolan guts displayed a significant heterogeneity, signifying the different biochar with different properties could perform various effects on ARGs. Meanwhile, most biochar amendments did not affect the RAs of ARGs in the collembolan guts and soil, but the addition of manure-derived biochar could obviously increase the abundance of ARGs in the collembolan guts, which may be attributed to the enhancement of heavy metal concentration in the soil after the introduction of manure-derived biochar (Fig. 2b). Furthermore, the dissolved biochar that come from different feedstock materials also has a distinct influence on the conjugated transfer of ARGs among bacteria. Particularly, the humic acid-like matter in dissolved biochar could dramatically affect the transfer of ARGs among bacteria (Liu et al., 2021). These findings indicated that the types and properties of biochar derived from different feedstock materials play a pivotal role in affecting the behavior of ARGs in different systems.

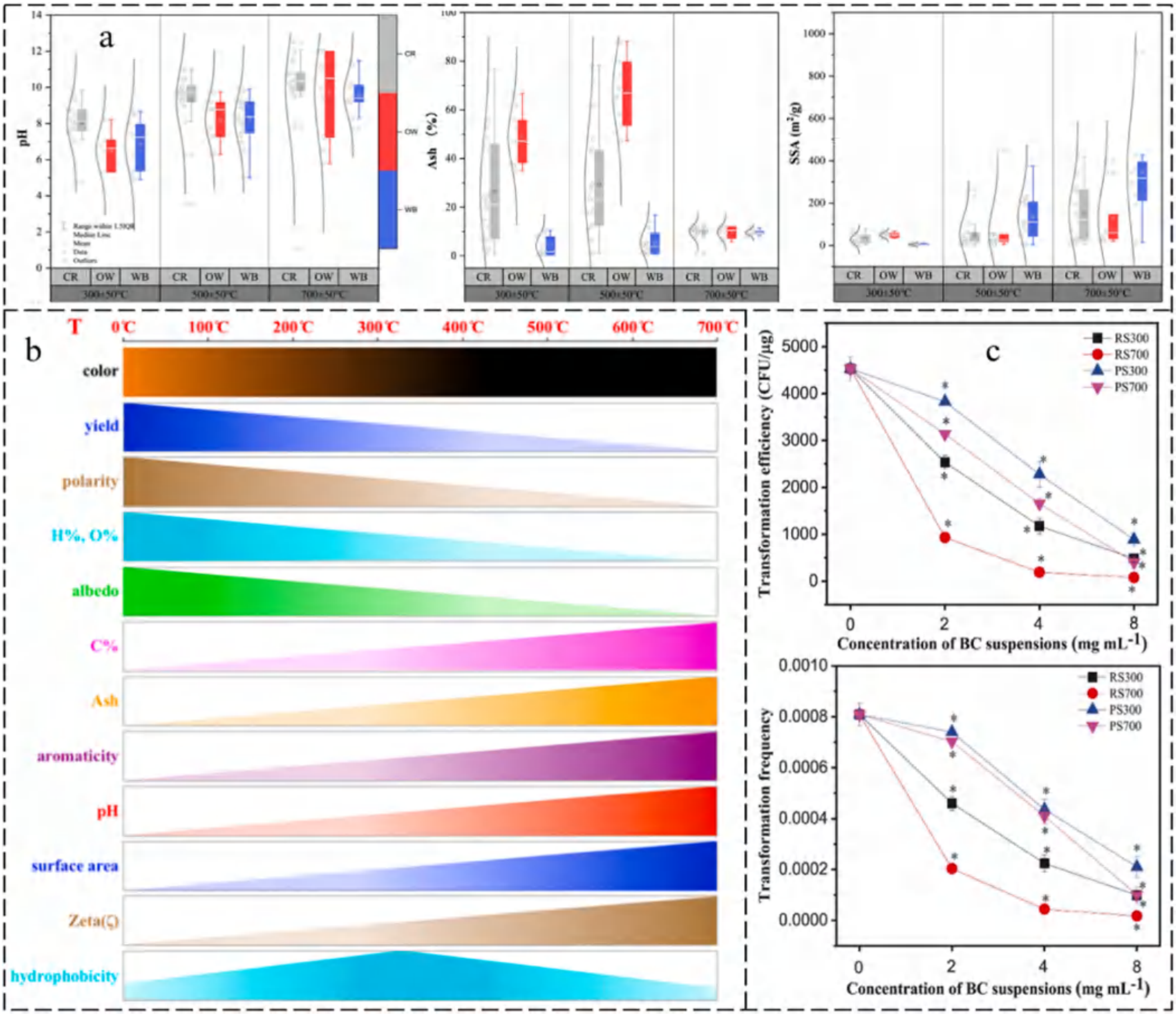

Fig. 8. (a) Differences in properties of biochar from different feedstocks (CR: crop residue, OW: organic wastes, WB: woody biomass) (Ji et al., 2022). (b) Biochar property variations with increased pyrolysis temperatures (Xiao et al., 2018). (c) Effects of biochar on the transformation efficiency and transformation frequency of eARGs in pUC18 (Fang et al., 2022).

4.2. Pyrolysis temperature of biochar

Pyrolysis temperature is another dominant factor influencing the physiochemical properties of biochar. According to previous studies, the carbon content, aromaticity, pH, ash content, SSA, stability, and pore size increase, while biochar yield, hydrogen content, oxygen content, H/C, and O/C ratios decrease, with increasing pyrolysis temperature (Fig. 8b). In particular, pyrolysis temperature is critical for tailoring desired functional groups and dissolutions in biochar, which plays a vital role in removing ARGs by biochar (Das et al., 2021; Hassan et al., 2020; Ortiz et al., 2020; Tomczyk et al., 2020). For example, the rice stalk biochar and peanut shell biochar could significantly inhibit the transformation of ARGs between plasmids to *E. coli*, and the inhibition degree increased with pyrolysis temperature (Fig. 8c). The inhibitory effect of biochar from low pyrolysis temperature (300 ℃) was largely caused by biochar dissolutions, while it was mainly attributed to biochar solids for high pyrolysis temperature (700 ℃). Biochar dissolutions would cause the intramolecular condensation and agglomeration of plasmids, and also reduce the permeability of cell membrane, thus impeding the transformation of ARGs between bacteria. While biochar solids could adsorb plasmids, thus restraining their transfer among bacteria. Meanwhile, biochar solids could also inactivate bacteria and thereby hindering their uptake of ARGs (Fig. 3a) (Fang et al., 2022). Liu et al., 2021a,c also found that the dissolved biochar varied with the pyrolysis temperature. The conjugative transfer frequency of ARGs between bacteria also changed significantly after treatment with dissolved biochar. The dissolved biochar could increase the transfer efficiency of ARGs at the low pyrolysis temperature (300 ℃), but could obviously inhibit the transfer of ARGs at the high pyrolysis temperature (700 ℃). Which could be due to there being a higher content of the humic acid-like substance in dissolved biochar at lower pyrolysis temperatures, while the humic acid-like substance can significantly improve the transfer efficiency of ARGs between bacteria. Lian et al. (2020) also reported that the rice straw biochar exhibited the higher adsorption removal efficiency for ampicillin ARGs (*ampC* and *ermB*) under high-temperature pyrolysis (700 ℃) than that of low-temperature pyrolysis (400 °C) in aqueous environments (Fig. 9a), which might be due to the different surface properties of biochar come from different pyrolysis temperatures. Therefore, it is of great significance to deeply analyze the effects of surface properties and dissolutions of biochar at different pyrolysis temperatures on ARGs removal.

4.3. Size of biochar

The size of biochar has an important effect on its properties of biochar. Many studies have manifested that the bulk-biochar can be broken into nanoscale particles (i.e., nano-biochar) by physical and/or chemical processes in the environment (Zha et al., 2022). Compared to the bulk-biochar, the nano-biochar possesses a better migration ability in the soil matrix, and can transport from terrestrial to aquatic environments via infiltration and surface runoff. Therefore, nano-biochar has more chance to interact with ARGs in real environmental media than bulk-biochar (He et al., 2018; Lian et al., 2020; Liu et al., 2018; Shen et al., 2020b). Furthermore, many researchers confirmed that the PFRs could be generated during the formation of biochar, while the size effect of biochar could be a crucial but neglected factor in determining the properties of PFRs, thus affecting the availability and reactivity of PFRs in biochar toward ARGs (He et al., 2018; Lian et al., 2020; Sigmund et al., 2017). As reported by Lian et al. (2020), the impacts of different sizes of biochar on ARGs in aqueous environments have been studied. The results showed that the binding capacity of nano-biochar to *ampC* and *ermB* ARGs was 50 – 100 times higher than that of bulk-biochar, which signifies the nano-biochar had better adsorption capacity for ARGs than that of bulk-biochar (Fig. 9a). Meanwhile, the *ampC* and *ermB* amplicons still exhibited lightful stripe when treated with bulk-biochar in the gel electrophoresis (B1 –B3 in Fig. 9b), indicating the bulk-biochar had little effect on the proliferation of adsorbed ARGs. In comparison, the lightful stripe could hardly be detected when treated with nano-biochar (N1 – N3 in Fig. 9b), manifesting the proliferation of *ampC* and *ermB* was significantly suppressed by nano-biochar. The gel electrophoresis was also employed to detect the liveness of potentially escaped ARGs from nano-biochar, and no lightful stripe was displayed for the free ARGs in the sample (N4 – N6 in Fig. 9b), suggesting the escaped ARGs from nano-biochar can be destroyed. The above results emphasize the significance of the size effect of biochar, which would play a crucial part in the interaction between ARGs and biochar, thus, more attention should be paid to future research and application.

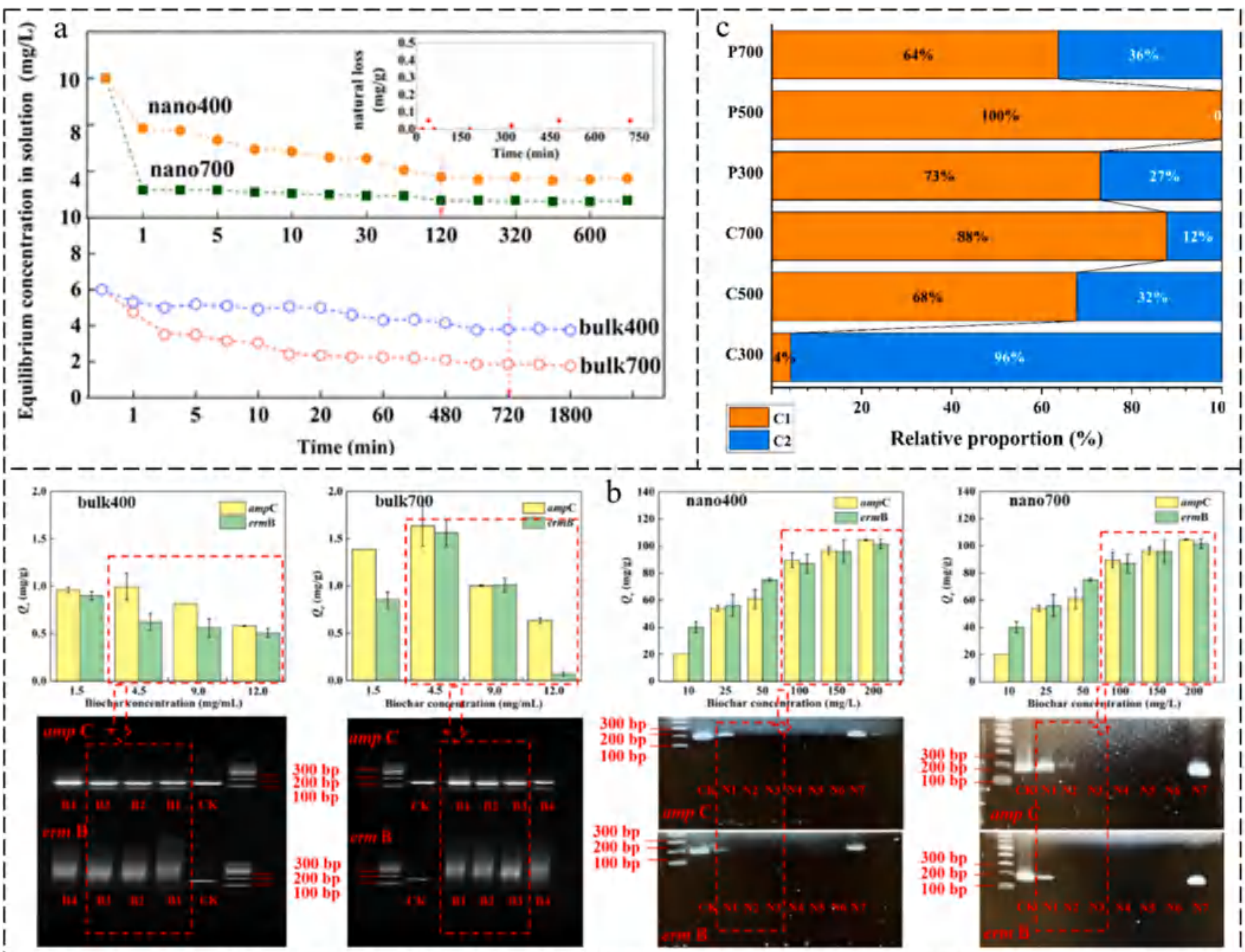

Fig. 9. (a) Adsorption kinetics of extracellular DNA on bulk-biochar and nano-biochar (Lian et al., 2020). Copyright 2020 Environmental Science & Technology. (b) Binding capacity of biochar toward *ampC* and *ermB* with different concentrations (Lian et al., 2020). Copyright 2020 Environmental Science & Technology. (c) Relative proportion of humic-like substances in dissolved biochar samples, the C1 represents UVC humic acid-like substance and the C2 represents UVC/UVA humic-like substance with large molecular size (X. Liu et al., 2021a; Z. Liu et al., 2021a). Copyright 2021 Environmental Pollution.

4.4. Concentration of biochar

The concentration of biochar also has an essential effect in its application in environmental governance process. Usually, the high concentration of biochar also tends to agglomeration, thus resulting in the activity decrease of biochar (Liu et al., 2016; Shen et al., 2020a). Meanwhile, the high concentration of biochar would cause the high levels of dissolved organic matter (DOM), humic acid substance, and some other substances to be released into the environmental system, thus affecting the behavior of ARGs (Akdeniz, 2019; Fang et al., 2022; Liu et al., 2016; Shen et al., 2020a). Besides, the physicochemical properties of environmental system would be changed to different degrees after adding biochar with different concentrations. As previously reported, when the addition concentration of dissolved biochar was $\leq$ 10 mg/mL, the humic acid-like substance substantially promoted ARGs transfer between bacteria. Whereas, the conjugative transfer efficiency was significantly suppressed when the concentration reached 100 mg/mL due to a high concentration of the humic acid-like substance (Fig. 9c) (Liu et al., 2021). Fang et al. (2022) also reported that the rice stalk biochar and peanut shell biochar could significantly inhibit the HGT of ARGs, and the transformation efficiency decreased markedly with increasing biochar concentration from 2 to 8 mg/mL due to the presence of different concentration DOM (Fig. 8c). Li et al. (2017) studied the impacts of bamboo biochar on ARGs variation in chicken manure composting, the average RAs of most ARGs (*tetC*, *tetG*, *tetW*, *tetX*, *drfA1*, *drfA7*, *ermB*, *ermF*, *ermQ, and ermX*) decreased by 0.85, 1.05, 1.08, and 1.15 logs with 0%, 5%, 10%, and 20% biochar addition, respectively (Fig. 3b). It is possible that a higher proportion of biochar could increase the temperature in the compost pile and maintain a high temperature for longer periods, thereby killing pathogenic bacteria and destroying plasmids more effectively, which could help to prevent the spread of ARGs. During the anaerobic digestion process, the different concentrations of biochar (0, 5, 20, and 50 g/L) also performed the different effects on the removal of ARGs. The results showed that the RAs of 5/13 ARGs were decreased when 5 g/L of biochar were added, while 20 g/L of biochar could obviously decrease the total RAs of ARGs in the digestion products. Biochar primarily influenced the abundance of ARGs by affecting the distribution of *Firmicutes* and *Proteobacteria*, and the effects of 20 g/L of biochar was more than that of 5 g/L (Sun et al., 2018). Therefore, controlling the concentration of biochar in the environmental system would play a significant impact on the behavior of ARGs.

5. Conclusions and Prospects

As an emerging contaminant, the ARGs would bring great risk and harm to the ecological environment and human health. Therefore, how to effectively and economically remove or damage ARGs and restrain the proliferation and dissemination of ARGs in the environment has become one of the hotspots and difficult points in the environmental field. Previous studies have proved that the biochar can control the ARGs in different environmental governance systems by affecting the system elements (including bacterial community, MGEs, and environmental factors), and/or direct adsorb and damage the ARGs. Although some achievements have been made, the use of biochar to restrain the proliferation and dissemination of ARGs in the environment is still in its infancy, and more efforts need to be paid to solve the related fundamental and technical gaps and challenges.

(1) The accurate and efficient detection of ARGs in the environment is a premise and essential for the further study of ARGs. The existing detection methods for ARGs include the detection of phenotype and genotype of drug-resistant bacteria. Among them, the polymerase chain reaction (PCR) based detection method is more mature, and the metagenomic sequencing method is more efficient and accurate. However, these methods are time-consuming, demanding, and inconvenient. Therefore, future research should focus on tracing ARGs in the source environment, finding indicative markers and potential hosts, and thus developing more convenient, shorter cycles, and higher accuracy detection methods. Meanwhile, it should develop more accurate detection methods for ARGs in different environmental media according to the characteristics of various environmental media, and establish the detection method database of ARGs.

(2) To further study the proliferation and dissemination of ARGs in the environment, and establish the distribution database of ARGs. The ARGs can be continuously disseminated and enriched in different environmental media, including soil, water, atmosphere, plant, and animal. However, most of the previous studies concentrated on ARGs in soil and water environment, and the proliferation and dissemination of ARGs in other systems is not well studied. Compared with ARGs in soil and water, the dissemination of ARGs in the air may bring more severe harm to ecological environment and human health (Just like the "COVID-19″). Therefore, it is essential to strengthen the research of ARGs in the air environment. Meanwhile, ARGs can be transmitted along the food chain, and finally accumulate at the top of the food chain, thus it is also crucial to investigate the behavior of ARGs in plants and animals. What's more, it is important to establish the distribution database of ARGs (such as the type, concentration, host bacterium, etc.) in different environmental media according to the research and detection results, which would provide the necessary basis for controlling and reducing the ARGs in the environment.

(3) To deeply investigate the processes and mechanisms of biochar removing and inhibiting the proliferation of ARGs in different environmental systems. Previous studies indicated that the removal and/or inhibition of ARGs by biochar is a very complex process, which depends on the properties of biochar, the types of ARGs, the factors of the environmental system, and so on. Although there are some relevant studies, there is no consensus and sometimes opposite results. Hence, it is necessary to enhance the study of related mechanism in the latter, which is essential for its practical application. In particular, the properties of biochar change significant with the change of raw materials, pyrolysis conditions, etc., thus the impacts of biochar properties on the removal and/or inhibition of ARGs in the different environmental systems should be deeply studied, and the influencing processes and mechanisms of biochar properties on ARGs should be summarized. Meanwhile, the preparation process of biochar should be optimized based on local conditions according to the action mechanism of ARGs by biochar in different environmental systems, to meet the needs of the practical application of biochar. Furthermore, for different environmental systems, their environmental factors are different, and the influence processes of biochar addition on them are also various, so, the process and mechanism of biochar removing and/or inhibiting ARGs are also different in different environmental systems, which also should be further investigated.

(4) To deeply study the co-selective pressure and mechanisms of xenobiotic pollutants on ARGs removal by biochar. The xenobiotic pollutants (e.g., heavy metals, dyes, ionic liquid, nanomaterials, etc.) often coexist with ARGs in the environmental system. Although many studies have evidenced those xenobiotic pollutants would enhance ARGs abundance by their continuing co-selective pressure on bacteria and/or the HGT, the related mechanisms remain unclear. Hence, it is necessary to deeply study the co-selective impacts and mechanisms of co-existing xenobiotic pollutants on ARGs removal by biochar. Meanwhile, at present, most studies on ARGs induced by co-selective pressure by using the single pollutant exposure experiments, while many kinds of selective pressures coexist in the environment. Hence,

the co-selective effects and mechanisms of multiple selective pressures on ARGs removal by biochar should be paid more attention. To more importantly, the optimization of the biochar utilization process should be intensively studied to achieve the simultaneous removal of xenobiotic pollutants and ARGs in the environmental system.

(5) To attach importance to explore the synergistic technologies of biochar and other treatment processes, thus improving the removal and inhibition of ARGs. Although the biochar has certain removal and/or inhibition effect on ARGs during different environmental media, the removal and/or inhibition efficiency of biochar on ARGs needs to be enhanced, one effective way is to couple biochar with other treatment processes. For example, the biochar can be combined with a catalytic oxidation process (such as Fenton catalysis, photocatalysis, electrocatalysis, etc.) by adding catalysts to the environmental system or loading catalysts on biochar, which can degrade ARGs and/or xenobiotic pollutants in the system, thus reducing the abundance of ARGs. Or adding functional microorganisms/loading functional microorganisms on biochar to alter the microbial community structure in the environmental system to change the HGT and restrain the proliferation of ARGs. Or combining biochar and membrane technology to enhance the removal efficiency of ARGs in water treatment. The coupling of biochar with other technologies not only significantly improves the removal of ARGs, but also enables the simultaneous removal of other pollutants in the system, which greatly improves the possibility of its practical application.

(6) The shortcoming of using biochar to control ARGs also should be concerned. Considering the complex sources, biochar is likely to become a carrier of potentially toxic substances, such as heavy metals, polycyclic aromatic hydrocarbons, furans, and dioxins. Meanwhile, the nanoscale biochar might enter plants, animals, and even humans through the food chain. Besides, the powdered biochar is not easily recycled, especially in soil remediation, which could result in biochar persisting in the environmental systems for a long time due to the high stability. Furthermore, biochar might become a new carrier of ARGs and some other pollutants if they exist in the environment for a long time. Therefore, these shortcomings of using biochar to control ARGs would pose many uncertain environmental and ecological risks, which should not be ignored and more systematic explorations are required.

(7) To deeply investigate the interface microbial chemistry of biochar for ARGs removal. Previous studies have shown that the microbial extracellular electron transfer (EET) between biochar and microbe interface plays an important role in environmental remediation, wastewater treatment, and resource regeneration, and biochar as a typical electron shuttle which can significantly promote the EET process and strengthen the transformation and removal of pollutants, thus the EET behavior between biochar and microbe interface during ARGs control should be studied in depth. Such as, the formation and community structure of biofilms on biochar, the effects of surface properties (e.g., the surface oxygen-containing functional groups) of biochar on EET efficiency, the effects of EET efficiency on the proliferation and abundance of ARGs, and whether the EET process could direct damage some ARGs.

Environmental implication

The unimaginable occurrence and proliferation of antibiotic resistance genes (ARGs) contaminants have brought great harm to ecological environment and human health, and it is very necessary to explore the efficient control technology of ARGs. In recent years, biochar, as a multifunctional, low-cost and environmentally-friendly material, has been widely used in the study of environmental pollution control, including the ARGs. However, it is still lack of a critical review about the impacts of biochar on the fate and dissemination of ARGs in environmental governance process, which would play a guiding role in the study of this field and is urgently needed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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