Aerobic composting: an efficient way to reduce antibiotic resistance genes in organic solid wastes

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~~Aerobic composting is as an efficient method way to reduce antibiotic resistance genesARGs in organic solid wastes~~

Abstract

The significant rise of Antibiotic Resistance Genes (ARGs) in organic solid wastes (OSWs) has emerged as a major threat to the food chain. Aerobic composting is a widely used technology for OSWs management.~~The sharp of Antibiotic r~~Resistance ~~g~~Genes~~resistance genes (ARGs) in organic solid wastes (OSWs) has become an important threat to the food chain. Aerobic composting, as a wildly used technology in dealing OSWs,~~ haswith ~~shown~~ the potential to influence the fate of AGRs. However, ~~due to~~ the variability of ~~the~~ results from different studies ~~which~~ has ~~exposed~~ highlighted the limitations of~~ a~~ ~~single~~ individual experiments~~,~~ and the effects~~effect~~ of composting on ARGs ~~is~~ are still uncertain. ~~To address this issue~~To address this gap~~Here~~, we ~~established~~ compiled a database ~~comprising~~including~~including~~ 4232 observations from ~~47~~ 42 published papers and conducted~~performed~~ a series of meta-~~analyses~~ analysis to ~~quantitative~~ quantify the impacts~~impact~~ of composting on ARGs. ~~The r~~Results~~results~~ ~~revealed that~~showed that aerobic composting ~~can~~ can~~ould~~ substantially~~significantly~~ reduce the abundances of ARGs and M~~m~~obile G~~g~~enetic E~~e~~lements (MGEs)~~ levels~~ by 74.3% and 78.8%, respectively. The 3rd to the 6th weeks was found to be the most stable and highest reduction period during the composting process, with a mitigation efficiency ranging

from 72.4% to 79.7%. ~~Additionally, the most stable and highest reduction period during the composting process was the 3^{rd} to the 6^{th} weeks,the 3^{rd} to the 6^{th} week was a stable and high reduction period during the composting process, with the mitigation efficiency ranging from 72.4% to 79.7%.~~ **Simultaneously, adjusting moisture content** ~~(MC)~~**, pH and C/N** of compost feedstock ~~-at~~to **60%-65%, 6-7 and 20-25** ~~before composting~~**, respectively**~~; —~~; u~~u~~**sing windrow composting and forced ventilation (vent rate: 0.25 to 0.35 L/min/kg D**M~~W~~**) as composting m**e~~e~~**th**o**ed**~~;~~; n~~n~~**ano-iron as additives may have the most significant ARGs mitigation efficiency. Eventually, MGEs and composting duration (CD) were the most important** ~~driven~~ **factors** ~~on in~~ driving **ARGs changes** during composting process**,** ~~which~~which CD ~~the importance of CD~~ **ha**~~d~~vings **been** previously **overlooked** ~~before~~**.** **The**se **findings** provide ~~of this meta-analysis may contribute to the~~ a **comprehensive insight** ~~of~~ into **the** effects~~effect~~ **of composting on ARGs reduction,** which may help prevent the transmission in food systems~~and interrupt the transmission of ARGs between livestock and food systems.~~ ~~one step further.~~

1 Introduction

The World Health Organization (WHO) has stated that Antibiotic Resistance become a major concern of 21^{st} century (Karkman et al., 2016; Pruden et al., 2006). The ability of Antibiotic Resistance Genes (ARGs) to transfer horizontally through conjugation, transformation and transduction, making it can be easily transferred to human pathogenic bacteria which significantly increases the risk of antibiotic failure and potential adverse effect on global public health (Ben et al., 2019; Huang et al., 2020; Allen et al., 2010; Huddleston, 2014). (Pei et al., 2019).

~~The World Health Organization (WHO) has stated that the propagation and prevalence of antibiotic resistance genes (ARGs) have become a major concern of 21st century with the abuse of~~ antibiotics~~antibiotic (Pei et al., 2019). Due to the ability of easily transferred in different ecosystems via horizontal gene transfer (HGT), ARGs has been found in various~~ environments~~environment (Ben et al., 2019; Huang et al., 2020; Allen et al., 2010; Huddleston, 2014). Whereas,~~ Once ~~the ARGs enter the human bodies through the food and water once, there will be an increased risk of antibiotic failure which~~ leads~~led to a potential adverse effect on global public health and ecological safety (Karkman et al., 2016; Pruden et al., 2006).~~

The administration of ~~According to the studies of absorption mechanism,~~ ~~a~~a~~A~~ntibiotics~~antibiotics~~ can promote the generation and accumulation of ARGs in gastrointestinal tract, ~~cannot be fully absorbed both in animals and human bodies, which~~ resulting in animal manures~~manure~~ and municipal sludges~~sludge~~ ~~have~~ becoming~~e~~ ~~important~~ crucial reservoirs for ARGs enrichment~~ARGs enrichment reservoirs~~ (Wohde et al., 2016; Du & Liu, 2012).~~).~~ Furthermore, the presence of antibiotics residues in organic solid wastes can also contributes to an increase in ARGs abundance (Du & Liu, 2012). ~~Meanwhile, l~~Livestock manures~~manure~~ and municipal sludges~~sludge~~ are commonly utilized ~~usually used~~ as soil amendments~~conditioners~~ due to their rich ~~organic matter~~carbon and nutrient contents, ~~so as~~ providing a realistic basis for the further spread of ARGs in agricultural systems

(Cao et al., 2020; Sardar et al., 2021；Wu et al., 2022). Therefore, much attention has been attracted in ~~the~~ recent years ~~by~~ on how to efficiently ~~removal~~eliminat~~eremoveal~~~~removal~~ ~~remove~~ the ARGs from manures and sludges during ~~the~~ organic solid wastes ~~organic solid wastes~~ treatment ~~process to cut off the spread of ARGs in ecosystemsecosystem~~.

Aerobic composting has been ~~wildly~~ widely used around the world due to~~because of~~ the ability to convert ~~organic solid waste~~ organic solid wastes~~OSW~~ into well-made fertilizer (Cao et al., 2021a). Meanwhile, thermophilic composting is also considered as an ~~In addition, dDue to the intense microbial activity and high temperature during the composting processing, make it is also~~ effective way in reducing the ecological risk of multiple pollutants (Cao et al., 2021b; Liao et al., 2019).

~~Therefore, with the sharp of ARGs level in manure and sludge,~~~~Accordingly,~~ ~~more and more researchers~~~~n~~Numerous studies have ~~have~~ been conducted to explore the efficiencies of composting on ARGs elimination ~~through aerobic composting~~ ~~have focused on the removal efficiency of composting about the ARGs in past two decades~~. ~~For example,~~ Wang et al. (2016) found that ARGs abundance ~~in swine manure~~ could be efficiently reduced after composting. ~~And~~ A~~a~~ 42-days experiment showed that the resistance genes levels of tetracycline and sulfonamide were much lower after composting (Selvam et al. (2012). However, in some studies, the level of ARGs was elevated trough composting.~~However,~~ Su et al. (2016) found that the composting process could significantly promote the increase of ARGs level. Qian et al. (2016a) stated that ~~during cow manure composting~~ the *tetQ*, *tetM*, and *tetW* abundance decreased but the level of *tetC*, *tetX*, *sul1*, and *sul2* were increased significantly~~.~~ ~~during cow manure~~after composting. Some certain ARGs could decrease in some composting system but the same genes clusters increased in other composting process (Johnson et al. 2016; Qin et al., 2016b). In addition, some studies have ~~found~~ revealed that the ARGs abundances~~abundance~~ may~~could~~ rebound in the cooling phase, but the rebound genes and time-points have~~were~~ varied~~ous~~ across~~from~~ different experiments (Pu et al., 2019; Wang et al., 2021).

~~Meanwhile, in order~~ T~~t~~o enhances the ~~improve the removal~~ efficiency of ARGs removal during composting, ~~many~~ some composting parameters and technologies have been investigated, such as additives, ventilation methods and composting methods (Liu et al., 2021). Cui et al., (2016) found that the removal rate of ARGs was increased by 0.86 log units compared with the control by adding biochar. However, the usage of corncob biochar enriched the levels of *sul2* after composting (Guan et al., 2021). Therefore, ~~due to~~ the high heterogeneity of ARGs changes during composting highlights the need for quantified analysis based on a large dataset~~which exposes the limitation of a single experiment, and the quantified analysis is urgently needed based on a large of data~~. (Danie et al., 2021；Wu et al., 2022).

~~Introduction 中的问题没有交代清楚，我们为什么要做一个综述，为什么要用整合分析因为现有的研究中存在着矛盾的结果：1）不同对堆肥~~

~~模式或者技术（条垛式、槽式、反应器堆肥等）下抗生素抗性基因的消减效率差异较大；2）不同的物料（混合物，starting materials）由于其特殊的性质导致其可能纯在较大的差异（pH、C/N）；3）不同的调控手段下（添加剂、通风、其他微电场等）也会对这个有影响；但是现在的研究没有一个初步整合的结果，什么物料采用什么技术在什么手段下取得更高的去除效率。所以，我们才会去做整合分析，为别人的研究提供参考。另外，现有是否有围绕抗性基因消减的综述性的文章，我们和他的区别是什么？这个是需要去更多的强调的。如果能够把这几部分写好，前面的关于抗生素如何的部分可以减少篇幅：ARGs 成为突出的问题；COMPOSTING 是处理的手段，堆肥中 ARGs 的高效去除能够避免问题，然后接上面的信息；最后就是回到我们的目标。~~

~~Meta-analysis is an excellent statistical tool to generate precise and systematic results about the fate of ARGs during composting and can reveal the effect of different parameters on ARGs changes (Zhou et al., 2022; Danie et al., 2021). (考虑到篇幅，这部分的结果可以不需要了，大家都知道整合分析能够有什么用)。).~~

~~Therefore~~Here, we ~~gathered~~ collected 4232 observations from ~~47~~ 42 published papers and performed a series of meta-analyses (or subgroup ~~meta-analyses~~meta-analysis) to investigate i) if aerobic composting significantly reduces ARG levels; ii), how the ARGs changes at different time points during the composting process; and iii) the effects~~effect~~ of different composting technical parameters on ARGs. Moreover, iv) the driven factors of ARGs change during composting. ~~**To the best of our knowledge, this is the first meta-analysis on aerobic composting and ARGs reduction**~~（表述不准确，类似的研究请看 Zhang et al., 2019; Journal of Hazardous Materials, 386, 121895（整合分析）；Oliver et al., 2019; Journal of Dairy Science, 103(2), 1051-1071（一般综述））.

2 Materials and Methods

2.1 Literature search

To investigate the fate of ARGs during composting, we searched publications in the database of China National Knowledge Infrastructure (CNKI) and Web O~~o~~f S~~s~~cience (WOS). The applied search term combinations were composting or aerobic composing and ~~antibiotics resistance gene (~~ARGs~~)~~. Additionally, the search was limited to studies online time on or before June 30, 2022.

The studies were included in this meta-analysis if they met the following criteria: i) the study was an original experiment. ii) the composting at least was lab-scale which means the work of the simulated composting process was not included. iii) the composting process was complete, as indicated by the compost temperature remaining stable at the ambient temperature. iv) the feedstock type was pig manure, chicken manure, cow manure or sewage sludge. v) only the qPCR was considered as Quantified method. vi) at least, the relative or absolute abundance of ARGs at the beginning and end of composting (end-~~-~~point data) were given directly or can be extracted from the figures.

2.2 Data collection

For the articles that we selected, the name and abundance of each ARG (end-point) were directly extracted from the tables, figures or supplementary materials. The ancillary data was also extracted from the selected article, including the physiochemical indexes of endpoints such as moisture content (MC), pH, ~~EC, total~~

~~organic carbon (TOC),~~ C/N ~~ratio and total nitrogen (TN)~~. Moreover, ~~in order to analyze the correlations more preciseness between physiochemical properties, composting duration and ARGs level,~~ when the physiochemical indexes and ARGs abundance of multiple time points (mid-point data) were reported during the composting, the data were also included in the ~~meta~~ database to investigate the effect of composting duration on ARGs changes and the driven factors. If figures were the only date recourse, the GetData software Version 2.22 was used to extract the data (Cao et al., 2019). Additionally, the composting methods and ventilation methods were also recorded in the meta-database. If additives were set as composting treatment in original experiment, the types of additives were recorded in the database to investigate the influence of additives on ARGs.

A total of 42 studies were found to meet the criteria mentioned in the methodology section, including 1242 end-point and 2990 mid-point observations (in total, 4232). Additionally, 232 end-point observations of MGEs were also extracted from the selected papers. The information of selected papers and the structure of meta-database both Endpoint observations and Total ARGs observations were described specifically in **Table S1** and **S2**, respectively. Additionally, the method to compare Ln(FC) produced by relative abundance and absolute abundance was given in **Text S1** and **Fig.S1**.

2.3 Meta analysis

The effect sizes (LnRR) of ARGs were evaluated by the natural log of the response ratio (RR) (Cao et al., 2019; Hedges et al., 1999). The primary dependent variable for this study was the fold-change (FC) of ARGs levels in the raw feedstock and sampling materials for each single ARGs, which was commonly used for describing the change of ARGs in the environment during a part of the time (Han et al., 2018).

$$LnRR = LnFC = \ln\frac{sampling}{initial}\,(1)$$

Where, *sampling* represents the ARGs levels of sampling time in composting material, and *initial* means the ARGs levels in the raw material. When *sampling* was the last sampling time, the ~~LnFC~~Ln (FC) of endpoint showed the ARGs change in the whole composting process.

In ~~meta-analyses~~meta-analysis, the standard deviation and the replication of each observation are usually taken to calculate the weight of effect sizes in a nonparametric way (Li et al., 2019). However, in our meta-database, most studies did not report any measure of variance for the response variables. Consequently, in order to include as many studies as possible, the observations were weighted equally and only replication-based weighting was adopted in the analysis using the following equation

（Cao et al., 2019；Quan et al., 2021）：

$$weight = \frac{nt \times n_i}{nt + n_i} = \frac{n}{2}\,(2)$$

Where n_t and n_i are the sample sizes for the treatment and control group in normal conditions. In this study, composting process as a whole treatment led to the sample sizes (n) are the same before and after the composting.

To better show the decline of ARGs during the composting, the weighted mean values of Ln(-FC) were transformed back to the percent changes (%) by the following exponentiation (Cao et al., 2019; Abdellah et al., 2022):

$$(\%)=\left[\exp\left(LnFC\right)-1\right]*100\left(3\right)$$

The results of meta-analysis (i.e., mean effect sizes and the 95% confidence intervals) were calculated by MetaWin 2.1 based on 5000 iterations of bootstrapping (Cao et al., 2019; Rosenberg et al., 2000). The effects were considered significant if the 95% CIs did not overlap with zero. Means of categorical variables were considered significantly different from each other if their 95% CIs did not overlap (Hedges et al., 1999).

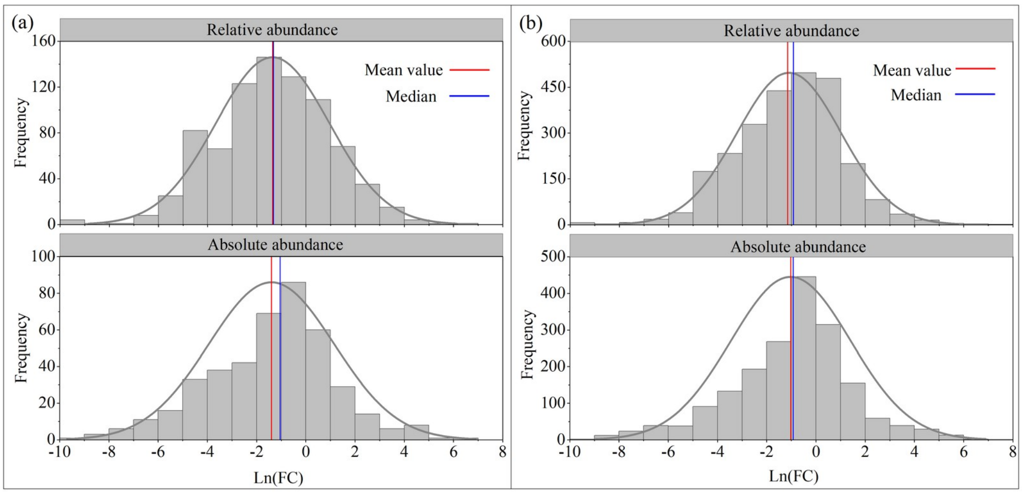
3 Results and Discussions~~（有结果，建议加入更多的讨论，从哪些因素影响堆肥开始，抓住重点描述）~~

~~3.1 Search results and general aspects of the data~~

~~A total 46 studies were found to meet the criteria mentioned in the methodology section, including 1242 end-point and 2990 mid-point observations (in total, 4232). Additionally, 232 end-point observations of mobile genetic elements (MGEs) were also extracted from the selected papers. Meanwhile, the structure of meta-database both Endpoint observations and Total ARGs observations were described specifically in~~

~~Table S1.~~

~~Due to the database including both relative and absolute abundancesabundance, the first question that needs to be answered before further analyses is whether the Ln(FC) produced by relative abundance and absolute abundance can be directly combined analysis. Therefore, the assessment of the distribution of Ln(FC) was approached, which Daniel et al., (2022) used the same way to compare the effect of different quantified technologies on ARGs level. As shown in Fig.1, the average and median of Ln(FC) that calculated by relative abundance were similar to the data which produced by absolute abundance both base on the Endpoint (Fig.1 (a))and Total ARGs database (Fig.1 (b)). Additionally, Q test was performed to evaluate the heterogeneity of components between these two groups of data. The results showed that there was no significant difference between the Ln(FC) calculated by relative abundance and absolute abundance in these two ARGs databases (p>0.05). Meanwhile, some previous studies have also suggested that relative abundance and absolute abundance could lead to similar conclusions about the changes in ARGs levels (Duan et al., 2019). Therefore, it is appropriate to directly combine and compare Ln(FC) calculated from relative and absolute abundance data for further analysis.~~



~~**Fig.1 The distribution of Ln(FC) calculated by relative abundance and absolute abundance based on the Endpoint database (a) and Total database (b). The red line and blue line indicate the Mean value and Median, respectively.**~~

3.1~~2~~ The effect of composting on ARGs

3.1.1 The overall impact of composting on ARGs changes

According to the meta-analysis results, ~~As indicated in ? , a~~a high reduction ratio ~~in the levels~~ of ARGs (74.3%) and MGEs (78.8%) abundances wa~~s~~ere achieved through composting. ~~According to the results of the meta-analysis, composting can significantly reduce the levels of ARGs and MGEs by 74.3% and 78.8%, on average.~~ However, a great variance was observed among different types of ~~organic solid waste~~OSWs.~~However, the effectiveness of composting in reducing ARGs and MGEs levels can vary depending on the type of organic solid waste being composted.~~ As shown in **Fig.~~2~~1(a)**, the ARGs levels in chicken manure and swine manure tended to have a higher reduction (81.7% and 78.0%, respectively) compared with cattle manure (52.3%) and sewage sludge (32.6%) during the composting process. The elevated of MGEs abundances in cattle manure (52.0%) and sewage sludge (26.3%), which can contribute to the spread of ARGs, may be the main reason that caused the lower ARGs reduction, as shown in **Fig.1(b)** (Wang et al., 2020).

~~(Wang et al., 2020)Based on the relations between MGEs and ARGs, as shown in Fig.2(b), the lower efficiency of reducing ARGs levels in cattle manure and sewage sludge during the composing process may be due to the increase elevated of MGEs (from which increased 52.0% and to 26.3% for cattle manure and sewage sludge,%, respectively) (Wang et al., 2020). 这里来了一个问题，MGE 和 ARG 的关系是什么？需要交代一下！~~

~~In addition, the difference in antibiotic residues in organic solid wastes may also be the main reason for the variation in the reduction efficiency of ARGs (Qian et al., 2018).~~

In order to specifically understand the effects of composting, all ARGs were classified according to antibiotic class and mechanism of action (Daniel et al., 2022). As depicted in **Fig.-1(c)**, composting had a higher removal effect for resistance genes of tetracycline, quinolone, macrocyclic lactone, β-lactam, and aminoglycosides ~~amino sugar~~ classes, with the removal rates of 79.6%, 88.0%, 79.9%, 76.3%, and 83.5% respectively. In contrast, the removal rate of sulfonamide resistance genes was only~~lower at~~ 35.3%. The high prevalence of *sul* gene-hosting microorganisms and the mobility of *sul* genes may be important reasons for the low reduction rate of sulfonamide resistance genes (Selvam et al., 2012). Based on the ARG mechanism classification (**Fig. 1~~2~~(d)**), composting had the best reduction effect on resistance genes in the target protection (TP) category (91.5%), followed by target alteration (TA, ~~(~~83.0%), antibiotic efflux (AE, ~~(~~67.7%), and antibiotic inactivation (AI, ~~(~~57.9%). The lowest reduction effect was observed for resistance genes ~~in the~~of target replacement (TR) category, at 32.4%. Since the action mechanism of sulfonamide resistance genes is mainly belong to TR~~target replacement~~, more attention should be paid to the effect of composting on this category in future research to improve the removal effect of sulfonamide resistance genes. ~~Overall, the results showed that composting can be an effective method for reducing ARGs in organic solid waste, but the effectiveness varies depending on the type of ARG and the type of organic waste being treated.（放到结论部分去讲）~~

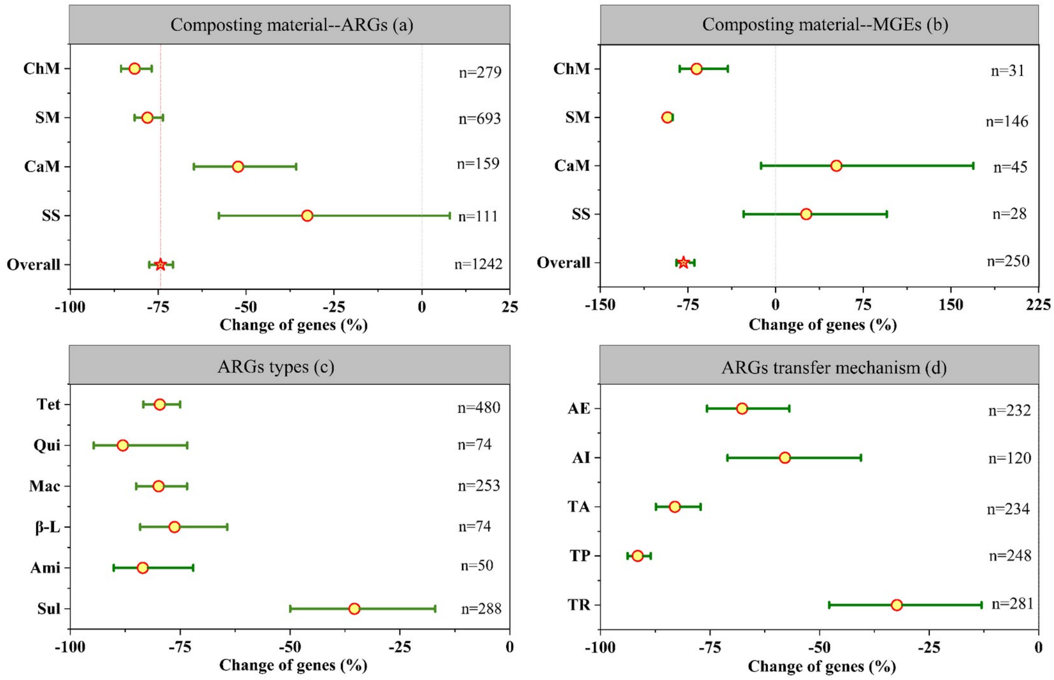


Fig.~~2~~ 1 The mitigation efficiency of ARGs (a) and MGEs (b) in different composting materials and the effect of composting on different ARGs types (c) and ARGs mechanism (d) reduction. The total number of observations for each category~~treatment~~ is displayed on the right-hand side of the results.

Error bars represent the 95% confidence scale. ChM, SM, CaM and SS represent chicken manure, s‘ine manure, cattle manure and sewage sludge. Tet, Qui, Mac, β-L, Ami and Sul indicate tetracycline, quinolone, macrocyclic lactone, β-lactam, aminoglycosides and sulfonamide antibiotics. AE, AI, TA, TP and TR mean antibiotic efflux, antibiotic inactivation, target alteration, target protection and target replacement.

~~（建议调整一下逻辑顺序，这部分内容和最开始的几种粪肥中抗生素消减的部分有重复，可以适当合并，这样逻辑更清晰，或者用小标题来描述）~~3.1.2 The impact of composting on the ARGs changes in different types of OSWs

Due to the heterogeneity of different organic solid waste components, the changes of various ARGs were investigated in chicken manure, swine manure, cow manure, and sludge, respectively. The results showed that composting was effective in reducing all types of ARGs in chicken manure~~, both classified by antibiotics and mechanism~~ (as shown in **Fig.~~3~~2(a) and (e)~~ Fig.3(eb)~~**). This may be because ~~during the marketing period of chicken,~~ most ARGs are selected ~~for~~ by the frequent use of high levels of antibiotics over a short period of time, resulting in low persistence and making them more prone to being eliminated when the selective pressure is released during the composting process (Qian et al., 2018). Additionally, the quinolone resistance genes were more effectively reduced compared to other ARGs types during composting of chicken manure. In contrast with the ARGs reduction in chicken manure composting, the level of sulfonamides resistance genes cannot be significantly reduced in either swine manure or cow manure composting, and similar results were seen in the change of TR category from the mechanism classification of ARGs (**Fig.~~3~~2(b) and ~~Fig.3~~(c)**). Moreover, the AE and AI categories also cannot be significantly mitigated in cow manure, which may because the ARGs ~~in cow manure~~ are selected over a long-time period so they have a high persistence, and thus they are more difficult to eliminate during the composting process (Qian et al., 2018). Compared with the ARGs changes in animal manures, the reduction effect of different types of resistance genes during sludge composting was highly variable, even resulting in the enrichment of some categories, as shown in **Fig.~~3~~2(d) and ~~Fig.3~~(h)**. This may be due to the components in sludge ~~is~~ are more complex and diverse, and the microorganisms present have a higher resistance to antibiotics and are more resistant to the adverse effects of the composting process (Su et al., 2016). In addition, the high moisture content of sludge may also inhibit the degradation of ARGs during composting by affecting microbial activity (Su et al., 2016). ~~Further research is needed to better understand the mechanisms behind the variability in ARG reduction during composting and develop strategies to improve the reduction of ARGs in all types of organic waste.~~

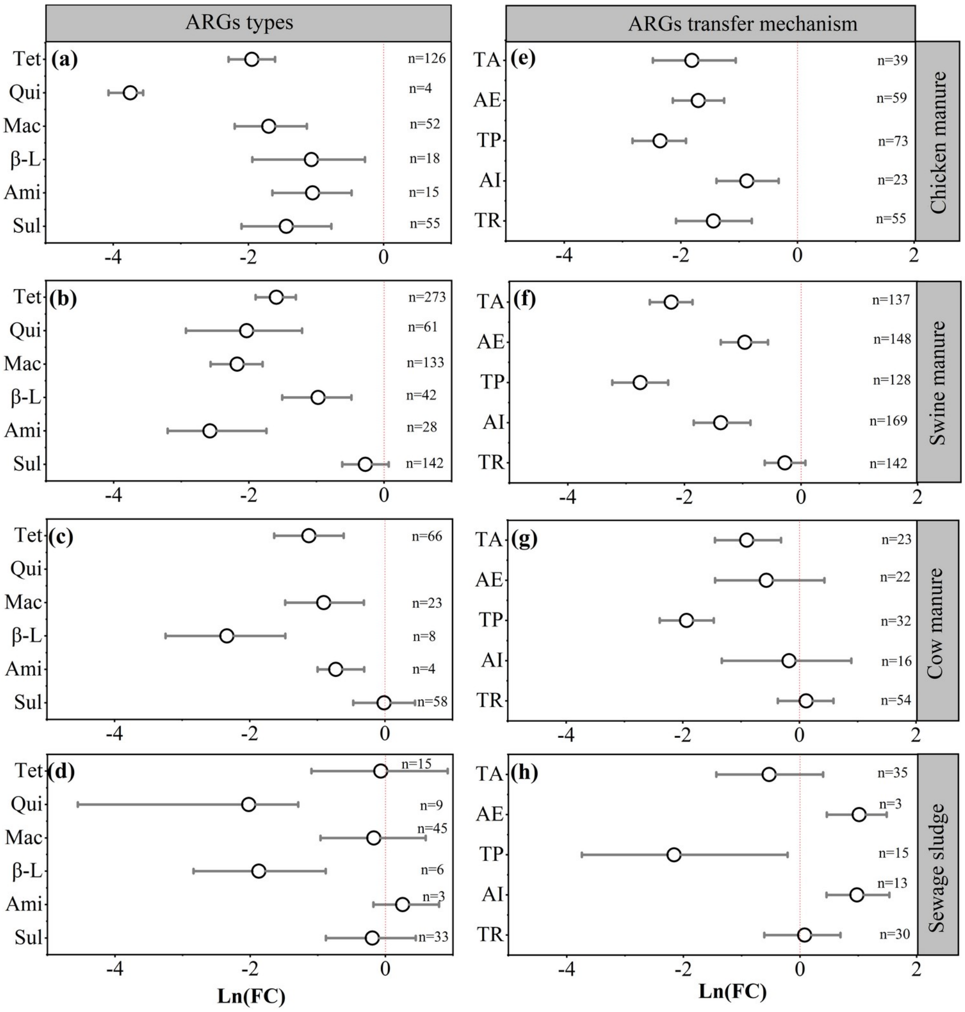


Fig.~~3~~ 2 The effect size ~~(LnFC)~~ of ~~ARGs types and mechanism in different organic solid wastes.~~ different ARGs types (a, b, c, d) and ARGs mechanism (e, f, g, h) changes in chicken manure, ~~; ARGs types (b) and ARGs mechanism (f) changes in~~

swine manure,~~; ARGs types (e) and ARGs mechanism (g) changes in~~ cow manure and~~; ARGs types (d) and ARGs mechanism (h) changes in~~ sewage sludge, respectively. The total number of observations for each treatment is displayed on the right-hand side of the results. Error bars represent the 95% confidence scale. Tet, Qui, Mac, β-L, Ami and Sul indicate tetracycline, quinolone, macrocyclic lactone, β-lactam, aminoglycosides and sulfonamide antibiotics. AE, AI, TA, TP and TR mean antibiotic efflux, antibiotic inactivation, target alteration, target protection and target replacement.

The effect size of each unique ARGs which sample number was more than five were calculated, in order to find out the persistence genes during composting. As shown in **Table ~~S2~~S3**, compared with average Ln(FC), the *sul1, sul2, dfrA1, tetA, tetC, tetG, tetX, qnrD, ermF, ermT, blaCTX-M, cmlA,* and *mefA* were identified as more difficult to degrade, should be the focus of future research.~~为什么这些更难被降解呢？~~

3.2~~3~~ The changes~~change~~ of ARGs during c~~C~~omposting~~Composting process~~

To investigate the changes of ARGs **at different time points during the** composting process, a database of total 4232 observation was established and analyzed in a time series. As shown in **Fig.~~4~~3(a)**, ~~the results indicated that~~ the reduction rate of ARGs during the first two weeks of composting was lower, at 46.2% and 47.1%, respectively. And then it increased to 74.6% in the third week (W3) and remained at this level until the sixth week (W6). After this, the mitigation efficiency entered a fluctuation period (W7 60.6%, W8 87.7% and W9- 46.9%). Hence, the stage of W3 to W6 is characterized by a stable and high rate of ARGs reduction. This is likely to result from the decreased prevalence of microorganisms that carry ARGs after exposure to high temperatures. ~~W3 to W6 is a stably high reduction rate stage of ARGs, which may due to the decrease in the abundance of microorganisms carrying ARGs after the high-temperature period~~ (Xie et al., 2021). ~~And t~~The~~the~~ fluctuation of the reduction efficiency is possibly caused by the changes in the dominant bacterial community in the composting materials (Ezugworie et al., 2021). In the early stages of composting, lower ARGs mitigation may be caused by the increase of antibiotic resistant bacteria ~~high microbial activity may be the main reason for the changes compared with high temperature directly degraded the extracellular ARGs~~ (Liu et al., 2021). Additionally, the mitigation rate of W9- reflected a significant rebound on the level of ARGs, compared with W8.

As different studies have reached different conclusions about the changing trends of different ARGs during the composting process. The different ARGs types were analyzed in time series based on the meta-analysis method. The results showed that only Tetracycline resistance genes could be significantly reduced at all various time points through the whole composting process (**Fig.~~4~~3(b)**), but the rebound of the genes happened after W8. The same rebound trend was also appeared in Quinolone resistance genes and Macrolide resistance genes (**Fig.~~4~~3(c) and (d)**). However, as shown in **Fig.~~4~~3(e)**, the β-lactam resistance gene showed a declining trend throughout the whole composting period. The significant enrichment of aminoglycoside resistance genes and sulfonamides resistance genes were presented after W6 (**Fig.4 3(f) and (g)**). In summary, except β-lactam resistance gene, the other 5 types of ARGs level all showed an increase at the end of composting. Therefore, it is important to confirm a suitable composting duration.

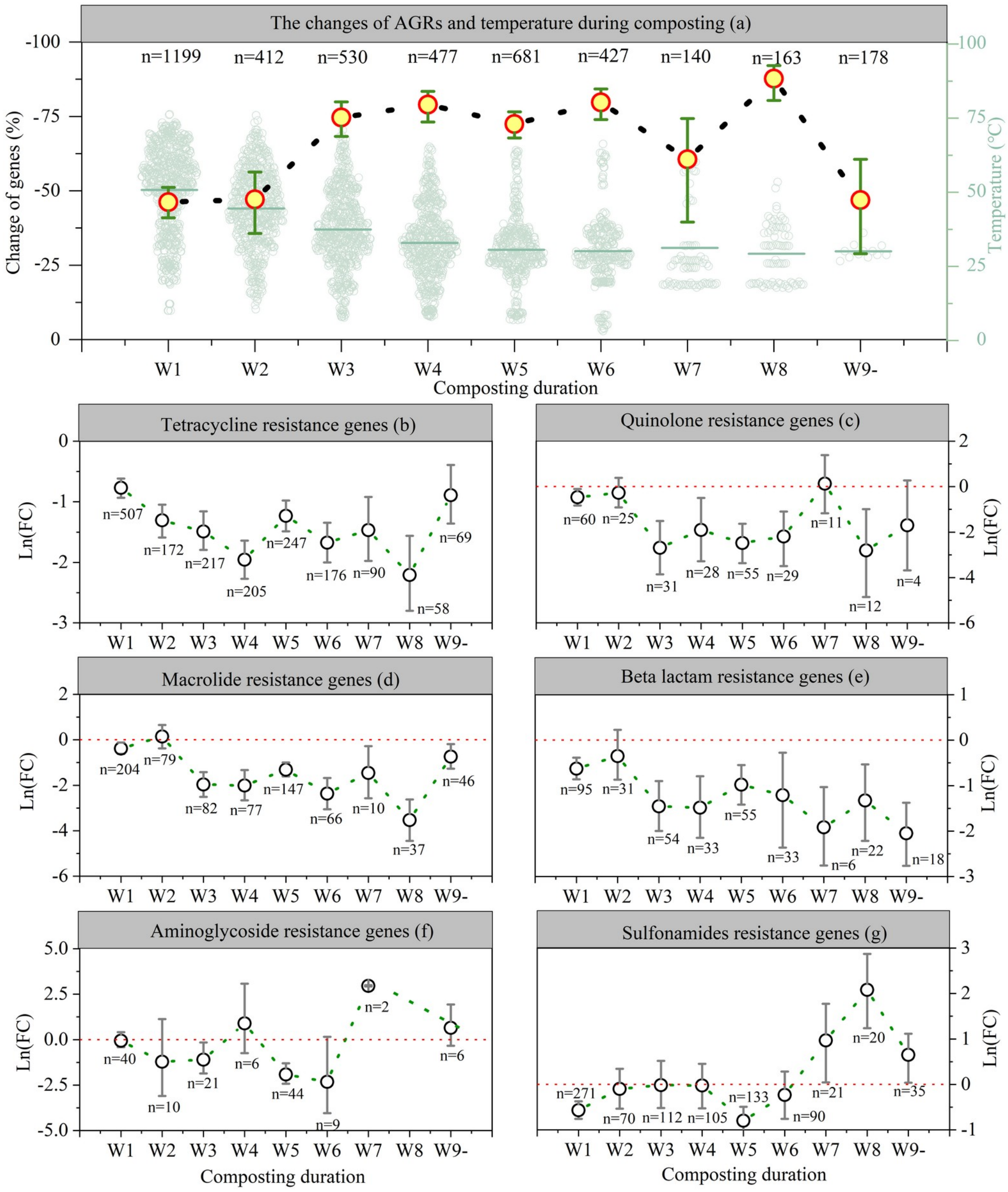


Fig.~~4~~ 3 The overall mitigation efficiency of ~~overall~~ ARGs at different composting periods (a); Tetracycline resistance genes (b), Quinolone resistance genes (c), Macrolide resistance genes (d), Beta lactam resistance genes (e), Aminoglycoside resistance genes (f) and Sulfonamides resistance genes (g) changes during the composting process at different composting period. The total number of observations for each treatment is displayed on the right-hand side of the results. Error bars represent the 95% confidence scale. W1 to W8 mean the first week to the 8th week during composting. W9- indicate the 9th week and after.

3.3~~4~~ The effects~~effect~~ of composting parameters on ARGs

~~As many~~Previous studies have shown~~,~~ that the fate of ARGs during the composting process is influenced by composting parameters ~~in some~~ ways~~way~~ (Liu et al., 2021; Guan et al., 2021). Therefore, common technological properties were analyzed to identify a set of composting strategies.

~~3.4.1 Initial physicochemical properties~~

Moisture content (MC), pH and C/N are the initial physicochemical properties of composting materials which will be adjusted to a suitable volume before composting to ensure the composting efficiency (Liang et al., 2003; Jiang et al., 2011; Sánchez-Monedero et al., 2001).

~~Moisture content~~

As some studies have indicated that ~~with~~ the increase of MC may promote proliferation of host bacteria and increases the risk of ARGs spread, while the low MC would limit the microbial activity during composting (Cheng et al., 2019; Gao et al. 2019). Therefore, these may be the reasons why that the MC from 60% to 65% achieved the highest ARGs mitigation efficiency, as shown in **Fig.~~5~~4(a)**.

~~(1) pH~~

pH is associated with ARGs through selective pressure on microorganisms

(Zhang et al., 2018). Duan et al. (2018) indicated that 16.8% variation in ARGs during the pig manure composting was due to pH. However, in contrast with some studies showed that high pH can mitigate the abundance of ARGs, our results indicated that adjusting the composting materials to a low or acidic pH (6-7) could promote the reduction of ARGs level (Gao et al. 2019). It may be due to the weak acid environment~~, which~~ is not conducive to the survival of ARGs host microorganisms.

~~C/N ratio~~

Wei et al. (2020) stated that the regulation of C/N in composting materials can change the fate of ARGs. ~~Compared~~According to this analysis, ~~C~~compared with the C/N from 25 to 30, ~~which appropriate reduce~~reduced ~~the~~ C/N (~~to~~ 20-25) could ~~increase~~ facilitate the reduction of ~~the~~ ARGs level, that may due to the different effects on populations of host microorganisms.

~~(4)~~

3.34.2 Composting technology and parameters

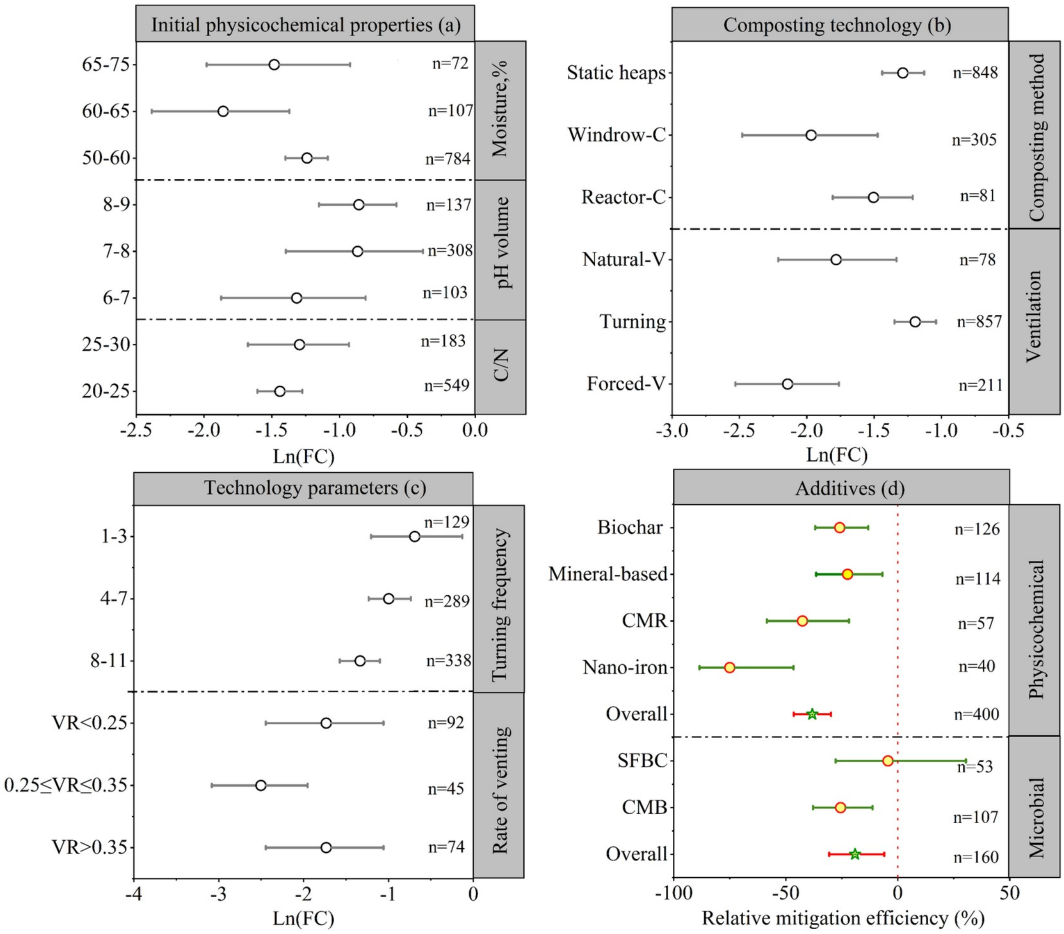
Different composting methods have performed various eco-efficiency, based on the same composting materials (Liu et al., 2022). However, the effect of composting method on the fate of ARGs was overlooked. As shown in **Fig.~~5~~ 4(b)**, compared with the traditional composting method (Static heaps), Windrow composting (Windrow-C) and Reactor composting (Reactor-C) had a better ARGs mitigation efficiency which may be because the prolong of high temperature period (HTP) and reduced composting duration (CD). ~~And t~~The difference of ARGs changes between Windrow-

C and Reactor-C is possibly due to the compost scale, that Windrow-C is applied to the industry company while Reactor-C ~~is~~ used in the lab-scale more often in the studies which we selected (Fan et al., 2021).

Fan et al. (2020) investigated the difference between vacuum-type composting and positive-pressure composting on the fate of ARGs which indicated ventilation method was an important technology to influence the ARGs variation. In ~~the~~ present study, the ventilation methods were divided into three types, i.e., Natural Ventilation (Natural-V), Turning and Forced Ventilation (Forced-V). As shown in **Fig.~~5~~ 4(b)**, the ~~the results indicated that~~ Forced-V had the best reduction efficiency, while ~~the mixing of materials~~T~~t~~urning led to the lowest mitigation rate which is possibly due to a sharp increase in the population of microorganisms after Turning (Getahun et al., 2012). The same reason is supported by the changes in ARGs levels with different Turning frequencies~~.~~, i.e., the more frequent heap turning, the lower ARGs reduction rate. Hence, if turning the materials is unavoidable, once every 8-11days ~~for once~~ can be more suitable~~.~~ (**Fig.~~5~~ 4(~~(~~c~~b~~))**. The rate of venting is an important parameter that can affect the composting microorganisms, temperature and MC (Gao et al.,2010). Therefore, the impact factors under different venting rate (VR) present a complex trade-off. T~~And t~~he results showed that the VR from 0.25 to 0.35 L/min/kg dry weight (DW) was more appropriate to the ARGs reduction compared with the other two venting rates (**Fig.~~5~~ 4(c)**).

3.3~~4~~.3 Additives~~（Additive 怎么分类的，是不是也需要描述一下？至少在材料方法部分~）~~

The relative change of genes~~ mitigation efficiency~~ was calculated to ~~find out~~investigate the effect of different additives on the ARGs reduction (The calculation method was described in **Text S2**.). All the additives were classified into physicochemical and microbial, and the overall relative mitigation efficiency of these two kinds of additives were 38.2% and 19.1%, respectively. Adding microbes may introduce new host bacterial populations carrying ARGs, which could potentially impact the reduction rate of ARGs during the composting process. However, complex microbial agent (CMA) was better than~~ a~~ single functional bacterial agent (SFBA) in the reduction of ARGs which the relative mitigation efficiency was 25.5% and 4.3%, respectively. This may be because the ~~complex microbial agents~~CMAs have a more balanced effect and can effectively degrade ARGs through the increase ~~in~~ of composting temperature. In addition, the CMA contains a diverse range of microorganisms, which could potentially increase the likelihood of ARGs degradation ~~through~~ by the interaction of different microbial populations (Liang et al., 2020). Nano-iron, a new type of physicochemical additive, achieved the highest relative reduction efficiency ~~for~~ of ARGs at 75.0%. As previous studies have reported that Nano-iron possibly influenced the ARGs by directly decease the MGEs (Wang et al., 2020; Qiu et al., 2022). However, the introduction of nanoparticles may also bring other ecological risks, and whether it should be widely promoted still requires further in-depth research.



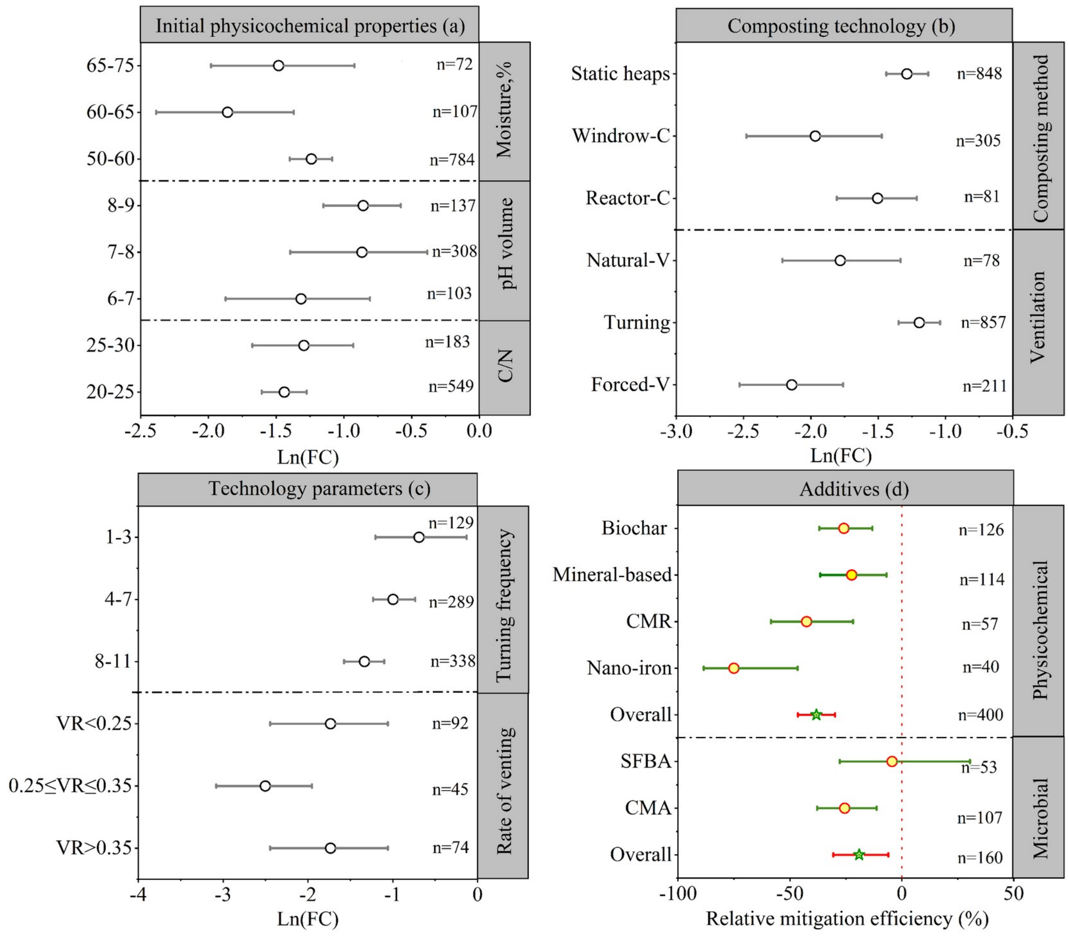


Fig.~~5~~ 4 the ~~Response ratios~~ effect size of Initial physicochemical properties (a) Composting technology (b) Technology parameters (c), and Additives (d) on the reduction of ARGs. The total number of observations for each treatment is displayed on the right-hand side of the results. Windrow-C, Reactor-C, Natural-V and Forced-V represents Windrow composting, reactor composting, natural ventilation and forced ventilation. CMR, SFBA and CMA indicate Chinese medicine residue, single functional bacterial agent and complex microbial agent. The unit of turning frequency and vent rate are days per turning time and L/min/kg dry weight (DW).

~~除了Additive是不是还有一些其他的，通风之类的？~~

3.4~~5~~ Driven~~r~~ factors of the ARGs changes during composting

In order to find out the driven factors of ARGs changes during the composting, 9 impact parameters were analyzed by random forest. As shown in **Fig.**~~6~~**5**, MGEs and

~~composting duration (~~CD~~)~~ had a higher relative importance with 28.3% and 25%, respectively. The importance of MGEs to ARGs fate has been mentioned by many other studies (Wang et al., 2020; Qiu et al., 2022). But the importance of ~~composting duration (~~CD~~)~~ was ignored over a long time, so identifying an appropriate composting time is crucial which have been specifically discussed in **3.3.** Additionally, future investigate should focus on different composting stages in ARGs reduction. Compared with Ventilation method (VM) and Composting method (~~-~~CM), The Initial MC, pH and HTP, may influence the fate of ARGs by directly change the microhabitat, have a higher relative importance at 20.3%, 20.2% and 16.9%, respectively. The lowest relative importance factors were Initial C/N and Additives with 9.5% and 8.4%.

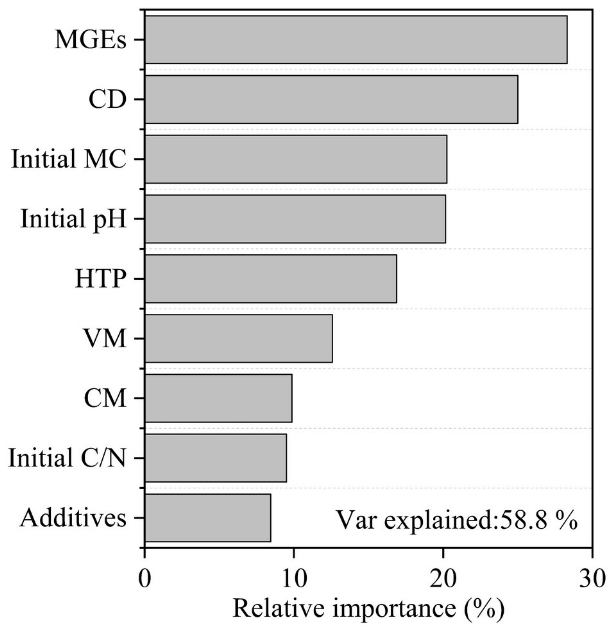


Fig.~~6~~ 5 The relative importance of composting parameters on ARGs changes. CD, HTP, VM and CM represent composting duration, high temperature period, ventilation method and composting method.

4 Conclusion~~（缩短 Conclusion 部分的占比，一段话，300 字足够）~~

This first meta-~~analyses~~analysies ~~establishes~~ established that the composting treatment can significantly reduce the ARGs (74.3%) and MGEs (78.8%) ~~levels~~abundances~~, and their effectiveness varies depending on the organic solids wastes types and ARGs types~~. ~~The changes in overall ARGs abundance at different time points indicates that~~Moreover, the 3rd to the 6th week is a stable and high reduction composting period, with the mitigation efficiency from 72.4% to 79.7% and ~~. Moreover,~~ the ARGs abundances rebound~~s~~ after the 8th week during the composting process. ~~But the β-lactam resistance genes show a declining trend throughout the whole composting period.~~

~~Compared with other categories, t~~The MC, pH and C/N are more effective in ARGs degradation when adjust to 60%-65%, 6-7 and 20-25 before composting, respectively. The windrow composting and forced ventilation (vent rate: 0.25 to 0.35 L/min/kg DW) are suitable technologies which can promote the reduction of ARGs. Nano-iron, as a new physicochemical additive, can mitigate ARGs by 75.0% compared with the control. Additionally, MGEs and CD are the most important driven factors that influence ARGs changes, with relative importance at 28.3% and 25%, respectively, which the significance of CD has been overlooked before.~~for a long time.~~

This meta-analysis offers a comprehensive insight into how composting and composting factors influence the response of ARGs. This information will aid the reduction of ARGs during composting, and cut off the spread in food systems.

Acknowledgement

References

1. Pei, M., Zhang, B., He, Y., Su, J., Gin, K., Lev, O., ... & Hu, S. (2019). State of the art of tertiary treatment technologies for controlling antibiotic resistance in wastewater treatment plants. Environment International, 131, 105026.
2. Ben, Y., Fu, C., Hu, M., Liu, L., Wong, M. H., & Zheng, C. (2019). Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. Environmental research, 169, 483-493.
3. Huang, Q., Chen, G., Wang, Y., Xu, L., & Chen, W. Q. (2020). Identifying the socioeconomic drivers of solid waste recycling in China for the period 2005–2017. Science of the total environment, 725, 138137.
4. Allen, H. K., Donato, J., Wang, H. H., Cloud-Hansen, K. A., Davies, J., & Handelsman, J. (2010). Call of the wild: antibiotic resistance genes in natural environments. Nature Reviews Microbiology, 8(4), 251-259.
5. Huddleston, J. R. (2014). Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. Infection and drug resistance, 7, 167.
6. Pruden, A., Pei, R., Storteboom, H., & Carlson, K. H. (2006). Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environmental science & technology, 40(23), 7445-7450.
7. Karkman, A., Johnson, T. A., Lyra, C., Stedtfeld, R. D., Tamminen, M., Tiedje, J. M., & Virta, M. (2016). High-throughput quantification of antibiotic resistance

genes from an urban wastewater treatment plant. FEMS microbiology ecology, 92(3), fiw014.

8. Wohde, M., Berkner, S., Junker, T., Konradi, S., Schwarz, L., & Düring, R. A. (2016). Occurrence and transformation of veterinary pharmaceuticals and biocides in manure: a literature review. Environmental Sciences Europe, 28(1), 1-25.
9. Du, L., & Liu, W. (2012). Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. Agronomy for sustainable development, 32(2), 309-327.
10. Cao, R., Wang, J., Ben, W., & Qiang, Z. (2020). The profile of antibiotic resistance genes in pig manure composting shaped by composting stage: Mesophilic-thermophilic and cooling-maturation stages. Chemosphere, 250, 126181.
11. Sardar, M. F., Zhu, C., Geng, B., Ahmad, H. R., Song, T., & Li, H. (2021). The fate of antibiotic resistance genes in cow manure composting: Shaped by temperature-controlled composting stages. Bioresource Technology, 320, 124403.
12. Wu, J., Wang, J., Li, Z., Guo, S., Li, K., Xu, P., ... & Zou, J. (2022). Antibiotics and antibiotic resistance genes in agricultural soils: A systematic analysis. Critical Reviews in Environmental Science and Technology, 1-18.
13. Cao, Y., Wang, X., Zhang, X., Misselbrook, T., Bai, Z., & Ma, L. (2021a). An electric field immobilizes heavy metals through the promotion of humic substance formation: an intrinsic bioremediation technique for composting. Bioresour Technol., 330, 124996.
14. Cao, Y., Wang, X., Zhang, X., Misselbrook, T., Bai, Z., & Ma, L. (2021). Nitrifier

denitrification dominates nitrous oxide production in composting and can be inhibited by a bioelectrochemical nitrification inhibitor. Bioresource Technology, 341, 125851.

15. Liao, H., Zhao, Q., Cui, P., Chen, Z., Yu, Z., Geisen, S., ... & Zhou, S. (2019). Efficient reduction of antibiotic residues and associated resistance genes in tylosin antibiotic fermentation waste using hyperthermophilic composting. Environment international, 133, 105203.
16. Wang, R., Zhang, J., Sui, Q., Wan, H., Tong, J., Chen, M., ... & Wei, D. (2016). Effect of red mud addition on tetracycline and copper resistance genes and microbial community during the full scale swine manure composting. Bioresource technology, 216, 1049-1057.
17. Selvam, A., Zhao, Z., & Wong, J. W. (2012). Composting of swine manure spiked with sulfadiazine, chlortetracycline and ciprofloxacin. Bioresource technology, 126, 412-417.
18. Qian, X., Sun, W., Gu, J., Wang, X. J., Zhang, Y. J., Duan, M. L., ... & Zhang, R. R. (2016a). Reducing antibiotic resistance genes, integrons, and pathogens in dairy manure by continuous thermophilic composting. Bioresource Technology, 220, 425-432.
19. Qian, X., Sun, W., Gu, J., Wang, X. J., Sun, J. J., Yin, Y. N., & Duan, M. L. (2016b). Variable effects of oxytetracycline on antibiotic resistance gene abundance and the bacterial community during aerobic composting of cow manure. Journal of hazardous materials, 315, 61-69.

20. Pu, C., Yu, Y., Diao, J., Gong, X., Li, J., & Sun, Y. (2019). Exploring the persistence and spreading of antibiotic resistance from manure to biocompost, soils and vegetables. Science of the total environment, 688, 262-269.
21. Wang, G., Li, G., Chang, J., Kong, Y., Jiang, T., Wang, J., & Yuan, J. (2021). Enrichment of antibiotic resistance genes after sheep manure aerobic heap composting. Bioresource Technology, 323, 124620.
22. Johnson, T. A., Stedtfeld, R. D., Wang, Q., Cole, J. R., Hashsham, S. A., Looft, T., ... & Tiedje, J. M. (2016). Clusters of antibiotic resistance genes enriched together stay together in swine agriculture. MBio, 7(2), e02214-15.
23. Liu, B., Yu, K., Ahmed, I., Gin, K., Xi, B., Wei, Z., ... & Zhang, B. (2021). Key factors driving the fate of antibiotic resistance genes and controlling strategies during aerobic composting of animal manure: A review. Science of the Total Environment, 791, 148372.
24. Cui, E., Wu, Y., Zuo, Y., & Chen, H. (2016). Effect of different biochars on antibiotic resistance genes and bacterial community during chicken manure composting. Bioresource Technology, 203, 11-17.
25. Fan, H., Wu, S., Woodley, J., Zhuang, G., Bai, Z., Xu, S., ... & Zhuang, X. (2020). Effective removal of antibiotic resistance genes and potential links with archaeal communities during vacuum-type composting and positive-pressure composting. Journal of Environmental Sciences, 89, 277-286.
26. Flores-Orozco, D., Levin, D., Kumar, A., Sparling, R., & Cicek, N. (2022). A meta-analysis reveals that operational parameters influence levels of antibiotic

resistance genes during anaerobic digestion of animal manures. Science of The Total Environment, 814, 152711.

27. Su, J. Q., Wei, B., Ou-Yang, W. Y., Huang, F. Y., Zhao, Y., Xu, H. J., & Zhu, Y. G. (2015). Antibiotic resistome and its association with bacterial communities during sewage sludge composting. Environmental science & technology, 49(12), 7356-7363.
28. Zhou, S., Kong, F., Lu, L., Wang, P., & Jiang, Z. (2022). Biochar—An effective additive for improving quality and reducing ecological risk of compost: A global meta-analysis. Science of The Total Environment, 806, 151439.
29. Cao, Y., Wang, X., Bai, Z., Chadwick, D., Misselbrook, T., Sommer, S. G., ... & Ma, L. (2019). Mitigation of ammonia, nitrous oxide and methane emissions during solid waste composting with different additives: a meta-analysis. Journal of Cleaner Production, 235, 626-635.
30. Hedges, L. V., Gurevitch, J., & Curtis, P. S. (1999). The meta-analysis of response ratios in experimental ecology. Ecology, 80(4), 1150-1156.
31. Han, X. M., Hu, H. W., Chen, Q. L., Yang, L. Y., Li, H. L., Zhu, Y. G., ... & Ma, Y. B. (2018). Antibiotic resistance genes and associated bacterial communities in agricultural soils amended with different sources of animal manures. Soil Biology and Biochemistry, 126, 91-102.
32. Quan, Z., Zhang, X., Davidson, E. A., Zhu, F., Li, S., Zhao, X., ... & Fang, Y. (2021). Fates and use efficiency of nitrogen fertilizer in maize cropping systems and their responses to technologies and management practices: A global analysis

on field 15N tracer studies. Earth's Future, 9(5), e2020EF001514.

33. Abdellah, Y. A. Y., Shi, Z., Luo, Y. S., Hou, W. T., Yang, X., & Wang, R. L. (2022). Effects of different additives and aerobic composting factors on heavy metal bioavailability reduction and compost parameters: A meta-analysis. Environmental Pollution, 119549.
34. Li, W., Wu, J., Bai, E., Guan, D., Wang, A., Yuan, F., ... & Jin, C. (2016). Response of terrestrial nitrogen dynamics to snow cover change: a meta-analysis of experimental manipulation. Soil Biology and Biochemistry, 100, 51-58.
35. Rosenberg, M. S. (2000). MetaWin: Statistical software for meta-analysis: version 2. Sinauer.
36. Cheng, D., Feng, Y., Liu, Y., Xue, J., & Li, Z. (2019). Dynamics of oxytetracycline, sulfamerazine, and ciprofloxacin and related antibiotic resistance genes during swine manure composting. Journal of environmental management, 230, 102-109.
37. Duan, M., Zhang, Y., Zhou, B., Wang, Q., Gu, J., Liu, G., ... & Li, Z. (2019). Changes in antibiotic resistance genes and mobile genetic elements during cattle manure composting after inoculation with Bacillus subtilis. Bioresource technology, 292, 122011.
38. Qian, X., Gu, J., Sun, W., Wang, X. J., Su, J. Q., & Stedfeld, R. (2018). Diversity, abundance, and persistence of antibiotic resistance genes in various types of animal manure following industrial composting. Journal of Hazardous materials, 344, 716-722.

39. Wang, Q., Gu, J., Wang, X., Ma, J., Hu, T., Peng, H., ... & Zhang, R. (2020). Effects of nano-zerovalent iron on antibiotic resistance genes and mobile genetic elements during swine manure composting. Environmental Pollution, 258, 113654.

40. Selvam, A., Xu, D., Zhao, Z., & Wong, J. W. (2012). Fate of tetracycline, sulfonamide and fluoroquinolone resistance genes and the changes in bacterial diversity during composting of swine manure. Bioresource technology, 126, 383-390.

41. Gao, Y., Lu, C., Shen, D., Liu, J., Ma, Z., Yang, B., ... & Waigi, M. G. (2019). Elimination of the risks of colistin resistance gene (mcr-1) in livestock manure during composting. Environment international, 126, 61-68.

42. Duan, M., Gu, J., Wang, X., Li, Y., Zhang, S., Yin, Y., & Zhang, R. (2018). Effects of genetically modified cotton stalks on antibiotic resistance genes, intI1, and intI2 during pig manure composting. Ecotoxicology and environmental safety, 147, 637-642.

43. Xie, J., Gu, J., Wang, X., Hu, T., Sun, W., Lei, L., ... & Guo, H. (2021). Insights into the beneficial effects of woody peat for reducing abundances of antibiotic resistance genes during composting. Bioresource Technology, 342, 125903.

44. Ezugworie, F. N., Igbokwe, V. C., & Onwosi, C. O. (2021). Proliferation of antibiotic-resistant microorganisms and associated genes during composting: An overview of the potential impacts on public health, management and future. Science of The Total Environment, 784, 147191.

45. Liang, C., Das, K. C., & McClendon, R. W. (2003). The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend. Bioresource technology, 86(2), 131-137.

46. Jiang, T., Schuchardt, F., Li, G., Guo, R., & Zhao, Y. (2011). Effect of C/N ratio, aeration rate and moisture content on ammonia and greenhouse gas emission during the composting. Journal of Environmental Sciences, 23(10), 1754-1760.

47. Sánchez-Monedero, M. A., Roig, A., Paredes, C., & Bernal, M. P. (2001). Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of the composting mixtures. Bioresource technology, 78(3), 301-308.

48. Zhang, R., Gu, J., Wang, X., Li, Y., Zhang, K., Yin, Y., & Zhang, X. (2018). Contributions of the microbial community and environmental variables to antibiotic resistance genes during co-composting with swine manure and cotton stalks. Journal of hazardous materials, 358, 82-91.

49. Wei, H., Ma, J., Su, Y., & Xie, B. (2020). Effect of nutritional energy regulation on the fate of antibiotic resistance genes during composting of sewage sludge. Bioresource Technology, 297, 122513.

50. Liu, Z., Wang, X., Li, S., Bai, Z., & Ma, L. (2022). Advanced composting technologies promotes environmental benefits and eco-efficiency: A life cycle assessment. Bioresource Technology, 346, 126576.

51. Fan, S., Li, A., ter Heijne, A., Buisman, C. J., & Chen, W. S. (2021). Heat potential, generation, recovery and utilization from composting: A review.

Resources, Conservation and Recycling, 175, 105850.

52. Getahun, T., Nigusie, A., Entele, T., Van Gerven, T., & Van der Bruggen, B. (2012). Effect of turning frequencies on composting biodegradable municipal solid waste quality. Resources, Conservation and Recycling, 65, 79-84.

53. Gao, M., Li, B., Yu, A., Liang, F., Yang, L., & Sun, Y. (2010). The effect of aeration rate on forced-aeration composting of chicken manure and sawdust. Bioresource Technology, 101(6), 1899-1903.

54. Liang, J., Jin, Y., Wen, X., Mi, J., & Wu, Y. (2020). Adding a complex microbial agent twice to the composting of laying-hen manure promoted doxycycline degradation with a low risk on spreading tetracycline resistance genes. Environmental Pollution, 265, 114202.

55. Qiu, X., Zhou, G., & Wang, H. (2022). Nanoscale zero-valent iron inhibits the horizontal gene transfer of antibiotic resistance genes in chicken manure compost. Journal of Hazardous Materials, 422, 126883.