agriculture

MDPI

Article

The Impact of Chicken Manure Biochar on Antibiotic Resistance Genes in Chicken Manure Composting

Kamalya Karamova, Natalia Danilova, Svetlana Selivanovskaya and Polina Galitskaya *

Institute of Environmental Sciences, Kazan Federal University, 420008 Kazan, Russia
* Correspondence: gpolina33@yandex.ru

Abstract: One way to increase yields in agriculture using organic alternative methods is the introduction of manure-based composts into the soil. However, the use of such composts carries a risk of soil contamination with antibiotic resistance genes (ARG) from the gut and manure of the livestock. The contamination of the composts with heavy metals or antibiotics can increase this risk, while the addition of porous materials, such as biochar, to the composts has the potential to decrease it. This study is devoted to revealing the fate of ARGs in bedding chicken manure composted with the addition of oxytetracycline (OTC), heavy metals, and chicken manure biochar. It was revealed that the additives did not affect the physicochemical parameters of the compost. The bacterial communities in different composting mixtures had similar structures and dynamics. It was revealed that the shifts of the bacterial compositions of the composting mixtures were mainly determined by the duration of the process. However, some minor differences in the OTU (operational taxonomic unit) levels were observed between the variants. The addition of biochar and metals led to 26.7% and 34.5% decreases, respectively, in the number of *tet*(A) gene copies, while the addition of oxytetracycline led to a 43.7% increase. The number of copies of the *int*1 gene increased by 45.9% after the addition of oxytetracycline. The correlation between the abundance levels of different bacterial OTU and ARG contents was estimated, and biochar's impact on those OTUs was analyzed. It was assumed that some OTUs might be carriers of ARGs (such as *Natronobacillus*, *Luteimonas*, and *Trichococcus*), and their abundance in the presence of the biochar decreased due to competitive exclusion by noncarriers (such as *Corynebacterium*, *Clostridia*, and *Halorhodospira*). The use of biochar in composting can be considered a way to reduce the contamination of the final composts with ARGs.

Keywords: oxytetracycline; heavy metals; respiration activity; horizontal transfer; microbial community

Citation: Karamova, K.; Danilova, N.; Selivanovskaya, S.; Galitskaya, P. The Impact of Chicken Manure Biochar on Antibiotic Resistance Genes in Chicken Manure Composting. *Agriculture* **2022**, *12*, 1158. https://doi.org/10.3390/agriculture12081158

Academic Editors: Francesco Primo Vaccari, Silvia Baronti, Nicolas Greggio and Diego Marazza

Received: 22 June 2022
Accepted: 30 July 2022
Published: 4 August 2022

1. Introduction

The quality of agricultural soil is very important because it is directly related to its fertility and the plant yield, which in turn is directly related to global food security. Many studies have demonstrated that the use of mineral fertilizers contributes to an increase in yields but leads to a drop in soil quality in the long term [1]. Therefore, alternative methods of fertilization have been discussed, with the use of livestock manure compost being one of them [2]. The use of compost as an organic soil fertilizer remains an integral practice in agriculture, as it improves the physicochemical and biological parameters and supplies plants with important nutrients, thereby increasing yields [3]. However, the manure from large-scale farms often contains antibiotics. These are antibacterial compounds that are widely used in veterinary and livestock husbandry in order to heal and prevent diseases and in order to stimulate livestock weight gain. Chicken meat is the main source of protein for the Earth's population, and the amount of antibiotics used in chicken husbandry exceeds the amounts used for other livestock types [4]. The most commonly used antibiotics in chicken husbandry are tetracyclines, sulfonamides, and macrolides [5,6].

Manure is usually contaminated by antibiotic compounds and their metabolites, since only a low proportion of antibiotics is absorbed in the chicken gut [5,6]. Antibiotics can

enter the soil from the compost and can negatively affect plant yields, since they inhibit the functioning of earthworms, reduce the diversity of bacteria, increase the proportion of plant pathogenic species, and reduce the bioavailability of nutrients [7].

Bacteria are able to quickly develop mechanisms to resist antibiotics. Therefore, manure and manure-based composts contain various microbes carrying antibiotic resistance genes (ARGs) able to be transferred to other generations through vertical transfer or other populations through horizontal transfer [5]. The problem of the ARG contamination of manures and fertilizers made of these manures is considered even bigger than the original problem of antibiotic contamination, since the resistance may be transferred and can last for months or even years, while antibiotic compounds usually decompose within several days or weeks [5,6]. The existing risks of ARG spread in soils and plants should be considered, as a threat to public health could be created by the consumption of agricultural products [8].

There are several mechanisms by which microorganisms have developed resistance to antibiotics. Those mechanisms can be encoded by one or several specific genes located in chromosomes or more often in plasmids [9]. In the case of tetracyclines, which are the most widespread antibiotics in poultry husbandry, about 40 different genes encoding resistance have been revealed. For example, the genes *tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E), *tet*(G), *tet*(H), *tet*(I), *tet*(J), and *tet*(Z) encode the active antibiotic efflux. The genes *tet*(M), *tet*(O), *tet*(S), *tet*(W), *tet*(Q), and *tet*(T) encode ribosome protection, and the *tet*(X) gene encodes antibiotic detoxification [10]. Bacteria may possess several ARGs encoding different ways to resist one and the same antibiotic compound, or to resist different antibiotics [11]. Many reports have demonstrated that composting leads to a decrease in the ARG content in manure, up to their total removal [12–14]. Some reports have underlined only a temporary decrease in ARG copy numbers in the thermophilic phase of composting, with regeneration in the following phases [15,16]. Rare reports have even demonstrated an increase in ARG copy numbers during the process of composting [17,18]. Those contradictory results were observed due to the complexity of the processes occurring during composting: on the one hand, ARG-carrying microbes might be eliminated in the thermophilic phase of composting, since they are not tolerant to high temperatures; on the other hand, the elevated temperatures might intensify the horizontal gene transfer and enlarge the number of ARG-carrying species [19]. Additional factors such as the manure origin, its level of contamination by antibiotics and AGRs, the type of antibiotics, the temperature and duration of the thermophilic phase, the biogenic elements, the moisture and other abiotic parameters involved in composting, and the presence of specific compounds such as heavy metals that are known to be intensifiers of ARG horizontal transfer may play significant roles in the fate of ARGs in the final compost [14,20–23]. Therefore, additional technologies for the improvement of ARG removal from compost are required. One of those is the addition of a porous material such as a zeolite or biochar to the composting mixture [24]. It is believed that the porous material may absorb antibiotic compounds, mitigating their selective pressure; absorb mobile genetic elements (plasmids, integrons, and transposases), mediating the horizontal gene transfer of ARGs; and create favorable conditions for some populations, causing bacterial succession in the direction of a nonresistant community [14,25–28]. However, the mechanisms of biochar influence on the compost bacterial community, including the ARG carriers, are not fully clear yet. In addition, the introduction of biochar into the soil as part of the compost can increase the plant biomass, and accordingly can contribute to the accumulation of organic matter in the soil, since its content is closely related to nutrient cycling, soil quality, and fertility. Previously, the best results have been obtained for biochar made of plant biomass, such as straw or wood [14,27,29–33]. Biochar made of chicken manure has high potential as a soil fertilizer, since it possesses not only common biochar characteristics such as high porosity and a large surface area, but also specific characteristics originating from the initial substrate—high nitrogen and phosphorous contents [34]. In the literature, data concerning this biochar type's influence on the processes of chicken manure composting, including the removal or enrichment of ARGs and the mechanisms of this process, are presented [34].

Based on our limited understanding of the fate of ARGs in chicken manure compost mixtures amended with chicken manure biochar, the overall objective of this study was to reveal the factors most affecting the genes *tet*(A) and *tet*(X) during the process of chicken manure composting. The genes *tet*(A) and *tet*(X) encode two different mechanisms of resistance of bacteria to tetracyclines, which are the most widespread group of antibiotics in poultry husbandry. For this, we composted chicken manure with and without biochar additions, as well as with and without the addition of oxytetracycline and heavy metals. The physicochemical parameters, as well as the respiration rates, ARGs and integron class 1 gene copy numbers, and the structure of the bacterial community, were monitored during the process of composting.

2. Materials and Methods

Bedding chicken manure was obtained from the poultry farm "Chelny-Broiler" (Naberezhnye Chelny, Tatarstan Republic). This is one of the 10 largest chicken farms in Russia, producing about 150,000 t chicken manure per year. The following variants of compost mixtures were prepared: control K (without additives), B (with the addition of 15% biochar, *w*/*w*), O (with the addition of the antibiotic oxytetracycline at a concentration of 300 mg kg^{-1}), M (with the addition of metals Ni, Cd, Fe, and Cu at concentrations of 70, 130, 1500, and 1000 mg kg^{-1}), MO (with the addition of metals and the antibiotic), and their combinations (variants BO, BM, and BMO). Since the presence of heavy metals is able to intensify the horizonal transfer of ARGs, and the purpose of the present study was to reveal the fate of ARGs in the composting mixtures, variants with heavy metals (raw and with the addition of biochar) were used in this study.

All composting variants were prepared in three replicates. Each replicate included 60 kg of raw bedding chicken manure and 18 kg of straw, which was used as a structuring agent. Some replicates contained additives (biochar, heavy metals, and oxytetracycline) in the amounts mentioned above. The composting was carried out for 120 days in rotary drum composters with a volume of 200 L with daily stirring for 1 h at a speed of 40 rpm (Supplementary Figure S1. The temperature in the compost mixtures was measured daily. On the 1st, 3rd, 7th, 14th, 21st, 28th, 35th, and 42nd days, and then once every two weeks, samples were taken for the analysis of the dissolved organic carbon; the respiration activity; the numbers of bacteria, fungi, and antibiotic-resistant genes; the *int*1 gene; and the structure of the microbial community. All measurements were carried out in three replicates.

The content of dissolved organic carbon was estimated using the wet oxidation method in accordance with ISO 14235:1998, using the process of oxidation of chromium ions Cr^{6+} to Cr^{3+} with pre-extraction with an aqueous solution of potassium sulfate [35]. The respiration activity of the microorganisms was evaluated based on the amount of carbon dioxide released during the incubation in hermetically sealed vessels according to ISO 16072:2002 [36].

The extraction of DNA from samples was performed immediately after sampling using a FastDNA™ Spin Kit for soil DNA (MP Biomedicals, Irvine, CA, USA). The resulting nucleic acid was used for the real-time PCR to assess the content of ARGs (*tet*(A), *tet*(X)), the *int*1 gene, and the total copy numbers of bacteria and fungi [37]. The structure of the bacterial community was assessed on the basis of 16S amplicon sequencing using the Illumina MiSeq method. To do this, the total DNA isolated from the samples was purified using the QIAquick PCR Purification Kit (Qiagen, Düsseldorf, Germany). The DNA concentration was determined on a Qubit Fluorometer (Invitrogen, Waltham, MA, USA) using a Quant-iTds DNA High-Sensitivity Assay Kit (Thermo Fisher, Waltham, MA, USA). The samples were further prepared using the MiSeq Reagen Kit v2 and a MiSeq device (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. The obtained sequence data were analyzed using the QIIME program [38]. The chimeras were excluded from analysis using the usearch61 algorithm. The bacterial operational taxonomic units (OTUs) were determined according to the Greengenes database. The taxonomic classification was performed using the RDP classifier and PyNAST [39]. The community

diversity analysis was performed using the R vegan software package (http://sortie-admin.readyhosting.com/lme/R%20Packages/vegan.pdf, accessed on 5 November 2020).

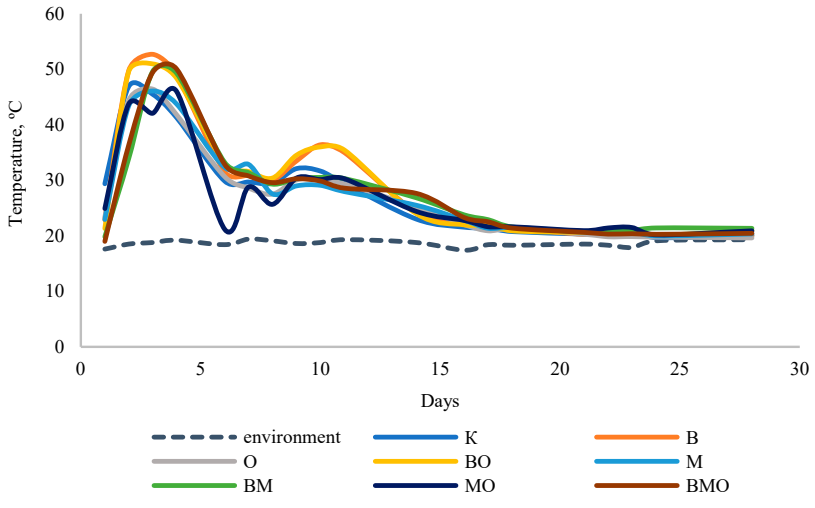
All studies were carried out at least in triplicate. The statistical processing of the obtained results was carried out using Microsoft Office Excel 2010 (Redmond, WA, USA). All tabular and graphical data contain average values and standard errors. To assess the significance of differences, Fisher's test was used at $\alpha = 0.05$. The beta diversity was calculated using the R software package (www.r-project.org, accessed on 5 November 2020). Multivariate scaling based on the Bray–Curtis coefficient was used to determine the beta diversity [40]. The mathematical modeling (robust linear model) and correlative analysis (RandomForest method) were carried out in the R software package (www.r-project.org, accessed on 5 November 2020).

3. Results

3.1. Physicochemical Parameters of Composting and Respiration Activity of Compost Mixtures

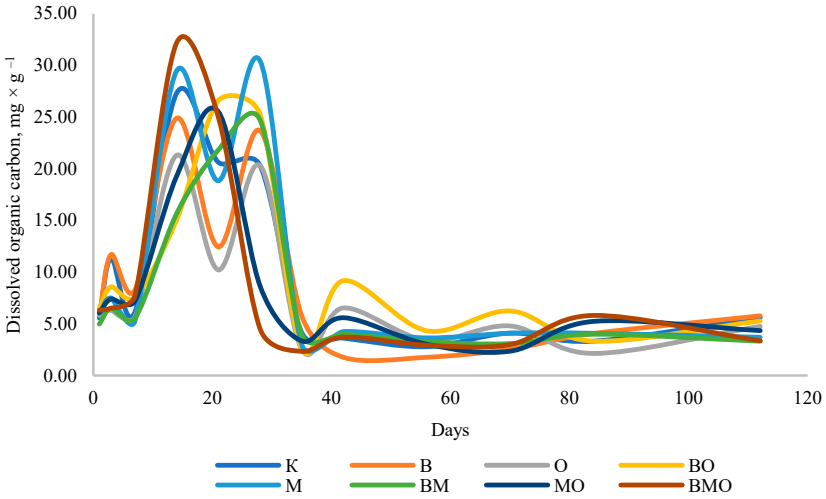
The results of the assessment of the temperature, dissolved organic carbon, and respiration activity are shown in Figure 1a–c. The temperature profiles of the compost mixtures generally coincided for all variants and did not depend on the addition of biochar, oxytetracycline, or heavy metals. Interestingly, a slight short-term increase in temperature was observed between days 2 and 5 in the variants B, BO, BM, and BMO, and between days 9 and 13 in the variants B and BO. Since this effect was observed only for the variants containing biochar, it can be assumed that the biochar played a role in the intensification of the microbes, enabling exothermic processes of decomposition of organic matter in the composting mixtures.

The dynamics patterns of the contents of dissolved organic carbon were alike for all variants of compost mixtures (Figure 1b). On the 1st day, the values were about 5–6 mg g^{-1}, then by the 14th day a sharp increase up to 21–32 mg g^{-1} was observed. The dissolved organic carbon levels remained high until the 21st day. Later on, a decrease to 2–5 mg g^{-1} was recorded. Starting on the 35th day, there were no significant changes in the content of dissolved organic carbon. All compost mixtures also showed similar dynamics of respiration activity during the entire composting process. On the 1st day, the values ranged from 0.002 to 0.008 mg CO_2 g h^{-1}. Furthermore, by the 3rd day, in all variants there was a sharp increase in the level of respiration to 0.008–0.013 mg CO_2 g h^{-1}, which coincided with the thermophilic phase of composting. Interestingly, the highest values for the respiration activity were noted for those variants of compost mixtures that contained the antibiotic and heavy metals (all samples except the control and B samples).

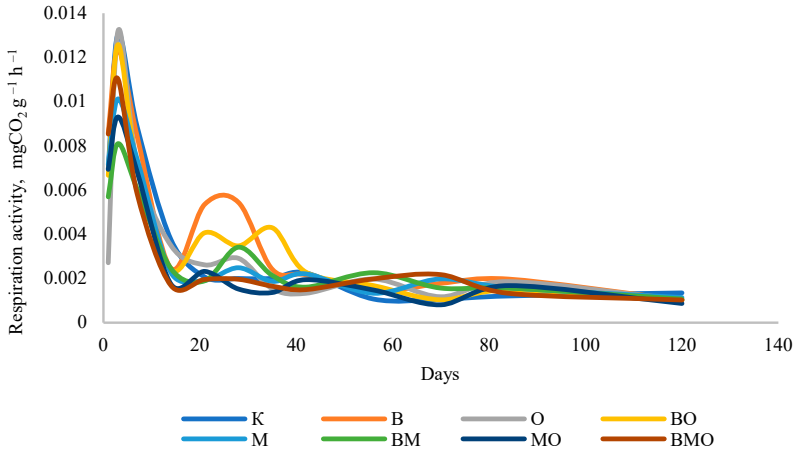


(**a**)

Figure 1. *Cont.*



(**b**)



(**c**)

Figure 1. Temperature (**a**), dissolved organic carbon content (**b**), and respiration activity (**c**) of the compost mixtures.

Thus, the addition of the biochar, antibiotics, and heavy metals to the compost mixtures did not generally have a significant effect on the physicochemical parameters during composting; however, it somehow affected the level of respiration of the composts.

3.2. Bacterial and Fungal Counts, and Copy Numbers of tet(A), tet(X), and int1 Genes

Figure 2a,b gives data on the number of copies of genes, reflecting the amounts of bacteria and fungi, on the 1st and 84th days of the experiment. On the 1st day, the bacterial gene copy numbers did not differ between the samples and ranged between 1.16 and 2.21×10^8 copies g^{-1}. By the end of the composting, the numbers of bacterial copies of genes decreased in all samples by 7–20-fold, and significant differences were observed between the numbers of samples; for example, the minimum value for this parameter was found in the BO sample ($6.25\ 10^6$ gene copies g^{-1}), which differed significantly from that of samples O, M, MO, and BMO. In the fungal community, on the contrary, a decrease in the number of copies of genes was not detected for any of the samples, while in the samples of BO, BM, and BMO there was a slight but statistically significant increase in this indicator (from 2.6×10^4–4.28×10^4 to 1.24×10^5–1.39×10^5 gene copies g^{-1}, respectively).

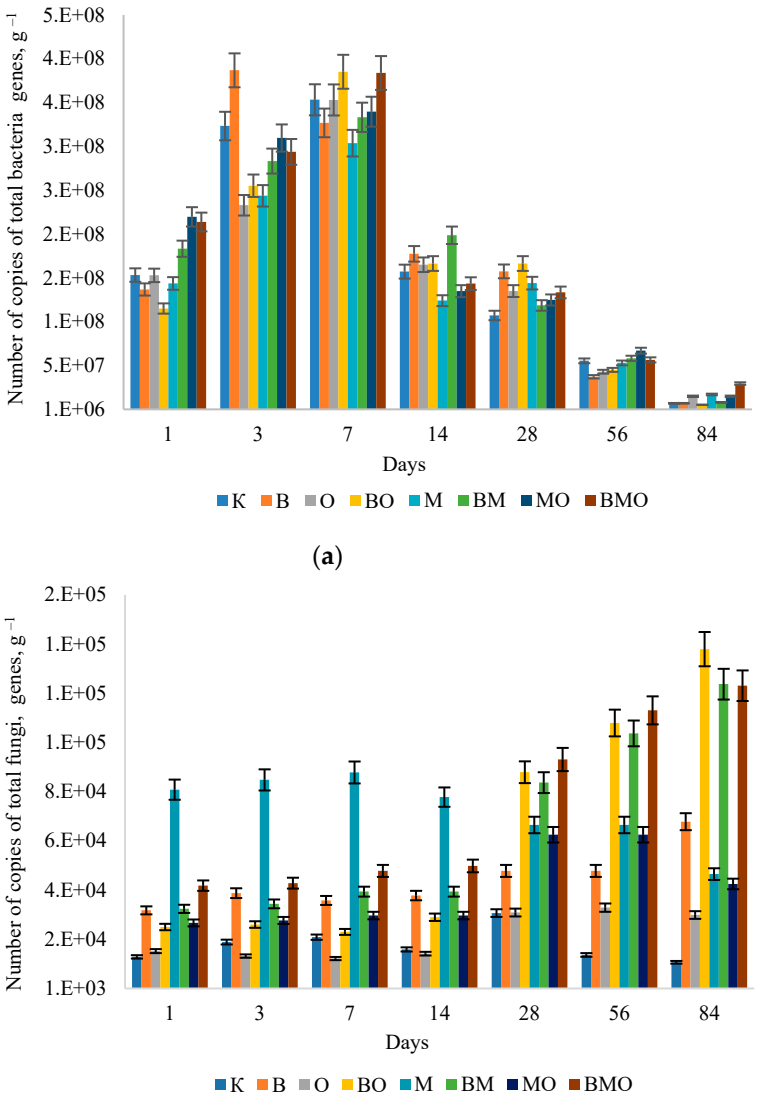


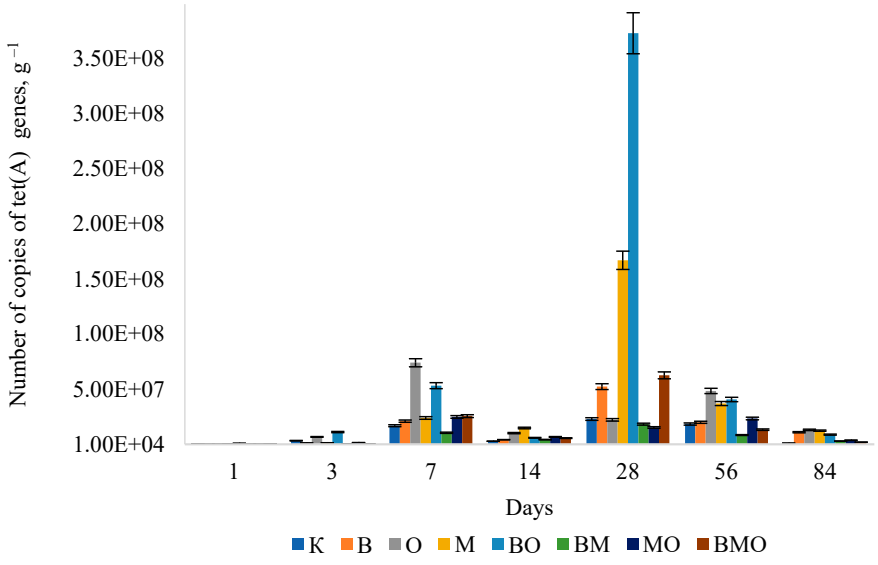
Figure 2. Bacterial (**a**) and fungal (**b**) gene copy numbers in the compost mixtures.

Furthermore, the content of tetracycline resistance genes was determined in the DNA samples. About 40 such genes are known from the literature; however, we chose two of them for a number of reasons: firstly, they are responsible for two different mechanisms of antibiotic resistance; secondly, they are among the most frequently analyzed by researchers; thirdly, as a result of our previous research (data not shown), the results obtained for these two genes were the most reproducible [41].

As follows from Figure 3a,b, the patterns of the dynamics of these genes for the control sample were different: at the beginning of composting, the number of copies of the *tet*(A) gene was 4.89×10^5 copies g^{-1}; by the 7th day it increased to 1.69×10^7 copies g^{-1}, then it decreased, increased, and fell again. The number of copies of the *tet*(X) gene was higher than that of *tet*(A) on all days. Moreover, the shape of the curve reflecting the gene copy numbers was different; on the 1st day it showed 1.61×10^7 copies g^{-1}, on the 3rd day it increased to 4.37×10^8 copies $\times g^{-1}$, and then it remained almost constant until the end of the experiment. The addition of biochar, oxytetracycline, and a mixture of metals into compost mixtures did not lead to changes in the nature of the fluctuations in the number of copies of the *tet*(A) gene. However, it mainly led to their slight increase. Thus, on the 28th day (on which the largest number of gene copies was observed in all samples), the minimum values were noted in samples O, BM, and MO; in other samples, the values

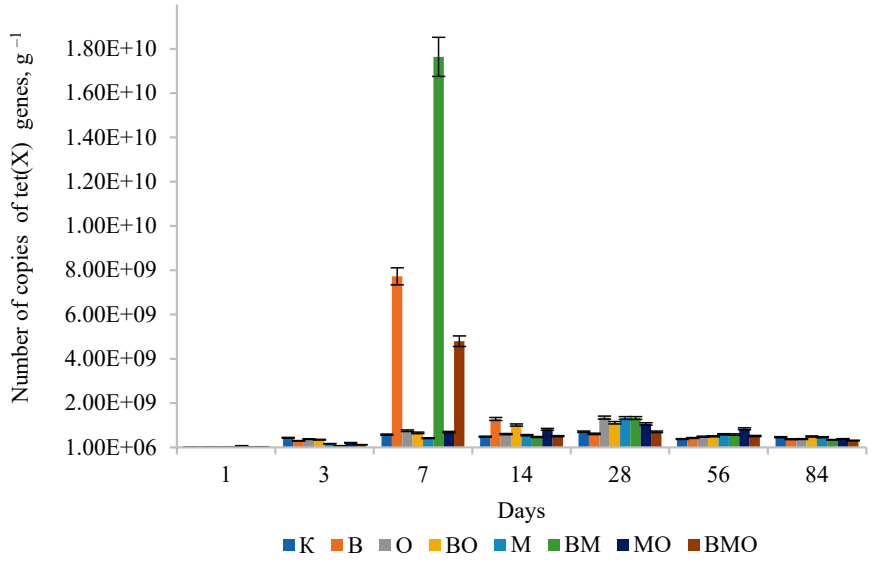
over the control were exceeded by 1.6 (sample BO), 2.7 (sample BMO), 2.3 (sample B), and 7.3 (sample M)-fold. At the end of the experiment, the lowest number of copies of the *tet*(A) gene was also found in the control sample. These differences did not allow us to unambiguously identify the factors influencing the number of copies of antibiotic-resistant genes; therefore, the data were subjected to further statistical processing. In the O, BO, M, and MO samples, the dynamics of the *tet*(X) resistance genes was similar to that in the control; on the 3rd day, the numbers of gene copies increased by 5–23-fold relative to the control and then remained unchanged until the end of the experiment. The "behavior" of the resistance genes *tet*(X) in samples B, BM, and BMO is noteworthy; on the 7th day, a significant increase in the number of their gene copies was observed, coinciding with the thermophilic phase. It can be assumed that the carriers of the *tet*(X) gene in these samples and at that time were thermophilic microorganisms, the number of which sharply decreased after the end of the thermophilic phase. Generally, for both *tet*(A) and *tet*(X) genes in all variants of compost mixtures, an increase in the number of gene copies was observed from the beginning of composting to its end.

Integrons of class 1 are mobile genetic elements involved in the horizontal transfer of ARGs. Therefore, assessments of the genes encoding these integrons are widely used to estimate the intensity of the process of horizontal transfer [42]. In the control sample, the number of *int*1 gene copies on the 1st day of the experiment was 7.26×10^6 copies g^{-1}; on the 14th day it slightly decreased (to 2.3×10^6 copies g^{-1}), and then did not change until the end of the experiment (Figure 3c). A similar pattern (maximum for the 1st day, then a decrease) was observed in all variants of compost mixtures. The highest number of copies of the *int*1 genes was detected on the 1st day in variants M, MO, and BMO, and on the 14th, 28th, and 84th days in samples O, BO, and MO. It should be noted that in the control variant and the variant with the addition of biochar, the *int*1 gene was present in slightly lower amounts compared to other composting variants. Additionally, in the sample with biochar we observed the lowest content of *int*1, and in the sample with heavy metals we observed a maximum drop in the content of *int*1. This suggests that the addition of antibiotics and metals intensifies the horizontal gene transfer, while the addition of biochar does not affect the transfer. This assumption needs to be verified further using statistics and modeling.

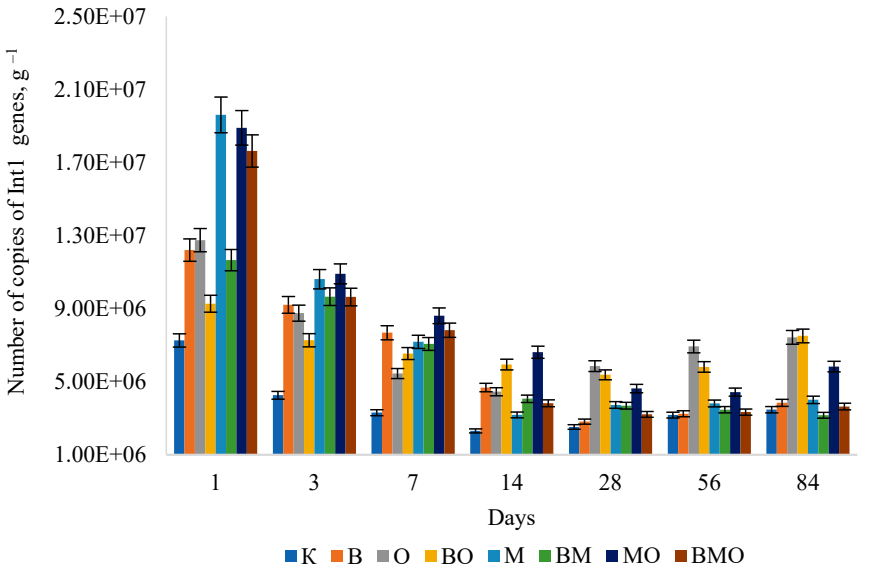


(**a**)

Figure 3. *Cont.*



(**b**)



(**c**)

Figure 3. Number of copies of the *tet*(A) gene (**a**), *tet*(X) gene (**b**), and the gene encoding integron class A (*int*1) (**c**) in the composting mixtures.

3.3. Bacterial Community Composition of the Composting Mixtures

Since bacteria are carriers of antibiotic-resistant genes, and the present study aims at studying the influence of various factors on the spread of such genes. In addition, it is known from the scientific literature that the fungal community during composting is quite stable and is less affected by factors such as the presence of antibiotics or the introduction of heavy metals, and the analysis of the structure of the bacterial community alone seems to be sufficient to achieve our goals [43].

In total, 283 bacterial OTUs were detected in the studied samples. There were no significant differences in the numbers of OTUs between the variants. On the 1st day, the numbers of OTU in the samples varied from 128 to 170, and by the 84th day, a gradual increase in their number by an average of 1.5–2-fold was observed, which amounted to 255–283 OTUs.

It was found that representatives of the *Firmicutes* and *Actinobacteria* phyla were the most abundant at the initial stages of composting. Starting from the 14th day, they were replaced by representatives of the *Proteobacteria* and *Bacteroidetes* phyla (Figure 4).

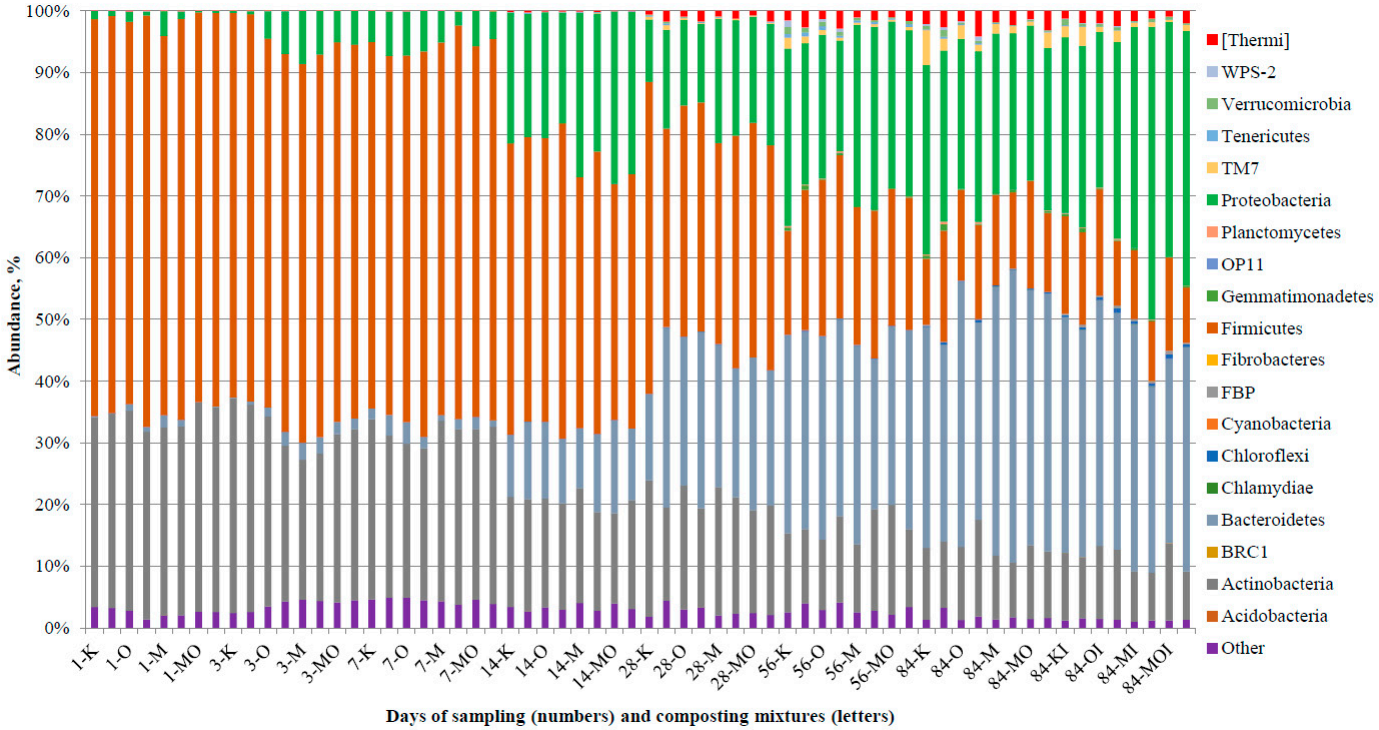


Figure 4. Bacterial community structure of the composting mixtures (phylum level).

Table 1 presents the OTUs, the abundance of which exceeded 5% on at least one of the sampling days. The similarity in the communities of different composting options turned out to be high. Thus, on the 1st–3rd days of composting, one and the same OTU—*Corynebacterium* sp.—dominated in all variants (abundance: 14–23%); on the 7th day its abundance decreased to 6–8%, and by the 84th day it was 0–2% in all composting variants. A similar trend towards a decrease in abundance in all variants was demonstrated by *Staphylococcus sciuri*; on the 1st–3rd days, its abundance was 6–12%, and on the 7th day the range was 3–4%. The peak abundance for species representing the families *Bacillaceae* and *Aerococcaceae* was observed on the 3rd day of composting (9–18% and 7–11%, respectively), probably due to their resistance to high temperatures and their ability to decompose organic compounds with heat release. For the OTU of the *Sphingobacteriaceae* family, an increase in abundance was noted from the beginning of composting (0% in all variants) to the end (4–6%). In general, representatives of the families *Corynebacteriaceae, Bacillaceae, Lactobacillales, Planococcaceae*, and *Staphylococcaceae*, found in both the control compost and in composts with various combinations of additives (biochar, antibiotic, and heavy metals), were maximally represented in the thermophilic phase (1–7 days), gradually decreasing in abundance up to the 84th day. Up to the 7th day, bacteria of the families *Flavobacteriaceae, Alcaligenaceae, Sphingobacteriaceae, Xanthomonadaceae*, and *Chitinophagaceae* were present in the samples in minimal amounts, but by the end of composting a gradual increase in their abundance was observed.

In general, no significant differences in the dynamics of the main bacteria's abundance were revealed between the variants (Figure 5). However, some differences were observed between the variants for other OTUs. Thus, on the 28th day, the abundance of *Aequorivita* sp. was lower in all variants containing heavy metals (M, BM, MO, and BMO) than in other variants. It can be suggested that heavy metals inhibited this species. On the 84th day of composting, differences were noted in the abundance of *Gelidibacter* sp. in the pairs O–BO and M–BM. Thus, in composts containing OTC and heavy metals (samples O and

M), the abundance of the species *Gelidibacter* sp. turned out to be lower (3%) than in the corresponding composts with the addition of biochar (9%). This was probably due to the mitigating effect of the biochar, which may have partly absorbed the metals.

Table 1. The list of dominating OTUs in the compost mixtures, and their abundance levels during the composting process.

No	Phylum	Class	Order	Family	Genus	Species
1	Actinobacteria	Actinobacteria	Actinomycetales	*Corynebacteriaceae*	*Corynebacterium*	
2	Firmicutes	Bacilli	Bacillales	*Bacillaceae*		
3	Bacteria	Firmicutes	Bacill	*Lactobacillales*	*Aerococcaceae*	
4	Bacteroidetes	Flavobacteriia	Flavobacteriales	*Flavobacteriaceae*		
5	Proteobacteria	Betaproteobacteria	Burkholderiales	*Alcaligenaceae*		
6	Firmicutes	Bacilli	Bacillales	*Staphylococcaceae*	*Staphylococcus*	*Staphylococcus sciuri*
7	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	*Sphingobacteriaceae*		
8	Firmicutes	Bacilli	Bacillales	*Staphylococcaceae*	*Staphylococcus;*	Other
9	Other	Other	Other	Other	Other	Other
10	Firmicutes	Bacilli	Bacillales	*Staphylococcaceae*	*Jeotgalicoccus*	
11	Firmicutes	Bacilli	Bacillales	*Planococcaceae*		
12	Bacteroidetes	Flavobacteriia	Flavobacteriales	*Flavobacteriaceae*	*Aequorivita*	
13	Actinobacteria	Actinobacteria	Actinomycetales	*Yaniellaceae*	*Yaniella*	
14	Firmicutes	Bacilli	Lactobacillales	*Leuconostocaceae*	*Weissella*	Other
15	Firmicutes	Bacilli	Lactobacillales	*Lactobacillaceae*	*Lactobacillus*	
16	Actinobacteria	Actinobacteria	Actinomycetales	*Dermabacteraceae*	*Brachybacterium*	*Brachybacterium conglomeratum*
17	Bacteroidetes	Flavobacteriia	Flavobacteriales	*Flavobacteriaceae*	*Gelidibacter*	
18	Actinobacteria	Actinobacteria	Actinomycetales	*Brevibacteriaceae*	*Brevibacterium*	
19	Firmicutes	Bacilli	Bacillales	*Staphylococcaceae*	*Staphylococcus*	
20	Actinobacteria	Actinobacteria	Actinomycetales	Other	Other	Other
21	Proteobacteria	Gammaproteobacteria	Alteromonadales	*Alteromonadaceae*		
22	Bacteroidetes	Flavobacteriia	Flavobacteriales	*[Weeksellaceae]*		
23	Firmicutes	Bacilli	Bacillales	*Bacillaceae*	Other	Other
24	[Thermi]	Deinococci	Deinococcales	*Trueperaceae*	B-42	
25	Proteobacteria	Gammaproteobacteria	Pseudomonadales	*Pseudomonadaceae*	*Pseudomonas*	
26	Bacteroidetes	Cytophagia	Cytophagales	*Flammeovirgaceae*		
27	Proteobacteria	Gammaproteobacteria	Xanthomonadales	*Xanthomonadaceae*	*Luteimonas*	
28	Proteobacteria	Gammaproteobacteria	Pseudomonadales	*Pseudomonadaceae*		
29	Proteobacteria	Gammaproteobacteria	Xanthomonadales	*Xanthomonadaceae*	*Rhodanobacter*	
30	Bacteroidetes	[Saprospirae]	[Saprospirales]	*Chitinophagaceae*		

Similarities and differences between the bacterial communities were assessed using the nonmetric multidimensional scaling (NMDS) method. Figure 6 demonstrates that in general, the bacterial communities of composts containing various combinations of biochar, antibiotic, and heavy metal additives had a high similarity to each other, since the points on the NMDS plot, which represent samples, are located close to each other. The main factor distinguishing bacterial communities from each other was the duration of composting—the

points characterizing the samples taken on the same day but for different variants are located close to each other, but far from the points characterizing other days of composting. Interestingly, the groups of points characterizing close sampling days (1–3, 3–7, and 56–84) are located closer to each other than the groups of points characterized by a significant difference in sampling time (for example, 1 and 84 days). Moreover, it can be seen that the similarity in the bacterial communities of different composting variants decreased over time, and the highest similarity was observed during the thermophilic phase of composting (days 3 and 7).

30	Bacteroidetes	[Saprospirae]	[Saprospirales]	*Chitinophagaceae*

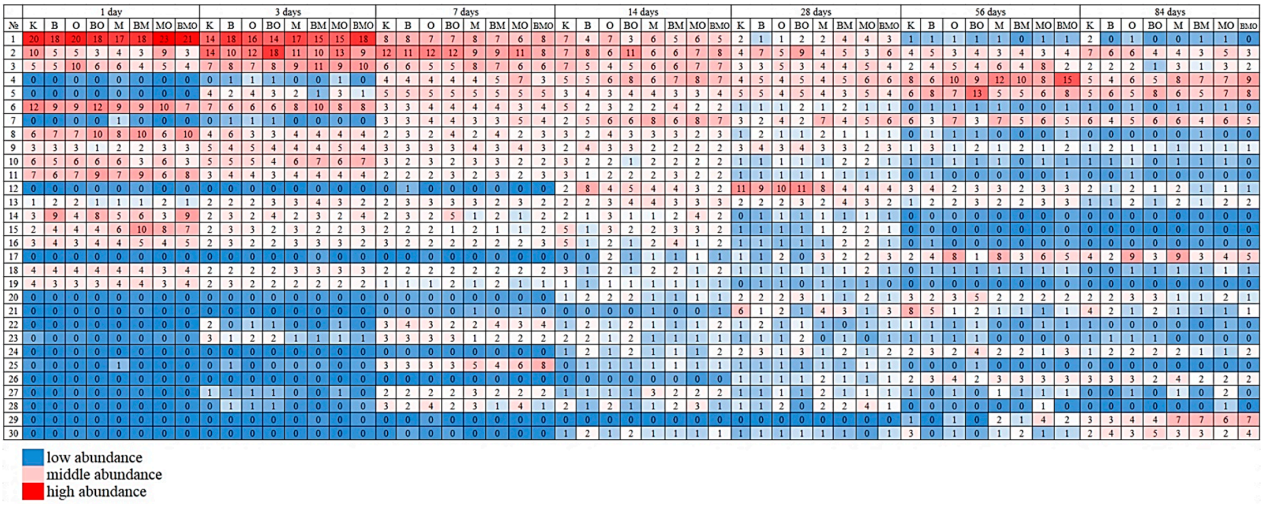


Figure 5. The dynamics of dominant OTUs in the compost mixtures during the composting process.

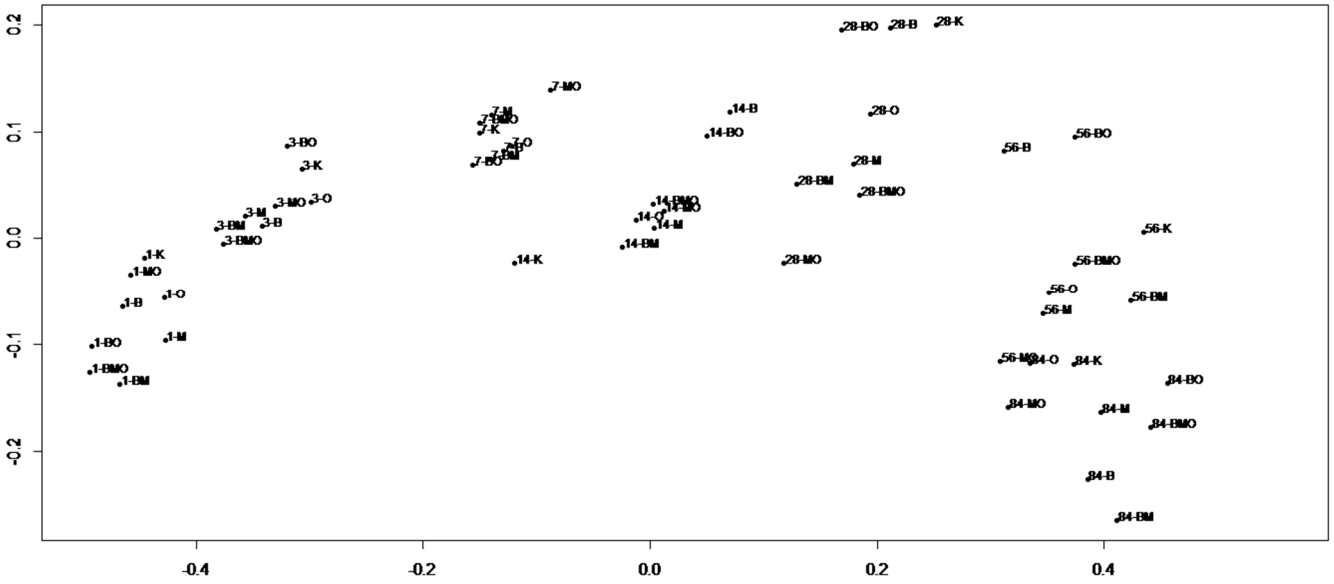


Figure 6. Beta-diversity of the bacterial communities of the compost mixtures (numbers represent the days of sampling and letters the names of the variants).

3.4. The Factors Influencing Bacterial Communities and ARG Copy Numbers in the Composting Mixtures

A robust linear model was used to analyze the significance of the effect of the biochar, oxytetracycline, metals, and their combinations on the number of copies of the *tet*(A), *tet*(X), and *int*1 genes. The influence was compared with the control to estimate not only the

presence or absence of the influence but also its strength. It was found that the introduction of additives was significant only for the *tet*(A) gene, but not for the *tet*(X) gene (Table 2). For the *tet*(A) gene, the introduction of biochar led to a decrease in the number of copies of genes relative to the control by 26.7%; the introduction of metals led to a decrease of 34.5%, while the introduction of oxytetracycline led to an increase of 43.7%. The number of copies of the *int*1 gene was significantly affected only by the addition of oxytetracycline, which led to a 45.9% increase.

Table 2. The influence of the additives (biochar, oxytetracycline, metals) on the number of copies of the *tet*(A), *tet*(X), and *int*1 genes.

Subject of Influence	Influencing Factor	Mean Changes in Values Relative to Control (V)	*p*-Value	Changes Expressed in % Relative to Control (y = 100 × (exp(−V) − 1))
tet(A)	Biochar addition	−0.31013	0.0246	−26.66491924
	Oxytetracycline addition	0.362413	0.0086	43.67922146
	Metals addition	−0.42241	0.0022	−34.45332489
tet(X)	Biochar addition	−0.05532	0.2467	-
	Oxytetracycline addition	0.088784	0.0632	-
	Metals addition	−0.04961	0.2989	-
*int*1	Biochar addition	−0.0637119	0.5848	-
	Oxytetracycline addition	0.37776208	0.0012	45.90157725
	Metals addition	0.1456025	0.2117	-

A correlation between the numbers of *tet*(A), *tet*(X), and *int*1 gene copies and the OTU abundance was carried out using the random forests method. The results are presented in Supplementary Table S1. The abundance of OTUs belonging to the *Natronobacillus*, *Ammoniphilus*, *Pseudomonadaceae*, and *Luteimonas* taxa was associated with the number of copies of the *tet*(A) resistance genes, and the abundance of *Natronobacillus*, *Ammoniphilus*, *Pseudomonadaceae*, *Luteimonas*, and *Trichococcus* was associated with the number of copies of the *tet*(X) resistance genes. It can be assumed that these OTUs were carriers of those resistance genes. Interestingly, within the same family and genus, there were OTUs with a high correlation with resistance genes and OTUs with a low correlation. Some examples of such families are *Bacillaceae*, *Planococcaceae*, *Pseudomonadaceae*, and *Xanthomonadaceae*. Furthermore, for each OTU, the influence (significant or not) of the introduction of the biochar was evaluated via robust linear modeling; in addition, this influence was quantified as a percentage of the control. The OTUs negatively affected by the biochar included representatives of the *Dietzia*, *Gordonia*, *Kocuria*, *Natronobacillu*, *Sporosarcina*, *Macrococcus*, *Carnobacteriaceae*, *Trichococcus*, *Clostridiaceae*, *Lachnospiraceae*, *Coprococcus*, *Ruminococcaceae*, *Tissierellaceae*, *Sporanaerobacter*, *Tissierella Soehngenia*, *Alcaligenes faeconaddomaceae*, and *Luteimonas* taxa. Furthermore, we compared the data obtained in two ways and identified those OTUs in which the number of resistance genes was associated with abundance (a coefficient above 70); the effect of the biochar was significant and negative (Table 3 and Supplementary Table S1). These OTUs included representatives of the genus *Natronobacillus* (association with *tet*(A) 79%, with *tet*(X) 86%; influence of biochar 37%), *Pseudomonadaceae* (association with *tet*(A) 75%, with *tet*(X) 80%; influence of biochar 46%), *Luteimonas* (association with tet(A) 100%, with *tet*(X) 94%; influence of biochar 34%), and *Trichococcus* (association with tet(A) 58%, with *tet*(X) 74%; biochar influence 44%). There were also OTUs with a significant increase in abundance after the addition of the biochar. These were from *Corynebacterium variabile* and representatives of the *Flavobacteriales*, *Rhodothermaceae*, *Clostridia*, GMD14H09, *Halorhodospira*, and *Piscirickettsiaceae* taxa (with increases of 334%, 231%, 292%, 222%, 423%, 1017%, and 287%, respectively). For all these OTUs, no association with the number of ARGs was found. This supports our hypothesis that the biochar creates favorable conditions for the development of species that are not carriers of antibiotic-resistant genes,

thereby indirectly influencing the number of carrier species, and accordingly the number of resistance gene copies.

Table 3. Correlation between *tet*(A) and *tet*(X) gene copy numbers and OTU abundance levels and the significance of the biochar addition to the composting mixtures (fragment). The entire table is presented in Supplementary Table S1.

OTE	Correlation between *tet*(A) Gene Copy Number and OTU Abundance, % from Maximum	Correlation between *tet*(X) Gene Copy Number and OTU Abundance, % from Maximum	Significance of Biochar Addition to the Composting Mixtures			
			p-Value	Mean Changes as Compared with Control (V)	Changes in % as Compared with Control y = 100 × (exp(−V) − 1)	Average Abundance, %
Natronobacillus sp.	79	86	0.013	−0.4564	−36.6	4
Ammoniphilus sp.	75	79	0.0173	0.4932	63.8	1
Pseudomonadaceae; g__; s__	75	80	0.032	−0.6208	−46.2	8
Luteimonas; s__	100	94	0.0314	−0.4157	−34.0	8
Trichococcus; s__	58	74	0.0033	−0.5886	−44.5	1

4. Discussion

4.1. Dynamics of the Main Parameters of Composting

The issue of animal waste composting remains relevant and requires further research, since new sequencing methods may reveal the specific characteristics of the composting process, such as the fate and spread of ARGs under the influence of different compost additives. In the present study, it was revealed that neither the biochar nor antibiotic and heavy metals added into compost mixtures have a significant effect on the main physicochemical parameters of composting; in addition, they affect the level of respiratory activity to some extent. The thermophilic phase in all variants of composts was noted on the 2nd–5th days of composting. In general, the obtained temperature profile corresponded to the traditional temperature dynamics during composting described in the literature [44–46]. The peak content of dissolved organic carbon was noted on the 14th–21st days for all composts and amounted to 21–32 mg g^{-1}; this result is also in line with what is presented in the scientific literature [47–49]. In the final composts, the content of dissolved organic carbon was 3.3–5.3 mg g^{-1}, which corresponds to a high degree of content when the compost is applied to the soil, which contributes to an increase in yield [48,49]. The highest respiration activity was noted on the 3rd day of composting and coincided with the thermophilic stage, which is characterized by a high activity of the microbial community [50,51].

4.2. Dynamics of Copy Numbers of Genes Characterizing Common Bacterial and Fungal Counts, Tetracycline Resistance, and Horizontal Gene Transfer

The number of bacterial gene copies generally corresponds to the scientific literature, as well as the downward trend in this amount during the composting process [52–54]. The absence of a decrease in fungal copies of genes revealed in our study was also confirmed by data from other studies [52,55].

For both the *tet*(A) and *tet*(X) resistance genes, the lowest copy numbers were found in the control sample. The numbers of both gene copies exceeded the control in samples B, O, BO, M, and MO and were similar to the control values in the other samples. However, the dynamics of the changes in the numbers of copies of these genes differed significantly in the control and experimental variants. Presumably, this was due to the confinement of these genes to one or another group of bacteria that inhabited the composts and changed their counts depending on the temperature, pH, the presence of substrates, and other environmental conditions [15,56,57]. In general, the numbers of copies of the tetracycline resistance genes *tet*(A) and *tet*(X) increased from the beginning to the end of the composting by 2–102- and 4–35-fold, respectively. This may have been due to the higher survival rate of the initially resistant species in an oxytetracycline-polluted environment, as well as the transfer of genes to resistant species [58,59]. We found a decrease in the number of copies

of the *int*1 gene encoding a class 1 integron involved in the horizontal transfer of ARGs on the 14th, 28th, and 84th days of composting compared to the 1st day in all variants of compost mixtures. The scientific literature provides contradictory information about the dynamics of the number of copies of the *int*1 genes during composting. Thus, in a number of works, an increase was noted. Other authors pointed to a downward trend [60,61].

It has been reported that the presence of heavy metals can increase the number of ARGs through co-selection mechanisms (co-resistance, cross-resistance, and co-regulation) [62–65]. It is also known that the addition of biochar to a compost mixture facilitates the decomposition of antibiotic residues, increasing the surface area for functional groups of bacteria. This results in a change in the number of ARGs in the compost mixtures. By reducing the number of ARGs, the biochar not only reduces its own problem, but also reduces indirect problems (various changes in the composition of bacterial community species) that can be caused by the presence of ARGs [66–68]. The addition of antibiotics to a compost mixture can exert selective pressure on the compost bacteria, thereby increasing the resistance of some bacteria and pathogens [69–71].

4.3. Composition of Bacterial Communities in the Composting Mixtures

At the phylum level, representatives of *Firmicutes* and *Actinobacteria* dominated at the beginning of composting, followed by representatives of *Proteobacteria* and *Bacteroidetes*. In general, such a change in the dominant types is typical for mixtures containing straw, sawdust, and chicken manure [72]. *Firmicutes* are known to produce various proteases and pectinases and can decompose complex carbohydrates such as cellulose. This type of bacteria may play a leading role in nitrogen-rich chicken manure composts [73]. Bacteria belonging to the *Actinobacteria* play a leading role in the degradation of organic matter and the humification of the composted substrate [74,75]. *Proteobacteria* and *Bacteroidetes* take part in the decomposition of complex organic protein compounds, as well as cellulose, starch, and chitin [76–78].

In our study, an increase in the number of bacterial OTUs in the process of composting by 1.5–2-fold was observed. The increase in bacterial OTU can be associated with the appearance in the composted substrate of available nutrients for various groups of microorganisms, such as soluble sugars and organic acids [47]. Representatives of the *Corynebacteriaceae*, *Bacillaceae*, *Lactobacillales*, *Planococcaceae*, and *Staphylococcaceae* families were most abundant at the beginning of composting, including in the thermophilic phase, in all variants of mixtures. These families are traditionally found in the microbial community of composts and play an active part in the composting process. Thus, bacteria of the *Lactobacillales* family enhance the degradation of organic substances, such as lignin and cellulose, and exhibit an antagonistic effect against various pathogens in manure by lowering the pH and producing peptides with antimicrobial activity [78]. Representatives of the *Flavobacteriaceae*, *Alcaligenaceae*, *Sphingobacteriaceae*, *Xanthomonadaceae*, and *Chitinophagaceae* families dominated at subsequent stages of composting. Bacteria of the *Xanthomonadaceae* family take part in the decomposition of cellulose. Bacteria of the *Alcaligenaceae* family are responsible for the degradation of organic acids and amino acids in composts. Bacteria of the *Chitinophagaceae* family produce enzymes to break down cellulose and chitin [73,78].

When comparing compost mixtures, a high similarity was found at the level of dominant OTUs. At the initial stage of composting (1–3 days), *Corynebacterium sp.* and *Staphylococcus sciuri* were characterized by having the highest abundance in all samples (abundance levels of 14–23% and 6–12%, respectively). Further, towards the end of composting, their abundance levels gradually decreased to 0–2% and 0–1%, respectively. Species representing the families *Bacillaceae* and *Aerococcaceae* had the maximum abundance levels during the thermophilic phase of composting in all variants (9–18% and 7–11%, respectively), since these species belong to the thermophilic group of microorganisms [75]. Representatives of the families *Corynebacteriaceae*, *Bacillaceae*, *Lactobacillales*, *Planococcaceae*, and *Staphylococcaceae*, found in both the control compost and in composts with various combinations of additives (biochar, antibiotic, heavy metals), were highly represented in the thermophilic

phase (1–7 days), gradually decreasing in abundance by the 84th day. Up to day 7, the bacteria of the families *Flavobacteriaceae*, *Alcaligenaceae*, *Sphingobacteriaceae*, *Xanthomonadaceae*, and *Chitinophagaceae* were present in the samples in minimal amounts, but by the end of composting a gradual increase in their numbers was observed. The various additions to the composts did not have a significant effect on the dynamics of the number of dominant bacterial taxa in the compost mixtures. However, on the 84th day, an increase in the abundance of *Gelidibacter* sp. in O and M samples containing OTC and metals, compared with BO and BM samples, was registered. This difference can be explained by the fact that the biochar, having a highly porous structure, contributed to the sorption of heavy metals and a decrease in their bioavailability, which affected the abundance of *Gelidibacter* sp. [79]. Indeed, the literature describes such changes in the structure and dynamics of the microbial community of composts pretreated with biochar [80,81].

The analysis of the beta diversity using the method of nonmetric multidimensional scaling (NMDS) made it possible to establish the high similarity between the bacterial communities in composts containing various additives. However, it was found that the differences between the bacterial communities of the same samples are significantly affected by the duration of composting. Indeed, in the process of composting, there is a natural shift in some bacterial communities by others, depending on the physicochemical characteristics of the composts and the availability of the nutrient substrates [82–85]. The most similar bacterial communities were observed on the 3rd and 7th days, belonging to the thermophilic phase of composting. This indicates that temperature is one of the main factors that shapes bacterial communities.

4.4. Revealing the Role of Biochar for ARG Fate in the Composting Mixtures

It is known that the use of highly porous substrates can significantly reduce the number of ARGs in the process of manure composting [31,86,87]. For this purpose, biochar is widely used in agriculture, being obtained from such plant materials as rice husks, straw, nut shells, bamboo, and mushrooms. [88,89]. The mechanism of action is mainly due to the high sorption capacity of the biochar in relation to the residual amounts of antibiotics in the composts and a decrease in their bioavailability [90–92]. In the present study, another biochar made of chicken manure was used. Using modeling, a significant effect of the introduction of this type of biochar on the number of copies of the *tet*(A) gene was revealed, whereby the latter decreased by 27% on average. The number of copies of the *tet*(X) gene was not significantly affected by the addition of biochar. The introduction of oxytetracycline also significantly affected only one of the resistance genes; the number of copies of the *tet*(A) gene increased by an average of 44%, as well as on the *int*1 gene, while the number of its gene copies increased by 46%. Interestingly, the addition of biochar, metals, and antibiotics had a much smaller impact on the species diversity at the level of large taxa (phyla, class, and order), but in some cases affected individual OTUs (*Pseudomonadaceae*, *Luteimonas*, and *Trichococcus*). It should be underlined that such a correlation was found specifically for individual OTUs but not for genera or families. Previously, authors have revealed ARG carriers at the genera [25,93,94] or family level [82,83,85].

5. Conclusions

It can be concluded that neither composting nor the addition of chicken manure biochar led to the complete elimination of oxytetracycline resistance genes from chicken manure. However, the addition of biochar caused a significant decrease in the number of ARG copies, including in conditions of contamination with metals and oxytetracycline; therefore, it can be recommended as a measure for the treatment of manure to solve the problem of ARG dissemination in soils and crops.

It was found that bacterial communities of the composted mixtures are very stable and do not depend on the addition of biochar, oxytetracycline, or metals, or on the time of sampling or the characteristics of each individual sample. The main aspect determining

the community structure is the composting phase, which is the result of biochemical and physicochemical processes.

It has been shown that the number of copies of ARG in the bacterial community positively correlates with the abundance of several bacterial taxa considered as carriers of ARG. Moreover, it was found that the addition of biochar had a positive effect on some taxa of bacteria (at the OTU level) and a negative effect on several others. Therefore, it has been suggested that the decrease in ARG copy numbers observed in the presence of biochar is due to the creation of more favorable conditions for those species that outcompete ARG carriers from the community.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12081158/s1, Figure S1: Rotary drum composters used for chicken manure composting; Table S1: Correlation between *tet*(A) and *tet*(X) gene copy numbers and OTU abundance and the significance of biochar addition to the composting mixtures.

Author Contributions: Conceptualization, S.S. and P.G.; methodology, P.G.; software, K.K.; validation, K.K., N.D., and P.G.; formal analysis, S.S.; investigation, K.K.; resources, N.D.; data curation, K.K.; writing—original draft preparation, K.K.; writing—review and editing, P.G.; visualization, S.S.; supervision, P.G.; project administration, N.D.; funding acquisition, P.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a subsidy allocated to Kazan Federal University from the Russian Foundation for Basic Research, project N°18-29-25054.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The work is carried out in accordance with the Strategic Academic Leadership Program "Priority 2030" of the Kazan Federal University of the Government of the Russian Federation.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Arunrat, N.; Kongsurakan, P.; Sereenonchai, S.; Hatano, R. Soil organic carbon in sandy paddy fields of Northeast Thailand: A review. *Agronomy* **2020**, *10*, 1061. [CrossRef]
2. Andreas, K.; Francesc, X.P.-B. Examining the perspectives of using manure from livestock farms as fertilizer to crop fields based on a realistic simulation. *Comput. Electron. Agric.* **2021**, *191*, 106486.
3. Jauregi, L.; Epelde, L.; Alkorta, I.; Garbisu, C. Antibiotic resistance in agricultural soil and crops associated to the application of cow manure-derived amendments from conventional and organic livestock farms. *Front. Vet. Sci.* **2021**, *8*, 633858. [CrossRef] [PubMed]
4. Al-Mnaser, A.; Dakheel, M.; Alkandari, F. Polyphenolic phytochemicals as natural feed additives to control bacterial pathogens in the chicken gut. *Arch. Microbiol.* **2022**, *204*, 253. [CrossRef] [PubMed]
5. Qiu, X.; Zhou, G.; Wang, H. Nanoscale zero-valent iron inhibits the horizontal gene transfer of antibiotic resistance genes in chicken manure compost. *J. Hazard. Mater.* **2022**, *422*, 126883. [CrossRef]
6. Danilova, N.; Galitskaya, P.; Selivanovskaya, S. Veterinary antibiotic oxytetracycline's effect on the soil microbial community. *J. Ecol. Environ.* **2020**, *44*, 10. [CrossRef]
7. Zhao, F.; Yang, L.; Li, G.; Fang, L.; Yu, X.; Tang, Y.; Li, M.; Chen, L. Veterinary antibiotics can reduce crop yields by modifying soil bacterial community and earthworm population in agro-ecosystems. *Sci. Total Environ.* **2020**, *808*, 152056. [CrossRef]
8. Liu, B.; Yu, K.; Ahmed, I.; Gin, K.; Xi, B.; Wei, Z.; He, Y.; Zhang, B. Key factors driving the fate of antibiotic resistance genes and controlling strategies during aerobic composting of animal manure: A review. *Sci. Total Environ.* **2021**, *791*, 148372. [CrossRef]
9. Reygaert, W. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiol.* **2018**, *26*, 482–501. [CrossRef] [PubMed]
10. Chopra, I.; Roberts, M. Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.* **2001**, *65*, 232–260. [CrossRef]

11. Blanco, P.; Hernando-Amado, S.; Reales-Calderon, J.A.; Corona, F.; Lira, F.; Alcalde-Rico, M.; Bernardini, A.; Sanchez, M.B.; Martinez, J.L. Bacterial Multidrug Efflux Pumps: Much More Than Antibiotic Resistance Determinants. *Microorganisms* **2016**, *4*, 14. [CrossRef] [PubMed]
12. Wang, J.; Gu, J.; Wang, X.; Song, Z.; Dai, X.; Guo, H.; Yu, J.; Zhao, W.; Lei, L. Enhanced removal of antibiotic resistance genes and mobile genetic elements during swine manure composting inoculated with mature compost. *J. Hazard. Mater.* **2021**, *411*, 125–135. [CrossRef] [PubMed]
13. Wang, R.; Zhang, J.; Sui, Q.; Wan, H.; Tong, J.; Chen, M.; Wei, Y.; Wei, D. Effect of red mud addition on tetracycline and copper resistance genes and microbial community during the full-scale swine manure composting. *Bioresour. Technol.* **2016**, *216*, 1049–1057. [CrossRef] [PubMed]
14. Zhou, G.; Qiu, X.; Wu, X.; Lu, S. Horizontal gene transfer is a key determinant of antibiotic resistance genes profiles during chicken manure composting with the addition of biochar and zeolite. *J. Hazard. Mater.* **2021**, *408*, 124883. [CrossRef]
15. Liu, Y.; Cheng, D.; Xue, J.; Weaver, L.; Wakelin, S.A.; Feng, Y.; Li, Z. Changes in microbial community structure during pig manure composting and its relationship to the fate of antibiotics and antibiotic resistance genes. *J. Hazard. Mater.* **2020**, *389*, 122082. [CrossRef]
16. Pu, C.; Liu, H.; Ding, G.; Sun, Y.; Yu, X.; Chen, J.; Ren, J.; Gong, X. Impact of direct application of biogas slurry and residue in fields: In situ analysis of antibiotic resistance genes from pig manure to fields. *J. Hazard. Mater.* **2018**, *344*, 441–449. [CrossRef]
17. Cao, R.; Ben, W.; Qiang, Z.; Zhang, J. Removal of antibiotic resistance genes in pig manure composting influenced by inoculation of compound microbial agents. *Bioresour. Technol.* **2020**, *317*, 123966. [CrossRef]
18. Zhou, Z.; Yao, H. Effects of composting different types of organic fertilizer on the microbial community structure and antibiotic resistance genes. *Microorganisms* **2020**, *8*, 268. [CrossRef]
19. Xie, W.Y.; Yang, X.P.; Li, Q.; Wu, L.H.; Shen, Q.R.; Zhao, F.J. Changes in antibiotic concentrations and antibiotic resistome during commercial composting of animal manures. *Environ. Pollut.* **2016**, *219*, 182–190. [CrossRef]
20. Wu, Y.; Wen, Q.; Chen, Z.; Fu, Q.; Bao, H. Response of antibiotic resistance to the co-exposure of sulfamethoxazole and copper during swine manure composting. *Sci. Total Environ.* **2022**, *805*, 150086. [CrossRef]
21. Bao, J.; Wang, X.; Gu, J.; Dai, X.; Zhang, K.; Wang, Q.; Ma, J.; Peng, H. Effects of macroporous adsorption resin on antibiotic resistance genes and the bacterial community during composting. *Bioresour. Technol.* **2020**, *295*, 121997. [CrossRef]
22. Ding, J.; An, X.L.; Lassen, S.B.; Wang, H.T.; Zhu, D.; Ke, X. Heavy metal-induced co-selection of antibiotic resistance genes in the gut microbiota of collembolans. *Sci. Total Environ.* **2019**, *683*, 210–215. [CrossRef]
23. Stepanauskas, R.; Glenn, T.C.; Jagoe, C.H.; Tuckfield, R.C.; Lindell, A.H.; King, C.J.; McArthur, J.V. Coselection for microbial resistance to metals and antibiotics in freshwater microcosms. *Environ. Microbiol.* **2006**, *8*, 1510–1514. [CrossRef] [PubMed]
24. Qiu, X.; Zhou, G.; Chen, L.; Wang, H. Additive quality influences the reservoir of antibiotic resistance genes during chicken manure composting. *Ecotoxicol. Environ. Saf.* **2021**, *220*, 112413. [CrossRef]
25. Wang, G.; Kong, Y.; Yang, Y.; Ma, R.; Li, L.; Li, G.; Yuan, J. Composting temperature directly affects the removal of antibiotic resistance genes and mobile genetic elements in livestock manure. *Environ. Pollut.* **2022**, *303*, 119174. [CrossRef] [PubMed]
26. Zhang, J.; Chen, M.; Sui, Q.; Tong, J.; Jiang, C.; Lu, X.; Zhang, Y.; Wei, Y. Impacts of addition of natural zeolite or a nitrification inhibitor on antibiotic resistance genes during sludge composting. *Water Res.* **2016**, *91*, 339–349. [CrossRef] [PubMed]
27. Su, J.Q.; Wei, B.; Ou-Yang, W.Y.; Huang, F.Y.; Zhao, Y.; Xu, H.J.; Zhu, Y.G. Antibiotic Resistome and Its Association with Bacterial Communities during Sewage Sludge Composting. *Environ. Sci. Technol.* **2015**, *49*, 7356–7363. [CrossRef] [PubMed]
28. Villaseñor, J.; Rodríguez, L.; Fernández, F.J. Composting domestic sewage sludge with natural zeolites in a rotary drum reactor. *Bioresour. Technol.* **2011**, *102*, 1447–1454. [CrossRef]
29. Nguyen, M.K.; Lin, C.; Hoang, H.G.; Sanderson, P.; Dang, B.T.; Bui, X.T.; Nguyen, N.S.H.; Vo, D.-V.N.; Tran, H.T. Evaluate the role of biochar during the organic waste composting process: A critical review. *Chemosphere* **2022**, *299*, 134488. [CrossRef] [PubMed]
30. Ogawa, M.; Okimori, Y. Pioneering works in biochar research, Japan. *Aust. J. Soil Res.* **2010**, *48*, 489–500. [CrossRef]
31. Qiu, X.; Zhou, G.; Wang, H.; Wu, X. The behavior of antibiotic-resistance genes and their relationships with the bacterial community and heavy metals during sewage sludge composting. *Ecotoxicol. Environ. Saf.* **2021**, *216*, 112190. [CrossRef] [PubMed]
32. Qiu, X.; Zhou, G.; Zhang, J.; Wang, W. Microbial community responses to biochar addition when a green waste and manure mix are composted: A molecular ecological network analysis. *Bioresour. Technol.* **2019**, *273*, 666–671. [CrossRef] [PubMed]
33. He, X.; Xiong, J.; Yang, Z.; Han, L.; Huang, G. Exploring the impact of biochar on antibiotics and antibiotics resistance genes in pig manure aerobic composting through untargeted metabolomics and metagenomics. *Bioresour. Technol.* **2022**, *352*, 127–138. [CrossRef]
34. Chaves, L.; Fernandes, J.; Dantas, E.; Guerra, C.; Tito, G.; Silva, A.; Laurentino, L.; Souza, F.; Lima, W.; Chaves, I. Characterization of poultry litter biochar for agricultural use. *Sylwan* **2020**, *164*, 468–487.
35. *ISO 14235*; 1998 Soil Quality—Determination of Organic Carbon by Sulfochromic Oxidation/ISO 14235. International Standard: Geneva, Switzerland, 1998.
36. *ISO 16072*; 2002—Soil Quality—Laboratory Methods for Determination of Microbial Soil Respiration. Available online: https://www.iso.org/standard/32096.html (accessed on 23 April 2019).
37. *ISO 17601*; 2016 Soil Quality—Estimation of Abundance of Selected Microbial Gene Sequences by Quantitative PCR from DNA Directly Extracted from Soil. Geoderma: Geneva, Switzerland, 2016.

38. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Pēa, A.G.; Goodrich, J.K.; Gordon, J.I. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [CrossRef]
39. Wang, Q.; Garrity, G.M.; Tiedje, J.M.; Cole, J.R. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **2007**, *73*, 5261–5267. [CrossRef] [PubMed]
40. Faith, D.P.; Minchin, P.R.; Belbin, L. *Compositional Dissimilarity as a Robust Measure of Ecological Distance*; Vegetatio Kluwer Academic Publishers: New York, NY, USA, 1987; Volume 69, pp. 57–68.
41. Cuihong, T.; Danyu, X.; Longfei, X.; Jintao, Y.; Ruonan, Z.; Jie, H.; Zhipeng, H.; Zhenling, Z.; Wenguang, X. Swine manure facilitates the spread of antibiotic resistome including tigecycline-resistant tet(X) variants to farm workers and receiving environment. *Sci. Total Environ.* **2022**, *808*, 152157.
42. De los Santos, E.; Laviña, M.; Poey, M.E. Strict relationship between class 1 integrons and resistance to sulfamethoxazole in Escherichia coli. *Microb. Pathog.* **2021**, *161*, 105206. [CrossRef]
43. Wu, X.; Ren, L.; Luo, L.; Zhang, J.; Zhang, L.; Huang, H. Bacterial and Fungal Community Dynamics and Shaping Factors During Agricultural Waste Composting with Zeolite and Biochar Addition. *Sustainability* **2020**, *12*, 7082. [CrossRef]
44. Arikan, O.; Mulbry, W.; Ingram, D.; Millner, P. Minimally managed composting of beef manure at the pilot scale: Effect of manure pile construction on pile temperature profiles and on the fate of oxytetracycline and chlortetracycline. *Bioresour. Technol.* **2009**, *100*, 4447–4453. [CrossRef]
45. Hu, Z.; Liu, Y.; Chen, G.; Gui, X.; Chen, T.; Zhan, X. Characterization of organic matter degradation during composting of ma-nure-straw mixtures spiked with tetracyclines. *Bioresour. Technol.* **2011**, *102*, 7329–7334. [CrossRef] [PubMed]
46. Ramaswamy, J.; Prasher, S.O.; Patel, R.M.; Hussain, S.A.; Barrington, S.F. The effect of composting on the degradation of a vet-erinary pharmaceutical. *Bioresour. Technol.* **2010**, *101*, 2294–2299. [CrossRef]
47. Bernal, M.P.; Alburquerque, J.A.; Moral, R. Composting of animal manures and chemical criteria for compost maturity as-sessment. A review. *Bioresour. Technol.* **2009**, *100*, 5444–5453. [CrossRef] [PubMed]
48. Kuryntseva, P.; Galieva, G.; Galitskaya, P.; Selivanovskaya, S. Co-Composting as A Method to Decrease Toxicity of Chicken Manure. *Int. J. Adv. Biotechnol. Res.* **2016**, *7*, 976–2612.
49. Sharon, Z.; Omer, M.; Jorge, T.; Yona, C. Dissolved organic carbon (DOC) as a parameter of compost maturity. *Soil Biol. Biochem.* **2005**, *37*, 2109–2116.
50. Guan, J.; Wasty, A.; Grenier, C.; Chan, M. Influence of temperature on survival and conjugative transfer of multiple antibiotic-resistant plasmids in chicken manure and compost microcosms. *Poult. Sci.* **2007**, *86*, 610–613. [CrossRef]
51. Kang, Y.; Li, Q.; Xia, D.; Shen, M.; Mei, L.; Hu, J. Short-term thermophilic treatment cannot remove tetracycline resistance genes in pig manures but exhibits controlling effects on their accumulation and spread in soil. *J. Hazard. Mater.* **2017**, *340*, 213–220. [CrossRef]
52. Chung, W.J.; Chang, S.W.; Chaudhary, D.K.; Shin JDu Kim, H.; Karmegam, N.; Govarthanan, M.; Chandrasekaran, M.; Ravindran, B. Effect of biochar amendment on compost quality, gaseous emissions and pathogen reduction during in-vessel composting of chicken manure. *Chemosphere* **2021**, *283*, 131129. [CrossRef]
53. Langarica-Fuentes, A.; Handley, P.S.; Houlden, A.; Fox, G.; Robson, G.D. An investigation of the biodiversity of thermophilic and thermotolerant fungal species in composts using culture-based and molecular techniques. *Fungal Ecol.* **2014**, *11*, 132–144. [CrossRef]
54. Meng, Q.; Yang, W.; Men, M.; Bello, A.; Xu, X.; Xu, B.; Deng, L.; Jiang, X.; Sheng, S.; Wu, X. Microbial Community Succession and Response to Environmental Variables During Cow Manure and Corn Straw Composting. *Front. Microbiol. Front.* **2019**, *10*, 529. [CrossRef]
55. Langarica-Fuentes, A.; Zafar, U.; Heyworth, A.; Brown, T.; Fox, G.; Robson, G.D. Fungal succession in an in-vessel composting system characterized using 454 pyrosequencing. *FEMS Microbiol. Ecol.* **2014**, *88*, 296–308. [CrossRef] [PubMed]
56. Alavi, N.; Sarmadi, K.; Goudarzi, G.; Babaei, A.A.; Bakhshoodeh, R.; Paydary, P. Attenuation of tetracyclines during chicken manure and bagasse co-composting: Degradation, kinetics, and artificial neural network modeling. *J. Environ. Manag.* **2019**, *231*, 1203–1210. [CrossRef] [PubMed]
57. Chen, C.; Pankow, C.A.; Oh, M.; Heath, L.S.; Zhang, L.; Du, P.; Xia, K.; Pruden, A. Effect of antibiotic use and composting on an-tibiotic resistance gene abundance and resistome risks of soils receiving manure-derived amendments. *Environ. Int.* **2019**, *128*, 233–243. [CrossRef]
58. Qian, X.; Sun, W.; Gu, J.; Wang, X.J.; Zhang, Y.J.; Duan, M.L.; Li, H.C.; Zhang, R.R. Reducing antibiotic resistance genes, integrons, and pathogens in dairy manure by continuous thermophilic composting. *Bioresour. Technol.* **2016**, *220*, 425–432. [CrossRef] [PubMed]
59. Tang, Z.; Xi, B.; Huang, C.; Tan, W.; Li, W.; Zhao, X.; Liu, K.; Xia, X. Mobile genetic elements in potential host microorganisms are the key hindrance for the removal of antibiotic resistance genes in industrial-scale composting with municipal solid waste. *Bioresour. Technol.* **2020**, *301*, 122723. [CrossRef]
60. Qian, X.; Gu, J.; Sun, W.; Wang, X.J.; Su, J.Q.; Stedfeld, R. Diversity, abundance, and persistence of antibiotic resistance genes in various types of animal manure following industrial composting. *J. Hazard. Mater.* **2018**, *344*, 716–722. [CrossRef]
61. Zhu, L.; Zhao, Y.; Yang, K.; Chen, J.; Zhou, H.; Chen, X.; Liu, Q.; Wei, Z. Host bacterial community of MGEs determines the risk of horizontal gene transfer during composting of different animal manures. *Environ. Pollut.* **2019**, *250*, 166–174. [CrossRef]

62. Wang, C.; Dong, D.; Wang, H.; Müller, K.; Qin, Y.; Wang, H.; Wu, W. Metagenomic analysis of microbial consortia enriched from compost: New insights into the role of Actinobacteria in lignocellulose decomposition. *Biotechnol. Biofuels* **2016**, *411*, 125–135. [CrossRef]
63. Yin, Y.; Song, W.; Gu, J.; Zhang, K.; Qian, X.; Zhang, X.; Zhang, Y.; Li, Y.; Wang, X. Effects of copper on the abundance and diversity of ammonia oxidizers during dairy cattle manure composting. *Bioresour. Technol.* **2016**, *221*, 181–187. [CrossRef]
64. Zhang, R.; Wang, X.; Gu, J.; Zhang, Y. Influence of zinc on biogas production and antibiotic resistance gene profiles during anaerobic digestion of swine manure. *Bioresour. Technol.* **2017**, *244*, 63–70. [CrossRef]
65. Baker-Austin, C.; Wright, M.S.; Stepanauskas, R.; McArthur, J.V. Co-selection of antibiotic and metal resistance. *Trends Microbiol.* **2006**, *14*, 176–182. [CrossRef] [PubMed]
66. Ezugworie, F.N.; Igbokwe, V.C.; Onwosi, C.O. Proliferation of antibiotic-resistant microorganisms and associated genes during composting: An overview of the potential impacts on public health, management and future. *Sci. Total Environ.* **2021**, *784*, 147191. [CrossRef] [PubMed]
67. Yu, H.; Xie, B.; Khan, R.; Yan, H.; Shen, G. The changes in functional marker genes associated with nitrogen biological transformation during organic-inorganic co-composting. *Bioresour. Technol.* **2020**, *295*, 122197. [CrossRef] [PubMed]
68. Guo, H.; Xue, S.; Nasir, M.; Lv, J.; Gu, J. Role of bentonite on the mobility of antibiotic resistance genes, and microbial community in oxytetracycline and cadmium contaminated soil. *Front. Microbiol.* **2018**, *9*, 2722. [CrossRef]
69. Chen, C.; Ray, P.; Knowlton, K.F.; Pruden, A.; Xia, K. Effect of composting and soil type on dissipation of veterinary antibiotics in land-applied manures. *Chemosphere* **2018**, *196*, 270–279. [CrossRef] [PubMed]
70. Xiong, W.; Wang, M.; Dai, J.; Sun, Y.; Zeng, Z. Application of manure containing tetracyclines slowed down the dissipation of tet resistance genes and caused changes in the composition of soil bacteria. *Ecotoxicol. Environ. Saf.* **2018**, *147*, 455–460. [CrossRef]
71. Mahmud, Z.H.; Shirazi, F.F.; Hossainey, M.R.H.; Islam, M.I.; Ahmed, M.A.; Nafiz, T.N.; Imran, K.M.; Sultana, J.; Islam, M.S.; Islam, M.A. Presence of virulence factors and antibiotic resistance among Escherichia coli strains isolated from human pit sludge. *J. Infect. Dev. Ctries.* **2019**, *13*, 195–203. [CrossRef]
72. Song, C.; Li, M.; Jia, X.; Wei, Z.; Zhao, Y.; Xi, B.; Zhu, C.; Liu, D. Comparison of bacterial community structure and dynamics during the thermophilic composting of different types of solid wastes: Anaerobic digestion residue, pig manure and chicken manure. *Microb. Biotechnol.* **2014**, *7*, 424–433. [CrossRef] [PubMed]
73. Partanen, P.; Hultman, J.; Paulin, L.; Auvinen, P.; Romantschuk, M. Bacterial diversity at different stages of the composting process. *BMC Microbiol.* **2010**, *10*, 94. [CrossRef]
74. Lacey, J. Actinomycetes in composts. *Ann. Agric. Environ. Med.* **1997**, *4*, 113–121.
75. Cai, L.; Gong, X.; Sun, X.; Li, S.; Yu, X. Comparison of chemical and microbiological changes during the aerobic composting and vermicomposting of green waste. *PLoS ONE* **2018**, *13*, e0207494. [CrossRef] [PubMed]
76. Huhe, J.C.; Wu, Y.; Cheng, Y. Bacterial and fungal communities and contribution of physicochemical factors during cattle farm waste composting. *Microbiology* **2017**, *6*, e00518. [CrossRef] [PubMed]
77. Zhang, Y.; Jewett, C.; Gilley, J.; Bartelt-Hunt, S.L.; Snow, D.D.; Hodges, L.; Li, X. Microbial communities in the rhizosphere and the root of lettuce as affected by Salmonella-contaminated irrigation water. *FEMS Microbiol. Ecol.* **2018**, *94*, fiy135. [CrossRef] [PubMed]
78. Hidalgo, D.; Corona, F.; Martín-Marroquín, J.M. Manure biostabilization by effective microorganisms as a way to improve its agronomic value. *Biomass Convers. Biorefinery* **2022**, *76*, 1–16. [CrossRef]
79. Chen, Z.; Zhang, J.; Huang, L.; Yuan, Z.; Li, Z.; Liu, M. Removal of Cd and Pb with biochar made from dairy manure at low temperature. *J. Integr. Agric.* **2019**, *18*, 201–210. [CrossRef]
80. Lehmann, J.; Joseph, S. Biochar for environmental management: Science and technology. In *Science, Technology and Implementation*; Crop and Soil Science: London, UK, 2009; pp. 1–976.
81. Xiao, R.; Awasthi, M.K.; Li, R.; Park, J.; Pensky, S.M.; Wang, Q.; Wang, J.J.; Zhang, Z. Recent developments in biochar utilization as an additive in organic solid waste composting: A review. *Bioresour. Technol.* **2017**, *246*, 203–213. [CrossRef] [PubMed]
82. Selvam, A.; Zhao, Z.; Wong, J.W.C. Composting of swine manure spiked with sulfadiazine, chlortetracycline and ciprofloxacin. *Bioresour. Technol.* **2012**, *126*, 412–417. [CrossRef]
83. Qian, X.; Sun, W.; Gu, J.; Wang, X.J.; Sun, J.J.; Yin, Y.N.; Duan, M.L. Variable effects of oxytetracycline on antibiotic resistance gene abundance and the bacterial community during aerobic composting of cow manure. *J. Hazard. Mater.* **2016**, *315*, 61–69. [CrossRef]
84. Zhang, Y.J.; Hu, H.W.; Gou, M.; Wang, J.T.; Chen, D.; He, J.Z. Temporal succession of soil antibiotic resistance genes following application of swine, cattle and poultry manures spiked with or without antibiotics. *Environ. Pollut.* **2017**, *231*, 1621–1632. [CrossRef]
85. Selvam, A.; Xu, D.; Zhao, Z. Fate of tetracycline, sulfonamide and fluoroquinolone resistance genes and the changes in bacterial diversity during composting of swine manure. *Bioresour. Technol.* **2012**, *126*, 383–390. [CrossRef]
86. Jia, X.; Wang, M.; Yuan, W.; Ju, X.; Yang, B. The Influence of Biochar Addition on Chicken Manure Composting and Associated Methane and Carbon Dioxide Emissions. *BioResources* **2016**, *11*, 5255–5264. [CrossRef]
87. Hagemann, N.; Subdiaga, E.; Orsetti, S.; de la Rosa, J.M.; Knicker, H.; Schmidt, H.P.; Kappler, A.; Behrens, S. Effect of biochar amendment on compost organic matter composition following aerobic compositing of manure. *Sci. Total Environ.* **2018**, *613–614*, 20–29. [CrossRef] [PubMed]

88. Lehmann, J.; Rillig, M.C.; Thies, J.; Masiello, C.A.; Hockaday, W.C.; Crowley, D. Biochar effects on soil biota—A review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836. [CrossRef]
89. Liu, N.; Zhou, J.; Han, L.; Ma, S.; Sun, X.; Huang, G. Role and multi-scale characterization of bamboo biochar during poultry manure aerobic composting. *Bioresour. Technol.* **2017**, *241*, 190–199. [CrossRef] [PubMed]
90. Czekała, W.; Malińska, K.; Cáceres, R.; Janczak, D.; Dach, J.; Lewicki, A. Co-composting of poultry manure mixtures amended with biochar The effect of biochar on temperature and C-CO_2 emission. *Bioresour. Technol.* **2016**, *200*, 921–927. [CrossRef]
91. Muhammad, N.; Dai, Z.; Xiao, K.; Meng, J.; Brookes, P.C.; Liu, X.; Wang, H.; Wu, J.; Xu, J. Changes in microbial community structure due to biochars generated from different feedstocks and their relationships with soil chemical properties. *Geoderma* **2014**, *226–227*, 270–278. [CrossRef]
92. Elzobair, K.A.; Stromberger, M.E.; Ippolito, J.A.; Lentz, R.D. Contrasting effects of biochar versus manure on soil microbial communities and enzyme activities in an Aridisol. *Chemosphere* **2016**, *142*, 145–152. [CrossRef] [PubMed]
93. Xu, X.; Ma, W.; Zhou, K.; An, B.; Huo, M.; Lin, X.; Wang, L.; Wang, H.; Liu, Z.; Cheng, G. Effects of composting on the fate of doxycycline, microbial community, and antibiotic resistance genes in swine manure and broiler manure. *Sci. Total Environ.* **2022**, *832*, 155–169. [CrossRef]
94. Li, Y.; Gu, J.; Wang, X.; Song, Z.; Hu, T.; Xie, J.; Guo, H.; Ding, Q.; Xu, L.; Wei, Y. The fate of antibiotic resistance genes and their influential factors in swine manure composting with sepiolite as additive. *Bioresour. Technol.* **2022**, *347*, 126–142. [CrossRef]