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Effect of Biochar-Containing Compost on Cucumber Quality and Antibiotic Resistance Genes Abundance in Soil–Cucumber System

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Abstract: The distribution of antibiotic resistance genes (ARGs) derived from compost in soil–plant systems is a serious issue. One possible remedy is the application of biochar-containing compost. In this study, cucumber quality and the abundance of ARGs in soil–cucumber systems under different compost treatments, namely, traditional pig manure and corn straw compost (PC); pig manure, corn straw, and large particle size biochar (5–10 mm) compost (PCLB); and pig manure, corn straw, and small particle size biochar (<2 mm) compost (PCSB); were investigated. The results showed that, under PCSB, the yield, vitamin C and soluble protein content of the cucumbers were the highest, while the absolute abundance of *intI2* was reduced in the soil. The amount of available potassium in the soil contributed the most to changes in cucumber yield and quality. The total absolute abundance of ARGs in the soil was highest in PC, followed by PCLB, PCSB, and CK (control treatment without fertilization) treatments. Compared to CK treatments, PCLB and PCSB applications to the soil decreased the abundance of *sul1* and *tetG* by 42–57% and 38–80%, respectively, in the cucumbers. In summary, the PCSB application was more beneficial in increasing soil nutrient content; improving cucumber yield, vitamin C, and soluble protein content; and reducing the risk of input and transport of ARGs in the soil–cucumber system.

Keywords: biochar-containing compost; cucumber; antibiotic resistance genes; soil

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Citation: Tong, Z.; Liu, F.; Rajagopalan, U.M.; Sun, B.; Tian, Y.; Zuo, Q.; Zhang, J.; Duan, J.; Bi, W.; Qin, J.; et al. Effect of Biochar-Containing Compost on Cucumber Quality and Antibiotic Resistance Genes Abundance in Soil–Cucumber System. *Sustainability* **2023**, *15*, 9563. https://doi.org/10.3390/su15129563

Academic Editor: Antoni Sánchez

Received: 3 May 2023
Revised: 9 June 2023
Accepted: 12 June 2023
Published: 14 June 2023

1. Introduction

To promote animal growth and prevent disease, antibiotics are used extensively in animal husbandry [1,2]. However, large amounts are not completely absorbed and metabolized by animal. Instead, they end up as residual in livestock animal manure [3], and their presence is capable of inducing the production and enrichment of antibiotic resistance genes (ARGs) [4]. On the other hand, the application of livestock manure on farmland can improve soil quality and increase crop yield [5–7]. However, its large-scale application may cause serious damage to the soil ecosystem as it is an important reservoir for antibiotics and ARGs [8,9].

Antibiotics and ARGs enter soil via manure application, resulting in the abundance of ARGs increasing significantly in soil and plants [10,11], with the antibiotics also continuing to induce the production of ARGs. Recent studies have shown that the transfer of ARGs from soil to plants has been accelerated by the application of manure [12,13], which may pose a further risk to human health. In addition, organic vegetables with high ARG levels are grown in manure-amended soils [14]. The spread of ARGs could, in turn, make the antibiotic therapy of human and animal diseases ineffective [15]. Approximately 10 million people worldwide will be infected with ARG-related diseases by 2050, and about 4.73 million people in Asia will die due to antibiotic resistance [16]. Therefore, the need to study the transport of ARGs from livestock manure to farmland ecosystems is crucial.

Composting is an effective way to obtain a stable substrate from livestock manure and is widely used in agriculture to produce organic fertilizer. In addition, composting is an effective way to remove antibiotics and ARGs from manure [17–19], further reducing their spread to soil. The application of compost to soil leads to more efficient utilization of nutrients [20] and better plant growth [21]. However, traditional composting shows limited ability in the removal of antibiotics and ARGs [22,23]. A few studies have been carried out to improve the removal efficiency of antibiotics and ARGs by adding additives during composting [24,25]. Biochar addition promotes the removal of antibiotics and ARGs during composting [26,27], which may further alleviate the risk of their input in soil. Cucumber is popular with consumers for its special flavor, and it also contains a wealth of nutrients needed by humans, such as protein and vitamin C [28]. Biochar-containing compost is an end product obtained by co-composting biochar with other raw materials, which has several advantages, such as higher nutrient content and humification, with lower ecological risk of antibiotics as compared to traditional compost application [29]. Thus, biochar-containing compost can be used in cucumber production and has the potential to improve cucumber yield and quality. However, the effect of the application of biochar-containing compost on cucumber yield and quality and on the abundance of ARGs in a soil–cucumber system has been less reported.

In this study, we focused on cucumber, and we conducted farmland experiments to investigate the effect of the application of biochar-containing compost on cucumber yield and quality and the transfer of ARGs in the soil–cucumber system. The study also aimed to identify the main factors responsible for the variation in cucumber yield and qualities and to clarify the correlation between bacterial communities, ARGs, and mobile genetic elements (MGEs) in soil.

2. Materials and Methods

2.1. Test Materials

The cucumber variety "Xinjin Chun No. 5" was used as the experimental material. The composts applied to the soil were PC (traditional pig manure and corn straw compost), PCLB (pig manure, corn straw, and large-particle-size biochar (5–10 mm) compost), and PCSB (pig manure, corn straw, and small-particle-size biochar (<2 mm) compost); they were all composted for 50 days. The biochar-containing compost was produced as described by Tong et al. [30]. The chemical fertilizers used in this study were urea ($N \geq 46\%$), calcium superphosphate ($P_2O_5 \geq 12\%$), and potassium sulfate ($K_2O \geq 60\%$). The physicochemical properties of composts are shown in Table 1.

2.2. Experimental Setup

The test farmland was located at Nongshengyuan Family Farm, Taigu District, Jinzhong City, Shanxi Province, China. This study started on 23 April 2022. The experiment was set up with four treatments: (1) CK treatment (control treatment without fertilization); (2) PC treatment (the application of 2.80 kg PC, 125.57 g urea, and 76.71 g potassium sulfate to soil); (3) PCLB treatment (2.80 kg PCLB, 136.82 g urea, 44.43 g calcium superphosphate, and 72.58 g potassium sulfate to soil); (4) PCSB treatment (2.80 kg PCSB, 131.06 g urea, 33.07 g calcium superphosphate, and 60.30 g potassium sulfate to soil). Due to the inconsistent nutrient levels of different composts, it is often necessary to introduce chemical fertilizers to ensure that the levels of nitrogen, phosphorus and potassium in the soil are consistent across all fertilization treatments [31–33]. The application of composts and chemical fertilizers was equivalent to adding 120 g N, 60 g P, and 60 g K to the soil of each plot (except for the CK treatment). The experiment was conducted in a randomized block design with a plot of 2 m × 3 m and three replications for each treatment.

Table 1. The physicochemical properties of composts.

Physicochemical Properties	Compost Samples		
	PC	**PCLB**	**PCSB**
pH	7.51 ± 0.04	7.63 ± 0.06	7.68 ± 0.04
EC mS/cm	4.19 ± 0.05	3.43 ± 0.13	3.88 ± 0.02
TN g/kg	22.14 ± 0.85	20.30 ± 0.84	21.24 ± 0.53
TP g/kg	21.35 ± 0.82	18.82 ± 0.21	19.46 ± 0.40
TK g/kg	7.15 ± 0.13	7.92 ± 0.30	10.19 ± 0.22
AP mg/kg	159.46 ± 0.83	165.06 ± 2.76	174.11 ± 0.93
AK mg/kg	13,192.45 ± 124.70	13,903.77 ± 64.46	14,654.30 ± 59.41
TOC g/kg	288.41 ± 0.91	296.47 ± 4.12	318.58 ± 2.16

(PC: traditional pig manure and corn straw compost; PCLB: pig manure, corn straw, and large-particle-size biochar (5–10 mm) compost; PCSB: pig manure, corn straw, and small-particle-size biochar (<2 mm) compost; EC: electric conductivity; TN: total nitrogen; TP: total phosphorus; TK: total potassium; AP: available phosphorus; AK: available potassium; TOC: total organic carbon).

2.3. Sampling and Analysis

2.3.1. Soil Sample Collections

Soil samples were collected at 0–20 cm depth on 15 June 2022. Some of them were stored at 4 °C for the measurement of NH_4^+-N and NO_3^--N. The air-dried soils were used for analysis of soil pH, EC, total organic carbon, total nitrogen, total phosphorus, total potassium, available phosphorus, available potassium, and soil aggregates. Other fresh soil samples were stored at −80 °C for the analysis of bacterial communities, antibiotics, ARGs, and MGEs. Soil samples collected using a ring knife with a volume of 100 cm^3 were used for the determination of soil bulk density.

2.3.2. Cucumber Sample Collections

Cucumber samples were first collected on 15 June 2022. The five cucumbers (Figure 1) selected from the first collection in each plot were used for indicator measurements. After washing the cucumbers, we used some of them for the determination of vitamin C, soluble protein, and nitrate content and some were used for the determination of ARGs and MGEs.

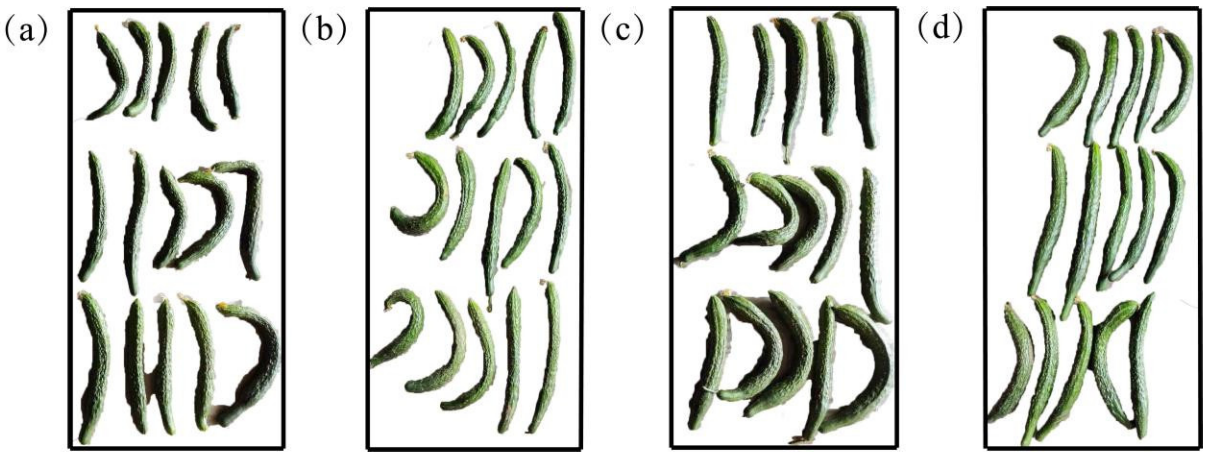

Figure 1. The photos of the five cucumbers selected for quality determination in CK (**a**), PC (**b**), PCLB (**c**), and PCSB (**d**) treatments.

2.3.3. Analysis Methods for Each Indicator of Soil and Cucumber

(1) Determination of Soil Physicochemical Properties

The soil physicochemical properties (pH, EC, organic matter, total nitrogen, total phosphorus, total potassium, available phosphorus, available potassium, NH_4^+-N, and NO_3^--N) were measured using the methods reported by Wang et al. [33]. 500 g of air-dried soil was weighed and dry-sieved to separate out three particle sizes of aggregates (large aggregates: >2 mm; small aggregates: 0.25–2 mm; microaggregates: <0.25 mm), then the percentage of each particle size aggregate to the total soil sample was calculated. Soil bulk density was measured according to Di et al. [34].

(2) Determination of Cucumber Yield and Quality

Cucumbers in each plot were weighed after each collection, and the total weight of the ten collected cucumbers was recorded as the cucumber yield. The vitamin C (Vc), soluble protein, and nitrate content in the cucumbers were measured using the methods of 2,6-dichlorophenol indophenol titration, Coomassie brilliant blue G-250 staining, and UV spectrophotometry [35].

(3) Determination of Antibiotics in Soil Samples

An LC-MS 8030 spectrometer (Shimadzu, Kyoto, Japan) was used to measure the concentrations of 11 antibiotics in the soil samples, including 1e tetracycline (doxycycline), 8 fluoroquinolones (ciprofloxacin, norfloxacin, ofloxacin, enrofloxacin, difloxacin, danofloxacin, pefloxacin mesylate, and flumequine), 1 macrolide (tilmicosin), and 1 lincoamide (lincomycin). Antibiotics were extracted from the soil samples according to the method reported by Wang et al. [36]. The parameter conditions of the LC-MS 8030 spectrometer and the recoveries of standard samples for antibiotics were in agreement with those reported by Tong et al. [26,30].

(4) DNA Extraction from Soil and Cucumber Samples and Q-PCR

Microbial genomic DNA was extracted from soil and cucumber samples using a rapid DNA extraction kit (TIANNAMP DNA Kit, TIANGEN, Beijing, China) according to the method described by the manufacturer. Each sample was repeated 3 times. This study selected 19 ARGs (11 tet genes: *tetA*, *tetB*, *tetC*, *tetG*, *tetM*, *tetO*, *tetQ*, *tetW*, *tetX*, *tetZ*; 2 sul genes: *sul1*, *sul2*; 1 gyr gene: *gyrA*; 4 erm genes: *ermB*, *ermC*, *ermF*, *ermT*; 1 mef gene: *mefA*; 1 mph gene: *mphA*) and 2 MGEs (*intI1* and *intII2*), which were quantified by the RT-qPCR system (ABI 7500, Thermo Fisher Scientific, Waltham, MA, USA). The primer sequences and thermal cycling procedures to amplify the ARGs were performed according to previous studies [26,37].

(5) Soil Bacterial Community Analysis

To analyze the variation in soil bacterial communities, the V3-V4 region of bacterial 16S rRNA was selected for amplification with the primers 341F (CCTACGGGNGGCWGCAG) and 806R (GGACTACHVGGGTWTCTAAT). The library was constructed using TruSeq® DNA PCR-Free Sample Preparation Kit, then quantified by Qubit 2.0 Fluorometer and q-PCR (ABI 7500, Thermo Fisher Scientific, Waltham, MA, USA) and sequenced on an Illumina MiSeq platform (MiSeq-PE300, Solexa, Hayward, CA, USA).

2.4. Statistical Analysis

SPSS version 25.0 was used for statistical analysis of the data. All data were analyzed in three replications. TB tools was used to generate a heat map of the abundance of the top 35 soil bacteria. Redundancy analysis (RDA) and network analysis were constructed with Canoco 5.0 and Cytoscape 3.9.0, respectively. Amos Graphics was used to establish a structural equation model.

3. Results and Discussion

3.1. Effect of Different Composts on Soil Physicochemical Properties

Table 2 shows the change in soil physicochemical properties under the influence of different composts. Compared to the CK treatment, the application of compost significantly increased the organic matter content in the soil. In fertilization treatments, the content of soil organic matter followed the order of PCSB > PCLB > PC treatments. This is due to the large amount of organic matter contained in compost (Table 1), and the application of compost results in additional organic matter input in the soil. The application of compost led to the input of large amounts of humic substances in the soil, thus contributing to an increase in soil organic matter content [29]. Previous studies found that composting with biochar and pig manure can significantly increase humic substances in compost products [26]. As shown in Table 2, the contents of available phosphorus and available potassium in the soil were in the order of PCSB > PCLB > PC > CK treatments. The reason may be that, on the one hand, the compost itself contains high levels of available phosphorus and available potassium (Table 1), while the application of compost restores available phosphorus and available potassium in the soil. On the other hand, the organic matter in compost provides a suitable habitat for microbial life in the soil, which effectively promotes the conversion of nutrients [38]. Biochar-containing composts were more effective in increasing the content of available nutrients and organic carbon in the soil [39,40]. In addition, the straw in compost may be an important factor in the increased organic matter, available phosphorus content and available potassium content of the soil. Previous studies have shown that straw can significantly increase the soil nutrient content [41].

Table 2. Soil physicochemical properties in different treatments.

Physicochemical Properties	Treatment			
	CK	PC	PCLB	PCSB
Organic matter (g/kg)	15.34 ± 0.15 c	16.32 ± 0.11 b	17.13 ± 0.41 b	18.71 ± 0.92 a
AP (mg/kg)	61.34 ± 1.96 c	96.71 ± 3.76 b	102.58 ± 1.24 b	113.86 ± 7.34 a
AK (mg/kg)	544.86 ± 7.71 d	609.26 ± 6.16 c	638.52 ± 2.96 b	671.99 ± 25.95 a
NH_4^+-N (mg/kg)	1.43 ± 0.10 d	6.15 ± 1.04 a	4.56 ± 0.97 b	2.96 ± 0.08 c
NO_3^--N (mg/kg)	35.90 ± 0.60 c	42.97 ± 0.74 b	47.08 ± 0.89 b	56.15 ± 5.47 a
pH	8.72 ± 0.02 a	8.70 ± 0.05 a	8.69 ± 0.03 a	8.67 ± 0.01 a
Large aggregates (%)	57.24 ± 0.87 c	61.34 ± 1.45 b	62.53 ± 0.52 b	67.24 ± 0.90 a
Small aggregates (%)	13.48 ± 0.25 ab	14.07 ± 1.51 a	11.97 ± 0.61 b	12.73 ± 0.57 ab
Micro-aggregates (%)	29.28 ± 1.00 a	24.59 ± 2.96 b	25.51 ± 1.00 b	20.03 ± 1.47 c
Bulk density (g/cm^3)	1.03 ± 0.01 a	1.01 ± 0.02 a	0.98 ± 0.01 b	0.97 ± 0.01 b

(CK: control treatment without fertilization; PC: traditional pig manure and corn straw compost; PCLB: pig manure, corn straw, and large-particle-size biochar (5–10 mm) compost; PCSB: pig manure, corn straw, and small-particle-size biochar (<2 mm) compost; AP: available phosphorus; AK: available potassium; Different lowercase letters indicate the signifcant difference in physicochemical properties under different treatments).

Moreover, the application of compost significantly increased the content of NH_4^+-N and NO_3^--N in the soil, and there was a significant discrepancy between fertilization treatments. The NH_4^+-N content varied in the order of PCSB < PCLB < PC treatments, while the NO_3^--N content varied in the order of PCSB > PCLB > PC treatments. The biochar showed a powerful adsorption capacity for NH_4^+ [42]. Small particle sizes of biochar with larger specific surface area were better for the absorption of NH_4^+-N. This, in turn, promoted the conversion of NH_4^+-N to NO_3^--N. Meanwhile, compared with the CK treatment, the application of compost decreased the pH of the soil and such variations in pH among different treatments were insignificant. The test farm in this study applied compost products for the first time, perhaps due to the short period of time compost products have been applied to the soil, and they did not have a significant effect on the pH in the soil.

Table 2 also shows the results for different aggregate sizes under each of the treatments used. Compared with the CK treatment, the proportion of large aggregates in the soil was increased by 7%, 9%, and 18% in the PC, PCLB, and PCSB treatments, respectively. In addition, the application of compost reduced the proportion of soil microaggregates.

Humic substances in compost contribute to improving soil porosity, forming aggregates, and enhancing soil structure [43]. Meanwhile, compared with the CK treatment, the bulk density of the soil was decreased by 2%, 4%, and 5% in the PC, PCLB, and PCSB treatments, respectively. These results indicate that the application of compost could lead to an increase in soil porosity. There was a significant positive correlation between large aggregates and organic matter ($p < 0.01$, $r = 0.938$), available phosphorus ($p < 0.01$, $r = 0.912$), and available potassium ($p < 0.01$, $r = 0.925$). The application of compost clearly promotes the formation of organic carbon, enhancing the aggregation of mineral particles and thus facilitating the formation of large agglomerates in the soil [44] which, in turn, could also reduce the loss of effective nutrients. This resulted in a higher proportion of large aggregates and a lower proportion of micro-aggregates in the soil.

3.2. Effect of Different Composts on Yield and Quality of Cucumber

Figure 2 shows the cucumber yield, Vc content, and soluble protein content after fertilization treatment. As can be seen, they were significantly higher than those after the CK treatment, with the application of PC, PCLB, and PCSB resulting in a 24%, 42%, and 50% increase in cucumber yield, respectively, and the discrepancy between PCSB and PCLB treatments being insignificant (Figure 2a). The soluble protein content shows significant discrepancy in different treatments. Compared with the CK treatment, the soluble protein content of the cucumbers increased by 15%, 25%, and 37% in the PC, PCLB, and PCSB treatments, respectively. The Vc and soluble protein content of the cucumbers were significantly higher in the PCSB treatment compared with the PCLB treatment.

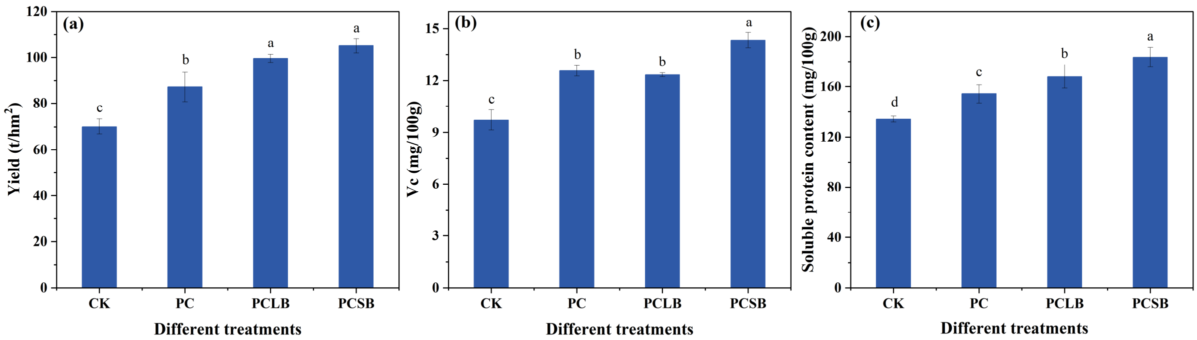

Figure 2. The yield (**a**), Vc content (**b**), and soluble protein content (**c**) of cucumbers in different treatments (Vc: vitamin C; Different lowercase letters indicate the signifcant difference in the yield, vitamin C and soluble protein content of the cucumbers under different treatments).

The presence of humic substances in composts as biostimulants can enhance the nutrient uptake capacity of plants [45]. The application of compost increased the content of organic matter, available potassium, and phosphorus in the soil (Figure 2a–c), enhancing its nutrient supply capacity and facilitating the absorption of nutrients by the cucumbers. Manure–biochar compost is more beneficial for increasing the utilization and plant uptake of soil nutrients than manure compost [46]. High bulk density would have resulted in low nutrient uptake capacity and reduced plant root growth [43]. The application of compost reduced the soil bulk density, with the lowest soil bulk density in the PCSB treatment. The application of compost improves the quality of the soil, and the improved soil quality may restructure the pore size distribution and aggregation processes in the soil to improve the water retention capacity of the soil, which can have a positive impact on plant growth or yield and fruit quality [47]. PCSB application had the most significant effect on soil quality improvement. Therefore, the application of PCSB is more effective in improving cucumber yield, Vc content, and soluble protein content. Meanwhile, the application of compost to the soil promoted the accumulation of nitrate in cucumbers, the content of which was highest in the PCSB treatment (72.29 mg/kg), followed by the PCLB treatment (64.81 mg/kg), the PC treatment (61.89 mg/kg), and the CK treatment (52.24 mg/kg). This may be due to the fact that the application of compost to the soil promotes the life activity of nitrifying bacteria, which increases the NO_3^--N content in the soil (Table 2) and promotes the formation of nitrate in cucumbers.

3.3. Relationship between Soil Physicochemical Properties and Cucumber Yield and Quality

RDA was used to evaluate the effect of soil physicochemical properties on cucumber yield and quality (Figure 3). Soil physicochemical properties explained 95.06% of the variation in cucumber yield, and Vc, soluble protein, and nitrate content. Available potassium shows the highest explanation of 91.10% for the change in cucumber yield, and Vc, soluble protein, and nitrate content, followed by NH_4^+-N (1.50%), NO_3^--N (1.10%), pH (0.80%), organic matter (0.40%), and available phosphorus (0.30%). The available potassium in soil is significantly related to the yield ($r = 0.958$, $p < 0.01$), as well as the Vc ($r = 0.948$, $p < 0.01$), soluble protein ($r = 0.949$, $p < 0.01$), and nitrate content ($r = 0.974$, $p < 0.01$) of cucumbers. This indicates that available potassium is essential for the growth and quality of cucumbers. The application of composts promoted the growth and quality of the cucumbers, mainly by increasing the content of the soil's available potassium.

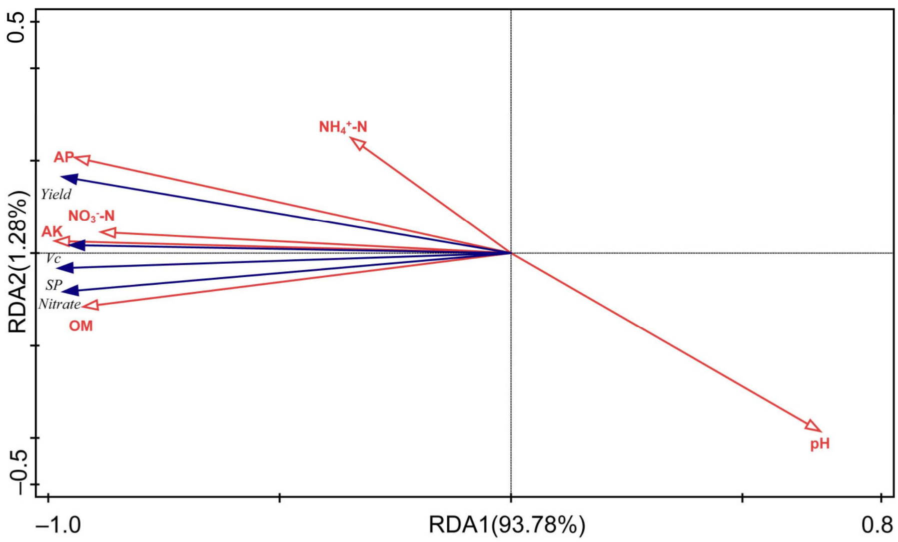

Figure 3. RDA of the relationship between soil physicochemical properties (red arrows) and the yield and qualities of cucumbers (blue arrows). OM: organic matter, AP: available phosphorus, AK: available potassium, Vc: vitamin C, SP: soluble protein.

3.4. Effect of Different Composts on Soil Bacterial Community

Figure 4a shows that the relative abundance of soil bacteria at the phylum level changes with the application of compost to the soil. *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Acidobacteria* are the predominant bacterial phyla in soil, accounting for 59.9–67.9% of the total bacterial community. *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes* are also the main phylum-level bacteria in composts applied to soil [30]. In compost-applied soils, the changes in bacterial communities are also reflected in the increase in abundance of these phylum bacteria. Compared with the CK treatment, the relative abundance of *Firmicutes* increased by 96%, 168%, and 208% in PC, PCLB, and PCSB treatments. Similarly, the application of compost improved the relative abundance of *Bacteroidetes* by 45–136%.

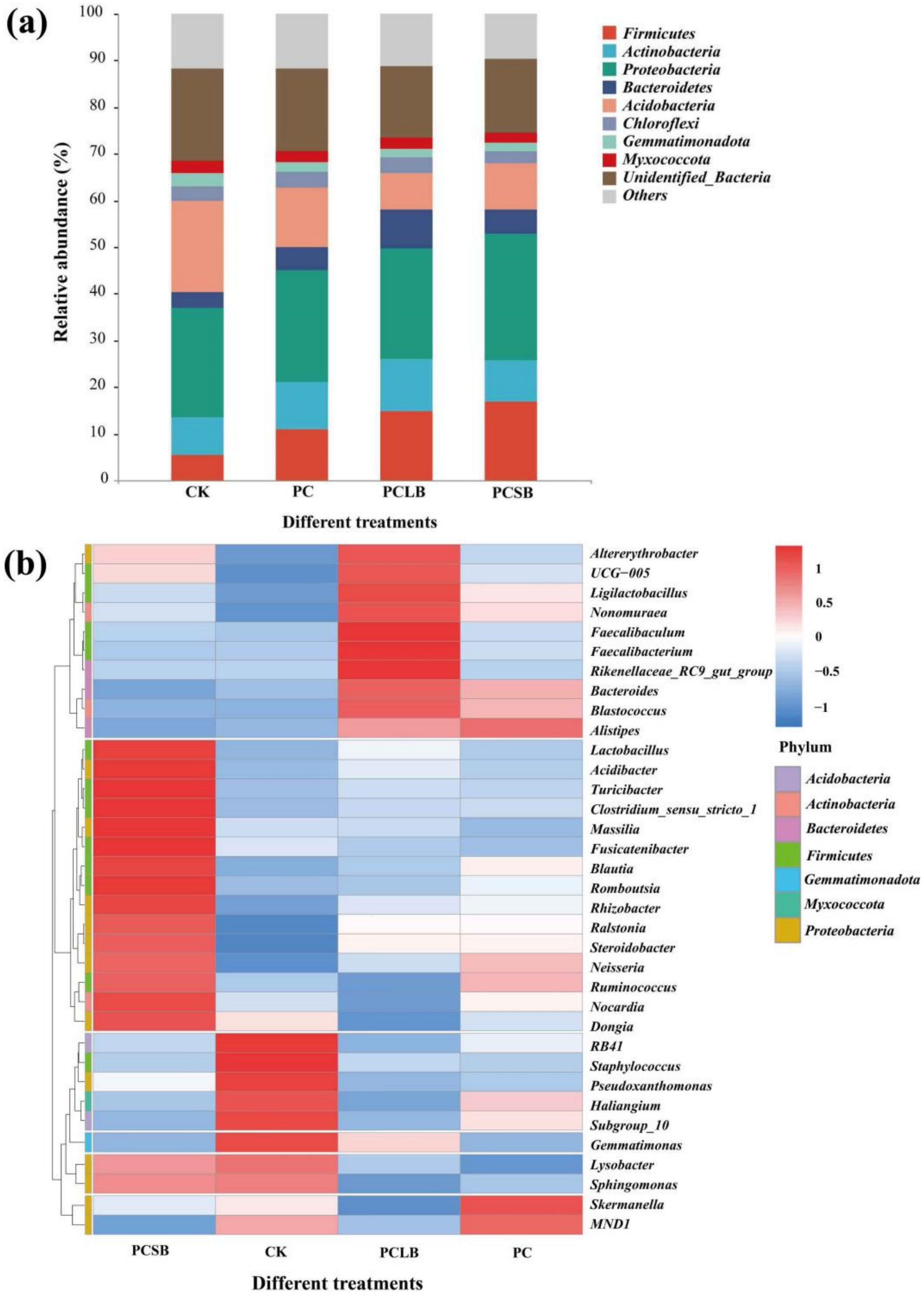

Figure 4. Relative abundance of microorganisms at phylum level (**a**) and heat map of the abundance of the top 35 soil bacteria at the genus level (**b**) in different treatments.

Firmicutes and *Bacteroidetes*, as organic decomposers, easily influenced soil nutrient availability [42]. The application of compost increased the relative abundance of *Actinobacteria* in the soil, which agrees with an earlier study on the improvement of soil nutrient availabilities, resulting in an increase in the proportion of *Actinobacteria* [48]. *Acidobacteria* is not a dominant bacteria phylum, and its relative abundance is less than 1% in compost. Compared with the CK treatment, the relative abundance of *Acidobacteria* in the soil decreased by 36–59% due to the application of compost. The compost caused a reduction in soil pH (Table 2), thereby inhibiting the activity of *Acidobacteria*. In contrast, *Acidobacteria* is suitable for survival in alkaline soil [49]. The compost also increased the relative abundance of *Proteobacteria* in the soil. This may be due to the fact that the compost contained a low level of antibiotics, and the soil with antibiotic exposure caused an increase in *Proteobacteria* relative abundance [50].

The compost increased the abundance of most bacteria genera in soil that contained a large amount of the nutrients necessary for their life activities. Figure 4b shows that the application of compost changed the relative abundance of the top 35 soil bacteria at the genus level. The highest abundances of nine and fifteen genera of soil bacteria were found in PCLB and PCSB treatments, and these genera primarily belong to *Proteobacteria*. Only eight bacteria—namely *Clostridium_sensu_stricto_1*, *Lactobacillus*, *Ligilactobacillus*, *Ralstonia*, *Romboutsia*, *Sphingomonas*, *Staphylococcus*, and *Turicibacter*—among the top 35 genera were relatively abundant in both soil and compost [30]. These bacteria (except *Sphingomonas*) are not the dominant genera in soil. This may be due to the fact that the bacteria in composts are not well adapted to the soil environment [51].

Supplementing oxygen can promote the growth and reproduction of *Proteobacteria* [52]. Compared with the CK treatment, the application of compost significantly increased the abundance of *Nocardia*, *Nonomuraea*, and *Blastococcus*, which belong to *Actinobacteria*. This may contribute to the removal of ARGs from soil. Previous studies found that *Actinobacteria* plays a key role in the degradation of resistant compounds [53]. *Sphingomonas* is the most dominant genus in soil, but not the dominant genus in compost. *Sphingomonas* can survive in alkaline environments [54], but the pH of compost is close to acidic and lower than the soil pH. Compared with the CK treatment, the application of compost reduced the relative abundance of *Sphingomonas* by 1–31%, due to compost application decreasing the soil pH.

3.5. Effect of Different Composts on Antibiotics and Antibiotic Resistance Genes in Soil

Antibiotics were not detected in soils of all treatments, indicating that the application of compost poses no risk of aggravating soil antibiotic contamination. When 2.8 kg of compost was introduced into 0–20 cm of soil in each plot, it was diluted approximately 470-fold, resulting in antibiotic concentrations in the soil that were below the detection limit. The soil adsorption, biodegradation, and plant uptake can reduce antibiotic levels in soil over time [55]. Previous studies have found that the antibiotics in soil can be removed by more than 90% within two months [56], and microorganisms are a crucial factor in their removal. *Actinobacteria* can decompose and metabolize complex organic compounds [57]. The addition of compost increased the abundance of *Actinobacteria* in the soil (Figure 4a), which contributes to the removal of antibiotics.

As shown in Figure 5a, the application of compost significantly increased the absolute abundance of ARGs in the soil, and there were significant differences between fertilization treatments. The application of compost with ARGs led to external ARGs being input in the soil. Moreover, compost with high nutrient levels can promote bacterial proliferation, leading to the enrichment of ARGs in soil [58,59]. Antibiotics in composts can also induce the production of antibiotic-resistant bacteria and ARGs in soil [60]. Compared with the PC treatment, the application of PCLB and PCSB decreased the total absolute abundance of ARGs in the soil by 26% and 40%, respectively. Biochar plays a key role in the removal of ARGs from soil [61]. According to a previous study, compared with PC and PCLB, PCSB contains fewer antibiotics and ARGs, so its application poses the lowest risk of input and proliferation of ARGs in soil [30]. Previous studies found that the abundance of ARGs did not decrease within 30 days of cultivation when biochar was present in the soil, but decreased significantly at day 90 [62]. The dissipation of ARGs in soil presents a time–response pattern. In this study, soil samples were collected up to day 50 of farmland tests, so the application of PCLB and PCSB did not significantly reduce the abundance of ARGs.

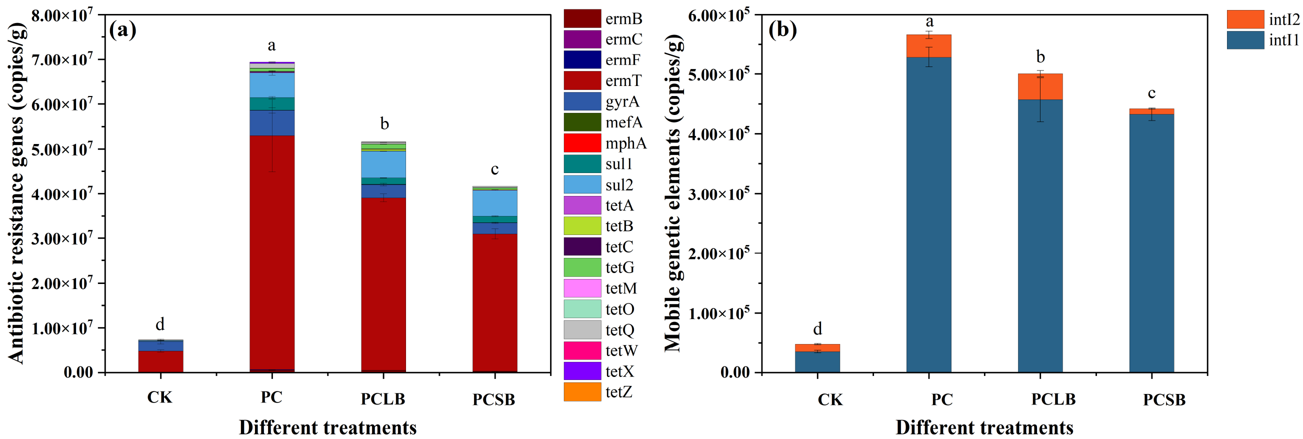

Figure 5. The absolute abundance of antibiotic resistance genes (**a**) and mobile genetic elements (**b**) of soil in different treatments. Different lowercase letters indicate the significant difference in the absolute abundance of antibiotic resistance genes and mobile genetic elements of soil under different treatments.

Figure 5b shows that, compared with the CK treatment, the application of compost significantly increased the total absolute abundance of MGEs in the soil, while the application of composts with MGEs led to external MGEs being input in the soil. This indicates that the application of compost promotes the horizontal gene transfer (HGT) of ARGs. Compared with the PC treatment, the application of PCLB and PCSB decreased the total absolute abundance of MGEs in the soil by 12% and 22%, inhibiting the HGT of ARGs. In addition, compared with the CK treatment, the application of PCSB decreased the absolute abundance of *intI2* in the soil by 29%. This indicates that the application of PCSB was able to inhibit the HGT of ARGs responsible by *intI2* in the soil. Biochar, containing a large number of pores on its surface, can increase the spatial distance between microorganisms, thus inhibiting the horizontal gene transfer of ARGs due to mutual contact between microorganisms [63]. Compared with large-particle-size biochar (5–10 mm) in PCLB, small-particle-size biochar (<0.074 mm) with a higher proportion of mesopores (2–50 nm) in PCSB is more conducive to reducing the chance of microorganisms being in contact with each other [30], thus facilitating the removal of ARGs in the soil.

3.6. Relationships between Soil Bacterial Community, Antibiotic Resistance Genes, and Mobile Genetic Elements

The significant positive correlation between ARGs and bacteria can be used to identify potential hosts for ARGs [14]. As shown in Figure 6, ARGs show a significant positive correlation with 16 species of bacteria at the genus level. These potential hosts are mainly distributed in four phyla, namely *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*. Compared with the CK treatment, the application of composts promoted the vital activity of potential hosts, resulting in the significantly increased abundance of ARGs in the soil. The evolution of host bacteria in ARGs is the main driver of change in ARGs [64]. Compared with the PC treatment, the application of PCLB and PCSB decreased the abundance of some potential hosts, thereby reducing the abundance of ARGs in soil. Meanwhile, this study found that a gene could be a host to various bacteria, indicating that ARGs perform HGT among different bacteria.

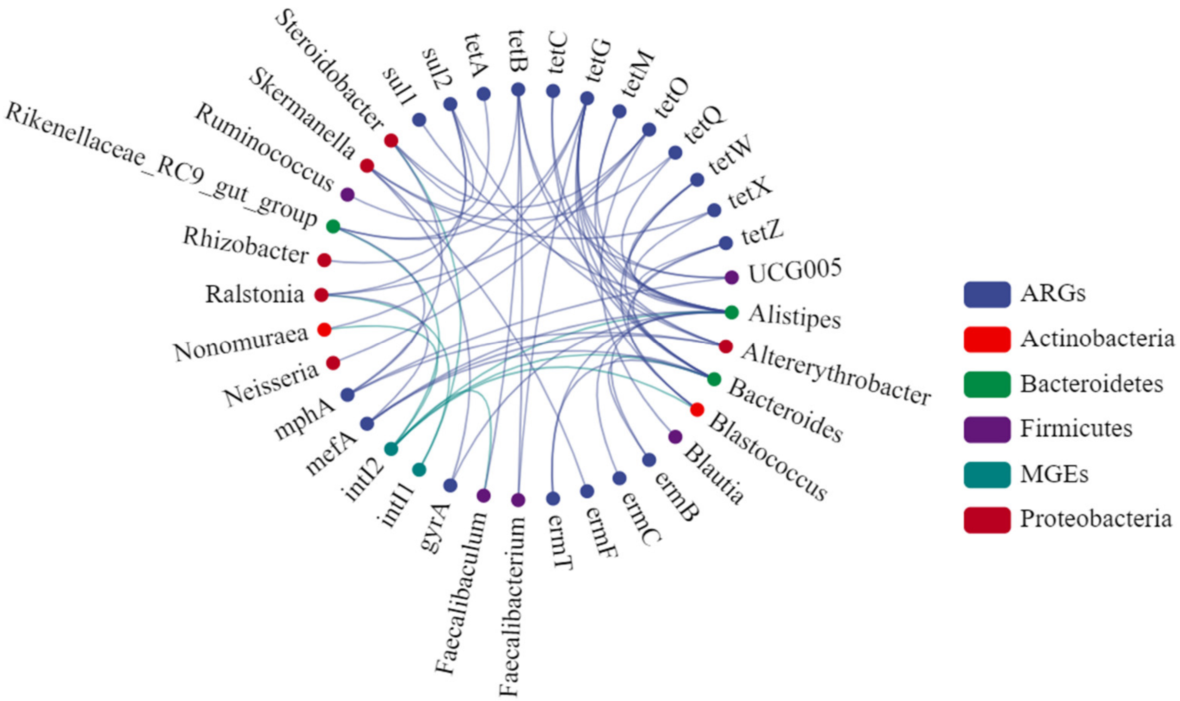

Figure 6. Co-occurrence network relationships between ARGs, MGEs, and bacteria at the genus level (the line between nodes represents a significant positive correlation, $p < 0.05$.

MGEs can promote the proliferation of ARGs in soil, thus increasing the ecological risk. Figure 6 shows MGEs hosting *Alistipes*, *Bacteroides*, *Blastococcus*, *Faecalibaculum*, *Nonomuraea*, *Ralstonia*, *Rikenellaceae _ RC9 _ gut _ group*, and *Steroidobacter*. These bacteria at the genus level are also the potential hosts of ARGs, suggesting that they are hubs for the HGT of ARGs. Compared with the CK treatment, the application of compost promoted the vital activities of potential host bacteria for MGEs, resulting in a significant increase in the total absolute abundance of MGEs in the soil. Compared with PC and PCLB treatments, the application of PCSB was more conducive to inhibiting the vital activities of potential hosts of MGEs, thus decreasing MGEs abundance and the risk of ARGs transmission in the soil.

3.7. Effect of Different Composts on Antibiotic Resistance Genes and Mobile Genetic Elements of Cucumber

As shown in Figure 7, compared with the CK treatment, the application of composts significantly increased the relative abundance of partial ARGs in the cucumbers. In all treatments, *tetC* and *tetQ* were not detected in the cucumbers. Compared with the PC treatment, the relative abundance of ARGs (expect *tetB*, *tetC*, *tetQ*, *tetZ*) decreased by 8–100% in the PCLB treatment and that of ARGs (except *tetB*, *tetC*, *tetM*, *tetQ*) reduced by 4–100% in the PCSB treatment. The application of PCLB and PCSB was more effective than PC in decreasing the relative abundance of ARGs in the cucumbers. Furthermore, compared with the CK treatment, the application of PCLB to the soil reduced the relative abundance of *sul1* and *tetG* in the cucumbers by 42% and 38%, respectively, while the application of PCSB reduced the relative abundance of *sul1* and *tetG* in the cucumbers by 57% and 80%, respectively.

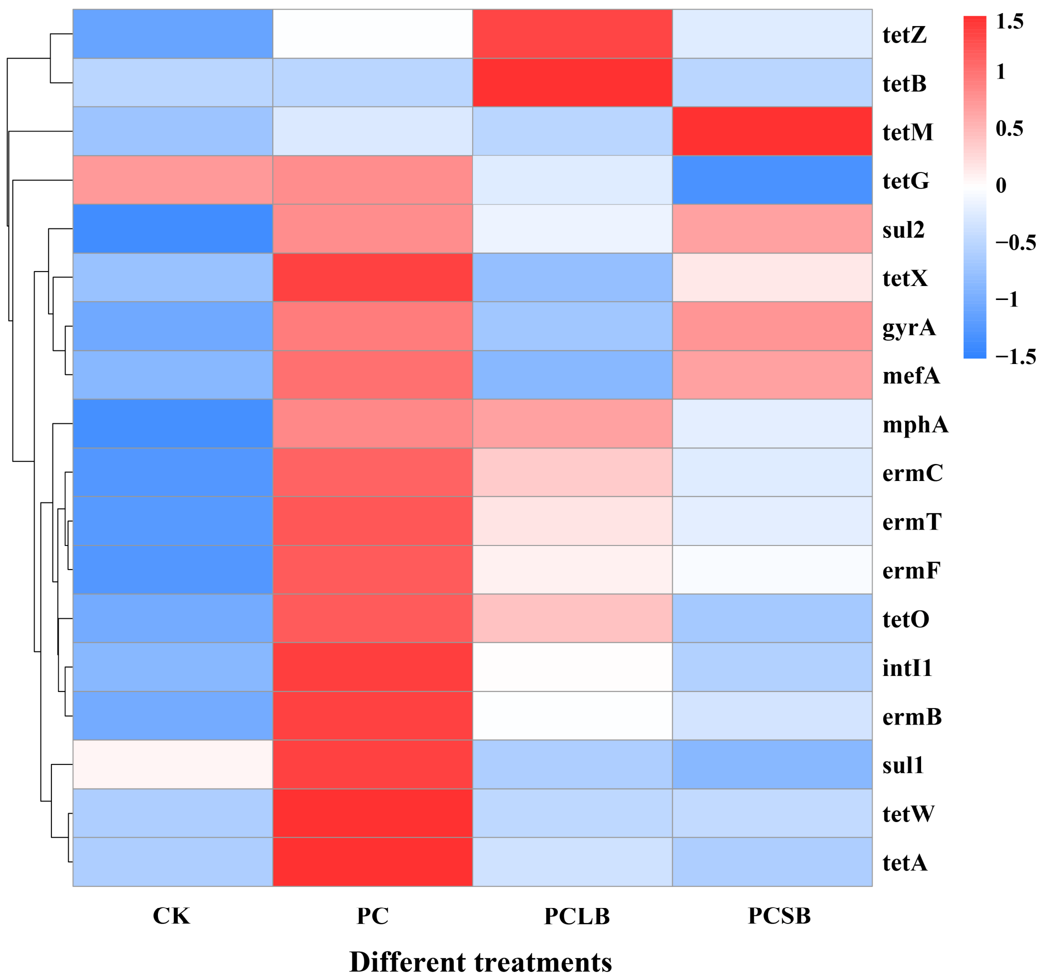

Figure 7. The relative abundance of ARGs and MGEs of cucumbers in different treatments.

Previous studies found that biochar could reduce the abundance of sul genes in vegetable tissues [65]. Biochar with a large specific surface area has a strong adsorption capacity for ARGs [66]. Compared with large-particle-size biochar (5–10 mm) in PCLB, small-particle-size biochar (< 0.074 mm) with larger specific surface area is more conducive to the adsorption of ARGs [30]. There is a significant correlation between *intI2* and *tetG* in the soil ($p < 0.01$). PCSB application reduced the absolute abundance of *intI2* in the soil (Figure 5b) and inhibited the proliferation of *tetG* in the soil, thus reducing the risk of tetG transfer from the soil to the cucumbers. In addition, *Alistipes*, *Bacteroides*, *Blastococcus*, and *Faecalibacterium* are potential host bacteria for *sul1* and *tetG* (Figure 6). The application of PCSB inhibited the life activity of these potential host bacteria in the soil. The migration of bacteria-carrying ARGs in the soil–plant system is an important factor in the transfer of ARGs from the soil to the plant [65]. The abundant pores of the biochar surface are strongly adsorbed to these bacteria, thus inhibiting the migration of *sul1* and *tetG* from the soil to the cucumber. Therefore, compared with PCLB, the application of PCSB is more beneficial in inhibiting the transfer of ARGs from soil to cucumber and decreasing the risk of transmission of ARGs to humans via the food chain.

Compared with the CK treatment, the application of compost significantly increased the relative abundance of *intI1* in the cucumbers. *IntI2* was not detected in cucumbers from all treatments, indicating that *intI1* is more likely to migrate from soil to cucumber. Compared with the PC treatment, the relative abundance of *intI1* in the cucumbers decreased by 55% and 78% in PCLB and PCSB treatments, respectively. PCSB was more efficient in inhibiting the transfer of *intI1* from soil to cucumber and suppressing the HGT of ARGs in the cucumbers.

3.8. Mechanisms of Variation in Antibiotic Resistance Genes in Soil–Cucumber Systems

The results of the structural equation model show the mechanism of interaction between soil physicochemical properties, soil bacterial communities, MGEs, and ARGs in the soil and cucumbers (Figure 8). Soil physicochemical properties have a significant correlation with changes in soil bacterial communities ($p < 0.001$). Nutrients are present in the soil that are partially available for microbial life activities [67]. The application of compost results in the input of external nutrients in the soil, thus promoting the life of microorganisms. Soil physicochemical properties show a significant correlation for MGEs ($p < 0.01$) and ARGs ($p < 0.05$). This may be due to nutrients in the soil providing energy material for the life activities of the host bacteria of ARGs and MGEs. At the same time, previous studies have shown that the application of compost can improve the availability of nutrients in soil [68], thus making it more conducive to microbial life. It is worth noting that, compared with soil bacterial communities ($p > 0.05$), MGEs in soil more significantly influence the change in ARGs in soil. Previous studies have identified bacterial communities in soil as a major factor influencing changes in ARGs [69]. The variation in ARGs in soil is mainly determined by the vital activity of their host bacteria. In this study, there are only 16 species of host bacteria for ARGs, and they account for 4–11% of the total bacterial community (Figure 4b). MGEs are responsible for the horizontal gene transfer of ARGs. The application of PCSB reduced the absolute abundance of *intI2* in the soil, indicating that PCSB application has the potential to inhibit horizontal gene transfer of ARGs, thereby facilitating their removal of ARGs.

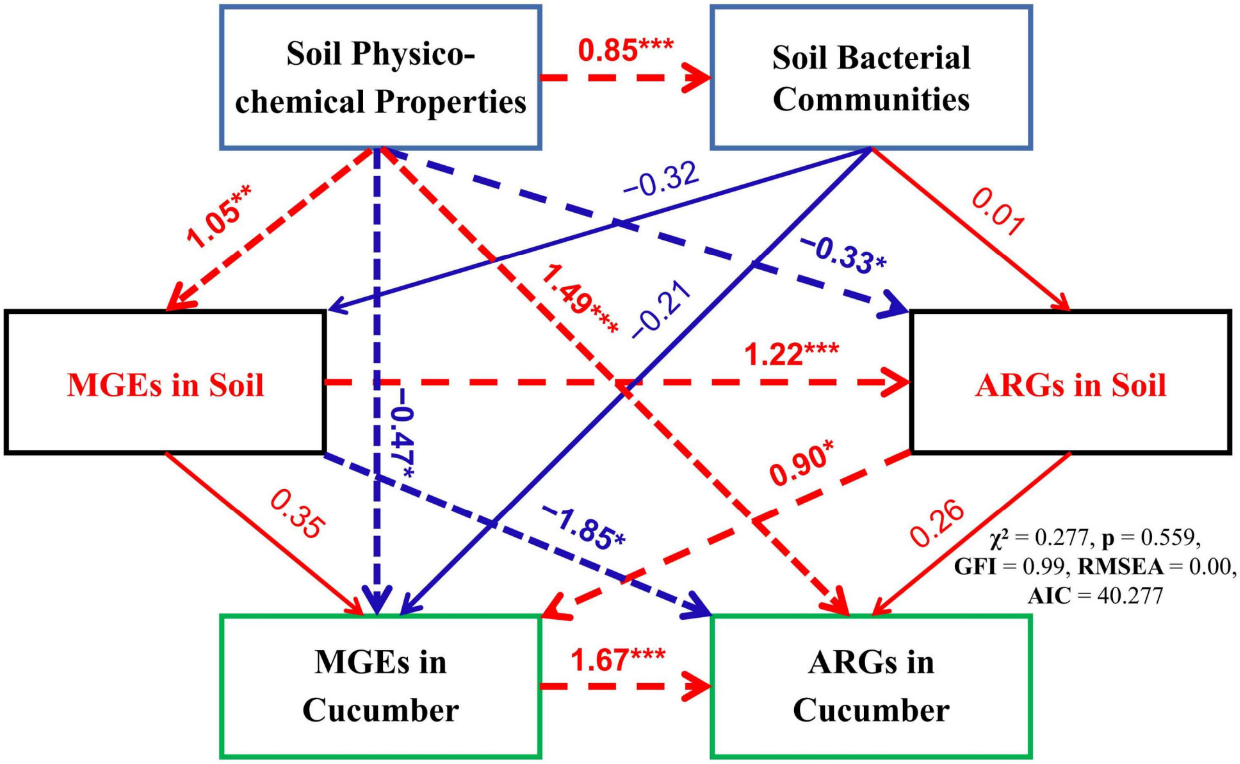

Figure 8. The direct and indirect relationships between soil physicochemical properties, soil bacterial communities, MGEs, and ARGs in soil and cucumbers. Significant levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Red arrows indicate positive correlations and blue arrows indicate negative correlations.

ARGs in the soil showed a significant correlation with MGEs ($p < 0.05$) in the cucumbers, and MGEs in the soil showed a significant correlation with ARGs ($p < 0.05$) in the cucumbers. This suggests that the accumulation of ARGs and MGEs in soil promotes ARGs transfer from soil to cucumber, and also promotes the horizontal gene transfer of ARGs in cucumbers. MGEs in the cucumbers had a significant effect on ARGs in the cucumbers ($p < 0.001$). The presence of *intI1* in the cucumbers contributed to the propagation of ARGs in the cucumbers, and similar phenomena occur in other plants [62]. ARGs in the soil had a significant direct effect on MGEs in the cucumbers and indirectly regulated the behavior of MGEs in the cucumbers. In addition, soil physicochemical properties had a significant effect on ARGs and MGEs in the cucumbers. The application of compost changes soil physicochemical properties, which further affects plant growth and development, and in turn influences the transferability of ARGs and MGEs in the soil–plant system [70]. Compost application improves cucumber yield and quality, which may stimulate the transfer of ARGs from soil to cucumber.

4. Conclusions

The results show that the application of biochar-containing compost significantly improved cucumber yield, Vc content, and soluble protein content, and the PCSB has the most significant improving effect. Available potassium is an important factor affecting cucumber yield and quality. Meanwhile, compared to PC and PCLB, PCSB application caused the least risk of ARG input in the soil and of its transfer from soil to cucumber. MGEs contribute most to changes in ARGs in the soil. The accumulation of MGEs and ARGs in the soil can facilitate the transfer of ARGs in the soil–cucumber system. Finally, the application of PCSB is more conducive to lowering the risk of ARGs spreading in the environment and harming human health.

Author Contributions: Methodology and writing—original draft, Z.T.; methodology, project administration, writing—review & editing, funding acquisition, and supervision, F.L.; writing—review & editing, U.M.R.; formal analysis, B.S., Y.T. and J.Z.; Resources, J.D.; Visualization, W.B. and J.Q.; Software, S.X. and Q.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Key R&D Program Projects, Shanxi Province, China (201903D221015); the "1331 Project", Shanxi Province, China (20211331-15); Demonstration Base for Joint Training of the Postgraduates on the Integration of Industry and Education, Shanxi Province, China (2022JD05); and Shanxi Agricultural University Horizontal Scientific Research Project, Shanxi Province, China (2022HX008).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data involved in this study are presented in the form of figures and tables in the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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