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# Effect of Mineral Fertilizers and Pesticides Application on Bacterial Community and Antibiotic-Resistance Genes Distribution in Agricultural Soils

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**Abstract:** Soils are a hotspot for the emergence and spread of antibiotic resistance. The effects of agrochemical treatments on the bacterial community of agricultural soils and the content of antibiotic-resistance genes (ARGs) were studied. Treatments included the following: control, mineral fertilizers (NPKs), pesticides, and the combined treatment of soils under soya (*Glycine max*), sunflower (*Helianthus annuus* L.), and wheat (*Triticum aestivum*). Bacterial community taxonomic composition was studied using 16S rRNA gene sequencing. The content of 10 ARGs and 3 integron genes (*intI1*, *intI2*, *intI3*) was determined using quantitative real-time PCR. The results showed that the treatments had little effect on the taxonomic composition and diversity of the soil bacterial community. The most significant factors determining differences in the microbial community were sampling time and soil physico-chemical parameters. A significant role of the bacterial community in ARG distribution in soils was demonstrated. Representatives of the *Pseudomonas*, *Bacillus*, *Sphingomonas*, *Arthrobacter* genera, and the *Nocardioidaceae* and *Micrococcaceae* families were likely ARG hosts. The presence of integron genes of all three classes was detected, the most numerous being *intI3*. This work provides important information on the role of agricultural soils in ARG transfer, and the findings may be useful for sustainable and safe agricultural development.

**Keywords:** agricultural soils; mineral fertilizers; pesticides; bacterial community; antibiotic-resistance genes



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## 1. Introduction

Agricultural soils play an essential role in food production. Unfortunately, intensive exploitation of such soils implies the widespread use of various chemical plant protection products (insecticides, herbicides, fungicides) and mineral fertilizers, which contribute to pollution and a decrease in soil quality. In addition to the traditionally considered soil pollutants (pesticides, heavy metals, PAHs), in recent years, more and more attention has been drawn to the study of the content of antibiotic-resistance genes (ARGs) which, in 2006, were recognized as a new class of pollutants [1]. Microbial resistance to antibiotics remains an unresolved problem, despite the efforts of the international community. It is gradually becoming clear that the emergence and spread of antibiotic resistance is a much more profound and complex phenomenon than just a consequence of uncontrolled antibiotic use, and natural ecosystems play a significant role here.

Soil serves as a habitat for a huge variety of bacteria, including antibiotic-resistant bacteria (ARB), and is a natural reservoir for many ARGs. Antibiotics serve as a tool

for communication and antagonism in bacteria, so the existence of antibiotic-resistance determinants in soil is quite logical. Different soil types have different microbial compositions. For agricultural soils, the predominance of the Actinobacteria, Proteobacteria, Acidobacteria, Verrucomicrobia, Bacteroidetes, and Firmicutes phyla has been shown [2]. Actinobacteria are widely known as producers of antibiotic compounds; it is reported that 64% of all antibiotics produced naturally come from actinomycete species [3]. The most common species of soil actinomycetes, Streptomyces, is the producer of 55% of antibiotics discovered between 1945 and 1978 [4]. Stress factors can affect the microbial community of soils, including producers of antibiotic substances and ARBs [5]. This can lead to ARGs from soil entering environmental strains through horizontal gene transfer, leading to the emergence of resistant clinical strains.

The issue of antibiotic resistance in agricultural soils has been studied quite well, but soil fertilization with manure or irrigation with wastewater is usually considered the main source of ARG contamination [6–11]. This is due to both the direct application of ARB and ARGs contained in manure and wastewater, and the proliferation of existing resistant bacteria due to the introduction of large amounts of organic matter to the soil.

However, much less attention has been paid to the influence of mineral fertilizers and pesticides widely used in traditional agriculture on the emergence and spread of antibiotic resistance. Insecticides (pyrethrins), fungicides (copper ammonium acetate), and herbicides (atrazine) have been shown to influence antibiotic resistance in *E. coli* [12]. The relative abundance of *intI1* and some ARB types in the wheat rhizosphere increased when chlorpyrifos was added to the soil [13]. Residues of the carbendazim, azoxystrobin, and chlorothalonil fungicides increased ARG content in greenhouse soils, especially *sul2*, *sul1*, *aadA*, *tet(L)*, *tetA(G)*, and *tetX2* [14]. Bacteria strains from contaminated agricultural soil that can degrade the monochrotophos insecticide (*Bacillus* sps, *Bacillus cereus*, *Bacillus firmus*, *Bacillus thuringiensis*) were found to be resistant to chloramphenyl, monochrotophos, ampicillin, cefotaxime, streptomycin, and tetracycline antibiotics [15].

Resistance to pesticides and antibiotics may be due to general strategies for protecting a microbial cell, for example, the formation of biofilms, increased expression of efflux pumps, and decreased synthesis of outer membrane porins [16]. Sublethal concentrations of pesticides can provoke oxidative stress and enhance mutagenesis in bacteria [17], which cause changes in antibacterial defense enzymes, among others [18]. Pesticides can also affect the soil bacterial community, reducing diversity and shaping a specific community of bacteria [19], including promoting ARG hosts. In this work, we tried to answer the question of whether mineral fertilizers and pesticide application affect the content of 10 clinically significant ARGs and genes of three classes of integrons in agricultural soils.

## 2. Materials and Methods

### *2.1. Study Area and Experimental Design*

The field experiment was carried out in 2022–2023 in the Rassvet village of the Rostov region, Russia (47°21′40″ N, 39°52′50″ E). The region has a temperate continental climate with an average annual rainfall of 530–550 mm, an average monthly temperature of from –5 °C to –9 °C in winter and from 22 °C to 24 °C in summer.

The soils are Calcic Chernozems (Loamic). Soy (*Glycine max*) and sunflower (*Helianthus annuus* L.) were grown in the experimental plots in 2022, and then, in 2023, wheat (*Triticum aestivum*) was grown after these predecessor plants.

Each plant was grown with four treatments—control (C), inorganic nitrogen–phosphorus–potassium fertilizer treatment (F), pesticide treatment (P), and combined fertilizer and pesticide treatment (FP). Detailed information on the introduced compounds is provided in Table A1 (Appendix A).

Each treatment was carried out in triplicate, the area of each plot was 20 × 12 m. Soil sampling was carried out twice—during the growing season before the application of pesticides and after their application at the end of the growing season at the time of harvest. Soil samples were taken from a depth of 0–20 cm by the envelope method [20,21],

and samples from three replicates were mixed into one composite sample. Soil samples were thoroughly mixed, placed into Falcon plastic tubes (50 mL), and stored at −20 °C. A list of soil samples is presented in Table A2 (Appendix A).

### *2.2. Analysis of Soil Physicochemical Properties*

Exchangeable ammonium was determined by extraction with potassium chloride and photoelectric colorimetry [22]. Nitrates were measured by the ionometric method [23]. Mobile compounds of phosphorus and potassium were extracted by ammonium carbonate solution and analyzed using a photoelectric colorimeter according to the Machigin method in modification [24]. The soil organic matter (SOM) in the soils was determined by the dichromate oxidation method using a photoelectric colorimeter [25]. The soil pH was measured in a 1:5 soil–water suspension using a pH meter, and the solid residue of the water extract was measured using a conductometer according to state instructional guidelines [26].

### *2.3. Total DNA Isolation and Metagenomic Analysis of 16S rRNA Genes*

The 16S rRNA sequencing method was used to study soil microorganisms. Total DNA was isolated from soil samples using the innuSPEED Soil DNA Kit (Analytik Jena, Jena, Germany) according to the manufacturer's instructions. A number of 16S rRNA sequencing libraries were constructed according to the 16S metagenomic sequencing library preparation protocol (16S metagenomic sequencing library preparation).

Amplification of the V3–V4 region of 16S rRNA was performed using prokaryotic primers: direct -TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG; reverse -GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC, followed by amplicon indexing. The sequences were analyzed by next-generation sequencing using the MiSeq system (Illumina, San Diego, CA, USA) in the 2 × 300 bp mode. The readings were processed and analyzed using the QIIME software version 1.9.1 (http://qiime.org/ (accessed on 10 October 2023)) [27]. After qualitative filtering, chimera removal, and sparsing steps, the sequences were grouped into operational taxonomic units (OTUs) with a sequence similarity threshold of 97%. The latest version of the GreenGenes 13.8 database was used [28].

### *2.4. Quantitative Measurement of Antibiotic Resistance Genes in the Studied Soils*

Isolation of total DNA from soils of plots with different treatments was carried out using the FastDNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. The content of genes of resistance to carbapenems (*bla*VIM-1), cephalosporins (*bla*CTX-M and *mecA*), glycopeptides (*vanA* and *vanB*), tetracyclines (*tetO*), macrolides (*ermB* and *mphA*), sulfonamides (*sul*2), aminoglycosides (*aadA*2), was measured, as well as the content of integron genes—*intI1*, *intI2*, *intI3*.

Quantitative PCR was carried out in a volume of 25 µL containing 1× reaction mixture with EVA Green dye (Synthol, Moscow, Russia), 0.2 µM of each primer, and 20 ng of template DNA. The primer sets and PCR conditions used in this study are shown in Table A3 (Appendix A). The specificity of the product was checked using melting curves, activity, $r^2$ values, and gel electrophoresis. Calibration curves for the quantification of target ARGs were plotted using tenfold dilutions of the plasmid containing the target genes ($10^2$–$10^8$ copies). Plasmid DNA standards were constructed by cloning based on the commercial vector pAL2-TA (Evrogen, Moscow, Russia) in accordance with the manufacturer's instructions. The concentration of plasmid DNA was determined using a Qubit 3.0 fluorimeter (Thermo Fisher Scientific, Waltham, MA, USA). The $R^2$ values of standard curves for 16S rRNA and ARGs were greater than 0.99, and amplification efficiencies ranged from 90% to 110%. To minimize the differences arising from analytical efficiency, differential extraction, and variations in background local amounts of bacterial genes, in this work, ARG abundance was expressed as a ratio of ARG abundance to 16S rRNA gene content.

### 2.5. Statistical Analysis

The Shannon index and Chao 1 index were calculated to estimate microbial community alpha diversity. One-way ANOVA and Tukey's HSD (Honestly Significant Difference) test were used to analyze the differences among samples. R (V 4.1.0) performing biomarker features using LEfSe [29], principal component analysis (PCA), and principal coordinate analysis (PCoA) were conducted to reveal the relationship between biomarker taxa and soil properties. The Spearman correlation matrix was selected to determine the correlation between ARGs and *intI1*, *intI2*, and *intI3*, between ARGs and soil properties, as well as the correlation between microbial taxa and *intI1*, *intI2*, and *intI3*. Statistical significance between the tested groups was assessed using the Kruskal–Wallis test. Beta diversity was assessed using the Bray–Curtis dissimilarity index, weighted-UniFrac, and unweighted-UniFrac metrics, and tested for statistical significance using the PERMANOVA test. The kruskal.test function from the stats R package was used to test for significant differences in transformed genera abundances between different plants and treatments. *p* values were corrected for multiple testing using the Benjamini–Hochberg (BH) FDR method implemented in the *p*.adjust function from the stats package. Figures were visualized using R (V 4.1.0).

## 3. Results

### 3.1. Soil Physicochemical Properties

The basic physicochemical properties of soils under different treatment conditions are shown in Table A4 (Appendix A).

### 3.2. Taxonomic Composition and Diversity of the Agricultural Soils Microbiome

High-throughput sequencing was used to determine the diversity and composition of the microbial community in soils with soya, sunflower, and wheat crops. After selection and filtration, an average of 4446 OTU was obtained per sample. At the phylum level, the soil bacterial community structure was similar in all studied samples (Figure 1). The dominant phyla were Actinobacteria (36.2–38.4%), Proteobacteria (18.3–23.3%), Planctomycetes (5.4–15.8%), Bacteroidetes (3.5–4.8%), Chloroflexi (5.4–6.5%), Acidobacteria (5.7–8.8%), Verrucomicrobia (2.7–4.4%), and Gemmatimonadetes (2.2–5.5%). In the soil containing soya, Cyanobacteria (2%) were also among the predominant phyla, the abundance of which in other soils was insignificant.

Common families for all studied soils were the following: actinobacteria *Gaiellaceae* (5.7–6.6%), *Geodermatophilaceae* 2.4–4.2%, *Micrococcaceae* 1.3–1.9%; *Micromonosporaceae* 1.4–1.6%, *Nocardioidaceae* 2.0–2.3%, *Rubrobacteriaceae* 1.6–3.7%, *Solirubrobacteraceae* 1.2–2.0%, *Chitinophagaceae* (Bacteroides) (1.6–2.8%), *Pirellulaceae* (Planctomycetes) (0.6–2.9%), proteobacteria *Bradyrhizobiaceae* (2.0–2.2%), *Hyphomicrobiaceae* (1.4–1.7%), *Rhodospirillaceae* (1.2–1.5%), *Sphingomonadaceae* (2.3–2.9%), and *Chthoniobacteraceae* (Verrucomicrobia) (1.1–2.0%) (Figure 2).

At the genus level, *Rubrobacter* (1.4–3.3%), *Bacillus* (0.6–1%), *Rhodoplanes* (1–1.2%), *Kaistobacter* (1.0–1.4%) predominated in the soil under all plants. Under soya and sunflower, the representation of the *Gemmata* genera (1.1–1.2%) was higher than under wheat (0.2–0.3%) (Figure 3).

In general, agrochemical treatments had little effect on changes in the abundance of individual bacterial taxa. Usage of mineral fertilizers for certain soils ($p < 0.05$) led to a decrease in the number of bacteria of the Firmicutes, OD1 phyla, the actinobacteria families *Gaiellaceae*, *Intrasporangiaceae*, and *Propionibacteriaceae*, and to an abundant increase in the *Sporichthyaceae* family. Pesticide application credibly ($p < 0.05$) reduced the abundance of the BRC1, Chlorobi, and Firmicutes phyla, and the *Gaiellaceae* family, and increased the abundance of the *Sphingomonadales* order, *Gemmataceae*, and *Burkholderiaceae* families. The combined treatment significantly ($p < 0.05$) influenced only some taxa at the genus level: a decrease in the abundance of the genera *Geodermatophilus*, *Nocardioides*, and *Nitrospira*, and an increase in the abundance of the *Solirubrobacter* and *Myxococcus* genera were observed (Figure 3).

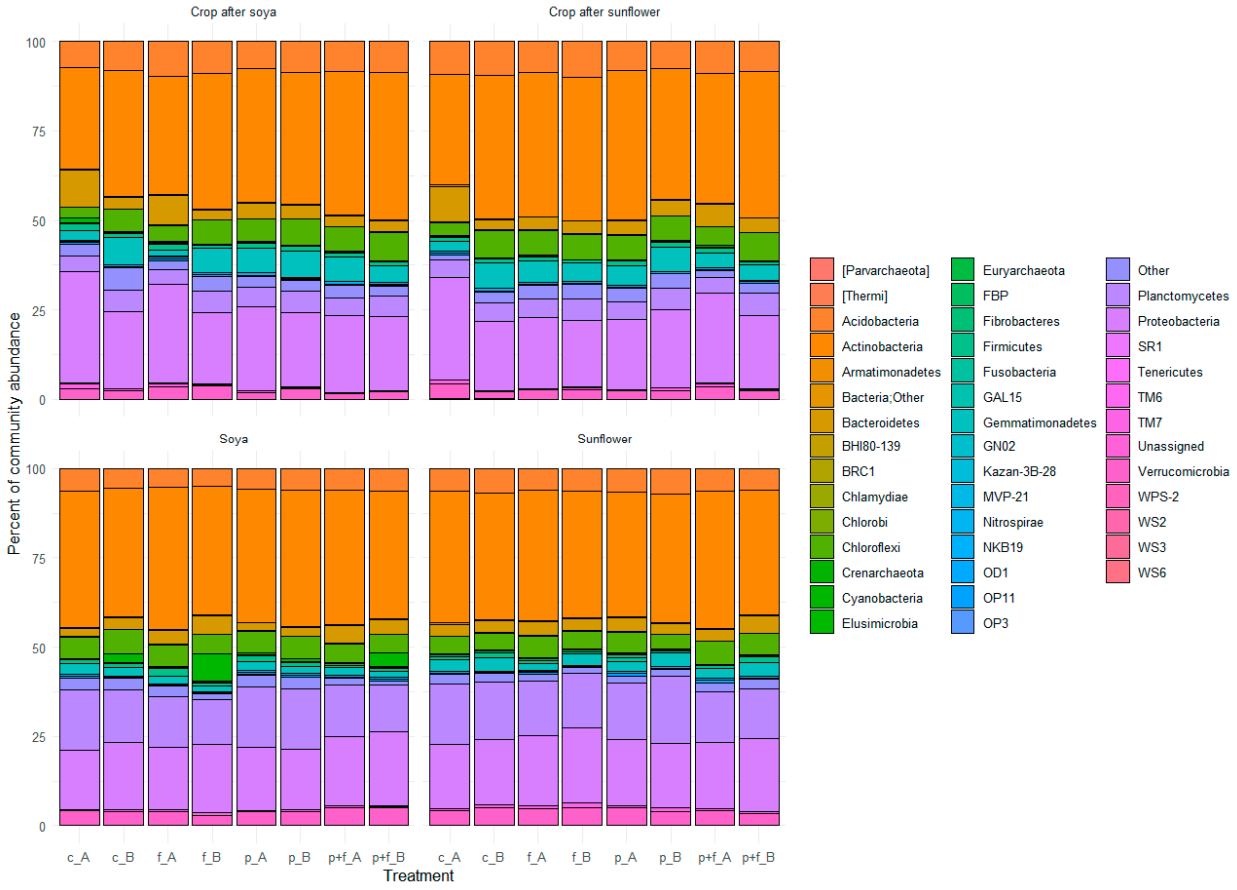


**Figure 1.** Taxonomic composition of agricultural soils microbiome under soya, sunflower, and wheat crops at the phylum level.

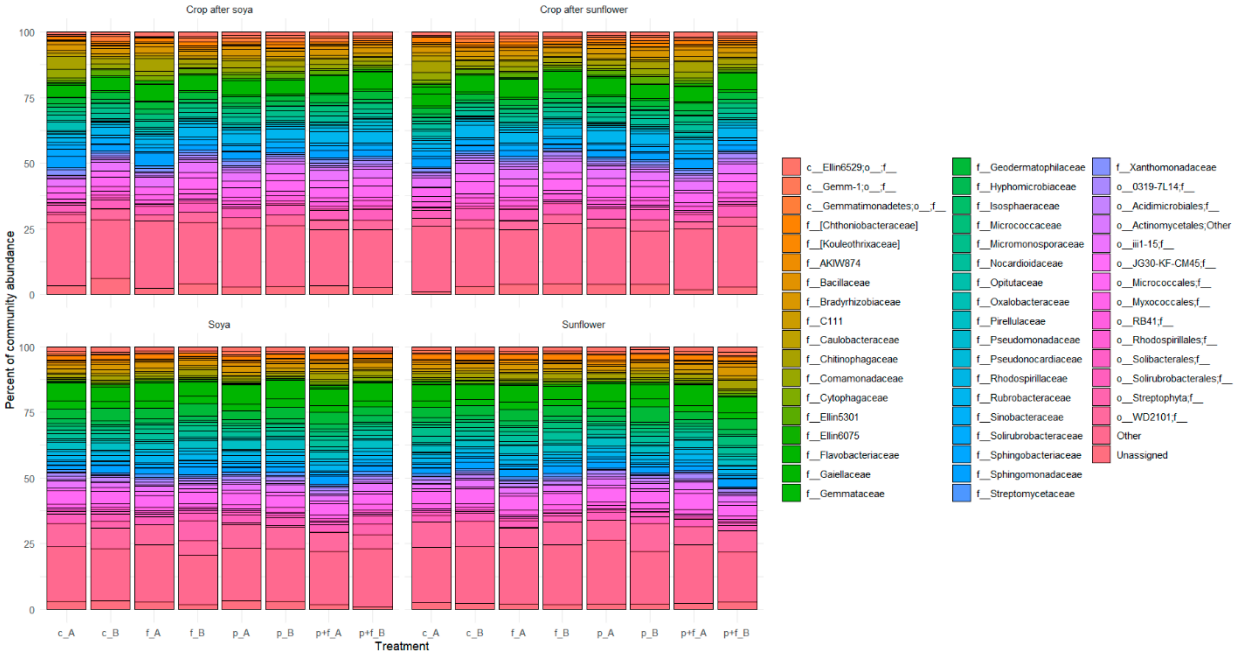


**Figure 2.** Taxonomic composition of agricultural soils microbiome under soya, sunflower, and wheat crops at the family level.

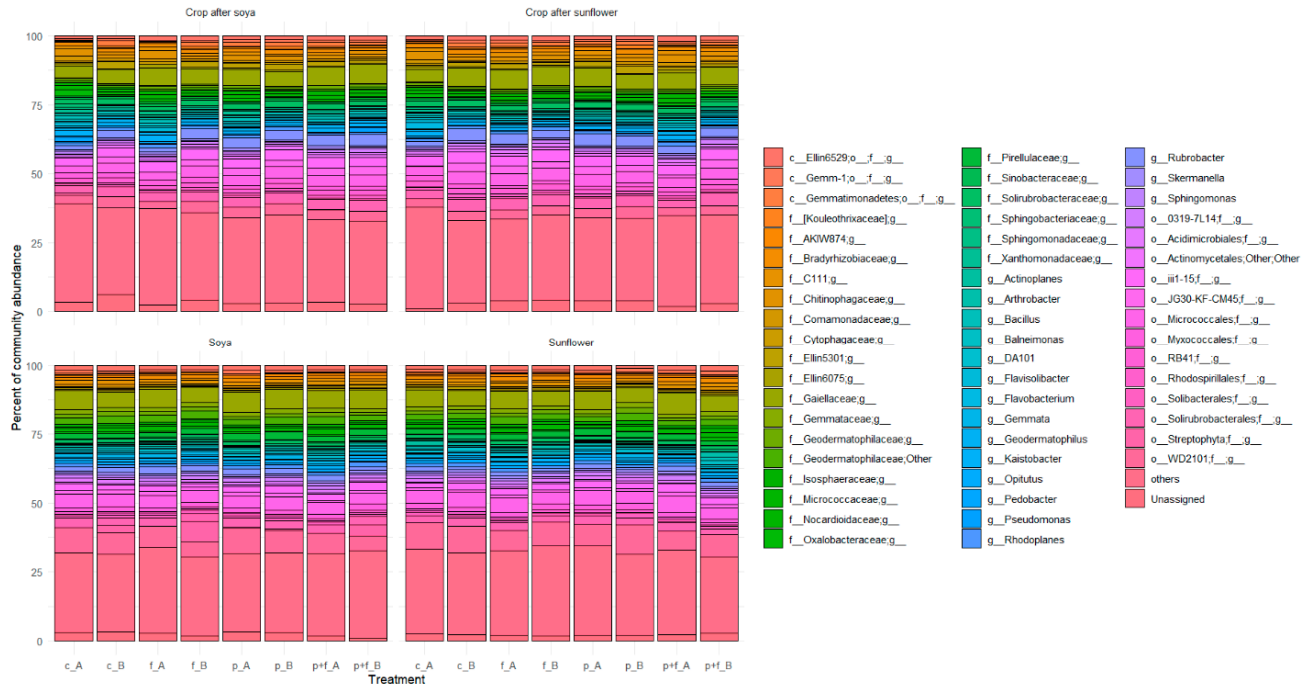


**Figure 3.** Taxonomic composition of agricultural soils microbiome under soya, sunflower, and wheat crops at the genus level.

Agricultural treatments had little effect on the α-biodiversity of the microbial community in agricultural soils (Figure 4). The Chao1, Shannon, and Simpson indices under wheat crops were, on average, higher than those under soya and sunflower crops (Table A5, Appendix A).

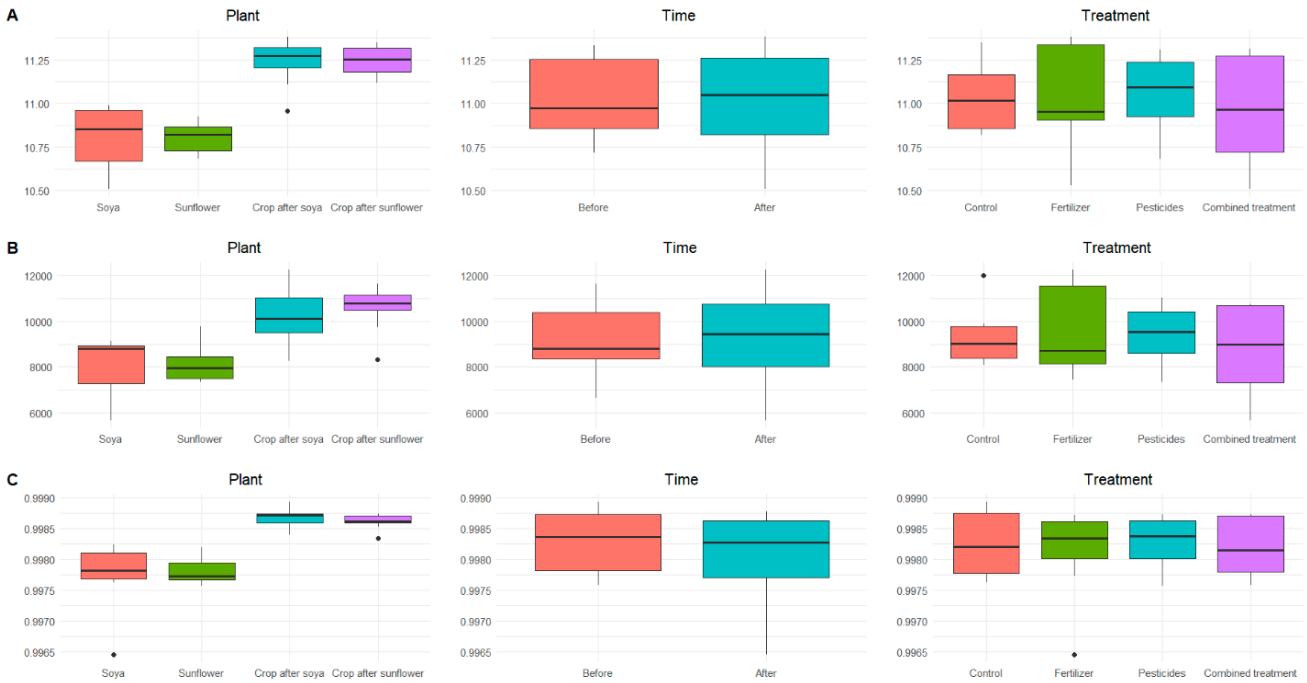


**Figure 4.** α-biodiversity of bacterial communities in soils. (**A**) Shannon index of soil bacterial communities; (**B**) Chao1 index of soil bacterial communities; (**C**) Simpson index of soil bacterial communities. No significant differences were observed for the group comparisons based on the Mann–Whitney U Test and Kruskal–Wallis ANOVA.

Sampling time was a significant factor influencing the taxonomic composition of agricultural soils, as confirmed by principal coordinate analysis (PCoA) (Figure 5). The samples are clearly grouped into three clusters, the first of which includes soils under soya and sunflower, and the second, soils under wheat. Interestingly, there is a third cluster that unites soils selected under wheat crops before the start of agrochemical treatments, after both predecessor plants (including control treatments). This indicates the influence of the previously cultivated crop as well.

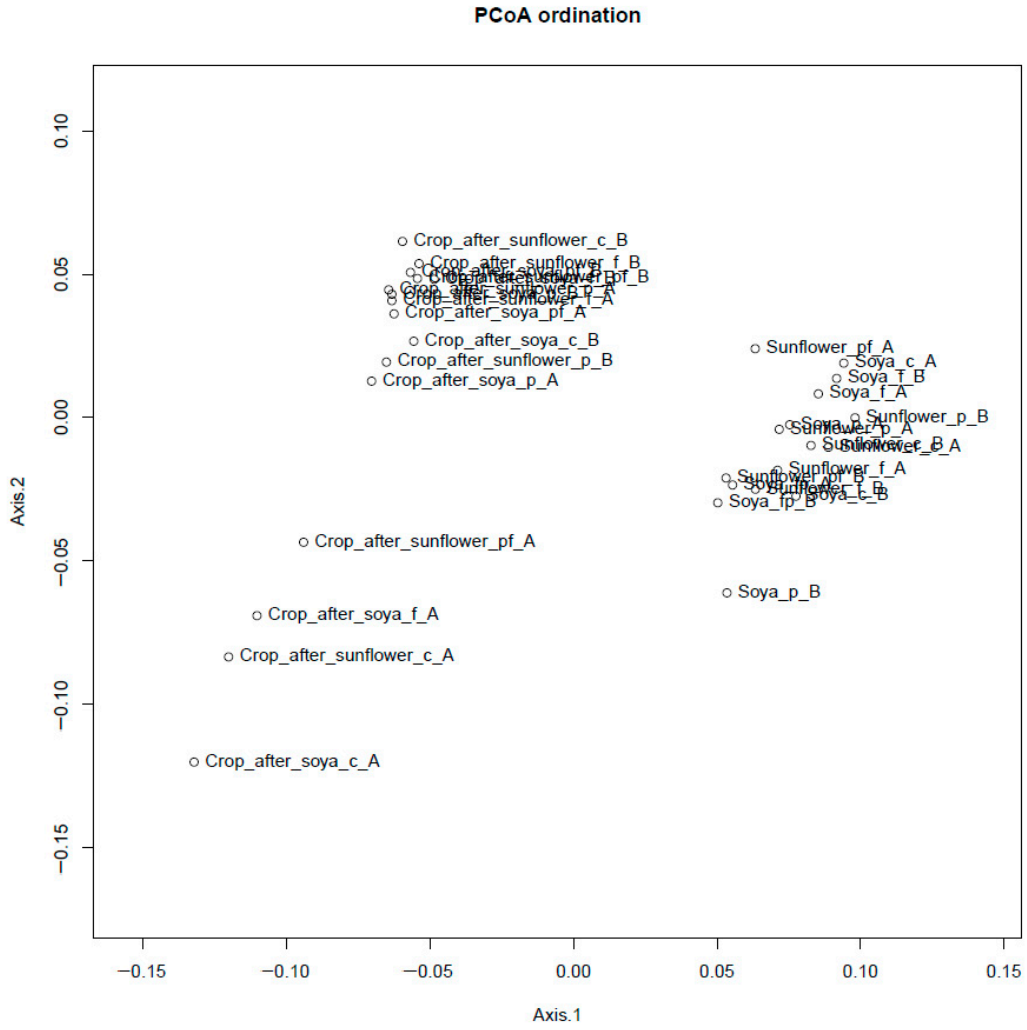


**Figure 5.** Principal coordinates analysis of the soil bacterial community based on Bray–Curtis dissimilarity (soil under soya crops (Soya), sunflower (Sun), wheat grown after soya (C(soya)), wheat grown after sunflower C(sun). C—control; f—fertilizers; p—pesticides, pf—pesticides + fertilizers. A—sampling before pesticide application, B—sampling after pesticide application).

The bacterial taxa making the greatest contribution to the differences between samples are presented in Figure 6. These include representatives of the phyla Planctomycetes, Acidobacteria, Proteobacteria, Gemmatimonadetes, and Actinobacteria. Grouping by the year of sampling is also visible here, which indicates a significant influence of climatic factors on the taxonomic composition of soils.

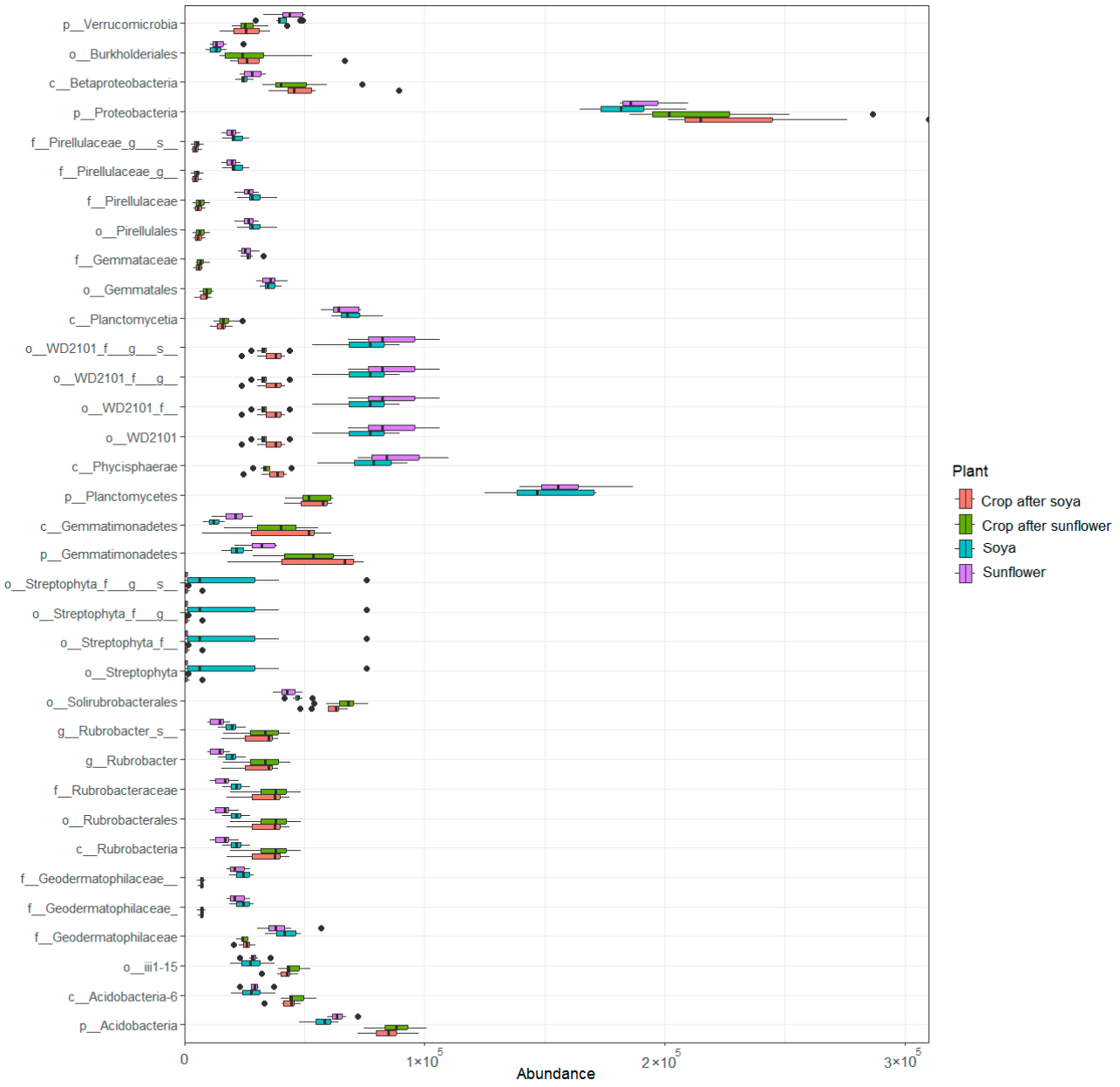


**Figure 6.** Bacterial taxa contributing the most to differences between soil samples, identified by the linear discriminant analysis effect size (LDA) (LEfSe) method. The figure shows only the taxa, the abundance of which differs statistically significantly ($p < 0.05$) between treatments.

The principal component method (PCA) revealed the interconnection between soil conditions and the structure of microbial communities (Figure 7). Two principal components (PCs) which describe 67.8% of the original data were identified. PC1 is the most significant as it accounts for 56.9% of the total variance. On PC1, Verrucomicrobia, Planctomycetes, *Rubrobacteriaceae*, *Pirellulaceae*, *Planctomycetia*, *Gemmatales*, and *Phycisphaerae* have strong positive loads; *Gemmataceae*, *Geodermatophilaceae*, Acidobacteria, *Solirubrobacteriales*, *Rubrobacteriales*, and *Gemmatimonadetes* have negative loads.

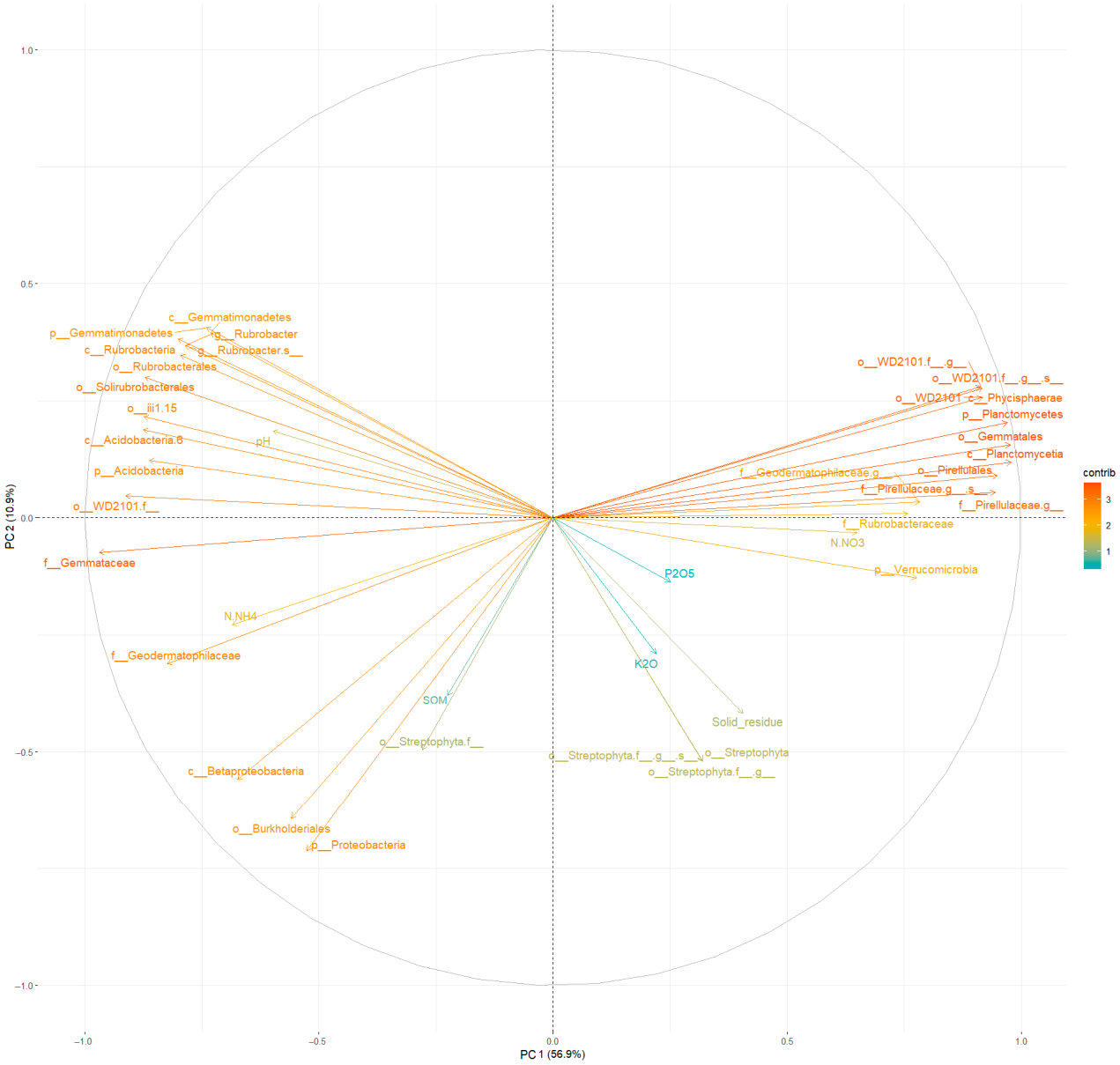


**Figure 7.** Factor loading plots of the abundance of bacterial phyla and the soil physicochemical properties of the main principal components. The length of the arrows approximates the variance of the variables, whereas the angles among them estimate their correlations.

Basic physicochemical soil properties were used as additional variables. Nitrates have a high positive correlation with PC1 (and with Verrucomicrobia, *Rubrobacteriaceae*, *Pirellulaceae*, *Planctomycetia*, *Gemmatales*, Planctomycetes, *Phycisphaerae*). Ammonium and pH have a negative relationship with PC1 and with *Gemmataceae*, *Geodermatophilaceae*, *Acidobacteria*, *Solirubrobacteriales*, *Rubrobacteriales*, and *Gemmatimonadetes*.

### *3.3. Content of ARGs and Integrons in Agricultural Soils*

Genes such as *bla*VIM, *vanB*, *tetO*, *ermB*, *sul2*, *aadA*2, and *vanA* were found in varying quantities in 100% of the studied soil samples, including control plots. Macrolide resistance genes *ermB* were found in the highest concentration under wheat crops, where their average amount was three orders of magnitude higher than under soya or sunflower (Figure 8).

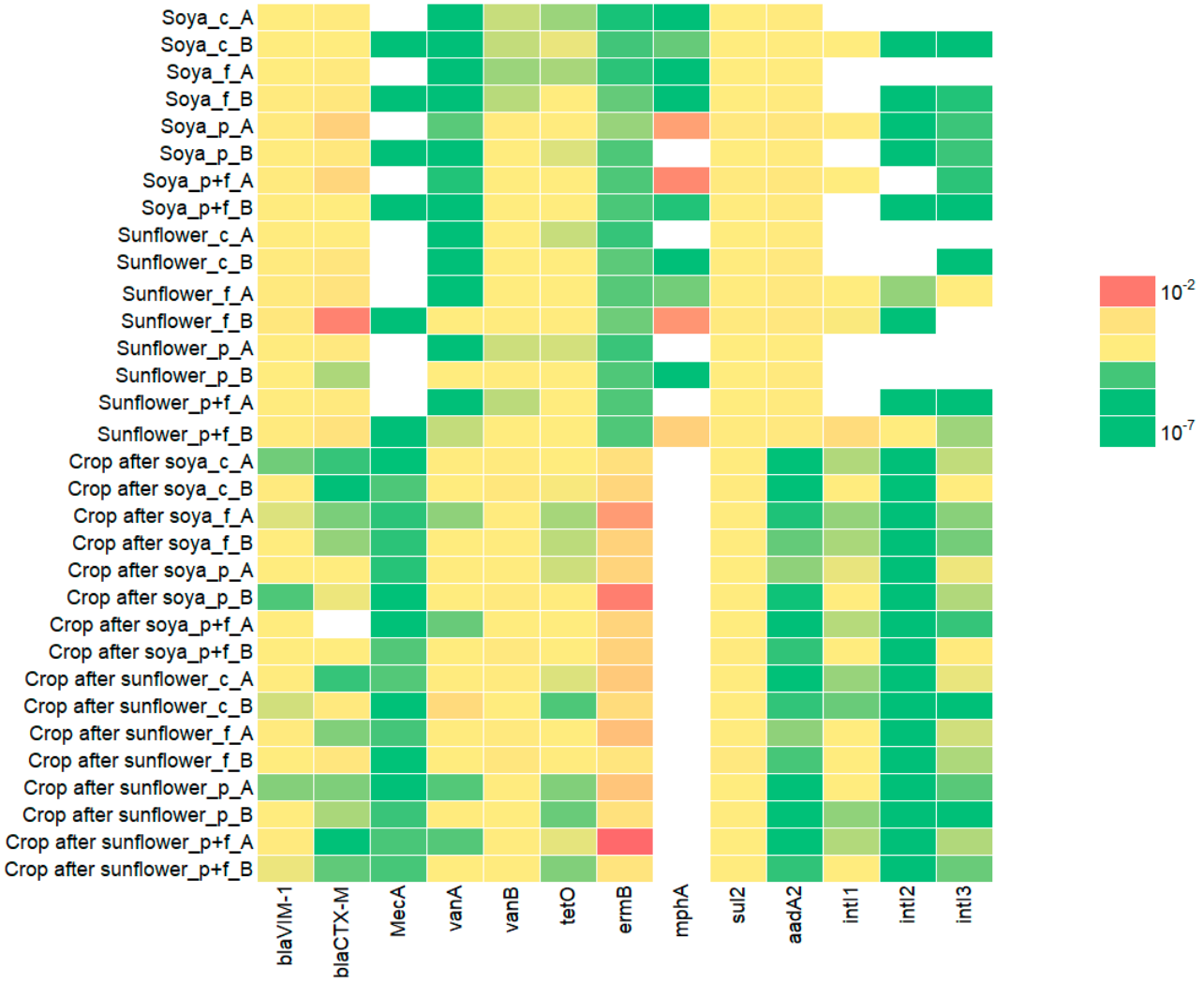


**Figure 8.** Content of ARGs and integrons in the studied agricultural soils (copies/copies 16S rRNA).

At the same time, another macrolide resistance gene, *mphA*, was found only in 31% of samples, mainly in soils under soya and sunflower crops, where its average concentration was also quite high ($1.51 \times 10^{-2}$–$8.01 \times 10^{-3}$ copies/copies 16S rRNA). The *mecA* gene in soya and sunflower was detected only in the samples taken after pesticide application (at the end of the plant growing season). With further cultivation of wheat, the *mecA* gene was found in every soil sample, at a concentration of 1–2 orders of magnitude higher.

All three classes of integrons were found in all soil samples under wheat in average concentrations of $2.24 \times 10^{-4}$–$2.41 \times 10^{-4}$ for *intI1*, $2.68 \times 10^{-6}$–$2.88 \times 10^{-6}$ for *intI2*, and $1.02 \times 10^{-4}$–$1.64 \times 10^{-4}$ for *intI3*. The *intI1* genes were least often found in soils under soya and sunflower, but their maximum concentration was found in the soil under sunflower crops after combined treatment ($5.85 \times 10^{-3}$). The *intI2* was detected more often but in low concentrations. The most common integron class in agricultural soils was *intI3*, occurring in 81% of samples. There was no statistically significant effect of agrochemical treatments on changes in the relative amount of ARGs in the soil (*t*-test).

Only the content of the tetracycline resistance gene, *tetO*, was significantly affected by the application of mineral fertilizers to agricultural crops ($p < 0.05$). There was no statistically significant effect of agrochemical treatments on changes in the relative amount of ARGs in the soil (t-criterion).

### *3.4. Correlation Analysis of ARGs Content and Taxonomic Composition*

To identify microorganisms that are potential hosts of ARGs in the soil, Spearman correlations were analyzed between the relative abundance of dominant taxa and the amount of ARGs and integrons in the soil under soya, sunflower, and wheat crops.

In the soil under soya, the most significant role as a host and carrier of ARGs was played by gamma-proteobacteria of the *Pseudomonas* genus (Figure A1 (Appendix A)). A close positive correlation ($r \geq 0.7$) was found between the abundance of the *Pseudomonas* genus and the content of 6 resistance genes out of 10 studied (*vanA*, *vanB*, *tetO*, *ermB*, *sul2*, *aadA*2), as well as integrons *intI1* and *intI2*. A moderately strong relationship is shown between the abundance of this genus and the abundance of the genes *bla*VIM-1 (r = 0.69), *bla*CTX-M (r = 0.68), and *mphA* (r = 0.57). Another important genus was *Bacillus*, the abundance of which was closely ($r \geq 0.7$) related to the abundance of *vanA*, *vanB*, and *ermB*, and the *intI1* and *intI2* integrons; a relationship of medium strength is shown with the amounts of *bla*VIM-1 (r = 0.61), *tetO* (r = 0.55), *sul2* (r = 0.57), *aadA*2 (r = 0.62), and *bla*CTX-M (r = 0.61).

A larger number of taxa, the abundance of which correlated with ARGs, were found in the soil under sunflower (Figure A2 (Appendix A)). *Sphingomonas* alpha-proteobacteria was a crucial genus, the abundance of which significantly correlated with all ARGs studied in this work (except for integrons). Close correlation ($r \geq 0.7$) is shown with the genes *bla*VIM-1, *vanB*, *ermB*, *sul2*, *aadA*2, and *bla*CTX-M; moderate correlation with the genes *mecA* (r = 0.62), *tetO* (r = 0.55), *mphA* (r = 0.67), and *vanA* (r = 0.64). The number of *Arthrobacter* genus bacteria closely correlated with the content of integrons *intI1* and *intI2*, *vanB*, *tetO*, *sul2*, and *aadA*2; less closely with *bla*VIM-1 (r = 0.55), *mecA* (r = 0.68), *ermB* (r = 0.51), and *mphA* (r = 0.56). Other probable hosts and carriers of ARGs were actinobacteria of the *Nocardioidaceae* and *Micrococcaceae* families, the abundance of which was significantly related to the abundance of all ARGs, including *intI1* and *intI2*. Taxa, the abundance of which closely correlated only with integrons *intI1* and *intI2,* were found—the *Flavisolibacter*, *Pedobacter* (Bacteroidetes), *Bacillus* (Firmicutes), *Balneimonas*, *Kaistobacter* (Proteobacteria) genera, and the *Bradyrhizobiaceae* and *Oxalobacteraceae* (Proteobacteria) families.

In soil under wheat grown after soya, the abundance of actinobacteria of the *Actinoplanes* genus and planctomycetes of the *Gemmata* genus was associated with the genes *vanB* and *sul2*, and all three integrons (Figure A3 (Appendix A)). In addition, the abundance of proteobacteria of the *Rhodoplanes* genus closely correlated with the content of the *ermB* gene (r = 0.75), and the abundance of actinobacteria *Geodermatophilus* with the content of *bla*VIM-1 (r = 0.76).

In the soil under wheat grown after sunflower, a close relationship ($r \geq 0.7$) was shown between the content of the macrolide resistance gene *ermB* and a large number of taxa, including the *Balneimonas*, *Rhodoplanes*, *Skermanella*, *Kaistobacter*, and *Pseudomonas* proteobacteria genera, and the *Micrococcaceae* and *Nocardioidaceae* actinobacteria families (Figure A4 (Appendix A)). The abundance of *Acidimicrobiales* acidobacteria closely correlated with the *intI1* integrons, the *Chitinophagaceae* and *Flavobacteriaceae* bacteroids with the *intI2* integrons, and *Sphingomonadacea*e and *Oxalobacteraceae* proteobacteria with *intI3* integrons. In general, a larger number (compared to other plants) of taxa associated with integron genes of classes 2 and 3 is shown.

### *3.5. Correlation between ARGs and Integrons in Agricultural Soils*

To investigate the potential for horizontal transfer of ARGs in soils under the three plants, Spearman correlations were calculated between the relative contents of *intI1*, *intI2*, *intI3,* and ARGs.

In soil under soya, the relative content of all studied resistance genes (except *mecA*) reliably correlated with the content of *intI1*, *intI2*, and *intI3* (Figure A5 (Appendix A)). A large number of close correlations between different ARGs were also found. Almost no connection between ARGs and integrons was found in the soil under sunflower (Figure A6 (Appendix A)). Only for tetracycline resistance, a correlation with the *intI1* (r = 0.62) and

*intI2* (r = 0.59) integron genes was shown. At the same time, all integrons, as well as various antibiotic-resistance genes, correlated with each other significantly.

In soil under wheat grown after soya, integron genes significantly correlated with each other. The content of ARGs, such as *vanB*, *bla*CTX-M, and *vanA,* correlated with the relative content of integrons of all three classes; *aadA*2 also correlated with *intI1* and *intI2* (Figure A7 (Appendix A)).

In soil under wheat grown after sunflower, the content of the resistance genes *mecA* (r = 0.73) and *vanA* (r = 0.76) correlated with *intI1* integrons; *vanA* (r = 0.58) with integrons *intI2*; *bla*VIM-1 (r = 0.95), *vanB* (r = 0.87), and *aadA*2 (r = 0.82) with *intI3* integrons (Figure A8 (Appendix A)).

## 4. Discussion

### *4.1. Effect of Agrochemical Treatments on the Bacterial Community of Agricultural Soils and Their Resistome*

Our study showed that physicochemical soil properties, such as ammonium ($NH_4^+$) and nitrate ($NO_3^-$) nitrogen amount, and pH, contribute to the formation of agricultural soils' taxonomic composition. Nitrate nitrogen and pH influence dominated all chemical factors. The abundance of bacteria of the *Rubrobacteraceae* family, which are known to inhabit arid soils [30], the Verrucomicrobia and Planctomycetes phyla, and planctomycetes of the *Pirellulales* and *Gemmatales* orders, was associated with the content of nitrate nitrogen. Planctomycetes are well known for their ability to perform anaerobic ammonium oxidation (the so-called anammox process, at which ammonium is oxidized and nitrite is consumed) [31], and are important participants in the soil nitrogen cycle. Soil properties influence both taxonomic composition and the content and behavior of ARGs. The soils studied in this work belong to chernozems, rich in organic matter and clay particles, with a generally neutral pH value. Actinobacteria from the *Gaiellaceae* family and alphaproteobacteria from the *Sphingomonadaceae* family (in particular, the *Kaistobacter* genus) predominant in the studied soils are characteristic inhabitants of chernozem soils [32].

It has been noted that clay minerals and soil organic matter can provide an increase in specific surface area, as well as essential nutrients for bacteria, which can further lead to the increased horizontal transfer of ARGs [33]. Lu et al. found that ARG content in three soil types increased in response to the same agronomical practices in the following order: clay > loam > sandy [34]. In this study, the taxonomic composition of soils under different plants significantly differed between the two sampling years. Seasonal and interannual variations in temperature, humidity, and other parameters can significantly influence microbial community composition and antibiotic-resistance profiles [35].

In general, we did not find a direct effect of agrochemical treatments on changes in the taxonomic composition of soil bacteriocenosis. However, individual taxa were found, the amounts of which significantly changed when agrochemicals were added. Thus, the abundance of bacteria of the *Sphingomonadales* order and the *Gemmataceae* and *Burkholderiaceae* families increased with pesticide application. A number of studies have shown that *Xanthomonadales*, *Sphingomonadales*, and *Pseudomonadales* act as destructors of chlorinated pesticides [36], and bacteria of the *Burkholderiales*, *Rhizobiales*, and *Acidobacteriales* orders are abundant in agricultural soils contaminated with chlorpyrifos [37]. In previous work [38], the relative abundance of sphingomonads increased following the combined application of fertilizers and pesticides (by 152% in peas and by 139% in chickpeas), but decreased following the separate application of inorganic fertilizers. A low content of bacterial taxa containing plant pathogens was noted. The main bacteria affecting soybean, sunflower, and wheat are *Pseudomonas syringae*, *Ps. solanacearum*, *Xanthomonas campestris*, and *Erwinia carotovora* [39,40]. In the studied soils, the content of the Xanthomonadaceae family was close to 1% in soils under wheat, and under soybean and sunflower bacteria of this family were practically absent; their numbers did not change significantly in response to the treatments. The content of *Pseudomonadaceae* was also low (0.11% in the soil under soybean, 0.02% in the soil under sunflower, 0.3% under wheat after soybean, and 0.06% under wheat after

sunflower); other pathogenic microorganisms (*Erwinia*, *Ralstonia*, etc.) were not detected. In general, bacterial diseases were not typical for plants in the study areas, in contrast to damage from fungal pathogens, insects, and weeds, which led to the use of fungicides, insecticides, and herbicides, but not bactericides.

The present study showed that the application of mineral fertilizers by itself did not affect the change in the relative amount of ARGs in agricultural soils. This is confirmed in the works of other researchers. For example, the application of mineral fertilizers (NPKs) in the paddy–upland crop rotation system had little or no effect on soil ARG content compared to the no-fertilizer control [41]. Han et al. [42] found that there was no significant enrichment of ARGs in rice soil both after 8 years of chemical fertilizer application and after short-term application. A 15-year field trial found that chemical fertilizers only affected ARG diversity moderately and did not have a significant effect on soil ARG levels [43]. Xie et al. found that NPK treatment reduced soil pH and caused significant changes in bacterial communities but had only a moderate effect on ARG diversity and abundance [44].

Additionally, our work did not show a direct effect of pesticides on changes in the amount of ARGs, although other studies show an increase in the content of some ARGs in response to the application of pesticides [45]. Nevertheless, the indirect effect of pesticides can be seen in the example of an increase in the abundance of the *Sphingomonadales* taxon, which is also the bacterial host of a number of ARGs. A total of 7 out of the 10 ARGs studied in this work were present in 100% of soil samples (including control ones) in varying quantities, forming a constant soil resistome that does not directly depend on agrochemical influences. The presence of permanent resistomes has been described for many habitats, including those not subject to anthropogenic impact. Thus, in intact forest soils, Willms et al. [46] found three sulfonamide resistance genes differing in taxonomic origin. Agricultural soils, tea plantations, and forest lands contained 68.2% of the total ARG subtypes, with forest soils showing a high abundance and quantity of ARGs [47]. Song et al. [48] discovered 30 ARGs causing resistance to modern antibiotics in virgin forest soils in China, and from 70 to 97 common ARGs were found between farmland and forest soils [49].

### *4.2. Relationship between Soil Bacterial Community and ARGs*

In this study, it was found that the main supposed bacterial hosts of ARGs were the proteobacteria genera *Pseudomonas*, *Sphingomonas*, and *Rhodoplanes*, and the actinobacteria *Arthrobacter*, *Nocardioidaceae*, *Micrococcaceae*, and *Actinoplanes*, the abundance of which correlated significantly with the content of several ARGs. Actinobacteria are well known as a group of bacteria that produce antibiotics or their derivatives [50]; accordingly, they also possess determinants of resistance to many antibiotics. Proteobacteria and Actinobacteria are among the most commonly predicted hosts of multidrug-resistant ARGs in soil metagenomic studies [51]. A strong correlation between bacterial taxa and ARG abundance during the anaerobic digestion of dairy manure was mentioned by Sun et al. [52], where the bacterial phyla Proteobacteria, Actinobacteria, and Bacteroides were the most widely known bacterial hosts that had a strong correlation with ARGs. Additionally, Proteobacteria, Actinobacteria, and Bacteroidetes have been found to increase their abundance in inorganically fertilized soils [53], and Proteobacteria carry broad host range plasmids that promote HGT in agricultural fields [54]. The role of actinobacteria in the spread of ARGs was also confirmed in the experiment of Lin et al. [55]. When treating soils fertilized with pig manure with fermentation broth, they observed a decrease in the number of ARGs due to actinobacteria inhibition (in particular *Nocardioides*). At the same time, Zhang et al. [14] showed that Actinobacteria were the main hosts of ARGs in mountain soils, while, in greenhouse soils, *Enterobacteriaceae* played the main role in the transfer of ARGs.

Many studies indicate that microbial phylogenetic and taxonomic structure is an important determinant of ARG composition [56,57]. Hu et al. [58] showed that ARG composition is closely correlated with the taxonomic structure of bacteria and herbs. Analyses of ARG distribution in the soil, rhizosphere, roots, leaves, and legumes of crops (tomato,

lettuce, beans) showed that microbiome composition and crop type were the main factors determining ARG distribution [59]. However, using the example of arable soils in China, Du et al. [60] noted a minor role of microbial phylogeny in soil resistome formation; the ratio of crop area, total crop yield, plastic film consumption, and pesticide usage had a greater influence.

In our work, we did not find a direct effect of agrochemical treatments on changes in the bacterial community taxonomic composition of soils under different plants; the sampling time and the physicochemical properties of soil made a greater contribution to diversity. At the same time, a large number of bacterial taxa were discovered, the abundance of which was associated with various ARGs, which indicates a significant role of soil bacteriocenosis taxonomic structure in the formation of soil resistome composition.

### *4.3. The Importance of Horizontal Gene Transfer (HGT) in Formation of Agricultural Soil Resistomes*

Horizontal gene transfer is an important mechanism for the spread of ARGs in the environment using mobile genetic elements (integrons, plasmids). In this study, *intI1* class 1 integron genes were detected less frequently compared to class 2 and 3 integron genes. Interestingly, when growing soya and sunflower, *intI1* genes were detected rarely and unsystematically, but, with further cultivation of wheat on these soils, they were present in all treatments, including the control ones. Usually, *intI1* integrons are considered an indicator of anthropogenic pollution, because they are associated with genes providing resistance to antibiotics, disinfectants, and heavy metals, and are found in a wide range of pathogenic and non-pathogenic bacteria [61]. The number of studies of class 3 integrons is still limited compared to *intI1* and *intI2*, but it is known, for example, that their number decreases when manure is added to soil (and *intI1* and *intI2* increased) [62]. A significant role of *intI2* in ARG transport and distribution has been shown in soils contaminated with Zn and OTC drugs [63].

In soil under soya, the content of almost all ARGs was associated with integrons of all three classes, which indicates a significant role of HGT in the resistome formation of these soils. It is interesting that in soil under sunflower, there was a large number of bacterial taxa, the abundance of which correlated with ARGs (and separately with integrons), but there was practically no connection between ARGs and integrons, that is, the role of HGT in ARGs distribution was minimal in these soils.

Significant participation of integrons (especially class 3 *intI3*) in ARG distribution under wheat crops was shown, where their content was associated with the content of several ARGs at once. Increased HGT was also described for the chlorpyrifos-contaminated wheat rhizosphere, where the relative abundance of *intI1* and several types of ARG-bearing bacteria (*Bacillus* and *Pseudomonas*) increased [55]. It should be noted that *Triticum aestivum* L. wheat can enrich more ARGs than other common crops during the early growth stage [64]. Thus, this study demonstrated that HGT is involved in soil resistome formation in agricultural soils (especially under soya and wheat crops).

## 5. Conclusions

This study found that agrochemical treatments, such as mineral fertilizers and pesticides, did not have a significant direct effect on the taxonomic composition and diversity of the bacterial community of agricultural soils under soya, sunflower, and wheat crops. The abundance of certain taxa (*Sphingomonadales*, *Gemmataceae*, *Burkholderiaceae*) was significantly increased in soils treated with pesticides. The main factors shaping the bacterial community structure were the physicochemical properties of soils and environmental factors.

Agrochemicals application did not affect the soil resistome directly. ARG spread in agricultural soils occurred due to both vertical inheritance (bacterial proliferation) and HGT involving three classes of integrons.

The main suggested bacterial hosts of ARGs were representatives of the *Pseudomonas*, *Bacillus*, *Sphingomonas*, *Arthrobacter* genera, *Nocardioidaceae*, and *Micrococcaceae* families. Determinants of resistance to carbapenems (*bla*VIM-1), glycopeptides (*vanA* and *vanB*), tetracyclines (*tetO*), macrolides (*ermB*), sulfonamides (*sul2*), and aminoglycosides (*aadA*2) were consistently present in agricultural soils.

The results of this study contribute to understanding the impact of agronomic management on agricultural soils in regard to ARG spread.

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**Data Availability Statement:** All data supporting the results of this study are available within the paper.

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## Appendix A

**Table A1.** Chemical plant protection products used in this study.

| Plant-Protecting Agent | Trade Name | Composition | Application | Dose (L ha$^{-1}$) | Treatment Method | Crop |
|---|---|---|---|---|---|---|
| Herbicides | Gardo Gold | 312.5 g L$^{-1}$ c-metolachlor 187.5 g L$^{-1}$ terbutylazine | SE | 4.0 | application to the soil before sowing | soya |
| | | | | 3.0 | | sunflower |
| | Benito | 300 g L$^{-1}$ bentazone | CC | 2.0 | spray to plant during vegetation | soya |
| | Reglon Super | 150 g L$^{-1}$ diquat | WS | 2.0 | spray to plant before harvesting (desiccant) | sunflower |
| Fungicides | Maxim | 25 g L$^{-1}$ fludioxonil | SC | 5.0 | pre-sowing seed treatment (protectant) | sunflower |
| | Optimo | 200 g L$^{-1}$ pyraclostrobin | EC | 1.0 | spray to plant during growing season | sunflower |
| | Ceriax Plus | 66.6 g L$^{-1}$ pyraclostrobin + 41.6 g L$^{-1}$ fluxapyroxad + 41.6 g L$^{-1}$ epoxiconazole | EC | 0.4 | spray to plant during growing season | winter wheat |
| Insecticides | Cruiser | 350 g L$^{-1}$ thiamethoxam | SC | 0.5 | pre-sowing seed treatment (seed dresser) | sunflower |
| | Ampligo | 50 g L$^{-1}$ lambda-cyhalothrin; 100 g L$^{-1}$ chlorantraniliprole | MS | 0.2 | spray to plant during growing season | sunflower |
| | Fascord | 100 g L$^{-1}$ alpha-cypermethrin | EC | 0.15 | spray to plant during growing season | winter wheat |

Notes: SE are suspension emulsions, CC are colloidal concentrates, WS are water solutions, SC are suspension concentrates, EC are emulsion concentrates, and MS are microencapsulated suspensions.

**Table A2.** Soil samples collected during a field experiment.

| № | Designation | Crop | Agrochemical Treatment | Sampling Time | Forecrop |
|---|---|---|---|---|---|
| Sampling before pesticide application | | | | | |
| 1 | Gc | soya | control | 14.06.2022 | – |
| 2 | Gf | soya | fertilizers | 14.06.2022 | – |
| 3 | Gp | soya | pesticides | 14.06.2022 | – |
| 4 | Gf + p | soya | fertilizers + pesticides | 14.06.2022 | – |
| 5 | Hc | sunflower | control | 14.06.2022 | – |
| 6 | Hf | sunflower | fertilizers | 14.06.2022 | – |
| 7 | Hp | sunflower | pesticides | 14.06.2022 | – |
| 8 | Hf + p | sunflower | fertilizers + pesticides | 14.06.2022 | – |
| 9 | T(g)c | winter wheat | control | 15.05.2023 | soya |
| 10 | T(g)f | winter wheat | fertilizers | 15.05.2023 | soya |
| 11 | T(g)p | winter wheat | pesticides | 15.05.2023 | soya |
| 12 | T(g)f + p | winter wheat | fertilizers + pesticides | 15.05.2023 | soya |
| 13 | T(h)c | winter wheat | control | 15.05.2023 | sunflower |
| 14 | T(h)f | winter wheat | fertilizers | 15.05.2023 | sunflower |
| 15 | T(h)p | winter wheat | pesticides | 15.05.2023 | sunflower |
| 16 | T(h)f + p | winter wheat | fertilizers + pesticides | 15.05.2023 | sunflower |
| Sampling after pesticide application | | | | | |
| 17 | Gc | soya | control | 07.07.2022 | – |
| 18 | Gf | soya | fertilizers | 07.07.2022 | – |
| 19 | Gp | soya | pesticides | 07.07.2022 | – |
| 20 | Gf + p | soya | fertilizers + pesticides | 07.07.2022 | – |
| 21 | Hc | sunflower | control | 22.09.2022 | – |
| 22 | Hf | sunflower | fertilizers | 22.09.2022 | – |
| 23 | Hp | sunflower | pesticides | 22.09.2022 | – |
| 24 | Hf + p | sunflower | fertilizers + pesticides | 22.09.2022 | – |
| 25 | T(g)c | winter wheat | control | 04.07.2023 | soya |
| 26 | T(g)f | winter wheat | fertilizers | 04.07.2023 | soya |
| 27 | T(g)p | winter wheat | pesticides | 04.07.2023 | soya |
| 28 | T(g)f + p | winter wheat | fertilizers + pesticides | 04.07.2023 | soya |
| 29 | T(h)c | winter wheat | control | 04.07.2023 | sunflower |
| 30 | T(h)f | winter wheat | fertilizers | 04.07.2023 | sunflower |
| 31 | T(h)p | winter wheat | pesticides | 04.07.2023 | sunflower |
| 32 | T(h)f + p | winter wheat | fertilizers + pesticides | 04.07.2023 | sunflower |

**Table A3.** Primers sets used in this study.

| Primer Name | Sequence, 5′-3′ | Amplicon Size, bp | PCR Conditions | Reference |
|---|---|---|---|---|
| *16S* | f: GTGSTGCAYGGYTGTCGTCA<br>r: ACGTCRTCCMCACCTTCCTC | 146 | 95 °C—3 min<br>95 °C—15 s<br>60 °C—60 s<br>72 °C—30 s<br>35 cycles | [65] |
| *intI1* | f: GCCTTGATGTTACCCGAGAG<br>r: GATCGGTCGAATGCGTGT | 196 | | [66] |
| *intII2* | f: TGCTTTTCCCACCCTTACC<br>r: GACGGCTACCCTCTGTTATCTC | 195 | | |
| *intI3* | f: GCCACCACTTGTTTGAGGA<br>r: GGATGTCTGTGCCTGCTTG | 138 | | |
| *bla*VIM-1 | f: ACTGTCGGATACTCACCACTC<br>r: GTTATGGAGCAGCAACGATGT | 189 | 95 °C—3 min<br>95 °C—10 s<br>57 °C—35 s<br>72 °C—30 s<br>40 cycles | [67] |

**Table A3.** *Cont.*

| Primer Name | Sequence, 5′-3′ | Amplicon Size, bp | PCR Conditions | Reference |
|---|---|---|---|---|
| *bla*CTX-M | f: ACCAACGATATCGCGGTGAT<br>r: ACATCGCGACGGCTTTCT | 101 | 95 °C—3 min<br>95 °C—15 s<br>58 °C—30 s<br>72 °C—30 s<br>40 cycles | [68] |
| *mecA* | f: GTGAAGATATACCAAGTGATT<br>r: ATGCGCTATAGATTGAAAGGAT | 147 | | [69] |
| *vanA* | f: CATGGCAAGTCAGGTGAAGA<br>r: CCACCGGCCTATCATCTTT | 187 | | [70] |
| *vanB* | f: AGACATTCCGGTCGAGGAAC<br>r: GCTGTCAATTAGTGCGGGAA | 220 | 95 °C—3 min<br>95 °C—40 s<br>56,5 °C—40 s<br>72 °C—40 s<br>35 cycles | [71] |
| *tetO* | f: ATGGCATACAGGCACAGACC<br>r: GGATGCTGCCCAACCTTTTG | 178 | 95 °C—3 min<br>95 °C—30 s<br>58 °C—40 s<br>72 °C—30 s<br>35 cycles | [72] |
| *sul2* | f: TCCGGTGGAGGCCGGTATCTGG<br>r: CGGGAATGCCATCTGCCTTGAG | 191 | | [73] |
| *ermB* | f: GCATTTAACGACGAAACTGGCT<br>r: TGGTGAATTAAAGTGACACGAATGT | 123 | 95 °C—3 min<br>95 °C—10 s<br>59 °C—30 s<br>72 °C—45 s<br>40 cycles | [72] |
| *mphA* | f: AGTTCGTGGTGAACGACAAG<br>r: AGTCGATCATCCCGCTGAC | 153 | 95 °C—3 min<br>95 °C—60 s<br>58 °C—60 s<br>72 °C—45 s<br>35 cycles | [74] |
| *aadA*2 | f: TAAGACGGGCTGATACTGG<br>r: CATAGCGTTGCCTTGGTAG | 251 | 95 °C—3 min<br>95 °C—10 s<br>53 °C—30 s<br>72 °C—30 s<br>40 cycles | [75] |

**Table A4.** The physicochemical characteristics of soil.

| Crop | Sampling Time | Treatment | Solid Residue (% *w*/*w*) | $NH_4^+$–N (mg $kg^{-1}$) | $NO_3^-$–N (mg $kg^{-1}$) | $P_2O_5$ (mg $kg^{-1}$) | $K_2O$ (mg $kg^{-1}$) | pH | SOM (%) |
|---|---|---|---|---|---|---|---|---|---|
| Soya | before applying pesticides | control | 0.065 | 12.54 | 13.2 | 17.6 | 449.3 | 6.63 | 4.04 |
| | | fertilizers | 0.072 | 9.58 | 11 | 33.1 | 430.2 | 7.25 | 4.16 |
| | | pesticides | 0.0595 | 6.72 | 9.1 | 20 | 420.7 | 7.37 | 4.22 |
| | | combined treatment | 0.0705 | 8.96 | 21.9 | 27.8 | 468.5 | 7.04 | 4.23 |
| | after applying pesticides | control | 0.08 | 9.07 | 13.2 | 21.1 | 497.1 | 6.9 | 4.16 |
| | | fertilizers | 0.0768 | 9.58 | 10.5 | 22 | 411.1 | 6.74 | 4.02 |
| | | pesticides | 0.0735 | 8.34 | 6.3 | 14 | 344.2 | 7.26 | 4.06 |
| | | combined treatment | 0.1075 | 8.79 | 11.8 | 27.2 | 473.3 | 7.33 | 4.17 |
| Sunflower | before applying pesticides | control | 0.0603 | 6.53 | 5.9 | 17.5 | 411.1 | 6.87 | 4.08 |
| | | fertilizers | 0.065 | 7.62 | 7.6 | 23.1 | 430.2 | 6.66 | 4.1 |
| | | pesticides | 0.06 | 5.21 | 14.8 | 27.8 | 363.3 | 7.18 | 3.87 |
| | | combined treatment | 0.0793 | 5.04 | 19.5 | 46.7 | 272.8 | 7.17 | 3.91 |
| | after applying pesticides | control | 0.0513 | 3.28 | 4.1 | 18.8 | 382.4 | 7.08 | 3.91 |
| | | fertilizers | 0.055 | 4.07 | 3.4 | 24.3 | 439.8 | 7.17 | 4.16 |
| | | pesticides | 0.057 | 2.53 | 9.6 | 24.3 | 401.5 | 6.9 | 3.88 |
| | | combined treatment | 0.065 | 3.39 | 14.8 | 35.1 | 420.7 | 6.9 | 3.99 |

**Table A4.** *Cont.*

| Crop | Sampling Time | Treatment | Solid Residue (% *w*/*w*) | $NH_4^+$–N (mg kg$^{-1}$) | $NO_3^-$–N (mg kg$^{-1}$) | $P_2O_5$ (mg kg$^{-1}$) | $K_2O$ (mg kg$^{-1}$) | pH | SOM (%) |
|---|---|---|---|---|---|---|---|---|---|
| Wheat grown after soya | before applying pesticides | control | 0.069 | 12.62 | 3.5 | 22.4 | 392 | 7.06 | 4.21 |
| | | fertilizers | 0.065 | 16.78 | 7.1 | 35.7 | 401.5 | 7.47 | 3.92 |
| | | pesticides | 0.059 | 12.12 | 6.2 | 26.4 | 392 | 7.23 | 4.2 |
| | | combined treatment | 0.051 | 14.2 | 7.2 | 28.1 | 411.1 | 7.25 | 4.09 |
| | after applying pesticides | control | 0.072 | 6.79 | 3.4 | 17.8 | 439.8 | 7.25 | 4.14 |
| | | fertilizers | 0.06 | 12.62 | 4.4 | 19.4 | 372.8 | 6.95 | 3.96 |
| | | pesticides | 0.0485 | 11.04 | 5.8 | 17.9 | 344.2 | 7.08 | 4.32 |
| | | combined treatment | 0.0465 | 10.29 | 5.9 | 17 | 420.7 | 7.81 | 3.98 |
| Wheat grown after sunflower | before applying pesticides | control | 0.0433 | 9.12 | 2.7 | 15.4 | 363.3 | 7.37 | 4.27 |
| | | fertilizers | 0.06 | 17.42 | 3.8 | 21.6 | 449.3 | 7.3 | 4.1 |
| | | pesticides | 0.0623 | 14.95 | 2.8 | 18.6 | 392 | 7.54 | 4.15 |
| | | combined treatment | 0.0693 | 15.2 | 4.6 | 23.7 | 449.3 | 7.41 | 4.2 |
| | after applying pesticides | control | 0.048 | 7.12 | 2.2 | 17.1 | 344.2 | 7.43 | 4.05 |
| | | fertilizers | 0.0593 | 12.45 | 3.1 | 16.5 | 382.4 | 8.02 | 3.93 |
| | | pesticides | 0.0628 | 12.37 | 1.4 | 14.8 | 369.8 | 7.31 | 4.06 |
| | | combined treatment | 0.0608 | 13.12 | 1.9 | 23.3 | 401.5 | 8.06 | 4.17 |

**Table A5.** Indices of the $\alpha$-diversity of bacterial communities.

| Crop | Sampling Time | Treatment | $\alpha$-Diversity Indices | | |
|---|---|---|---|---|---|
| | | | Shannon | Chao1 | Simpson |
| Soya | before applying pesticides | control | 8.75817 | 686 | 0.99666 |
| | | fertilizers | 9.50461 | 1336 | 0.99763 |
| | | pesticides | 9.33305 | 1172 | 0.99742 |
| | | combined treatment | 9.51581 | 1250 | 0.99769 |
| | after applying pesticides | control | 9.23950 | 1138 | 0.99677 |
| | | fertilizers | 8.50721 | 698 | 0.99339 |
| | | pesticides | 9.39649 | 1234 | 0.99743 |
| | | combined treatment | 9.81576 | 1599 | 0.99757 |
| Sunflower | before applying pesticides | control | 8.74802 | 682 | 0.99656 |
| | | fertilizers | 8.60386 | 622 | 0.99615 |
| | | pesticides | 9.79218 | 1616 | 0.99791 |
| | | combined treatment | 9.59351 | 1413 | 0.99768 |
| | after applying pesticides | control | 9.52641 | 1277 | 0.99767 |
| | | fertilizers | 9.46248 | 1196 | 0.99761 |
| | | pesticides | 8.27541 | 431 | 0.99573 |
| | | combined treatment | 9.07144 | 926 | 0.99682 |
| Wheat grown after soya | before applying pesticides | control | 9.18216 | 851 | 0.99762 |
| | | fertilizers | 9.18137 | 838 | 0.99756 |
| | | pesticides | 8.55761 | 576 | 0.99643 |
| | | combined treatment | 8.56796 | 615 | 0.99627 |
| | after applying pesticides | control | 8.77782 | 696 | 0.99684 |
| | | fertilizers | 9.26384 | 979 | 0.99767 |
| | | pesticides | 8.62682 | 635 | 0.99646 |
| | | combined treatment | 8.74083 | 667 | 0.99676 |
| Wheat grown after sunflower | before applying pesticides | control | 9.24749 | 900 | 0.99774 |
| | | fertilizers | 8.70664 | 660 | 0.99678 |
| | | pesticides | 8.71410 | 660 | 0.99670 |
| | | combined treatment | 9.69050 | 1310 | 0.99808 |
| | after applying pesticides | control | 8.83486 | 710 | 0.99701 |
| | | fertilizers | 8.93322 | 782 | 0.99719 |
| | | pesticides | 8.69292 | 632 | 0.99672 |
| | | combined treatment | 8.98178 | 819 | 0.99718 |

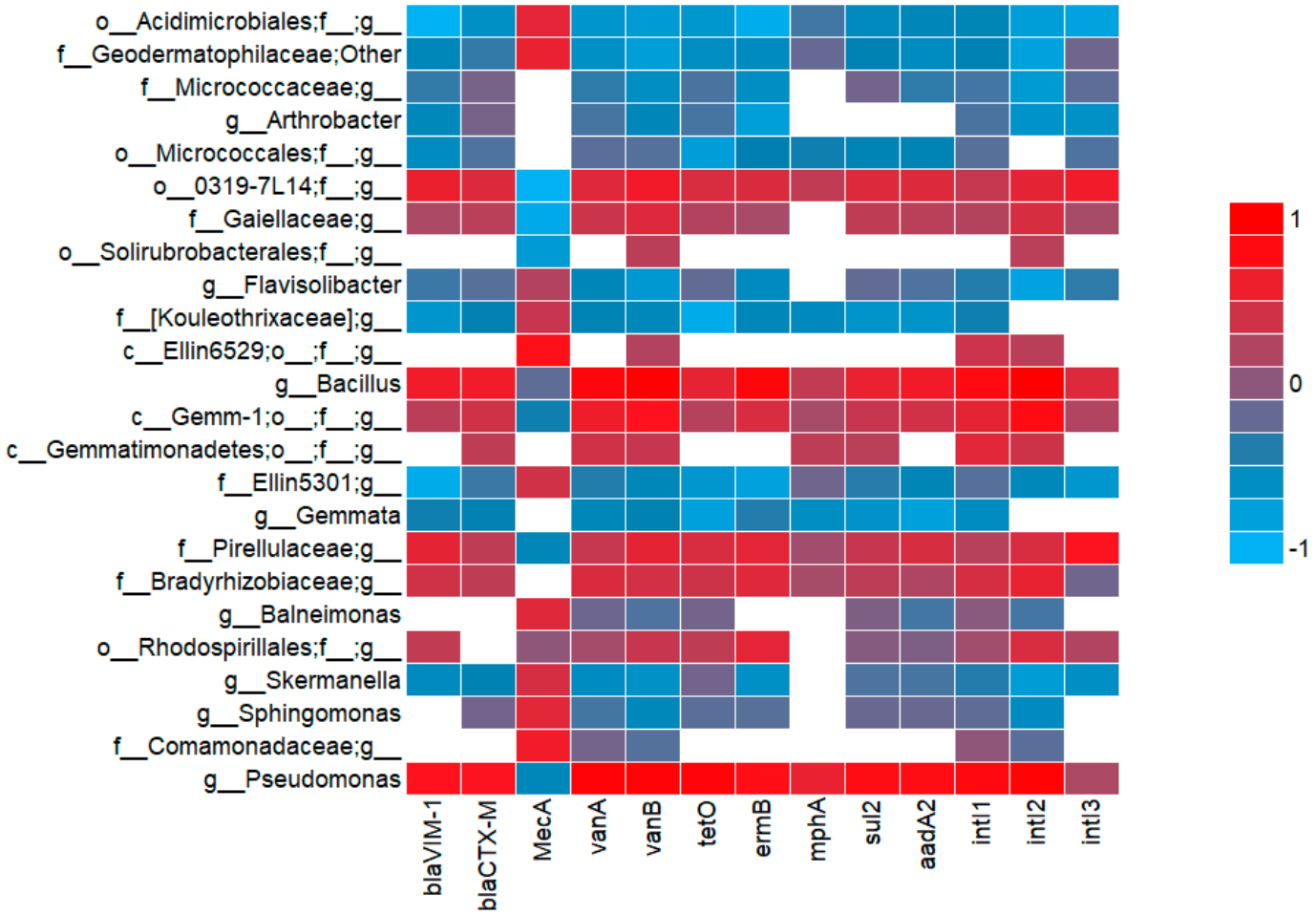


**Figure A1.** Correlation between the abundance of bacterial taxa and the relative abundance of ARGs and integrons in soil under soya.

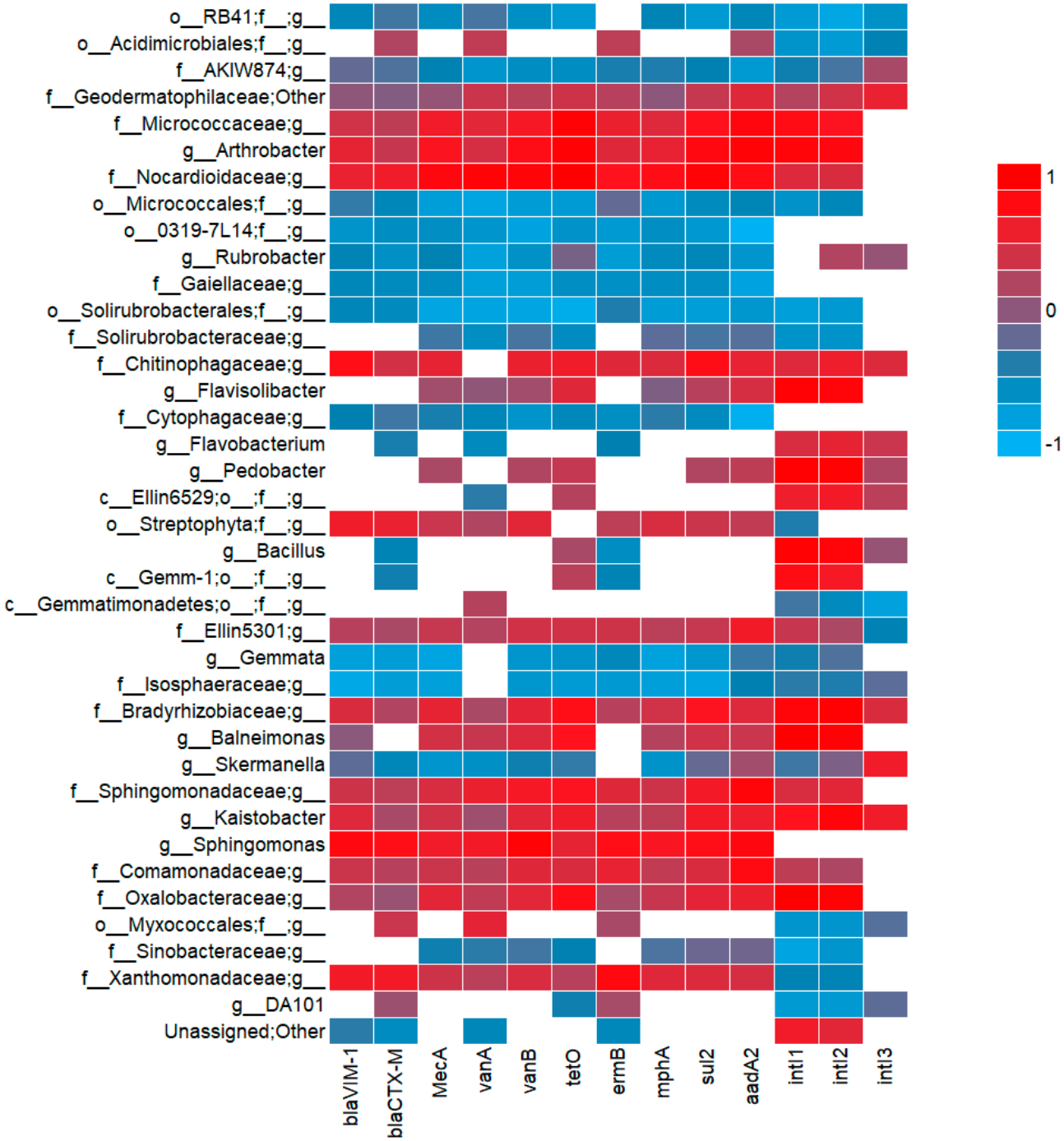


**Figure A2.** Correlation between the abundance of bacterial taxa and the relative abundance of ARGs and integrons in soil under sunflower.

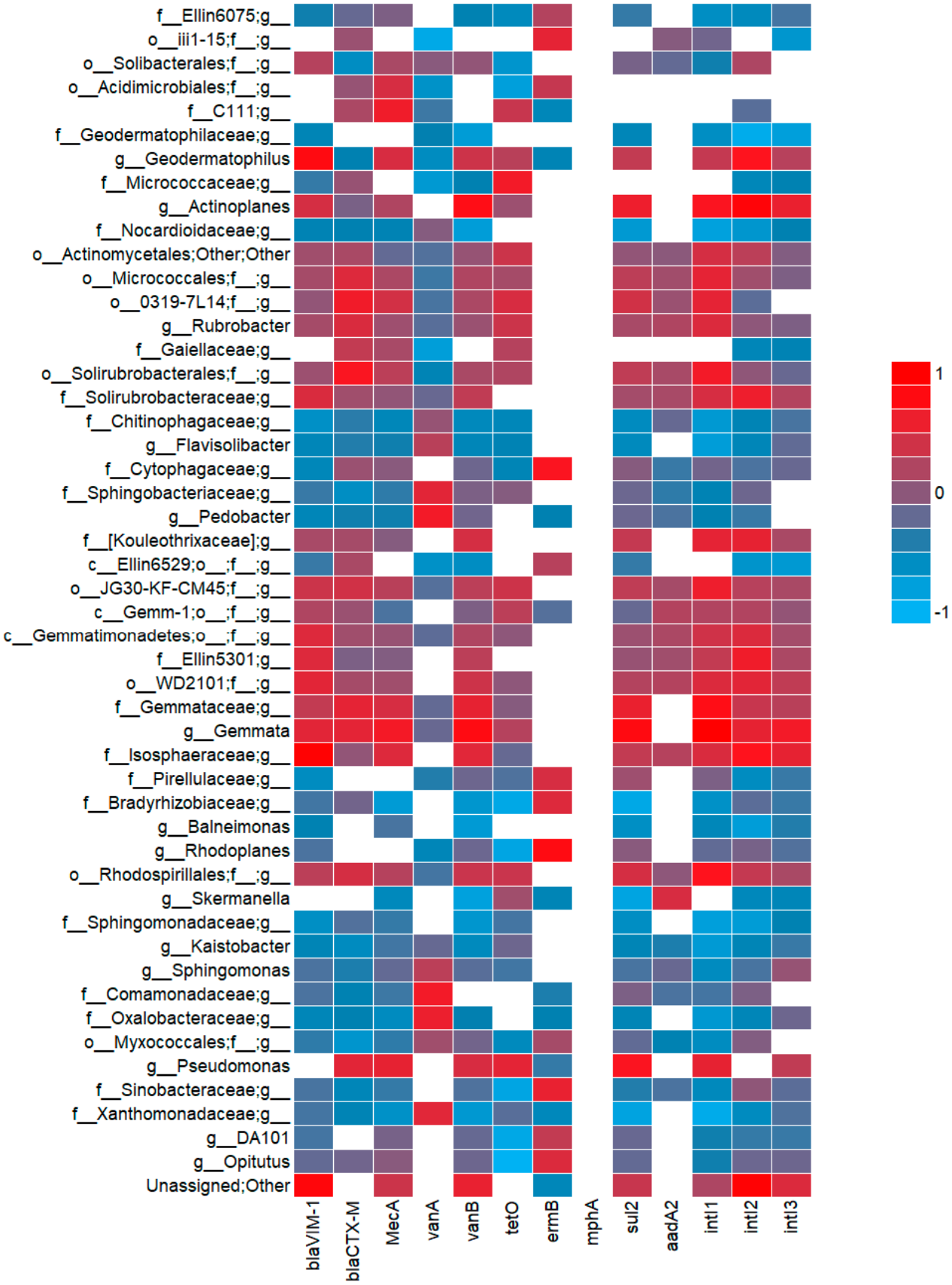


**Figure A3.** Correlation between the abundance of bacterial taxa and the relative abundance of ARGs and integrons in soil under wheat grown after soya.

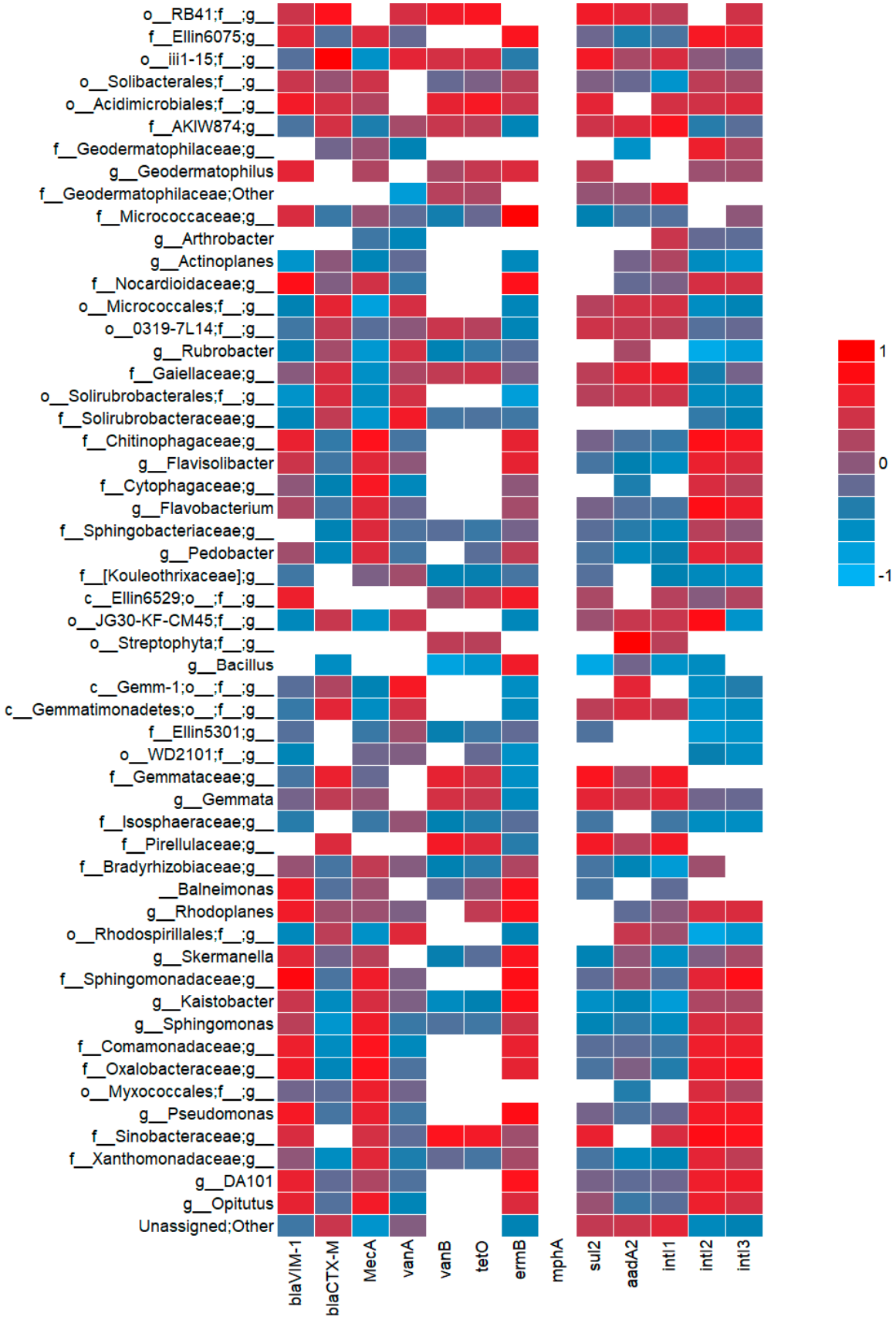


**Figure A4.** Correlation between the abundance of bacterial taxa and the relative abundance of ARGs and integrons in soil under wheat grown after sunflower.

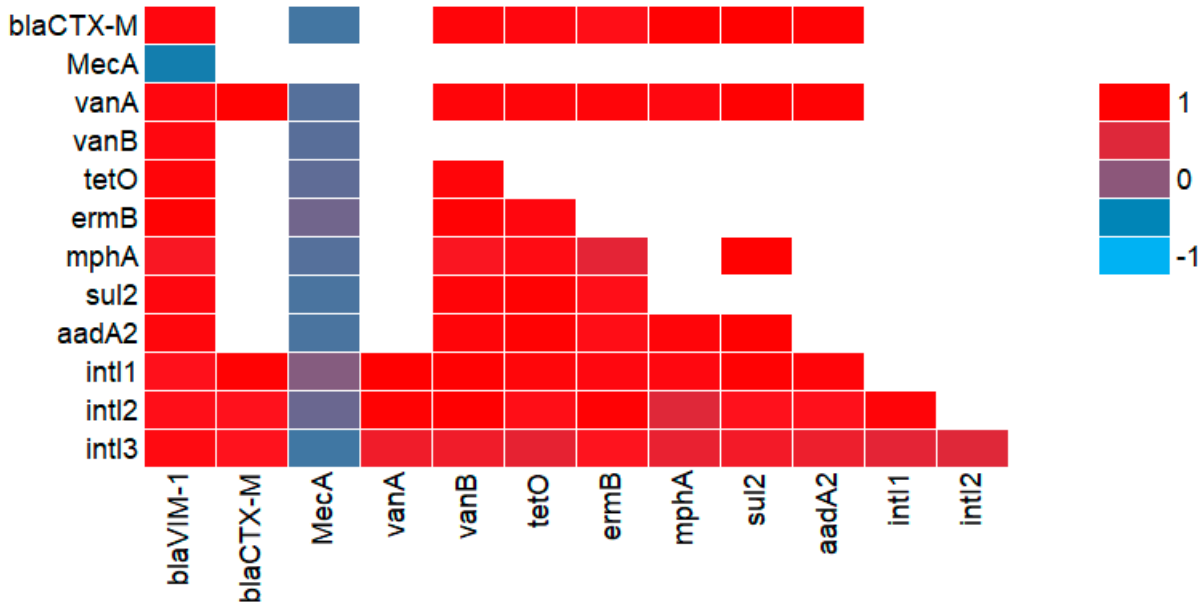


**Figure A5.** Correlation between the relative abundance of ARGs and integrons in soil under soya.

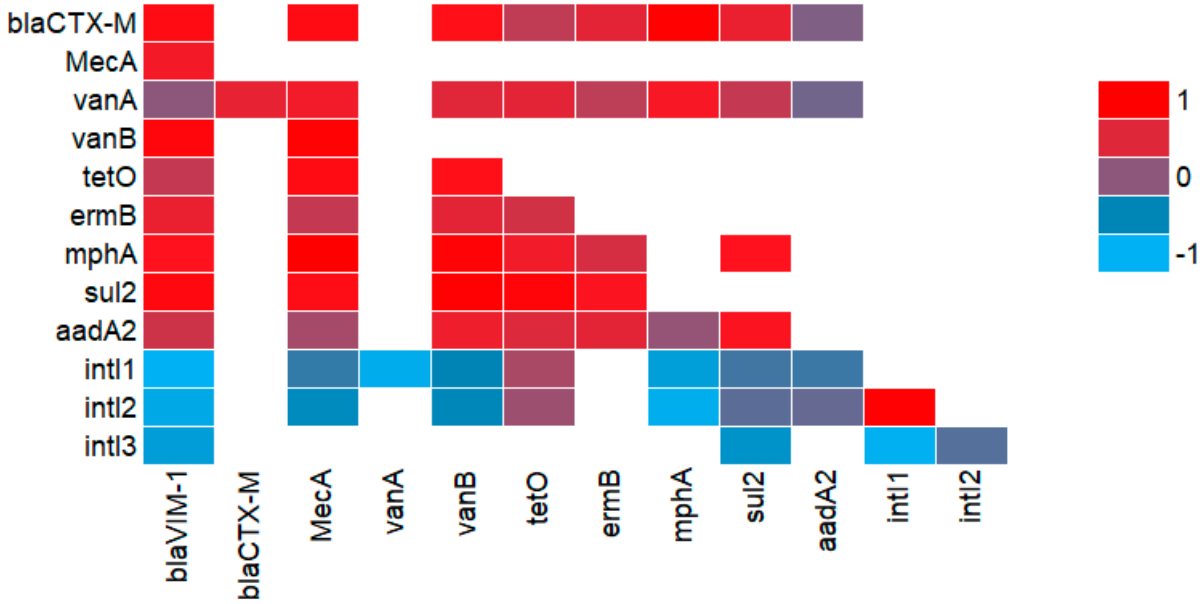


**Figure A6.** Correlation between the relative abundance of ARGs and integrons in soil under sunflower.

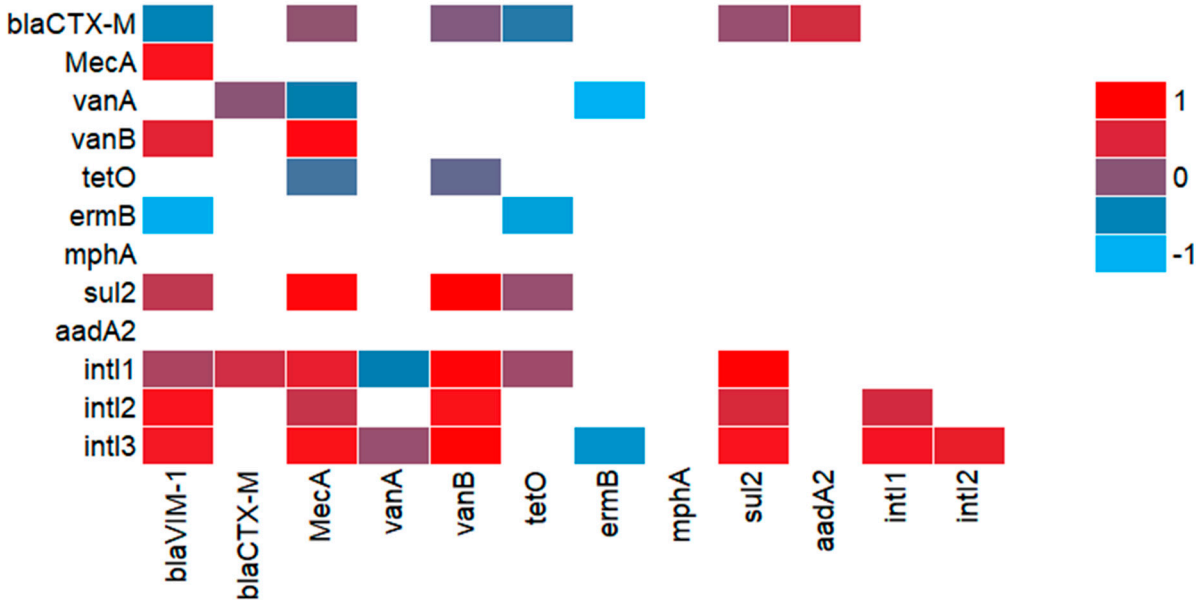


**Figure A7.** Correlation between the relative abundance of ARGs and integrons in soil under wheat grown after soya.

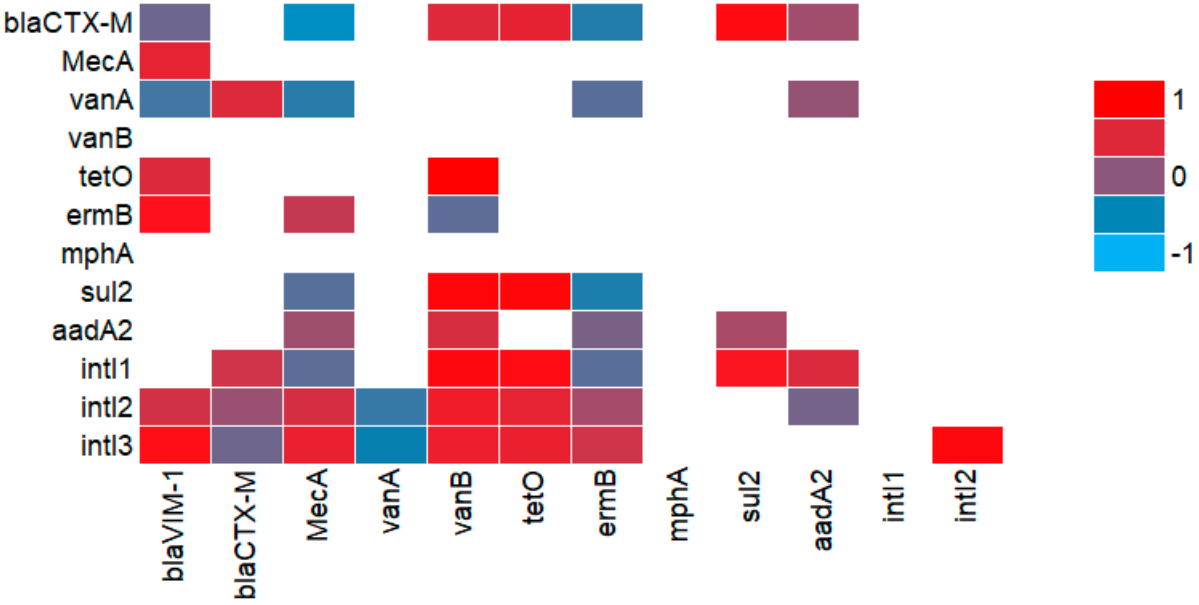


**Figure A8.** Correlation between the relative abundance of ARGs and integrons in soil under wheat grown after sunflower.

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