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Rhizosphere Microbiomes of *Amaranthus* spp. Grown in Soils with Anthropogenic Polyelemental Anomalies

Anna Muratova [1,*], Svetlana Gorelova [2], Sergey Golubev [1], Dilyara Kamaldinova [3] and Murat Gins [4,5]

[1] Institute of Biochemistry and Physiology of Plants and Microorganisms, Saratov Scientific Centre of the Russian Academy of Sciences (IBPPM RAS), Saratov 410049, Russia
[2] Natural Science Institute, Tula State University, Tula 300012, Russia
[3] Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Kazan 420021, Russia
[4] Agrarian and Technological Institute, Peoples' Friendship University of Russia, Moscow 117198, Russia
[5] Federal State Budgetary Scientific Institution "Federal Scientific Vegetable Center", Odintsovo 143080, Russia
* Correspondence: muratova_a@ibppm.ru; Tel.: +7-(8452)-970403

Abstract: Study of rhizospheric microbial communities of plants growing under different environmental conditions is important for understanding the habitat-dependent formation of rhizosphere microbiomes. The rhizosphere bacterial communities of four amaranth cultivars were investigated in a laboratory pot experiment. *Amaranthus tricolor* cv. Valentina, *A. cruentus* cv. Dyuimovochka, and *A. caudatus* cvs. Bulava and Zelenaya Sosulka were grown for six months in three soils with different anthropogenic polyelemental anomalies and in a background control soil. After the plant cultivation, the rhizosphere soils were sampled and subjected to metagenomic analysis for the 16S rRNA gene. The results showed that the taxonomic structure of the amaranth rhizosphere microbiomes was represented by the dominant bacterial phyla Actinobacteriota and Proteobacteria. A feature of the taxonomic profile of the rhizobiomes of *A. tricolor* cv. Valentina and *A. cruentus* cv. Dyuimovochka was a large abundance of sequences related to Cyanobacteria. The formation of the amaranth rhizosphere microbiomes was largely unaffected by soils, but cultivar differences in the formation of the amaranth rhizosphere microbial structure were revealed. Bacterial taxa were identified that are possibly selected by amaranths and that may be important for plant adaptation to various habitat conditions. The targeted enrichment of the amaranth rhizosphere with members of these taxa could be useful for improving the efficacy of amaranth use for agricultural and remediation purposes.

Keywords: *Amaranthus* spp.; rhizosphere microbial communities; rhizosphere bacterial diversity; technogenically contaminated soils; phytoremediation

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1. Introduction

In the past decade, research in rhizosphere biology has enjoyed an increased interest [1–4]. This interest is because the plant root zone is a unique niche that is saturated with physical, chemical, and biological interactions between macro- and microorganisms and between organisms and their environment. Studies on the functions of rhizosphere microorganisms have led researchers to understand their important role in plant life. Specifically, microbes improve plant nutrition through atmospheric nitrogen fixation, mobilization of hard-to-reach phosphorus, increased availability of trace elements, and siderophore production. Furthermore, microbes participate in plant growth regulation through the production of phytohormones and other phytoactive substances, and they increase plant adaptability through stimulation of the antioxidant defense, induction of systemic resistance, and protection from pathogens and organic/inorganic toxicants. Rhizosphere microbial communities are studied by both culture-based [2,5] and culture-independent methods [3,6–8], which helps to protect plants against diseases, improve yields, and increase the efficacy of plant use to restore disturbed soils (phytoremediation). Modern molecular technologies make it

possible to develop tools for the artificial modeling of rhizosphere microbiomes, which is of great importance for improving agricultural biotechnologies and predicting their results [9].

The main factors affecting the formation of plant-microbial complexes are the plant species [10] and the soil type [11]. Plant-root-associated microbiomes are considered an important extension of the plant genome itself [12]. The endosphere is the most plant-species-dependent and conserved compartment of rhizobacteria, which determines the so-called plant core microbiome [13]. Components of the core microbiome can be present in and can largely determine the composition of the microbiomes of other root zones, including the rhizoplane and the rhizosphere [10,14]. By contrast, the root-free edaphosphere (bulk soil) is the most plant-independent niche, whose microbiome is characterized by greater variability and is determined by the soil type. The rhizosphere microbiome is crucial for connecting plant and soil microbiomes [12,15]. Plants control the composition of their rhizosphere microbiome via root exudates and can modify it by selecting beneficial microorganisms, thereby contributing largely to the effectiveness of agricultural biotechnologies [1,14,16].

The organisms present in the rhizosphere include bacteria, fungi, oomycetes, nematodes, protozoa, algae, viruses, archaea, and arthropods [15], with bacteria being the most numerous. Bacterial diversity in the rhizosphere can be heavily influenced by abiotic and environmental conditions and can differ depending on the soil type and the plant genotype [7]. Studies on the metagenomes of plant rhizosphere communities are extremely relevant in modern science, because they carry specific information on the rhizobiomes of economically important plants and lead researchers to develop methods for their modification so that the efficiency of a particular agricultural biotechnology can be increased [7,15,17,18]. The rhizosphere microbiome composition has already been characterized for many plant species (e.g., [15,17,19,20]).

Amaranth is a widespread plant genus, many species of which are of great economic importance. *A. caudatus*, *A. cruentus*, and some other species are ancient grain and oilseed crops, which are grown in many countries. *A. tricolor* is also grown as a vegetable plant rich in essential amino acids and biologically active substances [21]. Many cultivars of *A. caudatus*, *A. hypochondriacus*, *A. tricolor*, and other species have richly colored leaves and hanging inflorescences and are used as ornamental crops. A number of *Amaranthus* plants are able to accumulate heavy metals [22–26] and radionuclides [27] and are, therefore, regarded as promising remediators. Soil phytoremediation from both organic and inorganic pollutants is known based on plant-microbial interactions [28], and the presence in the plant rhizosphere of microorganisms resistant to pollutants and able to promote plant growth is critical to the phytoremediation of polluted soils. In this context, the study of rhizosphere microbial communities may contribute to the characterization of the remediation potential of amaranth plants. Information on amaranth-associated rhizosphere microorganisms is extremely limited, but interesting. It was reported that rhizosphere microbiomes of a number of amaranth species are characterized by a pronounced abundance of representatives of Cyanobacteria [29].

The purpose of the study was to characterize the microbial communities of four *Amaranthus* cultivars grown on soils with anthropogenic polyelemental anomalies. Comparison of the rhizosphere microbiomes of plants growing under different environmental conditions will make it possible to better understand the habitat-dependent and species-(or cultivar)-dependent formation of microbiomes of these plants.

2. Materials and Methods

2.1. Soil Sampling

The soil samples used in the pot experiment were those of urban soils based on gray forest soils (WRB, 2006: Greyic Phaeozems), which were characterized by polyelemental anomalies [30]. The soils were collected in the sanitary protection zones near Kosaya Gora Iron Works (KGIW; Tula, Russia) and Tulachermet Co. (Tula, Russia) and on Tula's central avenue, Lenin Avenue. The background soil was collected near the Yasnaya Polyana

museum estate of Leo Tolstoy (Tula Region, Russia). The content of toxic substances in the background soil did not exceed the maximum permissible concentration (MPC) and approximate permissible concentration (APC); however, the iron content was quite high as compared with the world values, but minimal as compared with that of the urban soils used. Soil was sampled and prepared for the determination of toxic elements in accordance with the Russian State Standard [31]. Soil samples (~2 kg) from each location were taken from a depth of 0–25 cm from several sampling points using the envelope method. In total, at least 15 samples taken from each location were mixed and used for the pot experiment and analyses.

2.2. Soil Characterization

Soil used in our experiment was characterized on several parameters, such as type (on the basis of particle size distribution); pH; total carbon, water-soluble carbon, humus, N-NO_3, N-NH_4, and P_2O_5 contents; oil products; and metal(loid)s content. In the potassium chloride extract of soil, the potentiometric determination of pH using a glass electrode and a Mettler Toledo Delta 320 pH meter (Mettler-Toledo Instruments Shanghai Ltd., Shanghai, China) was carried out in accordance with the Russian State Standard [32]. Total organic carbon was determined according to [33]. The technique included the dichromate-wet combustion of soil organic matter by concentrated sulfuric acid and the quantitative colorimetric determination (at 590 nm) of the amount of Cr^{2+} generated by dichromate oxidation of soil organic matter. To determine the content of water-soluble carbon, aqueous (distilled water) extracts from the soil samples were obtained, and after drying, were subjected to the same procedure as the dried soil samples. Based on the data obtained, the total carbon, water-soluble carbon, and humus content were calculated. The content of mobile (available) phosphorus (P_2O_5) in mg/100 g was measured photocolorimetrically according to [34]. The method is based on the extraction of mobile compounds of phosphorus from the soil with a solution of ammonium carbonate and the subsequent photocolorimetric determination (at 710 nm) of phosphorus in the form of a blue phosphorus–molybdenum complex. Nitrates and water-soluble ammonium were measured by standard photocolorimetric methods [35,36]. The determination of nitrates included the extraction of nitrates from soil with a potassium chloride solution, reduction of nitrates to nitrites with hydrazine in the presence of copper as a catalyst, and photometric measurement (at 545 nm) of the colored diazo compound formed. The determination of exchangeable ammonium included the extraction of exchangeable ammonium from soil with a potassium chloride solution, generation of a colored indophenol compound formed by the interaction of ammonium with hypochlorite and sodium salicylate in an alkaline medium, and photometry of the colored solution (at 655 nm). All photocolorimetric measurements were carried out using an Evolution 60 UV-Vis Spectrophotometer (Thermo Scientific, Madison, WI USA). Oil products were measured gravimetrically [37]. The method is based on extraction of oil products with chloroform from air-dried soil, separation from polar compounds by liquid chromatography after replacing the solvent with hexane, and quantitative determination by gravimetric analysis. The determination of the elemental composition of soils was carried out using X-ray fluorescence analysis in the certified Laboratory of Chemical-analytical Research of GIN RAS. Soil preparation for analysis and quality control was carried out following certified methods and recommendations [30,38]. The concentrations of Mn, Fe, V, Cr, Ni, Cu, Zn, Pb, and As were determined using a serial wave XRF spectrometer "S4 Pioneer" (Bruker AXS GmbH, Karlsruhe, Germany) with a rhodium tube (capacity 4 kW). The obtained data were processed using the S4 Spectra Plus program using coefficients for correction of the routine samples matrix effects. Standard samples of the composition IAEA Soil-7, SChT-1.2 (soil), GBW-07404, 07405 (soil) were used as reference samples for soil analysis. The concentration of potentially toxic trace elements in the soil was compared with the MPCs and APCs of the metals by hygienic standards that meet international standards [39,40]. All chemical analyses of soil were performed in at least triplicate.

2.3. Experimental Design

We used four *Amaranthus* cultivars bred by the All-Russian Research Institute of Vegetable Breeding and Seed Production of the Federal Scientific Vegetable Center (VNIISSOK; Odintsovo District, Moscow Region, Russia). The specific cultivars were Valentina (*A. tricolor* L.), Dyuimovochka (*A. cruentus* L.), Bulava (*A. caudatus* L.), and Zelenaya Sosulka (*A. caudatus* L.). The description of the *Amaranthus* cultivars used is given in Table S1.

Plant seeds (~50 mg) were sown in soil-filled 2 L plastic pots. After one month, the seedlings were thinned out, leaving 10 plants per pot. Plants were cultivated under controlled conditions (day/night light cycle, 14/10 h; temperature, 22/24 °C) for 6 months. There were three replicates for each combination of *Amaranthus* cultivar and soil. At the end of growth, the plants were removed from the pots, and the soil was vigorously shaken off the roots. The rhizosphere soil adhering to young roots from 5 plants from each pot were combined from three replicates of one variant, and mixed samples were used for metagenomic analysis. The remaining soil adhering to the root surface as rhizosphere soil was carefully scraped off with sterilized tweezers.

2.4. A 16S rRNA Gene-Based Metagenomic Analysis of Rhizosphere Soil

Extraction and purification of soil DNA for metagenomic analysis was carried out using the Fast DNA®SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) and a Fast Prep®24 homogenizer (MP Biomedicals, Santa Ana, CA, USA), according to the manufacturer's instructions.

A 16S rRNA sequencing library was constructed, according to the 16S metagenomics sequencing library preparation protocol (Illumina, San Diego, CA, USA), targeting the V3 and V4 hypervariable regions of the 16S rRNA gene. The initial PCR was performed with template DNA using region-specific adapters shown to have compatibility with the Illumina index and sequencing primers (forward primer: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′; reverse primer: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′) [41]. Amplification was performed using the Veriti™ Thermal Cycle, 96-Well (Applied Biosystems™, Foster City, CA, USA), according to the Illumina protocol. After the first round of amplification, PCR products were visualized using gel electrophoresis. Then, the PCR products were purified with AMPure XT magnetic beads, and the second PCR was performed using primers from a Nextera XT Index Kit (Illumina). Subsequently, purified PCR products were quantified with a Qubit dsDNA HS Assay Kit (Thermo Scientific) on a Qubit 2.0 fluorometer. The sample pool (4 nM) was denatured with 0.2 N NaOH, diluted further to 10 pM, and combined with 20% (*v*/*v*) denatured 4 pM PhiX, prepared following Illumina guidelines. Sequencing of 16S rRNA gene V3–V4 variable regions was performed on the Illumina MiSeq platform in 2 × 300 bp mode.

2.5. Bioinformatics and Statistical Analysis

Reads were analyzed using QIIME2 software, version 2022.8 (http://qiime2.org/, accessed on 25 February 2023) [42]. Before filtering, there were 118,339 read pairs per sample on average. Raw reads were processed using the DADA2 algorithm implemented in QIIME [43]. After quality filtering, chimera and phiX sequences removal, we analyzed 17,870 joined read pairs per sample on average. The taxonomy was assigned to the sequences using the Naive Bayes classifier pre-trained on the latest SILVA 138 database 99% OTUs [44]. The number of observed features varied from 601 to 1030. To characterize the richness and evenness of the bacterial community, alpha diversity indices were calculated using Chao1, Shannon, and Simpson metrics. Similarities between microbial compositions of the samples were evaluated using the beta diversity characteristics, which were estimated using weighted and unweighted Unifrac measures with further non-metric multidimensional scaling (nMDS) visualization.

Venn diagrams were constructed with Creately software (https://creately.com/lp/venn-diagram-maker/, accessed on 5 February 2023; Cinergix Pty Ltd., Melbourne, VIC,

Australia). Spearman's rank correlation coefficients were calculated with Statistica software 13 (TIBCO Software Inc. 2017, Statsoft Russia, Moscow, Russia). Other calculations were done with Excel 2019 software (Microsoft, Redmond, WA, USA).

3. Results

3.1. Soil Characteristics

For all soils used in the experiment, the main characteristics were determined: type; pH; and the total content of (including water-soluble) carbon, biogenic forms of nitrogen (NH_4 and NO_3) and phosphorus (P_2O_5), heavy metals and metalloids (Fe, Mn, V, Ni, Cu, Zn, Pb, and As), and hydrocarbons. The results are given in Table 1.

Table 1. Characteristics of the soils used in the experiment.

Properties	Background Soil (Yasnaya Polyana)	KGIW	Tulachermet	Lenin Ave.
Soil type	Clay loam	Clay loam	Sandy loam	Clay loam
pH	6.20	7.26	7.35	7.29
Total carbon (% of air-dried soil)	4.87 ± 0.05	4.41 ± 0.11	4.84 ± 0.33	3.83 ± 0.06
Water-soluble carbon (% of total carbon)	0.14 ± 0.05	0.13 ± 0.02	0.14 ± 0.06	0.12 ± 0.03
Humus (% of air-dried soil)	8.12 ± 0.16	7.14 ± 0.14	7.62 ± 0.15	6.16 ± 0.12
Humus carbon (%)	4.72 ± 0.09	4.15 ± 0.08	4.43 ± 0.09	3.58 ± 0.07
N-NO_3 (mg/kg dw)	20.5 ± 1.2	28.2 ± 1.6	38.6 ± 3.5	41.1 ± 2.4
N-NH_4 (mg/kg dw)	12.9 ±1.2	6.9 ± 0.7	9.5 ± 0.3	2.3 ± 0.2
P_2O_5 (mg/kg dw)	51.0 ± 4.8	159.5 ± 3.6	98.4 ± 7.6	218.0 ± 6.7
Oil products (g/kg dw)	1.5 ± 0.6	**2.6 ± 0.4**	**4.1 ± 1.0**	**2.5 ± 0.7**
Metal(loid)s (mg/kg dw):				
Fe	15,600 ± 1860	**78,100 ± 1280**	**120,600 ± 5830**	**37,400 ± 2160**
Mn	1300 ± 67	**5700 ± 180**	1100 ± 87	**1600 ± 58**
V	57 ± 3.0	41 ± 4.1	**136 ± 7.2**	61 ± 2.9
Ni	25 ± 3.1	31 ± 4.2	**55 ± 3.3**	35 ± 2.8
Cu	29 ± 2.3	52 ± 4.1	**75 ± 0.8**	**378 ± 0.9**
Zn	47 ± 2.0	**310 ± 9.4**	**161 ± 3.6**	186 ± 5.3
Pb	18 ± 2.1	72 ± 6.0	26 ± 0.7	59 ± 1.1
As	5.1 ± 0.4	5.9 ± 0.5	**6.4 ± 0.2**	7.3 ± 0.3

Note: Data expressed as mean ± standard deviation ($n \geq 3$). Bold type means the permissible concentrations [29,30] were exceeded for: Fe (MPC_{total} 1500 mg/kg), Mn (MPC_{total}, 1500 mg/kg), V (MPC_{total}, 100 mg/kg), Ni (APC_{total}, sandy loam, 20 mg/kg; APC_{total}, clay loam, 80 mg/kg), Cu (MPC_{total} 55 mg/kg; APC_{total}, sandy loam, 33 mg/kg), Zn (APC_{total}, clay loam, 220 mg/kg; APC_{total}, sandy loam, 55 mg/kg), As (APC_{total}, sandy loam, 2 mg/kg; APC_{total}, clay loam, 10 mg/kg), and oil products (1.0 g/kg). The oil product content in soil is not regulated at the regional level; the recommended value is 1.0 g/kg.

All soil samples were represented by gray forest soils, had neutral or close to slightly acidic pH values, and were also characterized by a high content of iron. The soils of the sanitary protection zones near Kosaya Gora Iron Works (KGIW; Tula, Russia) and Tulachermet Co. (Tula, Russia) and the urban soil of Tula's central avenue, Lenin Avenue, all have an excessive content of heavy metals and oil products, indicating human-caused pollution. The KGIW soil had a high content of Mn, Zn, and petroleum products, which exceeded MPCs by 380, 140, and 260%, respectively. The Tulachermet soil had a high content of V, Ni, Cu, Zn, As, and oil products, exceeding MPCs and APCs by 36, 275, 127, 290, 300, and 410%, respectively. The Lenin Avenue soil had a high content of Mn, Cu, and oil products, exceeding the permissible concentrations by 690, 107, and 250%, respectively. The background soil was that collected near the Yasnaya Polyana museum estate of Leo Tolstoy (Tula Region). In it, the content of environmentally regulated elements did not exceed the MPCs or APCs.

3.2. Metagenomic Analysis of Rhizosphere Microbial Communities of Amaranthus spp.

3.2.1. Diversity of Rhizosphere Communities

Sequencing of the 16s rRNA gene from 16 rhizosphere samples resulted in 1,915,453 raw reads. After data denoising and chimera screening, 17,870 joined read pairs per sample on average were used for further identification. Rarefaction curves obtained with the normalized OTU number almost reached saturation levels for all samples, indicating that the bacterial communities were covered well by the sequence analysis (Figure S1). The sequences with >97% similarity were combined into operational taxonomic units (OTUs). The OTUs were assigned to 38, 124, 288, 449, and 804 taxa at the phylum, class, order, family, and genus levels, respectively.

To characterize the bacterial diversity and the richness of the microbial communities, we calculated the α- and β-diversity (Table 2 and Figure 1).

Table 2. The α-diversity indices for the rhizospheric microbial communities of amaranths grown on different soils.

Soil	Plant	Observed Features	Chao1	Shannon Index	Simpson Index	Faith PD
Background	*A. tricolor* cv. Valentina	743	748.000	8.6560	0.9964	81.03
	A. cruentus cv. Dyuimovochka	716	718.111	8.6309	0.9957	73.42
	A. caudatus cv. Bulava	890	898.347	8.5701	0.9930	86.78
	A. caudatus cv. Zelenaya Sosulka	910	916.949	8.7466	0.9955	97.51
Tulachermet	*A. tricolor* cv. Valentina	741	743.258	8.6019	0.9956	87.83
	A. cruentus cv. Dyuimovochka	823	829.343	8.8715	0.9970	87.65
	A. caudatus cv. Bulava	1010	1014.614	9.2749	0.9978	92.29
	A. caudatus cv. Zelenaya Sosulka	819	831.470	8.8402	0.9967	81.70
KGIW	*A. tricolor* cv. Valentina	807	807.786	8.9055	0.9970	82.02
	A. cruentus cv. Dyuimovochka	806	806.992	8.6081	0.9940	88.70
	A. caudatus cv. Bulava	990	994.844	9.1206	0.9970	97.06
	A. caudatus cv. Zelenaya Sosulka	964	974.350	9.1507	0.9974	88.53
Lenin Ave.	*A. tricolor* cv. Valentina	832	836.833	8.9232	0.9969	85.43
	A. cruentus cv. Dyuimovochka	601	602.600	8.4401	0.9959	63.57
	A. caudatus cv. Bulava	758	766.928	8.3750	0.9926	81.42
	A. caudatus cv. Zelenaya Sosulka	707	716.784	8.5888	0.9957	74.67

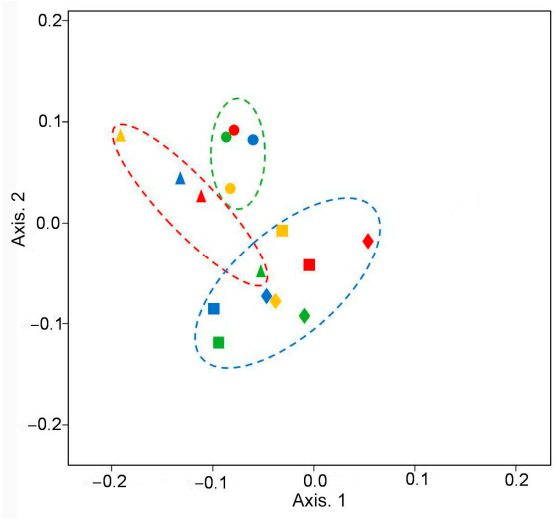

Figure 1. Principal coordinate analysis for the rhizosphere microbial communities of the *Amaranthus* cvs.: triangles (▲), *A. tricolor* cv. Valentina; circles (●), *A. cruentus* cv. Dyuimovochka; squares (■), *A. caudatus* cv. Bulava; diamonds (♦), *A. caudatus* cv. Zelenaya Sosulka; blue, background soil; yellow, Tulachermet soil; red, KGIW soil; green, Lenin Ave. soil.

The α-diversity was measured by using the species richness indices (Chao1, Shannon, and Simpson indices; Faith's phylogenetic diversity [PD], Table 2). Traditional (Shannon's and Simpson's) and phylogenetic (Faith's PD) indices of bacterial alpha-diversity in the rhizosphere communities of amaranths yielded similar conclusions: all communities were quite diverse and differed between plant species (cultivars) grown on the same soil.

A comparison of the rhizosphere microbiomes of different samples showed that the influence of soils on the formation of rhizosphere communities was not pronounced: the samples were not grouped according to soils. Yet, there were pronounced differences in rhizospheric samples according to the plant species studied (Figure 1). *A. caudatus* cv. Bulava and *A. caudatus* cv. Zelenaya Sosulka formed one cluster, which distinctly distanced itself from *A. tricolor* cv. Valentina, whereas the cluster of *A. cruentus* cv. Dyuimovochka had intersections with both *A. caudatus* cultivars.

3.2.2. Taxonomic Structure of Rhizosphere Communities

MiSeq sequencing showed that the amaranth rhizosphere communities included 804 genera of bacteria belonging to 449 families of 38 phyla.

Figure 2 illustrates the relative abundances of OTUs associated at the phylum level in the rhizosphere of the amaranth cultivars studied. In different soils in the rhizosphere communities of cv. Valentina, most OTUs were assigned to Actinobacteriota (29–39%), Proteobacteria (17–29%), Chloroflexi (8–18%), Cyanobacteria (4–18%), and Acidobacteriota (4–6%). In the rhizosphere cv. Dyuimovochka, the dominant phyla were also Actinobacteriota (25–42%), Proteobacteria (19–31%), Chloroflexi (6–12%), Cyanobacteria (3–19%), and Acidobacteriota (4–9%). In the rhizosphere cv. Bulava, the dominant phyla were Actinobacteriota (31–40%), Proteobacteria (19–25%), Chloroflexi (10–15%), and Acidobacteriota (7–12%). In the rhizosphere cv. Zelenaya Sosulka, the dominant phyla were Actinobacteriota (31–36%), Proteobacteria (21–27%), Chloroflexi (9–14%), and Acidobacteriota (8–11%). The percentages of OTUs assigned to other phyla were much smaller.

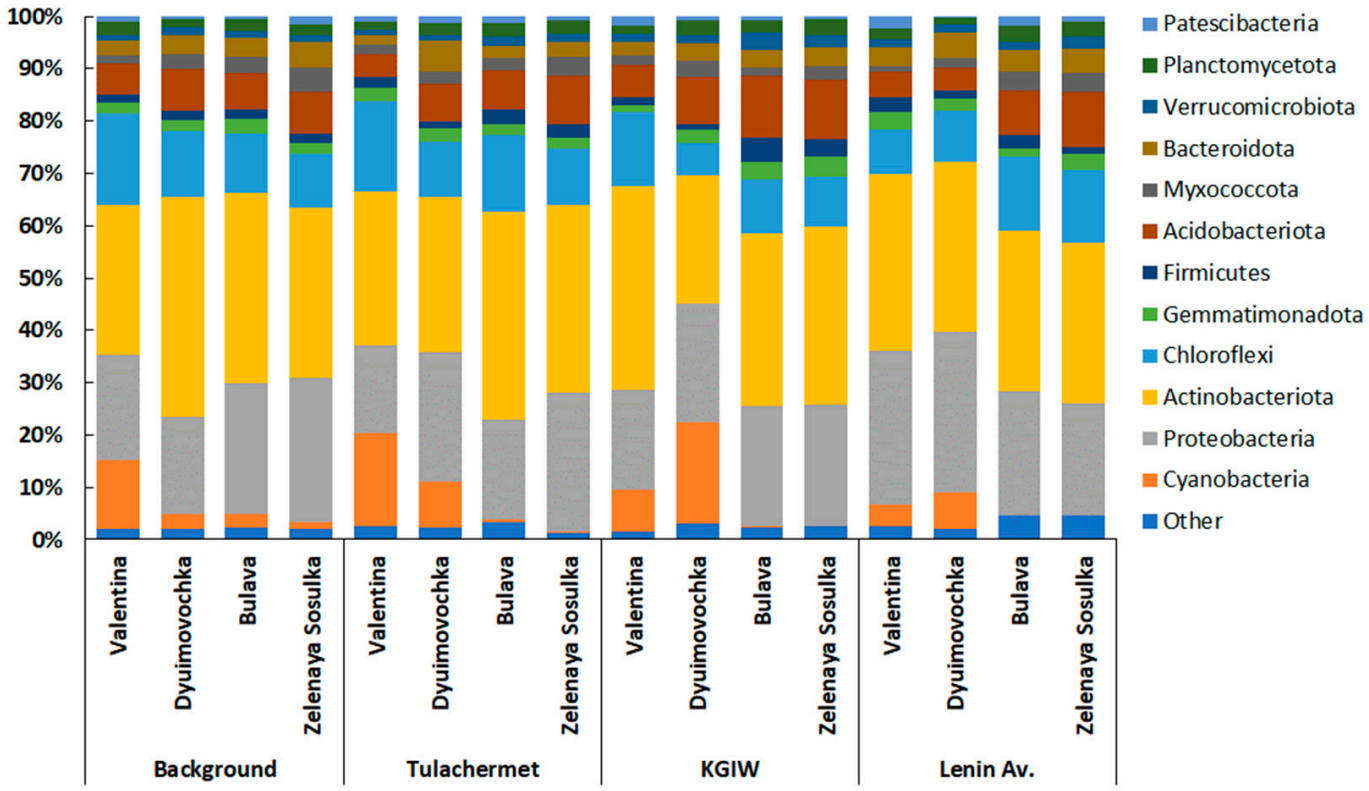

Figure 2. Relative abundances of OTUs associated at the phylum level of the microbial communities in the rhizospheres of four amaranth cultivars grown on different soils.

The list of taxa at the family level, for which the share of amaranths in the rhizosphere was ≥1%, is given in Table S2. Of the dominant Actinobacteriota phylum, 51 families were identified, of which 13 were most abundant (Table S2, Figure 3), making up 80 to 86% of all detected actinobacterial families in the amaranth rhizosphere. The family Gaiellaceae had the maximum share in the rhizospheric population of actinobacteria. Its abundance reached 1.8–3.0% in the rhizosphere of cv. Valentina, 1.5–4.6% in the rhizosphere of cv. Dyuimovochka, 2.1–3.7% in the rhizosphere of cv. Bulava, and 1.9–2.9% in the rhizosphere of cv. Zelenaya Sosulka. Other notable actinobacteria were members of the families Nocardioidaceae (1.9–4.8% of all actinobacteria) and Micromonosporaceae (0.8–5.4%) and members of the orders Solirubrobacterales (67-14 family) and MB-A2-108 (MB-A2-108 family).

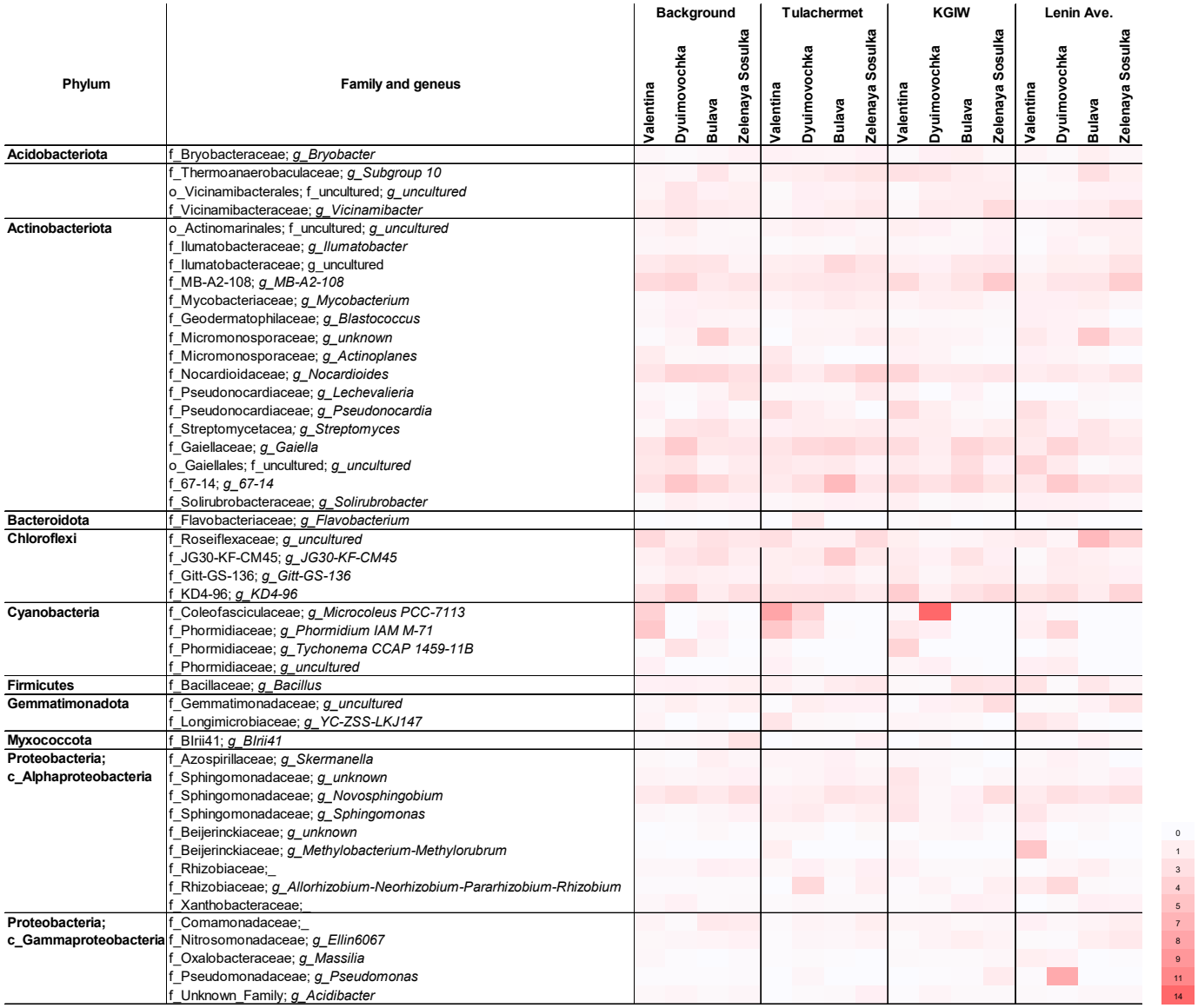

Figure 3. Heat map illustrating the relative abundances of OTUs associated at the genus level of the microbial communities in the rhizospheres of four amaranth cultivars grown on different soils.

In another dominant phylum, Proteobacteria, 93 bacterial families were identified. In total, 10 of them (Table S2, Figure 3) made the largest contribution to the structure of the amaranth rhizosphere microbiomes (54–76% of all detected families). Alphaproteobacteria was the major class of the phylum, accounting for 64–84% of all OTUs assigned to the

Proteobacteria. Among the Alphaproteobacteria, the dominant position was occupied by the families Sphingomonadaceae (3–7%) and Beijerinckiaceae (1–6%); a large proportion of OTUs also belonged to the Rhizobiaceae family (1–5%). The Gammaproteobacteria class accounted for 17 to 45% of all OTUs assigned to the Proteobacteria phylum, and the Nitrosomonadaceae and Comamonadaceae families were its predominant representatives. The contribution of the Betaproteobacteria and Deltaproteobacteria classes to the overall taxonomic structure of the amaranth microbiomes was not noticeable.

The rhizosphere microbiomes of two species of *Amaranthus* (*A. tricolor* cv. Valentina and *A. cruentus* cv. Dyuimovochka) were clearly enriched with members of the Cyanobacteria phylum, among which the Coleofasciculaceae and Phormidiaceae families dominated on all soils (Table S2, Figure 3), but were poorly represented in the rhizosphere of *A. caudatus* cv. Bulava and cv. Zelenaya Sosulka. The Phormidiaceae family was also abundant in the rhizosphere of *A. tricolor* cv. Valentina and *A. cruentus* cv. Dyuimovochka. This family was poorly represented in the rhizosphere of *A. caudatus* cv. Bulava and was not in the rhizosphere of cv. Zelenaya Sosulka. This may indicate that the maintenance of these bacterial taxa by host plants is species specific.

The species-specific changes in the taxonomic profile of the amaranth rhizosphere communities, as induced by the soil characteristics, can be seen in Figures 2 and 3. Spearman's rank correlation and principal component analysis did not reveal significant correlations between the kinds of soil used and the dominant taxa in the amaranth rhizosphere microbiomes. However, a close correlation was established between the cultivars ($r_s = 0.64$, $p < 0.05$) and the abundance of OTUs assigned to the Cyanobacteria phylum. In addition, moderate correlations were found between the cultivars and the abundance of the Acidobacteriota ($r_s = 0.55$, $p < 0.05$), Bacteroidota ($r_s = 0.52$, $p < 0.05$), and Planctomycetota ($r_s = 0.52$, $p < 0.05$) phyla in the rhizosphere communities.

3.2.3. Shared and Unique Taxa among Rhizosphere Microbial Communities

To determine which OTUs were shared by or were specific to the rhizosphere of each cultivar on the four soils, we did several comparative analyses (Figure 4).

In the background soil, the largest number of OTUs at the bacterial species level was found in the rhizosphere of *A. caudatus* cv. Zelenaya Sosulka, followed by *A. tricolor* cv. Valentina, *A. caudatus* cv. Bulava, and *A. cruentus* cv. Dyuimovochka. A total of 127 species were shared and accounted for from 30 to 39% of the rhizosphere communities of the 4 plant cultivars. The percentage of unique taxa ranged from 24 to 30%. The maximal number of unique species (125) was found for the rhizosphere of *A. caudatus* cv. Zelenaya Sosulka.

In the Tulachermet soil, the largest number of OTUs at the bacterial species level was found in the rhizosphere of *A. caudatus* cv. Bulava, followed by *A. cruentus* cv. Dyuimovochka, *A. tricolor* cv. Valentina, and *A. caudatus* cv. Zelenaya Sosulka. A total of 124 species were shared and accounted for from 28 to 34% of the rhizosphere communities of all plant cultivars. The percentage of unique taxa ranged from 23 to 29%. The maximal number of unique species (125) was found for the rhizosphere of *A. caudatus* cv. Bulava.

In the KGIW soil, the largest number of OTUs at the bacterial species level was found in the rhizosphere of *A. caudatus* cv. Bulava, followed by *A. cruentus* cv. Dyuimovochka, *A. caudatus* cv. Zelenaya Sosulka, and *A. tricolor* cv. Valentina. A total of 121 species were shared and accounted for from 28 to 33% of the rhizosphere communities of all plant cultivars. The percentage of unique taxa ranged from 25 to 30%. The maximal number of unique species (128) was found for the rhizosphere of *A. caudatus* cv. Bulava.

In the Lenin Ave. soil, the largest number of OTUs at the bacterial species level was found in the rhizospheres of *A. tricolor* cv. Valentina, followed by *A. caudatus* cv. Zelenaya Sosulka, *A. caudatus* cv. Bulava, and *A. cruentus* cv. Dyuimovochka. Only 88 species were shared and accounted for from 23 to 25% of the rhizosphere communities of the 4 plant cultivars. The percentage of unique taxa ranged from 24 to 29%. The maximal number of unique species (133) was found for the rhizosphere of *A. tricolor* cv. Valentina.

Among the unique taxa identified in the rhizosphere microbiome of each amaranth variety, those taxa that occur in at least two different soils were determined (Tables S3–S6). Overall, 53, 39, 50, and 52 such taxa were revealed in the rhizosphere of *A. tricolor* cv. Valentina, *A. cruentus* cv. Dyuimovochka, and *A. caudatus* cvs. Bulava and Zelenaya Sosulka, respectively. In addition, seven, two, eight, and six such unique taxa were revealed in the rhizosphere of cvs. Valentina, Dyuimovochka, Bulava, and Zelenaya Sosulka, respectively, grown in three different soils studied (Table 3). Most of those taxa were represented by uncultured bacteria.

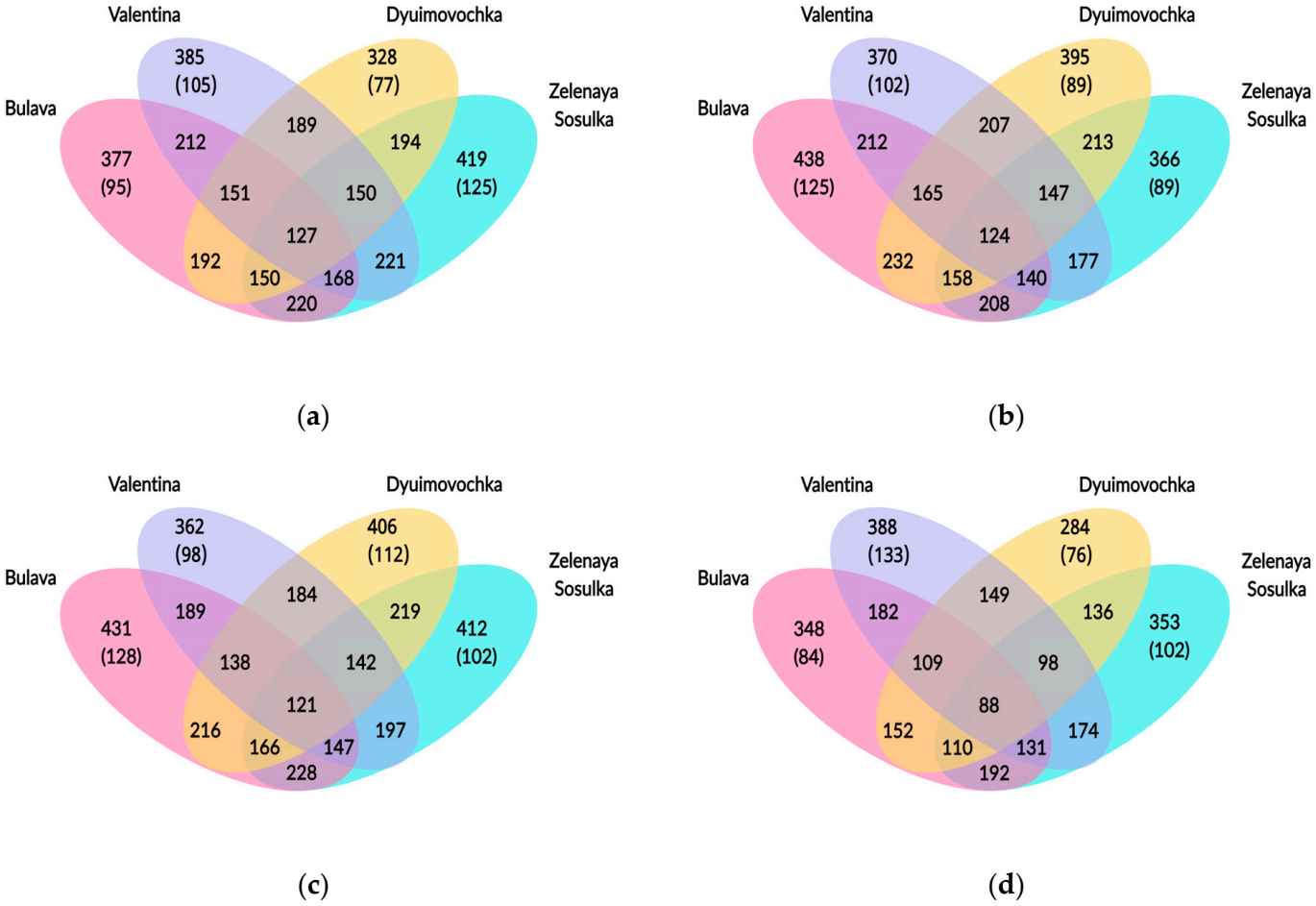

Figure 4. Venn diagram depicting shared and unique OTUs among the rhizosphere microbial communities of the amaranths grown on the background and polluted soils. The number of unique taxa is given in brackets. (**a**) Background; (**b**) Tulachermet; (**c**) KGIW; (**d**) Lenin Ave.

Table 3. Unique bacterial taxa revealed in the rhizosphere of *Amaranthus* plants and occurred in three different soils.

Plant Cultivars	Bacterial Taxa	
A. tricolor cv. Valentina	*g_Chloronema; s_Scytonema tolypothrichoides*	Background; Tulachermet; KGIW
	g_Leptolyngbya_VRUC_135;s_uncultured bacterium	Background; Tulachermet; Lenin Ave.
	g_Alsobacter; s_Alsobacter_metallidurans	Tulachermet; KGIW; Lenin Ave.
	g_C0119; s_uncultured_bacterium	Background; Tulachermet; Lenin Ave.
	g_Rhodocytophaga; s_uncultured Bacteroidetes	Background; Tulachermet; KGIW
	f_Rhodanobacteraceae; *g_uncultured; s_uncultured bacterium*	Background; Tulachermet; KGIW
	g__DS-100; s_unknown	Tulachermet; KGIW; Lenin Ave.

Table 3. *Cont.*

Plant Cultivars	Bacterial Taxa	
A. cruentus cv. Dyuimovochka	*g_Methylophilus; s_uncultured bacterium*	Tulachermet; KGIW; Lenin Ave.
	g_CENA518; s_uncultured_bacterium	Tulachermet; KGIW; Lenin Ave.
A. caudatus cv. Bulava	*g_Nakamurella; g_unknown; s_unknown*	Background; Tulachermet; KGIW
	g_Pir4_lineage; s_uncultured Pirellula	Background; Tulachermet; Lenin Ave.
	f_Moraxellaceae; *g_uncultured; s_uncultured gamma*	Background; KGIW; Lenin Ave.
	f_0319-7L14; *g_0319-7L14; s_uncultured bacterium*	Background; Tulachermet; Lenin Ave.
	o_Planctomycetales; f_uncultured; *g_uncultured*	Background; Tulachermet; KGIW
	f_Phycisphaeraceae; *g_uncultured*	Tulachermet; KGIW; Lenin Ave.
	f_Kapabacteriales; *g_Kapabacteriales*	Tulachermet; KGIW; Lenin Ave.
	f_Sericytochromatia; *g_Sericytochromatia*	Background; Tulachermet; KGIW
A. caudatus cv. Zelenaya Sosulka	f_Vicinamibacteraceae; *g_Vicinamibacteraceae;s_uncultured Geothrix*	Tulachermet; KGIW; Lenin Ave.
	f_Xanthomonadaceae; *g_unknown; s_unknown*	Background; Tulachermet; KGIW
	f_Rhodanobacteraceae; *g_Ahniella; s_uncultured bacterium*	Background; Tulachermet; Lenin Ave.
	f_Latescibacterota; *g_Latescibacterota; s_uncultured soil*	Background; Tulachermet; Lenin Ave.
	f_Blastocatellaceae; *g_Aridibacter; s_uncultured bacterium*	Background; KGIW; Lenin Ave.
	f_Subgroup 22; *g_Subgroup 22*	Tulachermet; KGIW; Lenin Ave.

The bacterial taxa listed in Table 3 are unique to each of the four plant cultivars studied and can be considered as plant-specific.

4. Discussion

Studies characterizing the rhizosphere microbiomes of *Amaranthus* plants are extremely scarce. Nambisan et al. [29] found that the Cyanobacteria phylum is distinctly enriched in the roots and the rhizosphere soil of the three grain amaranths—*A. hupochondriacus*, *A. cruentus*, and *A. caudatus*. Our study confirmed that the peculiarity of the rhizosphere microbial communities of two of the three *Amaranthus* species (*A. tricolor* cv. Valentina and *A. cruentus* cv. Dyuimovochka) is the distinct presence of cyanobacteria along with the dominant bacterial phyla, such as Proteobacteria, Actinobacteriota, and Chloroflexi. In addition, we obtained the first data on the rhizosphere microbiome of another amaranth species, *A. tricolor*. In Nambisan et al.'s research [29], the negative controls were other plant species (*Beta vulgaris*, *Cicer arietinum*, and *Solanum lycopersicum*), and in their rhizobiomes, no predominance of cyanobacteria was observed. In our study, besides the amaranth rhizosphere, we also analyzed the rhizosphere of other plants under the same experimental conditions—*Sorghum bicolor* cv. Sucro and *Sorghum bicolor* cv. Biomass. The rhizosphere of these plants showed no predominance of cyanobacteria either [20]. An analysis of the taxonomic profile of the cyanobacteria found in the amaranth rhizobiomes made it possible to identify members of the dominant *Microcoleus* and *Phormidium* genera. The presence of these genera in the rhizosphere of various plants was also noted earlier [4,45]. The accumulated data indicate that the amaranths may have specifically selected cyanobacteria from the surrounding soil microflora. In turn, cyanobacteria favor the growth of the amaranth plants, possibly through their plant-beneficial characteristics. It is known that cyanobacteria can produce plant-growth-promoting substances, including auxins, gibberellins, cytokinins, abscisic acids, vitamins, and amino acids [4,46–48]. Cyanobacteria can also add organic matter, synthesize and liberate amino acids and vitamins, reduce the content of soil oxidizable matter, provide oxygen to the submerged rhizosphere, ameliorate salinity, buffer the pH, solubilize phosphates, and increase the efficiency of fertilizer use in crop plants [46]. Cyanobacteria such as *Nostoc* and *Microcoleus* can form associations with cycads [49] and *Gunnera* [50] and can fix nitrogen [45], either as free-living organisms or in association with host plants, in which they reside in specific tissues. Cyanobacteria such as *Calothrix* and *Anabena* can be used as biofertilizers [51]. Rhizospheric cyanobacteria are still insufficiently studied, although interest in their use for soil fertilization and plant-growth promotion is increasing steadily [4]. The established abundance of cyanobacteria in the

amaranth rhizosphere requires further and deeper research on the part they play in plant vital activity.

To determine the contribution of different plant species (or cultivars) to the formation of their rhizosphere community in a particular soil, we compared their taxonomic composition, identifying shared and unique taxa (Figure 4). It was revealed that only about a third of taxa were shared between all four amaranths studied, and also about a third of taxa were unique. The contribution of *A. tricolor* cv. Valentina and *A. cruentus* cv. Dyuimovochka to the selection of cyanobacteria in their rhizosphere was confirmed. In addition, we revealed that two cultivars of *A. caudatus* selected different unique taxa in their rhizosphere, which suggests the cultivar-specific formation of rhizosphere microbiomes by amaranths (Tables S2–S5). No debated that plant root exudates are the principal connecting link between plant and plant-root-associated microbial communities [1,14,16]. In turn, the composition of root exudates is determined by plant physiology, which is different among plant species and even cultivars.

Current views hold that the relative content of individual taxa in the microbial community is a biological indicator of the soil status—for example, heavy metal pollution [52]. The urban soils used in our experiment were polluted by heavy metals. Many taxa identified in the amaranth rhizosphere microbiomes had previously been described as resistant to heavy metals or as oil-degraders, and they may be important for the resistance of amaranth to the human-caused pollution of soil. These taxa include (1) members of Proteobacteria, such as the genera *Pseudomonas* [18,53,54], *Novosphingobium* [55,56], *Sphingomonas* [57], *Rhizobium* [58,59], and *Massilia* [18,60]; (2) members of Actinobacteriota, such as *Mycobacterium*, *Nocardiodes*, *Streptomyces* [61], and the family Gaiellaceae [62]; (3) members of the Cyanobacteria phylum, such as the genus *Microcoleus* [63]; and (4) members of Firmicutes, such as the genus *Bacillus* [18]. In the rhizosphere of the amaranths used in this study, some of these taxa were present in large numbers (the genera *Gaiella*, *Nocardiodes*, *Microcoleus*, *Novosphingobium*, and *Pseudomonas*), whereas others were found in minor or single samples. Of note, increased numbers of members of Gaiellaceae had previously been found by Sun et al. [62] in the metal-polluted rhizosphere of crops. Those authors found a significant correlation between the increased abundance of Gaiellaceae-related bacteria and various metals and metalloids, and they concluded that these bacteria play a potentially active ecological part in the interaction with soil metals.

Rhizospheric microbial communities exert a great effect on the phytoremediation of metal-contaminated soils, not only by changing the bioavailability of metals [64], but also by promoting plant growth under pollutant stress through the fixation of nitrogen; production of phytohormones (indole-3-acetic acid, cytokinins, and gibberellins), siderophores, and enzymes (1-aminocyclopropane-1-carboxylate deaminase); and transformation of nutrients [65–67]. However, only 2–5% of rhizosphere microorganisms contribute to plant growth, and plants naturally select these beneficial microorganisms, which help them to grow and survive, especially under unfavorable conditions [3]. In the rhizospheric microbial communities of the amaranths used in this study, alongside cyanobacteria, we identified other groups of rhizobacteria with plant-growth-promoting potential (Figure 3). These included *Bacillus* (Bacillaceae, [68,69]), *Sphingomonas* (Sphingomonadaceae, [70]), *Streptomyces* (Streptomycetaceae, [69,71]), *Pseudomonas* (Pseudomonadaceae, [72]), and *Rhizobium* (Rhizobiaceae, [73]). Although the percentages of these taxa varied between cultivars and soils, their mere presence indicates that the rhizosphere microbiomes have the potential to promote amaranth growth on all urban soils tested.

We were unable to reveal any significant influence of the soils on the taxonomic structure of the amaranth rhizospheric microbiomes. Only about one third of the taxa identified in the cultivar rhizosphere microbiomes were common to all soil samples (Figure 4), which may indicate the maintenance of a specific plant microbiome, regardless of the kind of soil. We supposed that peculiarities and differences in the plant root exudate composition among plant cultivars had stronger differences than among soils, which resulted in the formation of different rhizosphere microbiomes depending more on the plants.

It is known that the composition of rhizosphere microbial communities is determined not only (and possibly not so much) by the soil type, but also by the plants. In our case, all soils studied were Greyic Phaeozems with slight differences, and all plants tested were Amaranthus belonging to different species. The compositions of the studied rhizosphere communities were quite similar, but the analysis of the obtained results revealed a greater influence of plants than soils.

For the final characterization of the amaranth core rhizobiomes, additional studies of the endosphere microbiome of each plant are required.

5. Conclusions

Limited data are available on the structure of the rhizosphere microbial communities of *Amaranthus* spp. We conducted a comparative study on the rhizosphere microbiomes of four *Amaranthus* cultivars (*A. tricolor* cv. Valentina, *A. cruentus* cv. Dyuimovochka, *A. caudatus* cv. Bulava, and *A. caudatus* cv. Zelenaya Sosulka). The cultivars were grown in an unpolluted (background) soil and in three polluted soils with polyelemental anomalies. The *A. tricolor* rhizosphere microbiome was characterized for the first time. The taxonomic structure of the amaranth rhizosphere microbiomes was represented by the dominant bacterial phyla Actinobacteriota, Proteobacteria, and Chloroflexi and by the phyla Cyanobacteria, Acidobacteriota, Planctomycetota, and Bacteroidota. In the taxonomic profile of the rhizobiomes of two *Amaranthus* species (*A. tricolor* cv. Valentina and *A. cruentus* cv. Dyuimovochka), there was a significant abundance of OTUs associated with the Cyanobacteria phylum. Bacterial taxa were identified that are possibly selected by amaranth plants during their coexistence and that may be important for plant adaptation to various habitat conditions, including polluted soils. The targeted enrichment of the amaranth rhizosphere microbiomes with members of these taxa could be useful for improving the efficacy of amaranth use for agricultural and remediation purposes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13030759/s1, Table S1: Characteristics of Amaranthus cultivars used in the research; Table S2: Taxonomic structure of the rhizosphere microbial communities of the amaranths studied; Tables S3–S6: Unique rhizosphere taxa occurred in at least two different soils planted with amaranths studied; Figure S1: Dependence of the number of detected taxa (OTUs) on the number of sequences obtained for amaranth rhizosphere soil samples.

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