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# Soil Bacterial Community and Soil Enzyme Activity Depending on the Cultivation of *Triticum aestivum*, *Brassica napus*, and *Pisum sativum* ssp. *arvense*

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**Abstract:** This study aims to determine the effects of crops and their cultivation regimes on changes in the soil microbiome. Three plant species were selected for the study: *Triticum aestivum*, *Brassica napus*, and *Pisum sativum* ssp. *arvense*, that were cultivated in soils with a similar particle size fraction. Field experiments were performed on the area of the Iławski Lake District (north-eastern Poland) at the Production and Experimental Station 'Bałcyny' (53°35′49″ N, 19°51′20″ E). In soil samples counts, organotrophic bacteria and actinobacteria were quantified, and the colony development index (CD) and ecophysiological diversity index (EP) were computed. In addition, a 16S amplicon sequencing encoding gene was conducted based on the hypervariable region V3–V4. Further analyses included an evaluation of the basic physiochemical properties of the soil and the activities of dehydrogenases, catalase, urease, acid phosphatase, alkaline phosphatase, arylsulfatase, and β-glucosidase. Analyses carried out in the study demonstrated that the rhizosphere of *Triticum aestivum* had a more beneficial effect on bacteria development than those of *Brassica napus* and *Pisum sativum* ssp. *arvense*, as indicated by the values of the ecophysiological diversity index (EP) and OTU abundance calculated for individual taxa in the soils in which the studied crops were grown. More OTUs of the taxa *Alphaproteobacteria*, *Gammaproteobacteria*, *Clostridia*, *Sphingomonadales*, *Rhodospirillales*, *Xanthomonadales*, *Streptomycetaceae*, *Pseudonocardiaceae*, *Acetobacteraceae*, *Solibacteraceae*, *Kaistobacter*, *Cohnella*, *Azospirillum*, *Cryptosporangium*, *Rhodoplanes*, and *Saccharopolyspora* were determined in the bacteriome structure of the soil from *Triticum aestivum* cultivation than in the soils from the cultivation of *Brassica napus* and *Pisum sativum* ssp. *arvense*. Also, the activities of most of the analyzed enzymes, including urease, catalase, alkaline phosphatase, β-glucosidase, and arylsulfatase, were the higher in the soil sown with *Triticum aestivum* than in those with the other two plant species.

**Keywords:** bacteria; diversity; OTU; enzymes activity

## 1. Introduction

Growing attention is being paid to the proper functioning of ecosystems [1,2]. This is due to numerous initiatives which have been undertaken globally to make the population aware of the need to protect soils and for their sustainable management. One of the first of these initiatives was the announcement of the European Soil Health Card, which has made both the population and policy makers aware of the significant role of the soil environment, and has introduced a new term, 'environmental services', which was later changed to 'ecosystem services' [3]. The concept of soil quality and health, often used interchangeably, is becoming increasingly recognized worldwide [4–6].

Legaz et al. [6] and Bünemann et al. [7] define 'soil quality' as the capability of soil to function in the framework of ecosystems, and also as land management and promotion of the biodiversity and health of plants and animals. According to Cardoso et al. [5] and Veum et al. [8], the term 'soil health' highlights the fact that soil is a live and dynamic being whose functions are determined by the diversity of living organisms. Therefore, the physical, chemical, and biological properties of soil may change according to biotic and abiotic factors, which consequently affect its functions and ecosystemic services [3,9].

Apart from elements such as profile morphology, physical and chemical properties, and microclimate, the microbiological activity of soil is another principal factor affecting its fertility [10]. Microorganisms and enzymes determine the transformation of organic matter introduced into soil, influence the humification process, and play a key role in the biogeochemical cycles of many macro- and micro- elements [7]. They are key participants in most of the important cycles, including carbon, nitrogen, phosphorus, and sulfur circulation. It is mainly the biogeochemical cycles of these elements that determines the quality of the natural environment, including the soil environment [9]. The role of microbes is invaluable in the transformation of postharvest residues, as well as natural and organic fertilizers [11,12], the detoxication of organic contaminants in soil [13,14], minimizing the prevalence of pests and pathogens [11], and finally, in establishing symbiotic systems with plants [15]. The enormous importance of microorganisms for the soil environment and their high metabolic activity are confirmed by the fact that the microbial biomass of soil accounts for approximately 85% of the total biomass of all living organisms colonizing this environment [11].

The main factors which affect the development of soil microorganisms include the abundance of organic and mineral colloids in the soil, the climate and microclimate, oxidation associated with humidity status [16–19], soil pH [20,21], soil tillage and plant cultivation systems [22–24], and fertilization systems [25]. An inseparable element in this case is soil temperature, which is affected by the climate and microclimate [16,26,27]. Significant are also contamination with heavy metals [28,29], various hydrocarbons [30–32], plant protection agents [33–35], and dioxins, as well as the salinity level [36], all of which determine the proliferation of various microbial communities for which an increase their diversity may reduce soil fertility and, ultimately, influence soil productivity [37].

All the aforementioned parameters determine the development of not only microorganisms, but plants as well. The connection between both of these groups of organisms is of the utmost importance, e.g., in the case of symbioses of certain bacteria and fungi species with plants. Noteworthy are also the microorganisms that live in association with plants and produce growth hormones, bind atmospheric nitrogen, and protect plants against pathogens. An important role is also ascribed to the microbes classified as plant growth-promoting rhizobacteria (PGPR) [38]. On the other hand, apart from bacteriorrhiza, mycrorrhiza, and microbe associations with plants, we cannot observe a beneficial effect of root secretions on microorganisms or a positive impact of root systems on the physical properties of soil, as it serves the function of drainage, which is essential for appropriate soil oxygenation [27,39]. Root secretions, which may contain organic acids, amino acids, carbohydrates, vitamins, and metal ions, modify the microenvironments of the rhizosphere [40]. They release ions, oxygen, water, and carbon-containing compounds [41]. They may both stimulate and inhibit the development of a soil microbiome [42], and act both as repellents [43] and attractants [44]. Volatile organic compounds (VOCs) emitted by soil microorganisms can affect root growth. Bacterial volatile compounds (BVCs) are used as a source of nutrients and information in plant–bacterium interactions [15]. Such interactions are the strongest in the rhizosphere [7,45], which is colonized by 10 to 100 times more microorganisms than the sphere that is distant from plant roots [46]. The beneficial bacteria of the rhizosphere compete with other microorganisms for organic compounds and colonize plant roots [47]. Microorganisms colonizing this microecosystem are affected by the root secretions of particular plant species and form specific microbial communities [48]. The 16S amplicon sequencing analysis, a small subunit ribosomal ribonucleic acid (SSU rRNA), is an important element in biological quality assessments of soil, as it may provide answers about the response of individual taxa to variable factors in different agricultural

ecosystems [49]. Despite the severity of the problem, investigations into the effects of plants on soil bacteria based on next-generation methods are still scarce.

The complex nature of the active rhizosphere has become a premise for undertaking a study aimed at determining the effect of a crop and its cultivation regime on changes in the soil microbiome. Three plant species were used in the study: winter wheat, winter rape, and field pea; these species were cultivated on soil with a similar fraction size. Analyses were performed to determine the structure and diversity of microorganisms and the activity of the soil enzymes participating in the metabolism of carbon, nitrogen, phosphorus, and sulfur. The coupled use of microbiological, biochemical, and physicochemical parameters form the basis for a better understanding of the interactions between the rhizosphere, microorganisms, and plants, which is extremely important from the viewpoint of soil fertility.

## 2. Materials and Methods

### *2.1. Sampling Area*

Field experiments were performed in the area of the Iławski Lake District (north-eastern Poland) at the Production and Experimental Station 'Bałcyny' (53°35'49" N, 19°51'20" E) of the University of Warmia and Mazury in Olsztyn (Poland). The Lake District stretches over 4230 km$^2$ and is one of the least polluted regions of Poland, called the Green Lungs of Poland. It is characterized by young glacial relief resulting from Pleistocene glaciations, and has a temperate warm transition climate due to the clash of continental and oceanic climates. According to the Institute of Meteorology and Water Management State Research Institute (IMGW), in this area in 2018, the average annual air temperature was +9.0 °C, with August being the warmest month (+20.4 °C), and February the coldest (−4.1 °C). The sunshine duration ranged from 240 h in July to 40 h in January. The growing season spanned approximately 206 days, and snow cover persisted for 70 days. The annual sum of precipitation was 550 mm, with the highest amount of precipitation being recorded in July (140.7 mm) and the lowest in February (2.0 mm). The average air humidity was approximately 81%.

The rhizosphere of the three following plant species was chosen for the study: winter wheat (*Triticum aestivum*) of Julius cultivar, winter rape (*Brassica napus*) of Garou cultivar, and field pea (*Pisum sativum* subsp. *arvense*) of Milwa cultivar. The plants were grown simultaneously on three plots, each with a surface area of 20,000 m$^2$. The fraction size distribution of the soil used in the experiment was established and is presented in Table 1.

**Table 1.** The granulometric composition of the soil used in the experiment.

| Plants | Particle Diameter, mm | | | | Granulometric Subgroups |
|---|---|---|---|---|---|
| | < 0.002 | 0.002–0.020 | 0.020–0.050 | 0.050–2.0 | |
| | % | | | | |
| *Triticum aestivum* | 3.9 $^a$ | 18.1 $^a$ | 17.7 $^a$ | 60.2 $^a$ | sandy loam |
| *Brassica napus* | 3.8 $^a$ | 16.9 $^b$ | 15.0 $^b$ | 64.4 $^a$ | sandy loam |
| *Pisum sativum* ssp. *arvense* | 4.0 $^a$ | 16.0 $^b$ | 14.0 $^b$ | 66.0 $^a$ | sandy loam |

Homogeneous groups denoted with letters (a, b) were calculated separately for each of the properties.

Winter rape was used as the previous crop of winter wheat, whereas winter wheat was used as the previous crop of winter rape and field pea. Winter wheat was sown on October 2, 2017, and the crop was harvested on August 2, 2018; winter rape was sown on August 19, 2017, and the crop was harvested on July 20, 2018, while pea was sown on April 7, 2018, and the crop was harvested on July 15, 2018. All plants were grown in accordance with the recommended technology for cultivating these plant species. All soil samples were collected the next day after the last crop harvest. In the case of winter wheat, fertilization was as follows: 66 kg N ha$^{-1}$, 20 kg P ha$^{-1}$, and 36 kg K ha$^{-1}$. The nitrogen dose was divided into three portions that were administered at stage 23 of plant development according

to the BBCH scale, in the form of an aqueous solution of $NH_4NO_3$ and $CO(NH_2)_2 + H_2O$, and at stages 32 and 52 according to the BBCH scale, in the form of $NH_4NO_3$. Potassium and phosphorus were applied once before sowing. Potassium was used in the form of KCl and phosphorus in the form of $Ca(H_2PO_4)_2$. In the case of winter rape, fertilization was as follows: 75.8 kg N $ha^{-1}$, 40 kg P $ha^{-1}$, and 60 kg K $ha^{-1}$. Nitrogen dose was divided into three portions that were administered before sowing in the form of $CO(NH_2)_2$ and at stages 30 and 50 of plant development according to the BBCH scale, in the form of $NH_4NO_3$. Phosphorus and potassium were applied once before sowing in the form of $Ca(H_2PO_4)_2$ and KCl, respectively. No fertilization was applied in the case of field pea. The plant density of winter wheat per 1 $m^2$ was 400 plants; that of winter rape was 45 plants, and that of field pea, 90 plants. Soil was cultivated in a conventional (ploughing) manner in the case of all of the studied plant species.

The area of 20,000 $m^2$ was divided into five plots, each measuring 4000 $m^2$. After the harvest, 15 soil samples were collected at random from a depth of 0–20 cm from each plot using the zigzag sampling method. All soil samples collected from each plot were homogenized and combined into one collective sample. Hence, a total of five samples of soil from the cultivation of each plant species studied were used for microbiological and biochemical analyses. Soil samples were collected using an Enger-Riehm probe.

### *2.2. Methodology of Microbiological Analyses*

#### 2.2.1. Bacterial Count

Counts of organotrophic bacteria (Org) and actinobacteria (Act) were determined in individual soil samples from the cultivation of each of the studied plant species (winter wheat, winter rape, field pea) with the serial dilution method, in three replications. Microbial counts were performed according the media and procedure described by Borowik et al. [50]. The composition of the microbiological media was as follows: organotrophic bacteria (Bunt and Rovira medium): agar medium (peptone 1.0 g, yeast extract 1.0 g, $(NH_4)_2SO_4$ 0.5 g, $CaCl_2$, $K_2HPO_4$ 0.4 g, $MgCl_2$ 0,2 g, $MgSO_4$ $7H_2O$ 0.5 g, salt Mo 0.03 g, $FeCl_2$ 0.01 g, agar 20.0 g, soil extract 250 $cm^3$, distilled water 750 $cm^3$, pH 6.6–7.0; Actinomycetes (Parkinson medium): soluble starch 10.0 g; casein 0.3 g; $KNO_3$ 2.0 g; NaCl 2.0 g; $K_2HPO_4$ 2.0 g; MgSO4·7H2O 0.05 g; $CaCO_3$ 0.02 g; $FeSO_4$ 0.01 g; agar 20.0 g; $H_2O$ 1 $dm^3$; 50 $cm^3$ aqueous solution of nystatin 0.05%; 50 $cm^3$ aqueous solution of actidione 0.05%; pH 7.0. Microorganisms were cultured on petri dishes at a temperature of 28 °C. The colony forming units (cfu) were counted every day for ten days using a colony counter. Counts of bacteria and actinobacteria isolated from the rhizosphere of particular plant species allowed us to compute the colony development index (CD), the ecophysiological diversity index (EP), and the microbial growth indexes at specific time intervals (Ks). Descriptions of the indexes and calculations are presented in De Leij et al. [51] and Tomkiel et al. 2015 [52]. The CD and EP were calculated from the following formulas [51]: CD = [N1/1 + N2/2 + N3/3 . . . N10/10] × 100, where N1, N2, N3, ...N10 are the sum of ratios of the number of colonies of microorganisms identified on particular days (1, 2, 3, ...10) to the total number of colonies identified throughout the study period, and EP = −Σ(pi·log10 pi), where pi is the ratio of the number of colonies of microorganisms identified on particular days to the total number of colonies identified throughout the study period. KS was determined with the use of the following formula [52]: Ks = (Nx/Nt) × 100, where Ks is the percentage of microbes cultured at specific time intervals, Nx is the number of colonies cultured at two-day intervals counted for ten days, and Nt is the total number of colonies cultured within ten days.

#### 2.2.2. DNA Extraction and Bioinformatic Analysis of Specific Bacterial Taxa

DNA was isolated from the rhizosphere of the three plant species using a "Genomic Mini AX Bacteria+" kit. According to the manufacturer's instructions, the PCR reaction was carried out using Q5 Hotstart Hight-Fidelity DNA Polymerase (NEBNext). In the case of amplicon libraries, the data

were pooled and normalized in the final stage of the library preparation. Afterwards, a 16S amplicon sequencing encoding gene was conducted for each DNA sample based on the hypervariable region V3–V4. The selected region was amplified and the library was developed using specific sequences of 341F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′) primers. The 16S library sequencing was performed on a MiSeq Reporter sequencer ver. 2.6 in the paired-end (PE) technology, 2 × 250 bp, using a v2 Illumina kit. The Illumina 16S Metagenomics workflow with MiSeq Reporter (MSR) software (San Diego, CA, USA) and Greengenes v13_5 software (South San Francisco, CA, USA) were used [53]. Preparation of the reference database included filtering low-quality, degenerate, and incomplete sequences, and then combining paired sequences based on the reference sequence database. The algorithm Uclust was assigned taxonomy, taking into account the ChimeraSlayer algorithm. Sequencing was performed by the Genomed S.A. Company (Warsaw, Poland).

### *2.3. Methodology of Biochemical Analyses*

The activities of dehydrogenases (EC 1.1), catalase (EC 1.11.1.6), alkaline phosphatase (EC 3.1.3.1), acid phosphatase (EC 3.1.3.2), arylsulfatase (EC 3.1.6.1), β-glucosidase (EC 3.2.1.21), and urease (EC 3.5.1.5) were determined in triplicate in individual soil samples from the cultivation of each plant species studied. A detailed procedure of enzymatic activity determination is provided by Borowik et al. [50] and Borowik et al. [13]. The substrates used to determine the enzymatic activity included aqueous solutions of the following chemical compounds: 2,3,5-triphenyl tetrazolium chloride (TTC) for dehydrogenases, urea for urease, disodium 4-nitrophenyl phosphate hexahydrate (PNP) for phosphatases, potassium-4-nitrophenylsulfate (PNS) for arylsulfatase, and 4-nitrophenyl-β-D-glucopyranoside (PNG) for β-glucosidase. The activities of all enzymes except for catalase were determined by measuring the reaction product extinction using a Perkin-Elmer Lambda 25 spectrophotometer (Massachusetts, USA). Catalase activity was analyzed with the titration method. The activity of dehydrogenases was expressed in μmol TFF (tri-fenylformazane), that of catalase in mol $O_2$, that of alkaline phosphatase, acid phosphatase, arylsulfatase, and β-glucosidase in mmol PN (p-nitrophenol), and that of urease in mmol N-$NH_4$ $kg^{-1}$ soil d.m. $h^{-1}$.

### *2.4. Methodology of Chemical and Physicochemical Analyses of Soil*

Fraction size of soil from cultivation of winter wheat, winter rape, and field pea was measured using a Malvern Mastersizer 2000 Laser Diffraction. The physicochemical analyses included the determination of soil pH in 1 mol KCl $dm^{-3}$ [54], hydrolytic acidity (HAC), and the sum of exchangeable base cations (EBC) according to the method outlined by Carter and Gregorich [55], whereas the chemical analyses included the determination of organic carbon content according to the method outlined by Tiurin [56], total nitrogen content according to the method outlined by Kjeldahl [57], available phosphorus and potassium contents according to the method outlined by Egner et al. [58], magnesium content with the atomic absorption spectrometry (AAS) according to the method outlined by Schlichting et al. [59], and exchangeable cations, $K^+$, $Ca^{2+}$, $Mg^{2+}$, and $Na^+$ according to the ISO 11260 [60] procedure. Determinations were carried out in the following solutions: HAC in 1 mol $(CH_3COO)_2Ca$ $dm^{-3}$, EBC in 0.1 mol HCl $dm^{-3}$; organic carbon in a mixture of 0.13 mol $K_2Cr_2O_7$ and concentrated $H_2SO_4$ in the ratio of 1,1; total nitrogen—wet mineralization in concentrated $H_2SO_4$; available phosphorus and potassium in a mixture of 0.03 $(CH_3CHOHCOO)_2Ca{\cdot}H_2O$ and 0.02 mol HCl; magnesium in 0.012 mol $CaCl_2{\cdot}6H_2O$; and exchangeable cations: $K^+$, $Ca^{2+}$, $Mg^{2+}$, and $Na^+$ in 1 mol $CH_3COONH_4$.

### *2.5. Statistical Analysis*

Counts of microorganisms, the activity of soil enzymes, and the physicochemical and chemical properties of soil were developed statistically using the Statistica 13.1 package (StatSoft, Tulsa, OK, USA) [61]. The results were compared with ANOVA and then with a post hoc Tukey test (HSD). Homogenous groups were computed at $p = 0.05$. The data meet assumptions of normality and similar

variance. Analyses of metagenomic profiles were performed on the STAMP 2.1.3. software (Australian Centre for Ecogenomics, School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia) [62] and the Circos 0.68 package (Canada's Michael Smith Genome Sciences Center, Vancouver, British Columbia V5Z 4S6, Canada) [63]. The abundance of each family and genus is directly proportional to the width of each band connecting bacterial taxa with an appropriate soil sample from the cultivation of: B—*Brassica napus*, T—*Triticum aestivum*, and P—*Pisum sativum* ssp. *arvense*. A specified color is assigned to each family and genus of bacteria. The outer ring represents the total percentage of 16S sequences, whereas the inner ring represents the number of 16S amplicon sequences assigned to a given taxon.

The relative abundance of bacteria was calculated using a two-sided test of statistical hypotheses, i.e., the G-test (w/Yates') + Fisher's, with the method of intervals confidence Asymptotic with CC [62]. The relative abundance of bacteria was visualized with the use of sequences whose percentage contribution was higher than 1%.

## 3. Results

### 3.1. Physicochemical and Chemical Properties of Soil

The pH value of soil from the cultivation of winter wheat, winter rape, and field pea ranged from 5.4 to 6.1 (Table 2). The most favorable pH value, the highest sorption capacity, and saturation with base cations were demonstrated for the soil sown with winter wheat, which offered the most beneficial conditions for the development of this crop (Table 3). This soil was also the richest in available phosphorus and potassium, and had the closest-to-optimal carbon to nitrogen ratio (14.8).

**Table 2.** The soil acidity and cation exchange capacity.

| Plants | $pH_{KCl}$ | HAC | EBC | CEC | BS % |
|---|---|---|---|---|---|
| | | mmol (+) $kg^{-1}$ d.m. of soil | | | |
| *Triticum aestivum* | 6.1 $^{a}$ | 19.4 $^{b}$ | 174.8 $^{a}$ | 194.2 $^{a}$ | 90.0 $^{a}$ |
| *Brassica napus* | 5.4 $^{b}$ | 21.2 $^{a}$ | 64.0 $^{b}$ | 85.2 $^{b}$ | 75.1 $^{b}$ |
| *Pisum sativum* ssp. *arvense* | 5.6 $^{b}$ | 19.2 $^{b}$ | 46.7 $^{c}$ | 65.9 $^{c}$ | 70.9 $^{c}$ |

Homogeneous groups denoted with letters (a, b, c) were calculated separately for each property.

**Table 3.** The content of carbon, phosphorus, nitrogen, potassium, sodium, calcium, and magnesium in soil.

| Plants | Content | | Available Forms | | Interchangeable Forms | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $N_{total}$ | $C_{total}$ | P | K | Mg | K | Ca | Na | Mg |
| | g $kg^{-1}$ d.m. of Soil | | mg $kg^{-1}$ d.m. of Soil | | | | | | |
| *Triticum aestivum* | 1.0 $^{a}$ | 14.8 $^{a}$ | 69.1 $^{a}$ | 224.1 $^{a}$ | 53.0 $^{b}$ | 272.0 $^{a}$ | 500.0 $^{b}$ | 20.0 $^{a}$ | 83.3 $^{b}$ |
| *Brassica napus* | 0.8 $^{c}$ | 13.8 $^{b}$ | 59.4 $^{b}$ | 128.7 $^{b}$ | 45.0 $^{c}$ | 184.0 $^{b}$ | 533.3 $^{a}$ | 20.0 $^{a}$ | 59.5 $^{c}$ |
| *Pisum sativum* ssp. *arvense* | 0.9 $^{b}$ | 14.3 $^{ab}$ | 43.1 $^{c}$ | 107.9 $^{c}$ | 62.0 $^{a}$ | 176.0 $^{b}$ | 350.0 $^{c}$ | 20.0 $^{a}$ | 92.9 $^{a}$ |

EBC—exchangeable base cations, HAC—hydrolytic activity, CEC—cation exchange capacity, BS—base saturation. Homogeneous groups denoted with letters (a, b, c) were calculated separately for each property.

### 3.2. Counts and Diversity of Microorganisms

The best conditions for microorganism proliferation were offered by the soil from field pea cultivation (Figure 1). The number of organotrophic bacteria in the field pea rhizosphere was 30% higher than in the rhizospheres of winter wheat and winter rape, whereas the number of actinobacteria was 98% and 110% higher than in the respective rhizospheres. The total bacteria count in the rhizosphere of winter wheat, winter rape, and field pea was not necessarily reflected in the proliferation rate and diversity of particular microorganisms. The analysis of the colony development index (CD) values demonstrated the fastest development of organotrophic bacteria in the rhizosphere of filed pea, and

the fastest development of actinobacteria in the rhizosphere of winter wheat. Within the first four days, as many as 87% of the total organotrophs and 49% of the total actinobacteria grew in the soil from winter wheat cultivation (Figure 2). The values of the ecophysiological diversity index (EP) determined for organotrophic bacteria ranged from 0.887 (winter wheat rhizosphere) to 0.715 (field pea rhizosphere), and those calculated for actinobacteria from 0.843 (field pea rhizosphere) to 0.741 (winter rape rhizosphere). Analyses of CD and EP values allowed us to conclude that regardless of the plant species, higher values of both indices were determined for organotroph bacteria than for actinobacteria (CD = 34.27 vs. CD = 22.50; EP = 0.82 vs. EP = 0.79, respectively).

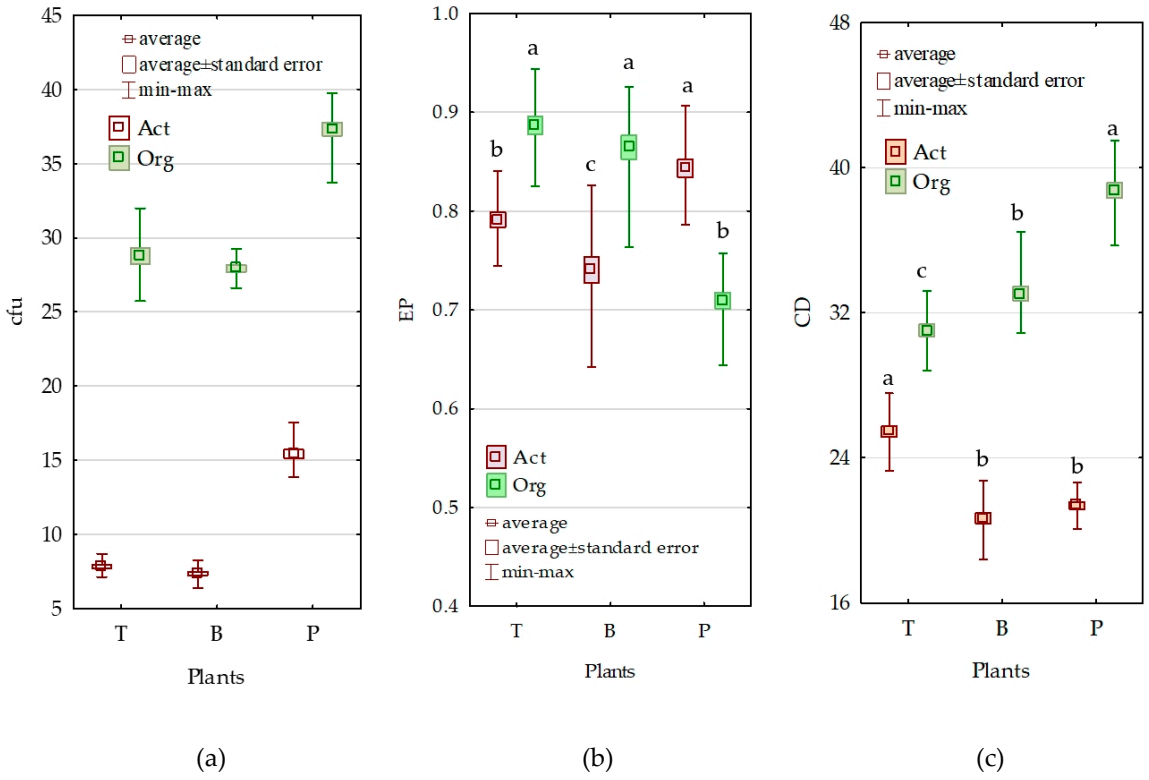


**Figure 1.** Microbiological properties of soil sown *Triticum aestivum* (T), *Brassica napus* (B), P*isum sativum* ssp. *arvense* (P); (**a**) count of soil bacteria; (**b**) physiological diversity index of bacteria (EP); (**c**) colony development index (CD). Homogeneous groups denoted with letters (a, b, c) were calculated separately for each microorganism. Org—organotrophic bacteria, Act—actinobacteria.

The crop and its cultivation regimes have a significant impact on the soil microbiome. The prevailing phylum in the rhizosphere of all plants turned out to be *Proteobacteria*, which accounted for 33.05% in the soil from winter rape cultivation and 35.24% in the soil from winter wheat (Figure 3). Other phyla identified in all soils were *Actinobacteria* and *Firmicutes.* The greatest differences in OTU numbers were determined in the case of phylum *Actinobacteria*, i.e., the OTU number in the soil from field pea cultivation was higher by 6.39% than in the soil from winter wheat cultivation, whereas the OTU number in the soil from winter rape cultivation was higher by 4.87% than in the soil from winter wheat cultivation.

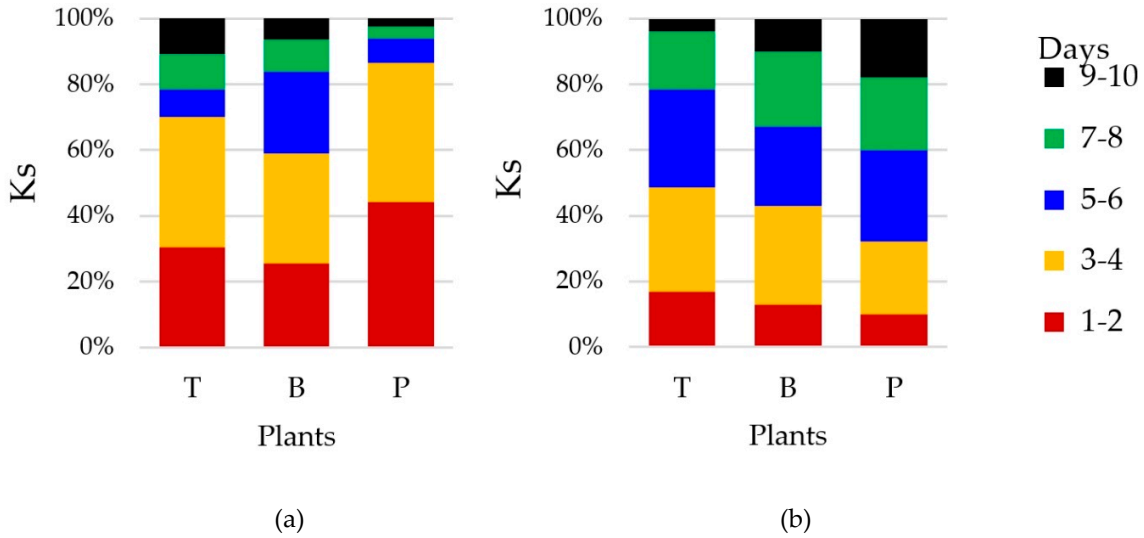


**Figure 2.** The growth and development index (Ks); (**a**) organotrophic bacteria; (**b**) actinobacteria.

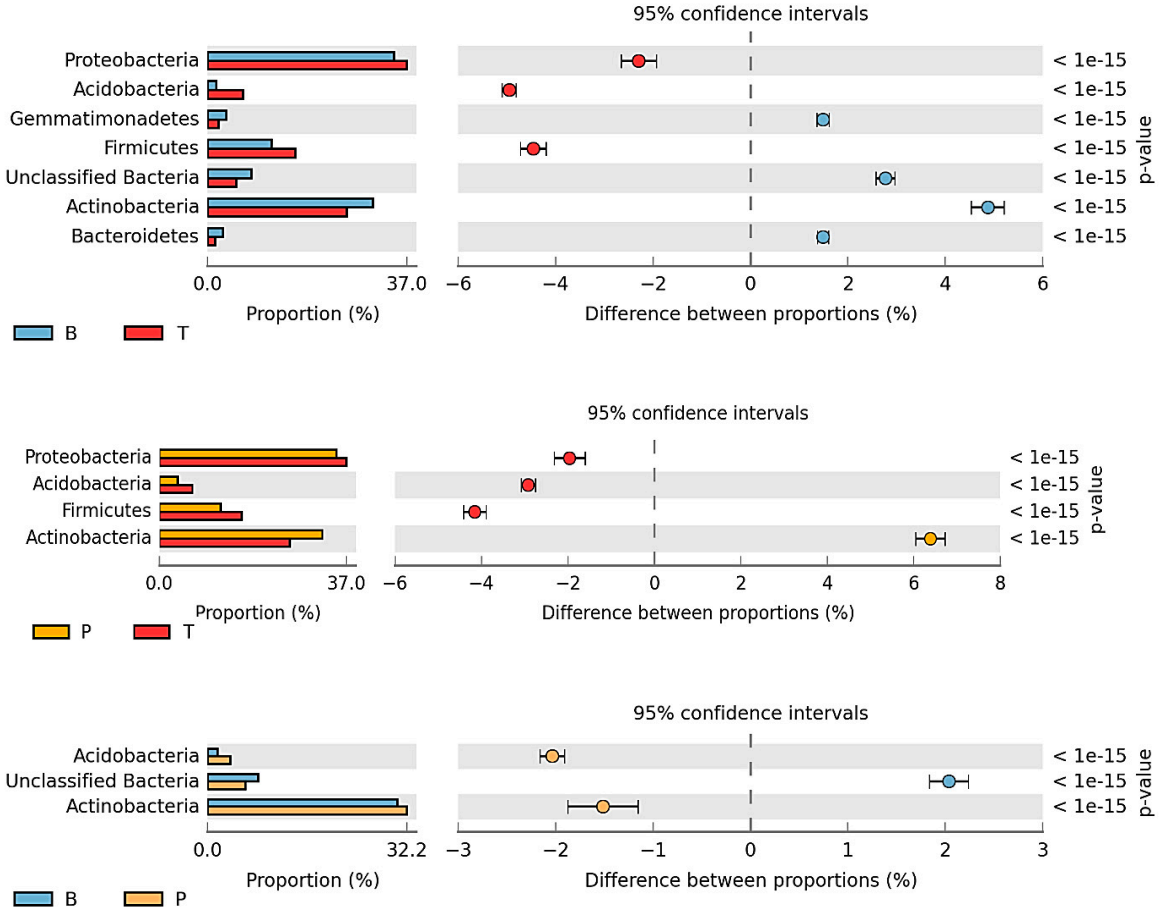


**Figure 3.** The relative abundance of dominant bacterial phyla in soil. Data on the number of readings greater than 1% of all OTUs. B—*Brassica napus*; T—*Triticum aestivum*; P—*Pisum sativum* ssp. *arvense*.

When comparing the effects of the discussed plant species on individual bacterial classes, it was found that the number of *Actinobacteria* OTUs determined in the field pea rhizosphere was higher by 6.82% than in the rhizosphere of winter wheat, and by 3.15% compared to the rhizosphere of winter

rape (Figure 4). The second largest phylum was that of *Alphaproteobacteria*, whose OTU abundance was similar in soils from the cultivation of all of the studied plants.

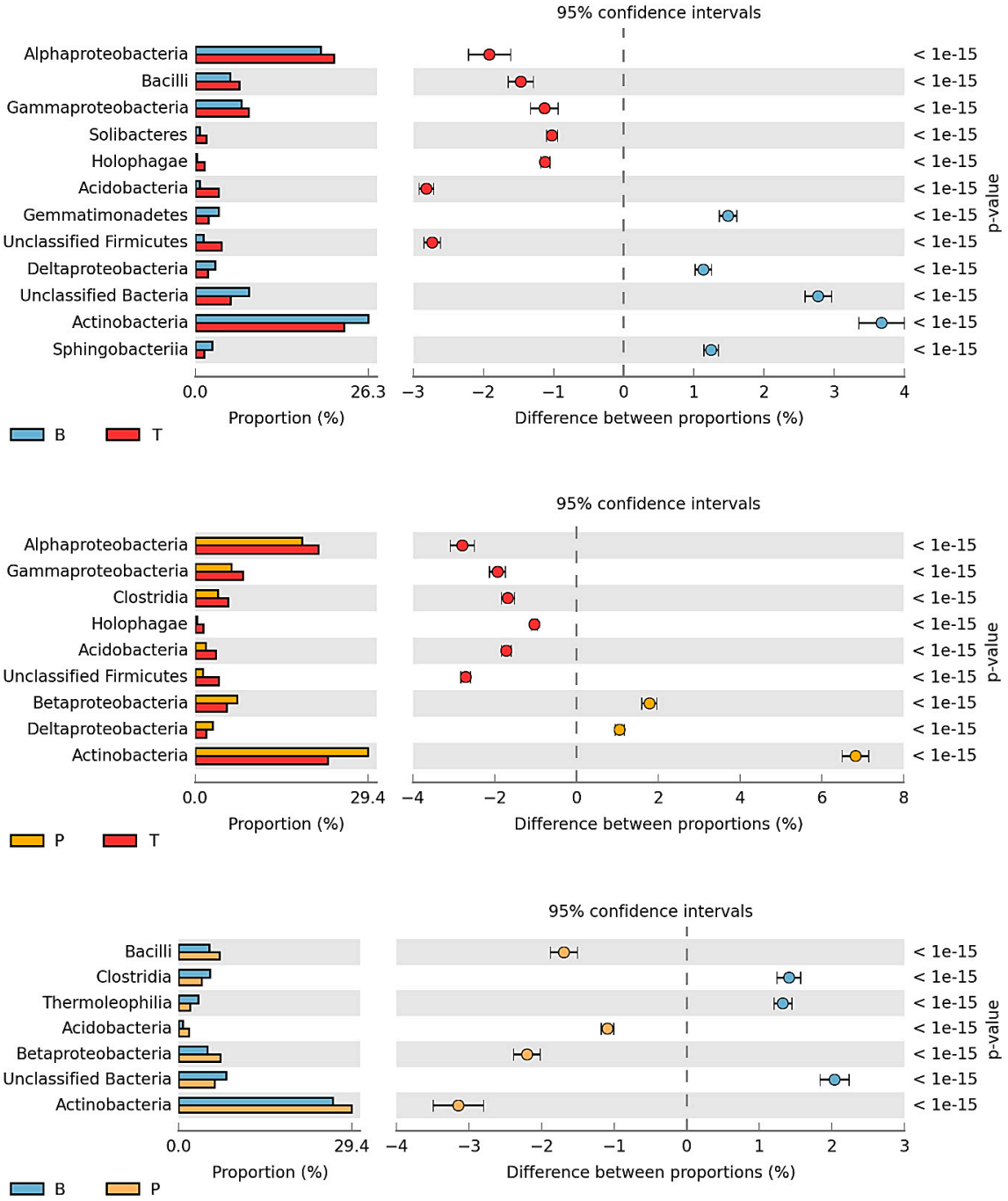


**Figure 4.** The relative abundance of dominant class bacteria in soil. Data on the number of readings greater than 1% of all OTUs. B—*Brassica napus*; T—*Triticum aestivum*; P—*Pisum sativum* ssp. *arvense*.

The greatest OTU abundance in order of rank, on average, and regardless of the cultivated plant species, was as follows: *Actinomycetales* (34,738 OTU) classified in the class *Actinobacteria*, phylum Actinobacteria; *Bacillales* (8083 OTU) classified in the class *Bacilli*, phylum *Firmicutes*; *Sphingomonadales* (11846 OTU); *Rhizobiales* (6897 OTU); *Rhodospirillales* (5897 OTU) classified in the class *Alphaproteobacteria*, phylum *Proteobacteria*; and for *Xanthomonadales* (5154) classified in the class *Gammaproteobacteria*, phylum *Proteobacteria* (Figure 5). Differences in the OTU abundance of particular classes in the soils from

the cultivation of field pea, winter wheat, and winter rape usually did not exceed 3%. An exception was the order *Actinomycetales*, whose OTU number in the soil from field pea cultivation was higher by 6.94% than in the soil from winter wheat cultivation, and by 3.25% than in the soil from winter rape cultivation.

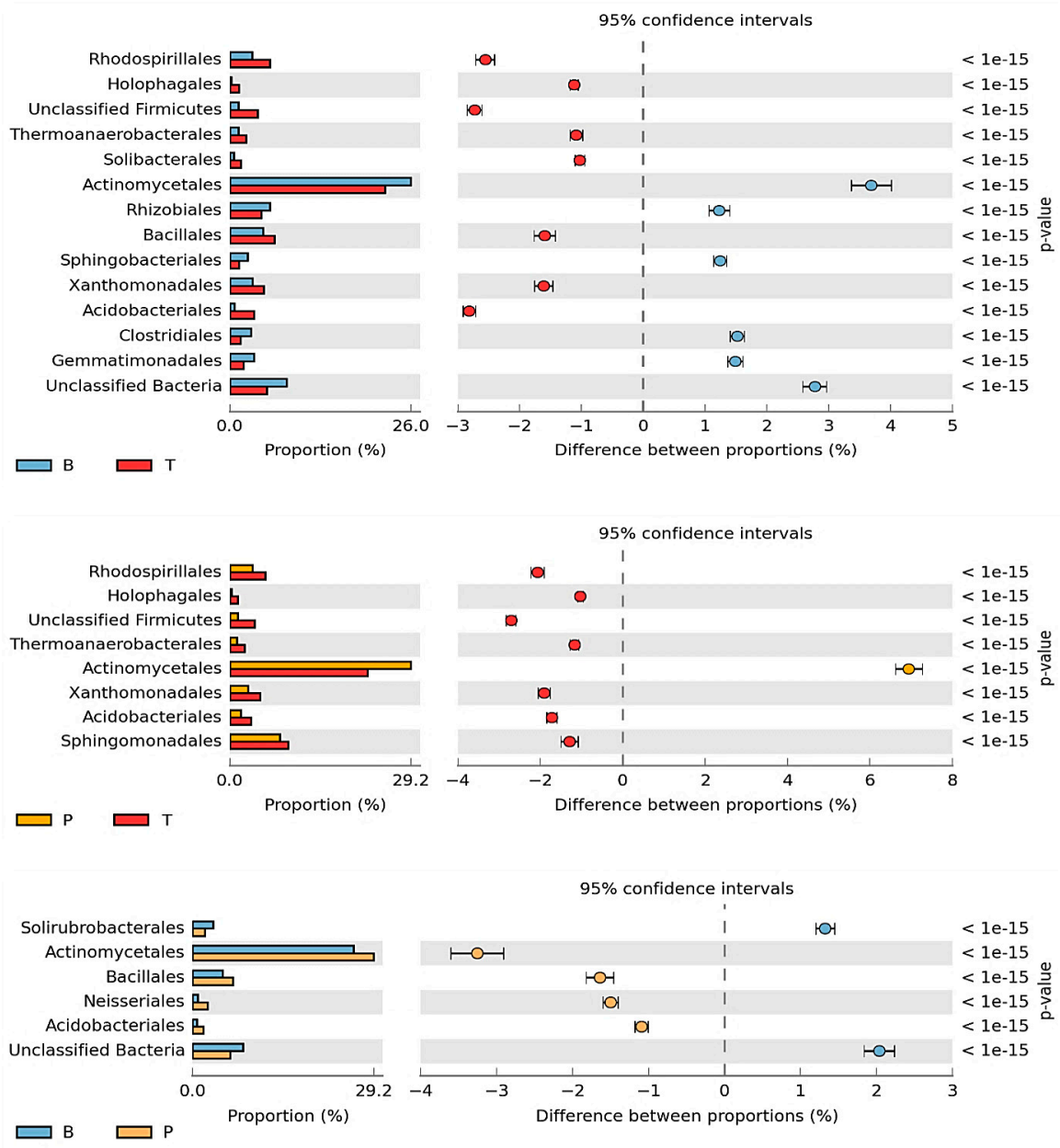


**Figure 5.** The relative abundance of dominant order bacteria in soil. Data on the number of readings greater than 1% of all OTUs. B—*Brassica napus*; T—*Triticum aestivum*; P—*Pisum sativum* ssp. *arvense*.

The cultivation of plants modified the soil microbiome also at the family level (Figure 6). In the soil sown with winter wheat, the highest numbers of OTUs were found for the following families:

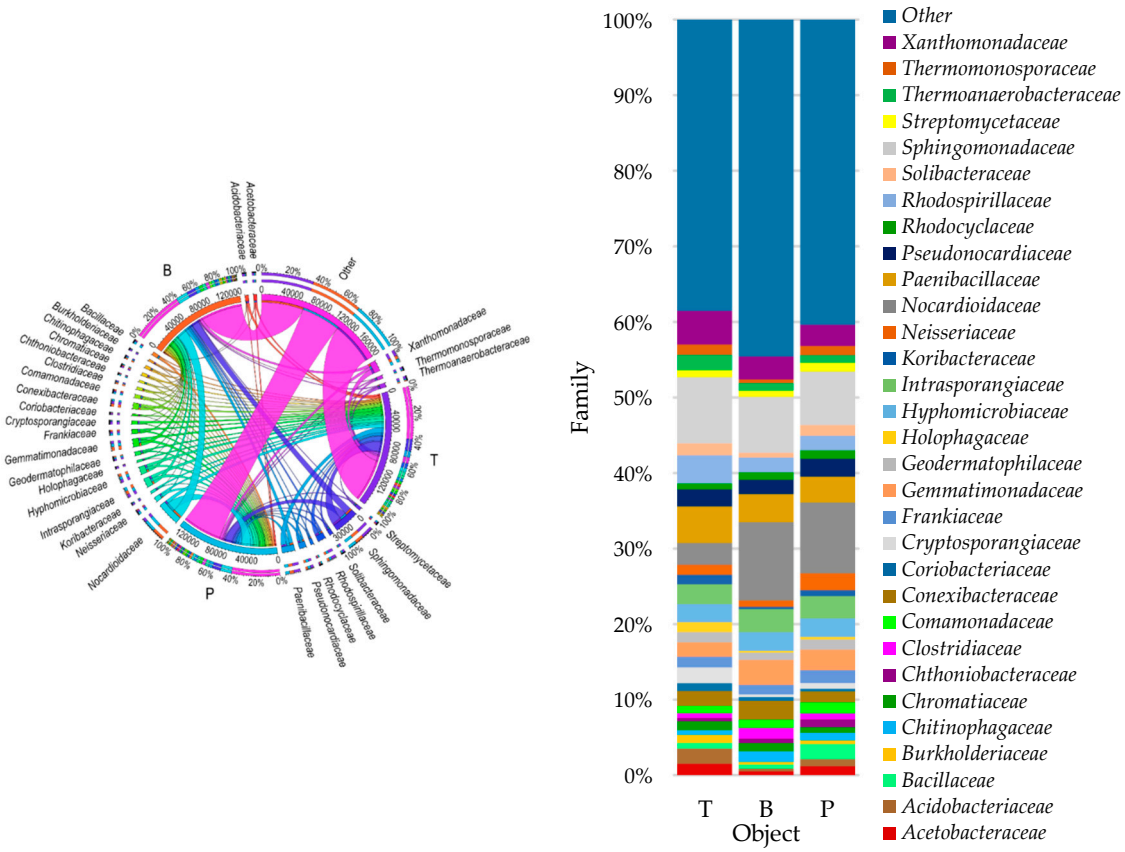


**Figure 6.** The relative abundance of dominant family bacteria in soil. Data on the number of readings greater than 1% of all OTUs. B—*Brassica napus*; T—*Triticum aestivum*; P—*Pisum sativum* ssp. *arvense*.

*Paenibacillaceae* (4.81%), *Xanthomonadaceae* (4.43%), and *Rhodospirillaceae* (3.71%), and in the soil from winter rape cultivation, for the families: *Nocardioidaceae* (10.37%), *Sphingomonadaceae* (7,40%), *Paenibacillaceae* (3,70%), *Gemmatimonadaceae* (3,31%), *Intrasporangiaceae* (3.06%), and *Xanthomonadaceae* (3.01%); and finally, in the soil from field pea cultivation, for the families: *Nocardioidaceae* (9.36%), *Sphingomonadaceae* (7.08%), and *Paenibacillaceae* (3.43%). At this taxonomic level, the greatest differences in the effects of individual plant species were noticeable in the abundance of OTUs from the *Nocardioidaceae*.

In the soils from the cultivation of winter rape and field pea, there were by 7.93% and 6.91% more OTUs, respectively, than in the soil from winter wheat cultivation. It is worthy of notice that the highest number of genera were classified in the soil sown with winter wheat, and the lowest in the soil sown with winter rape (Figure 7). In the soils from winter rape and field cultivation, the prevailing genus turned out to be the *Nocardioides*, which accounted for 7.45% and 6.63% of all identified bacteria, respectively, whereas in the soil from winter wheat cultivation, it was the *Kaistobacter* genus (6.32%).

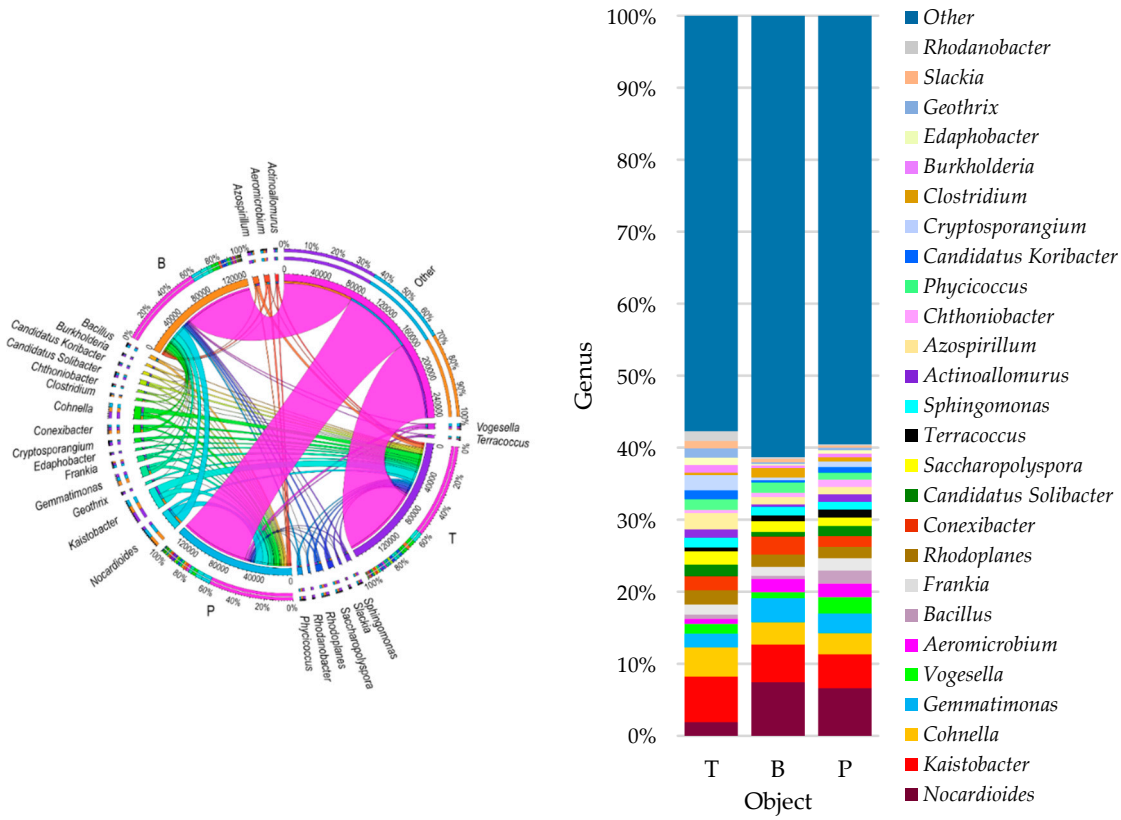


**Figure 7.** The relative abundance of dominant genus bacteria in soil. Data on the number of readings greater than 1% of all OTUs. B—*Brassica napus*; T—*Triticum aestivum*; P—*Pisum sativum* ssp. *arvense*.

### 3.3. Enzymatic Activity of Soil

Apart from the numbers and diversity of microorganisms, the biological activity of soil and, thus, its fertility, is determined by the activity of soil enzymes (Table 4). In the present study, the enzymatic activity of soil was significantly affected by the plant crop and its cultivation regimes. The highest activities of dehydrogenases and acid phosphatase were determined in the soil from field pea cultivation, and those of catalase, urease, alkaline phosphatase, β-glucosidase, and arylsulfatase in the soil from winter wheat cultivation. Especially big differences between plant species were noticeable in the activities of alkaline phosphatase and arylsulfatase, which, in the soil sown with winter wheat, were respectively 6.2 times and 2.7 times higher than in the soil from winter rape cultivation, and 3.5 times and 1.9 times higher than in the soil from field pea cultivation. This activity was strongly correlated with the productivity of individual plant species (Figure 8). Considering the total activity of all analyzed enzymes taking part in carbon, nitrogen, phosphorus, and sulfur metabolism, the highest biochemical activity was demonstrated for the rhizosphere of winter wheat, followed by that of field pea and winter rape.

The activities of catalase, urease, alkaline phosphatase, β-glucosidase, and arylsulfatase were positively significantly correlated with the sum of exchangeable base cations and with the degree of soil saturation with base cations (Figure 9). The sorption properties of soil had no effect on the activities of dehydrogenases and acid phosphatase. A similar response of the enzymes was observed regarding the contents of available and exchangeable phosphorus in the soil. The activities of dehydrogenases, urease, phosphatases (both, acid and alkaline), and arylsulfatase were also positively significantly correlated with total nitrogen content of the soil.

**Table 4.** The soil acidity and the cation exchange capacity.

| Plants | Deh | Cat | Pal | Pac | Aryl | Glu | Ure |
|---|---|---|---|---|---|---|---|
| | μM TFF M $O_2$ | mM PNP | mM PNP | | | | mM N-$NH_4$ |
| *Triticum aestivum* | 5.67 $^{b}$ | 3.45 $^{a}$ | 2.41 $^{a}$ | 3.29 $^{b}$ | 0.51 $^{a}$ | 1.16 $^{a}$ | 2.07 $^{a}$ |
| *Brassica napus* | 5.18 $^{c}$ | 3.25 $^{b}$ | 0.39$^{C}$ | 2.73 $^{c}$ | 0.19 $^{c}$ | 0.97 $^{b}$ | 0.94$^{C}$ |
| *Pisum sativum* ssp. *arvense* | 6.16 $^{a}$ | 3.49 $^{b}$ | 0.69 $^{b}$ | 3.49 $^{a}$ | 0.27 $^{b}$ | 0.84$^{C}$ | 1.47 $^{b}$ |

Homogeneous groups denoted with letters (a, b, c) were calculated separately for each enzyme.

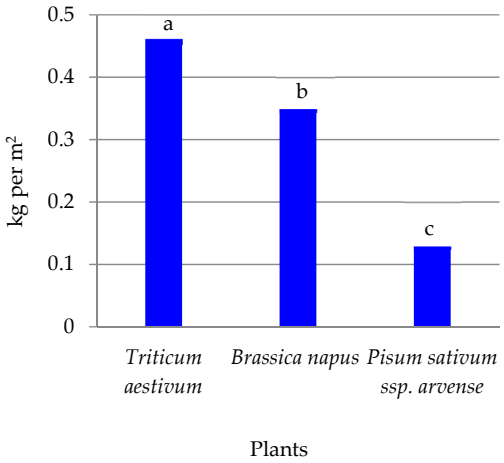


**Figure 8.** The yield of plants in kg per $m^2$.

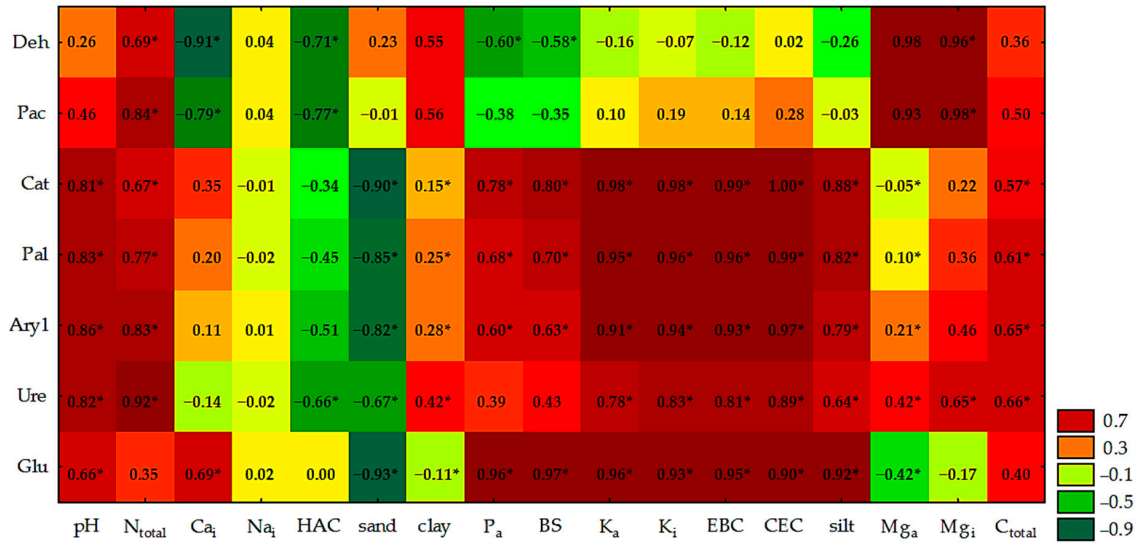


**Figure 9.** Pearson correlation coefficients between enzyme activity and soil physicochemical and chemical properties, $n = 15$, $p = 0.05$, * significant differences.

## 4. Discussion

### *4.1. Physicochemical and Chemical Properties of Soil*

The abundance of available phosphorus and potassium in the soil, as well as the highest sorption capacity and soil saturation with base cations, were probably the main factors which affected the yields of the tested plants (Figure 8). Grain yield produced from 1 $m^2$ of the plot sown with winter wheat reached 0.462 kg grains, that achieved from the plot sown with winter rape reached 0.350 kg grains, and that for field pea reached only 0.130 kg grains. Next to the natural plant features, the physicochemical properties of the soil are closely related to the development of the root system. Besides the varied

chemical composition of the plant species grown in the study, the size of the root system was probably the main determinant of the differences observed in the microbiological and biochemical properties of soil, because plant species and soil fertility are the key indicators of the biological activities of soil [64]. The superiority of one factor over another is affected by multiple habitat factors, like, e.g., soil tillage system [65] the contents of phosphorus and $Ca^{2+}$, $Mg^{2+}$, $Al^{3+}$ cations, and the pH value of soil [66].

### *4.2. Counts and Diversity of Bacteria*

The physicochemical properties of the soil from winter wheat cultivation should, theoretically, be more favorable to the development of organotrophic bacteria and actinobacteria than the soil used for winter rape and field pea cultivation. This is due to, among other things, more favorable fraction size distribution, a higher sorption capacity, and lower acidification of the soil. However, the experimental data also pointed to some other dependencies influenced by the species of the cultivated plant. In spite of the fact that the physicochemical properties of the soil sown with field pea were far from being the most favorable, it was its rhizosphere that was the most abundant in organotrophs and actinobacteria. This can be explained by the difference in the root system morphology between winter wheat and field pea, in favor of the latter, as well as by the capability of field pea roots for symbiosis with atmospheric nitrogen-binding bacteria from the genus *Rhizobium*, and by the difference in the chemical composition of root secretions. The aforementioned factors contributed to a more dynamic development of organotrophic bacteria of r strategy than of k strategy, which was indicated by the values of their colony development index (CD). Usually, the greater inflow of nutrients to the natural environment aids the development of fast-growing microorganisms [34]. The k strategists, i.e., the slow-growing bacteria, are more stable in this respect. They are responsible for maintaining soil homeostasis, and are also resistant to the adverse effects of environmental conditions [67], as well as being typical of soil ecosystems [15,48].

Generally, organotrophic bacteria proliferate faster in the soil than actinobacteria as a result of organic matter inflow to the natural environment [13]. Such a response of microorganisms is not always associated with their ecological diversity [48]. In the present study, the highest value of the ecophysiological diversity index (EP) of organotrophic bacteria was determined in the winter wheat rhizosphere (0.877) and that of actinobacteria in the field pea rhizosphere. In the case of the latter microorganisms, the EP values reached 0.843 in the soil from pea field cultivation, 0.791 in the soil from winter wheat cultivation, and 0.684 in the soil from winter rape cultivation.

Under natural conditions, microbiome stability is largely affected by the species of growing plant and, especially, by its root system [41,48]. By providing water-soluble compounds to plants, including organic acids, sugars, or amino acids, the roots of plants stimulate the microbiological activity of soil [68,69], whereas plant growth promoting bacteria (PGPR) colonize roots and increase the root system biomass [40,70]. The PGPR modify the root architecture through the production of phytohormones, siderophores, and hydrogen cyanide, as well as by nitrogen uptake and mechanisms of phosphates stabilization [15,47,71]. Therefore, the rhizosphere is characterized by greater diversity of the population of microorganisms than the soil distant from the root system of plants [42,46,72].

Another important factor which determines soil health, and thus, soil quality and productivity, is the structure of microbial communities [73], because these communities affect the stability of the soil ecosystem [7,11,74]. There is a strong correlation between the soil microbiome and the plant microbiome [75,76]. It is the soil bacteriome that often determines the quality features of cultivable plants [77]. According to Bakker et al. [78] and Xu et al. [76], the prevailing phyla in the arable soil include *Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes*, and *Acidobacteria.* According to Xu et al. [76], proteobacteria comprised 40% and 43% in the pot and the field experiments, respectively. Proteobacteria can quickly respond to nutrient changes in the rhizosphere. Maron et al. [67] and Pascault et al. [79] have emphasized that *Acidobacteria, Actinobacteria, Planctomycetes, Chloroflexi*, and *Gemmatimonadetes* are classified among slow-growing microorganisms (k-strategists), whereas *Proteobacteria* (mainly those

from classes *Alphaproteobacteria* and *Gammaproteobacteria*) and *Firmicutes* are among the fast-growing ones (r-strategists).

The dependency between a plant species and the microbiological communities in the rhizosphere that was observed in the present study was also reported by Huang et al. [80]. According to Maron et al. [67], diminished diversity of microorganisms retards the degradation of both autochthonous and allochthonous carbon sources, thereby reducing the global emission of $CO_2$ by as much as 40%, whereas the importance of the diversity effect increases along with increasing the availability of nutrients. Bacteria from various families differ significantly in their genetics, which determine their functions in the soil environment [81]. For instance, bacteria representing the families *Solibacteraceae* and *Acidobacteriaceae* play an active part in protein and carbohydrate mineralization; those from the family *Baciliaceae* degrade chitin and cellulose, and participate in the biosynthesis of plant growth hormones and secondary metabolites [82]; bacteria from the family *Burkholderiaceae* are active participants of bioplastics biodegradation [83]; and *Streptomycetaceae*, *Pseudonocardiaceae*, and *Promicromonosporaceae* exhibit robust activity against carboxymethyl cellulose, xylan, chitin, and pectin substrates [84].

According to Chaparro et al. [66], the secretions of the roots of various species or even ecotypes of plants, which determine the soil microbiome, differ in their chemical compositions. They manifest plant responses to the chemical signals emitted by soil microorganisms. Their secretion process can proceed both at the passive and active transport pathways. Root secretions determine interactions between plants and the soil microbiome, and therefore, regardless of the soil type, the crop and its cultivation regime is the key determinant of the soil microbiome [64]. The above factors contribute to a more favorable structure in the bacteriome of the soil from winter wheat cultivation compared to the soils from winter rape and field pea cultivation.

### *4.3. Enzymatic Activity of Soil*

The results of ample investigations [10,85–87] have demonstrated that the analysis of the activity of soil enzymes is of great importance to the evaluation of soil quality and productivity, due to its high sensitivity, ease of measurements, and a high correlation with plant yield. The enzymatic activity of soil is usually strongly associated with soil colonization by microorganisms. In the reported study, this effect was observed only in the case of dehydrogenases and acid phosphatase. Their activities were highest in the soil from cultivation of field pea, i.e., in the rhizosphere had the greatest effect on the promotion of soil colonization by cultivable bacteria (organotrophic bacteria and actinobacteria). The positive correlation between the abundance of microbial communities and the activity of dehydrogenases has been shown by many authors [88,89], who explained this phenomenon by the localization of these enzymes in viable microorganism cells [86]. According to Merino et al. [90], intracellular, rather than extracellular, enzymes provide information about the potential activity of a community of soil microorganisms. In the present study, the activities of the other tested enzymes, in particular of urease, alkaline phosphatase, β-glucosidase, and arylsulfatase, were higher in the soil sown with winter wheat than in those of field pea and winter rape. The activities of extracellular enzymes are positively correlated with the sorption capacity of soil [91], whereas the soil from the cultivation of winter wheat was characterized by the best physicochemical properties. The activities of these enzymes may, however, be suppressed by the excessive sorption capacity caused by, e.g., biocarbon supplementation [92].

## 5. Conclusions

The crop and its cultivation regimes contributed to the development of specific conditions which modify soil microbiome. The rhizosphere of winter wheat had a more beneficial effect on bacteria development and enzyme activity than those of winter rape and field pea, as indicated by the values of the ecophysiological diversity index (EP) in the soils sown with the tested plants. Microbiological indices, including bacterial count and diversity, as well activities of soil enzymes, are reliable indicators

of soil environment conditions, and are also helpful parameters in the evaluation of soil fertility and productivity.

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