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Effect of Previous Crop on the Structure of Bacterial and Fungal Communities during the Growth of *Vicia faba* L. spp. minor

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Abstract: The aim of this study was to assess how soil use and the cultivation of *Triticum aestivum* spp. *vulgare* L. (Sw), *Triticum aestivum* spp. *spelta* L. (Ww), *Zea mays* L. (M), and *Brassica napus* L. (Wr) impacts soil microbiota. This study consisted of a pot experiment over 120 days, until *Vicia faba* spp. minor seeds and pods reached the developmental stage of growth. This study showed that *T. aestivum* spp. *vulgare* L. grown in the soil sown with faba beans had a beneficial effect on the development of organotrophic bacteria, actinobacteria, and fungi. Regardless of the previous crop and soil cultivation method, r-strategists were found among the organotrophic bacteria and fungi, whereas K-strategists were found among the actinobacteria. All soils sown with faba beans were primarily colonized by bacteria belonging to the phylum Actinobacteriota (represented by the genus *Cellulosimicrobium*) and fungi belonging to the phylum Ascomycota. In the soil sown with field faba beans from the cultivation of Sw and Wr, the soil was dominated by *Mortierella* genus fungi; that of Ww was dominated by *Cladosporium*, and that of M was dominated by *Alternaria*. The results of this study provide new insights into the influence of previous crops and further cropping with faba bean on the quantitative and qualitative composition of the soil microbiota.

Keywords: previous crop; soil; rhizosphere; diversity of microorganisms; metagenomics

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1. Introduction

Vicia faba L. is a crop that plays an important role in maintaining high soil fertility, contributing to the sustainable development of agriculture [1]. It is one of the most widely cultivated Fabaceae plants across the globe and ranks fourth in the world in terms of growing area (after peas, chickpeas, and lentils) [2]. Its largest acreage is in China, accounting for 60% of the total global production, followed by Ethiopia and Egypt [3]. Legumes in general are the primary food source in human nutrition and animal feeding, while also playing a key role in crop rotation. Not only do they improve soil fertility but also reduce weeds, pests, and pathogens [4]. Previous studies [5,6] have demonstrated that *V. faba* L. improves the physical conditions and biodiversity of soil, boosts the immobilization of phosphorus, and increases the nitrogen content of the soil through biofixation.

There are certain biochemical processes that take place in the soil during the growth and development of plants which have a significant impact on soil microbiota. Plants, through the varied morphological structure of their roots, the production of root secretions, and the presence of dead root cells, can influence the soil microbiome. De Vries [7] and Steinauer et al. [8] have reported that plant root secretions are the primary determinants of rhizosphere microbial activity and diversity due to their nutritional, antibiotic, and signaling properties. Their quantitative and qualitative composition depends on the plant species and the type and moisture content of the soil. According to Philippot et al. [9], the more diverse the plant community is, the more diverse the composition of plant root secretions, which translates to higher microbial diversity in the soil. A study conducted by

Eisenhauer et al. [10] demonstrated that plant diversity promotes the growth of bacteria and fungi, as root secretions from the plants enrich the soil with organic substances. In this manner, root secretions may significantly affect the correlations between the composition of plant communities and the composition and structure of soil microbial assemblages [8,11]. Plants may modify the rhizosphere microbiome to gain certain benefits by selectively stimulating those microorganisms that possess the specific traits which promote plant growth and development [12]. The structure of microbial communities in the soil environment is also affected by the pH and by redox processes [13]. Each previous crop modifies the physical, chemical, and biological properties of the soil, thus leaving behind a distinct and different habitat for the successive crop. Such modifications may entail increased organic carbon content; better nutrient availability; and improved soil aggregation, porosity, and soil density, as well as reduced evaporation of water from the soil (thus preserving soil moisture for the next plants to grow) [14–17]. Different root systems draw water and nutrients from different levels of the soil, which can improve the physical and chemical properties of the soil. In addition, crop residues remain in the soil after cultivation and form a new humus layer—the more diverse the crops, the richer the composition of the resultant humus [18].

Marschner et al. [19] have reported that soil microorganisms play an essential role in the nutrient cycle by transforming and mineralizing organic matter, as well as by releasing and converting mineral nutrients. Microbes may also regulate nutrient uptake by plants through the oxidation, reduction, chelation, and solubilization of the nutrients. Plant growth-promoting microorganisms colonize the rhizosphere and play a key role in increasing soil fertility, boosting yields, and preventing diseases [20]. Microorganisms that promote plant growth and development include bacteria of the genera *Pseudomonas*, *Azospirillum*, *Enterobacter*, *Azotobacter*, *Rhizobium*, *Bacillus*, *Serratia*, *Klebsiella*, and *Azoarcus*. Their diversity in soil varies depending on soil type, environmental conditions, plant type, and nutrient availability in the rhizosphere [21]. Microbial diversity also reflects disturbances in the soil ecosystem, which makes it an important indicator for assessing changes in the soil environment and nutrient content [22]. Interactions between rhizosphere microorganisms and the root system of plants have a beneficial effect on plant health, yields, and soil quality. These positive effects may be attributed to microbial metabolites that promote phosphate solubilization, nitrogen fixation, and nutrient availability. In addition, they enhance crop adaptability to adverse environmental conditions, strengthen plant resistance through induced systemic immunity, and control phytopathogens [23].

Soil use can change soil environmental factors, nutrient contents, and biological interactions, thereby affecting the soil microbiome [24], which in turn can lead to the disruption of nutrient cycling in the environment [25]. A change in the plant community can alter the supply of carbon to the soil through the litter or root secretions produced by the plants, thus also affecting the diversity and activity of soil microbiota [26]. Ortiz and Sansinenea [27] have reported that intensive crop farming decreases soil nutrients, resulting in poorer and lower-quality yields of successor crops. The soil microbiome can vary depending on the soil cultivation method applied. This is illustrated, for example, by Biederbeck et al. [28], who observed that soil used to grow legumes (*Lens culinaris Medikus*, *Lathyrus tingitanus* L., *Lathyrus sativus* L., and *Pisum sativum* L.) in a crop rotation system had larger populations of fungi and bacteria than a monoculture and a wheat rotation crop. Similarly, Song et al. [29] examined the structures of ammonia-oxidizing bacteria in the rhizospheres of faba bean, corn, and wheat and found significant differences between intercrop and monoculture systems across different plant growth stages but no differences between the crops themselves. During the anthesis growth phase, the co-cultivation modified the composition of the ammonia-oxidizing bacterial community in the faba bean soil and, to a lesser extent, the wheat soil. Another study, by Granzow et al. [30], reported differences in the abundance and diversity of bacteria and fungi in soil used to grow beans and wheat in different systems (monoculture, row intercrop, mixed intercrop). The bacteria and fungi were significantly more diverse in pooled soil samples from the mixed system

compared to the inter-row. In addition, the microbial make-up varied depending on the crop species and the plant intercrops, meaning that the diversity of microorganisms in the soil was affected by the cultivation system. Finally, Li et al. [20] pointed to the beneficial effect of long-term crop rotation with alfalfa and soybean on the microbial diversity of the corn rhizosphere. Specifically, they noted an increase in the relative population number of Proteobacteria, Actinobacteriota, and Acidobacteriota phyla, which promoted maize development and yield.

The response of microorganisms to diversified soil use is highly complex and, so far, insufficiently explored. The research hypothesis is that the experimental factors, i.e., the previous crop and the further cultivation of field faba beans, as well as the interaction of these factors, cause changes in the quantitative and qualitative composition of the soil microbiota. Therefore, the aims of this study were to establish the effect of the previous crop (*T. aestivum* spp. *vulgare* L., *T. aestivum* spp. *spelta* L., *Z. mays* L., and *B. napus* L.) and soil use (unsown and sown with *V. faba* L. spp. minor) on the abundance, diversity, and structure of bacterial and fungal communities.

2. Materials and Methods

2.1. Soil Material

The experiment was conducted using soil collected from the arable soil layer from a depth of 0 to 20 cm, sourced from a site in the Elbląg Fens (Żuławy Elbląskie) in the village of Krzyżanowo (Poland NE, 54.0242° N, 19.1216° E) in the commune of Stare Pole, Malbork county, in the eastern part of the Pomeranian Voivodeship, on which spring wheat (*T. aestivum* spp. *vulgare* L. cultivar "Tybalt"), winter wheat (*T. aestivum* spp. *spelta* L. cultivar "Apostel"), maize (*Z. mays* L. cultivar "SY Calo"), and winter rapeseed (*B. napus* L. cultivar "Kuga F1") were grown. The previous crops were fertilized as follows (values per pure ingredient in kg ha^{-1}): *T. aestivum* spp. *vulgare* L. and *T. aestivum* spp. *spelta* L.—198 kg N, 60 kg P, and 90 kg K; *Z. mays* L.—310 kg N, 62 kg P, and 93 kg K; and *B. napus* L.—264 kg N, 80 kg P, and 120 kg K. This area is located within the Vistula Fens (Żuławy Wiślane), part of the Gdańsk Coast macroregion, South Baltic Coast subprovince, and more specifically in its eastern part called the Elbląg Fens. The relief of the area was shaped by the cumulative activity of the Vistula River mouth. The Vistula Fens are a plain area with a very even topography, in the shape of a flat cone. There is a type of natural landscape here—a coastal delta landscape—which covers an area of 7949 ha (of which 332 ha is forests and 6257 ha is agricultural land). Almost 90% of the soils in the Vistula Żuławy are very fertile (very light soils account for 2.0%, light soils account for 3.6%, medium soils account for 22.8%, heavy soils account for 50.8%, and very heavy soils account for 16.9%). There are also sandy podsolic soils and peat soils, located mainly along rivers [31]. The soil used in this experiment was classified as Fluvisols type [32], and its characteristics are presented in Table 1.

Table 1. Characteristics of the soil with further cropping with *V. faba* L. spp. minor.

Previous Crop	Soil Kind	Granulometric Fraction (%)			pH_{KCl}	HAC	EBC	CEC	BS (%)	C_{org}	N_{total}	C_{org}/N_{total}
		Sand	Silt	Clay		$mmol^{(+)}\ kg^{-1}$ d.m. of soil				$g\ kg^{-1}$ d.m. of soil		
Sw	SiL	30.00	69.00	1.00	6.233	26.750	238.000	264.750	89.896	18.220	2.460	7.406
Ww	SiL	34.00	65.00	1.00	6.367	18.750	298.000	316.750	94.080	18.770	2.630	7.137
M	SiL	29.00	69.00	2.00	6.233	21.250	386.667	407.917	95.645	17.170	2.625	6.541
Wr	SiL	38.00	61.00	1.00	5.933	28.000	210.000	238.000	88.236	18.290	2.745	6.663

Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.; SiL—silt loam; pH_{KCl}—soil reaction; HAC—hydrolytic acidity; EBC—sum of exchangeable bases; CEC—sorption capacity; BS—base saturation; C_{org}—organic carbon content; N_{total}—total nitrogen content; C_{org}/N_{total}—ratio of organic carbon content to total nitrogen content.

2.2. Physical and Chemical Soil Tests

Before setting up the experiment, basic physical and chemical analyses of the soil were performed in 3 replications. To this end, the soil was dried in the open air and then

passed through a 2 mm sieve. The following soil tests were carried out: granulometric composition with laser diffraction using Mastersizer 2000 analyzer (Malvern Instruments Ltd., Malvern, UK), pH using the potentiometric method in 1 mol KCl dm^{-3}, hydrolytic acidity and the sum of exchangeable base cations with titration, soil sorption capacity, degree of soil saturation with base cations, and organic carbon and total nitrogen contents using a Vario Max Cube CN device (Elementar Analysen systeme GmbH, Langenselbold, Germany). The methodology for the physical and chemical soil tests can be found in Wyszkowska et al. [33].

2.3. Experimental Procedure

The pot-growing experiment was conducted from May to September in 2021 in the greenhouse of the Didactic and Experimental Center of the Faculty of Agriculture and Forestry, University of Warmia and Mazury in Olsztyn (north-eastern part of Poland). The research was conducted in two stages. The first stage involved the collection of soil material collected in March 2021 used to grow *T. aestivum* spp. *vulgare* L., *T. aestivum* spp. *spelta* L., *Z. mays* L., and *B. napus* L. The second stage of the research was carried out in the greenhouse, where *Vicia faba* L. spp. minor was cultivated. Prior to setting up the experiment, the soil was sifted through a 5 mm sieve, after which 5 kg of each soil (used to grow spring wheat, winter wheat, maize, and winter rapeseed) was weighed into plastic pots with a capacity of 7 dm^3. The tests were carried out in two series on unsown soil (3 replications) and on soil sown with faba beans (3 replications) (*V. faba* L. spp. minor). All soil samples received the same fertilization with phosphorus and potassium, calculated as a pure component: 91.65 mg kg^{-1} phosphorus (in KH_2PO_4 form) and 36.14 mg kg^{-1} potassium (in KH_2PO_4 + KCl form). Then, 10 seeds of the Albus variety of faba bean were sown in each pot, and the soil moisture was brought to 50% of the capillary water capacity. When the faba beans were in the germination stage (stage 0), the plants were thinned, and 5 plants were left in one pot. Throughout the entire experiment, soil moisture was maintained at a constant level, and water losses were replenished every day with deionized water. On day 120 of the experiment, when *V. faba* L. spp. minor reached developmental stage 8 (ripening of seeds and pods) according to the BBCH scale (Biologische Bundesanstalt, Bundessortenamt and Chemische Scale), soil samples were collected for microbiological tests. The soil samples were taken from each pot (with the unsown soil and soil sown with faba bean), with 700 g soil taken from each combination. The greenhouse-based part of the experiment was conducted under the following conditions: average temperature of 16.8 °C, average humidity of 70.0%, and average day length of 10.2 h.

2.4. Microbiological Soil Tests

Once *V. faba* L. spp. minor was harvested (day 120), microbiological analyses of the soil were performed in 4 replications using the serial dilution method. The abundance of organotrophic bacteria, actinobacteria, and fungi was determined according to the procedure provided in Kucharski et al. [34] and Wyszkowska et al. [35]. From each combination, appropriate dilutions of the soil suspension (for organotrophic bacteria and actinobacteria, solutions of 10^{-5} and 10^{-6}, while for fungi, solutions of 10^{-3} and 10^{-4}) were introduced into Petri dishes in parallel in 4 replications. Organotrophic bacteria were cultured on Bunt and Rovir medium with soil extract, actinobacteria on Küster and Williams medium with antibiotics (nystatin and actidion), and fungi on Martin's glucose–peptide medium with Rose Bengal and the antibiotic aureomycin. The microbial material was incubated in the thermostat for 10 days (temperature 28 °C), with the developing microbial colonies counted every day. The number of microorganisms was expressed in cfu per 1 kg d.m. of soil.

Soil microorganisms (bacteria and fungi) were identified with Next-Generation Sequencing (NGS). Genomic DNA was isolated from the faba bean-sown soil samples by the company A&A Biotechnology (Gdańsk, Poland) using the Genomic Mini AX Bacteria+ kit. Universal primers 1055F (5′-ATGGCTGTCGTCAGCT-3′) and 1392R (5′-ACGGGCGGTGTGTAC-3′) were used in this study to amplify the bacterial 16S rRNA

and fungal ITS gene region. Genomic DNA isolation was performed according to the method provided in Zaborowska et al. [36]. The next stage of analyses included sequencing genomic DNA of bacteria and fungi, followed by bioinformatic analysis of the obtained sequences, carried out by the company Genomed S.A. (Warsaw, Poland). The sequencing of bacterial amplicons was performed based on the V3-V4 hypervariable region of the 16S rRNA gene using the specific primers 314F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′). Fungal amplification was performed based on the ITS1 region using the specific primers ITS1FI2 (5′-GAACCWGCGGARGGATCA-3′) and 5.8S (5′-CGCTGCGTTCTTCATCG-3′). Preliminary data analysis was performed on a MiSeq instrument using MiSeq Reporter (MSR) v2.6 software. Bacterial sequence reads were performed using QIIME 2 software based on the Silva 138 sequence database, while QIIME software based on the UNITE v8 sequence database was used to read fungal sequences. The sequencing procedure and bioinformatic analysis are described in Baćmaga et al. [37] and Wyszkowska et al. [38]. The bacterial and fungal sequences are deposited in the NCBI GenBank under the following accession numbers: bacterial 16S rRNA (https://www.ncbi.nlm.nih.gov/nuccore/?term=OR652689:OR652778[accn]) (16 October 2023) and fungal ITS (https://www.ncbi.nlm.nih.gov/nuccore/?term=OR652779:OR653394[accn]) (16 October 2023).

2.5. Calculations and Statistical Analyses

Based on the number of microorganisms, the colony development index (CD) and the ecophysiological diversity index (EP) of microorganisms were computed according to the formula provided in Wyszkowska et al. [38]. The number of microorganisms grown in specific time intervals (Ks) was calculated according to the formula provided in Wyszkowska et al. [35]. The number of microorganisms was also used to calculate the rhizosphere effect (R:S), which is the ratio of the number of microorganisms in the soil sown with faba beans (R) to the number of microorganisms in the unsown soil (S). The Shannon–Wiener index (H′) and the Simpson index (D) were calculated from the number of bacterial and fungal OTUs according to the formulas described in Zhang et al. [39].

The results were statistically processed using the Statistica 13.3 package [40] based on multivariate ANOVA at $p = 0.05$. Homogeneous groups were calculated using Tukey's post hoc test (HSD). Changes in the number of microorganisms were presented in dendrogram form using Ward's multivariate cluster analysis (CA). A two-factor analysis of variance (ANOVA) at $p = 0.05$ was conducted to determine the significance of the effect of the factors studied (previous crop and further cropping with faba bean). Metagenomic data at the phylum and genus taxonomic level are presented for OTUs $\geq 1\%$ of the sequences obtained. The dominant phylum of bacteria and fungi was statistically derived using the G test (w/Yates) + Fisher test using STAMP 2.1.3 software [41]. The dominant genera of bacteria and fungi were presented in heat map form using the RStudio v1.2.5033 software [42], the gplots library [43], and the v3.6.2 system [44]. Unique and common genera of bacteria and fungi were presented as a Venn diagram using InteractiVenn software (https://www.interactivenn.net) [45].

3. Results

3.1. Cultured Bacteria and Fungi

The abundance of organotrophic bacteria and actinobacteria was significantly modified by the previous crop ($p < 0.001$) and the interactions of the factors studied: the previous crop and further cropping with faba bean (organotrophic bacteria $p < 0.001$ and actinobacteria $p = 0.001$). The abundance of fungi was modified by all the factors analyzed: the previous crop ($p < 0.010$), further cropping with faba bean ($p < 0.001$), and the interaction of the factors ($p < 0.001$) (Tables 2 and 3). Sw soil sown with faba bean had the highest abundance of organotrophic bacteria (2.089×10^{10} cfu kg^{-1} d.m. of soil), actinobacteria (1.529×10^{10} cfu kg^{-1} d.m. of soil), and fungi (0.675×10^{8} cfu kg^{-1} d.m. of soil). The number of organotrophic bacteria was 1.9-fold higher than in the unplanted soil, and the

number of fungi was 2.7-fold higher than in the unplanted soil. On the other hand, the number of actinobacteria in the Sw soil sown with faba bean was at a similar level to that in the unseeded soil. In the unplanted soil, the number of organotrophic bacteria was highest in the Ww site (1.354×10^{10} cfu kg^{-1} d.m. of soil) and the number of actinobacteria was highest in the Sw site (1.493×10^{10} cfu kg^{-1} d.m. of soil), while the number of fungi was highest in the Wr site (0.450×10^{8} cfu kg^{-1} d.m. of soil).

Table 2. The level of significance of the studied factors determined with two-factor ANOVA (*p* value).

Factors	Number of Microorganisms (L)			Colony Development Index (CD)			Ecophysiological Diversity Index (EP)		
	Org	Act	Fun	Org	Act	Fun	Org	Act	Fun
Pc	0.000 *	0.000 *	0.004 *	0.000 *	0.069	0.000 *	0.956	0.010 *	0.018 *
Fc	0.084	0.371	0.000 *	0.004 *	0.394	0.389	0.043 *	0.001 *	0.849
Pc × Fc	0.000 *	0.001 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *

Pc—previous crop; Fc—further cropping with faba bean; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi; *—significant at $p = 0.05$.

Table 3. The number of microorganisms in the soil depending on the previous crop and further cropping with faba bean, cfu 10^{n} kg^{-1} d.m. of soil.

Previous Crop	Org × 10^{10}	Act × 10^{10}	Fun × 10^{8}
Unsown soil			
Sw	1.078 ± 0.140^{e}	1.493 ± 0.602^{a}	0.252 ± 0.015^{e}
Ww	0.529 ± 0.046^{g}	0.430 ± 0.017^{e}	0.371 ± 0.042^{d}
M	0.906 ± 0.431^{f}	0.845 ± 0.283^{d}	0.366 ± 0.011^{b}
Wr	1.187 ± 0.053^{d}	1.347 ± 0.076^{b}	0.450 ± 0.041^{c}
Sown soil			
Sw	2.098 ± 0.644^{a}	1.529 ± 0.050^{a}	0.675 ± 0.084^{a}
Ww	1.354 ± 0.074^{c}	0.835 ± 0.053^{d}	0.391 ± 0.043^{cd}
M	1.505 ± 0.049^{b}	0.991 ± 0.165^{c}	0.600 ± 0.044^{b}
Wr	1.550 ± 0.054^{b}	1.510 ± 0.033^{a}	0.404 ± 0.063^{c}

Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi. Homogeneous groups are denoted with letters (a–g) separately for each group of microorganisms (two-way ANOVA with Tukey's test at significance level of $p = 0.05$).

Two clusters of microorganisms were identified based on a dendrogram (Figure 1) which grouped soil microorganisms according to their response to the previous crop (both in the soil sown with *V. faba* L. spp. minor and in the unsown soil). The first cluster consisted of organotrophic bacteria and actinobacteria, while the second one consisted only of fungi.

Analysis of variance showed (Table 2) that the index of microbial colony development was determined to the greatest extent by the interaction of the factors studied, namely the previous crop and further cropping with faba bean ($p < 0.001$). The value of the CD index of organotrophic bacteria (Figure 2) in the soil sown with faba bean was the highest in the Sw site (51.892) and in the unplanted soil in the Wr site (48.195). On the other hand, the CD index of organotrophic bacteria in the soil sown with faba bean was the lowest in soil M (34.359), while in the unseeded soil, it was the lowest in soil Ww (22.329). The CD index of actinobacteria in the soil sown with faba bean was the highest in the Ww site (26.053), while in the unseeded soil, it was the highest in the Sw site (24.140). The lowest CD value for actinobacteria was recorded in soil sown with faba bean at sites M (21.622) and Wr (21.737) and in unseeded soil at site Sw (24.140). In the case of fungi, the highest CD value in soil sown with faba bean was found at the Sw site (44.546), while in the unseeded soil, it was highest at the M site (40.121). The opposite trend was observed for the lowest CD value, that is, in the soil sown at site M (35.735) and in the unseeded soil at site Sw (28.216).

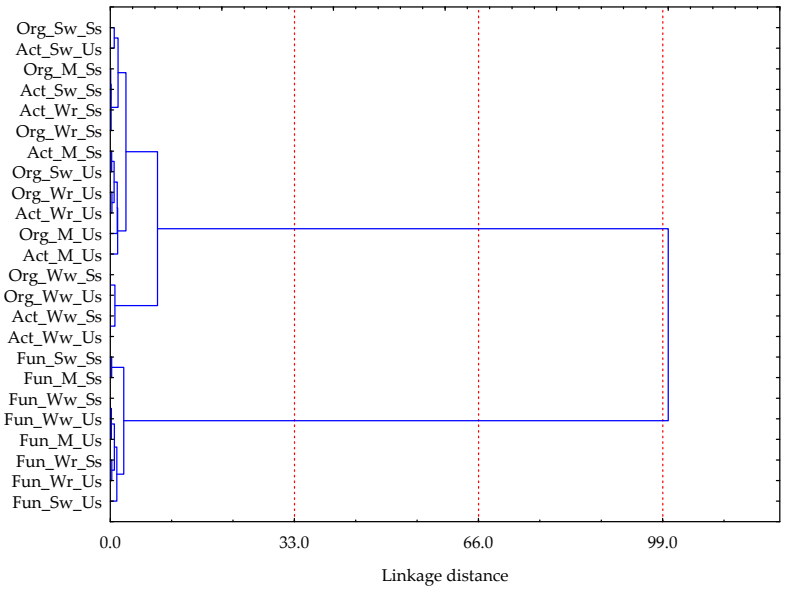


Figure 1. The numbers of microorganisms in soil, depending on the previous crop and further cropping with faba bean, presented as a dendrogram (Ward's method). Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.; Us—unsown soil; Ss—sown soil; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi.

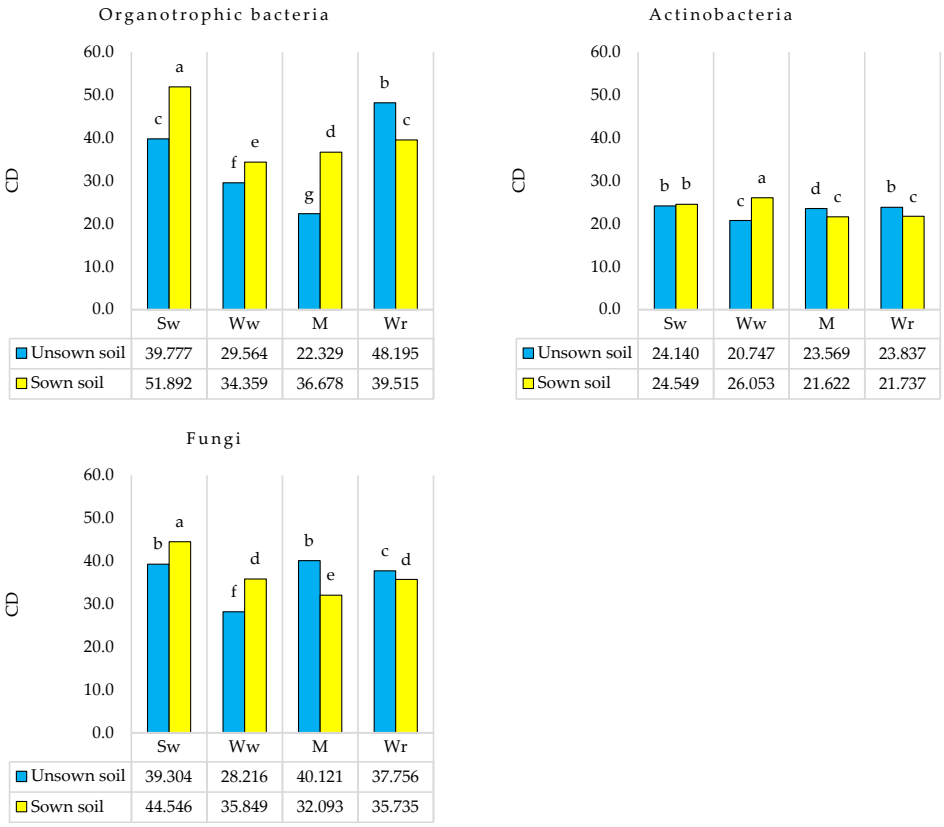


Figure 2. Colony development index (CD) of microorganisms in soil, depending on the previous crop and further cropping with faba bean. Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.; Org—organotrophic bacteria, Act—actinobacteria, Fun—fungi. Homogeneous groups are denoted by letters (a–g) (two-way ANOVA with Tukey's test at significance level of $p = 0.05$).

Organotrophic bacteria and fungi (Table 4) proliferated the fastest in the *V. faba* L. spp. minor-sown soil and in the unsown soil. In the first two days of incubation, the greatest increase in organotrophic bacterial and fungal counts in the *V. faba* L. spp. minor-sown soil was recorded in variant Sw (Ks of 60.44% and 64.16%, respectively). In the unsown soil, the highest increase in the colony number of organotrophic bacteria was determined in soil Wr (Ks = 55.03%), and that of fungi was in soil M (Ks = 61.94%). The proliferation of actinobacteria in the soil was slightly slower compared to organotrophic bacteria and fungi. The greatest increase in their number was recorded from day 3 to day 8 of incubation in both sown and unsown soil. In the same period, the most intense proliferation of actinobacteria was observed in soil Wr (the total Ks value was 90.77% in the sown soil and 90.52% in the unsown soil).

Table 4. The number of microbial colonies (in %) cultured at different time intervals (Ks) in the soil depending on the previous crop and further cropping with faba bean.

Previous Crop	Organotrophic Bacteria					Actinobacteria					Fungi				
	Days of Culture Incubation														
	1–2	3–4	5–6	7–8	9–10	1–2	3–4	5–6	7–8	9–10	1–2	3–4	5–6	7–8	9–10
Unsown soil															
Sw	50.227	22.500	8.636	15.455	3.182	9.360	36.289	22.660	28.571	3.120	42.718	25.243	26.214	5.825	0.000
Ww	36.094	38.187	12.031	12.467	1.221	14.427	39.038	18.670	26.733	1.132	39.275	33.837	21.148	5.740	0.000
M	11.458	23.177	31.771	23.958	9.635	14.804	27.374	30.168	18.156	9.497	61.935	10.968	9.032	13.548	4.516
Wr	55.025	21.523	10.761	9.036	3.655	7.692	38.104	23.614	28.801	1.789	41.823	38.606	10.188	9.383	0.000
Sown soil															
Sw	60.438	19.377	6.920	11.419	1.845	10.127	33.386	33.861	20.095	2.532	64.158	23.297	9.677	2.867	0.000
Ww	23.256	30.233	19.535	23.721	3.256	5.143	26.857	30.286	29.714	8.000	20.530	13.907	37.086	28.477	0.000
M	44.219	27.656	13.125	13.125	1.875	4.038	35.629	24.703	26.841	8.789	40.784	18.431	18.431	22.353	0.000
Wr	46.494	31.707	8.079	12.043	1.677	2.660	38.498	31.142	21.127	6.573	41.520	25.146	18.129	15.205	0.000

Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.

It was found that the studied factors significantly influenced the index of ecophysiological diversity of microorganisms (Table 2, Figure 3). The previous crop most significantly affected the EP of actinobacteria ($p = 0.010$) and fungi ($p < 0.05$), and further cropping with faba bean most significantly affected the EP of organotrophic bacteria ($p < 0.05$) and actinobacteria ($p = 0.001$), while the interaction of these factors most significantly affected the EP of organotrophic bacteria, actinobacteria, and fungi ($p < 0.001$). The value of the EP index of organotrophic bacteria in soil sown with *Vicia faba* L. spp. minor ranged from 0.741 (Sw) to 0.791 (M), while in unplanted soil, it ranged from 0.768 (Sw) to 0.872 (Ww). The EP of actinobacteria was at a similar level and ranged from 0.827 (Sw) to 0.870 (M) in faba bean-sown soil and from 0.827 (Wr) to 0.858 (Sw) in unseeded soil. The EP of fungi in soil sown with faba bean was highest at the Wr site (0.784) and lowest at the Sw site (0.594), while in unseeded soil, the EP value was highest at the Sw site (0.777) and lowest at the M site (0.643). Considering the analyzed groups of microorganisms, the highest EP value of organotrophic bacteria was recorded in the unplanted soil from under *T. aestivum* spp. *spelta* L. (Wr).

The combination of *V. faba* L. spp. minor (Figure 4) with soil previously cropped with winter wheat proved to be the best promoter of organotrophic bacteria and actinobacteria growth, as evidenced by the highest values of the rhizosphere effect index (R:S reached 2.572 and 1.939, respectively). In turn, fungi thrived in the *V. faba* L. spp. minor-sown soil previously cropped with spring wheat (R:S = 2.666).

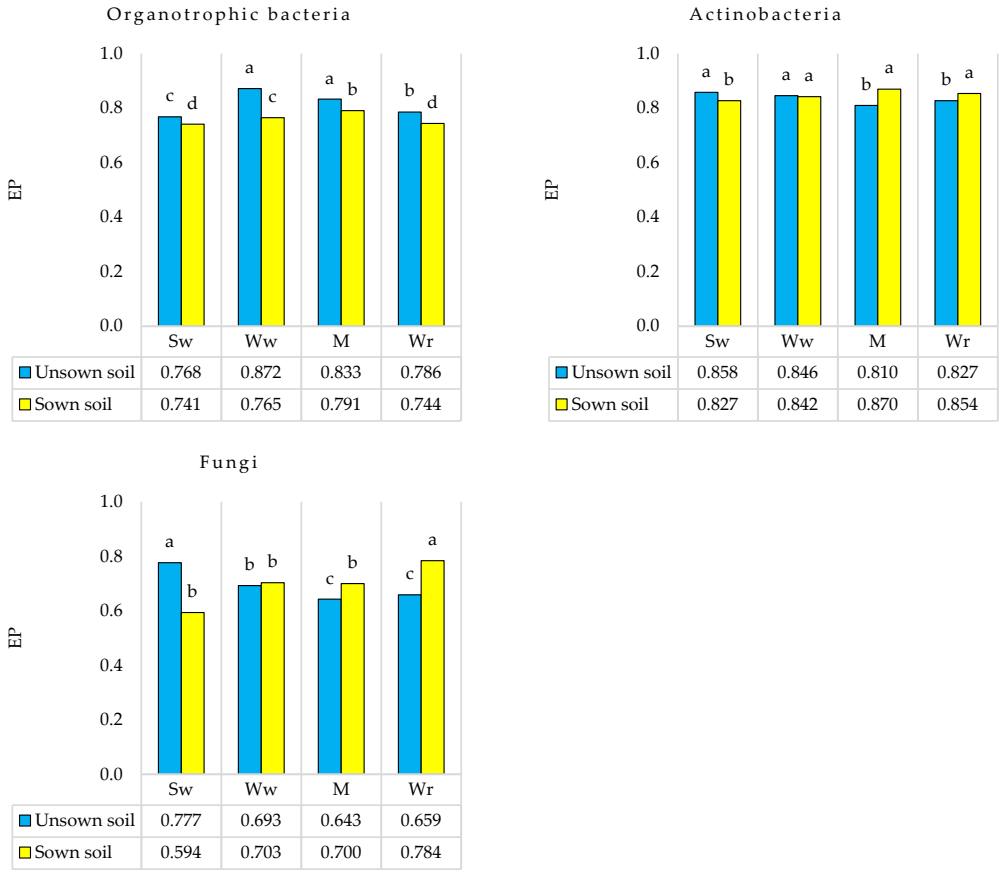


Figure 3. Ecophysiological diversity (EP) index of microorganisms in soil, depending on the previous crop and further cropping with faba bean. Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi. Homogeneous groups are denoted by letters (a–d) (two-way ANOVA with Tukey's test at significance level of $p = 0.05$).

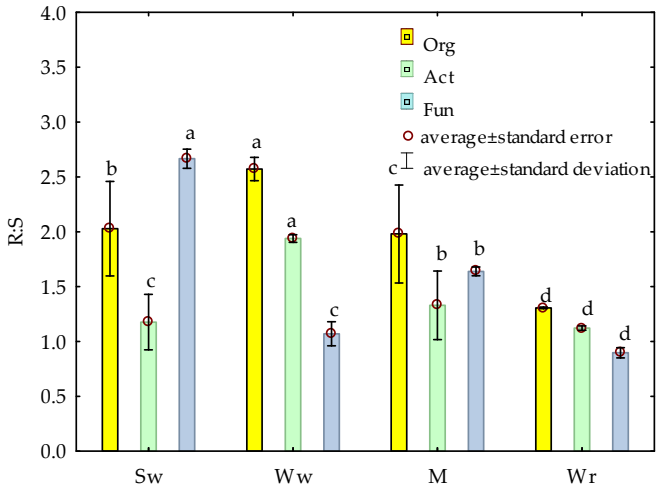


Figure 4. Rhizosphere effect (R:S) based on the number of microorganisms in the soil with further cropping with *V. faba* L. spp. minor. Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi. Homogeneous groups are denoted by letters (a–d) separately for each group of microorganisms (one-way ANOVA with Tukey's test at significance level of $p = 0.05$).

3.2. Bacterial and Fungal Communities

The majority of the bacteria in the soils belonged to the phylum Actinobacteriota (they accounted for 38.62% to 46.44%), with their highest abundance noted in soil M (Figure 5). Their number was higher compared to that in soils Sw, Ww, and Wr by 7.82%, 5.49%, and 6.60%, respectively. The soils were also densely populated by Proteobacteria, which accounted for 16.43% to 29.33%. The highest number of bacteria belonging to this phylum was found in soil Wr—2.59% more than in soil Sw, 12.90% more than in soil Ww, and 6.52% more than in soil M. A slightly lower abundance was noted for bacteria belonging to Acidobacteriota, Chloroflexi, Gemmatimonadota, Myxococcota, Bacteroidota, Verrucomicrobiota, Planctomycetota, Firmicutes, Desulfobacterota, and Patescibacteria. The total number of bacterial OTUs (phylum-level) in the soil at the sites was as follows: Sw—61,600; Ww—53,494; M—60,168; and Wr—63,294.

In terms of fungi, soils Sw, Ww, and M were mostly colonized by the phylum Ascomycota (relative abundance of 62.56%, 69.17%, and 77.68%, respectively), while soil Wr was mainly colonized by phylum Mortierellomycota (constituting 47.20%) (Figure 6). The greatest differences in fungal proportions were noted for phyla Ascomycota and Mortierellomycota. Ascomycota in soil Wr was 24.19% less abundant compared to in soil Sw, 30.80% less compared to in soil Ww, and 39.31% less compared to in soil M. In the case of Mortierellomycota, the greatest disproportions were found between soils Wr and M (Mortierellomycota counts were 42.07% higher in Wr than in M) and between Wr and Ww (Mortierellomycota counts 32.01% higher in Wr than in Ww). In addition, the analyzed soils were also colonized by Basidiomycota, which were most abundant in soil M (accounting for 15.73%), and Mucoromycota, which were the most abundant in soil Wr (accounting for 7.71%). In soil Sw, the total number of OTUs of fungi at the phylum level was as follows: Sw—98,643; Ww—74,196; M—85,282; and Wr—85,334.

The genus *Cellulosimicrobium* (Figure 7) was the prevailing one across all analyzed soil samples, with the highest *Cellulosimicrobium* OTUs found in soil Sw (16,545 OTUs) and the lowest found in soil Ww (13,301 OTUs). The soils were also extensively populated by the genera *Sphingomonas* (from 613 OTUs to 2546 OTUs), SC-I-84 (from 434 OTUs to 1472 OTUs), KD4-96 (from 1075 OTUs to 1265 OTUs), and *Gemmatimonas* (from 812 OTUs to 1567 OTUs). In addition, soil Ww was also heavily populated by *Vicinamibacteraceae* (1207 OTUs), RB41 (1045 OTUs), and MB-A2-108 (1230 OTUs), whereas soil M was heavily populated by bacteria from the *Lysobacter* (1033 OTUs) and *Nanomurea* (1344 OTUs) genera. The total count of bacteria in soil Sw was 30,498 OTUs; in Ww, it was 25,011 OTUs; in M, it was 28,424 OTUs; and in Wr, it was 28,953 OTUs.

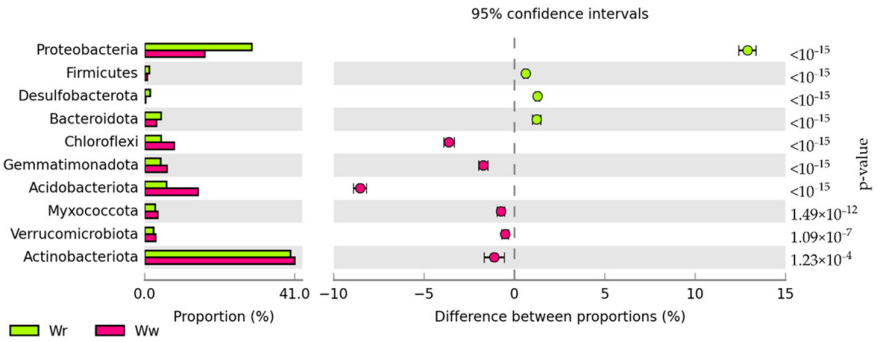


Figure 5. *Cont.*

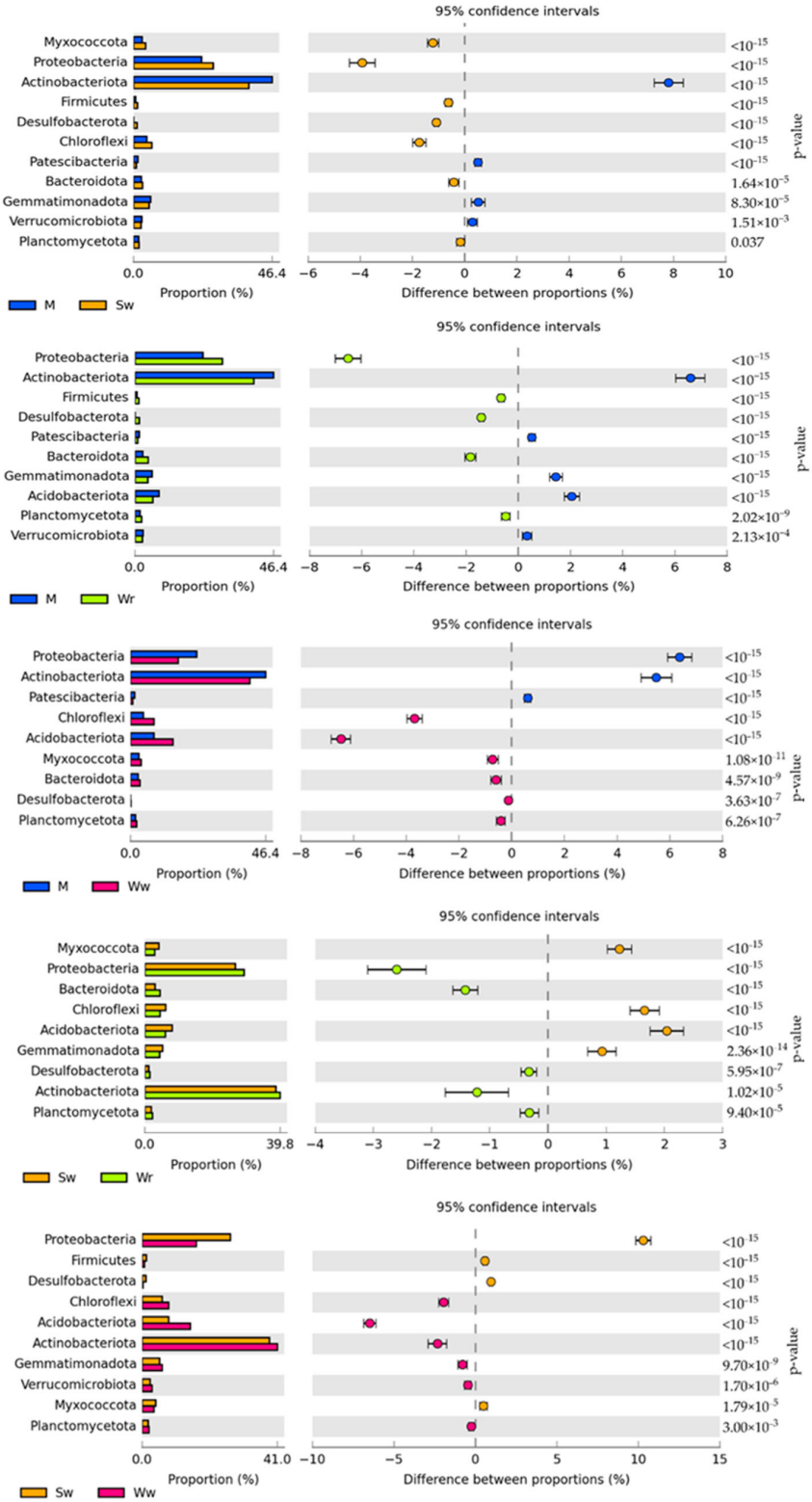


Figure 5. Relative abundance of the dominant phylum of bacteria in the soil with further cropping with *V. faba* L. spp. minor (OTU $\geq$ 1%). Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.

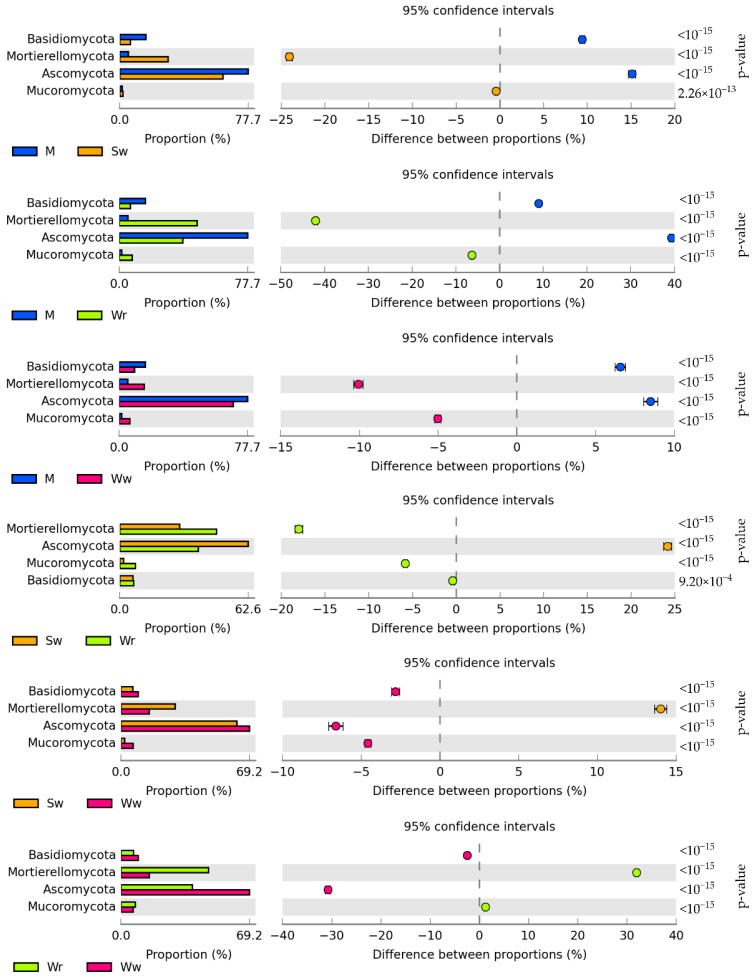


Figure 6. Relative abundance of the dominant phylum of fungi in the soil with further cropping with *V. faba* L. spp. minor (OTU ≥ 1%). Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.

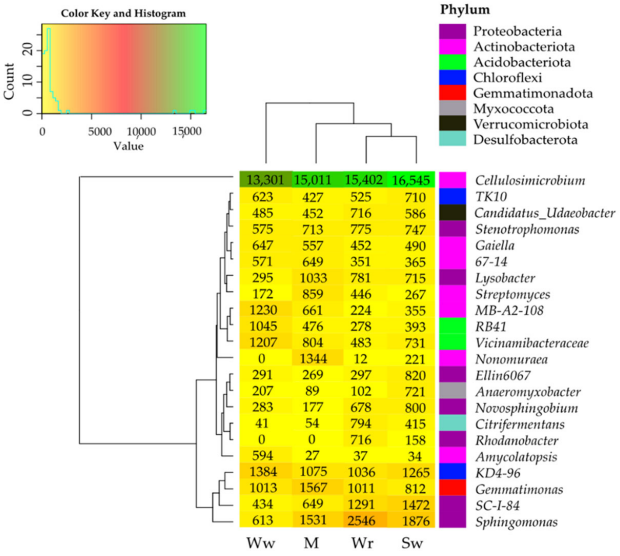


Figure 7. Dominant genera of bacteria in the soil with further cropping with *V. faba* L. spp. minor (OTU ≥ 1%). Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.

Soils Sw and Wr were primarily populated by fungi of the genus *Mortierella* (OTUs of 22,531 and 33,137, respectively), soil Ww was primarily populated by *Cladosporium* (16,862 OTUs), and soil M was primarily populated by *Alternaria* and *Stemphylium* (OTUs of 19,832 and 19,510, respectively) (Figure 8). In addition, fungi of the genus *Penicillium* extensively colonized soil Sw, with their relative abundance reaching 21,627 OTUs. Fungal OTUs were as follows: Sw—65,483; Ww—52,673; M—59,144; and Wr—60,603.

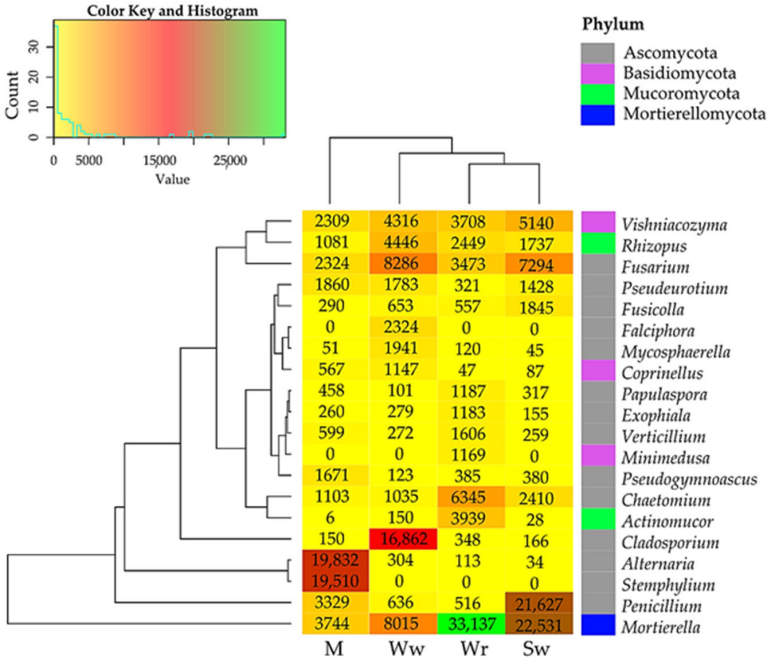


Figure 8. Dominant genera of fungi in the soil with further cropping with *V. faba* L. spp. minor (OTU ≥ 1%). Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.

Twenty common bacterial genera were identified in all analyzed soils (Figure 9), i.e., *Cellulosimicrobiu*, *Sphingomonas*, SC-I-84, KD4-96, Ellin6067, *Gemmatimonas, Novosphingobium, Stenotrophomonas, Vicinamibacteraceae, Anaermyxobacter*, *Lysobacter*, TK10, *Candidatus_Udaeobacter*, *Gaiella*, *Citrifermentans*, RB41, 67-14, MB-A2-108, *Streptomyces*, and *Amycolatopsis*.

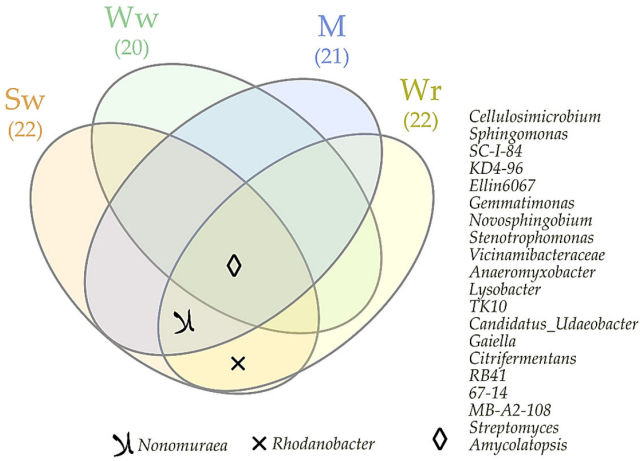


Figure 9. Unique and common genera of bacteria in the soil with further cropping with *V. faba* L. spp. minor (OTU ≥ 1%). Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.

According to the Venn diagram (Figure 10), 17 common genera of fungi were identified in all soils: Mortierella, Penicillium, Fusarium, Vishniacozyma, Chaetomium, Fusicolla, Rhizopus, Pseudeurotium, Pserudogymnoascus, Papulaspora, Verticillium, Cladosporium, Exophala, Coprinellus, Mysospharella, Alternaria, and Actinomucor. Unique fungal genera were identified as well, with these being Falciphora in the Ww soil, Stemphylium in soil M, and Minimedusa in soil Wr.

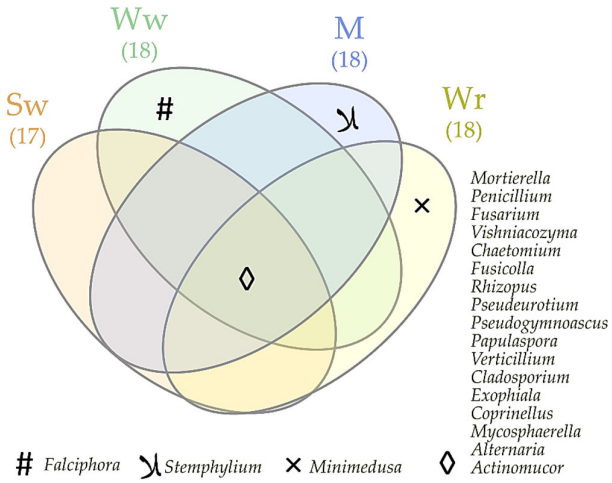
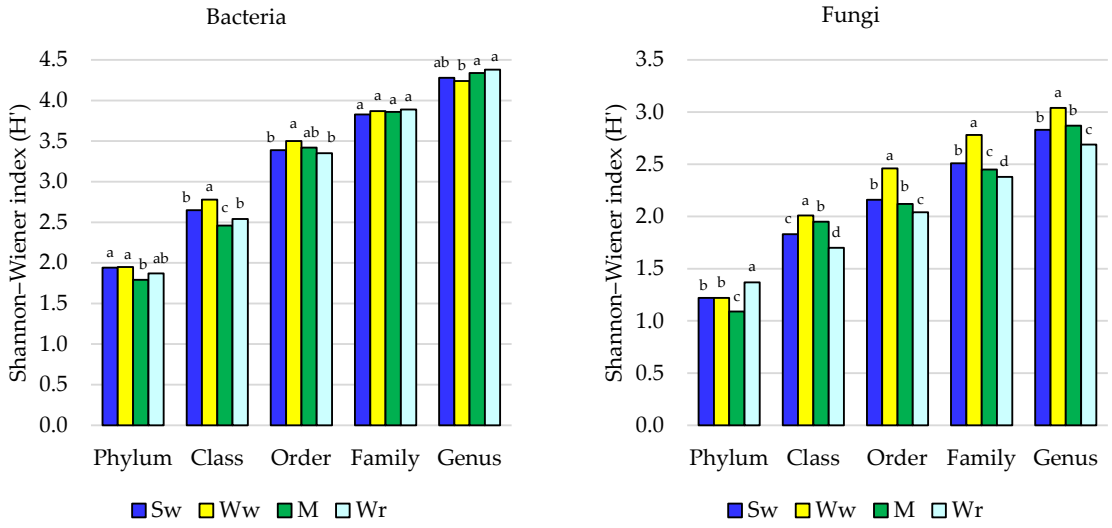


Figure 10. Unique and common genera of fungi in the soil with further cropping with *V. faba* L. spp. minor (OTU ≥ 1%). Sw—*T. aestivum* spp. *vulgare* L.; Ww–*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L.

The computed values of the Shannon–Wiener (H) and Simpson (D) indices (Figure 11) indicate that previous cropping caused greater changes in the diversity of fungi than in that of bacteria, with greater significant differences noted between the soil samples in the former. In the case of bacteria, the greatest changes were recorded at phylum and class levels, whereas in the case of fungi, these changes were significant across all analyzed taxonomic levels from phylum to genus. The highest values of the Shannon–Wiener and Simpson indices from all the studied sites were computed at the genus level. The Shannon–Wiener index values for bacteria ranged from 4.240 (Ww) to 4.380 (M), while those computed for fungi ranged from 2.690 (Wr) to 3.040 (Ww). The Simpson index values for bacteria ranged from 0.930 (Sw) to 0.950 (Wr), and for fungi, they ranged from 0.900 (Ww) to 0.960 (Wr).



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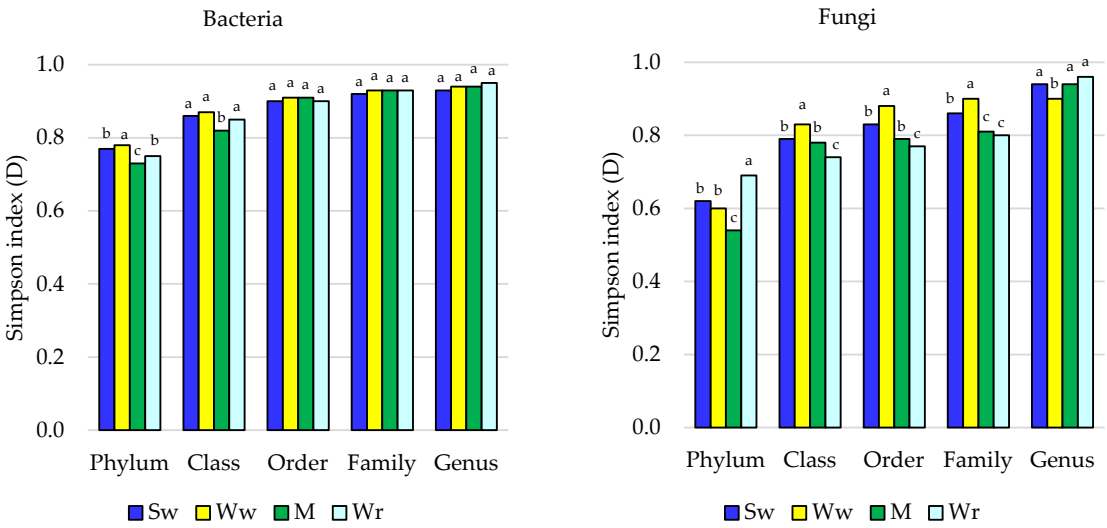

Figure 11. Shannon–Wiener (H′) and Simpson (D) indices for the soil with further cropping with *V. faba* L. spp. minor calculated from the abundance of operational taxonomic units (OTUs) of bacteria and fungi. Sw—*T. aestivum* spp. *vulgare* L.; Ww—*T. aestivum* spp. *spelta* L.; M—*Z. mays* L.; Wr—*B. napus* L. Homogeneous groups denoted with the same letters (a–d) were calculated separately for each taxonomic group of microorganisms (one-way ANOVA with Tukey's test at significance level of $p = 0.05$).

4. Discussion

4.1. Cultured Bacteria and Fungi

Our results demonstrate that the number of microorganisms in the soil was significantly affected by the previous crops farmed and the cultivation method. Among the soils sown with *V. faba* L. spp. minor, organotrophic bacteria and fungi showed the highest growth when spring wheat alone was used as the previous crop, whereas actinobacteria were the most successful in the soil with spring wheat and winter wheat as the previous crops. For unsown soil, the highest abundance of organotrophic bacteria and fungi was recorded in the post-winter rapeseed soil, whereas the highest number of actinobacteria was noted in the post-spring wheat soil. The higher population numbers of the microorganisms in the faba bean soil previously used to grow spring wheat may be due to the greater abundance of nutrients, which allowed the microbes to colonize the soil easier than the other soil types examined. In addition, the post-spring wheat soil could have also contained chemoattractants which attracted specific groups of microorganisms [46,47]. The intercrop cultivation of wheat and faba beans may affect the structure of rhizosphere microorganisms by modifying the physiological characteristics of plants and root secretions and may also improve soil quality by increasing the total number of microorganisms [48]. Ghorbi et al. [16] reported that *V. faba* L. spp. minor promotes the activity of soil microorganisms by fixing large amounts of atmospheric nitrogen. Changes in crop combinations may modify the structure of mycorrhizal fungi and rhizobia, which help the plants absorb and transport less mobile nutrients in the soil while also increasing their tolerance to drought and resistance to pathogens. This root–microbe symbiosis promotes the production of root secretions, with a beneficial effect on other rhizosphere microorganisms [49]. A study by Mbuthia et al. [50] showed a higher microbial abundance in uncultivated soils compared to conventionally farmed soils. In particular, uncultivated soils had higher counts of Gram-positive bacteria, actinobacteria, and arbuscular mycorrhizal fungi compared to the soil from under continuous cotton sown with *Vicia villosa* and *T. aestivum* L. According to these authors, these variations in the microbial population may have resulted from changes in soil moisture, C and N levels, and soil pH.

Furthermore, we observed changes in the microbial growth rate in the soil, as evidenced by the microbial colony development index (CD) values. The *V. faba* L. spp. minor

rhizosphere had the fastest growth of organotrophic bacteria and fungi when previously used to grow spring wheat, whereas actinobacteria grew the fastest in the post-winter wheat soil. The growth rate of the analyzed microorganisms could be expressed as follows: organotrophic bacteria = fungi > actinobacteria. This particular hierarchy may result from the presence of those nutrients in the soil that promote the development of rapidly growing microorganisms, which in our study included organotrophic bacteria and fungi [34,35]. The soil used to grow maize and then *V. faba* L. spp. minor exhibited the greatest diversity of organotrophic bacteria and actinobacteria, whereas the post-winter wheat soil showed the greatest diversity of fungi. These results show that changes in the structure of microorganisms are not always associated with their diversity [51].

4.2. Bacterial and Fungal Communities

The soil management system can significantly affect the physicochemical properties of soil, including the carbon and nutrient levels, texture, and pH, which in turn cause changes in the diversity and functioning of the soil microbiota [52,53]. Soil microorganisms participate in nutrient cycling, regulate gas exchange, cause microaggregation, and carry out biochemical processes in the soil environment, which is why their diversity is seen as a key component of quality and health in soil tillage systems [54]. Fox et al. [55] reported that crop species, i.e., grasses, forbs, legumes, and their mixtures, can have different impacts on the soil microbial community make-up. These differences are primarily determined by the physiological characteristics of the plant (including mass and length) and the chemical composition of their roots, especially root secretions. Crop residues and root secretions can persist in the soil matrix for extended periods of time, influencing the growth and development of the successor plants.

The taxonomic composition and diversity of soil microorganisms is an important biological indicator reflecting the formation and evolution of soil fertility. Among soil microorganisms, bacteria are most abundant and play an irreplaceable role in improving the ecological environment [56]. Our results indicate that there is a relationship between the changes in the structure of the bacterial community and the type of the previous crop grown. According to Jalali et al. [57], Soliman et al. [58], and Bünemann et al. [59], the structure of microbial communities significantly affects the stability, quality, and productivity of soil ecosystems. Granzow et al. [30], Bakker et al. [60], and Wemheur et al. [61] reported that the arable soils are mostly colonized by bacteria of the phyla Actinobacteriota, Acidobacteriota, Proteobacteria, Bacteroidetes, and Firmicutes. In our study, Actinobacteriota consistently prevailed over all other analyzed bacteria; however, their exact abundance varied depending on the previous crop. The number of Actinobacteriota was 1.2-fold higher when maize was used as the previous crop rather than spring wheat. Actinobacteriota are generally the dominant bacteria in the soil environment due to their important role in biogeochemical cycles and their metabolic capacity to produce various bioactive substances, including those with antibacterial and antifungal effects. In addition, they produce extracellular hydrolytic enzymes, which can decompose plant residues, animal residues, and other organic compounds from which soil humus is formed [62,63]. Zhang et al. [64] and Xu et al. [65] have shown that the structure of the Actinobacteriota communities is significantly affected by the type of vegetation. Zhang et al. [66] have claimed that the organic matter and root secretions of soil overgrown with diverse vegetation provide a good microenvironment for these bacteria, which may in turn significantly affect the structure of their communities. The predominance of Actinobacteriota in our study may stem from the adequate nutrients supplied by the previous crop, which fueled rapid Actinobacteriota growth in the soil. In addition, these particular microbes play an important role in the transformation of plant residues, which explains why they were more abundant than any other identified bacteria in the analyzed soils [67,68]. On the genus level, we found that *Cellulosimicrobium* bacteria (a genus belonging to the phylum Actinobacteriota) were the most successful in the soils. Indeed, Park and Shim [69] have demonstrated that *Cellulosimicrobium* are widespread in soil, grasses, and decomposing plant material. The genus produces multiple

enzymes that degrade plant cell walls and can convert lignocellulosic biomass. Thus, it may be speculated that the previous crops used on the examined soils may have become an appropriate substrate for the growth and development of *Cellulosimicrobium.* The phylum Proteobacteria was the second largest found in the analyzed soils and was the most prolific in the post-winter rapeseed soil, with counts 1.8-fold higher than in the post-winter wheat. Such differences in the proportions between the bacteria may be due to changes in the chemical and physical properties of the soil, which in turn affected the structures of these bacterial communities [53,70]. According to Spain et al. [71], Proteobacteria are the prevailing phylum in soil capable of withstanding extreme conditions and, therefore, are claimed to reliably reflect the structure of the bacterial communities in the soil ecosystem. Additionally, Rodrigues et al. [72] and Wang et al. [73] count Proteobacteria among the fast-growing copyotrophs that grow well in soil rich in easily degradable organic matter. The opposite is true for oligotrophic bacteria, including for example Acidobacteriota, Planctomycetota, Gemmatimonadota, Verrucomicrobiota, and Chloroflexi, which respond negatively to the increased carbon in the soil, which may explain their lower growth rate in the soils analyzed by us. This is corroborated by Niewiadomska et al. [74], who explored the effect of the cultivation method on soil microbiological parameters and similarly found Actinobacteriota and Proteobacteria to be the most abundant phyla.

Fungi play a very important role in soil ecosystems, contributing to organic matter degradation and to the carbon, nitrogen, and phosphorus cycle [75], promoting plant growth and development, and increasing plant resilience against disease [76]. They are involved in the formation and stabilization of soil aggregates (mainly macroaggregates) and therefore play a central role in the processes of soil structure formation. The stabilization of soil aggregates by fungi varies depending on the species and the nature of the available substrates and the soil cultivation method [77,78]. Ma et al. [79] believe that the specific extracellular enzymes of fungi make them more effective in degrading plant residues in the soil than bacteria. He et al. [80] have found that the amendment of soil with nitrogen or organic matter led to modifications in the structure of fungi either directly, by providing them with necessary nutrients, or indirectly, by changing the edaphic conditions. Therefore, Frąc et al. [81] consider that soil fungi, due to their functions in the environment, can be divided into three functional groups, namely, as biological regulators (regulate the development of diseases and organisms harmful to plants); ecosystem regulators (shape the soil structure and habitat for other organisms by stabilizing physiological processes occurring in the soil environment); and those involved in the decomposition of organic matter and the transformation of other compounds.

Delgado-Baquerizo et al. [82] and Yang et al. [83] reported that changes in the structure of fungal assemblages can potentially impact the function of the entire soil among other things through interactions with other soil organisms. In our study, most of the soils (spring wheat, winter wheat, and maize as previous crops) were primarily colonized by fungi of the phylum Ascomycota. The one exception was the soil previously used to grow winter rapeseed, which proved more accommodating to Mortierellomycota fungi. In addition, the soils were also colonized by Basidiomycota and Mucoromycota but not as extensively as by Ascomycota and Mortierellomycota. Different previous crops also produced different numbers of fungi from the prevailing phylum. In the soil with maize as the previous crop, the relative number of Ascomycota was two times higher compared to that in soils previously cropped with winter rapeseed. In turn, the winter rapeseed batch had a higher relative abundance of Mortierellomycota. These differences in fungal community make-up may result from changes in taxonomic structures [84]. Ji et al. [76] have indicated that Ascomycota are saprophytic copyotrophs classified as r-strategists, which can break down hard-to-decompose organic substances, such as lignin and keratin. Indeed, this phylum plays an important role in the carbon and nitrogen cycle, plant biomass degradation, and endophytic interactions with plants. Furthermore, Ascomycota species can form symbiotic associations or act as latent saprotrophs or pathogens living in plant tissues [85,86]. Challacombe et al. [85] have reported that lignin degradation

was mainly associated with Basidiomycota fungi, whereas Ascomycota are considered to be scarcely capable of breaking down lignin as they do not possess the genomes and oxidases responsible for its degradation. However, there are some species which can grow on lignin, whose genomes encode lignin-oxidizing lacases and enzymes. Ascomycota thrive in dry meadow soils, whereas Basidiomycota prefer organic-rich soils [79,80,85,86]. Our study demonstrated significant differences in the proportions of Ascomycota and Mortierellomycota, which may be related to the soil environment stabilization, the impact of soil conditions, and the availability of the substrate used by these fungi. The number of fungi belonging to Ascomycota may decrease as the organic matter in the soil is degraded. Other fungi, for example, Mortierellomycota or Basidiomycota, may take up these organics for growth, resulting in nutrient depletion and the inhibition of Ascomycota growth [87]. In addition, our study showed that spring wheat and spring rapeseed used as the previous crops positively affected the development of the *Mortierella* genus fungi, winter wheat positively affected the development of *Cladosporium* fungi, and maize positively affected the development of *Alternaria* fungi. Soil used to grow *V. faba* L. spp. minor after previous cropping with spring wheat or maize stimulated the growth of fungi of the genus *Penicillium* and *Stemphylium*, respectively. Finally, *Mortierella* and *Penicillium* exhibited antagonism against plant pathogens—an effect that may bolster plant development [5]. In contrast, Ali et al. [88] have reported that many species of the genus *Alternaria* led to significant losses in crop yields. These species mainly attack the aboveground parts of plants, causing a variety of symptoms from necrotic leaf spots to infested shoots, which ultimately leads to defoliation and a decrease in the quality and quantity of crop yields.

5. Conclusions

Previous cropping produced changes in the proliferation and structure of bacterial and fungal communities in soil used to grow *V. faba* L. spp. minor. Where *T. aestivum* spp. *vulgare* L. was used as the previous crop prior to *V. faba* L. spp. minor cultivation, our analyses demonstrated that organotrophic bacteria and fungi had the highest population numbers of all the microbial groups and the highest values of the colony development index. The highest values of the ecophysiological diversity index for organotrophic bacteria and actinobacteria were computed for the soil sample previously cropped with *Z. mays* L., whereas *B. napus* L. produced the highest EP for fungi. In the unsown soil, the highest numbers of organotrophic bacteria and fungi were observed in the soil previously used to grow *B. napus* L., while the highest numbers of actinobacteria were found in the soil previously cropped with *T. aestivum* spp. *vulgare* L. The CD index for organotrophic bacteria, actinobacteria, and fungi peaked in the *B. napus* L., *T. aesttivum* spp. *spelta* L., and *Z. mays* L. soils, respectively. In contrast, the EP index for organotrophic bacteria was the highest in the *Z. mays* L. soil, while that of actinobacteria and fungi was the highest in the *T. aestivum* spp. *vulgare* L. soil. Both in the unsown soil and soil sown with faba bean, the organotrophic bacteria and fungi were mostly represented by the fast-growing species, while actinobacteria were mostly represented by the slow-growing ones. The samples of soil sown with *V. faba* L. spp. minor were primarily colonized by the bacteria belonging to the phylum Actinobacteriota, with *Cellulosimicrobium* being their most abundant representative. In terms of fungi, the phylum Ascomycota was the most prolific one, with the exception of the soil previously cropped with *B. napus* L., in which Mortierellomycota was the predominant phylum. The *T. aestivum* spp. *vulgare* L. and *B. napus* L. soils were most heavily populated by *Mortierella*, the *T. aestivum* spp. *spelta* L. soil was most heavily populated by *Cladosporium*, and the *Z. mays* L. soil was most heavily populated by *Alternaria*. Unique fungal genera were also identified, these being *Falciphora* in the *T. aestivum* spp. *Spelta* soil, *Stemphylium* in the *Z. mays* L. soil, and *Minimedusa* in the *B. napus* L. soil. These results show that these previous crops can modify the number and structure of bacterial and fungal communities in soil sown with *V. faba* L. spp. minor, which may have an indirect impact on the growth and development of crops.

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