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Microbial Activity and Diversity in Soil Sown with *Zea mays* and *Triticosecale*

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Abstract: The ongoing scientific debate on the selection of the best bioindicators to reflect the quality of arable soils indicates both their microbiome and biochemical parameters. Consideration has also been given to the fact that *Zea mays* has achieved the status of a crop used in the feed industry and for energy purposes, and *Triticosecale* is attracting increasing interest in this area. Therefore, the aim of this study was to determine the wide range of effects of *Zea mays* and *Triticosecale* cultivation on soil microbial and biochemical activity. The assessment of these parameters was based on the determination of microbial abundance, colony development index (CD), ecophysiological index of microbial diversity (EP), soil enzyme activities (dehydrogenases, catalase, urease, acid phosphatase, alkaline phosphatase, *β*-glucosidase, and arylsulfatase) as well as soil physicochemical properties. The innovative nature of the research was achieved by extending the pool of analyses to include both microbial biodiversity and analysis of soil samples at three depths: 0–20 cm; 21–40 cm; and 41–60 cm. It was found that the highest activities of soil enzymes and the abundance of organotrophic bacteria and fungi, as well as their colony development indices (CD), occurred within the rhizosphere and that their values decreased with increasing depth of the soil profile layers. Two phyla, *Actinobacteria* and *Proteobacteria*, representing the microbiome of arable soils, were identified independently of soil management practices. Unique bacterial genera in the soil under *Triticosecale* cultivation were *Pseudonocardia*, whereas *Rhodoplanes*, *Nocardioides*, and *Rhodanobacter* were found under *Zea mays* cultivation. The activity of all enzymes, especially urease and arylsulfatase, was significantly higher in the soil under *Triticosecale*. This was influenced by the more favorable physicochemical properties of the soil.

Keywords: triticale; maize; quality of soil; diversity of the microbiome; biochemical parameters

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1. Introduction

In the European Union, Poland is the second largest producer of cereals after France. It accounts for about 74% of the total agricultural area. It is also the third largest producer of cereals, after France and Germany, with a harvest of 26.5–31.8 million tonnes [1]. The most commonly grown cereals in the country are maize and winter triticale, the area of which is steadily increasing. The popularity of these crops is determined by their tolerance to the country's climate and soil conditions and their ability to be used for food, feed, and energy purposes alike [2,3]. Significantly, on a global scale, production of *Zea mays* exceeds an impressive 1147.7 million tonnes per year [4], with Poland, Germany, and France being the largest producers of triticale in the world. Global production is 17 million tonnes per year, and 90% of this cereal is produced in Europe [5].

One aspect of soil fertility is its ability to meet the nutritional needs of plants [6]. It can be described as the ability to provide plants with essential nutrients and water [7,8]. The main soil properties that help to express its fertility are the abundance of macro- and microelements, the pH, the availability of soil air, and the prevailing moisture conditions in

the environment [8,9]. Soil fertility and productivity are largely determined by the chemical composition of the parent rock, the particle size distribution, which determines its structure, water-holding, and sorptive properties, as well as the content of organic matter and the diversity of soil microorganisms and their biochemical activity [8,10].

There is no doubt that the loss of biodiversity in agricultural soils is a threat to human well-being [11]. To mitigate the ongoing trend and its consequences, Global Biodiversity Ecosystem Services Models (GBESM) have been developed, incorporating land use data and classification of both green and agricultural areas [12]. Biodiversity conservation also provides an opportunity to achieve the 17 sustainable development goals, which ensure environmental and social well-being and are expected to be achieved by 2030 [13,14]. Importantly, the concept of biodiversity is increasingly included in regional and global policy frameworks and is represented in the United Nations (UN) Sustainable Development Goals (SDGs) [13] and the Convention on Biological Diversity (CBD) [15]. Importantly, it is now also part of the European Union (EU) Green Deal [16].

A fundamental element of agricultural development is the soil, the quality of which depends on the health and vitality of the organisms that inhabit it [17,18]. Therefore, assessing the current state of soil biological properties is of great importance [19].

This is important, if only because the availability of elements such as nitrogen, phosphorus, and sulfur to plants is enabled by microbial control of soil fertility, which ultimately benefits the overall productivity of agroecosystems [20–23]. Soil microorganisms contribute significantly to nutrient cycling in the environment and possess biotransformation capabilities that enhance cycles that support human life processes that, in turn, depend on natural ecosystems and, consequently, plant development and growth [24]. They also participate in the formation of suitable soil structures and enhance plant resistance to stress factors [25,26]. Soil microorganisms play an important role in the transformation of organic and mineral nutrients as well as in the detoxification of pollutants [27]. They can also indicate plant productivity and their resilience to stressful conditions [28].

Achieving an ecological balance, which is undeniably linked to the maintenance of biodiversity, is only possible by improving knowledge of the soil microbiome, as bacteria are generally considered to be universal indicators of the current state of a given soil environment [29]. The microbiome exhibits an interdependence with various environmental properties. Among the most important factors are the geographical location, the flora, and the organic matter content of the site. Additionally, microorganisms respond to soil nutrient levels, and changes in water and temperature conditions [30–32]. The composition of soil microbial communities is also influenced by soil pH [33–36]. Furthermore, it has also been demonstrated that soil depth rather than geographic location, dominates the structure of bacterial communities [37]. Despite the large number of studies conducted, this knowledge is still limited and there is a continuing need to understand the relationships and effects of environmental characteristics on microbial community diversity [36].

The impact of microbial biodiversity of the microbiome in the soil environment is also determined by biochemical activity [38]. Soil enzyme activity is influenced by many factors. To ensure the biochemical properties of the soil, attention should be paid to management practices, including organic fertilization, use of crop residues, and proper crop rotation design. Additionally, attention should also be paid to the appropriate use of plant protection products [39–41]. Soil enzyme activity is considered to be an indicator of soil fertility [42–44]. Soil enzymes respond to changes in soil management, anthropogenic activities, and environmental pollution caused by heavy metals or toxic substances long before changes are detected by other soil quality indicators [45]. Additionally, climate change should be considered as it directly and indirectly affects soil, cultivated vegetation, livestock, and crop pests [46]. Projections indicate that climate change will affect soil processes and the restoration of soil productivity and fertility [47]. The impact of current, strong climate change on the productivity of the soil environment can be estimated by monitoring the enzymatic activity of soil microorganisms and observing changes in soil

properties. Therefore, it is necessary to deepen the knowledge of soil biochemical activity in order to practice sustainable planning and management of crop production [41,48–50].

The ongoing scientific debate regarding the selection of the best bioindicators to illustrate soil quality is highlighted by a bibliometric analysis carried out by Bonilla-Bedoya et al. [51]. They identified 14,970 keywords from scientific articles, of which 25% were related to the definition of soil enzymatic activity and the potential of microorganisms as important indicators of the biodiversity of arable soils. Particularly important biochemical parameters indicating soil condition include dehydrogenases, ureases, and phosphatases [52]. Based on these parameters, including the activity of catalase, arylsulfatase, and β-glucosidase, indicators of soil fertility are established [53]. The sensitivity of enzymes to soil changes arises from the fact that their matrix serves as a space for competition or inhibitory effects [54]. A comprehensive analysis of the structural diversity of soil microbiomes can be achieved by understanding their nucleotide sequences. Based on these sequences, it has been shown that the dominant bacterial taxa in arable soils at the phylum level are mainly *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Chloroflexi* [55,56]. It is also worth noting that according to Alami et al. [57] and Rao et al. [58], the highest abundances, accounting for more than 10%, are mainly generated by *Proteobacteria*, *Acidobacteria*, and *Actinobacteria*.

Along with agricultural intensification, land-use change is the main cause of global biodiversity loss [59]. However, optimizing the activity of soil microorganisms and future research aimed at expanding knowledge in this area can ensure stable agricultural productivity by verifying the microbial potential for ecosystems [24].

Considering the above trends, the aim of this study was to determine the broad effects of maize and triticale cultivation on soil microbiological and biochemical activity, including microbial abundance and biodiversity and the activity of seven soil enzymes. The innovative nature of the experiment was achieved by verifying the above parameters also in deeper soil layers (0–25 cm), which, according to Hao et al. [60], receive the most attention. Two research hypotheses were tested: (1) there is a strong correlation between the crop species and microbial diversity and soil enzyme activity; (2) soil enzyme activity is a function of microbial diversity.

2. Materials and Methods

2.1. Soil Sampling Area

The agricultural land surveyed is located in the Mazury Plain in the southern part of Szczytno County (northeastern Poland), in the heart of the region known as the 'Green Lungs of Poland' (Figure 1).

The soil samples were collected in the southern part of Szczytno County, which is characterized by flat terrain and poor-quality soils for agricultural production, covering an area of 3 ha [61], where maize and winter triticale were cultivated. Soil samples were collected from three different depths: 0–20 cm; 21–40 cm; and 41–60 cm. Subsequently, the soil samples were transported to the laboratory, where they were divided into two parts: one part was spread out to dry to determine its grain size distribution and basic chemical and physicochemical properties, while the other part was stored at 4 °C for the determination of the microbiological and biochemical properties of the soil.

The cultivation of both analyzed plants was carried out on poor podzolic soils characteristic of the northeastern region of Poland. Both plant species were grown on soils composed of loose sand (Table 1).

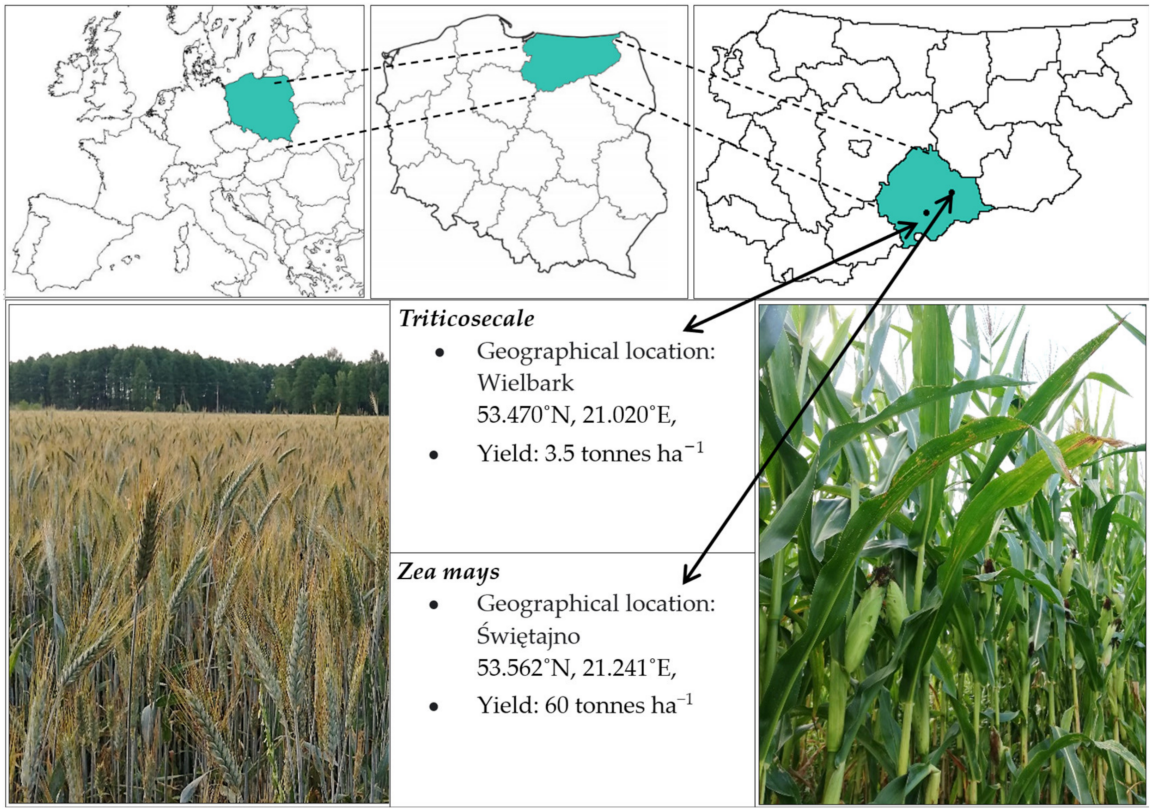

Figure 1. Geographic location of the analyzed sites.

Table 1. Soil granulometric composition.

Fraction Diameter (mm)	Depth (cm)					
	0–20	21–40	41–60	0–20	21–40	41–60
	Plants					
	Zea mays			*Triticosecale*		
	%					
2.0–1.0	0.00	0.00	0.00	0.00	0.00	0.00
1.0–0.5	0.00	0.00	0.00	0.00	0.00	0.00
0.5–0.25	0.22	0.28	0.00	1.23	0.27	1.66
0.25–0.1	26.48	34.24	22.34	35.81	37.29	41.73
0.1–0.05	47.46	48.74	71.64	49.53	51.54	52.06
0.05–0.02	14.05	6.77	6.02	9.62	5.35	3.43
0.02–0.006	7.34	5.62	0.00	2.86	4.03	1.08
0.006–0.002	4.41	4.32	0.00	0.95	1.52	0.04
<0.002	0.04	0.03	0.00	0.00	0.00	0.00
Granulometric Subgroup	loamy sand	loamy sand	sand	sand	sand	sand
Symbol	LS	LS	S	S	S	S

On the agricultural site where winter triticale was sown, sand is present in the upper layers of the overburden. In the case of the maize site, the layer from 0 to 40 cm depth consisted of loamy sand (Figure 2). Both sand and loamy sand belong to poorly fertile soils. The productive value of such soils can be increased by proper saturation of the sorption complex with base ions and by correct agronomy practices. The quality of sandy sites is largely influenced by the prevailing water conditions in the area [8,62,63].

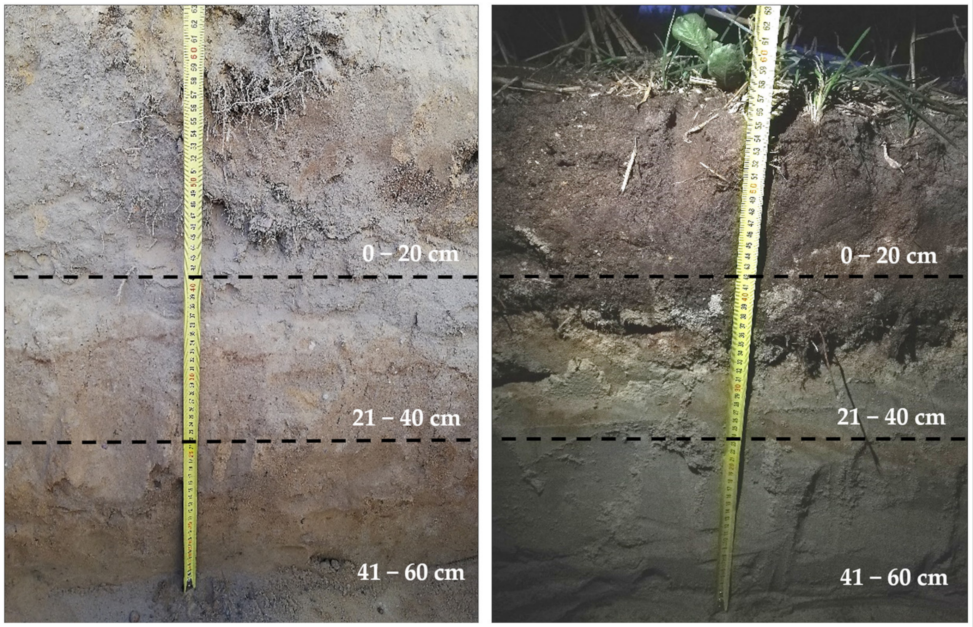

Figure 2. Photographs of excavated soil pits: *Zea mays* (**a**); *Triticosecale* (**b**).

Szczytno County is characterized by unfavorable conditions for the production of agricultural products. The average annual temperature in the district in the multiannual period (1991–2020) was 8 °C. The vegetation period in this region lasts approximately 219 days, while the annual precipitation total from 1991 to 2020 amounted to 600 mm [64]. In 2022, this region experienced a drought. The average annual temperature was 8.72 °C, while the total annual rainfall was 479.82 mm [65] (Figure 3).

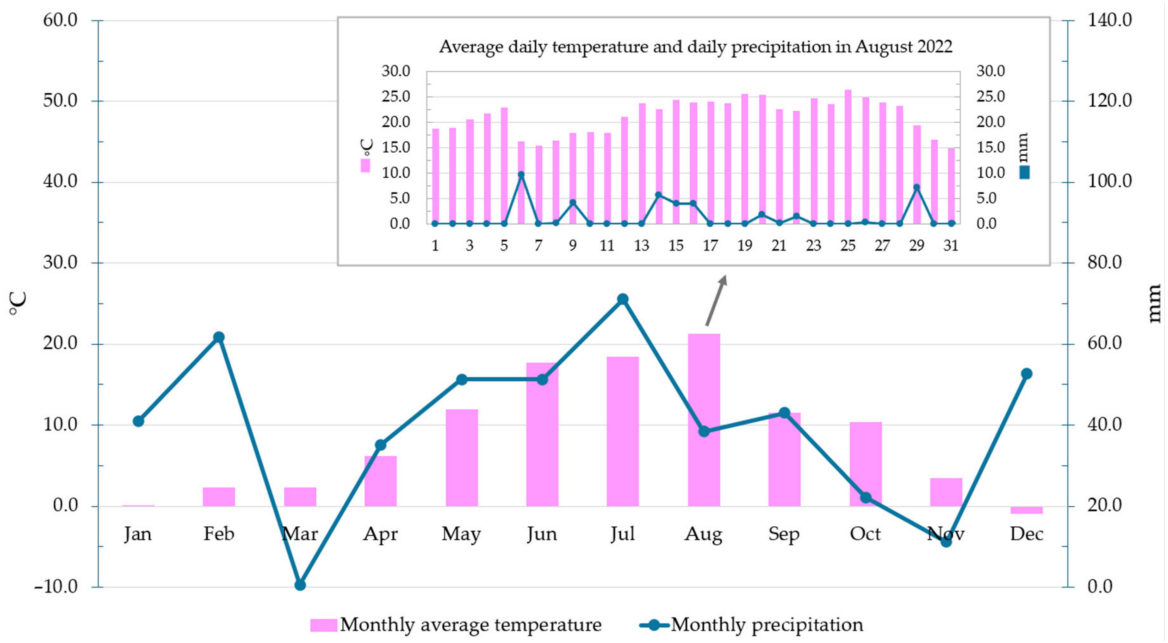

Figure 3. The average monthly temperatures and the monthly precipitation in the year 2022 in Szczytno County.

2.2. The Cultivation Technology of *Zea mays* and *Triticosecale*

Most of the farms located in the discussed county are focused on dairy cattle farming. Both *Zea mays* and *Triticosecale* are used in feed production, to meet the nutritional needs of cattle. Hence, the choice of crop plants for research purposes.

Selected species of plants have been used in agricultural production in the region in recent years and are often selected by farmers in the area. They are characterized by high yield, resistance to disease, and lodging. The winter triticale was preceded by a green crop. Corn was grown in monoculture. Maize was sown at a rate of 9 seeds per m^2, while triticale had 600 plants per 1 m^2. Natural and mineral fertilizers were used for maize fertilization. Their doses are given in Table 2 and their chemical composition in Table 3.

Table 2. Plant fertilization.

Cultivated Plant Species	Fertilizer	Dose	Fertilization Date
Zea mays	Cattle manure Polyphosphate 6	30 tons ha^{-1} 200 kg ha^{-1}	pre-sowing sowing fertilization
Triticosecale	Cattle liquid manure	26 thousand dm^3 ha^{-1}	pre-sowing

Table 3. Chemical composition of fertilizers, %.

Fertilizer	Chemical Composition, %
Cattle manure	N—0.50; P—0.13; K—0.57; Ca—0.11; Mg—0.36
Cattle liquid manure	N—0.34; P—0.09; K—0.31; Ca—0.15; Mg—0.08
Polyphosphate 6	N—6.0; P—8.73; K—25.0; S—2.8

Herbicides were applied to the maize plantation: Ikanos 040 OD at a rate of 1 dm^3 ha^{-1}; Mocarz 75 WG at a rate of 0.2 kg ha^{-1}; and Hydrotek at a rate of 0.2 dm^3 ha^{-1}, while for the winter triticale, Esteron 600 EC at a rate of 1 dm^3 ha^{-1} and Raxade at a rate of 50 g ha^{-1} were used.

2.3. Microbiological Analysis

A microbiological analysis was carried out to determine the abundance of soil-dwelling microorganisms, taking into account the plant species cultivated and the depth of soil sampling for each of the samples obtained. The serial dilution method was used to determine the abundance of organotrophic bacteria and mold fungi. The study was carried out in six replicates. The microorganism cultures were incubated in a Memmert Ine 550 incubator (Schwabach, Germany) at a constant temperature of 28 °C. Both groups of microorganisms were cultured for a period of 8 consecutive days. The abundance of analyzed bacteria and fungi was expressed using the colony forming unit (cfu) count per 1 kg of soil d.m. Additionally, the obtained results were used to calculate the colony development index (CD) and the ecophysiological diversity index (EP). These indices were described in detail in the manuscripts by Lipińska et al. [66] and Zaborowska et al. [67].

DNA was isolated from soil samples using the Genomic Mini AX Bacteria+ kit (A&A Biotechnology, Gdańsk, Poland). Universal primers 1055F (5′-ATGGCTGTCGTCAGCT-3′) and 1392R (5′-ACGGGCGGTGTGTAC-3′) were used in the reaction. Isolation was performed according to the procedure described by Ferris et al. [68]. Sequencing was performed based on the highly variable V3-V4 region using the Illumina MiSeq sequencer (Genomed S.A., Warsaw, Poland). Primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′) were used for amplification. Bioinformatic analysis was performed using QIIME 2 (Quantitative Insights Into Microbial Ecology) software based on the GreenGenes reference sequence database version 13_8. The results

of the relative abundance of identified sequences at five selected taxonomic levels with an OTU count ≥1% are presented.

2.4. Soil Biochemical Analysis

The activity of seven enzymes was determined from soil samples in three replicates (Table 4). The activity of all enzymes analyzed, except catalase, was measured using a Perkin Elmer Lambda 25 spectrophotometer (Waltham, MA, USA) by measuring the absorbance of the reaction products.

Table 4. The analyzed soil enzymes (kg^{-1} d.m. of soil h^{-1}) along with their reaction products and wavelengths.

Enzyme	Reaction Product	Wavelength (nm)	Unit	Literature
Dehydrogenases	triphenyl formazan (TFF)	485	µmol	[69]
Catalase	O_2	-	mol	[70]
Urease	N-NH_4	410	mmol	
Acid phosphatase	4–nitrophenol (PN)	410	mmol	
Alkaline phosphatase	4–nitrophenol (PN)	410	mmol	[71]
β-glucosidase	4–nitrophenol (PN)	400	mmol	
Arylsulfatase	4–nitrophenol (PN)	420	mmol	

The activity of soil enzymes can objectively reflect the condition of the soil under investigation. Therefore, in this study, we used the biochemical activity index (BA) proposed by Wyszkowska et al. [53], which combines the total activity of all enzymes and thus reflects the quality status of the soils under study.

2.5. Analysis of Physicochemical and Chemical Properties

For a more detailed characterization of the soils studied, their particle size distribution was determined using a Malvern Mastersizer 3000 Laser Diffraction (Malvern, Worcestershire, UK), pH, hydrolytic acidity (HAC), exchangeable basic cations (EBC), total nitrogen content, and organic carbon content. All measurements were made on air-dried soil sieved through a 2 mm mesh sieve. Soil pH was determined potentiometrically in 1 mol KCl solution using a pH meter HI 2221 (Hanna Instruments, Washington, UK), and hydrolytic acidity and exchangeable basic cations were determined using the Kappen method. The obtained HAC and EBC results allowed the determination of the soil cation exchange capacity (CEC) and the degree of base saturation (BS), as described in their manuscript [72]. Organic carbon and total nitrogen content were determined using a Vario Max Cube CN analyzer and expressed as g C_{org} kg^{-1} dry soil and g N_{Total} kg^{-1} dry soil, respectively. All analyses were carried out in triplicate.

2.6. Statistical Analysis of Results

The research results obtained were statistically analyzed using the Statistica package [73]. Homogeneous groups were calculated using the post-hoc analysis of Tukey's HSD test. The analysis performed was two-factorial, with the first factor being crop species and the second factor being the depth of soil sampling. Additionally, analysis of variance (ANOVA) was used to determine the percentage contribution of the independent variables (η^2). The results were also examined using Principal Component Analysis (PCA). STAMP 2.1.3 software was used to graphically represent the types of microorganisms identified by next-generation sequencing (NGS), with data statistically compared using the G-test (with Yates') + Fisher's [74]. Classes of microorganisms were visualized using the Circos tool [75], while orders and families of microorganisms were represented using a heat map generated by R v1.2.5033 software supplemented with R v3.6.2 and the gplots library [76,77]. Common and unique types of microorganisms were illustrated using InteractiVenn [78].

3. Results

3.1. Microorganisms

Using the percentage of variance in the dependent variable explained by the independent variable (η^2), it was shown that the depth of the soils analyzed significantly influenced changes in the abundance of organotrophic bacteria (96.93%) and the colony development index (CD) (79.96%) more than the species of cultivated plants themselves. However, the ecophysiological diversity (EP) of the bacteria was more related to the crop species (48.22%) than to the soil depth (32.85%) (Figure 4a).

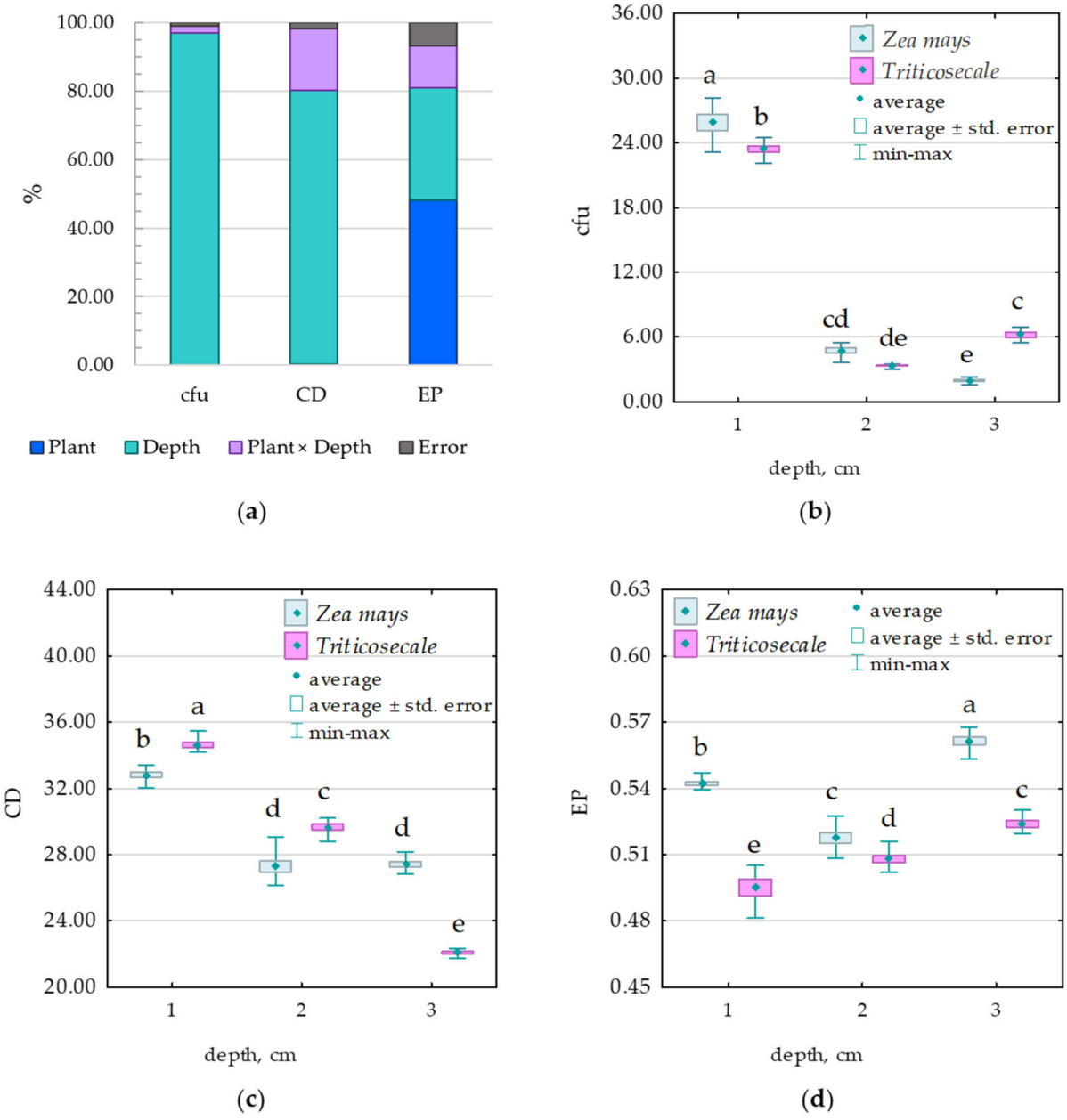

Figure 4. The percentage of independent variables (η^2) influencing the abundance of organotrophic bacteria in soil under *Triticosecale* and *Zea mays* cultivation (**a**); abundance of organotrophic bacteria (**b**), presented in cfu kg^{-1} d.m. of soil; colony development index (CD) of organotrophic bacteria in the soil (**c**); ecophysiological diversity index (EP) of organotrophic bacteria in the soil (**d**). 1, 2, 3—depth of soil samples: 0–20 cm; 21–40 cm; 41–60 cm. Homogeneous groups denoted with letters (a–e) were calculated separately for each enzyme. For all independent variables, homogeneous groups were determined using Tukey's test at $p = 0.05$.

When analyzing the abundance of organotrophic bacteria in the soils of both cereal sites, it was found that regardless of the crop species, the highest abundance of this group of microorganisms and their development index was recorded in the surface layer of the soil profile (Figure 4b,c).

Regardless of the crop species, bacteria responded similarly to changes in depth. In deeper layers of the soil profile, the values of the discussed characteristic decreased statistically. The highest values of the EP index of bacteria were recorded in the deepest parts of the soil profile of both cereals. Regardless of the soil profile studied, the EP index of organotrophic bacteria reached higher values in soils under *Zea mays* than under *Triticosecale* (Figure 4d).

Most of the bacteria found in the soils belonged to the *Actinobacteria* type. These bacteria constituted between 40.22% to 46.51% (Figure 5). Their abundance was higher in soils cultivated with *Zea mays* (Zm) than with *Triticosecale* (Tr). The relative abundance of these bacteria was 6.29% higher in *Zea mays* soils. The second most abundant phylum of bacteria was *Proteobacteria*. These bacteria ranged from 24.40% (Tr) to 27.76% (Zm). Their relative abundance, similar to *Actinobacteria*, was higher in soils under *Zea mays* cultivation (by 3.36%). These soils were also densely populated by *Acidobacteria*, *Chloroflexi*, and Gemmatimonadetes. However, their relative abundance was higher under *Triticosecale* cultivation than under *Zea mays* cultivation, by 5.45%, 4.85%, and 1.34%, respectively.

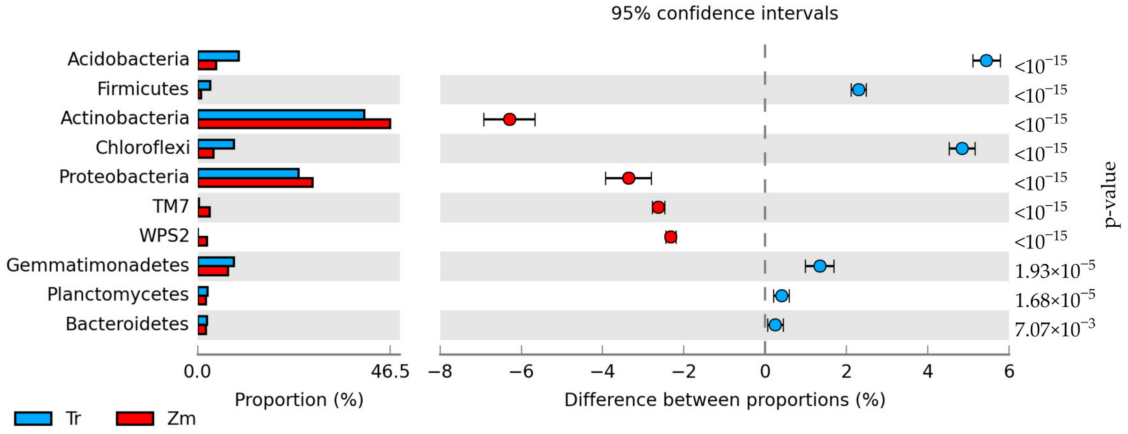

Figure 5. Differences in the relative abundance proportions of dominant bacterial types, presented using the statistical analysis software STAMP 2.1.3. Tr—*Triticosecale*; Zm—*Zea mays*.

The dominant bacterial classes in the soils were *Actinobacteria*, *Thermoleophilia*, and *Alphaproteobacteria* (Figure 6). They accounted for 15.43% to 28.79% (*Actinobacteria*), 8.68% to 31.37% (*Thermoleophilia*), and 13.24% to 17.23% (*Alphaproteobacteria*), respectively. The abundance of *Thermoleophilia* and *Alphaproteobacteria* was higher in the soil under Zm cultivation, while the abundance of *Actinobacteria* was higher in the soil under Tr cultivation.

The predominant bacterial family in both soils was *Gaiellaceae*, classified in the order *Gaiellales*, class *Thermoleophilia*, phylum Actinobacteria (Figure 7). Families such as *Nocardioidaceae*, *Sphingomonadaceae*, *Intrasporangiaceae*, *Xanthomonadaceae*, *Hyphomicrobiaceae*, and *Rhodospirillaceae* were less abundant. *Nocardioidaceae* and Intrasporangiaceae belong to the order *Actinomycetales*, class *Actinobacteria*, phylum Actinobacteria, while *Sphingomonadaceae*, *Xanthomonadaceae*, *Hyphomicrobiaceae*, and *Rhodospirillaceae* belong to the orders *Sphingomonadales*, *Xanthomonadales*, *Rhizobiales*, and *Rhodospirillales*, classes *Alphaproteobacteria* and *Gammaproteobacteria*, phylum Proteobacteria. *Gaiellaceae* and *Xanthomonadaceae* were more abundant in soils from *Zea mays* cultivation, whereas *Sphingomonadaceae*, *Nocardioidaceae*, and *Intrasporangiaceae* were more abundant in soils from *Triticosecale* cultivation.

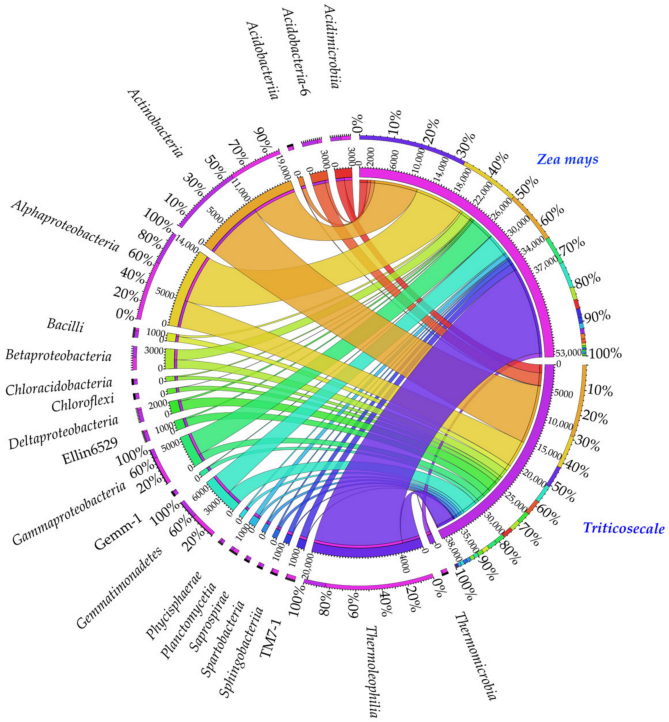

Figure 6. The dominant bacterial classes visualized using the Circos software, OTU ≥ 1%. Tr—*Triticosecale*; Zm—*Zea mays*.

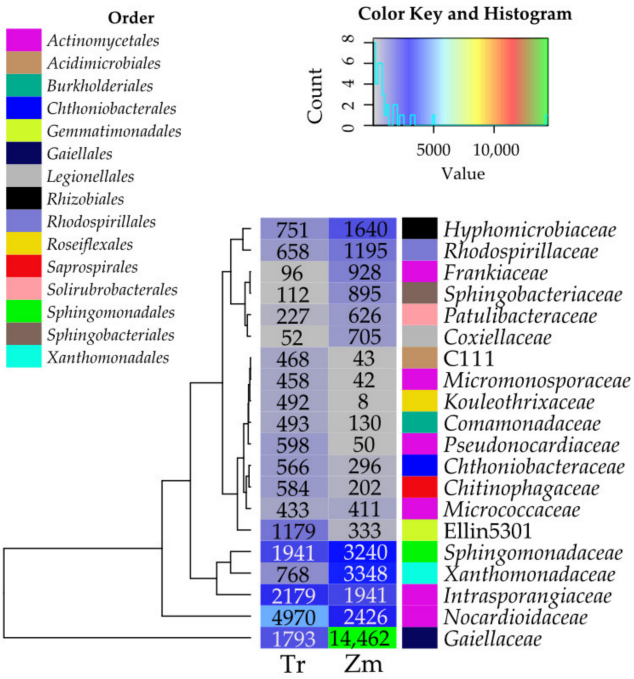

Figure 7. The predominant families of bacteria, depicted on the heat map, OTU ≥ 1%. Tr—*Triticosecale*; Zm—*Zea mays*.

Six bacterial genera with OTU ≥ 1% were identified from the data obtained (Figure 8). *Kaistobacter* and *Terracoccus* constituted the common soil microbiome. However, their OTU numbers differed significantly with respect to cultivated plants. *Rhodoplanes*, *Nocardioides*, and *Rhodanobacter* were unique genera in soils under *Zea mays* cultivation, while *Pseudonocardia* was the unique bacterial genus inhabiting soils under *Triticosecale* cultivation.

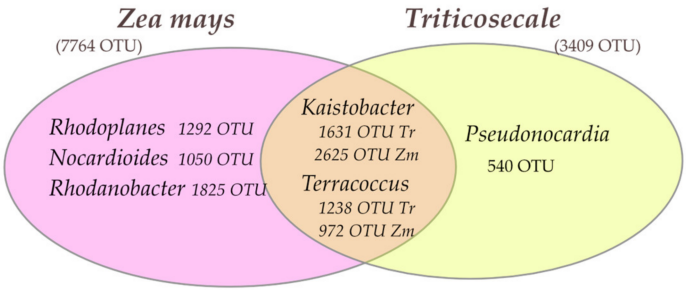

Figure 8. Venn diagram illustrating unique and shared genera of identified bacteria, OTU ≥ 1%. Tr—*Triticosecale*; Zm—*Zea mays*.

The analysis of η^2 did not reveal a clear relationship between the variables and the parameters analyzed for mold fungi. The abundance of fungi was more dependent on the depth of sampling (52.25%), similar to that of the organotrophic bacteria. Conversely, a different effect can be observed when assessing the percentage contribution of the colony development index of fungi, where the crop species significantly influenced the parameter studied (63.84%). However, the index of ecophysiological diversity of fungi was unequivocally influenced by the correlation of both variables (Figure 9a).

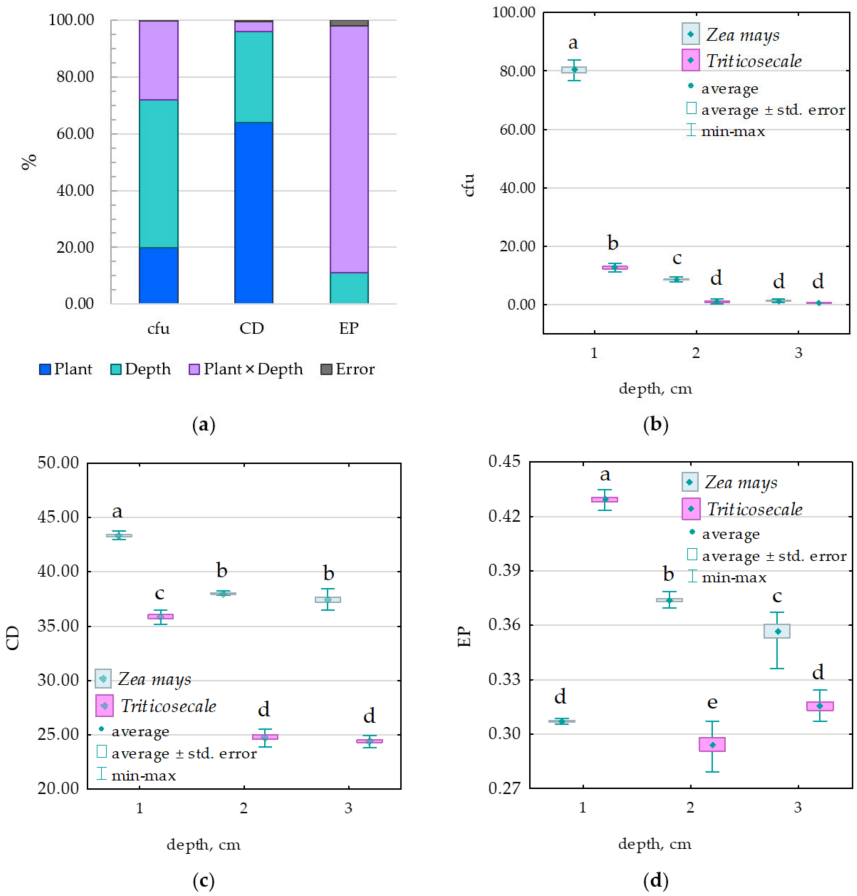

Figure 9. The percentage contribution of independent variables (η^2) affecting the abundance of fungi in soil under cultivation of *Triticosecale* and *Zea mays* (**a**); fungal abundance (**b**); presented in 10^6 cfu kg^{-1} d.m. of soil; colony development index (CD) of fungi in the soil (**c**); ecophysiological diversity index (EP) of fungi in the soil (**d**). 1, 2, 3—depth of soil samples: 0–20 cm; 21–40 cm; 41–60 cm. Homogeneous groups denoted with letters (a–e) were calculated separately for each enzyme. For all independent variables, homogeneous groups were determined using the Tukey test at $p = 0.05$.

The surface layer of the soil under the *Zea mays* cultivation exhibited the highest fungal abundance (Figure 9b). The abundance of these microorganisms in the topsoil under *Triticosecale* cultivation was 84.24% lower than under *Zea mays* cultivation. In both analyzed arable soils, the lowest fungal abundances were recorded in the soil at a depth of 41–60 cm, and compared to the surface layer of the soil, they were lower by 98.29% in the *Zea mays* cultivated soil and by 94.93% in the *Triticosecale* cultivated soil, respectively. The soil cultivated with *Zea mays* showed the highest values of the fungal colony development index (CD) at each of the depths analyzed (Figure 9c). Comparing the maize and winter triticale sites, it can be observed that these crop species responded similarly to changes in soil depth. The highest value of the ecophysiological diversity index of mold fungi was obtained in the soil layer from 0 to 20 cm under *Triticosecale* cultivation and was 0.429, while in the soil at the same depth but under *Zea mays* cultivation, it was 0.307 (Figure 9d).

3.2. Enzymes

The obtained values of η^2 unequivocally indicate that the biochemical activity of the soil is determined more by the depth of the soil profile than by the crop species (Figure 10a). The soil depth shaped the enzyme activity ranging from 97.33% (Deh) to 54.97% (Ure), while the plant species ranged from 31.17% (Ure) to 1.36% (Glu). On both arable soils analyzed, dehydrogenase activity was highest in the shallowest parts of the soil profile (Figure 10b). The highest dehydrogenase activity was recorded on the winter triticale site and was 19.38% higher than the soil from the same depth but under *Zea mays* cultivation. At other depths, this activity decreased drastically. Similarly, the catalase activity decreased with increasing depth of the soil profile at both sites. The highest catalase activity was recorded on the winter triticale site and was 23.40% higher compared to the *Zea mays* cultivated soil at the shallowest of the depths considered (Figure 10c). The soils cultivated with *Triticosecale* showed, similar to the case of oxidoreductases, a higher activity of the hydrolases analyzed. Comparing the hydrolase activity in the soil at a depth of 0–20 cm, the activity of urease was 70.83%, *β*-glucosidase 69.13%, alkaline phosphatase 56.48%, acid phosphatase 45.92%, and arylsulfatase 37.76% higher in soil samples from *Triticosecale* cultivation than in those from *Zea mays* cultivation (Figure 10d–h).

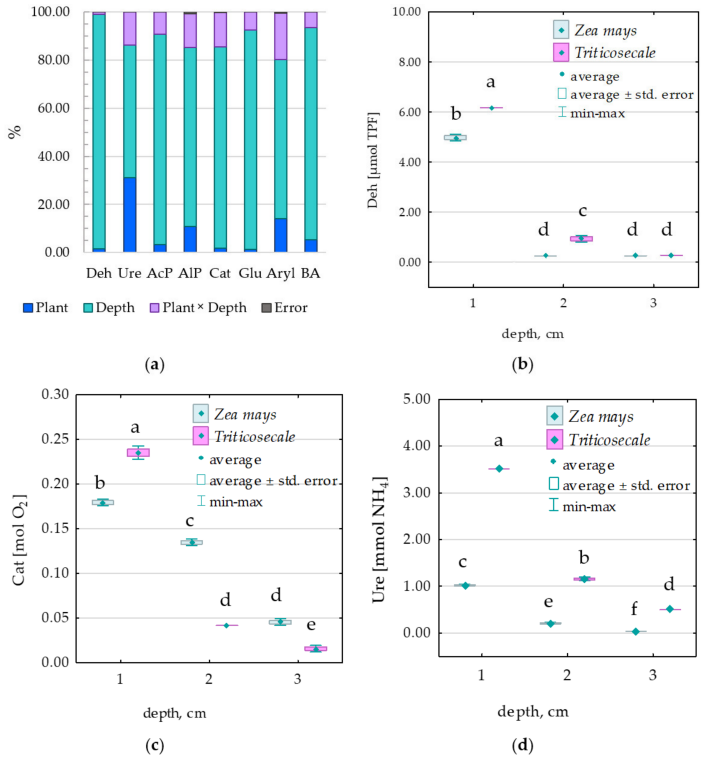

Figure 10. *Cont.*

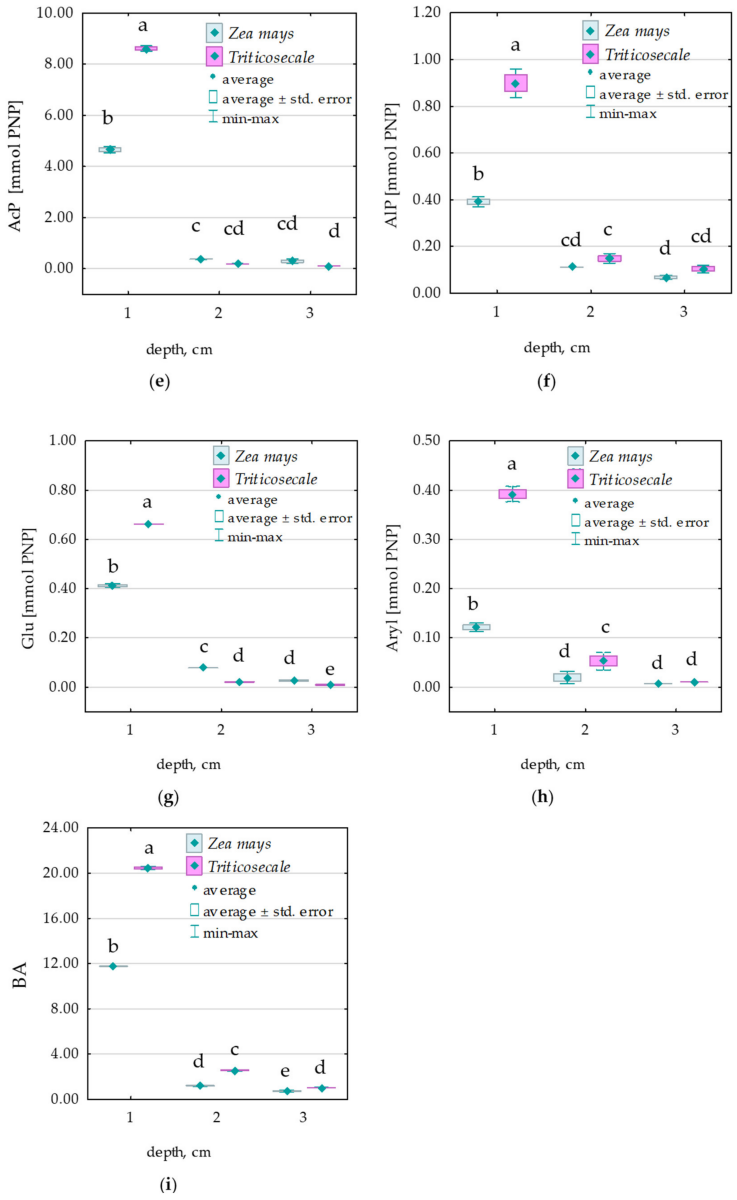

Figure 10. The percentage contribution of independent variables (η^2) affecting the activity of soil enzymes under *Triticosecale* and *Zea mays* cultivation and soil enzyme activity, presented in 1 kg dm of soil h^{-1}. 1, 2, 3—depth of soil samples: 0–20 cm; 21–40 cm; 41–60 cm. (**a**) the percentage contribution of independent variables (η2); (**b**) Deh—dehydrogenases; (**c**) Cat—catalase; (**d**) Ure—urease; (**e**) AcP—acid phosphatase; (**f**) AlP—alkaline phosphatase; (**g**) Glu—*β*–glucosidase; (**h**) Aryl—arylsulfatase; (**i**) BA—biochemical activity index. Homogeneous groups denoted with letters (a–f) were calculated separately for each enzyme. For all independent variables, homogeneous groups were determined using Tukey's test at $p = 0.05$.

3.3. Physicochemical and Chemical Properties of Soil

The physicochemical properties of the soil (pH—74.11%, HAC—72.81%, EBC—42.43%, CEC—56.56%, and BS—41.64%) were more dependent on the cultivated plant species, while the content of organic carbon (84.65%) and total nitrogen (85.42%) were more dependent on the soil depth (Figure 11).

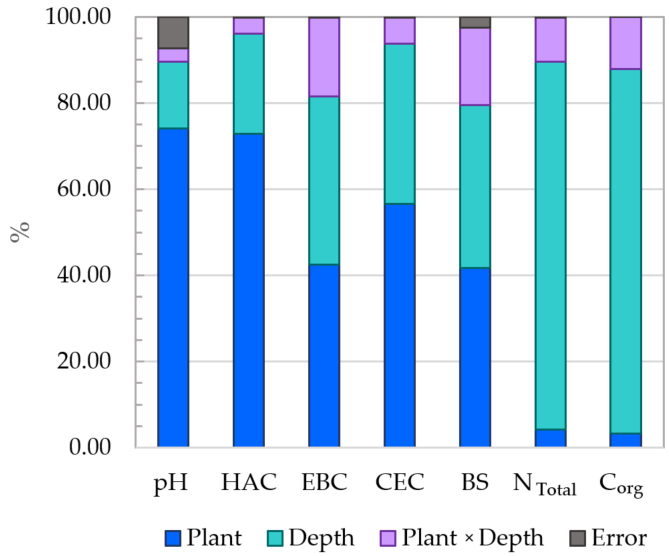

Figure 11. Percentage contribution of independent variables (η^2) affecting selected physicochemical and chemical properties of soils under cultivation of *Triticosecale* and *Zea mays*.

The soil under *Zea mays* cultivation exhibited very strong acidification (pH 4.03–4.37), whereas the soil pH under *Triticosecale* cultivation (4.73–5.48) could be classified as acidic (Table 5). In both arable soils, an increase in pH was observed with increasing depth in the soil profile. Changes in soil pH were reflected in hydrolytic acidity, which ranged from 18.75 to 40.13 mmol H+ kg^{-1} soil (*Zea mays*) and from 46.88 to 55.88 mmol H+ kg^{-1} soil (*Triticosecale*), and in the sum of exchangeable basic cations, which ranged from 8.00 to 21.00 mmol H+ kg^{-1} soil (*Zea mays*) and from 24.00 to 94.00 mmol H+ kg^{-1} soil (*Triticosecale*). The sorption capacity and the degree of saturation of the basic elements in the soil sown with *Triticosecale* soils were higher than in the soil sown with *Zea mays* soils. Each of the physicochemical properties analyzed, with the exception of pH, reached its highest values in the surface layer of the soil, while the remaining properties decreased proportionally with increasing depth in the soil profile.

Table 5. The physicochemical properties of soils.

Depth	Properties							
	pH	HAC	EBC	CEC	BS	N_{Total}	C_{org}	C:N
		mM (+) kg^{-1} dm			%	g kg^{-1} dm		
				Zea mays				
1	4.033 c	40.125 d	21.000 c	61.125 d	34.356 c	1.490 b	10.950 b	7.349 b
2	4.167 c	25.125 e	10.000 d	35.125 e	28.470 c	0.640 c	2.070 c	3.235 c
3	4.367 bc	18.750 f	8.000 d	26.750 f	29.907 c	0.458 d	0.957 d	2.088 d
				Triticosecale				
1	4.733 b	55.875 a	94.00 a	149.875 a	62.719 a	2.410 a	21.370 a	8.874 a
2	5.283 a	49.125 b	35.00 b	84.125 b	41.605 b	0.460 d	0.630 de	1.370 e
3	5.483 a	46.875 c	24.00 c	70.875 c	33.862 c	0.598 c	0.359 e	0.600 f

HAC—hydrolytic acidity; EBC—exchangeable basic cations; CEC—soil cation exchange capacity; BS—degree of base saturation. Homogeneous groups denoted with letters (a–f) were calculated separately for each physicochemical properties of soils.

In the surface layer of the soil profile under *Triticosecale* cultivation, the total nitrogen content in the 0–20 cm profile was 2.41 g N_{Total} kg^{-1}, and the organic carbon content was 21.37 g C_{org} kg^{-1}, while in the soil under *Zea mays* cultivation, they were, respectively, 1.49 g N_{Total} kg^{-1} and 10.95 g C_{org} kg^{-1}. The content of both analyzed parameters de-

creased with increasing depth of the soil profile. The determination of N_{Total} and C_{org} allowed the calculation of the C:N ratio. All the results obtained were statistically different from each other and were in the range below 10, indicating intense mineralization and humification of organic matter at both cereal sites.

3.4. Interactions between Microbiological, Biochemical, and Physicochemical Properties of Soil

The PCA analysis (Figure 12) demonstrated that the activity of Deh, Cat, Ure, AcP, AlP, Glu, Aryl, and EP_{Fun} was highest in the soil under *Triticosecale* cultivation (0–20 cm), whereas the abundance of Fun bacteria, as well as the CD_{Fun} and EP_{Org} indices, was highest in the soil under *Zea mays* cultivation (0–20 cm). Conversely, the abundance of organotrophic bacteria and their development index were comparable in both soils. All parameters describing the microbiological properties, except for EP_{Org}, and the biochemical properties of the soil significantly decreased with increasing depth in the profiles of both soils analyzed. The first principal component accounted for 68.50% of the variation in microbiological and soil enzyme variables, while the second component accounted for 20.52%.

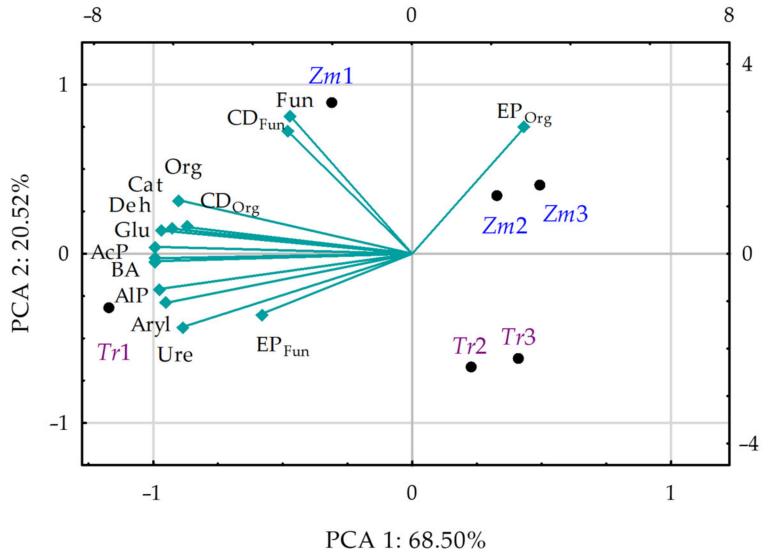

Figure 12. Soil enzyme activity along with the abundance, colony development index, and ecophysiological diversity index of bacteria and fungi were presented using Principal Component Analysis (PCA). Deh—dehydrogenases; Cat—catalase; Ure—urease; AcP—acid phosphatase; AlP—alkaline phosphatase; Glu—*β*–glucosidase; Aryl—arylsulfatase. Tr—*Triticosecale*; Zm—*Zea mays*; CD—colony development index; EP—ecophysiological diversity index; 1, 2, 3—depth of soil sample: 0–20 cm; 21–40 cm; 41–60 cm.

The depth of the soil profile was negatively correlated with all the parameters studied, except for EP_{org} and pH, but statistically significant negative correlations occurred only between the depth of the soil profile and the abundance of Org, CD_{org} index, the activity of Deh, Cat, AcP, Glu, BA index, and C_{org} content (Table 6). Bacteria were significantly positively correlated with the activity of Deh, Cat, AcP, AlP, Glu, and BA index, as well as with the content of N_{Total} and Corg. For fungi, a significant negative correlation was found between their colony development index and the soil pH value. There was a significant positive correlation between the activity of the soil enzymes studied. An exception was the lack of correlation between the activity of Ure and Cat. All studied soil enzymes and the BA index were also significantly positively correlated with the content of N_{Total} and C_{org}. Additionally, the activity of Ure, AlP, and Aryl showed significant positive correlations with EBC, CEC, and BS, while AcP showed positive correlations with EBC and BS.

Table 6. Pearson's simple correlation coefficients between the study variables, $p < 0.050$, N = 6.

Variable	Depth	Org	CD_{Org}	EP_{Org}	Fun	CD_{Fun}	EP_{Fun}	Deh	Ure	AcP	AlP	Cat	Aryl	Glu	BA	pH	HAC	EBC	CEC	BS	N_{Total}	C_{org}
Depth	1.000	−0.856	−0.898	0.452	−0.652	−0.504	−0.281	−0.881	−0.703	−0.815	−0.778	−0.898	−0.743	−0.851	−0.839	0.405	−0.468	−0.581	−0.575	−0.585	−0.807	−0.814
Org	−0.856	1.000	0.749	−0.208	0.765	0.539	0.266	0.961	0.679	0.898	0.820	0.844	0.759	0.922	0.902	−0.354	0.488	0.569	0.573	0.553	0.898	0.881
CD_{Org}	−0.898	0.749	1.000	−0.288	0.514	0.547	0.380	0.860	0.727	0.821	0.789	0.830	0.779	0.836	0.839	−0.444	0.332	0.617	0.556	0.652	0.778	0.818
EP_{Org}	0.452	−0.208	−0.288	1.000	0.267	0.398	−0.340	−0.326	−0.698	−0.403	−0.539	−0.366	−0.578	−0.380	−0.441	−0.438	−0.750	−0.734	−0.778	−0.713	−0.439	−0.427
Fun	−0.652	0.765	0.514	0.267	1.000	0.667	−0.240	0.611	0.090	0.433	0.280	0.533	0.189	0.497	0.442	−0.587	0.061	−0.065	−0.027	−0.062	0.420	0.404
CDF_{un}	−0.504	0.539	0.547	0.398	0.667	1.000	0.364	0.473	0.038	0.440	0.299	0.677	0.242	0.501	0.395	−0.971	−0.452	−0.088	−0.212	−0.096	0.421	0.445
EP_{Fun}	−0.281	0.266	0.380	−0.340	−0.240	0.364	1.000	0.396	0.565	0.595	0.640	0.627	0.669	0.577	0.542	−0.287	−0.002	0.592	0.428	0.552	0.611	0.634
Deh	−0.881	0.961	0.860	−0.326	0.611	0.473	0.396	1.000	0.830	0.970	0.927	0.872	0.890	0.977	0.979	−0.283	0.556	0.736	0.715	0.734	0.958	0.958
Ure	−0.703	0.679	0.727	−0.698	0.090	0.038	0.565	0.830	1.000	0.894	0.957	0.705	0.975	0.862	0.913	0.136	0.762	0.986	0.964	0.985	0.892	0.898
AcP	−0.815	0.898	0.821	−0.403	0.433	0.440	0.595	0.970	0.894	1.000	0.983	0.891	0.961	0.996	0.996	−0.247	0.538	0.829	0.776	0.816	0.996	0.998
AlP	−0.778	0.820	0.789	−0.539	0.280	0.299	0.640	0.927	0.957	0.983	1.000	0.849	0.995	0.967	0.984	−0.111	0.621	0.915	0.866	0.902	0.982	0.986
Cat	−0.898	0.844	0.830	−0.366	0.533	0.677	0.627	0.872	0.705	0.891	0.849	1.000	0.816	0.923	0.877	−0.560	0.271	0.607	0.528	0.583	0.897	0.905
Aryl	−0.743	0.759	0.779	−0.578	0.189	0.242	0.669	0.890	0.975	0.961	0.995	0.816	1.000	0.939	0.964	−0.061	0.627	0.944	0.889	0.935	0.958	0.967
Glu	−0.851	0.922	0.836	−0.380	0.497	0.501	0.577	0.977	0.862	0.996	0.967	0.923	0.939	1.000	0.991	−0.315	0.501	0.784	0.732	0.770	0.993	0.995
BA	−0.839	0.902	0.839	−0.441	0.442	0.395	0.542	0.979	0.913	0.996	0.984	0.877	0.964	0.991	1.000	−0.202	0.593	0.845	0.807	0.837	0.990	0.992
pH	0.405	−0.354	−0.444	−0.438	−0.587	−0.971	−0.287	−0.283	0.136	−0.247	−0.111	−0.560	−0.061	−0.315	−0.202	1.000	0.600	0.258	0.384	0.262	−0.227	−0.257
HAC	−0.468	0.488	0.332	−0.750	0.061	−0.452	−0.002	0.556	0.762	0.538	0.621	0.271	0.627	0.501	0.593	0.600	1.000	0.774	0.889	0.772	0.554	0.523
EBC	−0.581	0.569	0.617	−0.734	−0.065	−0.088	0.592	0.736	0.986	0.829	0.915	0.607	0.944	0.784	0.845	0.258	0.774	1.000	0.978	0.995	0.831	0.835
CEC	−0.575	0.573	0.556	−0.778	−0.027	−0.212	0.428	0.715	0.964	0.776	0.866	0.528	0.889	0.732	0.807	0.384	0.889	0.978	1.000	0.974	0.784	0.776
BS	−0.585	0.553	0.652	−0.713	−0.062	−0.096	0.552	0.734	0.985	0.816	0.902	0.583	0.935	0.770	0.837	0.262	0.772	0.995	0.974	1.000	0.809	0.820
N_{Total}	−0.807	0.898	0.778	−0.439	0.420	0.421	0.611	0.958	0.892	0.996	0.982	0.897	0.958	0.993	0.990	−0.227	0.554	0.831	0.784	0.809	1.000	0.996
C_{org}	−0.814	0.881	0.818	−0.427	0.404	0.445	0.634	0.958	0.898	0.998	0.986	0.905	0.967	0.995	0.992	−0.257	0.523	0.835	0.776	0.820	0.996	1.000

Explanations of abbreviations are provided in Figures 4 and 10 and Table 5. Red color—statistically significant, black color—statistically insignificant.

4. Discussion

4.1. Abundance and Diversity of Microorganisms

Soil, whose quality depends on the activity of the organisms that inhabit it [18,79], is a key element in agricultural development. In the studies carried out, the highest abundance of the microbiome, based on organotrophic bacteria and fungi, was found in the 0–20 cm soil depth. This is probably due to the fact that soil at this depth is characterized by the highest nutrient richness [80,81]. At this depth, regardless of the cultivated plant species, the highest values of colony development indices (CD) were also recorded. Under maize cultivation, the values were lower for organotrophic bacteria (CD = 32.82) and higher for mold fungi (CD = 43.32). In the soil sown with triticale, the values were slightly different, with 34.64 for organotrophic bacteria and 35.89 for mold fungi, respectively. As reported by Mundra et al. [81], this is likely due to the decreasing nutrient content with soil depth and, consequently, the response of soil microorganisms to these changes. A similar relationship can be observed in the soils from the study sites where soil aeration tillage was applied in the surface layer of the soil. Li et al. [82] suggested that such practices may increase soil microbial activity but also induce enzymatic activity.

Additionally, the results obtained showed that it was the soil used for winter triticale where the crop rotation was carried out that had a higher abundance of organotrophic bacteria and, at the same time, a significantly lower community of mould fungi. The abundance of this group of microorganisms was even 84.18% lower in the soil under triticale. The trends observed also confirm the results obtained by Yin et al. [83], Andam et al. [84], and Shan et al. [85]. However, in our study, it was the cultivation of *Triticosecale* that significantly enhanced the proliferation of organotrophic bacteria and *Zea mays* fungi. Plant root secretions, which optimize the soil microbial community, undoubtedly contributed to this [86,87]. The structure of root secretions consists mainly of proteins and polysaccharides. They also contain amino acids, alcohols, or organic acids [88]. The latter are secreted by triticale in the form of citrates and malates, and this process is a plant response to abiotic stress [89–91]. The winter triticale site studied was characterized by low soil pH, which undoubtedly affected the structure of root secretions and, ultimately, microbial activity. According to Wu et al. [92], organic acids from plant root secretions have a significant moderating effect on the soil microbiome. Crops also have the potential to attract beneficial bacteria to the rhizosphere [93]. The increase in organotrophic bacteria observed in our study is probably due to the colonization of the rhizosphere zone of crop plants by members of this group, mainly bacteria of the genera *Bacillus* and *Pseudomonas.* Their high activity is due to their response to a large pool of compounds contained in root secretions, if only because these microorganisms are equipped with MCPs (methyl methyl-accepting chemotaxis proteins) [94]. For bacteria of the genus *Bacillus*, Liu et al. [95] listed eight types of proteins: McpA; McpB; McpC; McpR; TlpA; TlpB; YfmS; and HemAT. In contrast, Sampedro et al. [96] demonstrated chemotactic responses of *Pseudomonas* sp. to up to 140 compounds synthesized by plants.

The cultivation techniques of *Zea mays* and *Triticosecale* also played an important role. According to Rayne and Aula [97], the plowing of crop residues and the use of natural fertilizers also improve the biological properties of the soil.

A significant difference of 84.18% in the abundance of mold fungi in the soil under *Zea mays* cultivation was undoubtedly related to the long-term monoculture management practiced in the maize field, which contributed to the increased activity of this group of microorganisms [98]. Conversely, Wu et al. [92] demonstrated that root exudates from monocultures lead to a decrease in soil condition, which corresponds to the lower abundance of organotrophic bacteria compared to fungi obtained in our study.

The soil samples analyzed showed a predominance of two types of microorganisms: *Actinobacteria* and *Proteobacteria*. These are representatives of bacteria commonly found in agricultural soils [55,56]. *Actinobacteria* were the dominant type inhabiting the soils studied. According to Mitra et al. [99], the microbiome represented by *Actinobacteria* contributes to the improvement in both soil condition and plant health. These microorganisms also serve

to remove pollutants [100], but more importantly, they have the ability to decompose crop residues [101]. Therefore, the presence of crop residues in the soil, as remnants of previous crops, which increase the soil reproduction coefficient through their decomposition, also contributed to the increase in *Actinobacteria* abundance. The highest abundance of *Proteobacteria* was found in soils where *Zea mays* was grown. This plant is characterized by a strong root system that extends 2 m into the soil profile, and *Proteobacteria* proliferate more abundantly in the rhizosphere, which is rich in soil organic matter [102]. Additionally, soils derived from corn cultivation were characterized by very high acidification. Eldridge et al. [103] observed a strong correlation between low pH and the occurrence of *Proteobacteria* in the soil environment. In both the winter triticale and maize soils, members of the *Gaiellaceae* family predominated. In the studies by Sun et al. [104], Gorelova et al. [105], and Rogozhina et al. [106], it was recognized as a family tolerant to environmental requirements and forming the native rhizosphere microbiome. Both study sites were characterized by unfavorable habitat conditions. The presence of unique bacterial genera was observed under maize cultivation. *Rhodoplanes* and *Nocardioides* are relatively stress-tolerant microorganisms [107,108]. Moreover, both genera of microorganisms discussed are characterized by the ability to degrade environmentally harmful substances [108,109]. The presence of one of the less common bacterial genera—*Pseudonocardia*—has been recorded in the soil of winter triticale cultivation [110]. These microorganisms are involved in the stimulation of plant development. They are recognized as a source of antibacterial and antifungal substances [111–113].

4.2. Enzymatic Activity

Enzyme activity is considered to be a particularly sensitive indicator of environmental change, especially in soil ecosystems [114]. The results of the biochemical analysis illustrated that the response of all the soil enzymes tested was moderated by soil depth, similar to that of the microorganisms, and was also negatively correlated with this parameter. The highest activity values for the seven enzymes tested, together with the corresponding biochemical soil fertility index (BA), were obtained at depths between 0 and 20 cm. In studies by other researchers comparing the fertility of surface and subsurface soil layers (>40 cm), multiple declines in soil enzyme activity were also observed under cultivated crops, including maize [115–118]. Piotrowska-Długosz et al. [119] also reported that urease activity was higher at Ap levels than at the deepest soil levels (30–150 cm). These tendencies were mainly influenced by edaphic factors, and the organic carbon content determined about 50% of the changes in soil enzyme activity [120]. It should be emphasized that winter triticale cultivation induced a much higher increase in soil biochemical activity than maize. In the surface layer of the soil alone, the activities of all the enzymes tested were 19.38% higher for dehydrogenases, 23.40% for catalase, 37.76% for arylsulfatase, 45.92% for acid phosphatase, 56.48% for alkaline phosphatase, 69.13% for β-glucosidase, and 70.83% for urease in the soil sown with winter triticale. As reported by Wolińska et al. [121] and Jaskulska et al. [122], sowing *Triticosecale* has a favorable effect on the soil's biological properties of soils compared to other cereal crops or soils adjacent to the discussed species. Moreover, the previous crop in this position was green manure, which probably resulted in higher values of soil biochemical parameters [123–125]. These findings are confirmed by the research results of Woźniak and Kawecka-Radomska [126]. In soils under a crop rotation system, the activity of dehydrogenases and two hydrolases, urease and phosphatase, was higher than in monoculture soils. It should also be emphasized that high values of dehydrogenase activity were expected, as these enzymes are found in living microbial cells and are, therefore, significantly correlated with the microbiological activity of the soils studied. They are considered to be common and reliable indicators of microbial respiratory metabolism [127].

4.3. Physicochemical Properties

Both the biological and physicochemical properties of soil influence their quality and are referred to as indicators of their fertility [128,129]. In our own research, soils from the shallowest layers of the soil profile were found to be more acidic. Except for soil pH, the values of all physicochemical properties analyzed decreased with increasing depth in the soil profile. The winter triticale site showed more favorable physicochemical properties compared to soils under maize cultivation. Rational soil management, including appropriate fertilization, is mainly responsible for their proper formation [130]. The application of lime probably contributed to increased concentrations of alkaline ions and neutralization of acidification in the surface layers of the soil (0–20 cm). The introduction of sustainable agricultural practices, including the application of lime to degraded sites, ensures their proper regeneration [131,132].

An important parameter estimated in our research for all depths of the soil profile was the C:N ratio, defined as a sensitive indicator of soil environmental quality [31]. Its lower value in the surface soil layer (0–20 cm) under *Zea mays* cultivation (7.349) compared to *Triticosecale* cultivation (8.874) significantly moderated both the activity and structure of soil microbiomes, as well as their biochemical parameters. According to Xu et al. [133], the soil organic carbon content, and especially the C:N ratio, are the main determinants of their biological properties.

5. Conclusions

Both the microbial diversity and biochemical activity proved to be reliable bioindicators of soil quality. Based on this, it was demonstrated that soil depth, associated changes in physicochemical properties, and the species of cultivated plants influence soil conditions. Soil depth exerts the greatest moderating influence on all the parameters analyzed, with the highest values in the surface layer (0–20 cm). Both the microbiological properties, except for EP_{Org}, and the activity of the enzymes tested decrease significantly with increasing depth of the soil profile. These trends correspond to a deterioration in physicochemical properties with depth, except for pH, which is more favorably affected by *Triticosecale* cultivation than by *Zea mays*. In the research conducted, this was also reflected in the higher activity of seven verified enzymes, especially urease and arylsulfatase, in the soil under winter triticale. Another consequence of *Triticosecale* cultivation is the stimulation of organotrophic bacteria activity, while *Zea mays* promotes the proliferation of mold fungi. Regardless of the species of cultivated plants, the microbiome of arable soils represented two phyla, *Actinobacteria* and *Proteobacteria*. However, the relative abundance of representatives of the *Acidobacteria*, *Chloroflexi*, and *Gemmatimonadetes* phyla was higher under *Triticosecale* cultivation than under *Zea mays*. A valuable achievement of the study was the identification of unique bacterial genera *Rhodoplanes*, *Nocardioides*, and *Rhodanobacter* in maize soil and *Pseudonocardia* in winter triticale soil. The research results obtained provide inspiration to compare the microbiological activity of more soil types with the same cultivated plant species and the identification of their basic and unique microbiome.

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