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Impact of Various Grass Species on Soil Bacteriobiome

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Abstract: Today, various grass species are important not only in animal feeding but, increasingly often, also in energetics and, due to esthetic and cultural values, in landscape architecture. Therefore, it is essential to establish the roles various grass species and their functional forms play in modifying soil bacteriobiome and enzymatic activity. To this end, a pot experiment was conducted to examine effects of various fodder grass and lawn grass species on the bacteriobiome and biochemical properties of soil. Nonsown soil served as the control for data interpretation. Analyses were carried out with standard and metagenomic methods. The intensity of effects elicited by grasses depended on both their species and functional form. More favorable living conditions promoting the development of soil bacteria and, thereby, enzymatic activity were offered by fodder than by lawn grass species. Among the fodder grasses, the greatest bacteriobiome diversity was caused by sowing the soil with *Phleum pratense* (Pp), whereas among lawn grasses in the soil sown with *Poa pratensis* (Pr). Among the fodder grasses, the highest enzymatic activity was determined in the soil sown with *Lolium* x *hybridum* Hausskn (Lh), and among the lawn grasses—in the soil sown with *Lolium perenne*. Sowing the soil with grasses caused the succession of a population of bacterial communities from r strategy to k strategy.

Keywords: fodder grasses; lawn grasses; soil bacteria; soil enzymes

1. Introduction

Interactions between soil, plants, and soil microbiome are complex in character and require extended research. Determination of changes in soil stability and identification of associations between microbiological diversity of soil and plants occurring in agricultural ecosystems are difficult because they are affected by plant root secretions [1–4], climatic changes [5–7], and various pollutants [2,7]. As the key component of life on Earth, soil is capable of meeting most of plant demands. Its traits, including abundance of nutrients, productivity, and fertility, are a measure of the strength of plant growth and crop yield [8–11]. Plant productivity largely depends on soil culture [9], count of soil bacteria and fungi colonizing the rhizosphere [12,13], count of epiphytic microorganisms occurring on the surface of plants and endophytic ones colonizing their tissues [14], presence of pathogens [15,16], humus content [17,18], soil pH [19], water-air balance [20–22], soil fraction size [23,24] as well as the microbiological and biochemical activity of soil [10,25–27].

For agricultural sustainability, 176 cultivars have been shortlisted by the Research Center for Cultivar Testing (Słupia Wielka, Poland, 52.227° N 17.218° E) of which 19 are grasses species of monocotyledonous flowering plants from the *Poaceae* (*Gramineae*) family, commonly known as grasses, have been used in contemporary agriculture: x*Festulolium* Asch. & Graebn., *Festuca rubra* L., *Festuca pratensis* Huds., *Festuca filiformis* Pourr., *Festuca ovina* L., *Festuca trachyphylla* (Hack.) Krajina, *Festuca arundinacea* Schreber, *Dactylis glomerata* L., *Agrostis gigantea* Roth, *Agrostis capillaris* L.,

Agrostis stolonifera L., *Arrhenatherum elatius* (L.) P. Beauv.ex J. Presl & C. Presl, *Bromus catharticus* Vahl, *Phleum pratense* L., *Poa pratensis* L., *Poa trivialis* L., *Lolium x hybridum* Hausskn, *Lolium perenne* L., and *Lolium multiflorum* Lam. Pursuant to EU regulations, all cultivars submitted to the national register are evaluated for distinctness, uniformity and stability (DUS), whereas crops are additionally evaluated for their value for cultivation and use [28]. Grasses from the *Poaceae* family, i.e., from the family of monocotyledoneous flowering plants, represent one of the most important and the most abundant group of plants on the entire Earth. This family includes crops and monocotyledoneous fodder plants. These plants constitute the source of feed to both, wild and domesticated animals. They possess therapeutic and health-promoting properties, and are able to adapt to various climatic zones and various habitats. The form assemblages of savannas, steppes, prairies and pampas, as well as lowland, mountain, and arctic meadows. They have been accompanying man for years. They have been and are used most often in animal feeding due to their high nutritive value, resulting from the chemical composition of plants. Grasses are rich in dietary fiber digestible protein, minerals, and vitamins [29]. According to Peeters [30], grasses have a higher nutritive value for animals than fodder beet. They represent complete feeds rich in organic and mineral compounds. Their leaves and stems may be easily ingested by animals and effectively digested by microorganisms colonizing their rumens. In addition, they are valuable energetic feed.

For sustainability, modern agricultural practices need to include every effort not to deplete the soil's organic matter, because the use of chemicals together with intensive cultivation can lead to soil sterilization and microbiological imbalance [11,31,32]. The development of soil edaphon is at risk of the impairment of decomposition and humification processes due to organic matter accumulating in the soil [33].

Soil microorganisms and enzymes take part in the mineralization of organic substances [34–39], in retention of heavy metals [40–42], and in degradation of plant protection agents [43–45] and polycyclic aromatic hydrocarbons (PAHs) [34,36,46]. They are the driving force of the geochemical cycle of elements, and participate in transformations of simple and complex organic compounds [47]. Diversity of microorganisms influences the functioning of ecosystems, biological homeostasis as well as chemical and physical properties of soil, and by this means determines its productivity [35,48,49].

Microorganisms that colonize soil and other environments synthesize intra- and extracellular enzymes indispensable for depolymerization and hydrolysis of organic macromolecules which serve as sources of carbon and energy [50]. Determination of enzymatic activity of soil is essential to the understanding of the functional dynamics of a soil ecosystem. According to Moeskops et al. [51], Zhan et al. [52], and Knight and Dick [53], it is also a good indicator of the biological status of soil because the activity of enzymes from the class of oxidoreductases (dehydrogenases or catalase) is strictly responsible for respiration of microorganisms in the soil. A reliable indicator of changes undergoing in the soil is also the activity of urease. Although this is an extracellular enzyme related to a lesser extent with the condition of microorganisms, it is highly sensitive to various xenobiotics [52]. In turn, β-glucosidade is responsible for cellulose transformation to glucose [53], while phosphatases—for transformations of phosphorus compounds [54], and, inter alia, arylsulfatase—for the metabolism of organic sulfur [55]. It can therefore be concluded that the geochemical transformations proceeding in the soil are strongly associated with its biological activity.

Due to the small amount of research into the effects of grasses on soil biodiversity, research was undertaken to compare (1) the soil bacteriobiome of six grasses species (three fodder and three lawn grasses); (2) the effect of grasses on colony development and ecophysiological diversity index of soil bacteria; (3) the grass yield of fodder and lawn grasses; and (4) the enzymatic activities of soil with grasses and without grass.

2. Materials and Methods

2.1. Soil Characteristics

The experiment was conducted with eutric cambisol soil sampled from the topsoil, from a depth of 0 to 20 cm of the arable lands from the Olsztyn Lake District situated in the northeast of Poland (NE Poland, 53.7161° N 20.4167° E). It contained 74.93% of the sand fraction, 22.85% of the silt fraction, and 2.22% of the clay fraction. In terms of fraction size, this was loamy sand [56]. The physicochemical and chemical properties of soil are presented in Table 1. They were conducted according to the procedures presented in the manuscript by Borowik et al. [57].

Table 1. Physicochemical and chemical soil properties.

pH_{KCl}	HAC	EBC	CEC	BS %	Content		Available Forms			Interchangeable Forms			
	mmol (+) kg^{-1} d.m. of Soil				N_{total}	C_{total}	P	K	Mg	K	Ca	Na	Mg
					g kg^{-1} d.m. of Soil		mg kg^{-1} d.m. of Soil						
6.70	11.40	49.00	60.40	81.10	0.62	9.30	93.68	141.10	42.00	156.00	623.50	40.00	59.50

HAC—hydrolytic activity, EBC—exchangeable base cations, CEC—cation exchange capacity, BS—base saturation, d.m.—dry matter.

2.2. Plant Characteristics

The study focused on plants having a well-developed root system, including three species of lawn grasses and three species of fodder grasses (Table 2).

Table 2. Characteristics of grasses used in the study.

Grasses Kind	Common Name	Botanical Name	Abbreviation	Variety	Photosynthesis Kind
Fodder	Hybrid ryegrass	*Lolium* x *hybridum* Hausskn	Lh	Gala	C3
	Tall fescue	*Festuca arundinacea*	Fa	Rahela	C3
	Timothy	*Phleum pratense*	Pp	Kaba	C3
Lawn	Perennial ryegrass	*Lolium perenne*	Lp	Bajka	C3
	Smooth-stalked meadowgrass	*Poa pratensis*	Pr	Sójka	C3
	Red rescue	*Festuca rubra*	Fr	Dark	C3

2.3. Experimental Design

The study was conducted in a pot experiment, at the teaching-experimental station of the University of Warmia and Mazury in Olsztyn (NE Poland, 53.760° N 20.454° E). It was accomplished in two series: with nonsown (without grasses) soil and with soil sown with the selected grass species. The experiment (pots sown with six different species of grass and soil without grasses) was performed in four replications in 10 dm^3 Kick–Brauckman pots, each filled with 9 kg of soil. Before the experiment had been established, the soil was sieved through a screen with mesh diameter of 5 mm, then thoroughly mixed, weighed into 9-kg portions, carefully mixed with mineral fertilizers, and poured into the pots. With soil sowing, 22 seeds were sown to each pot. The same mineral fertilization was applied for all grass species and control soil (not sown with grasses). The pre-sowing fertilization included, in mg kg^{-1} soil d.m. (dry matter): N—80, P—20, K—40, and Mg—10, whereas, after the harvest of the first and the second re-growth, the plants were additionally fertilized with nitrogen in the amount of 40 mg N kg^{-1} soil d.m.. All grass species emerged evenly and at the same time. After emergence, 20 plants were left in each pot. The experiment spanned for 105 days. Within this period, soil humidity was kept at a level of 50% of the maximum water capacity. The grasses were cut three times. Each time,

the biomass of aerial parts was determined. In the last term of cutting (day 105 of experiment). Plants were removed from the pots and then the soil from each pot was mixed thoroughly.

2.4. Determination of Bacterial Count and Activity of Soil Enzymes

In soil samples, microbiological and biochemical analyses were carried out using standard methods, which are given in Table 3. These methods are described in detail in the manuscript of Borowik et al. [57] and Wyszkowska et al. [58]. Analyses were performed in four replications.

Table 3. Parameters for determining the number of organotrophic bacteria and actinobacteria, calculating colony development index and ecophysiological diversity index, and determining enzyme activity.

Tested Feature	Medium/Formula/Substrat	References
	Medium	
Organotrophic bacteria (Org)	peptone 1.0 g, yeast extract 1.0 g, $(NH_4)_2SO_4$ 0.5 g, $CaCl_2$, K_2HPO_4 0.4 g, $MgCl_2$ 0,2 g, $MgSO_4$ $7H_2O$ 0.5 g, salt Mo 0.03 g, $FeCl_2$ 0.01 g, agar 20.0 g, soil extract 250 cm^3, distilled water 750 cm^3, pH 6.6–7.0	[59] [58]
Actinobacteria (Act)	soluble starch 10.0 g; casein 0.3 g; KNO_3 2.0 g; NaCl 2.0 g; K_2HPO_4 2.0 g; $MgSO_4 \cdot 7H_2O$ 0.05 g; $CaCO_3$ 0.02 g; $FeSO_4$ 0.01 g; agar 20.0 g; H_2O 1 dm^3; 50 cm^3 aqueous solution of nystatin 0.05%; 50 cm^3 aqueous solution of actidione 0.05%; pH 7.0	[60] [58]
	Formula	
Colony development index (CD)	CD = [N1/1 + N2/2 + N3/3 + … + N10/10] × 100, where: N1, N2, N3,..., N10—the sum of ratios of the number of colonies of microorganisms identified in particular days (1, 2, 3,..., 10) to the total number of colonies identified throughout the study period	[61]
Ecophysiological diversity index (EP)	EP = −Σ(pi·log10 pi), where: pi—the ratio of the number of colonies of microorganisms identified in particular days to the total number of colonies identified throughout the study period.	
	Substrat	
Dehydrogenases (Deh)	$C_{19}H_{15}ClN_4$	[62]
Catalase (Cat)	H_2O_2	[63]
Urease (Ure)	CON_2H_4	[64]
β-glucosidase (Glu)	$C_{12}H_{15}NO_8$	
Acid phosphatase (Pac)	$O_2NC_6H_4OP(O)(ONa)_2$ $6H_2O$	
Alkaline phosphatase (Pal)		
Aryosulphatase (Aryl)	$NO_2C_6H_4OSO_2OK$	

2.5. Metagenomic Analysis

Taxon of bacteria in soil samples was determined using analysis of the 16S rRNA encoding gene based on the hypervariable region V3–V4. Two primers were used for amplification: 1055F (5′-ATGGCTGTCGTCAGCT-3′) and 1392R (5′-ACGGGCGGTGTGTAC-3′). Polymerase chain reaction (PCR) was performed in real time in an Mx3000P thermocycler (Stratagene) and sequencing was in an Mx3000P thermocycler (Stratagene) at Genomed S.A. Warsaw, Poland.

2.6. Bioinformatic Analysis

The classification of bacteria was carried out with the QIIME package based on reference sequences database Greengenes v13_8 [65]. Sequences shorter than 1250 base pairs (bp), incomplete sequences and sequences containing more than 50 degenerated bases were omitted in the analysis and reference databases were prepared. The sequences were grouped in operational taxonomic units (OTUs).

2.7. Statistical Analysis

The Statistica 13.1 package [66] was used for statistical analyses. Homogenous groups were determined with the Tukey's test, at p = 0.05, and respective results were presented graphically using principal component analysis (PCA) and graphs categorized for dependent variables (category X) and the grouping variable (category Y). Using the analysis of variance (ANOVA), F and P values were calculated for all parameters tested (Table S1). Relative abundance of microorganisms in soil samples was visualized with the use of STAMP 2.1.3 software, using a two-way test for statistical hypotheses: G-test (w/Yates') + Fisher's and Asymptotic with CC confidence interval method [67]. Genomic data were presented in the circular system using Circos 0.68 package [68]. Visualization of relative abundance data was performed using sequences with contribution exceeding 1%. The read-outs below 1% were summed up with the other nonclassified ones in a sample. To determine bacterial diversity at the level of each taxonomic group, Shannon–Wiener (H) and Simpson (D) indices were calculated using all metagenomic data. In addition, in order to consider not only the role of individual grass species in soil bacteriobiome modification but also to emphasize functional types of grasses, the fodder grasses: *Lolium perenne* (Lp), *Festuca arundinacea* (Fa), and *Phleum pratense* (Pp) were grouped and marked as T1, whereas the lawn grasses: *Lolium perenne* L.× *hybridum* (Lh), *Poa pratensis* (Pr), and *Festuca rubra* (Fr)—as T2.

3. Results

3.1. Grass Yield

The growth and development of grasses were significantly affected by their species (Figure 1). Generally, regardless of cutting term and grass species, the yield of fodder grasses (T1) was higher by 71.68% on average than that of the lawn grasses (T2). Among the fodder grasses, in the first and third terms of harvest—the greatest biomass yield was obtained from *Lolium* x *hybridum* Hausskn (Lh), whereas in the second term—from *Festuca arundinacea* (Fa). In the case of lawn grasses, the best yield was produced by *Lolium perenne* (Lp) in all three terms of harvest. To sum up, regardless of the harvest date, the largest biomass among fodder grasses was obtained in the case of *Lolium* x *hybridum* Hausskn (Lh), and, among lawn grasses, *Lolium perenne* (Lp).

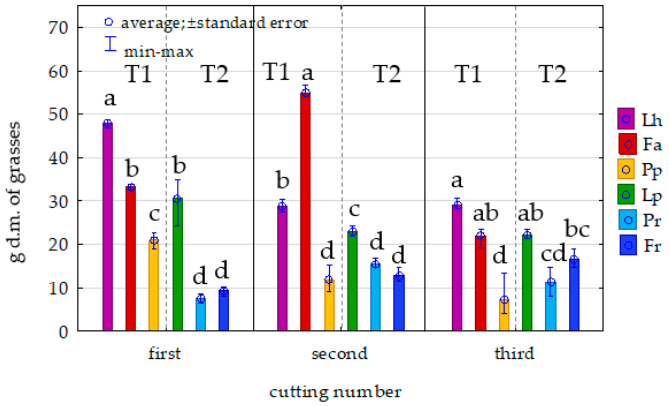

Figure 1. The yield of grasses in g dry matter (d.m.) per pot. T1—fodder grasses; T2—lawn grasses; homogeneous groups denoted with letters (a–d) were calculated separately for every cutting; Lh—*Lolium* x *hybridum* Hausskn; Fa—*Festuca arundinacea*; Pp—*Phleum pratense*, Lp—*Lolium perenne*, Pr—*Poa pratensis*; Fr—*Festuca rubra*.

3.2. Counts and Diversity of Soil Bacteria

Cultivation of grasses ambiguously modified soil bacteriobiome (Figure 2). Sowing the soil with *Lolium* x *hybridum* Hausskn (Lh) caused a significant increase in the population number of both organotrophs and actinobacteria, whereas cultivation of *Festuca arundinacea* (Fa), *Lolium perenne* (Lp),

and *Festuca rubra* (Fr)—only in the population number of actinobacteria. In turn, sowing *Phleum pratense* (Pp), *Lolium perenne* (Lp), *Poa pratensis* (Pr), and *Festuca rubra* (Fr) on soils significantly reduced the proliferation of organotrophic bacteria. Regardless of grass species, but considering their functional character, it was demonstrated that the fodder grasses (T1) increased the count of organotrophic bacteria by 26.57% and that of actinobacteria by 156.49% compared to the control soil (not sown with grasses), whereas the lawn grasses (T2) increased the count of actinobacteria by 47.43% and decreased that of organotrophs by 37.91% compared to the control soil.

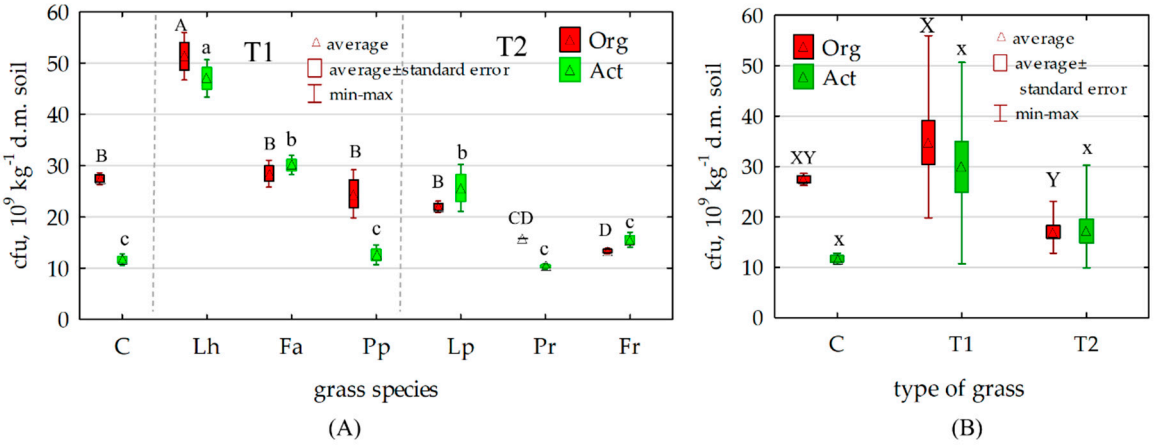

Figure 2. Count of soil organotrophic bacteria (Org) and actinobacteria (Act), cfu 10^9 kg^{-1} d.m. of soil: (**A**) depending on the species of grass, (**B**) depending on grass type (fodder or lawn). Homogeneous groups denoted were calculated separately for each microorganisms, groups denoted with letters (a–d) were calculated for the species of grass and groups denoted with letters (x–y) were calculated for the type of grass. C—unsown soil; Lh—*Lolium* x *hybridum* Hausskn; Fa—*Festuca arundinacea*; Pp—*Phleum pratense*, Lp—*Lolium perenne*, Pr—*Poa pratensis*; Fr—*Festuca rubra.*

The positive impact of the fodder grasses on the proliferation of soil microorganisms was not reflected in their ecophysiological diversity index (EP), because cultivation of Lh, Fa, and Pp not only did not increase the EP index of organotrophs but decreased its value by 7.9% on average, and also did not change the EP index of actinobacteria (Figure 3). Also the cultivation of the lawn grasses caused insignificant changes in the value of EP index of organotrophic bacteria, whereas Lp and Fr had the same effect also on actinobacteria. Only Pr decreased EP index of actinobacteria by 8.14%.

Sowing the soil with both fodder and lawn grasses caused a significant decrease in the values of the colony development index (CD) calculated for the organotrophic bacteria (Figure 4). A decrease in CD value calculated for organotrophic bacteria ranged from 27.80% (Fa) to 36.88% (Lh) in the case of fodder grass species, and from 30.02% (Fr) to 37.73% (Pr) in the case of lawn grass species. A lesser decrease in CD value was observed in the case of actinobacteria, i.e., from 3.97% (Fa) to 16.26% (Pp) in the soils used to cultivate fodder grasses, and from 6.23% (Fr) to 16.93% (Pr) in the soils sown with lawn grasses.

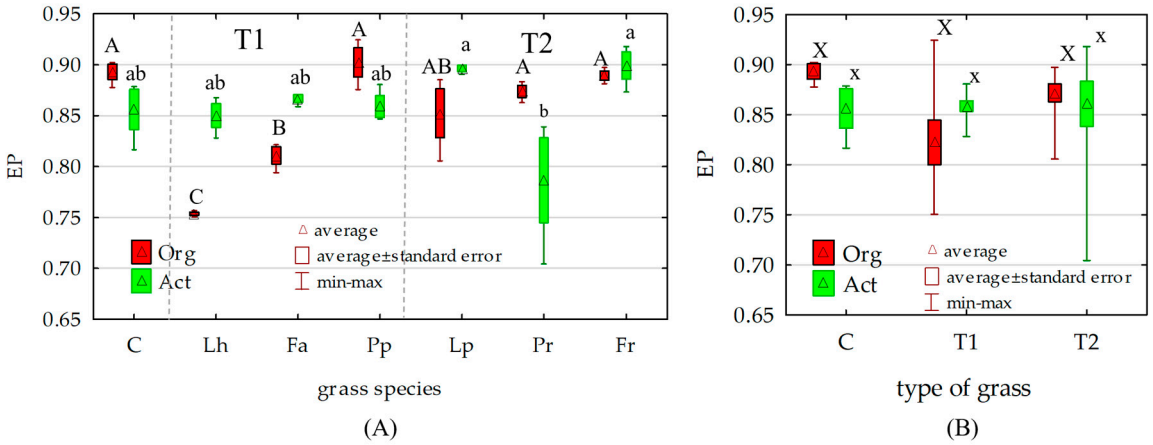

Figure 3. Ecophysiological diversity index (EP) of organotrophic bacteria (Org) and actinobacteria (Act): (**A**) depending on the species of grass, (**B**) depending on grass type (fodder or lawn). Homogeneous groups denoted were calculated separately for each microorganisms, groups denoted with letters (a–c) were calculated for the species of grass and groups denoted with letters (x) were calculated for the type of grass. C—unsown soil; Lh—*Lolium* x *hybridum* Hausskn; Fa—*Festuca arundinacea*; Pp—*Phleum pratense*, Lp—*Lolium perenne*, Pr—*Poa pratensis*; Fr—*Festuca rubra.*

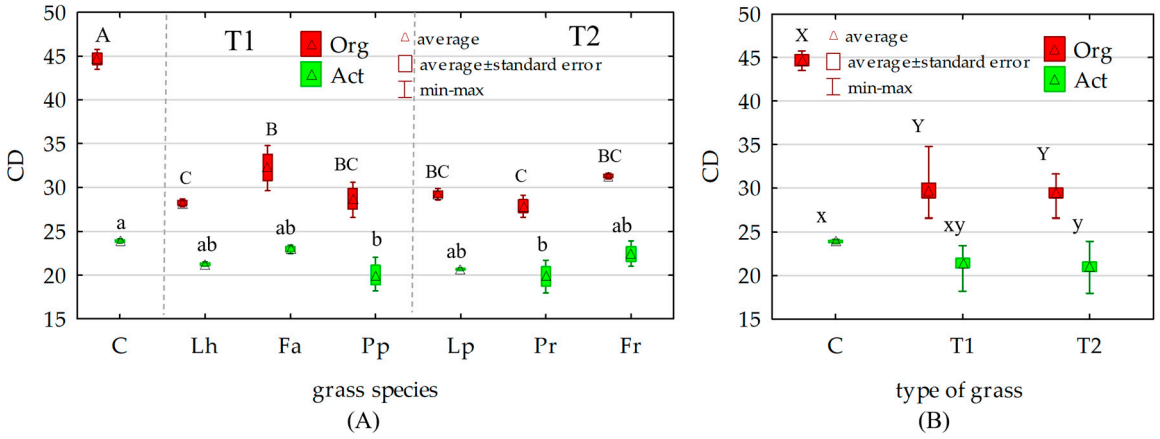

Figure 4. Colony development index (CD) of organotrophic bacteria (Org) and actinobacteria (Act): (**A**) depending on the species of grass, (**B**) depending on grass type (fodder or lawn). Homogeneous groups denoted were calculated separately for each microorganisms, groups denoted with letters (a–d) were calculated for the species of grass and groups denoted with letters (x–y) were calculated for the type of grass. C—unsown soil; Lh—*Lolium* x *hybridum* Hausskn; Fa—*Festuca arundinacea*; Pp—*Phleum pratense*, Lp—*Lolium perenne*, Pr—*Poa pratensis*; Fr—*Festuca rubra.*

At all plots, the prevailing phyla included *Proteobacteria* and *Actinobacteria* (Figure S1). In the control soil, *Proteobacteria* accounted for 28.78% of total bacteria, whereas this fraction was 38.56% in the soil sown with fodder grasses and 32.93% in that sown with lawn grasses. In the control soil, *Actinobacteria* accounted for 23.55%, in the soil sown with fodder grasses, for 19.77%, and, in the soil sown with lawn grasses, for 21.32% of total bacteria. The OTU number of *Proteobacteria* in the soil sown with fodder and lawn grasses was higher by 9.8% than in the control soil, whereas the OTU number of *Actinobacteria* decreased by 3.4% in the soil sown with fodder grasses and by 2.9% in soil sown with lawn grasses, compared to the control soil.

Apart from *Proteobacteria* and *Actinobacteria*, taxa identified at the phylum level included: *Acidobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Firmicutes* and *Planctomycetes*, *Bacteroidetes*, *Verrucomicrobia*, *Cyanobacteria*, as well as OD1 and TM7 (Figure 5A,B). The OTU number of bacteria classified as 'others' reached 4.3% in the control soil, 3.38% in the soil sown with fodder grasses, and 3.80% in the soil

sown with lawn grasses. Cultivation of all grass species facilitated the proliferation of *Proteobacteria*, *Bacteroidetes*, and *Verrucomicrobia* bacteria, which resulted in higher OTU numbers of these bacteria compared to the control soil. The OTU number of *Actinobacteria* increased only in the sample of soil sown with *Lolium* x *hybridum* Hausskn (Lh) and *Lolium perenne* (Lp); the OTU number of *Chloroflexi* increased in the soils sown with *Lolium perenne* (Lp), *Poa pratensis* (Pr), *and Festuca rubra* (Fr); the OTU number of Firmicutes increased in the soil sown with *Poa pratensis* (Pr), whereas the OTU number of *Planctomycetes* rose in the soil sown with *Poa pratensis* (Pr), and *Festuca arundinacea* (Fa).

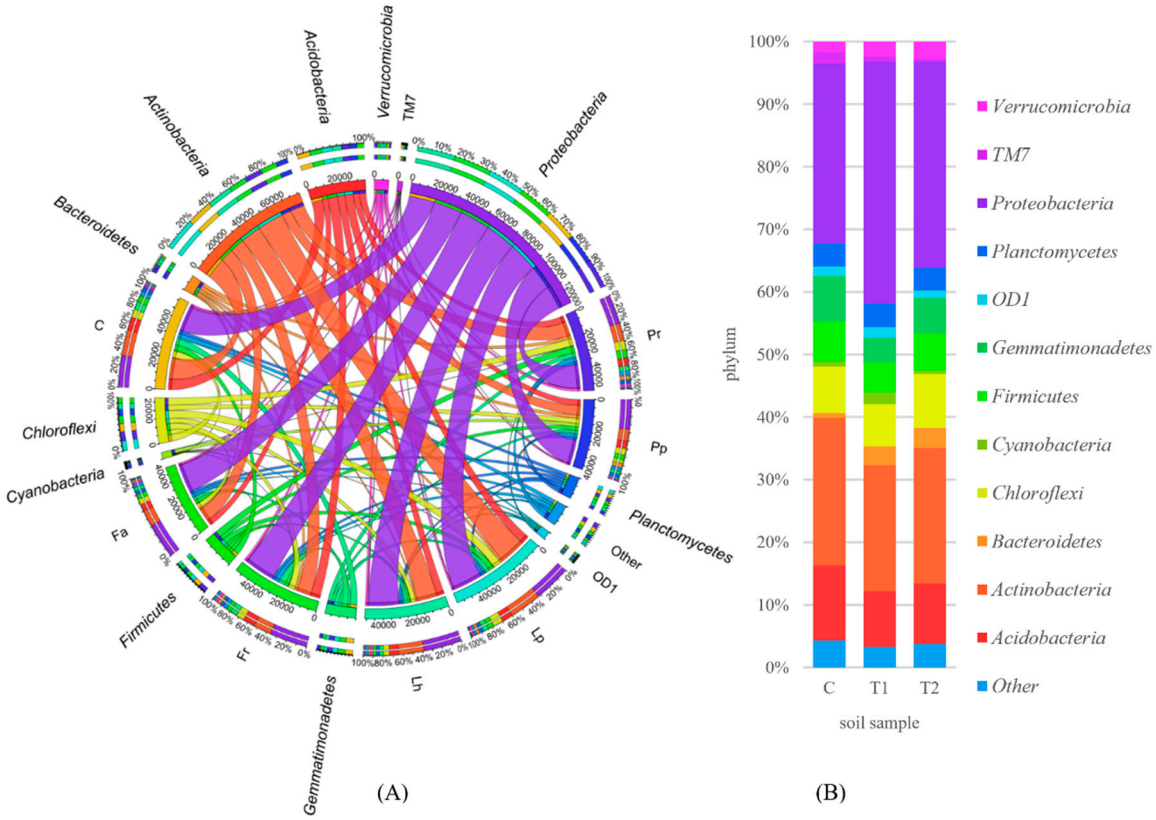

Figure 5. Bacterial communities at the phylum level (**A**) depending on the species of grass, (**B**) depending on grass type (fodder or lawn). Abundances <1% are gathered into the category "other". C—unsown soil; T1—average bacteria abundance in soils sown with fodder grasses; T2—average bacteria abundance in soils sown with lawn grasses. Lh—*Lolium* x *hybridum* Hausskn; Fa—*Festuca arundinacea*; Pp—*Phleum pratense*, Lp—*Lolium perenne*, Pr—*Poa pratensis*; Fr—*Festuca rubra.*

Both, in the control soil and soils sown with grasses, the prevailing class of bacteria was *Alphaproteobacteria*, which in the control soil accounted for 19.32% of total bacteria, in the soil sown with fodder grasses—for 19.99%, and in the soil sown with lawn grasses—for 16.50% (Figure S2). The second prevailing class was *Actinobacteria*, which in the structure of all bacterial classes represented 12.98% in the control soil, 13.88% in the soil used to cultivate fodder grasses, and 13.98% in the soil used to grow lawn grasses. Sowing the soils with fodder grasses caused the greatest changes in the abundance of bacterial classes: *Gammaproteobacteria*, *Betaproteobacteria*, and *Acidobacteria*-6, whose OTU numbers increased by 6.37%, 2.64%, and 1.99%, respectively, compared to the control soil.

Also, sowing the soil with lawn grasses increased OTU numbers of these bacteria in the range from 3.56% (*Gammaproteobacteria*) to 1.92% (*Acidobacteria*-6). Differences in changes in the bacterial structure were also noticeable between the rhizospheres of the fodder and lawn grasses. The OTU number of *Alphaproteobacteria* in the soils sown with fodder grasses was higher by 3.49% and that of *Gammaproteobacteria* by 2.82% than in the soils sown with lawn grasses. Regardless of grass species and functional designation, apart from the two prevailing classes *Alphaproteobacteria*

and *Actinobacteria*, all soils contained also (in a descending order of OTUs): *Betaproteobacteria*, *Thermoleophilia*, *Gammaproteobacteria*, *Bacilli*, *Acidobacteria*-6, *Gemmatimonadetes*, *Deltaproteobacteria*, *Planctomycetia*, *Solibacteres*, *Acidimicrobiia*, *Acidobacteriia*, Gemm-1, C0119, Ellin6529, *Chloracidobacteria*, *Clostridia*, *Phycisphaerae*, *Saprospirae*, *Ktedonobacteria*, *Pedosphaerae*, *ZB2*, *Thermomicrobia*, *Spartobacteria*, *Sphingobacteriia*, *Verrucomicrobiae*, *Chloroflexi*, *Chloroplast*, *TM7-1*, *Flavobacteriia*, and *Nostocophycideae* (Figure 6A,B).

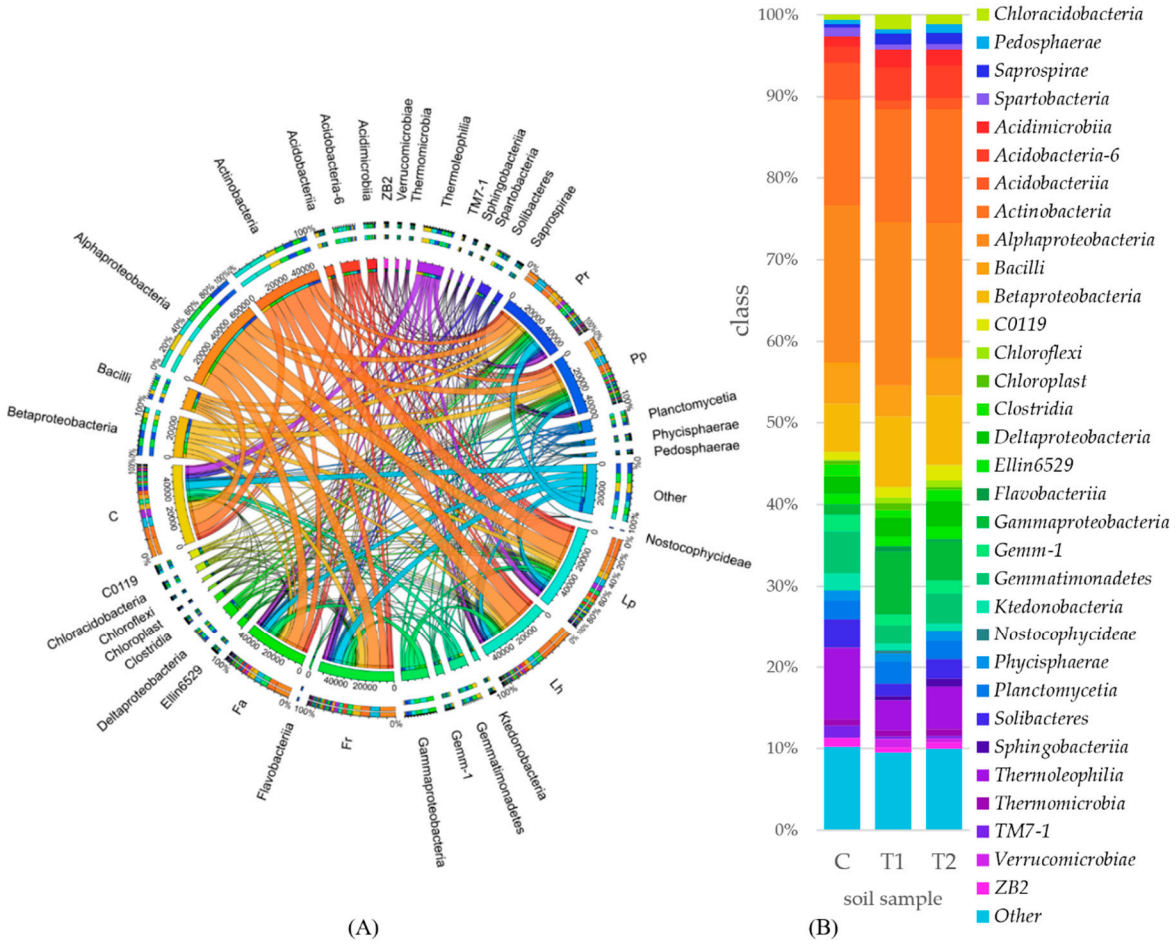

Figure 6. Bacterial communities at the class level (**A**) depending on the species of grass, (**B**) depending on grass type (fodder or lawn). Abundances <1% are gathered into the category "other". C—unsown soil; T1—average bacteria abundance in soils sown with fodder grasses; T2—average bacteria abundance in soils sown with lawn grasses. Lh—*Lolium* x *hybridum* Hausskn; Fa—*Festuca arundinacea*; Pp—*Phleum pratense*, Lp—*Lolium perenne*, Pr—*Poa pratensis*; Fr—*Festuca rubra.*

The effect of grasses on soil bacteriobiome was also noticeable at the order level. Among the 37 identified orders with OTU numbers above 1%, the greatest abundance was demonstrated for bacteria classified to *Actinomycetales*, *Sphingomonadales*, and *Rhizobiales* (Figure S3). When comparing effects of various functional types of grasses, it was found that sowing the soils with fodder grasses increased OTU numbers of *Xanthomonadales* by 4.67%, *Sphingomonadales* by 3.18%, *Burkholderiales* by 2.41%, *Solibacterales* by 2.04%, and *Actinomycetales* by 1.12%, whereas sowing the soils with lawn grasses increased OTU numbers of *Burkholderiales* by 2.60%, *Xanthomonadales* by 1.85%, *Alteromonadales* by 1.39%, *Actinomycetales* by 1.23, and *Rhizobiales* by 1.02%, compared to the nonsown control soil.

The highest OTU number of *Actinomycetales* bacteria was determined in the soils sown with *Lolium* x *hybridum* Hausskn (Lh) and *Lolium perenne* (Lp); whereas that of *Sphingomonadales*—in the soils sown with *Lolium* x *hybridum* Hausskn (Lh), *Festuca arundinacea* (Fa), *Festuca rubra* (Fr), and *Lolium perenne*

(Lp) (Figure 7A,B). Higher OTU numbers were also determined in the soils with growing grasses than in the control soil for the bacteria from the following orders: *Burkholderiales*, *Xanthomonadales*, *Saprospirales*, and *Sphingobacteriales*.

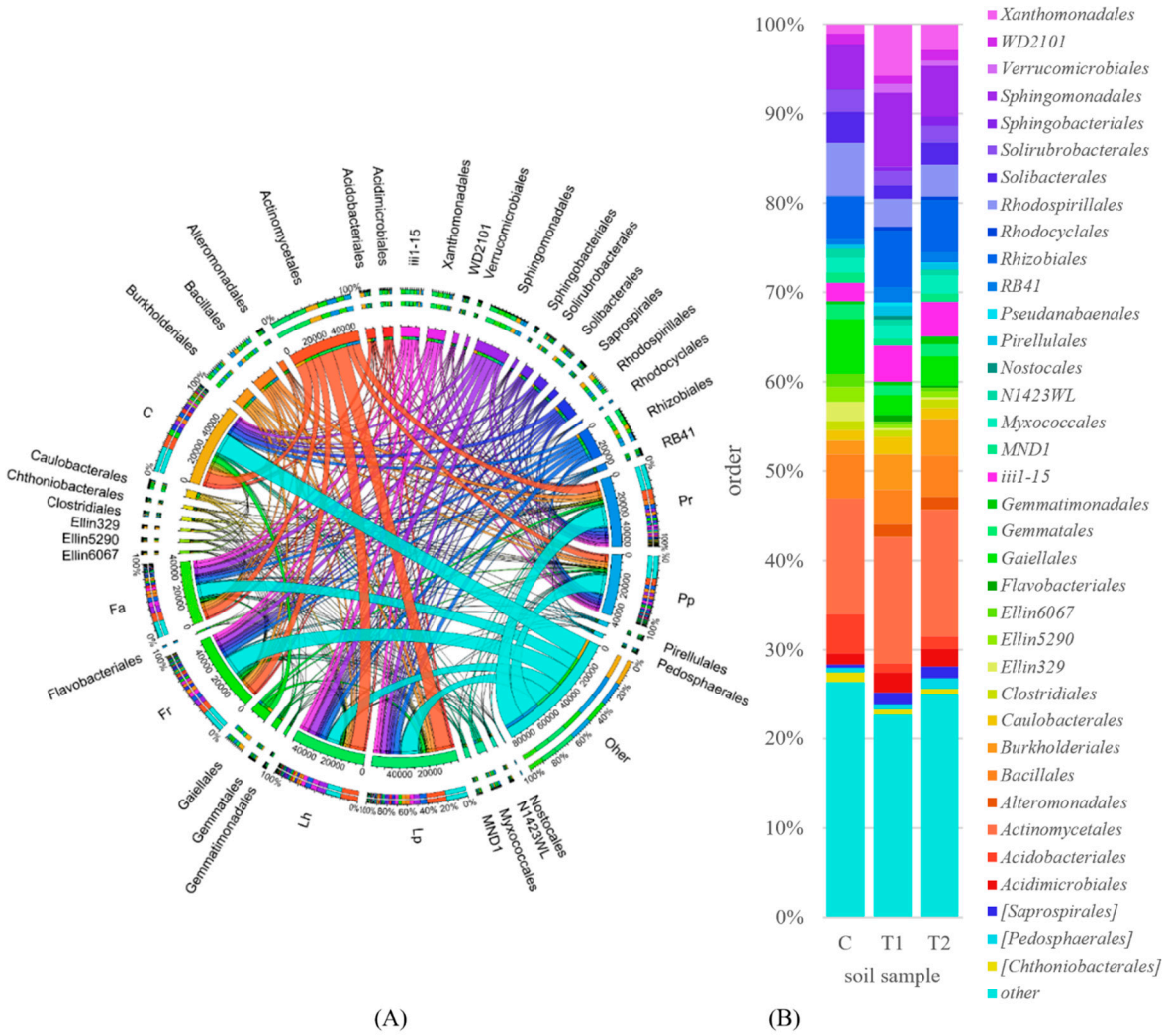

Figure 7. Bacterial communities at the order level (**A**) depending on the species of grass, (**B**) depending on grass type (fodder or lawn). Abundances <1% are gathered into the category "other". C—unsown soil; T1—average bacteria abundance in soils sown with fodder grasses; T2—average bacteria abundance in soils sown with lawn grasses. Lh—*Lolium* x *hybridum* Hausskn; Fa—*Festuca arundinacea*; Pp—*Phleum pratense*, Lp—*Lolium perenne*, Pr—*Poa pratensis*; Fr—*Festuca rubra.*

Differences in the abundance of bacterial populations were also observed at the family level. Compared to the control soil, the greatest changes in the structure of bacteria classified to families, after soil sowing with both fodder and lawn grasses, occurred in the families of *Gaiellaceae* (decrease by 3.16% and 2.57%, respectively), *Koribacteraceae* (decrease by 2.77% and 2.54%), *Intrasporangiaceae* (increase by 2.14% and 1.38%), *Xanthomonadaceae* (increase by 3.54% and 1.13%), and *Comamonadaceae* (increase by 2.61% and 2.43) (Figure S4).

When comparing effects of individual grass species on OTU number of bacteria classified to families, it can be concluded that they were inexplicit (Figure 8A,B). All species increased OTU numbers of the following families: *Intrasporangiaceae*, *Bradyrhizobiaceae*, *Xanthomonadaceae*, *Sinobacteraceae*, *Comamonadaceae*, *Pirellulaceae*, *Chitinophagaceae*, *Ellin5301*, *Nostocaceae*, and *Rhodocyclaceae*, but decreased OTU numbers of *Gaiellaceae*, *Rhodospirillaceae*, *Koribacteraceae*, *Solibacteraceae*, *Acetobacteraceae*, *Pseudonocardiaceae*, *Paenibacillaceae*, *Frankiaceae*, and *Chthoniobacteraceae*. Noteworthy is the fact that

sowing the grasses onto soils resulted in the appearance of families: *Alteromonadaceae*, *Methylophilaceae*, *Flavobacteriaceae*, and *Verrucomicrobiaceae*, that were not identified in the control soil.

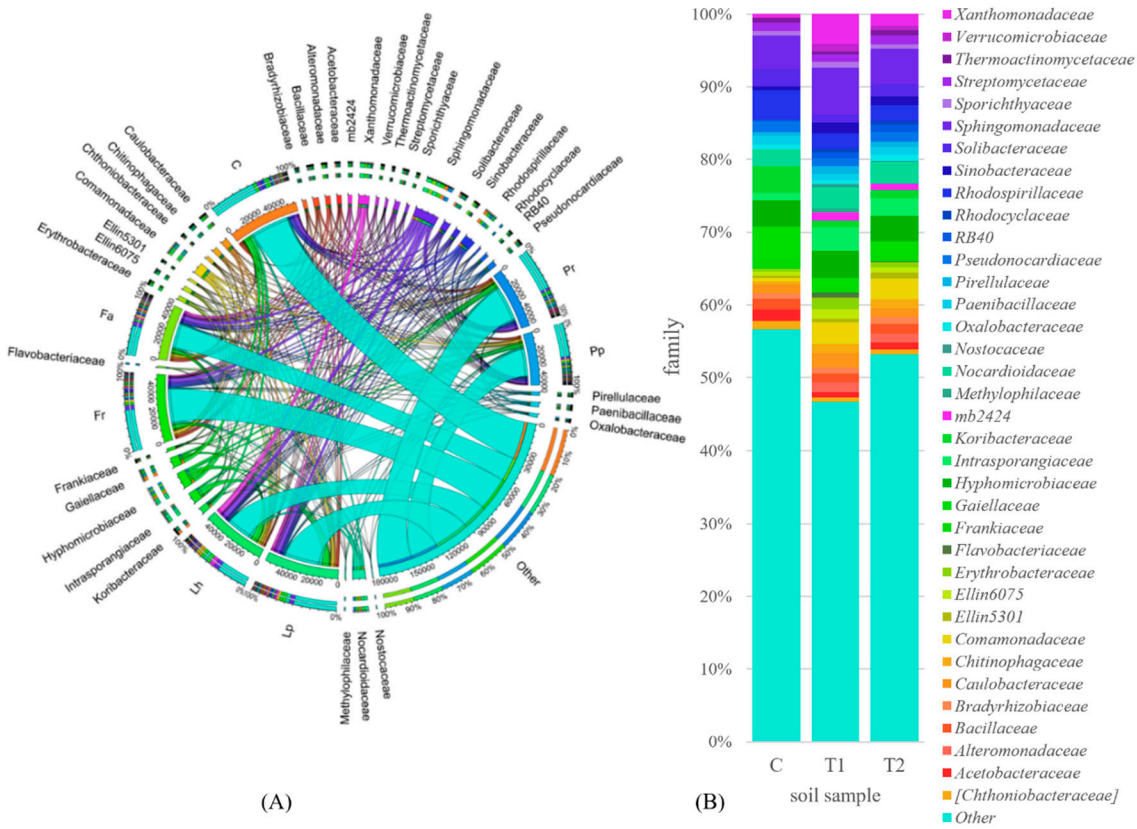

Figure 8. Bacterial communities at the family level (**A**) depending on the species of grass, (**B**) depending on grass type (fodder or lawn). Abundances <1% are gathered into the category "other". C—unsown soil; T1—average bacteria abundance in soils sown with fodder grasses; T2—average bacteria abundance in soils sown with lawn grasses. Lh—*Lolium* x *hybridum* Hausskn; Fa—*Festuca arundinacea*; Pp—*Phleum pratense*, Lp—*Lolium perenne*, Pr—*Poa pratensis*; Fr—*Festuca rubra*.

Considering OTU numbers above 1%, only 11 bacterial genus were identified in the control soil not sown with grasses, whereas 17 genus in the soil sown with Pp and Pr, 16 genus in the soil sown with Fr and Lp, 15 genus in the soil sown with Fa, and 14 genus in the soil sown with Lh (Figure 9). The contribution of the identified bacteria in the genus structure ranged from 13.86% in the soil sown with Pr to 20.34% in the soil sown with Lh. The genus *Kaistobacter* was found to predominate on all plots. Regarding OTU number, it was followed by *Rhodoplanes* in the control soil and soil sown with Pr, by *Terracoccus* in the soil sown with Lh and Lp, by HB2-32-21 in the soil sown with Fa and Fr, and by *Flavobacterium* in the soil sown with Pp. Compared to the soils overgrown with grasses, no OTUs of the following genera were identified in the control soil: *Arenimonas, Dechloromonas, Flavobacterium, Methylotenera*, and *Mycoplana.*

The analysis of values of Shannon and Simpson diversity indices points to a richer microbiome of the soils sown with grasses compared to the control soil (Table 4). The greatest abundance among the fodder grasses was found in the rhizosphere of Pp, and, among the lawn grasses, in the rhizosphere of Pr.

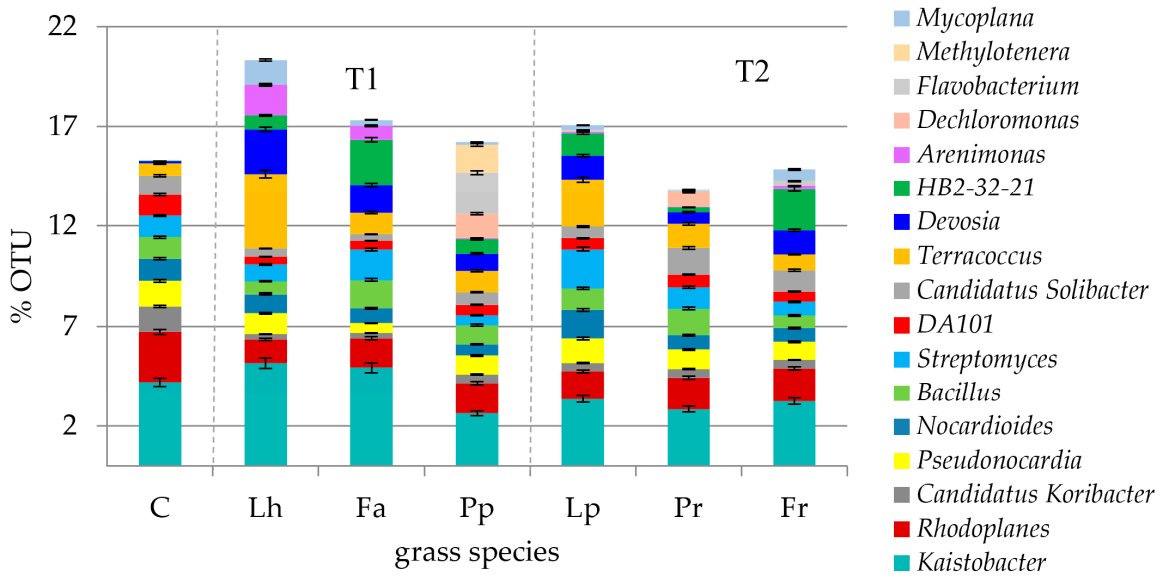

Figure 9. The operational taxonomic units (OTUs) structure of identified genus of bacteria in the total number of OTUs. C—unsown soil; T1—fodder grasses; T2—lawn grasses. Lh—*Lolium* x *hybridum* Hausskn; Fa—*Festuca arundinacea*; Pp—*Phleum pratense*, Lp—*Lolium perenne*, Pr—*Poa pratensis*; Fr—*Festuca rubra.*

Table 4. Shannon and Simpson indices calculated from abundance of OTU.

Taxon	C	Lh	Fa	Pp	Lp	Pr	Fr	C	T1	T2
					Shannon-Wiener index					
phylum	2.05 ^{ab}	1.86 ^{d}	1.92 ^{cd}	2.10 ^{ab}	1.90 ^{d}	2.12 ^{a}	2.01 ^{ac}	2.05 ^{x}	1.96 ^{x}	2.01 ^{x}
class	2.81 ^{bc}	2.67 ^{c}	2.80 ^{bc}	2.97 ^{a}	2.82 ^{abc}	2.96 ^{ab}	2.90 ^{ab}	2.81 ^{x}	2.81 ^{x}	2.89 ^{x}
order	2.77 ^{b}	2.75 ^{b}	2.90 ^{a}	2.91 ^{a}	2.81 ^{ab}	2.79 ^{ab}	2.93 ^{a}	2.77 ^{x}	2.86 ^{x}	2.84 ^{x}
family	2.00 ^{d}	2.49 ^{a}	2.43 ^{ab}	2.23 ^{c}	2.34 ^{bc}	2.04 ^{d}	2.26 ^{c}	2.00 ^{z}	2.38 ^{x}	2.21 ^{y}
genus	0.76 ^{c}	0.97 ^{a}	0.86 ^{b}	0.87 ^{b}	0.87 ^{b}	0.74 ^{c}	0.78 ^{c}	0.76 ^{z}	0.90 ^{x}	0.80 ^{y}
					Simpson index					
phylum	0.84 ^{ab}	0.78 ^{c}	0.78 ^{c}	0.83 ^{ab}	0.80 ^{b}	0.85 ^{a}	0.82 ^{ab}	0.84 ^{x}	0.80 ^{x}	0.82 ^{x}
class	0.92 ^{ab}	0.89 ^{b}	0.92 ^{ab}	0.93 ^{a}	0.92 ^{ab}	0.94 ^{a}	0.93 ^{a}	0.92 ^{x}	0.91 ^{x}	0.93 ^{x}
order	0.94 ^{ab}	0.91 ^{b}	0.94 ^{ab}	0.96 ^{a}	0.92 ^{b}	0.95 ^{a}	0.95 ^{a}	0.94 ^{x}	0.94 ^{x}	0.94 ^{x}
family	0.67 ^{ef}	0.82 ^{a}	0.78 ^{ab}	0.70 ^{de}	0.75 ^{bc}	0.66 ^{f}	0.72 ^{cd}	0.67 ^{y}	0.77 ^{x}	0.71 ^{y}
genus	0.28 ^{cd}	0.36 ^{a}	0.32 ^{b}	0.30 ^{c}	0.31 ^{b}	0.26 ^{d}	0.28 ^{cd}	0.28 ^{y}	0.33 ^{x}	0.28 ^{y}

Homogeneous groups denoted were calculated separately for each taxon groups denoted with letters (a–f) were calculated for the species of grass and groups denoted with letters (x–z) were calculated for the type of grass. C—unsown soil; Lh—*Lolium* x *hybridum* Hausskn; Fa—*Festuca arundinacea*; Pp—*Phleum pratense*; Lp—*Lolium perenne*, Pr—*Poa pratensis*; Fr—*Festuca rubra.*

3.3. Activity of Soil Enzymes

Study results demonstrated the highest enzymatic activity in the soil sown with Lh and Lp (Table 5, Figure 10), and the lowest one in the control soil. Activities of all enzymes were expressed in activity units per dry matter of 1 kg of soil within 1 h, and so the activity of dehydrogenases ranged from 10.292 µmol TFF (triphenyl formazan) in the soil sown with Lh to 0.654 µmol TFF with Pr; that of catalase, from 0.205 mol O_2 in the soil sown with Pr to 0.091 mol O_2 in the control soil; that of urease, from 0.880 mmol N-NH_4 in the soil sown with Pp to 0.302 in the control soil; that of acid phosphatase, from 1.302 mmol PNP (p-nitrophenyl) in the soil sown with Lp to 0.785 mmol PNP in the control soil; that of alkaline phosphatase, from 0.397 mmol PNP in the soil sown with Lh to 0.138 mmol PNP in the soil sown with Fr; that of β-glucosidase, from 0.348 mmol PNP in the soil sown with Lh to 0.298 mmol PNP in the soil sown with Pr; and that of arylsulfatase, from 0.164 mmol PNP in the soil sown with Pp

to 0.082 mmol PNP in the control soil. The average enzymatic activity of soil sown with fodder grasses was 161% higher than in nonsown soil, and of soil sown with lawn grasses was 83% higher than in nonsown soil. Generally, the enzymatic activity of soils sown with fodder grasses was higher (by 30%) than that of the soils overgrown with the lawn grasses. The lowest enzymatic activity was determined in the control soil not sown with grasses (Figure 10); there was a significantly higher one in the soils sown with Pr, Fr, and Fa; and the highest one was in the soils sown with Lh, Lp, and PP.

Table 5. Enzymatic activity in 1 kg d.m. of soil per 1 h.

Grass Species	Deh μmol TFF	Cat mol O_2	Ure mmol N-NH_4	Pac	Pal	Glu	Aryl
				mmol PNP			
C	1.38 ^{e}	0.09 ^{e}	0.30 ^{b}	0.79 ^{d}	0.16 ^{c}	0.30 ^{b}	0.08 ^{d}
Lh	10.29 ^{a}	0.18 ^{b}	0.47 ^{ab}	1.20 ^{ab}	0.40 ^{a}	0.35 ^{a}	0.15 ^{a}
Fa	2.38 ^{d}	0.14 ^{c}	0.60 ^{ab}	1.18 ^{ab}	0.32 ^{b}	0.30 ^{b}	0.14 ^{ab}
Pp	3.25 ^{c}	0.19 ^{ab}	0.88 ^{a}	1.08 ^{bc}	0.33 ^{b}	0.31 ^{b}	0.16 ^{a}
Lp	6.87 ^{b}	0.15 ^{c}	0.68 ^{ab}	1.30 ^{a}	0.35 ^{ab}	0.34 ^{a}	0.11 ^{bc}
Pr	0.65 ^{f}	0.21 ^{a}	0.48 ^{ab}	0.98 ^{c}	0.17 ^{c}	0.30 ^{b}	0.09 ^{cd}
Fr	1.74 ^{de}	0.11 ^{d}	0.63 ^{ab}	1.27 ^{a}	0.14 ^{c}	0.31 ^{b}	0.11 ^{cd}
C	1.38 ^{z}	0.09 ^{z}	0.30 ^{y}	0.79 ^{y}	0.16 ^{z}	0.30 ^{y}	0.08 ^{y}
T1	5.31 ^{x}	0.17 ^{x}	0.65 ^{x}	1.15 ^{x}	0.35 ^{x}	0.32 ^{x}	0.15 ^{x}
T2	3.09 ^{y}	0.16 ^{y}	0.60 ^{x}	1.18 ^{x}	0.22 ^{y}	0.32 ^{x}	0.10 ^{y}

Homogeneous groups were calculated separately for each enzyme groups denoted with letters (a–f) were calculated for the species of grass and groups denoted with letters (x–z) were calculated for the type of grass. C—unsown soil; Lh—*Lolium* x *hybridum* Hausskn; Fa—*Festuca arundinacea*; Pp—*Phleum pratense*, Lp—*Lolium perenne*, Pr—*Poa pratensis*; Fr—*Festuca rubra.*

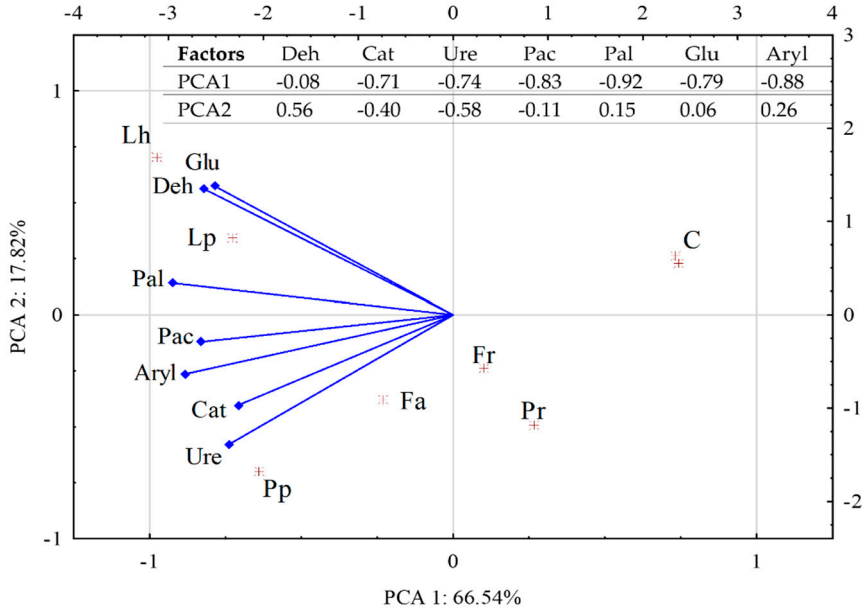

Figure 10. Activity of soil enzymes presented with the principal component analysis (PCA) method. Deh—dehydrogenases; Cat—catalase, Ure—urease; Pac—acid phosphatase; Pal—alkaline phosphatase; Glu—β-glucosidase; Aryl—arylsulfatase. C—unsown soil; Lh—*Lolium* x *hybridum* Hausskn; Fa—*Festuca arundinacea*; Pp—*Phleum pratense*, Lp—*Lolium perenne*, Pr—*Poa pratensis*; Fr—*Festuca rubra.*

4. Discussion

4.1. Grass Yield

The genotype of grasses turned out to be the main factor which differentiated their yield. Conditions mentioned by Broadbent et al. [69] that model plant growth and development, such as,

climatic zone, fraction size, composition of soil, nutrients content in soil, as well as climatic and anthropogenic stresses, could not affect grass growth and development because the pot experiment was performed under controlled conditions. In addition, mineral fertilization was the same for all grass species; therefore this was the genotype that determined the higher biomass produced by the fodder than by the lawn grasses. According to Shukla et al. [70], plants used in agriculture are often grown for green forage and energetic biomass, hence they are increasingly exploited for other purposes than feeds.

4.2. Counts and Diversity of Soil Bacteria

The present study demonstrated that the analyzed grass species modified the soil bacteriobiome to various extents, which was mainly due to the development of their root system [71–73] and chemical composition of their root secretions [13]. According to Berg and Smalla [74] and to Murphy [75], plants may contribute to the establishment of unique communities of soil microorganisms. Among all analyzed grass species, *Lolium* x *hybridum* Hausskn (Lh) contributed to the greatest increase in the population number of organotrophic bacteria compared to the control soil, whereas *Poa pratensis* (Pr), *Lolium perenne* (Lp), *Phleum pratense* (Pp), and *Festuca rubra* (Fr) significantly suppressed their proliferation. The analysis of study results demonstrates that the fodder grasses had a more beneficial effect on the proliferation of organotrophic bacteria and actinobacteria than the lawn grasses had. As reported by Deru et al. [71] and Saleh et al. [73], this could be due to the genetic determinants of individual grass species, which affect development of their root system; whereas the root system influences the development of rhizosphere microbiome by the mineral and organic compounds it secretes [13,74]. Singh et al. [76] emphasized that greater amounts of root secretions produced by young plants contribute to a better availability of carbon and energy sources to microorganisms. In addition, these secretions facilitate rhizosphere colonization by microorganisms [77]. This, in turn, leads to cooperation between the plant and the bacteriobiome, because part of rhizospheric bacteria penetrate inside plant tissues through damaged tissue or due to the release of enzymes capable of increasing solubility of nonabsorbable elements [73].

Although fodder and lawn grasses sown onto the soil elicited changes in the counts of organotrophs and actinobacteria, they did not improve their ecophysiological diversity index (EP). A shift could, however, be noticed in bacteria development towards the k strategists, i.e., slow-growing bacteria, which was indicated by decreased values of the colony development index (CD) caused by both fodder and lawn grasses. These results confirm earlier findings reported by De Leij et al. [61], and Murphy et al. [75], who also observed that the microbiome of the rhizosphere of plants changes along with the prolonging growing season, and that the population of r-strategists turns into k-strategists. Marschner et al. [78], Murphy et al. [75], and Kielak et al. [79] demonstrated that bacterial communities of the rhizosphere are initially predominated by *Proteobacteria* r-strategists. Also in our study, the *Proteobacteria* and *Actinobacteria* were the prevailing phyla on all pots; whereas the prevailing classes included: *Alphaproteobacteria* and *Actinobacteria*; the prevailing orders were: *Actinomycetales*, *Sphingomycetales*, and *Rhizobiales*; the prevailing families included: *Sphingomonadaceae* and *Hyphomicrobiaceae*; and the predominating genera were: *Kaistobacter*, *Rhodoplanes*, *Teracoccus*, and *Flavobacterium.* These results correspond with literature data [22,33,80–83]. In general, a richer bacteriobiome in terms of diversity was demonstrated in the soils sown with grasses than in the control soil without grasses. In the case of the fodder grasses, the greatest diversity occurred in the rhizosphere of *Poa pratensis* (Pr), whereas, in the case of lawn grasses, it was in the rhizosphere of *Phleum pratense* (Pp).

The response of *Proteobacteria* and *Actinobacteria* to sowing grasses onto soil varied. Greater OTUs of *Proteobacteria* were demonstrated in the soils sown with grasses, regardless of their functional type, than in the control soil, whereas the OTU number of *Actinobacteria* in the soil was reduced by both groups of grasses. Changes at the level of phylum and other taxonomic units in the soil sown with various species of legumes and grasses were also observed by Zhou et al. [47] and Singh et al. [76].

A special trait of *Actinobacteria* is their resistance to extreme environmental conditions [22,33,82]. This phylum was described as a promising taxon of plant growth promoters [83].

According to Delgado-Baquerizo et al. [80], the most abundant class of bacteria in soils of the world is *Alphaproteobacteria*, which includes *Bradyrhizobium*, *Sphingomonas*, *Rhodoplanes*, *Devosia*, and *Kaistobacter* genera; whereas among *Actinobacteria*, there are the *Streptomyces*, *Salinibacterium*, and *Mycobacterium* genera. In our study also, the *Kaistobacter* and *Rhodoplanes* genera were found to prevail, but other major genera included *Terracoccus, Candidatus Koribacter*, and *Devosia*.

Both the results of this study and literature data [21,22,33,80,82] indicate that investigations addressing the genetic biodiversity of bacteria should be continued in various soil ecosystems.

4.3. Activity of Soil Enzymes

Being sensitive indicators of soil quality, enzymes are strongly associated with the microbiological activity and species colonization of plants [4,84]. In the present study, grasses stimulated the biochemical activity of soil. This is due to their beneficial effect on the soil bacteriobiome, as indicated by results of this study and by literature data [51,52,84–86]. The association between the activity of soil enzymes and microbiome quality is due to the origin of enzymes [38,50,53,84]. In soil ecosystems, they are mainly derived from microorganisms and, to a lesser extent, from plants and other soil organisms [87–90]. The positive correlation between the activity of soil enzymes and the activity of microorganisms has been demonstrated by many experts in soil science [91–93]. In our own research, the higher activity of soil enzymes in soil sown with fodder grass is mainly associated with a greater diversity of bacteria at the family and genus level in soil from below these plants than in soil from below lawn grasses. The values of the Shannon-Wiener and Simpson indicators prove this. Nevertheless, the more beneficial effect elicited by the fodder than by the lawn grasses on the biochemical properties of soil proves that, by activating the microbiome, the plants can intermediately affect enzymatic activity. This hypothesis was corroborated by other authors [3,86,91–94].

5. Conclusions

The analyzed grass species from the family *Poacea* had a beneficial effect on soil microbiome and activity of soil enzymes. The intensity of their effect was determined by both their species and their functional type. More favorable conditions for the growth and development of soil bacteria, and thereby for the enhanced enzymatic activity, were offered by the fodder than by the lawn grasses. Among the fodder grasses, the greatest bacteriobiome diversity was demonstrated in the soil sown with *Poa pratensis* (Pp), whereas, among the lawn grasses, it was in soil sown with *Phleum pretense* (Pr). The highest enzymatic activity was determined also. Considering the fodder grasses, this was in the soil with *Lolium* x *hybridum* Hausskn (Lh), and in the soil with *Lolium perenne* (Lp) in the case of lawn grasses. The sowing of soils with grasses caused the succession of bacterial communities from r strategy to k strategy. In all pots, the prevailing phyla included *Proteobacteria* and *Actinobacteria*; the prevailing classes were *Alphaproteobacteria* and *Actinobacteria*; the prevailing orders included *Actinomycetales*, *Sphingomycetales*, and *Rhizobiales*; the prevailing families were *Sphingomonadaceae* and *Hyphomicrobiaceae*; and the prevailing genera included *Kaistobacter*, *Rhodoplanes*, *Teracoccus*, and *Flavobacterium.*

Supplementary Materials: The following are available online at http://www.mdpi.com/1424-2818/12/6/212/s1, Table S1: One-way significance tests carried out using the analysis of variance (ANOVA), Figure S1: The relative abundance of dominant phylum bacteria in soil. Data on the number of readings greater than 1% of all OTUs, Figure S2: The relative abundance of dominant class bacteria in soil. Data on the number of readings greater than 1% of all OTUs, Figure S3: The relative abundance of dominant order bacteria in soil. Data on the number of readings greater than 1% of all OTUs, Figure S4: The relative abundance of dominant family bacteria in soil. Data on the number of readings greater than 1% of all OTUs.

Author Contributions: A.B. conceived and designed the ideas and. wrote the manuscript with the help of J.W. and J.K.; A.B. conducted the experiments, collected and analyzed the data, conducted the bioinformatic analysis and visualization of data; all authors contributed to the final version of this manuscript. All authors have read and agreed to the published version of the manuscript.

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