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The Reaction of Cellulolytic and Potentially Cellulolytic Spore-Forming Bacteria to Various Types of Crop Management and Farmyard Manure Fertilization in Bulk Soil

Jakub Dobrzyński [1], Paweł Stanisław Wierzchowski [1,*], Wojciech Stępień [2] and Ewa Beata Górska [3]

[1] Institute of Technology and Life Sciences, Falenty Al. Hrabska 3, 05-090 Raszyn, Poland; j.dobrzynski@itp.edu.pl
[2] Department of Agricultural Chemistry, Institute of Agriculture, Warsaw University of Life Sciences SGGW, Nowoursynowska 166, 02-787 Warsaw, Poland; wojciech_stepien@sggw.edu.pl
[3] Department of Biochemistry and Microbiology, Institute of Biology, Warsaw University of Life Sciences SGGW, Nowoursynowska 166, 02-787 Warsaw, Poland; ewa_gorska@sggw.edu.pl
* Correspondence: s.wierzchowski@itp.edu.pl

Abstract: The ecology of cellulolytic bacteria in bulk soil is still relatively unknown. There is still only a handful of papers on the abundance and diversity of this group of bacteria. Our study aimed to determine the impact of various crop management systems and farmyard manure (FYM) fertilization on the abundance of cellulolytic and potentially cellulolytic spore-forming bacteria (SCB). The study site was a nearly 100-year-old fertilization experiment, one of the oldest still active field trials in Europe. The highest contents of total carbon (TC) and total nitrogen (TN) were recorded in both five-year rotations. The abundances of SCB and potential SCB were evaluated using classical microbiological methods, the most probable number (MPN), and 16S rRNA Illumina MiSeq sequencing. The highest MPN of SCB was recorded in soil with arbitrary rotation without legumes (ARP) fertilized with FYM (382 colony-forming units (CFU) mL^{-1}). As a result of the bioinformatic analysis, the highest values of the Shannon–Wiener index and the largest number of operational taxonomic units (OTUs) were found in ARP-FYM, while the lowest in ARP treatment without FYM fertilization. In all treatments, those dominant at the order level were: Brevibacillales (13.1–43.4%), Paenibacillales (5.3–36.9%), Bacillales (4.0–0.9%). Brevibacillaceae (13.1–43.4%), Paenibacillaceae (8.2–36.9%), and Clostridiaceae (5.4–11.9%) dominated at the family level in all tested samples. Aneurinibacillaceae and Hungateiclostridiaceae families increased their overall share in FYM fertilization treatments. The results of our research show that the impact of crop management types on SCB was negligible while the actual factor shaping SCB community was the use of FYM fertilization.

Keywords: crop management; next generation sequencing; soil microbiome; fertilization; cellulolytic spore-forming bacteria

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1. Introduction

In recent decades, we have observed a rapid increase in agricultural production. The pursuit of maximum crop yielding leads to changes in the chemical, physical, and biological properties of soil [1,2]. This phenomenon is caused by the use of various agrotechnical practices, including the use of crop protection agents, long-term mineral fertilization, and long-term cropping in monoculture [3,4]. Continuous cropping can lead to decreased yields, an increase in the number of fungal phytopathogens, and a biological imbalance in soil [5,6]. To a large extent, microorganisms are responsible for maintaining biological balance and determining the direction of biochemical processes occurring in soil [7]. Therefore, knowledge about the impact of agrotechnical practices on the abundance and biodiversity of microorganisms in soil is crucial.

Cellulose is the most common biopolymer in soil. Thus, an important group of microorganisms involved in the circulation of elements in the soil are cellulose-degrading

microorganisms [8]. Soil properties such as pH, moisture, and soil organic matter content influence microbial cellulose degradation. This process requires a complex of enzymes belonging to the class of O-glycoside hydrolases, including endo-β-1,4-glucanases (EC 3.2.1.4), exoglucanases, syn. 1,4-β-glucan cellobiohydrolase (EC 3.2.1.91), and cellobiase, syn. β-glucosidase (EC 3.2.1.21) [9,10]. In soil, cellulolytic enzymes are primarily produced by fungi and bacteria. Cellulolytic microorganisms include relatively anaerobic, spore-forming bacteria (SCB). This group includes bacteria of the phylum Firmicutes, among others from the Bacilliaceae, Paenibacilliaceae, Clostridriaceae families which numerously occur in different types of bulk soil [11,12]. Moreover, relatively anaerobic, spore-forming bacteria can be used as plant growth promoters. SCB may promote plant growth through direct and indirect mechanisms. Direct mechanisms include the secretion of phytohormones, e.g., auxins (e.g., indoleacetic acid—IAA) and gibberellins, fixation of atmospheric nitrogen (nitrogenase production), and solubilization of nutrients such as phosphates. Indirect mechanisms of plant growth promotion based on protection against phytopathogens largely depend on enzymes degrading fungal cell walls, e.g., chitinases and glucanases [13–17].

The ecology of cellulolytic bacteria in arable soils is still relatively poorly understood. Thus, the study aimed to evaluate the impact of monoculture and rotation systems as well as farmyard manure fertilization on the abundance of potential cellulolytic and cellulolytic spore-forming bacteria in soil from nearly a century-old fertilization experiment. It is worth mentioning that currently there are only a few other similar experiments in Europe, for instance in Rothamsted (Great Britain) conducted since 1843, and in Halle (Germany) conducted since 1894. Such a long-term monoculture, crop rotation, and farmyard manure fertilization field trial allowed for a unique assessment of the bacterial community of tested microorganisms.

2. Materials and Methods

2.1. Site Characteristics

The research was carried out in in the fields of the Institute of Agriculture, University of Life Sciences in Skierniewice, Poland (51°57′54.8″ N 20°09′27.4″ E). The experiment was established in 1922 on loamy sand textured Luvisol and from the beginning to the present, it is conducted in triplicate. On plots without liming pH was approximately 4.5. The average annual temperature for the period 1921–2017 was 8.0 °C, and the annual precipitation was 530 mm. The size of a single plot was 4 m × 9 m. The buffer zone between plots was 2 m wide. The presented study included seven crop rotation and soil management treatments with or without farmyard manure (FYM) fertilization (Table 1).

Table 1. Experimental treatments.

Abbr.	Crop Management	Crops	FYM Fertilization
ARP	Arbitrary rotation without legumes (since 1923)	Potato *-winter wheat- spring barley	No
ARP-FYM	Arbitrary rotation without legumes (since 1992 with FYM)	Potato *-winter wheat-spring barley	Yes (30 t ha^{-1})
LRL	Rotation with legumes (since 1924)	Lupine *-spring triticale-barley	No
FRR	Five-year rotation (since 1924)	Lupine-winter wheat-rye *-potato-barley	Yes (30 t ha^{-1})
FRP	Five-year rotation (since 1924)	Lupine-winter wheat-rye-potato *-barley	Yes (30 t ha^{-1})
MP	Monoculture (since 1923)	Potato	Yes (20 t ha^{-1})
MR	Monoculture (since 1923)	Rye	Yes (20 t ha^{-1})

* Crop present during sampling.

Sample-source plots were limed since 1976 with doses 1.6 t CaO ha every 4 years on arbitrary rotation without legumes (ARP), ARP with farmyard manure (FYM), rotation with legumes (LRL) and every 5 years on potato monoculture (MP), rye monoculture (MR), rye five-year rotation (FRR), and potato five-year rotation (FRP).

2.2. Sampling and Chemical Analysis

Samples of bulk soil were taken by a sampler probe in July 2017. Soil samples per experimental treatment were collected from a depth of 0–20 cm from three separate plots. Each plot sample was a composite sample made out of soil collected from three random points. Soil for chemical analysis was then air-dried, ground, and passed through a 2 mm sieve. The following chemical parameters were determined in soil samples: total carbon (TC) [18] and total nitrogen (TN) by the direct method [19], pH in 1M KCl [20]. Soil for most probable number (MPN) analysis was passed through a sterile 2 mm sieve and stored at 4 °C (MPN analysis lasted 2 days).

2.3. Microbiological Analyses

Most probable number of SCB was determined using the dilution method with three replicates. Ten grams of soil were suspended in 100 mL of sterilized water and then tenfold serial dilutions were prepared. Next, one mL of dilutions 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} was added to Park medium ($(NH_4)_2SO_4$—0.5 g; KH_2PO_4—1.0 g; KCl—0.5 g; $MgSO_4$—0.2 g; $CaCl_2$—0.1 g, with the addition of 0.5 cm × 8 cm filter papers). To ensure that only spore-forming bacteria are present in the samples, cultures were pasteurized for 15 min at 85 °C, then incubated for 21 days at 28 °C. Filter paper degradation was evaluated macroscopically, and later the readout was carried out using McCrady's statistical tables [21], and expressed as colony forming units (CFU) per ml.

2.4. DNA Extraction and 16S rRNA Sequencing

The genetic material for sequencing analysis came from 21-day cultures of SCB. 7 representative samples were made by merging 6 repeated culture samples per treatment (5 mL each): three repetitions of cultured samples from a dilution of 10^{-1} (0.1 g soil) and three from 10^{-2} (0.01 g soil). Then the representative samples were shaken at 200 rpm for 30 min, 2 mL aliquots were frozen in liquid nitrogen and stored at −80 °C.

DNA isolation was carried out with a method based on the Genomic Mini AX Bacteria + kit (A&A Biotechnology, Gdynia, Poland). After isolation, the DNA was further purified using an Anti-Inhibitor kit (A&A Biotechnology, Gdynia, Poland).

Analysis of the genes encoding 16S rRNA was carried out based on the hypervariable V4 region of the 16S rRNA gene. Specific 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVHHHTWTCTAA-3′) primer sequences were used to amplify the selected region and prepare the library. The polymerase chain reaction (PCR) was carried out using the NEBNext® High-Fidelity 2X PCR Master Mix. Sequencing took place on the MiSeq sequencer, in paired-end (PE) technology, 2 × 250 nt, using Illumina v2. Sequencing data was deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under BioProject accession PRJNA665141.

The 16S rRNA gene sequence reads were processed with mothur [22]. Paired-end reads were merged and assembled using the make.contigs command. Pairs shorter than 290 bp, longer than 300 bp, or with an average Phred score quality below 25 were discarded. Chimaeras were removed using the vsearch algorithm. The final reads were clustered into operational taxonomic units (OTUs) using dist.seqs and cluster commands (opticlust algorithm) with a 0.03 distance cut off. A taxonomic identity was attributed to each OTU via the SILVA 134 rRNA database [23] using an 80% homogeneity cut off. Rarefaction curves along with data used for figure generation are available in the Supplementary Materials (Figure S1, Table S1).

2.5. Statistical Analysis and Data Visualisation

Statistical analysis and data visualization was undertaken using R 3.5.3 statistical programming language [24]. The Shannon–Wiener diversity index was calculated using mothur's built-in functionality. Bray–Curtis dissimilarities between OTU compositions of individual samples were calculated and plotted for principal coordinate analysis (PCoA) using the phyloseq package [25]. One-way analysis of variance (ANOVA) was used for the analysis of soil chemical parameters and MPN (n = 3) among seven treatments. Differences between treatments were tested using the Tukey–Kramer honest significant difference (HSD) test at α = 0.05 [26]. Variance homogeneity was examined using Levene's test. The Wilk–Shapiro normality statistic was calculated to determine if residual values conformed to a normal distribution.

3. Results and Discussion

3.1. Soil Parameters and Most Probable Number (MPN)

In most treatments, the pH values were similar, small differences between ARP-FYM and FRR (Table 2) may have been caused by the use of liming. C:N ratio was at a similar level. The exception being soil from the LRL treatment, where significantly lower C:N ratio (9.70) was observed. This result may be explained by the presence of lupine in the year of study, thanks to symbiosis with atmospheric nitrogen-fixing bacteria the TN content in bulk soil increased. The highest values of TC were recorded in FRR and FRP soils, respectively 0.7% and 0.68%. Similar patterns were observed for total nitrogen values, the highest TN contents were recorded in FRR (654 mg/kg) and FRP (648 mg/kg) soils, along with slightly lower values in LRL soil (532 mg/kg), compared to monoculture, higher TN contents were also observed in other rotations. Long-term organic residue accumulation may have been responsible for changes in TN concentration. These results are consistent with the previous study of Stępień and Kobiałka [27] conducted at the same experimental station. The authors showed higher TC and TN contents in crop rotation soils compared to monoculture soils. Moreover, Adamiak and Adamiak [28] showed slightly lower TC content in five-year rotation soil (rye in the study year) compared to rye monoculture soil after 18 years of FYM fertilization. Values obtained were also similar to those in the study of Congreves, et al. [29], where authors found higher soil organic carbon (SOC) and TN content in corn–soybean–wheat rotation soil compared to corn continuous cropping soil at the 11-year-old long-term fertilization experiment located in Canada. In contrast, Kaiser et al. [30] obtained lower SOC content in rye-potato rotation soil in comparison with long-term rye monoculture soil—long-term trials in Halle (Germany). The authors explained this phenomenon by crop rotation-specific soil organic matter mineralization.

Table 2. Soil chemical properties and most probable number (MPN) of cellulolytic spore-forming bacteria (SCB).

Treatment	pH	TC (%)	TN (mg kg^{-1})	C:N Ratio	MPN (CFU mL^{-1})
ARP	6.10 ± 0.04 ab	0.47 ± 0.15 ab	427.00 ± 20.00 c	10.80 ± 0.40 ab	45.00 ± 12.16 d
ARP-FYM	6.00 ± 0.13 b	0.53 ± 0.10 ab	479.00 ± 34.00 bc	11.00 ± 0.90 ab	382.00 ± 19.67 a
LRL	6.10 ± 0.10 ab	0.56 ± 0.13 ab	532.00 ± 16.00 b	9.70 ± 0.32 b	25.00 ± 6.80 d
MP	6.10 ± 0.09 ab	0.37 ± 0.10 ab	324.00 ± 20.00 d	11.40 ± 0.70 a	30.00 ± 6.11 d
MR	6.20 ± 0.11 ab	0.36 ± 0.13 b	329.00 ± 32.00 d	10.90 ± 0.60 ab	50.00 ± 10.00 d
FRR	6.30 ± 0.06 a	0.70 ± 0.11 a	654.00 ± 30.00 a	10.70 ± 0.63 ab	293.00 ± 17.08 b
FRP	6.20 ± 0.09 ab	0.68 ± 0.12 ab	648.00 ± 28.00 a	10.50 ± 0.40 ab	249.00 ± 17.90 c
Prob > F	0.038	0.020	<0.001	0.089	<0.001

Different letters behind the mean values and SD (n = 3) indicate significant differences (Tukey–Kramer HSD test, α < 0.05).

Compared to monoculture treatments MP (CFU mL^{-1}) and MR (50 CFU mL^{-1}), significantly higher MPN of SCB was recorded in FRR (293 CFU mL^{-1}) and FRP (249 CFU mL^{-1}) crop rotations. Obtained results may be explained by the impact of greater amounts of crop residues which induced spore-forming cellulolytic bacteria growth in crop rotation treatments. Available literature provides scant information on the impact of crop management and FYM fertilization on the MPN of SCB. Gregorutti and Caviglia [31] found no connections between the crop residues and the MPN of cellulolytic bacteria in the field experiment conducted in Argentina. Moreover, Pankhurst et al. [32] showed a lack of significant differences between the activity of cellulose-decomposing microorganisms in crop rotation soil (wheat-sown pasture) and continuous wheat soil in long-term experiments in South Australia. The authors explained their results by low winter rainfall and the dry soil conditions. The discrepancy between our results and these studies could possibly be explained by differences in soil properties and climate conditions.

In addition, significantly higher numbers of SCB in arbitrary rotation without legumes with FYM fertilization (382 CFU mL^{-1}) compared to ARP without FYM fertilization (45 CFU mL^{-1}) were noted. Most likely, the long-term FYM fertilization caused an increase in the amount of N and C in soil, which led to an increase in the most probable number of tested bacteria. Similar patterns were observed by Górska et al. [33].

3.2. 16S rRNA Sequencing

Analysis of 16S rRNA genes was carried out on cultures of cellulolytic SCB. Hence, our bacterial group is referred to as cellulolytic spore-forming bacteria and potentially cellulolytic spore-forming bacteria. The central dogma of microbiology claims that 90% of microorganisms found in all environments of our globe are non-cultivable [34]. Development of next-generation sequencing (NGS) has contributed to the hypothesis that this value may be overstated, as confirmed by some researchers. Van Insberghe et al. [35] have isolated 1264 isolates (from 8 different culture media) from forest soils that have previously been sequenced. Comparative analysis showed that the isolates constituted about 22% of OTUs obtained from bioinformatic analysis of 16S rRNA genes from this soil. Moreover, Bai et al. [36] created a comprehensive collection of more than 2000 isolates derived from *Arabidopsis* root extract and sequenced the 16S rRNA genes of these isolates. Then, compared the 16S rRNA of the *Arabidopsis* root extract and found out that about 60% of OTUs coincide with the 16S rRNA gene sequences of the studied isolates. Referring to this data, the analysis of 16S rRNA genes was carried out in bacterial cultures. This type of analysis gave an insight into this particular physiological group of microorganisms which are SCB and potentially cellulolytic SCB.

A total of 608,042 16S bacterial raw sequences were obtained from 7 samples. After quality filtering, a total of 407,526 sequences were obtained (53,096 unique), with an average of 58,218 sequences per sample (Table 3). A total of 5029 unique OTUs were formed after binning with 97% similarity rate.

Table 3. Reads after processing, operational taxonomic unit (OTU) numbers, Shannon biodiversity index, and coverage.

Treatment	Sequences	OTUs	Shannon	Coverage
ARP	43,800	804	2.73	98.80%
ARP-FYM	59,097	1288	3.52	98.50%
LRL	81,093	1089	2.91	99.10%
FRR	62,032	1205	2.95	98.70%
FRP	40,610	1150	3.25	98.10%
MP	53,464	1270	3.30	98.40%
MR	67,430	975	3.51	99.10%

As a result of sequencing SCB cultures, in all samples about 100% of OTUs were assigned to the phylum Firmicutes. Firmicutes is the dominant bacterial phylum in arable

soils. Its abundance in the soil bacterial communities ranges from 3% to 18% depending on agrotechnical practices [37,38]. Shannon index and OTU results are partly consistent with results obtained by the classical microbiology analysis. Most OTUs were observed in ARP-FYM treatment (1288), and slightly fewer OTUs were found in MP treatment (1270). While the lowest OTU numbers (less than 1000) were noted in MR and ARP treatments, this observation is in line with lower MPN values obtained for these treatments. The Shannon index was partly consistent with OTU numbers. The highest Shannon values were recorded in ARP-FYM and MR treatment, 3.52 and 3.51, respectively, while the lowest was observed in ARP treatment (2.73). Opinions about the impact of crop management systems on OTU numbers and diversity indexes of whole soil bacterial communities are divergent. For instance, Yin et. al. [39] observed the decrease in richness and the Shannon index in rotation soil (wheat and soybeans compared to wheat monoculture). Similar patterns were detected by Mayer et al. [40]. Soman et al. [41] did not report differences in bacterial diversity and OTU numbers in soil from two different rotations (two-year corn and soybean, and three-year corn-oat-alfalfa)—long-term trails at the Morrow Plots (Urbana, Illinois, United States). Venter et al. [42] based on the meta-analysis of richness and biodiversity of bacteria in soil from different crop managements, documented that higher values of bacterial richness and biodiversity occurred in soils from crop rotation. In our work based on the phylum Firmicutes, we did not notice major differences in these parameters. Zhao et al. [38] recorded significantly increased abundances of the phylum Firmicutes in bacterial communities in soil from 15- and 22-year-old cucumber monoculture soil compared to the cucumber planted for one season. Previously, Zhao et al. [3] detected the same patterns in coffee monoculture. However, these authors did not explain their findings in detail. Overall, the phylum Firmicutes was more abundant in crop rotation soils compared to monoculture soils [43–46]. This occurrence was explained by the impact of crop residues and decaying roots accumulating over time, and more generally by soil health resulting from crop rotation. The discrepancies between the studies are not clear and may be caused by other factors such as micronutrient content or soil electrical conductivity (EC). Thus, further research is needed to explain this phenomenon, e.g., determining the correlation between more detailed soil physicochemical properties, and the abundance of the phylum Firmicutes in treatments. For instance, previous studies investigating the impact of cotton monoculture (20 years) on soil bacterial communities showed positive correlation between the abundance of phylum Firmicutes and soil EC [43]. Finally, differences between studies may have also been caused by the heterogeneity of agricultural practices, as previously noted by Soman et al. [41].

The PCoA analysis showed that FYM fertilization in comparison to crop management was a stronger factor modulating bacterial communities in analysed treatments. Figure 1 shows significant distances between bacterial communities from treatments with or without FYM fertilization.

PCoA results were consistent with higher values of OTUs, Shannon index and MPN of tested bacteria in ARP-FYM treatment. Cellulose present in FYM straw may have been responsible for stimulating the increase in SCB and potentially SCB abundance. Additionally, most members of Firmicutes have generally been described as copiotrophs which are fast-growing microorganisms that prefer environments rich in organic matter [47]. Francioli et al. [48] observed relatively more Firmicutes in farmyard manure fertilized soils in a long-term fertilization experiment. Similarly, Hartmann et al. [49] observed an increased percentage of Firmicutes in long-term FYM fertilization when compared to mineral fertilization. This phenomenon was additionally confirmed by a study investigating the impact of different cropping practices on bulk soil and rhizosphere microbiomes [50]. Moreover, the beneficial effect of FYM on richness and biodiversity of whole soil bacterial communities was recorded several times [51–53].

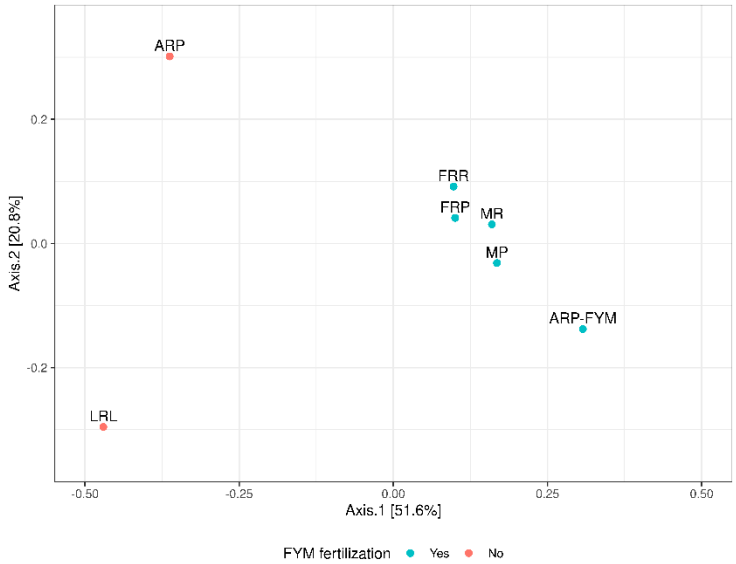

Figure 1. Principal coordinate analysis (PCoA) of OTU compositions between different samples.

Eleven bacterial orders were identified in the treatments, the dominants at order level (Figure 2) were Brevibacillales (13.1–23.4%), Paenibacillales (5.3–36.9%), Bacillales (4.0–30.9%). The most abundant order Brevibacillales was recorded in ARP soil, where this order accounted for nearly 50% of all covered taxons. Moreover, a large abundance of Brevibacillales was observed in FRR (23.6%) and FRP (22.7%) treatments. The abundance of order Paenibacillales was quite varied in the treatments, high OTU numbers belonging to this order were recorded in LRL (36.9%) and ARP (22.8%) treatments. The highest abundance of Bacillales was observed in LRL treatment (30.9%). The percentage values of Aneurinibacillales were similar in most treatments and ranged from 16.7 to 20.1%, exceptions were LRL and ARP treatments (below 2%). Higher values were also obtained for Clostridia_or (3.0–21.7%) and Clostridiales (5.6–11.3%) orders.

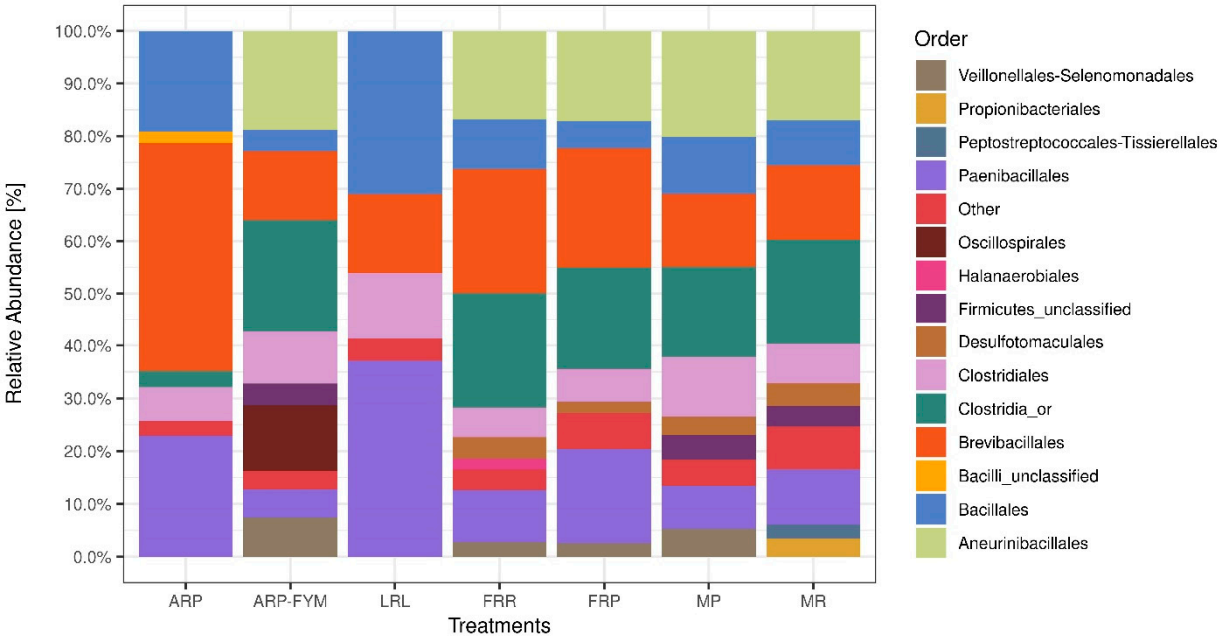

Figure 2. Bacterial abundances at the order level. ARP—arbitrary rotation without legumes, ARP-FYM—arbitrary rotation without legumes fertilized with farmyard manure, LRL—rotation with legumes, FRR—rye five-field rotation, FRP—potato five-field rotation, MP—potato monoculture, MR—rye monoculture. Taxons below 2% abundancy and single observation are represented by the "Other" category in the figure.

The studied bacterial community patterns at the family level were similar to those recorded for orders (Figure 3). Brevibacillaceae (13.1–43.4%), Paenibacillaceae (8.2–36.9%), and Clostridiaceae (5.4–11.9%) dominated at the family level in all treatments. Currently there are no data available in other studies that describe possible changes in communities of cellulolytic and potentially cellulolytic SCB at the order and family level depending on crop management and FYM fertilization. Higher abundance of the family Paenibacillaceae in LRL and ARP treatments, in comparison to all other treatments, may be connected with the lack of FYM fertilization in those treatments. So far, bacteria belonging to the family Paenibacillaceae have been isolated from a variety of soil environments [54,55]. The Paenibacillaceae family is one of the best-described Firmicutes families. A large number of *Paenibacillus* strains are capable of producing direct plant growth promoters, including phytohormones, phosphate solubilization, and nitrogen fixation [56,57]. In addition, bacteria belonging to the genus *Paenibacillus* can help control phytopathogens by triggering induced systemic resistance (ISR) or by producing a variety of biological compounds including lipopeptides with antibiotic properties [54,58,59]. Thus, the presence of these family members may help improve plant and soil health.

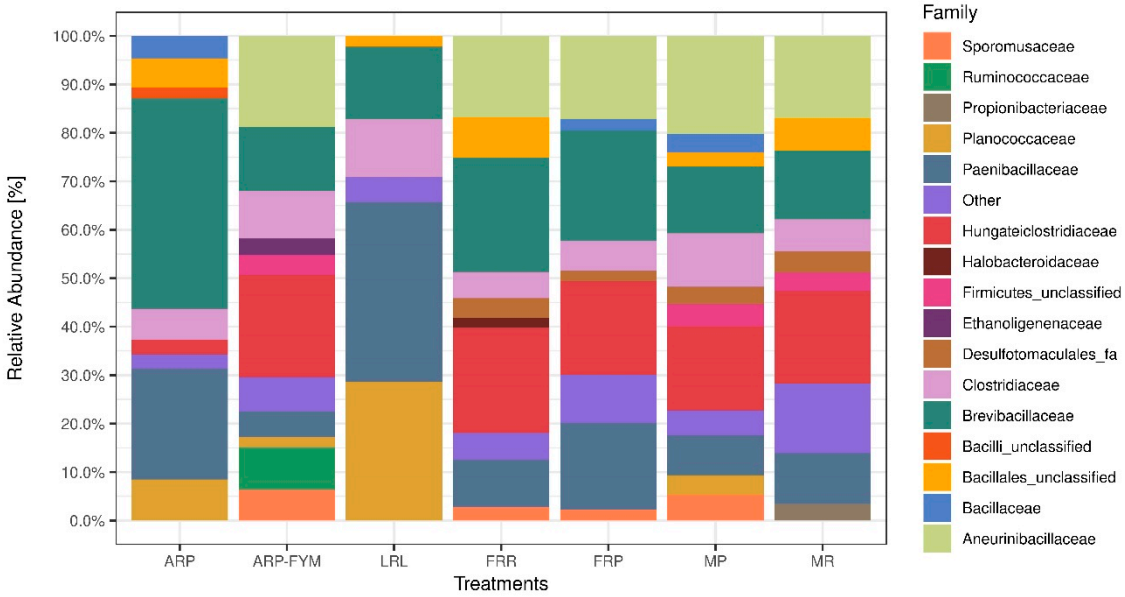

Figure 3. Bacterial abundances at the family level. ARP—arbitrary rotation without legumes, ARP-FYM—arbitrary rotation without legumes fertilized with farmyard manure, LRL—rotation with legumes, FRR—rye five-field rotation, FRP—potato five-field rotation, MP—potato monoculture, MR—rye monoculture. Taxons below 2% abundancy and single observation are represented by the "Other" category in the figure.

Results obtained at the family level also confirmed lower abundance of Aneurinibacillales in the ARP and LRL bacterial communities. The large abundance of the family Aneurinibacillaceae in most treatments may be explained by FYM fertilization, but the reason for this observation seems unclear and its detailed explanation requires further research. Bacteria belonging to the family Aneurinibacillaceae have been isolated from a variety of environments. Several strains of Aneurinibacillaceae were capable of promoting plant growth. Chauhan et al. [60] detected the production of nitrogenases, IAA, and the ability to solubilize phosphates in a new strain *Aneurinibacillus aneurinilyticus* CKMV1. The authors also detected antifungal activity against a few phytopathogens, e.g., *Fusarium oxysporum*, *Alternaria* sp., and *Rhizoctonia solani*. Alenezi et al. [61] also showed the potential of biocontrol against plant diseases in the strain *Aneurinibacillus migulanus* that produced a new gramicidin. Antifungal activity of *Aneurinibacillus migulanus* was also detected by Schuster and Schmitt [62]. Additionally, three nitrogenases genes were found in the genome of *Aneurinibacillus terranovensis* [63]. Thus, the presence of bacteria

belonging to Aneurinibacillaceae may have a positive impact on soil health in treatments with FYM fertilization.

The family Planococcaceae belonging to the order Bacillales was a definite dominant in crop rotation with legumes—LRL treatment (28.5%). This observation may be explained by the impact of decaying legume roots. The presence of Planococcaceae members in legumes' rhizosphere was also described by other authors [64,65].

Additionally, Figure 3 shows some information about anaerobic bacteria. The family Hungateiclostridiaceae abundance in LRL treatment was less than 2%, while the abundance of Hungateiclostridiaceae in ARP reached 3%. The relatively low abundance of this taxon, in comparison to other treatments, may be connected with no FYM fertilization in these treatments. However, this hypothesis is difficult to explain since the abundance of the related anaerobic family of bacteria Clostridaceae was relatively high in all tested treatments. Hungateiclostridiaceae is a novel family of anaerobic bacteria belonging to the phylum *Firmicutes*. So far, researchers have isolated members of this family from various environments and described a few strains: *Defluviitalea raffinosedens*, *Hungateiclostridium mesophilum*, and *Hungateiclostridium thermocellum*. These strains were able to produce cellulolytic and xylanolytic enzymes [66–68].

4. Conclusions

In summary, results obtained in this study answered some questions about the impact of long-term crop management and FYM fertilization on the studied bacterial group. Biodiversity and OTU numbers of SCB and potentially SCB showed that crop rotation and long-term monoculture had a relatively negligible impact on the analyzed bacterial communities. The differences between ARP-FYM and ARP indicated that farmyard manure was a more potent factor in shaping SCB communities, most likely due to straw present in FYM. A crucial difference in the community structures was an increased abundance in the Aneurinibacillaceae and Hungateiclostridiaceae families in FYM fertilization treatments. Thanks to their plant growth promoting capabilities, members of these families can have a positive impact on arable soil health. However, further research is needed to determine the exact mechanisms that increase their abundance in FYM fertilization treatments.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11040772/s1, Table S1: Data used for Figures 2 and 3, Figure S1: Rarefaction curves.

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