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Bacterial Communities: Interaction to Abiotic Conditions under Effect of Anthropogenic Pressure

Monika Vilkiene, Ieva Mockeviciene *, Grazina Kadziene, Danute Karcauskiene, Regina Repsiene and Ona Auskalniene

Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Akademija, 58344 Kėdainiai, Lithuania; monika.vilkiene@lammc.lt (M.V.); grazina.kadziene@lammc.lt (G.K.); danute.karcauskiene@lammc.lt (D.K.); regina.repsiene@lammc.lt (R.R.); ona.auskalniene@lammc.lt (O.A.)
* Correspondence: ieva.mockeviciene@lammc.lt

Abstract: Relationships between different microorganisms' groups and the soil environment are reversible, and the state of the soil and its provided services can also change the structure and abundance of microorganisms as well as that microorganisms can affect soil conditions. The aim of our research was to analyze the physical and chemical properties of differently formed agroecosystems, which are affected by different anthropogenic pressures and to compare how bacterial composition differ in totally different environments. It was established that different soil microorganisms' physiological groups significantly correlated with chemical and physical soil properties: atmospheric nitrogen-fixing bacteria showed a positive correlation with soil pH_{KCl}, N_{sum}, P_2O_5, and soil bulk density; meanwhile, soil porosity, and the K_2O amount in the soil negatively affected the population of atmospheric nitrogen-fixing bacteria. The same tendencies were inherent to actinomycetes and ammonifying bacteria. Micromycetes showed a negative trend with soil pH_{KCl}, showing that soils with lower pH_{KCl} are characterized by a higher abundance of micromycetes. Analysis of the taxonomic diversity of soil microbes reveals that the bacterial communities were dominated by two main species of bacteria: *Betaproteobacterium* and *Candidatus Saccharibacteria*. Bacterial identification shows that the main bacterial species were the same in all analyzed sampling places despite the different anthropogenic activities, parent material, and other abiotic conditions. Only a few species were identified in different soil groups, and it may be assumed that those groups could be potential bioindicators for specific soil types, but more in depth research is needed to confirm this hypothesis.

Keywords: agricultural ecosystems; anthropogenic intensity; *Cambisol*; next generation sequencing; soil microbial community; *Retisols*

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Citation: Vilkiene, M.; Mockeviciene, I.; Kadziene, G.; Karcauskiene, D.; Repsiene, R.; Auskalniene, O. Bacterial Communities: Interaction to Abiotic Conditions under Effect of Anthropogenic Pressure. *Sustainability* **2023**, *15*, 11366. https://doi.org/10.3390/su151411366

Academic Editor: Surendra Singh Bargali

Received: 12 May 2023
Revised: 18 July 2023
Accepted: 19 July 2023
Published: 21 July 2023

1. Introduction

Microbial communities play an essential role in ecosystems. Microorganisms are extremely important in supporting soil health as they are key factors in many processes [1,2]. Microorganisms and their distribution in the soil significantly affect the functions of soil ecosystems. As a result of the soil microbiome managing biogeochemical cycles, macro- and micronutrients such as copper, iron, nitrogen, and carbon, as well as other essential components for the survival of both plants and animals, have a major impact on climate change and plant and soil health [3]. It is crucial to comprehend the trends and forces influencing bacterial diversity, in order to understand how ecological systems of soil behave to perturbations in environmental conditions. The breakdown of organic matter, the maintenance of soil structure, carbon mineralization, and other processes belong to the functions of the soil microorganism communities [4]. These essential microorganisms activities can easily be impacted by human activities transforming soil chemical properties and interfere with soil bacterial communities both directly and indirectly [5]. Furthermore, changes in the dominant bacterial taxa and their interactions with other soil bacterial

communities also alter the structure, function, and stability of microbial ecosystems [6]. Significant changes in soil bacterial communities' composition and functioning due to different intensity anthropogenic activity were associated with changes in soil pH and nutrient availability [7].

The soil environment can change the structure and abundance of microorganisms, and associations among bacterial populations and the soil environment are able to be reversed. Bacterial diversity is also related to physical and chemical soil properties [8,9]. Different land use types and management intensities indirectly or directly influence the soil bulk density and alter changes in soil chemical characteristics and increase soil [10]. The microbial community has been regarded as a key indicator for determining how agricultural practices affect soil quality and soil ecosystem services as well, because changes in their community structures are correlated with changes in the soil quality. Although there are many soil characteristics that affect soil quality, biological processes are the most delicate and important ones [11]. Because soil organisms are the main drivers of organic matter decomposition, nitrogen cycling, soil fertility, and soil quality, the maintenance of a high level of microbial diversity in the soil is of the utmost significance for sustainable agriculture [12–14]. The amendment of organic fertilizers can also have an impact on the population, content, and activity of soil microorganisms even though their main purpose is to increase the availability of nutrients to plants [15]. In soil, there exist enormous numbers of soil microbiota which have an immense cumulative mass and activity. The understanding of the causal connections between changes in microbe communities and soil ecosystem services is limited, but based on the studies, we can predict that a greater variety of microorganisms communities might indicate improved soil quality [16].

In recent years, research on soil microorganisms has received considerable attention [17–22]. The structure of soil microorganism communities is particularly essential for soil ecosystems to function at maximum efficiency [23–25], where soils characterized by a high abundance and diversity of microorganisms are considered to provide the needed ecosystem services [26,27]. Studies conducted by many scientists have identified increasing soil functional diversity and microbiome complexity as one of the mechanisms by which organic amendments contribute to soil health [28–32]. However, it is often unclear whether organic amendments have consistent or long-term effects on soil microbial communities, restricting our capacity to foresee how soil conditioners may affect different types of soils' microbial populations. Long-term studies of the soil microbiome have revealed a lot of knowledge about its diversity. Metagenomics and high-throughput sequencing studies of the soil microbiome have revealed new information about the phylogenetic structure of soil microbial communities and opened new perspectives on soil microbial diversity and functioning. However, despite ongoing research, soil microbial diversity is underestimated. The role of different microbial species in the biochemical cycles that significantly influence the responses of the soil ecosystem depending on situations in the environment has not been sufficiently studied and evaluated [33]. In addition, metagenomic studies conducted during this research will allow a more detailed assessment of the role of microorganisms in the soil ecosystem functioning under different management intensity, which is very important now that the climate is changing. Furthermore, soil microbiome research will enable soil fertility assessment and sustainable agricultural development to realistically predict how soil ecosystems will respond to anthropogenic environmental changes.

The aims of this study were (1) to identify bacterial diversity in ecosystems which have diverse anthropogenic activities; (2) to identify how anthropogenic activities affect bacterial diversity; (3) and to verify how different physical and chemical properties in soil affect soil microorganisms' physiological groups in differently formed agricultural ecosystems. These aims were reached by evaluating the main physical and chemical soil properties and their relationship with soil microorganism groups as well as employing next generation sequencing (NGS) analysis to identify all bacterial diversity in the analyzed soils. This concept allows us to better understand the anthropogenic activity effect in different types of soil.

2. Materials and Methods

2.1. Experimental Soil Samples and Properties

Soil samples for analysis were collected from 2018 to 2020 during the plant vegetation period: for chemical parameters in spring, when plant vegetation emerges. Samples for physical analysis were taken two times: firstly, in spring, when plant growth begins, and, secondly, in the autumn, (after harvest). The coordinates of the soil sampling places are presented in Table 1 and marked in Figure 1. The selected sites are in two agri-pedological different places. Sites marked as 1, 2, 3, and 6 soil types are *Dystric Bathygleyic Glossic Retisol* with a texture of moraine loam (clay content 13–15%). Sites 4 and 5—*Endocalcari—Epihypogleyic Cambisol* have a texture of light loam. All sampling places were evaluated according to the intensity of anthropogenic activities (0 score to low anthropogenic activity places, 3 to high anthropogenic activity places). Place 6 was picked as a control treatment, because there is spontaneous birch approximately 25 years old, so there is no anthropogenic activity. According to anthropogenic activity all sampling places were ranked from 0 (no anthropogenic activity) to 3 points (high anthropogenic activity) (Table 1).

Figure 1. Soil sampling locations of the study.

The description of general soil characteristics is presented in Table 1.

Table 1. General characteristics of sampling places (mean ± standard deviation).

Sampling Place No.	Coordinates	Type of Soil	pH_{KCl}	C_{org}	N_{sum}	Main Applicable Agri-Technics	Intensity of Anthropogenic Activities
1.	N 55°41′, E 21°29′ (Vėžaičiai, Klaipėda dist.)	*Bathygleyic Dystric Glossic Retisol*	3.97 ± 0.1	1.32 ± 0.04	0.11 ± 0.003	traditional tillage, no liming, every year NPK	1
2.	N 55°41′, E 21°29′ (Vėžaičiai, Klaipėda dist.)	*Bathygleyic Dystric Glossic Retisol*	6.75 ± 0.2	1.30 ± 0.06	0.12 ± 0.01	periodical liming ×2.0 rate every 3–4 years, conventional tillage, every year NPK	2
3.	N 55°43′, E 21°27′ (Vėžaičiai, Klaipėda dist.)	*Bathygleyic Dystric Glossic Retisol*	5.71 ± 0.1	1.55 ± 0.15	0.15 ± 0.01	periodical liming using 1.0 of the liming rate and 60 t ha^{-1} FYM (farmyard manure) every 5 years, traditional tillage, every year NPK	3
4.	N 55°38′, E 23°86′ (Dotnuva, Kėdainiai dist.)	*Endocalcari-Epihypogleyic Cambisols*	6.93 ± 0.1	1.38 ± 0.18	0.16 ± 0.01	conventional tillage, no liming, every year NPK	1
5.	N 55°38′, E 23°86′ (Dotnuva, Kėdainiai dist.)	*Endocalcari-Epihypogleyic Cambisols*	7.1 ± 0.2	1.19 ± 0.13	0.15 ± 0.01	conventional tillage, no liming, every year NPK + cover crop management	3
6.	N 55°41′, E 21°30′ (Vėžaičiai, Klaipėda dist.)	*Bathygleyic Distric Glossic Retisol*	4.35 ± 0.2	1.08 ± 0.13	0.08. ± 0.01	immature spontaneous forest (~25 years birch)	0

2.2. The Investigation of Soil Microorganisms

The abundance of individual physiological groups of soil microorganisms is evaluated by the method of agar plates seeding of natural moisture soil suspension, and counting the colony forming units (CFU) per gram of completely dry soil. Soil samples for analyses were taken from a 0 cm to 20 cm upper soil layer, three times a year: in spring (when the plant vegetation begins), in the middle of summer (in approximately the middle of June) and in the autumn (after plant harvest). According to the soil, the suspension dilution method was determined: (1) the number of colonies forming units of ammonifying soil microorganisms (contamination on the plates of the protein medium, with peptone, agar (Thermo Scientific™, Vilnius, Lithuania); (2) the number of colonies forming units of soil actinomycetes (contamination on the plates of the protein medium, with peptone, agar (Thermo Scientific™); (3) the number of colonies forming units of soil micromycetes (contamination on the plates of acid beer mush agar (Thermo Scientific™); (4) the number of colonies forming units of nitrogen-fixing bacteria (contaminating on the plates of Noris agar (Thermo Scientific™).

Mixed soil samples have been gathered from a layer of arable soil (0–20 cm) in the autumn of 2020 for the metagenomic examination of soil microorganisms. Six soil samples were used to extract total genomic soil DNA using the ZR Soil Microbe DNA MiniPrepTM (50) (Zymo Research, Irvine, CA, USA), using a DNA extraction kit as directed by the manufacturer. In the "BaseClear" company, the metagenomics analysis had been carried out. Illumina MiSeq platform's next generation sequencing, which is based on 16S, was the method employed for the analysis. Using sequence overlap and USEARCH version 9.2, paired-end sequence reads have been compressed into so-called pseudoreads. When defining these pseudoreads, bacteria are assigned to a category using the RDP database [34] and fungi are identified using the UNITEITS gene database [35], based on the findings of alignment with SNAP version 1.0.23 [36].

2.3. Investigation of Soil Chemical and Physical Properties

Seeking to analyse topsoil chemical parameters, a steel auger was used to collect three replicate samples to 20 cm depth. After each sample was air-dried, visible plant remains and roots were physically excluded. Then, the soil was squashed and sifted through a 2-mm diameter sieve and homogeneously intermingled. Chemical soil characteristics were performed at the Agrochemical Research Laboratory of LAMMC. The following methods were applied for the determination of soil chemical characteristics: soil pH—ISO 10390:2005; SOC—ISO 10694:1995; soil total nitrogen (N_{sum})-Kjeldahl method (ISO 11261:1995); mobile aluminum—ISO 14254:2018; and plant-available phosphorus (P_2O_5), as well as potassium (K_2O) using the Egner–Riehm–Domingo (A-L) method (LVP D-07). The soil bulk density was determined by the Kaczynski method (ISO 11272:2017) in three replicates.

2.4. Meteorological Conditions

The humidity and temperature conditions varied throughout the research, according to the analysis of weather data collected during the plant's growing season (Table 2).

Lithuania is situated in the northern part of the moderate climate zone. The country is small compared to its area, but the climatic conditions of the research years were strictly different. The total amount of precipitation during the research period was less than the long-term mean, according to an analysis of data on the amount of rainfall during the plant growing season (Table 2). The exception was in 2020 in Dotnuva, where the overall precipitation was higher compared to the long-term mean; because of the July rainy period—rainfall amount was higher 2.5 times compared to the long-term mean. The average monthly air temperature was higher compared to the long-term mean during the research period. Data fixed in the Vėžaičiai meteorological station were higher by 1.4 °C and in Dotnuva by 0.4 °C, compared to the long-term mean.

Table 2. Rainfall and air temperatures in 2018–2020.

Month	Total Monthly Rainfall mm								Average Monthly Air Temperature °C							
	Year						Long-Term Mean 1981–2010		Year						Long-Term Mean 1981–2010	
	2018		2019		2020				2018		2019		2020			
	Vėž	Dot	Vėž	Dot	Vėž	Dot	Vėž	Dot	Vėž	Dot	Vėž	Dot	Vėž	Dot	Vėž	Dot
March	25.5	17.3	85.9	37.8	54.5	31.7	57.3	34.8	−1.8	−1.9	2.7	3.3	3.2	3.5	0.6	1.9
April	47.9	52.1	1.4	0.0	6.9	9.4	37.4	31.1	8.8	9.9	8.9	8.9	6.3	6.8	6.3	8.2
May	38.8	40.2	78.0	55.4	26.9	50.1	47.7	48.8	16.5	16.9	11.5	12.9	10.0	10.6	11.7	13.8
June	35.5	34.1	37.9	16.1	90.6	165.9	71.1	63.9	16.3	17.5	19.1	20.6	17.8	18.9	14.7	17.0
July	61.2	83.3	99.4	66.0	80.9	65.6	84.3	75.9	20.1	20.5	16.5	17.3	16.4	17.4	17.3	18.0
August	67.7	37.0	29.8	107.0	74.4	47.7	97.0	62.6	18.7	19.5	17.5	18.2	18.2	18.5	16.8	18.0
September	92.2	19.1	104.0	48.5	48.6	14.8	98.7	44.6	14.4	15.0	12.9	12.8	14.7	15.0	12.2	14.2
Overall	368.8	283.1	436.4	330.8	382.8	385.2	493.5	361.7	13.3	13.9	12.7	13.4	12.4	12.9	11.4	13.0

Note: Vėž—Vėžaičiai meteorological station, Dot—Dotnuva meteorological station.

2.5. Statistical Analysis

The differences in the investigated parameters between the treatments were estimated using one-way analysis of variance (ANOVA) from the Vegan package of the R programme 2.6-4 with the data on microbial abundance being presented as mean and a standard error of the mean. The significance of differences between treatment means was examined using the least significant difference method (LSD) at the 5% and 1% probability levels [14]. Based on the quantity of operation taxonomy units (OTUs), the taxonomic diversity of microorganisms was determined. The Chao1 and Shannon alpha diversity indices were employed to evaluate the composition of the soil microbial community. The Shannon index determines the diversity of species in a given community, whereas the Chao1 index evaluates the abundance of species.

3. Results and Discussion

3.1. The Variation of Soil Chemical Properties under Different Anthropogenic Intensity

Because of the many different characteristics that define it and how they relate to the determinants of land use, soil quality is a complex factor in the management of agricultural ecosystems [14]. Concerning applied agri-techniques related to the chemical properties variation, soil organic carbon (SOC) and nitrogen (N) takes an essential function in preserving soil fertility. Changes in soil chemical characteristics play a critical role in maintaining the soil microbial community and biodiversity. Intensity of anthropogenic activities, with the aim to optimize soil C and N cycles can work in two different ways: (1) applying sustainable, optimal agricultural measures can improve soil productivity and reduce negative environmental impacts, or (2) in the presence of excessive, unbalanced soil anthropogenic load, applied measures can disrupt nutrient cycles and thus promote soil degradation [37]. Six treatments from two soils with different anthropogenic intensity possessed various physical and chemical characteristics. The most intensively managed treatment in *Retisol*, where executed anthropogenic activity was ranked for 3 points, increased the soil pH_{KCl}, total nitrogen and plant-available phosphorus content, as well as being a crucial factor affecting SOC content (Figure 2). Limed *Retisol* with added farmyard manure (FYM), where the most intensive anthropogenic activity was carried out, resulted in the highest amount of organic carbon (1.59% of SOC). SOC content in this treatment was 0.33% higher compared to the naturally acid soil, in which the intensity of anthropogenic activity was ranked as 1. The much more labile carbon sources that come from manure can be employed to clarify these results. The incorporation of FYM with a combination of liming might have a greater positive effect on SOC accumulation compared to manure application only because it stimulates not only labile carbon accumulation but also recalcitrant carbon too [38]. Opposite results were obtained in other tested soil, such as *Cambisol*, where the intensification of anthropogenic activities led to a decrease in SOC content. Comparing the results under different management intensity in this type of soil, it was determined that

the SOC content decreased by 0.12 percentage points under the cultivation of cover crops, which was assigned as intensive anthropogenic activity. Growing cover crops encourages the flow of atmospheric carbon to the soil and actively contributes to the stability of SOC by supplying organic carbon derived from plant residues [39,40]. Many soil intensification studies found that cover crops can effectively increase total SOC and promote C sequestration. But the research data of our study are contrary. The results confirm the statements of Vicente-Vicente and colleagues [41], who found that the first years of cover crops growing induced the increase of non-protected SOC fraction, which could be easily decomposed by microorganisms resulting in a loss of total SOC content. They concluded that catch crop incorporation could increase SOC content, but it will be influenced by soil microbial communities. According to other scientists, the overall effect of cover crops on SOC could be seen after 3–5 years of growing [42,43].

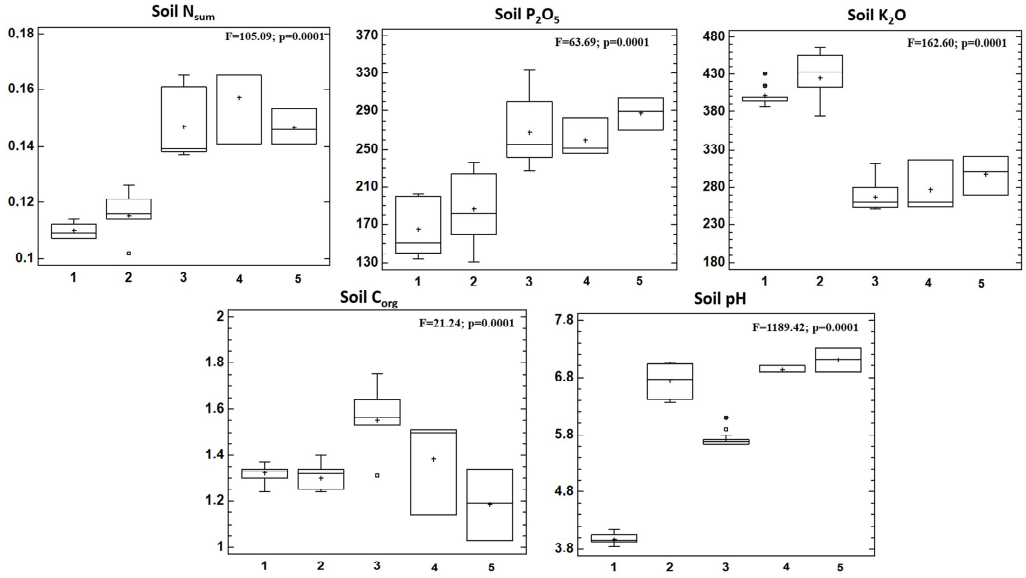

Figure 2. The variation of soil chemical properties under different anthropogenic intensity: 1—naturally acid *Retisol*, 2—limed *Retisol*, 3—limed *Retisol* with additionally added farmyard manure, 4—unlimed *Cambisol*, 5—unlimed *Cambisol* with cover crops. Note: N_{sum}—total nitrogen, P_2O_5 and K_2O—plant available phosphorus and potassium, C_{org}—soil organic carbon. Error bars in the boxplot indicate the standard deviation of the mean.

Intensive soil management strongly affected the nutrient amount (nitrogen and plant-available phosphorus and potassium) in the soil. The most intensive anthropogenic activities, applied in *Retisol*, were equal in impact to the least active activities applied in *Cambisol*. These differences could be explained by the differences of the parent material and sorption properties. In *Cambisol*, more clay particles are found in the upper layers, creating favorable conditions for accumulation and retention of moisture. This means that SOC can accumulate, and due to geochemical barriers, both phosphorus and nitrogen are retained. Differently, in *Retisol* due to the low amount of clay particles and carbonates, which causes the environment without carbonates to become more acid in nature, the absence of clay particles makes it difficult to accumulate and retain moisture, and at the same time does not accumulate SOC and nutrients. Moreover, due to the different meteorologic conditions (higher precipitation amounts in west Lithuania), the accumulated substances are washed out faster and are not retained.

Management practices such as cover crops growing and fertilization (manure) application have an essential effect on the accumulation of N, plant-available phosphorus and potassium. Intensive soil management (additionally added farmyard manure and cover crops growing) promoted the increase of the content of N in both types of soil. It could be largely explained by N storage by providing an extra available N [44]. Additionally,

it is conceivable that a higher overall N content would encourage the microbial breakdown of labile organic materials, in this way resulting in a decrease of SOC in *Cambisol* with intensive management (cover crops) treatment. The distribution of soil nitrogen and P_2O_5 content paralleled that of SOC since other nutrients, including nitrogen, are synthesized from organic material, and are released incrementally throughout mineralization. Plant-available phosphorus (P_2O_5) content was greatly increased by intensive management—additionally added farmyard manure and cover crop growth (334 g kg^{-1} and 287.67 g kg^{-1}, respectively). It is also anticipated that the addition of plant residues to the soils will have influenced the formation of SOC and the total nitrogen in this soil. Based on the data received, it could be stated that intensive anthropogenic activities are crucial for increasing the nutrient amount in the soil, because in undisturbed, especially naturally acid, soil at low pH, most of the nutrients are "locked" in the soil and are not available to plants [45]. This statement is also confirmed by comparing data from immature spontaneous forest (~25 years birch), in which all the investigated chemical indicators were lower compared to those treatments in which anthropogenic activities were carried out.

3.2. The Effect of Intensive Soil Management on Physical Properties

Physical and chemical soil characteristics and microbiological parameters can be used as indicators of soil health due to their quick response to management and soil environmental changes, understanding the connection between soil characteristics and microorganisms under various anthropogenic activity intensities is crucial [46–49]. Even a small change in the soil's physical properties, such as bulk density or moisture are well-known to be important factors for soil microorganisms and can cause various changes in microbial populations [50,51]. Parent material, climate, and anthropogenic activity mostly affect not only a soil's physical properties, but also influence the soil's bacterial community [52,53]. Soil compaction is a "hidden" threat, not only for soil quality, but also for the abundance of microorganisms. It occurs belowground and affects root morphological traits whereas microbial communities are also affected as there are dependent on soil properties and root performance [54]. The optimal value of soil bulk density for plants growing is <1.4 Mg m^{-3}, the critical value of the bulk density restricting root growth is 1.6 g cm^{-3} for clay loam and 1.63 g cm^{-3} for sandy loam and loam soils [55]. The result of our experiment reveals, as we expected, that physical conditions in different sampling places were different and were affected by anthropogenic pressure. The same tendency can be reported for total and air-filled porosity (Figure 3). This tendency mostly can be explained by parent material properties [55].

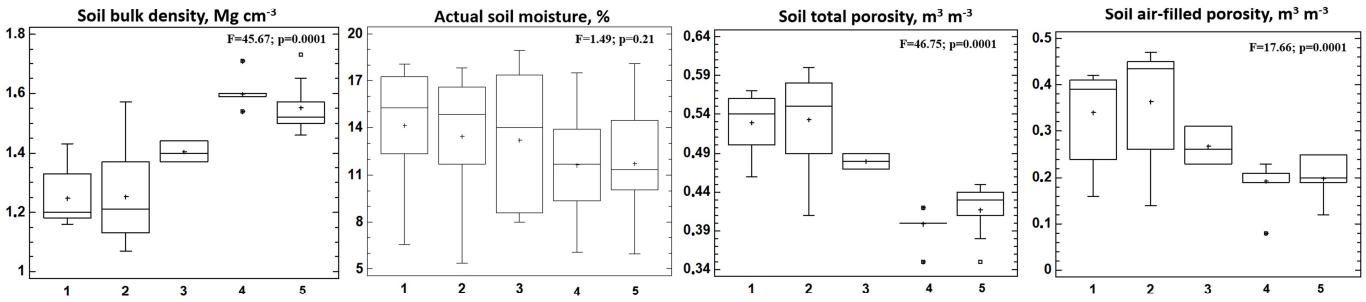

Figure 3. The variation of physical properties under different anthropogenic intensity: 1—naturally acid *Retisol*, 2—limed *Retisol*, 3—limed *Retisol* with additionally added farmyard manure, 4—unlimed *Cambisol*, 5—unlimed *Cambisol* with cover crops. Note: The boxplot's error bars show the mean's standard deviation.

Overall, different anthropogenic intensity had no statistically significant effect in the current experiments, because soil samples were collected from the 1 and 4 locations where tillage was the same but parent material and climate differed.

3.3. The Assessment of the Richness of Soil Microorganisms' Physiological Groups

Soil regulatory services including nutrient cycling, the biological control of pests and diseases, carbon storage, and the regulation of greenhouse gas emissions, are controlled by microorganisms that play a key part in each of these functions [56,57]. The relative abundance of the experiment soil microorganisms' physiological groups is presented in Figure 4.

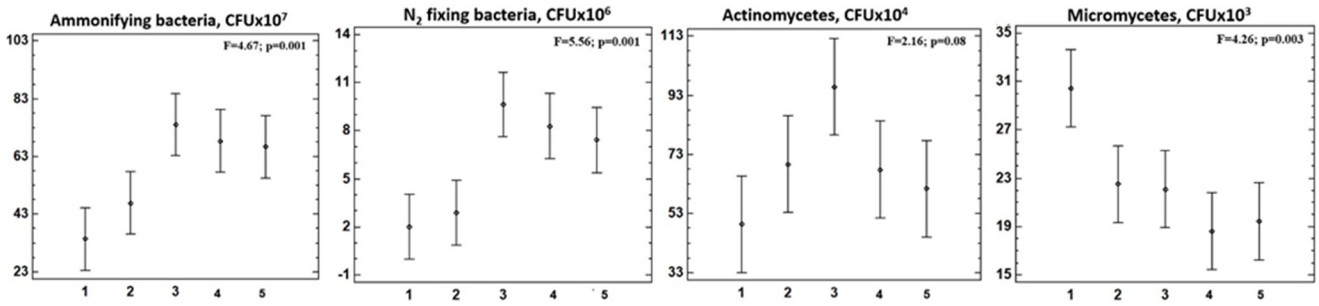

Figure 4. The richness of soil microorganisms' physiological groups in different agroecosystems: 1—naturally acid *Retisol*, 2—limed *Retisol*, 3—limed *Retisol* with additionally added farmyard manure, 4—unlimed *Cambisol*, 5—unlimed *Cambisol* with cover crops. Note: Mean ± standard deviation.

The results indicate that different anthropogenic pressures have altered soil microorganisms' physiological group. The statistically significant highest rate of ammonifying, nitrogen-fixing bacteria and actinomycetes was determined in *Retisol* (sampling place No. 3), where the most intensive anthropogenic activity was carried out (periodically liming and additionally added farmyard manure). According to the richness of the previously mentioned microorganisms' group, the second group was *Cambisol* (sampling places No. 4 and 5), but there were no statistical differences among the remaining sample locations. *Micromycetes* data shows the opposite situation. In the undisturbed *Retisol* where no anthropogenic activities were applied, the statistically significant highest richness of micromycetes was determined. Meanwhile, in the sampling places, where soil pH_{KCl} was higher than 6.4 (sampling places No. 3, 4, and 5), the micromycetes population was significantly lower. This trend can be related to soil pH changes, and it supports the finding that higher soil pH (in the pH range of 4–7) and greater bacterial diversity are positively correlated [58,59].

3.4. The Determination of Soil Microorganisms' Physiological Group Seasonal Changes

Soil is described as a habitat for biodiversity, but soil organisms can be described as the foundation of soil food webs as well as being responsible for plant biodiversity, and these roles can be referred to as soil regulation services [56,60–62]. It was analyzed how different microorganism physiological groups vary during the plant vegetation period in differently formed agroecosystems to identify what processes are active in the soil. Figure 5 presented the data that reveal differences between seasonal fluctuation in different sampling places. After analyzing the changes in the ammonifying bacteria population, two scenarios can be identified. In the *Retisol* (sampling places No. 1, 2, and 3), regardless of the intensity of anthropogenic activity, ammonifying bacteria population increased and reached its peak in autumn. Meanwhile, in *Cambisol* (sampling places No. 4 and 5), independently of management intensity, the peak was observed in spring, and the population was declining during the plant vegetation period.

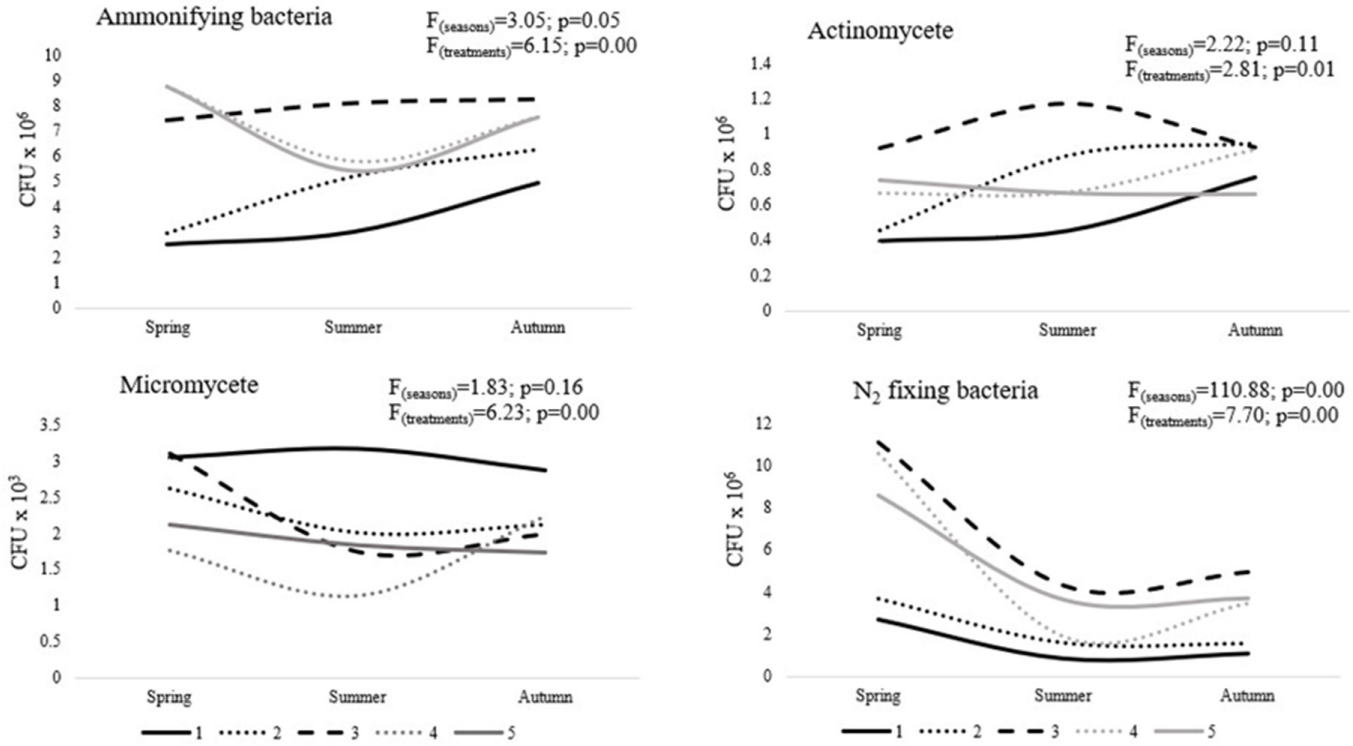

Figure 5. Variation of soil microorganisms' physiological groups in different agroecosystems during plant vegetation season: 1—naturally acid *Retisol*, 2—limed *Retisol*, 3—limed *Retisol* with additionally added farmyard manure, 4—unlimed *Cambisol*, 5—unlimed *Cambisol* with cover crops.

There were no statistical differences between the seasonal fluctuation of the actinomycetes population. In *Retisol*, whereas intensive anthropogenic activity was carried out (sampling place No. 3), the population was highest; meanwhile, in naturally acidic *Retisol* without anthropogenic activities, the actinomycetes population was lowest. A similar amount of micromycetes was determined in the spring and autumn periods. Except in summertime, the decline of the richness of this microorganism's group was seen. An exception is the naturally acid *Retisol* and *Cambisols* (sampling places No. 1 and 5) without anthropogenic activities, where during almost the entire plant vegetation period, the same level of micromycetes population was found.

The nitrogen-fixing bacteria population peak was determined in spring. Despite differences between the sampling places, this tendency can be explained by the morphological cycle of nitrogen-fixing bacteria because they are the most active (highest nitrogenase activity) in the plant flowering stage [63–66].

As one of the study aims was to verify how different soil physical and chemical properties affect soil microorganisms' physiological groups in differently formed agricultural ecosystems. The relation between the mentioned parameters is reflected in the principal component analysis biplot (Figure 6).

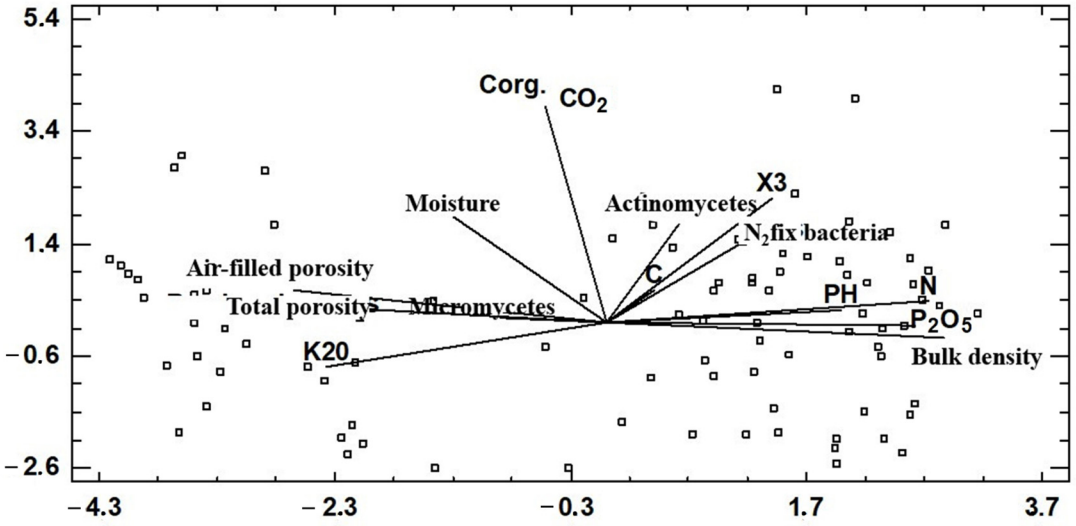

Figure 6. Principal component analysis (PCA) of soil microorganism physiological group fluctuation based on different soil properties.

Different soil microorganisms' physiological groups correlated with the analyzed soil properties, e.g., atmospheric nitrogen-fixing bacteria have a positive correlation with soil pH, N_{sum}, P_2O_5, and soil bulk density; meanwhile, soil porosity and K_2O negatively affect the population of atmospheric nitrogen-fixing bacteria. The same tendencies are inherent to actinomycetes and ammonifying bacteria. Whereas micromycetes have a negative correlation with soil pH value, results show that in soils with lower pH values a higher abundance of micromycetes (Figure 4) and plant-available phosphorus content were detected. This also positively correlates with soil moisture, porosity, and potassium content.

3.5. The Determination of Soil Microorbial Diversity and Abundance under Different Anthropogenic Pressure

The next generation sequencing method is designed to estimate total microbial biodiversity, not only its active fraction. With the advancement of next generation sequencing techniques, microbial life-history traits have been widely applied in various ecosystems [67]. However, microbial activity level and taxonomic diversity are not always interrelated, because only 0.1 to 0.2% are identified as active microorganisms [68]. Data in Figure 7 represented the relative abundance of the five most common bacterial species in our analyzed soil samples. Two major bacterial species predominated in the bacterial communities, according to an analysis of the taxonomic diversity of soil microbes: *Betaproteobacterium* and *Candidatus Saccharibacteria.* The highest number of *Betaproteobacterium* was in the limed *Retisol* with additionally added FYM (sampling place No. 3) (25.84%). The amount of *Candidatus Saccharibacteria* was 28.66% in naturally acid *Retisol* with silver birch stand (sampling place No 6). The most common species were the same in the analyzed places, but there were two species which were common to different soil groups: bacteria species *Holophaga* was identified only in *Retisol*; meanwhile delta proteobacteria was found only in *Cambisol*.

Moreover, one of our research goals was to identify how anthropogenic activity affects bacterial diversity in differently affected ecosystems. Hierarchical cluster analysis results, presented in Figure 8, were not very distinguishable, revealing the bacterial diversity that was most similar in the same group soils (*Cambisol*), but providing the interesting results in that third place, according to similarity, the was the periodically limed *Retisol* with additionally added FYM (sampling location No. 3), and fourth was the naturally acid *Retisol* with immature spontaneous forest (~25 years birch).

Figure 7. Relative abundance of most common bacteria species in analyzed soil samples.

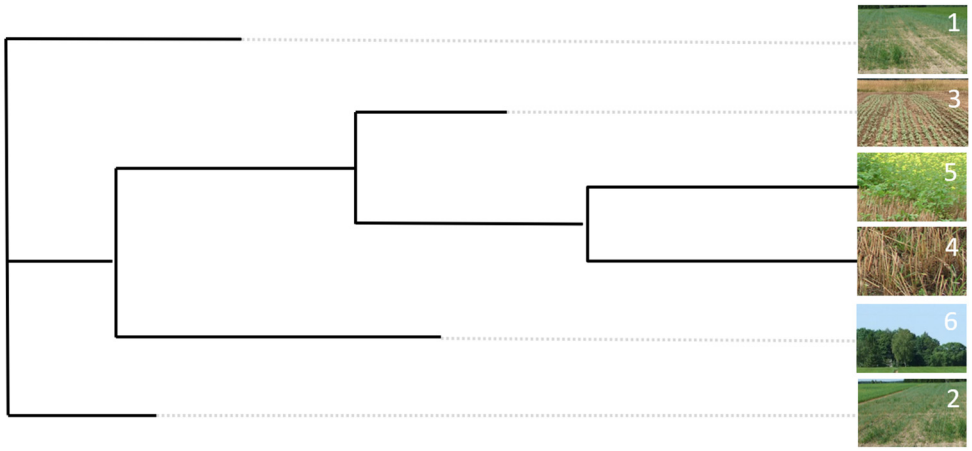

Figure 8. Hierarchical cluster analysis results of bacteria species in analyzed soil samples: 1—naturally acid *Retisol*, 2—limed *Retisol*, 3—limed *Retisol* with additionally added farmyard manure, 4—unlimed *Cambisol*, 5—unlimed *Cambisol* with cover crops, 6—naturally acid *Retisol* with immature spontaneous forest (~25 years birch).

4. Conclusions

Soil parent material and climate are the main drivers affecting a soil's physical, chemical, and microbiological properties and bacterial diversity. Our research results show, that natural forests can restore and improve soil bacterial diversity. Bacterial identification shows that the main bacterial species were the same in all analyzed sampling locations despite the anthropogenic activities, parent material, or other abiotic conditions. Only a few species were identified in different soil groups; it may be an assumption that those groups could be potential bioindicators for specific types of soil, but there is a need for

more detailed research for the confirmation of this hypothesis. However, there is still a lack of knowledge; hence, in future studies different soil groups should be analyzed to get a broader view of soil microbial diversity.

Author Contributions: Conceptualization, I.M. and M.V.; methodology, M.V., I.M., D.K., G.K., O.A. and R.R.; software, M.V. and I.M.; validation, I.M. and M.V.; formal analysis, I.M. and M.V.; investigation, I.M. and M.V.; resources, D.K.; data curation, M.V.; writing—original draft preparation, M.V.; writing—review and editing, I.M.; visualization, M.V.; supervision, D.K.; project administration, M.V.; funding acquisition, M.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data used to support the findings of this study will be available from the corresponding authors upon request.

Conflicts of Interest: The authors declare no conflict of interest.

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