sustainability

MDPI

Article

Biological Indicators of Soil Quality under Different Tillage Systems in *Retisol*

Monika Vilkiene [1,*], Ieva Mockeviciene [1], Danute Karcauskiene [1], Skaidre Suproniene [2], Modupe Olufemi Doyeni [2] and Dalia Ambrazaitiene [1]

[1] Vezaiciai Branch, Research Centre for Agriculture and Forestry, Institute of Agriculture, 96216 Vezaiciai, Lithuania; ieva.mockeviciene@lammc.lt (I.M.); danute.karcauskiene@lammc.lt (D.K.); dalia.ambrazaitiene@gmail.com (D.A.)

[2] Lithuanian Research Centre for Agriculture and Forestry, Institute of Agriculture, Akademija, 58344 Kėdainiai, Lithuania; skaidre.suproniene@lammc.lt (S.S.); modupe.doyeni@lammc.lt (M.O.D.)

* Correspondence: monika.vilkiene@lammc.lt; Tel.: +370-622-64633

Abstract: Soil microorganism diversity has a close relation with soil function, and the changes in the composition of the soil microbial population can directly affect it. The aim of this study was to identify the bacterial community composition and determine the main soil chemical and physical properties formed by the different tillage systems. In the experiment, we analyzed the combination of three tillage systems and four organic fertilizers. Soil samples were taken from the two layers of the soil profile: the upper 0–10 cm and the lower 10–20 cm. The composition and diversity of soil bacterial communities were assessed by the sequencing of 16S rRNA genes. Results revealed that the highest biodiversity was found in the soil with shallow ploughless tillage and enriched with farmyard manure. *Actinobacteria* and *Proteobacteria* were the dominant bacterial species across all treatments. Their total abundance varied between 26% and 36% in the different analyzed agroecosystems. For the *Dystric Bathygleyic Glossic Retisol*, shallow ploughless tillage is the most suitable tillage system, as it creates favorable conditions for the accumulation of organic carbon in the soil under the Western Lithuania climate conditions.

Keywords: bacterial communities; biodiversity; bio-indices; 16S rRNA sequencing; *Dystric Glossic Retisol*

Citation: Vilkiene, M.; Mockeviciene, I.; Karcauskiene, D.; Suproniene, S.; Doyeni, M.O.; Ambrazaitiene, D. Biological Indicators of Soil Quality under Different Tillage Systems in *Retisol*. *Sustainability* **2021**, *13*, 9624. https://doi.org/10.3390/su13179624

Academic Editor: Helvi Heinonen-Tanski

Received: 20 July 2021
Accepted: 24 August 2021
Published: 26 August 2021

1. Introduction

To meet increasing human food demands, scientists face new challenges that result from significant ongoing climate changes, which include mild winters, a prolonged vegetation period, extreme temperatures, and intense rain [1–3]. Another challenge is growing globalization, due to international cooperation, competition, and volatile markets amid limited land resources [4,5]. These factors encouraged the need to improve the current agricultural systems to increase their production volume and the quality of the same unit area. Soil is a non-renewable natural resource, which is characterized by high degradation level and extremely low regeneration capacity [6]; hence, anthropogenic activity has a negative impact on the stability and long-term productivity of the entire ecosystem [1]. Applying intense agricultural technology leads to a decrease in soil organic matter [7]. The regularity of organic material accumulation in short-term studies is very difficult to evaluate, as the main factors that can influence this process are climate, terrain, type of organic residues inputted, soil group, and its granulometric composition. Therefore, long-term studies are needed to assess this process [7,8].

One of the most important indicators of soil quality is the accumulation of a common biomass of microorganisms in the arable soil layer. The activity of soil microorganisms determines the ecosystem's stability, metabolism, and soil fertility. Microorganisms in the soil carry out an essential transformation of substances and maintain its fertility. Physical degradation in the soil reduces the number of microorganisms, slows down biochemical

processes, and leads to crop losses [9–11]. Based on research, we can say that a greater variety of microorganism communities may indicate better soil quality, but there is little research to accurately assess the regularities associated with changes in microorganism communities and processes in the soil ecosystem [11,12].

The analysis of literature sources showed that the accumulated research data on soil microorganisms and their activities did not reveal all the processes that happen over many years in the ecosystem. Therefore, we deal with them in just 1–2% of the total population [13–15]. However, during the last decade, the development of high biotechnology processes and its application to soil microorganism studies allowed for the identification of the complete genome of soil microorganisms and to explore their functions that determine the role and importance of various natural processes [16]. The resulting contribution from the knowledge gained provided an insight to the improvement of agrotechnical measures to improve and maximize yield with minimal impact on the environment [17–20].

In this study, we hypothesized that the consistency of organic carbon storage in soil is determined not only by the amount of organic matter added but also by its chemical composition, time of insertion, and the intensity of mineralization processes. The accumulation of organic carbon stock is influenced by agricultural methods and the physicochemical properties that have formed over a long period of time. It is likely that bioindicators can be found during the study, which will allow for the estimation of the organic matter mineralization intensity in the soil. Therefore, the goal of this study was to evaluate the regularities of soil organic carbon accumulation in the long-term application of agrotechniques and to determine the genetic diversity of microorganisms actively involved in the process of mineralization.

2. Materials and Methods

2.1. Site Location

The field experiments were carried out at the Vėžaičiai Branch of Lithuanian Research Centre for Agriculture and Forestry (geographical coordinates 55°43′38″ N, 21°27′43″ E) during the period between the years 2015 and 2019. The soil of the experimental site is *Dystric Bathygleyic Glossic Retisol* with a texture of moraine loam (clay content 13–15%) [21]. The chemical properties of the soil in the initial stage of the experiment were (Table 1): the soil pH range at the beginning of the experiment was 5.2–5.6, the total organic carbon range was 1.49–1.59%, the total nitrogen content range was 0.16–0.17%, there was moderate mobile phosphorus (140–204 mg kg^{-1} soil) content, and there was high mobile potassium (228–289 mg kg^{-1} soil).

Table 1. Characteristics of soil before the experiment conduction.

	Primary Soil Tillage		Organic Fertilizers			
	Deep Ploughing	Shallow Ploughless Tillage	Plant Residue	Straw	Green Manure 1st Cut	Manure 40 t ha^{-1}
pH_{KCl}	5.6	5.2 *	5.5	5.4	5.6	5.6
K_2O, mg kg^{-1}	289	228 *	260	271	265	283
P_2O_5, mg kg^{-1}	204	140 **	176	188	181	202 **
N_{total}, %	0.17	0.16 **	0.17	0.17	0.16 *	0.16 *
$C_{org.}$ %	1.59	1.46 **	1.56	1.58	1.54	1.53

Note: * and **—significant differences from control ($p < 0.05$) and ($p < 0.01$).

2.2. Meteorological Conditions

During the four years of the experiment, it was noted that there were only a few days with lower temperature, and it lasted for a short time. The average annual positive temperature of the experimental period was 9.5 °C, which was observed for almost the entire four years. The highest precipitation was observed in autumn (October–December), with higher values in December. In 2015 and 2017, it was particularly drizzly in July

and August, with precipitation levels of 135.3 and 179.6 mm, respectively. Such mild weather conditions had a particularly favorable effect on the activity of soil microorganisms. Under such meteorological conditions lasting the entire year, soil microorganisms could intensively degrade organic matter or participate in its transformation processes.

2.3. Experimental Design and Treatments

The experiment was designed to assess the influence of long-term tillage and organic fertilization on the intensity of mineralization. The experiment was set up in the year 2013 and had a split-plot design where the whole-plot treatments were laid out in a randomized design with three replicates. The whole-plot treatments consisted of three tillage methods—deep ploughing (22–25 cm) (DP), ploughless tillage (7–10 cm) (PT), and ploughless tillage (7–10 cm) with additional deep loosening (up to 40 cm), which was applied every 4 years (PTS). The split-plot treatments involved four types of additional organic fertilizers: stubble (S), chopped straw + N10 (ChS), chopped grass (1st cut) + N10 (G), and farmyard manure 40 t ha^{-1}(M). Soil samples were taken from the two layers of the soil profile: the upper 0–10 cm and the lower 10–20 cm. The experiment had a total of 36 plots (10 m × 5 m) with 12 different treatment combinations. The soil samples for metagenomic analysis were taken in October, in the year 2018, after harvesting.

2.4. The Evaluation of the Intensity of the Soil Organic Matter Mineralization

For the evaluation of soil organic matter mineralization intensity, rye straw (cellulose) samples were used. The straw was air-dried, chopped to the length from 3 to 5 cm, divided into 5 g stocks, and put in plastic net bags. The diameter of the plastic bag cells was 0.05 mm. The size of bags was 5 × 12 cm. The edges of the bags were soldered, the top was closed with metal fasteners, and the bags were sterilized (170 °C ± 3 °C, 4 h). Different color metal wires were added to the bags (the blue ones were put 7 cm deep, and orange/brown ones were put 20 cm deep). In the autumn of 2015, straw samples were buried in all experimental treatments (864 bags in total). For the evaluation of mineralization intensity, the bags were dug up at 3 different times as highlighted: (1) when the temperature of soil in 20 cm was lower than +5 °C for longer than 3 days; (2) in spring, when the temperature of soil in 20 cm was higher than +5 °C for longer than 3 days; (3) before harvesting. The experiment was repeated in 2015 and 2019.

The mineralization intensity was determined according to the weight method. From the dug up decomposed straw samples, the soil was carefully removed. Then, the samples were washed through with running water. After that, the samples were dried in the thermostat (40 °C) for about 12 h. Then, the samples were weighed and dried again for 20–30 min. They were weighed again, and if the mass was the same, it was fixed as real mass after decomposition. The mineralization intensity was estimated according to the first formula.

$$v = \frac{m_0 - m_1}{t} \quad (1)$$

where v—mineralization intensity over one day; m_0—the weight of decomposing samples (5 g); m_1—the weight of decomposed samples after digging (g); t—number of the days while the samples were buried. Only the mass of the straw (without the weight of the bag) was used for the evaluation of the mineralization intensity. Qualitative mineralization analysis (the concentration of lignin, celluloses, and hemicelluloses in decomposed straw samples) was carried out in the Chemical Research Laboratory of the Lithuanian Research Centre for Agriculture and Forestry.

2.5. Investigation of Soil Chemical and Physical Properties

After harvesting, soil samples were taken using a steel auger from the three replicates of the topsoil (0–20 cm). All samples were air-dried, and visible roots and plant residues were manually removed. Chemical analyses were carried out at the Agrochemical Research Laboratory of Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. Soil pH was determined in 1 M KCl according to the standard ISO 10390:2005

(soil/solution ratio 1:2.5) using a pH meter IONLAB. Soil organic carbon (%) content was determined according to ISO 10694:1995 using spectrophotometer UV/VIS Cary 50 Conc (Varian, Germany). Dissolved organic carbon (DOC) was analyzed using an ion chromatograph SKALAR (Skalar Analytical B.V., Breda, The Netherlands). The generated results (mg C/l) were recalculated into the g kg^{-1} of soil. Soil total nitrogen (N_{total}) was determined by the Kjeldahl method, and the plant-available phosphorus (P_2O_5) as well as potassium (K_2O) were determined by the A–L (Egner–Riehm–Domingo) method. All measurements were performed in three laboratory replicates.

2.6. Evaluation of Nutrients Transformation Processes

To assess the changes of soil nutrients in time, the relative annual change of 'X' nutrient content (g kg^{-1} per year) and nutrient sequestration potential (Mg ha^{-1}) were calculated in accordance with the following formula:

$$M = \left(\frac{C_t - C_0}{t} \right) \quad (2)$$

where, M is the annual change of 'X' nutrient content (g $kg^{-1}y^{-1}$); C_0 and C_t are the X nutrient contents of the initial and final year of the experiments, respectively; and t is the period of each experiment.

2.7. The Investigation of Soil Microorganisms

The prevalence of individual physiological groups of soil microorganisms is determined by the method of agar plates seeding of natural moisture soil suspension, calculating the number of colony-forming units (CFU) per gram of completely dry soil. Soil samples were taken from the selected variants of the fields of two arable soil layers: 0–10 cm and 10–20 cm. These samples were taken three times a year: in autumn, by burying cellulose bags; in spring, after the second bag digging; and in summer, after harvesting the last cellulose bag. By using the soil samples, the suspension dilution method was determined: (1) the number of colony-forming units of ammonifying soil microorganisms (isolation on the plates of the protein medium, with peptone agar; (2) the number of colony-forming units of soil actinomycetes (isolation on the plates of the protein medium, with peptone agar; (3) the number of colony-forming units of soil spore-forming bacteria (isolation on the plates of a mix of agar and neutral beer mush agar); (4) the number of colony-forming units of soil micromycetes (isolation on the plates of acid beer mush agar); (5) the number of colony-forming units of soil cellulolytic bacteria (isolation on the plates of Hutchinson agar).

2.8. The Metagenomic Analysis of Soil Microorganisms

For the extraction of soil microorganism DNA, soil samples were taken from an arable soil layer (0–20 cm) in the autumn of the year 2018 after harvesting (pooled sample from 3 replications). For the sampling, the treatments were selected based on the preliminary results of soil microorganisms' abundance and mineralization intensity. The soil microorganisms DNA was extracted from the samples of 4 treatments: (1) deep ploughing and chopped straw; (2) deep ploughing and farmyard manure; (3) shallow ploughless tillage and chopped straw; and (4) shallow ploughless tillage and farmyard manure.

The soil microorganisms' identification was done in the following steps. (1) For the extraction of soil microorganisms DNA, a D6005 Fungal/bacterial Miniprep kit (Zymo Research® Freiburg im Breisgau, Germany) was used. DNA from the soil samples was extracted 4 times according to the manufacturer protocol, except when the homogenization speed was modified. For optimal performance, beta-mercaptoethanol was added to the fungal/bacterial DNA Binding buffer to a final dilution 0.5% (v/v) i.e., 500 µL per 100 mL. (1.1). Then, 200 mg of tissue to ZR Bashing Bead™ Lysis Tube (Zymo Research® Freiburg im Breisgau, Germany) and 750 µL Lysis Solution was added. (1.2). Then, it was secured in a bead beater fitted with a 2 mL tube-holder assembly and processed at 6 ms^{-1}

speeds for 4 s. We repeated the cycle 2 times. (1.3). We centrifuged the ZR Bashin Bead™ Lysis Tube in a microcentrifuge at 10,000× *g* for 1 min. (1.4). Then, we transfered up to 400 µL supernatant to a Zymo-Spin™ IV Spin Filter in a Collection Tube and centrifuged at 7000 rpm (≈7000× *g*) for 1 min. (1.5). We added 1200 µL fungal/bacterial DNA Binding Buffer to the filtrated in the collection tube from step 1.4. (1.6). We transfered 800 µL of the mixture from step 1.5 to a Zymo-Spin™ II Column in a collection tube and centrifuged 10,000× *g* for 1 min. (1.7). We discarded the flow from the collection tube and repeated step 1.6. (1.8). We added 200 µL DNA Pre-Wash Buffer to the Zymo-Spin™ IIC column in a new collection tube and centrifuged at 10,000× *g* for 1 min. (1.9). We added 500 µL fungal/bacterial DNA Wash Buffer to the Zymo-Spin™ IIC column and centrifuged at 10,000× *g*, 1 min. (1.10). We transfered the Zymo-Spin™ IIC column to a clean 1.5 mL microcentrifuge tube and added 100 µL (minimum 25 µL) DNA Elution Buffer directly to the column matrix. Then, we centrifuged at 10,000× *g* for 1 min, after which the centrifugation was repeated for 30 s to elute the DNA. The elution was added to the replicated column and centrifuged again. The metagenomics analysis was done in "BaseClear" company (Leiden, The Netherlands). For analysis, next-generation sequencing based on 16S by an Illumina MiSeq platform was employed.

2.9. Statistical Analysis

Statistical analysis was done using the computer program ANOVA (analysis of variance) from the R program Vegan package [22]. Then, two-way analysis of variance was used to estimate the differences in the tested parameters among the treatments (chemical and physical soil parameters and soil microorganisms physiological group abundancy). The least significant difference method (LSD) at the 5% and 1% probability levels was used to test the significance of differences between treatment means. Biodiversity indexes were estimated by the Estimate 9.1.0 package [23].

3. Results

3.1. The Alternation of Soil Chemical and Physical Properties in Different Agroecosystems

For many years, diverse agroecosystems have developed through different tillage methods and the addition of organic matter. The different tillage methods affected the accumulation of chemical elements in the arable soil layers. In the deep ploughed soil, pH values were highest (5.53–5.58), with a higher content of plant available phosphorus (P_2O_5)—189.96 mg kg^{-1} (Table 2). Furthermore, in the shallow ploughless tillage, the lowest pH values were observed ranging from 5.16 to 5.25, while a higher content of mobile potassium (K_2O)—327.88 mg kg^{-1} and organic carbon (1.74%) were observed compared to the other treatments. The type of organic fertilizers embedded in the soil did not show statistically significant changes in the main biogenic elements in the soil.

Soil temperature data showed that the average annual soil temperature in the lower layer of arable soil was 0.31 °C higher than in the upper layer. The different tillage methods did not have a significant influence on soil temperature fluctuations. Soil bulk density is probably the most frequently measured soil quality parameter in tillage experiments. Over the course of the study, the deep ploughing and incorporation of organic fertilizers tended to increase the bulk density compared with the shallow ploughless tillage type. The lowest bulk density was observed in the shallow ploughless tillage with deep loosening. Shallow ploughless tillage incorporated with straw as an organic fertilizer significantly increased the soil moisture content. On the contrary, the incorporation of plant residue and manure (40 t ha^{-1}) had no effect on the moisture content. The soil porosity and organic matter content played critical roles in the biological productivity. Overall, shallow ploughless tillage with and without deep loosening produced the highest total porosity (49.01 and 49.57, respectively), while deep ploughing and the incorporation of organic fertilizers showed lower total porosity.

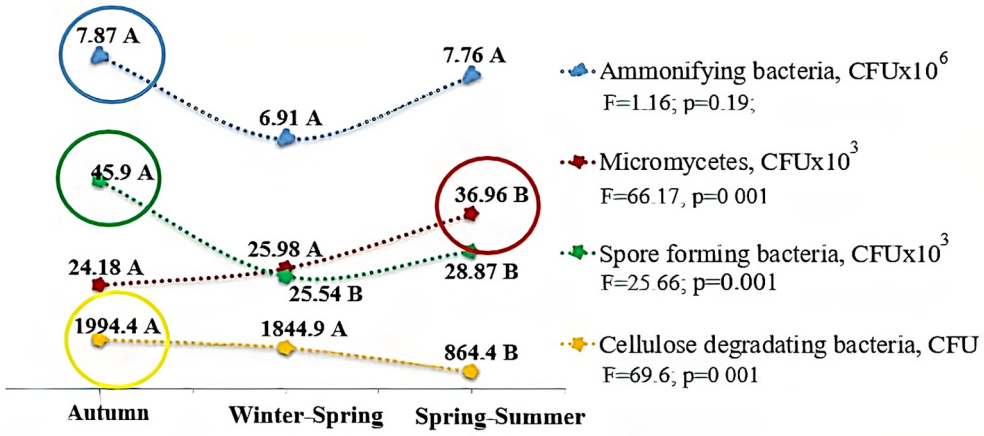
Table 2. Characteristics of soil samples (mean ± standard deviation).

Treatments	Primary Soil Tillage			Organic Fertilizers			
	Deep Ploughing	Shallow Ploughless Tillage	Shallow Ploughless Tillage + Deep Loosening	Plant Residue	Straw	Green Manure 1st Cut	Manure 40 t ha^{-1}
pH	5.51 ± 0.2	5.25 ± 0.3	5.37 ± 0.2	5.34 ± 0.3	5.43 ± 0.2	5.34 ± 0.2	5.39 ± 0.2
K_2O, mg kg^{-1}	284.25 ± 82	327.88 ± 116	273.58 ± 59	284.17 ± 110	294.78 ± 59	304.78 ± 103	300.22 ± 90
P_2O_5, mg kg^{-1}	189.96 ± 36	174.17 ± 55	136.92 ± 36	171.50 ± 44	169.50 ± 51	153.17 ± 43	175.22 ± 56
N_{total}, %	0.13 ± 0.01	0.14 ± 0.02	0.14 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.14 ± 0.02	0.14 ± 0.01
Corg., %	1.59 ± 0.2	1.74 ± 0.2	1.47 ± 0.2	1.66 ± 0.2	1.53 ± 0.3	1.64 ± 0.2	1.59 ± 0.3
Moisture, %	18.85 ± 0.9	20.49 ± 0.9	19.11 ± 1.3	18.83 ± 0.7	20.06 ± 1.3	19.79 ± 1.3	19.12 ± 1.1
Porosity, %	47.88 ± 6.6	49.01 ± 3.1	49.57 ± 3.5	47.51 ± 7.7	47.97 ± 1.9	51.19 ± 3.0	48.79 ± 3.3
Bulk density, Mg m^{-3}	1.37 ± 0.07	1.35 ± 0.09	1.34 ± 0.09	1.36 ± 0.09	1.39 ± 0.06	1.30 ± 0.08	1.37 ± 0.09

3.2. The Dynamic of Soil Microorganism Physiological Groups

The experiment also paid attention to the abundance of individual physiological groups of soil microorganisms and their variation at separate stages of the study throughout the year. To determine the predominant processes in the soil, proper identification was made when each group of soil microorganisms reached their population peak within one year.

In Figure 1, we observed that ammonifying bacteria were found mainly in autumn—indicating that the representatives of this group are usually abundantly identified in October–December months, when there are many easily degradable organic compounds in the soil environment. In addition, spore-forming bacteria also reached their peak during this autumn period, although with no significant differences between the different microbial group. However, the third most popular group in this period, cellulose-degrading bacteria, did not reveal a significant difference between the representatives of this group in autumn and winter–spring periods. Micromycetes were mostly found in the spring–summer period and are significantly different from the ammonifying bacteria during this period.



* letters label significantly different between averages(P>0.005) for each treatment apart.
** circles notice the peak of every physiological group of soil microorganisms

Figure 1. The dynamic of different soil microorganism physiological groups (CFU × 10^n × 1 ga.d.s.) during the experiment (letters A and B identify significant differences between CFU numbers during the seasons).

3.3. Mineralization Intensity of Soil Organic Matter in Different Agroecosystems

According to the results of the experiment, the mineralization process of soil organic matter takes place all year round, but in *Dystrict Bathygleyic Glossic Retisol*, with the climatic conditions of Western Lithuania, it intensifies in the autumn (Figure 2). The mineralization process in the autumn was 99% more active compared to the winter period and there was

an almost 73% mineralization rate during the spring. The mineralization process test result revealed that the intensity of the process differs in various soil layers.

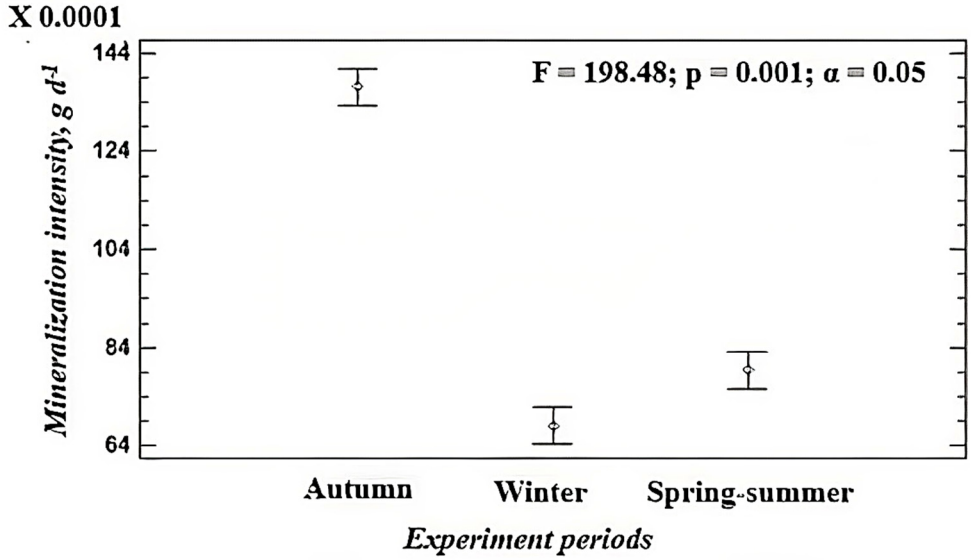


Figure 2. The variation of mineralization during seasons (avg. ± standard deviation).

Straw samples mineralized at a rate of 4.7% faster in the lower arable soil layer (10–20 cm) than the samples in the upper layer (0–10 cm). The different organic fertilizers did not have a significant influence on the intensity of the mineralization process. However, positive tendencies were noticed especially in the soils where freshly cut grass and farmyard manure were used with 8.3% of the samples mineralized faster compared to amendment with other organic fertilizers. Additionally, the different tillage methods did not have a significant influence on the rate of the mineralization process. However, it was observed that the mineralization process in the deep ploughed soil was slightly slower (2.2%) compared to the shallow ploughless tillage.

3.4. The Alternation of Soil Bacterial Community in Different Agroecosystems

For the next-generation sequencing, which was based on 16S RNA analyses from all the experiment scheme, we picked out four treatments: (1) deep ploughed soil with chopped straw; (2) deep ploughed soil with farmyard manure; (3) shallow ploughless soil with chopped straw; and (4) shallow ploughless soil with farmyard manure. We arrived at the decision to choose the two tillage methods because deep ploughing is the traditional soil tillage type, while the shallow ploughless tillage is a more environmentally friendly alternative. In selecting organic fertilizers, we followed the "no waste" technology logic with husbandry—where farmyard manure in the literature is widely described as organic fertilizer, and straw is a good alternative. The tillage type influenced the number of taxonomic units in the soil (Figure 3). In the deep ploughed soil with farmyard manure, the number of taxonomic units was higher (154,904 OTU); meanwhile, in the ploughless tillage, we can see a similar situation with a higher operation taxonomy units number in the treatment with straw (161,917 OTU). According to these results, we can assume that the different combinations of tillage practices (for example, shallow ploughless tillage compared to deep ploughing) and organic fertilization (straw compared to farmyard manure) create similar, more favorable environmental conditions for microorganisms.

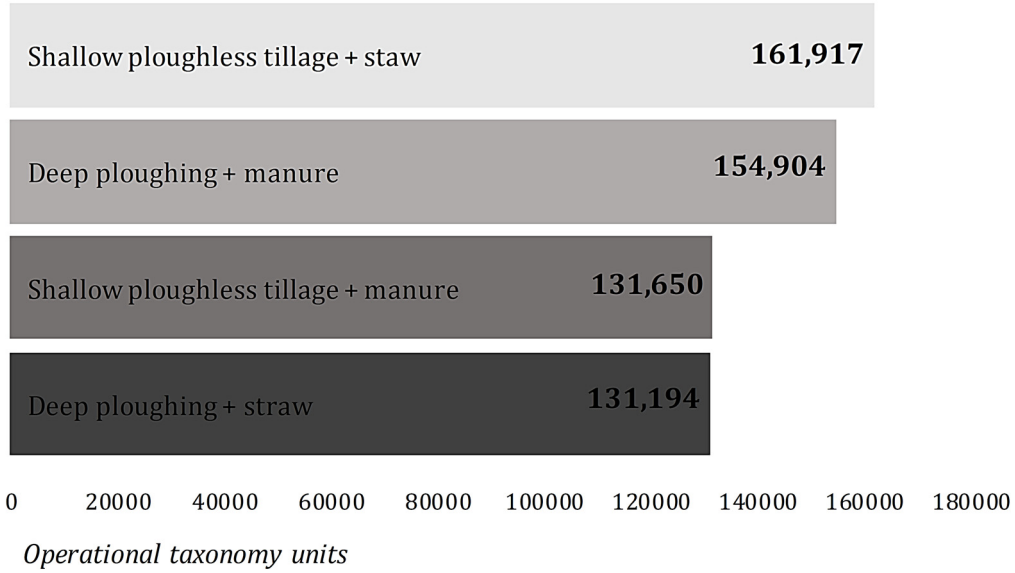


Figure 3. The distribution of operational taxonomy units (OTUs) under different tillage and fertilization.

The number of taxonomic units in different agroecosystems showed the abundance of microorganisms but does not allow for the evaluation of the biodiversity. Therefore, five different biodiversity indices were calculated (Figure 4). Shannon (5.89–5.93) and Simpson (0.991–0.993) biodiversity indices found that in all the studied soils, their values were similar. Hence, we can conclude that the 10 most abundant species were the same in all tested variants. The ACE, Chao1, and JackKnife biodiversity indices varied in the analyzed soils—the highest indices were calculated for shallow ploughless soils with farmyard manure (respectively 3302; 3446; 3413), which leads us to the assumption that biodiversity was the largest in this scenario.

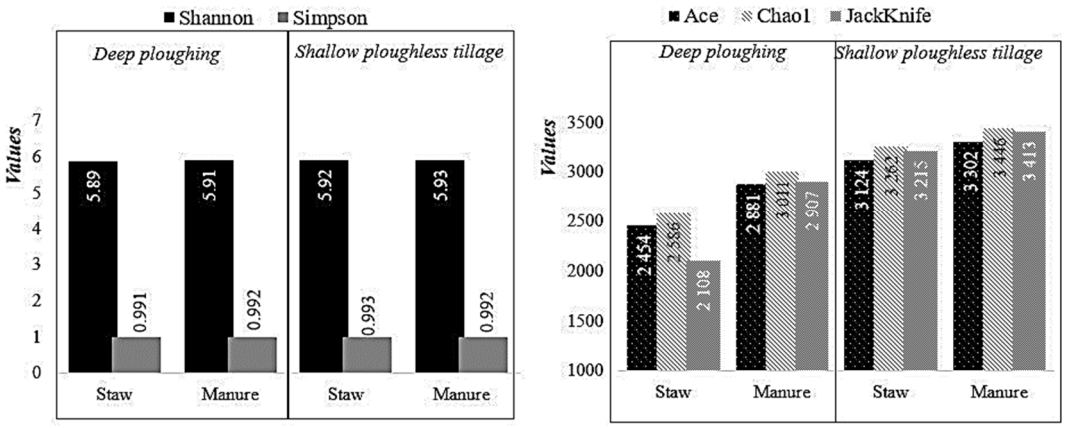


Figure 4. The biological diversity indices.

The lowest values of these indices (2454, 2586, 2108) were determined for deep ploughed soils, where straw is incorporated as a source of organic matter. These results showed poorer biodiversity. However, from an agronomic point of view, it is important to note not only a higher diversity but also a rich diversity comprising of bacteria and their function in the general system of soil–plant. For this purpose, we used the next-generation sequencing. In all analyzed soils, two types of bacteria were dominant: *Actinobacteria* and *Proteobacteria*. Their relative abundance in different agroecosystems ranged from 30% to 36% and 26% to 28%, respectively. The abundance of other identified types of bacteria in all the soils tested does not exceed the 10% limit. The most widespread family is *Micrococcaceae*,

which is classified as the species of *Actinobacteria*. Its abundance in different agroecosystems was from 4% to 9% of the total population of bacteria and *Hyphomicrobiaceae*, which belongs to the *Proteobacteria* type, and it accounts for 3% of the total bacterial population in all the analyzed samples.

In Figure 5, it was noted that 10 of the most abundant species were the same in all tested variants. The identified bacteria comprised 25–28% of all the microbial community. The most common types, *Arthrobacter* and unclassified *Candidatus Saccharibacteria*, make up 8.4–3.6% and 3.2–3.7% of the total population, respectively.

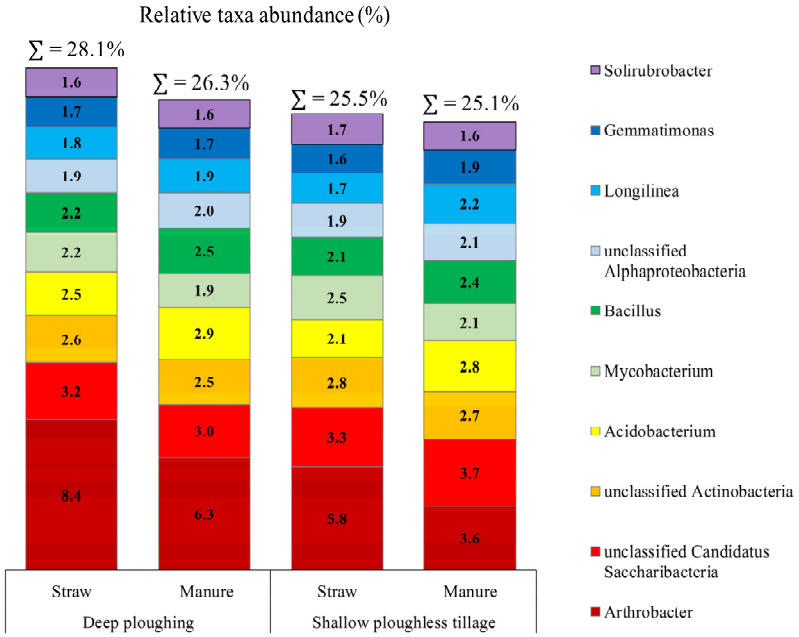


Figure 5. Ten most abundance species in soil samples.

3.5. Carbon Stock in the Soil under Different Combination of Tillage and Organic Fertilization

During the experiment, soil chemical analysis showed that different intensity of tillage affects organic carbon accumulation in the soil. We observed that accumulation processes happened in the deep ploughed soil with a lower arable layer (10–20 cm) despite the organic fertilizers type (Figure 6). The average relative organic carbon content in the lower layer was 0.07 higher compared to the upper layer. Meanwhile, in shallow ploughless tillage, these processes were noticed in the upper soil layer (0–10 cm), with the organic carbon content showing 1.8% in contrast to 1.51% observed in the 10–20 cm layer. However, shallow ploughless tillage with additional deep loosening brought about organic carbon losses in all arable soil layers. The soil organic carbon content decreased from 1.64% in upper layer to 1.35% in the lower 10–20 cm soil layer during the experiment period.

The highest positive yearly change in dissolved organic carbon (Figure 7) was established in the shallow ploughless soil compared to the negative change observed in the deep ploughed soil. The application of additional deep loosening reduces the dissolved organic carbon content compared to shallow ploughless tillage respectively from 0.194 to 0.173 g kg^{-1}. Organic fertilizer has no statistically significant effects on soil organic carbon and dissolved organic carbon accumulation in soil.

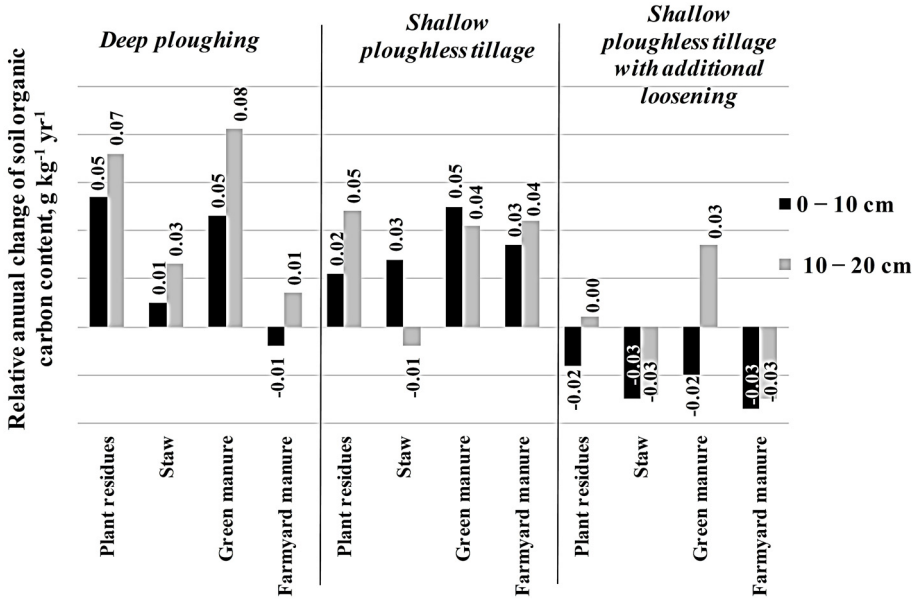


Figure 6. Mean annual change (g kg^{-1} yr^{-1}) in soil organic carbon, applying different tillage and organic fertilizers.

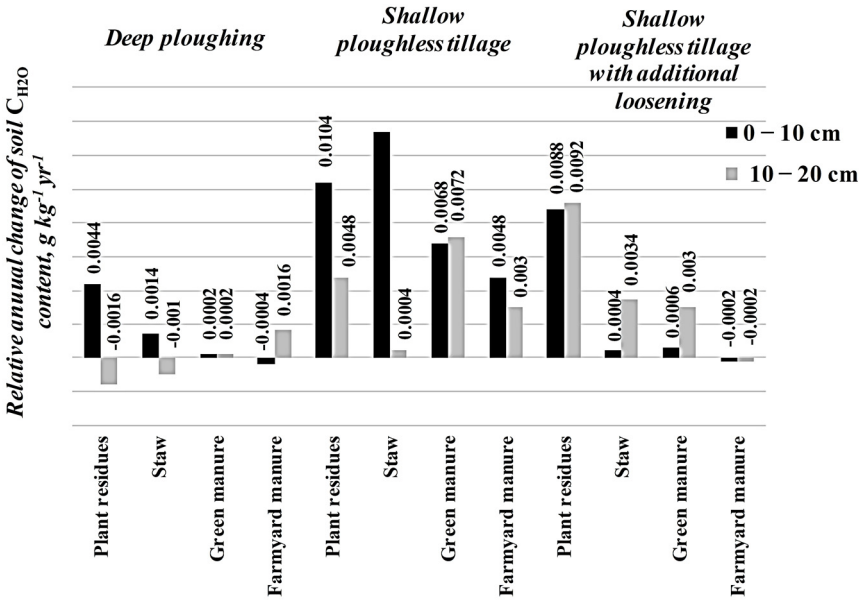


Figure 7. The mean differences in soil dissolved organic carbon (g kg^{-1} yr^{-1}) rate, applying different tillage and organic fertilizers.

In the deep ploughed soil (Figure 8), the organic carbon (1.5%) and dissolved organic carbon content (0.19 g kg^{-1}) was similar to those observed in shallow ploughless soil with additional deep loosening (1.49% and 0.17 g kg^{-1}, respectively). However, carbon lost as carbon dioxide in this treatment is significantly lower (0.033 (mg (g abs. dry soil)$^{-1}$ day^{-1}) and 0.035 (mg (g abs. dry soil)$^{-1}$ day^{-1}).

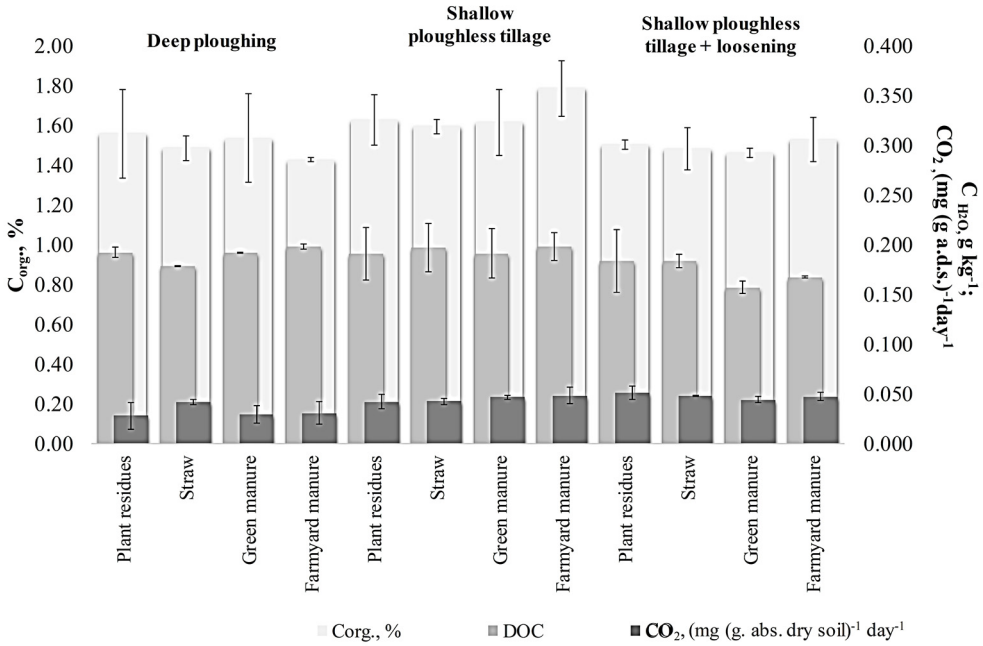


Figure 8. The mean difference in carbon fraction applying different tillage (mean ± standard deviation).

This indicated that the mineralization processes are not very active. Consequently, there is no rapid decomposition of organic compounds, and we can assume that under the conditions of the western Lithuanian climate, deep ploughing is a more favorable context for the accumulation of stable carbon compounds in the soil when compared to the application of shallow ploughless tillage with additional loosening. In the soil with applied shallow ploughless tillage, both organic carbon (1.66%) and dissolved organic carbon (0.194 g kg^{-1}) were higher in comparison to the other two tillage types. Carbon content lost as carbon dioxide (0.045(mg (g abs. dry soil)$^{-1}$ day^{-1}) was moderate compared to the other two treatments. We can assume that the soil with applied shallow ploughless tillage forms favorable conditions for carbon transformation.

4. Discussion

4.1. Soil Properties in Differently Formed Agroecosystems

In the present study, the results indicated that soil properties were affected under different tillage management conditions. The shallow ploughless tillage with additional loosening significantly increased soil density and moisture, but it adversely affected the aeration and total porosity of the soil. The results on soil density under the Lithuanian weather conditions supported previous research studies of a similar nature in other countries [24–26]. It was determined that the addition of different organic fertilizers did not have a significant influence on the physical properties of the soil, except for aeration porosity. The highest porosity was in the treatment where the plant residues were ploughed. Experiment results also showed that shallow ploughless tillage soil provides optimal soil physical conditions for microorganism activity and plant growth. This is in consonance with earlier reports by Acir [27] and Salem [28] where it was noted that decreased soil disturbance positively affected soil bulk density and other physical properties, consequently leading to the stimulation and accumulation of soil microbial biomass and an improved C sequestration under the conservational tillage practices.

4.2. Soil Microbiota Activity in Differently Formed Agroecosystems

Field management practices can influence soil microbial activity through changes in soil environment. Previously conducted studies showed that the long-term application of organic fertilizers leads to the formation of favorable conditions for the development of microorganisms, resulting in an improvement of soil biological activity and a substantial

increase in crop yield, which can be sustainable for years [29,30]. During our study period, it was determined that the mineralization process is most intensive in autumn. During this period, Lithuania has the highest precipitation levels and relatively high air temperature. These conditions provide a possibility for the transport of nutrients from the upper soil layers to the lower layer of soil where optimal conditions for microbial activities are reached [31,32]. The findings of the study of Francioli et al. [33] provided the basis for research related to the result of an amendment on the soil microorganism population actions, effects, and structure. Organic manure amendment favored a significant improvement in soil microorganism biomass and variety, superior to mineral fertilizers, given that organic manure is richer in micro- and macronutrients. In addition, specific amendments may indicate that groups of microbes in soil are either beneficial or harmful, and the obtained results indicate that organic amendment favors some beneficial microorganisms, restraining the detrimental ones. So, it is very important to identify the dominant microorganisms in soil and to determine their functions. Accordingly, our research results align with other published data where different combinations of tillage and organic fertilization create similar and more favorable environmental conditions for microorganisms [34].

The experiment also paid attention to the abundance of individual physiological groups of soil microorganisms and their variation at the separate stage of the study throughout the year. The class of microorganisms identified is abundantly available in months when growth conditions in the environment are at an optimum, which has been reported in previous studies [35,36]. It is important to state that while spore-forming bacteria also reached their peak during this period, the cellulose-degrading bacteria did not reveal a significant difference between the representatives of this group in the autumn and winter–spring periods. The cellulose-degrading bacteria was the most prevalent in February, with the possibility of associating with a high level of organic carbon and nitrogen in the soil, arising from the death of microorganisms at extremely low ambient temperatures. This is confirmed by the significant decrease in the number of ammonifying and spore-forming bacteria in this period [37,38]. Micromycetes were mostly found in the spring–summer period. Such representatives of this micromycetes group are associated with the chemical composite of the substrate and are actively involved in the breakdown of the easily degradable compounds. The micromycetes release complex enzymes that facilitate the breakdown of more resistant compounds that are not easily susceptible to the initial degrading microbial actions [39–41].

For a long time, it has been taken for granted that the main plant cell-forming compounds (lignin, hemicellulose) are inert and highly degradable cell components of plants [42,43]. Recent studies showed that these compounds degrade under the influence of soil microorganisms, but they are believed to be the main sources of stabilized carbon in the soil, and only a small fraction is converted to a labile carbon fraction or immobilized in microorganism biomass [44–46]. There is a need to understand the diversity of microorganisms participating in the degrading process of the substrates; hence, we determined the abundance of individual physiological groups on the surface of the degradable substrate as well as its variation with different combinations of tillage and organic fertilization. The results of the experiment revealed that the abundance of microorganisms on the decomposing substrate samples varies in the different soil arable layer. Spore-forming and cellulolytic bacteria were found to be substantially higher on samples in the lower soil arable layer at 42.76% and 19.5%, respectively. Seasonality has had a significant influence on the changes in the abundance of microorganism physiological groups on substrate samples—ammonifying bacteria, micromycetes, and spore-forming bacteria reached their peak during the summer months, while cellulolytic bacteria were most noticed during the spring period.

The accumulation of organic matter and inorganic nutrients in the surface layer of unplowed soil creates an environment in which biological activity is strongly stimulated [47]. Tillage type significantly influenced the abundance of physiological groups of microorganisms on the decomposing substrate. Shallow ploughess tillage proved to be a great

environment for the richness of ammonifying and spore-forming bacteria. Tillage type was not a statistically significant factor for the abundance of micromycetes. Other researchers determined that by deep plowing, organic residues and mineral fertilizers are introduced in depth, thus intensifying the microbiological processes throughout the mass of the arable layer [47,48]. In our research, deep loosening of the soil significantly reduced micromycetes and cellulose-degrading bacterial populations on the decomposable substrate.

4.3. Carbon Stock in the Soil under Different Combination of Tillage and Organic Fertilization

Many decades of research have shown that SOC plays an important role in a wide range of soil properties and processes. The cessation of ploughing or reduction of tillage intensity generally reduces disturbance and provides better protection for SOC against decomposition, but the changes in the total SOC content become evident after long-term application of less intensive tillage [49]. In the present study, the sequestration of organic carbon was influenced by the varying tillage intensity. In the deeper soil layers, SOC concentrations were higher under deep ploughing. However, by applying shallow ploughless tillage, SOC accumulated in the upper soil layer (0–10 cm). The higher SOC content under shallow ploughless tillage could be a result of increased biomass production on the soil surface associated with the enrichment of organic fertilizers. A similar result was reported stating that shallow ploughless tillage leaves more than 30% of residue on the soil surface [50]. This result can be attributed to the increase of the total carbon content in the soil but also to the increase of the determined enzymatic activities. Instead, increasing the crops facilitates the abundance of plant residues returned in the soil, which increases the total carbon content and improves the activity of soil enzymes that decrease the C/ N ratio by decomposing abundant residues resulting from the application of long-term mineral fertilization. Thus, it can be concluded that the optimal proportion of organic fertilizers is the ideal approach to optimize the quality of the soil and implicitly its productivity as well as the abundance of bacterial strains [47,51]. Additionally, similar data were found in a long-term study, when Dhillon et al. [52] also reported greater SOC content in upper soil layers under shallow ploughless tillage compared to other tillage types. In assessing the SOC content under shallow ploughless tillage with additional deep loosening and shallow ploughless tillage, the SOC content was similar. These data suggest that there was a positive effect of reduced tillage on SOC accumulation in upper soil layers due to low soil disturbance and slow decomposition of surface-placed plant residues, which has been established in several experiments carried out in temperate climate conditions [53,54]; however, several studies have shown that tillage has an insignificant effect on SOC content, though can change the distribution of SOC in soil profile.

Based on the data of previous studies, the average relative annual change rate of SOC and DOC could be used as an index to assess the SOC sequestration potential. Other researchers concluded that the reduced tillage intensity and the use of additional improvers (fertilizers) have increased the average relative annual change rate of SOC. As proposed by Segnini et al. [55], the plant residues on the soil surface under less-intensive tillage would be only partially decomposed, resulting in an accumulation of dissolved organic matter. This led to changes in the organic matter transformation processes generally and the humification of SOC specifically [56].

5. Conclusions

An acidification process was determined in all analyzed soils except in the soil with shallow ploughless tillage and additional loosening; however, this method significantly increased soil density and humidity but adversely affected the aeration and general porosity of the soil. The highest biodiversity was found in the soil with shallow ploughless tillage and enriched with farmyard manure. *Actinobacteria* and *Proteobacteria* were the dominant bacterial species. In the conditions of the western Lithuanian climate, the process of mineralization in *Dystric Bathygleyic Glossic Retisol* takes place all year round but becomes the most intensive in autumn. Neither the application of different organic fertilizers nor

tillage methods have any significant effect on the rate of mineralization of the substrate samples. Shallow ploughless tillage is the most suitable tillage technology, as it creates favorable conditions for the accumulation of organic carbon in the soil. Additional loosening promotes the loss of organic carbon in soil and increases the number of unsaturated compounds, which leads to soil degradation. These experimental results showed that great opportunities exist to change deep ploughing with alternative and more environmentally friendly techniques, but further research is encouraged to determine the soil fungal and mesofauna biodiversity.

Author Contributions: Conceptualization, D.A. and M.V.; methodology, M.V., D.A. and I.M.; software, M.V., I.M.; validation, M.V., I.M. and D.K.; formal analysis, M.V., I.M.; investigation, M.V., I.M. and S.S.; resources, M.V., I.M. and S.S.; data curation, M.V., I.M. and S.S.; writing—original draft preparation, M.V., I.M. and M.O.D.; writing—review and editing, M.V., I.M., D.A., D.K., M.O.D. and S.S.; visualization, M.V., I.M.; supervision, D.A. 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 that they have no conflict of interest to publish this manuscript.

References

1. Merrill, S.D.; Liebig, M.A.; Tanaka, D.L.; Krupinsky, J.M.; Hanson, J.D. Comparison of soil quality and productivity at two sites differing in profile structure and topsoil properties. *Agric. Ecosyst. Environ.* **2013**, *179*, 53–61. [CrossRef]
2. de Moraes Sá, J.C.; Lal, R.; Cerri, C.C.; Lorenz, K.; Hungria, M.; de Faccio Carvalho, P.C. Low-carbon agriculture in South America to mitigate global climate change and advance food security. *Environ. Int.* **2017**, *98*, 102–112. [CrossRef]
3. Rahimzadeh, A. Political ecology of climate change: Shifting orchards and a temporary landscape of opportunity. *World Dev. Perspect.* **2017**, *6*, 25–31. [CrossRef]
4. Cassman, K.G. Ecological intensification of maize-based cropping systems. *Better Crop.* **2017**, *101*, 4–6.
5. Juhola, S.; Klein, N.; Käyhkö, J.; Neset, T.-S.S. Climate change transformations in Nordic agriculture? *J. Rural. Stud.* **2017**, *51*, 28–36. [CrossRef]
6. Kadžienė, G. *Dirvožemių Savybės Kaitos Integruotas Vertinimas Skirtingose Žemės Dirbimo-Tręšimo Sistemose*; Akademija: Kaunas, Lithuania, 2009; p. 119.
7. Kochiieru, M.; Feiziene, D.; Feiza, V.; Volungevičius, J.; Velykis, A.; Slepetiene, A.; Deveikyte, I.; Seibutis, V. Freez-ing-thawing impact on aggregate stability as affected by land management, soil genesis and soil chemical and physical quality. *Soil Tillage Res.* **2020**, *203*, 1047–1055. [CrossRef]
8. Sollins, P.; Gregg, J.W. Soil organic matter accumulation in relation to changing soil volume, mass, and structure: Concepts and calculations. *Geoderma* **2017**, *301*, 60–71. [CrossRef]
9. Janušauskaitė, D.; Velykis, A.; Satkus, A. Sunkaus priemolio dirvožemio armens ir poarmenio gerinimo priemonių povei-kis mikrobiologiniams procesams. *ZEMDIRBYSTE* **2008**, *2*, 45–60.
10. Xue, P.; Minasny, B.; McBratney, A.B. Land-use affects soil microbial co-occurrence networks and their putative functions. *Appl. Soil Ecol.* **2022**, *169*, 104–118. [CrossRef]
11. Hemkemeyer, M.; Schwalb, S.A.; Heinze, S.; Joergensen, R.G.; Wichern, F. Functions of elements in soil microorganisms. *Microbiol. Res.* **2021**, 126832. [CrossRef]
12. Adetutu, E.M.; Ball, A.S.; Weber, J.; Aleer, S.; Dandie, C.E.; Juhasz, A.L. Impact of bacterial and fungal processes on 14C-hexadecane mineralization in weathered hydrocarbon contaminated soil. *Sci. Total. Environ.* **2012**, *414*, 585–591. [CrossRef] [PubMed]
13. Stark, C.; Condron, L.; Stewart, A.; Di, H.; O'Callaghan, M. Influence of organic and mineral amendments on microbial soil properties and processes. *Appl. Soil Ecol.* **2007**, *35*, 79–93. [CrossRef]
14. Li, Y.; Lee, C.G.; Watanabe, T.; Murase, J.; Asakawa, S.; Kimura, M. Identification of microbial communities that assimilate substrate from root cap cells in an aerobic soil using a DNA-SIP approach. *Soil Biol. Biochem.* **2011**, *43*, 1928–1935. [CrossRef]
15. Orgiazzi, A.; Dunbar, M.B.; Panagos, P.; de Groot, G.A.; Lemanceau, P. Soil biodiversity and DNA barcodes: Opportunities and challenges. *Soil Biol. Biochem.* **2015**, *80*, 244–250. [CrossRef]
16. Vieira, A.; Moura, M.; Silva, L. Soil metagenomics in grasslands and forests—A review and bibliometric analysis. *Appl. Soil Ecol.* **2021**, *167*, 104047. [CrossRef]

17. Santos, S.; Nielsen, T.K.; Hansen, L.H.; Winding, A. Comparison of three DNA extraction methods for recovery of soil pro-tist DNA. *J. Microbiol. Methods* **2015**, *115*, 13–19. [CrossRef]
18. Mocali, S.; Benedetti, A. Exploring research frontiers in microbiology: The challenge of metagenomics in soil microbiology. *Res. Microbiol.* **2010**, *161*, 497–505. [CrossRef] [PubMed]
19. Thomas, T.; Gilbert, J.; Meyer, F. Metagenomics—A guide from sampling to data analysis. *Microb. Inform. Exp.* **2012**, *2*, 3. [CrossRef]
20. Hiraoka, S.; Yang, C.-C.; Iwasaki, W. Metagenomics and Bioinformatics in Microbial Ecology: Current Status and Beyond. *Microbes Environ.* **2016**, *31*, 204–212. [CrossRef]
21. Repsiene, R.; Karcauskiene, D. Changes in the chemical properties of acid soil and aggregate stability in the whole profile under long-term management history. *Acta Agric. Scand. Sect. B-Plant Soil Sci.* **2016**, *66*, 671–676. [CrossRef]
22. Oksanen, J.; Blanchet, G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; et al. Vegan. Community Ecology Package; 2015. Available online: http://CRAN.Rproject.org/package=vegan (accessed on 20 July 2021).
23. Colwell, R.K. EstimateS: Statistical Estimation of Species Richness and Shared Species from Samples. Version 9. 2013. Available online: http://purl.oclc.org/estimates (accessed on 20 July 2021).
24. Draycott, A.P. *Sugar Beet*; Blackwell Publishing Ltd.: Oxford, UK, 2006; pp. 114–129.
25. Volungevicius, J.; Feiza, V.; Amaleviciute-Volunge, K.; Liaudanskiene, I.; Slepetiene, A.; Kuncevicius, A.; Vengalis, R.; Vėlius, G.; Prapiestiene, R.; Poskiene, J. Transformations of different soils under natural and anthropogenized land management. *ZEMDIRBYSTE* **2019**, *106*, 3–14. [CrossRef]
26. Volungevicius, J.; Amalevičiūtė-Volungė, K.; Feiziene, D.; Feiza, V.; Slepetiene, A.; Liaudanskienė, I.; Versuliene, A.; Vaisvalavicius, R. The effects of agrogenic transformation on soil profile morphology, organic carbon and physico-chemical properties in Retisols of Western Lithuania. *Arch. Agron. Soil Sci.* **2018**, *64*, 1910–1923. [CrossRef]
27. Acir, N.; Günal, H.; Çelik, I.; Barut, Z.B.; Budak, M.; Kılıç, S. Effects of long-term conventional and conservational tillage systems on biochemical soil health indicators in the Mediterranean region. *Arch. Agron. Soil Sci.* **2020**, 1–14. [CrossRef]
28. Salem, H.M.; Meselhy, A.; Elhagarey, M.; Ali, A.M.; Wu, W. Soil erosion control and wheat productivity are improved by a developed ridge-furrow and reservoir tillage systems. *Arch. Agron. Soil Sci.* **2020**, 1–10. [CrossRef]
29. Samuel, A.D.; Bungau, S.; Tit, D.M.; Melinte Frunzulica, C.E.; Purza, L.; Badea, G.E. Effects of long-term application of organic and mineral fertilizers on soil enzymes. *Rev. Chim.* **2018**, *69*, 2608–2612. [CrossRef]
30. Samuel, A.D.; Bungau, S.; Fodorm, I.K.; Tit, D.M.; Blidar, C.F.; David, A.T. Effects of liming and fertilization on the dehydro-genase and catalase activities. *Rev. Chim.* **2019**, *70*, 3464–3468. [CrossRef]
31. Holub, P.; Klem, K.; Tůma, I.; Vavříková, J.; Surá, K.; Veselá, B.; Urban, O.; Záhora, J. Application of organic carbon affects mineral nitrogen uptake by winter wheat and leaching in subsoil: Proximal sensing as a tool for agronomic practice. *Sci. Total Environ.* **2020**, *717*, 137058. [CrossRef] [PubMed]
32. Kögel-Knabner, I.; Amelung, W. Soil organic matter in major pedogenic soil groups. *Geoderma* **2021**, *384*, 114785. [CrossRef]
33. Francioli, D.; Schulz, E.; Lentendu, G.; Wubet, T.; Buscot, F.; Reitz, T. Mineral vs. Organic Amendments: Microbial Community Structure, Activity and Abundance of Agriculturally Relevant Microbes Are Driven by Long-Term Fertilization Strategies. *Front. Microbiol.* **2016**, *7*, 1446. [CrossRef] [PubMed]
34. Tang, H.; Xiao, X.; Li, C.; Cheng, K.; Shi, L.; Pan, X.; Li, W.; Wen, L.; Wang, K. Tillage and crop residue incorporation effects on soil bacterial diversity in the double-cropping paddy field of southern China. *Arch. Agron. Soil Sci.* **2021**, *67*, 435–446. [CrossRef]
35. Tripolskaja, L.; Bagdanavičienė, Z.; Romanovskaja, D. Mineralinio azoto ir dirvožemio mikrobinis aktyvumas irstant or-ganinėms trąšoms rudens–žiemos laikotarpiu. *Žem. Ūkio Moksl.* **2002**, *2*, 2–12.
36. Zhao, X.; Zhao, Y.; Xi, B.; Wei, Z.; Wu, J.; Zhao, T. Seasonal population changes in the ammonia-oxidizing bacteria commu-nity structure of Songhua Lake, China. *Process Saf. Environ. Prot.* **2016**, *104*, 523–530. [CrossRef]
37. Brockett, B.F.T.; Prescott, C.E.; Grayston, S.J. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven bio geoclimatic zones in western Canada. *Soil Biol. Biochem.* **2012**, *44*, 9–20. [CrossRef]
38. Jirout, J. Nitrous oxide productivity of soil fungi along a gradient of cattle impact. *Fungal Ecol.* **2015**, *17*, 155–163. [CrossRef]
39. Kutateladze, L.; Zakariashvili, N.; Jobava, M.; Burduli, T.; Sadunishvili, T. Microscopic fungi spread in different types of soils in Western Georgia. *Ann. Agrar. Sci.* **2016**, *14*, 227–232. [CrossRef]
40. Bohacz, J. Lignocellulose-degrading enzymes, free-radical transformations during composting of lignocellulosic waste and biothermal phases in small-scale reactors. *Sci. Total. Environ.* **2017**, *580*, 744–754. [CrossRef] [PubMed]
41. Antongiovanni, M.; Sargentini, C. Variability in chemical composition of straws. *CIHEAM* **1991**, *16*, 49–53.
42. Tuomela, M.; Oivanen, P.; Hatakka, A. Degradation of synthetic 14C-lignin by various white-rot fungi in soil. *Soil Biol. Biochem.* **2002**, *34*, 1613–1620. [CrossRef]
43. Tan, B.; Yin, R.; Yang, W.; Zhang, J.; Xu, Z.; Liu, Y.; He, S.; Zhou, W.; Zhang, L.; Li, H.; et al. Soil fauna show different degradation patterns of lignin and cellulose along an elevational gradient. *Appl. Soil Ecol.* **2020**, *155*, 103673. [CrossRef]
44. Torres, I.F.; Bastida, F.; Hernandez, T.; Bombach, P.; Richnow, H.; García, C. The role of lignin and cellulose in the carbon-cycling of degraded soils under semiarid climate and their relation to microbial biomass. *Soil Biol. Biochem.* **2014**, *75*, 152–160. [CrossRef]
45. Mirzavand, J.; Asadi-Rahmani, H.; Moradi-Talebbeigi, R. Biological indicators of soil quality under conventional, reduced, and no-tillage systems. *Arch. Agron. Soil Sci.* **2020**, 1–14. [CrossRef]

46. Šimon, T.; Javurek, M.; Mikanova, O.; Vach, M. The influence of tillage systems on soil organic matter and soil hydrophobi-city. *Soil Tillage Res.* **2009**, *105*, 44–48. [CrossRef]
47. Bungau, S.; Behl, T.; Aleya, L.; Bourgeade, P.; Aloui-Sossé, B.; Purza, A.L.; Abid, A.; Samuel, A.D. Expatiating the impact of anthropogenic aspects and climatic factors on long-term soil monitoring and management. *Environ. Sci. Pollut. Res.* **2021**, *28*, 1–23. [CrossRef]
48. Kakabouki, I.; Folina, A.; Efthimiadou, A.; Karydogianni, S.; Zisi, C.; Kouneli, V.; Kapsalis, N.C.; Katsenios, N.; Travlos, I. Evaluation of Processing Tomato Pomace after Composting on Soil Properties, Yield, and Quality of Processing Tomato in Greece. *Agron.* **2021**, *11*, 88. [CrossRef]
49. Paustian, K.; Lehmann, J.; Ogle, S.; Reay, D.; Robertson, G.; Smith, P. Climate-smart soils. *Nat. Cell Biol.* **2016**, *532*, 49–57. [CrossRef] [PubMed]
50. Dhillon, M.R.; Del Corso, B.; Figueiredo, E.; Nambi, W.R. Soil organic carbon, total nitrogen, and soil pH, in a long-term continuous winter wheat (*Triticum aestivum* L.) experiment. Commun. *Soil Sci. Plant Anal.* **2018**, *49*, 803–813. [CrossRef]
51. Chen, J.; Wei, X. *Controlled-Release Fertilizers As a Means to Reduce Nitrogen Leaching and Runoff in Container-Grown Plant Production Nitrogen in Agriculture—Updates*; IntechOpen: London, UK, 2018; pp. 33–52. [CrossRef]
52. Pospisilova, L.; Fasurová, N.; Petrášová, V. Humus content and quality under different soil tillage systems. *Soil Water Res.* **2010**, *5*, 90–95. [CrossRef]
53. Villamil, M.B.; Little, J.; Nafziger, E.D. Corn residue, tillage, and nitrogen rate effects on soil properties. *Soil Tillage Res.* **2015**, *151*, 61–66. [CrossRef]
54. Hábová, M.; Pospíšilová, L.; Hlavinka, P.; Trnka, M.; Barančíková, G.; Tarasovičová, Z.; Takáč, J.; Koco, Š.; Menšík, L.; Nerušil, P. Carbon pool in soil under organic and conventional farming systems. *Soil Water Res.* **2019**, *14*, 145–152. [CrossRef]
55. Segnini, A.; Carvalho, J.L.N.; Bolonhezi, D.; Pereira Milori, D.M.B.; da Silva, W.T.L.; Simões, M.L.; Cantarella, H.; de Maria, I.C.; Neto, L.M. Carbon stock and humification index of organic matter affected by sugarcane straw and soil man-agement. *Sci. Agric.* **2013**, *70*, 321–326. [CrossRef]
56. Litvinovich, A.; Pavlova, O.; Lavrishchev, A.; Bure, V.; Saljnikov, E. Empirical models of transformations of humic acids and humin in Umbric Albeluvisol Abruptic as influenced by liming. *Zemdirb.-Agric.* **2017**, *104*, 115–122. [CrossRef]