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Soil Chemical Properties and Microbial Behavior under Short-Term Organic and Mineral Fertilization within Different Crops

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Abstract: Agronomic practices can have a negative impact on soil health and quality and ecosystem resilience. The objectives of the study were (1) to evaluate the soil chemical properties and microbial abundance under short-term application of organic and mineral fertilizers and different cultivated crops and (2) to observe the antifungal efficacy of microorganisms isolated from the studied soil. A field trial was conducted in the 2021–2022 period on a preluvosoil-type soil in four randomized blocks with eight fertilizer treatments based on manure compost (MC) and MC + mineral fertilizer (V1—control-soil; V2—NPK only; V3—15 t/ha MC; V4—15 t/ha MC + NPK; V5—30 t/ha MC; V6—30 t/ha MC + NPK; V7—60 t/ha MC; and V8—60 t/ha MC +NPK) and four crops (winter wheat—*Triticum aestivum* L., maize—*Zea mays* L., soybean—*Glycine max* L., and a mixture of perennial grasses and legumes). In almost all treatments, the soil pH decreased during the summer–autumn period. The organic carbon (C_{org}) and humus contents increased compared to the initial state of the soil after the application of different doses of MC and MC + NPK fertilization in almost all treatments. The microbial load of the soil was influenced by the fertilization regime and crop species, but there were no significant differences between the variants. The highest bacterial load was recorded in soil cultivated with a mixture of perennial grasses and grain legumes, i.e., in the variant with 15 t/ha MC, followed by soil cultivated with maize and fertilized with 30 t/ha and 60 t/ha MC. A higher number of fungi was observed in the mixture of perennial grasses and legumes, and *Rhizobium* population was higher, especially in the winter wheat plots, despite the fertilization regime. The antifungal efficacy of the microorganisms isolated from the samples was medium to low, except in the winter wheat experiment, where the efficacy against *Fusarium culmorum* was medium to high and against other pathogens was medium. In the other crops within the experiment, the antagonistic activity of the soil microorganisms was medium to low.

Keywords: soil chemical properties; organic amendments; chemical fertilizers; microbial abundance; antagonistic activity; ecosystem sustainability

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1. Introduction

Soil plays a crucial role in maintaining ecosystem functions and services [1,2], as there is a link between soil properties and the productivity and sustainability of natural and agricultural systems [3,4].

Agricultural practices can disrupt soil trophic networks and negatively affect soil health and quality, as well as ecosystem integrity and resilience [5]. More than 1/3 of

soil resources are degraded by intensive agricultural activities and land management practices [6]. Therefore, the assessment of soil quality indicators (e.g., soil organic matter content, nitrogen supply, pH, microbial community and diversity, etc.) has become increasingly important over the last decade [7] in order to develop management strategies that ensure a good and sustainable soil quality [8].

Land use and management practices can slowly change soil properties. Therefore, it is a challenge to monitor the changes in the provision of ecosystem services and to find a set of sensitive soil indicators that can reflect the dynamics of soil function [9]. The microbial community can be a good indicator for assessing soil quality, as any change in community structure can be correlated with changes in soil quality, with biological processes being the most sensitive [10]; their diversity, abundance, and function can be influenced by cultivated crops, climatic conditions, or agricultural management practices such as tillage, fertilization, and pesticide use [11].

Fertilization can influence soil quality, but its effects depend on the type of fertilizer (organic or mineral) [12], soil properties, fertilization regime, crops grown, and climatic conditions of the area [13]. Excessive use of mineral fertilizers can contribute to soil acidification [14,15], ammonia volatilization, and denitrification [16], which changes the soil pH and reduces the soil organic carbon (C_{org}) content [14,15]. Ammonium fertilizers can lower the pH of the soil due to nitrification processes and the uptake of ammonium by plants, which leads to soil acidification and thus to a reduction in crop yields and soil fertility [17]. Intensive mineral fertilization also has a negative impact on the soil microbial community [18,19], which performs important functions in the soils, from the decomposition of organic matter to the soil recycling of nutrients or the formation of aggregates [20,21]. The long-term application of nitrogen fertilizers, for example, influences the nitrogen cycle and thus the corresponding bacterial population [18]. The changes in microbial populations or activity led to changes in soil physico-chemical properties, which can be used to determine the improvement or deterioration of the soil [22].

In line with the objectives of the circular economy, there is great interest in the reuse of organic waste and by-products as soil conditioners [23]. Organic amendments such as organic manure can have beneficial effects on both crops and soil because organic matter is mineralized throughout all seasons and nutrients are absorbed more gradually and slowly by plants compared to mineral fertilizers [24]. Hua and Zhu [25] reported that the application of organic amendments can improve soil organic matter content and increase phosphorus (P) utilization efficiency by increasing microbial biomass, phosphatase activity, and fungal activity, thereby accelerating the biochemical cycling process of P availability in soil. For example, cattle manure can increase C_{org} content in soil, stabilize organic aggregates, and reduce organic matter decomposition [26], while pig and chicken manure can increase soil bacterial abundance and total nitrogen (Nt) content. Environmental problems can occur when organic amendments are applied incorrectly or excessively (e.g., ammonia volatilization, greenhouse gas emissions, nitrate leaching, or P runoff) [27]. Thus, an adequate amount of fertilizer is not only necessary to meet the nutrient requirements of crops, but also to achieve higher nutrient use efficiency and lower environmental costs [28].

Crop diversification is also important for improving chemical nutrients and microbial activity in the soil and for improving soil health in agroecosystems [29]. Thus, soil microorganisms depend on organic carbon for their growth and development, which can be provided by plants mainly through root exudates and secretions into the rhizosphere [30]. In addition, each plant species can influence the microbial communities and structure through root distribution in soil depth, root respiration, or crop residues [31,32]. Turner et al. [33] found that the structures of bacterial and fungal communities in the rhizosphere differ between pea and cereal crops. In addition, beneficial bacteria such as *Actinobacteria* and *Rhizobia* were found in the soil at the early stage of maize development, while at the late stage of development, the soil may be enriched with saprophytic fungi [34]. Moreover, perennial grassland legumes (e.g., alfalfa or clover) are usually associated with

soil microorganisms involved in symbiotic and non-symbiotic N fixation and mycorrhizal associations [35].

Crop species can also have a major impact on soil chemical properties by supplying organic matter through litter, roots, or root exudates [36]. Compared to cereals, grain legumes and root crops can improve the chemical composition of the soil mainly through their residues [37]. Grain legumes (especially peas) increase the soil C_{org} content by supplying organic carbon and nitrogen and increasing the humus content [38] as well as the availability of N, P, K, and Mg [38,39]. Dhakal and Islam [30] showed that a 50–50% mixture of grasses and legumes increased soil mineralizable carbon and nitrogen compared to 100% grasses.

Most previous studies have focused on investigating separately the soil chemical and microbiological properties affected by fertilizers. Of particular interest is the effect of fertilizer regime on soil microbial population, which also has a major impact on nutrient cycling, soil structure, or plant health. Moreover, many studies deal with the effects of long-term organic and mineral fertilizers application on soil chemical and biological properties. Given the above context, the main objectives of our study were (1) to assess the changes in chemical properties and microbial abundance of the analyzed soil induced by short-term organic and mineral fertilization and cultivation of four different crops (i.e., winter wheat, a mixture of legumes and grasses, maize, and soybean) and (2) to observe the inhibitory effect of the microorganisms isolated from the soil on some plant pathogens, such as *Fusarium graminearum* and *Fusarium culmorum* (major pathogens for cereals), *Sclerotium bataticola* (pathogen for grain legumes), and *Sclerotinia sclerotiorum* (soil pathogen with a diverse host range).

The novelty of this study lies in the detailed comparison of chemical parameters and microbial abundance in the same soil type, when different organic and mineral fertilization dozes were applied, and different crops were grown. Understanding the organic or NPK fertilizers effects on microbial abundance is necessary for understanding the microbiological processes, to preserve soil ecosystem functions. This research theme is an important part of soil ecology, and its results can help to ensure a sustainable coexistence of ecosystems and agroecosystems in an area that is strongly anchored in agricultural production in Romania, namely the sylvosteppe area of the Romanian Plain. Also of great importance is the suppressive effect of microorganisms isolated from preluvosoil against different phytopathogens that can cause various diseases in cultivated crops and can be used as biocontrol agents.

2. Materials and Methods

2.1. Site Description

A field trial with different doses of manure compost (MC), mineral fertilizers, and crop species was conducted in the period 2021–2022 at the Research and Development Station for Agronomy (RDSA) of Moara Domnească (44°29′36″ N 26°15′29″ E), belonging to the University of Agronomic Sciences and Veterinary Medicine (UASVM) and located in the northeastern part of Bucharest (in the Romanian Plain and a transition zone from steppe to sylvosteppe), in Ilfov County, about 17 km from Bucharest (Figure 1).

The experimental field was established in the southwestern part of the Moara Domnească RDSA, near Lake Pasarea, on a slightly sloping land where alfalfa was previously grown. There is a forest edge in the immediate vicinity.

The climate in the region is temperate with harsh winters and hot summers. In the period from September 2021 to October 2022, the climatic conditions (especially the pluviometric ones) from the Moara Domnească area influenced crop growth. The coldest months were January and March, and the hottest month was July. September 2021 was very dry, as only 3.1 mm of precipitation was recorded and the average amount of precipitation during this growing season was 30.29 mm (Table S1, Figure S1). April 2022, which coincides with the spring crops sowing, was characterized by a very good pluviometric regime, with a monthly rainfall of 71.5 mm.

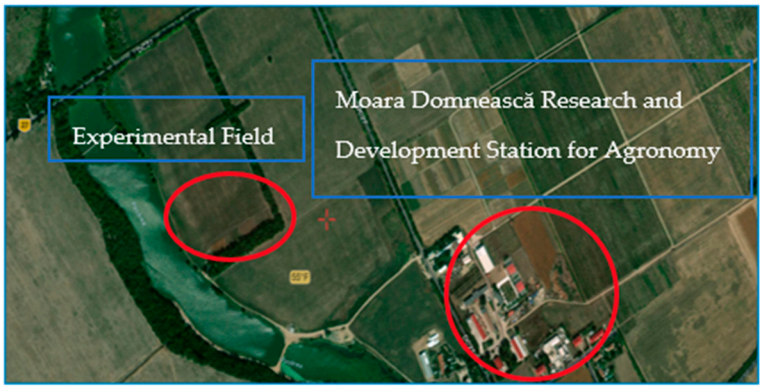

Figure 1. Spatial map of the Experimental Field from Research and Development Station for Agronomy (RDSA) of Moara Domnească [40].

2.2. Soil Analysis

2.2.1. Soil Physicochemical Characteristics Analysis

The soil in Moara Domnească is a red preluvosoil with a specific B-argic horizon (Bt) characterized in the upper layer (Ao) by a loamy-clayey texture, a pH between 5.2 and 5.4, a base saturation level between 65 and 70%, and a low differentiation of the soil profile [41].

To characterize the soil in the different horizons (Ap–Ao–A/B–Bt_1–Bt_2), a soil profile (Figure 2) was made, and soil samples were taken to determine their main physicochemical properties in the laboratory. In addition, soil samples were taken from a 0–20 cm deep layer before and during the experiment from each plot to investigate the initial state of the soil and the change in its chemical properties.

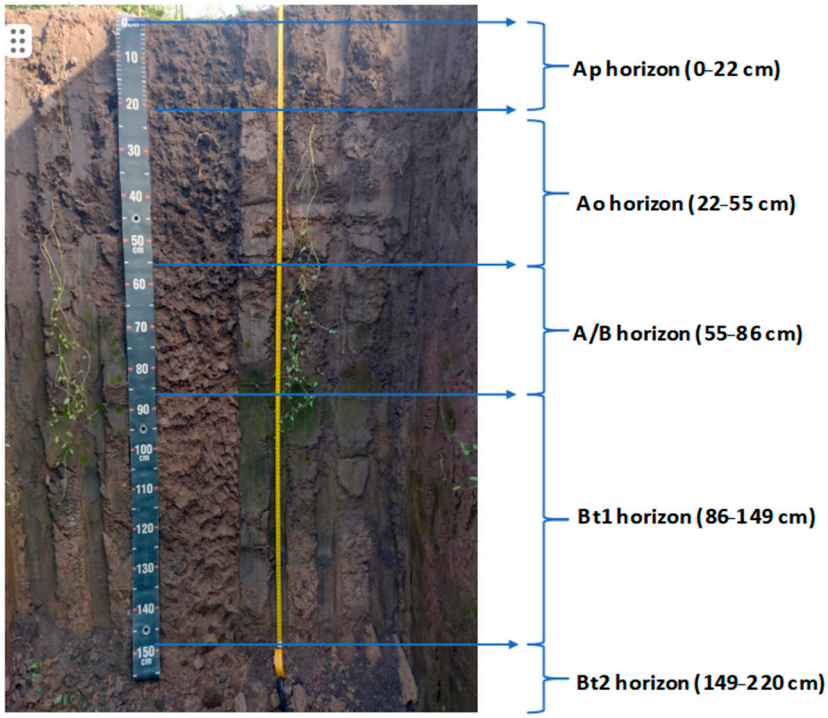

Figure 2. Moara Domnească soil horizons at a 0–220 cm depth.

The soil bulk density (BD) was determined using metal cylinders with a volume of 100 cm^3. Cylinders filled with soil were weighed in the field and oven-dried at 105 °C until they had a constant weight. The BD was estimated by dividing the dry weight of the soil material by the volume of the soil [42]. Total porosity (TP) was measured based on soil

density and BD values according to STAS 7184/5-78 [43]. The degree of compaction (GD) was calculated using the following formula:

$$GD\ (\%\ v/v) = (PMN - TP)/PMN \times 100 \quad (1)$$

where PMN is the minimum required porosity, calculated based on the following relation: PMN = 45 + 0.163 × C, (%v/v); C is the colloidal clay content, with a diameter of <0.002 mm (%g/g), and TP is total porosity, (%v/v) [44].

Chemical analyses were also carried out for soil samples taken from different depths of the horizons and from a layer of 0–20 cm to perform a comprehensive soil analysis. Soil pH was determined by the potentiometric method in aqueous suspension (1:2.5 w/v, soil:water) [45] using a Mettler Toledo pH meter (Mettler Toledo, Greifensee, Switzerland) after shaking and resting for 1 h. The soil C_{org} was determined on 0.2 mm ground soil samples by dichromate oxidation and subsequent titration with ferrous ammonium sulfate [46]. The humus content was estimated from the C_{org} content by multiplying it by a coefficient (1.7241), knowing that the average percentage of carbon content of humus is 58% [47].

Available phosphorus and potassium in the soil were extracted with ammonium acetate lactate (AL extractable) at a pH of 3.75 [48] and analyzed by flame photometry (for potassium content) and UV/Vis spectrometry (for phosphorus content). The N content of the soil was determined using the Kjeldahl method. The soil was digested in concentrated sulfuric acid in the presence of a catalyst mixture, which made it possible to regulate the boiling temperature until complete dissolution and oxidation. The organic N contained in the sample was oxidized to ammonium (as ammonium sulfate). The ammonium (NH_4^+-N) in the digest was quantified by capturing the ammonia (NH_3) released by steam distillation of the digest using alkalis and titrating with a volumetric acid solution, according to the methodological rules in STAS 7184/2-85 [49].

The nitrate and ammonium nitrogen content of the soil was determined in the fresh soil samples after extraction with a K_2SO_4 (0.1 N) solution at a ratio of 1:3 soil/solution (w/v). The nitrate concentration in the solution was determined by the potentiometric method using an ion-selective electrode. NH_3 was determined by the distillation and titration method. The extraction solution was placed in a distillation unit and the extracted NH_3 was distilled off by steam distillation and retained in the form of NH_4^+-N in a sample cup containing boric acid (H_3BO_3-2%) and then titrated with sulfuric acid (H_2SO_4) [50].

2.2.2. Soil Microbiological Characteristics Analysis

Biological analyses were carried out on soil samples taken from the top layer of 0–20 cm and the microbial load and antifungal efficacy of some soil microorganisms were determined to assess the initial and current microbiological status of the soil. The samples were collected from the field and brought to the microbiology laboratory on the same day. In the laboratory, they were kept refrigerated (at 4 °C) for 1 to 4 days until microbial analysis. Serial decimal dilutions were prepared from soil samples to quantify the microbial load and to perform quantitative and qualitative microbiological tests. For the first dilutions (from 10^{-1}), 10 g of soil was processed and infused for 1 h at room temperature with stirring at 120 rpm and left overnight. Depending on the type of analysis, $10^{-4} \div 10^{-6}$ dilutions were then used. A volume of 100 µL of the final dilutions was applied in replicates to solid culture media.

Different growth media were used depending on the type of microorganisms to be detected: (i) Plate Count Agar (PCA) medium was used for cultivable bacteria. After proper incubation, only bacterial colonies were counted, including *Actinomycetes* [51]; (ii) for fungi, Rose-Bengal Chloramphenicol (RBC) Agar medium was used and only fungal colonies were counted after incubation [52]; (iii) for *Enterobacteriaceae*, Eosin Methylene Blue (EMB) Agar medium was used. Several culture media were also used for the detection of specific microorganisms: (I) For the detection of fluorescent *Pseudomonas* spp., KingB agar medium was used and after incubation, the plates were irradiated with UV light. The fluorescent colonies with smooth appearance were considered as *Pseudomonas* [53];

(II) for the differentiation of microorganisms that can solubilize inorganic phosphorus from poorly soluble compounds, NBRIP (National Botanical Research Institute's Phosphate) growth medium was used. After incubation, the colonies surrounded by a clear halo were considered phosphate-solubilizing microorganisms [54]; (III) for chitinolytic microorganisms, a similar protocol was used and the growth medium was chitin agar [55]; (IV) for the detection of cellulolytic microorganisms, carboxymethylcellulose agar (CMC-Agar) was used. After incubation, the plates were flooded with 0.1% Congo red solution for 15 min and then washed with 1 M sodium chloride (NaCl). The colonies surrounded by a clear halo were considered as cellulose-degrading microorganisms [56]; (V) skim milk agar (SMA) medium was used for the caseinase-producing proteolytic microorganisms [57] and (VI) *Rhizobium*-like bacteria were detected on Yeast Mannitol Agar (YMA) medium with Congo red. The colonies that showed a smooth, glistening, and raised appearance, full margins and a whitish or (semi-) translucent feature were counted [58–60]. The concentration of microorganisms in 1 g of soil was calculated according to the following formula:

$$\text{Colony-forming units (CFU)/g} = \text{Colony count/plate} \times \text{dilution factor/plate} \times \text{serial dilution factor} = \text{colony count/plate} \times 10 \times 10^{\text{number of serial dilution}} \quad (2)$$

The antifungal efficacy (E%) of the microbial communities in the tested soil samples was evaluated by biometric measurements to calculate the efficacy of inhibition of plant pathogenic mycelial growth. In the current study, we performed the antifungal activity against four plant pathogens i.e., *Fusarium graminearum* (DSM 4527 reference strain), *Fusarium culmorum* (FC46 strain), and two Romanian isolates, *Sclerotium bataticola* (sinonim *Macrophoma phaseolina*, current name *Tiarosporella phaseolina*) and *Sclerotinia sclerotiorum*. The double culture method was used against these pathogens. For microbial antagonism, soil infusions (1:9 *w*/*v*) were inoculated in Potato Dextrose Agar (PDA) growth medium using the pour plate method. For this, the soil infusion was first added to the Petri dish, then molten agar at 45 °C was poured into the dish and swirled. After solidification, the plate was inoculated in the center with a mycelial plug from a freshly grown culture. Control plates for each fungal strain were prepared by inoculating sterile PDA plates with the same amount of fungal inoculum. Test and control plates were then incubated, and the fungal growth analysis was comparatively assessed. Efficacy was calculated using the following formula:

$$\text{E\%} = (\text{RC} - \text{RT})/\text{RC} \times 100 \quad (3)$$

where E (%) is the inhibitory efficacy of the soil microbial community against the phytopathogenic fungi; RC is the phytopathogenic fungal growth in the control plates (average radius of the phytopathogenic colony in the control plates); and RT is the phytopathogenic fungal growth (as average radius) in the test plates with soil suspension [61].

2.3. Manure Compost Chemical and Microbiological Characteristics Analysis

The compost used in all experiments was obtained from cattle manure collected at the RDSA Moara Domnească livestock farm and straws. For the chemical analyses of the manure compost, samples were taken from 6 points of the pile.

The pH of the compost samples was determined by the potentiometric method in aqueous suspension (1:5 *v*/*v*, soil: water) according to SR ISO 10390:2022 using a Mettler Toledo pH meter, after shaking and resting for 1 h [62]. The C and N content of the compost was measured with an elemental analyzer vario Macro Cube (Elementar, Langenselbold, Germany) using the Dumas method (combustion at 900 °C). Acetanilide was used as a calibration standard. The total phosphorus and potassium content was measured by the wet digestion method using an HNO_3-$HClO_4$ mixture and then analyzed by flame photometry (for the potassium content) and UV/Vis spectrometry (for the phosphorus content) [48].

The manure compost was microbiologically analyzed before it was applied to the experimental field. For this purpose, 10 g of the compost sample was mixed with 50

mL of sterile distilled water and stirred for 1 h using a magnetic stirrer (Velp Scientifica, Europe, Usmate Velate, MB, Italy). After sedimentation, dilutions were prepared from the supernatant and CompactDry Disks (NISSUI Pharmaceutical Co., Ltd., Tokyo, Japan) were inoculated according to the manufacturer's instructions. For identification and quantification of pathogens in the manure compost (i.e., *Salmonella* sp., *Escherichia coli*, Coliforms, and *Enterobacteriaceae*), samples were incubated at different temperatures specified by the manufacturer (for *Salmonella* sp. at 37 °C/41 °C and for *Escherichia coli,* Coliforms, and *Enterobacteriaceae* at 37 °C). CompactDry EC disks were used for the enumeration of *Escherichia coli* and coliforms. An amount of 1 mL of the previously collected supernatant was dropped onto the center of the CompactDry EC plate. The sample is automatically and uniformly diffused into the medium. The samples were incubated for 24 h at 37 °C. *Escherichia coli* formed blue to blue-violet colonies; coliforms showed a red to pink coloration. The red/pink and blue/blue-violet colonies together represented the total number of coliform groups. CompactDry ETB disks, containing a medium with glucose and selective agents, were used to differentiate and enumerate *Enterobacteriaceae.* An amount of 1 mL of the previously obtained supernatant was dropped onto the center of the CompactDry ETB plate. The sample diffused automatically and evenly into the medium. The samples were incubated for 24 h at 37 °C. Red/violet-colored colonies were *Enterobacteriaceae.* Red/violet and other colored colonies together were the total Gram-negative bacteria. CompactDry SL disks were used for *Salmonella* sp. detection based on the combination of three different test principles: alkalinization of the medium and color change from blue-violet to yellow; greening of the colony, caused by the decomposition of the chromogenic substrate with a specific enzyme of *Salmonella* (black colonies were formed by hydrogen sulfide-producing *Salmonella*); and the motility of *Salmonella* sp. [63]. For the pre-enrichment phase, 25 g of the compost sample was mixed with 225 mL of sterile BPW medium (Buffered Peptone Water: enzymatic digestion of casein 10 g/L, sodium chloride 5 g/L, disodium hydrogen phosphate 3.5 g/L, potassium dihydrogen phosphate 1.5 g/L, final pH 7.0 $\pm$ 0.2) and incubated at 37 °C for 18 h. After the incubation period, 0.1 mL of the pre-enriched sample was added to 10 mL of RVS broth (Rappaport-Vassiliadis soy broth: peptone from soy flour 4.5 g/L; magnesium chloride hexahydrate 29.0 g/L; sodium chloride 8.0 g/L; dipotassium hydrogen phosphate 0.40 g/L; potassium dihydrogen phosphate 0.60 g/L; malachite green oxalate 0.036 g/L) and incubated at 41.5 °C for 24 h. For "*Salmonella* positive", black to green isolated or fused colonies must be visible and the medium around the colonies must turn yellow. For "*Salmonella* negative", no black or green colonies must be present. The medium must not turn yellow [64].

Serial decimal dilutions were prepared to quantify the microbial load in the manure compost samples. For 10^{-1} dilutions, 10 g of manure compost was processed, melted for 1 h at room temperature, stirred at 120 rpm, and then allowed to stand overnight. Dilutions of $10^{-4} \div 10^{-6}$ were then used depending on the type of analysis. The same growth media were used as for the microbiological analysis of the soil.

The antifungal efficacy of soil microbial communities against various plant pathogenic fungi was also analyzed. The same method was used against *Fusarium graminearum*, *Fusarium culmorum*, *Sclerotium bataticola*, and *Sclerotinia sclerotiorum*. The antimicrobial activity was evaluated by measuring the growth rays of the plant-pathogenic fungus and by determining the efficacy of inhibition of plant-pathogenic mycelial growth. The efficacy was measured based on the same formula as for the antimicrobial efficacy of the soil microorganism [61].

2.4. Experimental Design

The experiment was conducted in a 4 randomized block design with four crops (i.e., winter wheat—*Triticum aestivum* L.; a mixture of perennial grasses and legumes—ryegrass—*Lolium perenne* L., bluegrass—*Poa pratensis* L., meadow fescue—*Festuca pratensis* L., white clover—*Trifolium repens* L., and bird's foot trefoil—*Lotus corniculatus* L.; soybean—*Glycine*

max L.; and maize—*Zea mays* L.) and eight fertilization treatments for each crop species (Table 1). There were 32 plots within each block.

Table 1. Treatment variants and mineral fertilizers fractions applied to crops during the 2021–2022 vegetation period.

Treatment	Mixture of Perennial Grasses and Legumes		Winter Wheat		Maize			Soybean		
	Doses of Fertilizers									
	Fraction (kg/ha)		Fraction (kg/ha)		Fraction (kg/ha)			Fraction (kg/ha)		
	1	2	1	2	1	2	3	1	2	3
	BBCH stages for different fertilizer dose applications *									
	22	29	27	31	00	13	16	00	12	19
V1—soil (Control)	-	-	-	-	-	-	-	-	-	-
V2—NPK	40 N; 40 P_2O_5; 0 K_2O	40 N; 40 P_2O_5; 0 K_2O	57 N; 57 P_2O_5; 0 K_2O	40 N;40 P_2O_5; 0 K_2O	28 N; 28 P_2O_5 0 K_2O	46 N; 46 P_2O_5; 0 K_2O	29 N; 29 P_2O_5; 0 K_2O	18 N; 18 P_2O_5 0 K_2O	20 N; 20 P_2O_5; 0 K_2O	13 N; 13 P_2O_5; 0 K_2O
V3—15 t/ha MC	-	-	-	-	-	-	-	-	-	-
V4—15 t/ha MC + NPK	29 N; 29 P_2O_5; 0 K_2O	29 N; 29 P_2O_5; 0 K_2O	42 N; 42 P_2O_5; 0 K_2O	21 N; 21 P_2O_5; 0 K_2O	29 N; 29 P_2O_5 0 K_2O	34 N; 34 P_2O_5; 0 K_2O	21 N; 21 P_2O_5; 0 K_2O	16 N; 16 P_2O_5; 0 K_2O	18 N; 18 P_2O_5; 0 K_2O	11 N; 11 P_2O_5 0 K_2O
V5—30 t/ha MC	-	-	-	-	-	-	-	-	-	-
V6—30 t/ha MC + NPK	18 N; 18 P_2O_5; 0 K_2O	18N; 18 P_2O_5; 0 K_2O	27 N; 27P_2O_5; 0 K_2O	13 N; 13 P_2O_5; 0 K_2O	19 N; 19P_2O_5; 0 K_2O	22 N; 22 P_2O_5; 0 K_2O	14 N;14 P_2O_5; 0 K_2O	13 N;13 P_2O_5; 0 K_2O	15 N 15 P_2O_5; 0 K_2O	9 N; 9 P_2O_5; 0 K_2O
V7—60 t/ha MC	-	-	-	-	-	-	-	-	-	-
V8—60 t/ha MC + NPK	According to dose calculation, in V_8, for the mixture of perennial grasses and legumes, winter wheat, and maize, the amount of MC should have ensured the nutrient requirements (NPK) and it was decided not to supplement it with chemical fertilizers							8 N; 8 P_2O_5; 0 K_2O	10 N 10 P_2O_5 0 K_2O	6 N; 6 P_2O_5; 0 K_2O

Abbreviations: Biologische Bundesanstalt, Bundessortenamt, and Chemical Industry (BBCH); chemical fertilizers only (NPK); manure compost (MC); nitrogen (N); phosphorus pentoxide (P_2O_5); potassium oxide (K_2O). * in the mixture of perennial grasses and legumes, there were considered the BBCH stages for the perennial grasses.

Plot size was 15 m^2 (5 m long × 3 m wide) with four replicates and a 1 m wide border between plots. Three doses of MC (15 t/ha; 30 t/ha and 60 t/ha) were applied at the end of September 2021 (Figure 3), either alone or in combination with NPK complex fertilizers 20:20:0, which were applied fractionally according to the nutrient requirements of each crop at different stages of vegetation (Table 1).

Figure 3. Different doses of manure compost applied in Moara Domnească experimental plots in the autumn of 2021.

The drought from the beginning of September 2021 did not allow plowing, but the soil was scarified twice at a depth of 40 cm. The seedbed was prepared by two passes with the cultivator, the average soil moisture being 19.5%.

The seeds of winter wheat (Jaguar variety), purchased from the company ITC Seeds, and those of the mixture of perennial grasses and legumes were sown on 22 October 2021. The percentages of species within the mixture were as follows: 30% *Lolium perenne* (Mara variety); 10% *Poa pratensis* (Fima variety); 20% *Festuca pratensis* (Transilvan variety); 20% *Trifolium repens* (Danitim variety); and 20% *Lotus corniculatus* (Dacia 1 variety). The seeds of the mixture were obtained from the Research and Development Institute for Grassland in Brașov and the Research and Development Institute for Agriculture in Turda.

The soybean seeds (Daciana variety), obtained from the Research Institute for Cereals and Industrial Crops in Fundulea, and the maize seeds (Carioca hybrid), obtained from Syngenta, were sown on April 6 and 15, in the optimal season for the region, after a very good preparation of the seedbed by running over it twice with a cultivator. Soil moisture before sowing averaged 20.5%. The soybean seeds were treated with Nitragin and mycorrhiza before sowing to improve symbiotic activity.

2.5. Statistical Analysis

Statistical analysis of the data obtained was performed using Analyse-it software for Microsoft Excel version 5.66 One-way analysis of variance (ANOVA) was used to test all parameters and to evaluate the statistically significant differences between treatments ($p < 0.05$). In addition, least significant differences (LSD) at the 5%, 1%, and 0.1% probability levels were used with Microsoft Excel and the following significances were determined:

- ns: non-significant differences;
- * significant differences between the fertilized variants and the control;
- ** distinct significant differences between the fertilized variants and the control;
- *** very significant differences between the fertilized variants and control.

For the differences with negative values, the notifications are as follows:

- o—significant negative differences between the fertilized variant and control;
- oo—distinctly significant negative differences between the fertilized variant and the control;
- ooo—very significant negative differences between the fertilized variant and the control.

3. Results

3.1. Manure Compost Chemical and Microbiological Characteristics

The chemical analysis of the MC showed that the pH value was slightly alkaline and the contents of C_{org} and total nitrogen (Nt) contents averaged 21.58% and 1.76%, respectively. It also had a high content of mobile N forms ($N\text{-}NO_3$; $N\text{-}NH_4$) (Table 2).

Table 2. Chemical characteristics of manure compost used in all experiments (October 2021).

Manure Compost Chemical Properties	Measurement Unit	Mean Value ± SD
pH	-	8.00 ± 0.097
C_{org}	%	21.58 ± 0.359
Nt	%	1.76 ± 0.030
C: N Ratio	-	12.26 ± 0.190
$N\text{-}NO_3$	mg/kg d.m.	431.5 ± 206.7
$N\text{-}NH_4$	mg/kg d.m.	763.5 ± 158.5
P_t	%	0.603 ± 0.019
K_t	%	2.575 ± 0.148

Abbreviations: standard deviation (SD); organic carbon (C_{org}); total nitrogen (Nt); carbon:nitrogen ratio (C:N); nitrate nitrogen ($N\text{-}NO_3$); ammonia ($N\text{-}NH_4$); total phosphorus (Pt); total potassium (Kt); milligrams (mg); dry matter (d.m.).

The microbiological analyses of the MC showed a slight increase in *Enterobacteriaceae* load, with the limits set by the Italian Fertilizer Law (Law 748/84, amended by Decree 27/3/98) being $<1 \times 10^3$ [65]. The load of *Escherichia coli*, coliforms, and *Salmonella* sp. was

in accordance with the guidelines for compost quality, according to which the maximum values for *Escherichia coli* and coliforms are ≤1000 most probable number (MPN)/g D.M., and *Salmonella* sp. must not be present [66].

In MC, high loads of cultivable bacteria and fluorescent *Pseudomonas* were found and the microorganisms responsible for the decomposition of compost and cellulolytic microorganisms were also dominant (Table 3).

Table 3. Microbial composition and load in the manure compost before application on the experimental field (October 2021).

Microbial Composition	Measurement Unit	Average Microbial Load
Enterobacteriaceae	CFU/mL	11×10^3
Escherichia coli and coliforms	CFU/mL	8.37×10^3
Salmonella	CFU/mL	nd
Cultivable bacteria	CFU/g soil	4.25×10^8
Fungi	CFU/g soil	9.14×10^4
Fluorescent *Pseudomonads*	CFU/g soil	3.53×10^7
Microorganisms that solubilize inorganic phosphorus	CFU/g soil	2.12×10^7
Cellulolytic microorganisms	CFU/g soil	6.22×10^7
Proteolytic microorganisms	CFU/g soil	4.18×10^7

Abbreviations: colony-forming units (CFU); not detected (nd).

The microorganisms isolated from the manure compost had an inhibitory effect on the in vitro development of some tested microorganisms such as *Fusarium graminearum*, *Fusarium culmorum*, *Sclerotium bataticola*, or *Sclerotinia sclerotiorum* (Table 4).

Table 4. Antifungal efficiency of the microbial communities in the manure compost samples.

Phytopathogenic Fungus	Measurement Unit	Antifungal Efficacy (E) (Mean Values)
Fusarium graminearum	%	79.98 ± 6.89
Fusarium culmorum	%	83.32 ± 9.36
Sclerotium bataticola	%	79.76 ± 4.46
Sclerotinia sclerotiorum	%	73.58 ± 4.02

3.2. Variation of Soil Chemical Properties under Organic and Chemical Fertilizer Application and Different Crop Cultivation

The physical and chemical analyses carried out on the soil samples taken from different soil layers made it possible to characterize the red preluvosoil from the Moara Domnească experimental field. Thus, the Ap horizon (0–22 cm) has a dark brown color (7.5YR 3/2) when wet and a brown (7.5YR 4/1) color when dry, a granular structure, clayey texture, and fine sand grains; it is poorly compacted and has many roots. The soil had a high BD, a very low TP, and a medium GD.

The Ao horizon (22–55 cm) has a dark brown color (7.5YR 3/2) in the upper part when wet and a brown color (7.5 YR 4/1) when dry, and a brown color (7.5YR 4/2) in the lower part when wet and a brown color (7.5 YR 5/2) when dry. It is moderately compacted and has many roots in the upper part, a loamy-sandy texture, sand grains, and a large polyhedral structure. The soil BD was very high, the TP had a medium value, and the GD was 25.86%.

The A/B horizon (55–86 cm) is brown in color (7.5YR 4/2) when wet, with a loamy-clayey texture, moderately compacted in the upper part, highly compacted in the lower part, has rare roots, and a poorly developed polyhedral structure. The BD was high, TP was very low, and GD was very high.

The Bt_1 horizon (86–149 cm) has a dark brown color (7.5YR 5/2) in the wet state, with a loamy-clayey texture, a polyhedral structure, is highly compacted, and has few sparse roots. The BD value was very high, and the TP value was very low.

The Bt_2 horizon (149–220 cm) has a brown color (7.5YR 5/3) when wet, with fine sand grains, a loamy-clayey texture and a polyhedral structure; it is moderately compacted and has manganese oxides. This soil had a very high BD value, low TP values, and a high GD value (Table S2).

The pH value is moderately acidic in the Ap horizon and very slightly alkaline in the Bt_2 layer, with the value determined in the top soil layer (Ap) being lower than the values characterizing the preluvosoil (6.2–6.7) [67]. The C_{org} and humus contents decrease with the depth of the layer, with the values in the upper horizon (Ap) being higher. The humus content in the preluvosoil is generally between 2 and 4% [42]. In the arable layer (0–22 cm), the Nt content indicates a medium fertility of the soil. The soil has a high supply of available P in all soil horizons, with values ranging from 158 mg/kg in Ap and Ao to 148 mg/kg in Bt. The K supply of the soil is also very good in the Ap horizon and good in the deeper layers (Table S2).

The analyses of soil samples taken from a depth of 0–20 cm before the establishment of the experiment (October 2021) showed that the soil in its initial state had a medium acidic pH and the mean value of the C_{org} content in the soil was 1.94%. The soil was well supplied with Nt and very well supplied with P and K (Table 5).

Table 5. Chemical properties of soil before starting the experiment (October 2021).

Soil Chemical Properties	Measurement Unit	Value ± SD
pH	-	5.89 ± 0.176
C_{org}	%	1.94 ± 0.113
Humus	%	3.34 ± 0.196
Nt	%	0.20 ± 0.012
N-NO_3	mg/kg d.m.	11.85 ± 4.80
N-NH_4	mg/kg d.m.	12.14 ± 3.60
Available P	mg/kg d.m	65.50 ± 13.60
Available K	mg/kg d.m	231.70 ± 30.20

Abbreviations: standard deviation (SD); organic carbon (C_{org}); total nitrogen (Nt); nitrate nitrogen (N-NO_3); ammonia (N-NH_4); milligrams (mg); dry matter (d.m.).

Compared to the initial state of the soil in October 2021, some of the soil chemical properties changed in spring. For example, the pH value of the soil averaged 5.89 and did not change over the winter. The C_{org} and humus content recorded higher values and the Nt value was lower on average than in October 2021 (Table 6).

Table 6. Soil chemical properties in the spring (March 2022).

Soil Chemical Properties	Measurement Unit	Mean Values ± SD
pH	-	5.89 ± 0.148
C_{org}	%	2.11 ± 0.078
Humus	%	3.65 ± 0.135
Nt	%	0.17 ± 0.006

Abbreviations: standard deviation (SD); organic carbon (C_{org}); total nitrogen (Nt).

The application of different doses of MC and chemical fertilizers resulted in a variation of some soil chemical properties over the growing season and from one variant to another in the plots cultivated with a mixture of perennial grasses and legumes, winter wheat, maize, and soybean. In June, in the soil from the mixture of perennial grasses and legumes, the lowest pH value was recorded in V2 (moderately acidic), where only NPK was applied, whereby the differences compared to the control were distinctly significantly negative, and the highest values were recorded in V3 and V5, which indicates slightly acidic soil.

The C_{org} content also varied slightly, recording values of 2.01% in variant V1 and 2.3% in V7, with a very significant difference compared to the control, with an increase of 0.29% when 60 t/ha MC was applied. In the other variants, the values did not vary from one treatment to another and were distinctly significant, except in V3, where the difference was significant, and the increase was only 0.16% compared to the control. The highest humus content in the soil was also determined in variant V7 (3.96%), followed by V8, whereby the differences compared to the control were statistically significant ($p < 0.05$). For V3, V4, V5, and V6, the differences were significant ($p < 0.05$). The Nt content in the soil ranged from 0.154% (V1) to 0.180% (V7), and except for V2, where the differences were significant, the values recorded in the other treatments were very significant (Table S3).

In winter wheat plots, the lowest pH value was recorded in V4 and the highest value in V5. Small differences were found between V3, V5, and V7, but compared to the control, they were distinctly significant. The C_{org} values in the soil ranged from 1.99% (V4) to 2.36% (V6). Significant ($p < 0.05$) differences were observed between V3 and V6 compared to the control, while insignificant differences were observed between the other variants. Humus content was influenced by soil C_{org}, with the obtained values varying as follows: V6 > V7, V3 > V5, and V8 > V2 > V4 > V1. The Nt content ranged from 0.159% (V4) to 0.184% (V6). Compared to V1, the results in V6, V7, and V3 were very significant (Table S3).

Differences between treatments were also observed in the maize plots in June. The pH ranged from 5.46 (V4) to 5.90 (V3), a difference that was distinctly significant compared to the control. The highest C_{org} value, which was distinctly significant, was determined in V5 followed by V6 and V7. The lowest C_{org} value of the soil was found in the control. The soil humus content was related to the C_{org} content; the lowest value was found in the control variant and the highest value in V5, a difference that was also distinctly significant. The Nt content in the soil ranged from 0.168% (V1) to 0.189% (V5), with the value being distinctly significant.

Under soybean cultivation, the values of the soil chemical properties differed between the treatments and compared to the control. Regarding soil pH, Table S3 shows that the differences between the control, the variants where different doses of MC were applied, and the variants where MC was applied in combination with NPK were insignificant and ranged between 5.82 (V7) and 6.21 (V3). The lowest value of C_{org} in the soil samples analyzed was recorded in V1 and the highest value in V4. In V4 and V3 the differences compared to the control were very significant. The humus and Nt contents were influenced by the different doses of MC in combination with mineral fertilizers, the highest values being determined in V4 (Table S3).

In the autumn of 2022, chemical analyses were also performed to determine whether there were seasonal changes in the soil chemical properties. The results varied under the different treatment conditions (Table S4). For example, the pH values in the mixture of perennial grasses and legumes varied from one treatment to another ($p < 0.05$). In variant V6, the pH had the highest value (6.06), indicating a slightly acidic soil, and the lowest value was observed in V2 (NPK only), where the soil was described as acidic. The soil C_{org} content showed lower but very significant values compared to the summer period, ranging from 1.88% in V1 to 2.26% in V6. The soil humus content varied between treatments, with the highest value recorded in V6. The Nt content was higher in autumn than in the summer. V3 had the lowest Nt content compared to the control, and V6 had the highest Nt content.

In the winter wheat plots, soil pH values were lower in the treated variants than in the control, indicating an acidic soil. The Corg content was higher in V8, followed by V4 and V5, but these values were insignificant compared to the control variant. The Nt and humus contents also varied between treatments ($p < 0.05$) and the highest values were recorded in variant V8 (60 t/ha MC and NPK fertilizers), namely 4.31% and 0.240%, respectively.

After maize cultivation, pH values in the treated variants were generally lower than in the control, and the lowest value was observed in variants V4 and V7, which characterize an acidic soil, with slight variations between treatments. In almost all variants, C_{org} and humus contents were slightly lower than in summer, except in variants V6 and V8, where

values were slightly higher, i.e., 2.37% and 2.30% for C_{org} and 4.09% and 3.97% for humus, respectively (Table S4). In the autumn, the values of Nt content did not differ much between treatments, but they were higher than in the growing season. The highest value was recorded in V6, with a significant positive difference compared to the control. Variant V6 was followed by V8 with 0.222%, which was also significant.

In the fall, there were differences between the treatments in terms of pH in the soybean plots ($p < 0.05$). A maximum value of 6.27 was determined in V3, which indicates a slightly acidic soil. This value was significant compared to the control. The C_{org} content of the soil also varied, and the maximum value was recorded in V6, followed by V4 and V8. Compared to the control, the values in the variants V6 and V4 were very significant. In addition, the variants V6 and V4 were the ones with the highest values for humus and Nt content (Table S4).

3.3. Variation of Soil Microbial Abundance and Their Antifungal Efficiency under Organic and Mineral Fertilization and after Different Crop Cultivation

The soil samples analyzed before the start of the experiment showed a bacterial load of 10^7 CFU/g soil and a fungal load of 10^5 CFU/g soil. In addition, microorganisms that are beneficial to agricultural crops and are involved in atmospheric nitrogen fixation or biological pest control (e.g., fluorescent pseudomonads, rhizobia, or microorganisms that solubilize phosphorus from mineral compounds) were identified (Table 7).

Table 7. Initial microbial load and the component of microbial communities of Moara Domnească red preluvosoil.

Microbial Composition	Measurement Unit	Average Microbial Load
Cultivable bacteria	CFU/g soil	3.7×10^7
Fungi	CFU/g soil	2.0×10^5
Fluorescent pseudomonads	CFU/g soil	6.5×10^6
Microorganisms that solubilize inorganic phosphorus	CFU/g soil	4.0×10^6
Chitinolytic microorganisms	CFU/g soil	7.5×10^6
Cellulolytic microorganisms	CFU/g soil	4.5×10^6
Proteolytic microorganisms	CFU/g soil	8.0×10^6
Rhizobia	CFU/g soil	1.3×10^6

Abbreviations: colony-forming units (CFU).

In our experiment, in the autumn, under a mixture of perennial grasses and legumes and different doses of organic and mineral fertilizers, cultivable bacteria (10^7 CFU/g soil), chitinolytic, proteolytic, and cellulolytic microorganisms (10^6–10^7 CFU/g soil), and rhizobia (10^6 CFU/g soil) were the most abundant groups in the soil samples analyzed. The fungal load ranged from 1.8×10^5 CFU/g soil (V7) to 7.4×10^5 CFU/g soil in V4. The same observations can also be made for the cultivable bacteria, with the highest load being counted in V3 and the lowest load, in V7. The microorganisms that solubilize the inorganic P were abundant in V4 (15 t/ha MC + NPK), while a high number of chitinolytic microorganisms was found in V2 and V3. Also, in V2 (NPK only), high loads of cellulolytic microorganisms and proteolytic microorganisms were observed. Rhizobia colonies were more common in V3 and V8 (Table 8).

In the winter wheat plots, the fertilization regime also influenced the abundance of microorganisms in the soil. For example, the number of fungi was higher when 30 t/ha MC was applied to the soil, followed by the variant with 60 t/ha MC in combination with NPK fertilization. The number of cultivable bacteria was higher in variant V6, followed by V5 and V4. Fluorescent pseudomonads colonies were low in all treatments and the activity of microorganisms involved in the mineralization of inorganic phosphorous was increased by the application of 30 t/ha of MC and NPK fertilizer (V6). The highest chitinase and cellulolytic activity was observed in the variants with high doses of MC (V7) and MC combined with NPK fertilization (V8). Proteolytic microorganisms were more abundant

in the soil treated with 60 t/ha MC and in V2 (NPK only). The number of rhizobia did not vary much between treatments. However, the highest load was found in variant V5 (30 t/ha MC).

Table 8. Microbial composition and abundance in soil cultivated with winter wheat, a mixture of perennial grasses and legumes, soybean, and maize under different fertilization treatments in autumn (October 2022).

Microbial Composition	V1—Control	V2—NPK	V3—15 t/ha MC	V4—15 t/ha MC + NPK	V5—30 t/ha MC	V6—30 t/ha MC + NPK	V7—60 t/ha MC	V8—60 t/ha MC + NPK
Mixture of perennial grasses and legumes experiment								
Fungi (CFU/g)	4.9×10^5	3.5×10^5	5.3×10^5	7.4×10^5	3.4×10^5	4.2×10^5	1.8×10^5	5.1×10^5
Cultivable bacteria (CFU/g)	4.0×10^7	4.5×10^7	7.5×10^7	4.0×10^7	5.0×10^7	5.5×10^7	2.0×10^7	4.5×10^7
Fluorescent pseudomonads (CFU/g)	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$
Microorganisms that solubilize inorganic phosphorus (CFU/g)	3.5×10^6	3.5×10^6	5.5×10^6	7.5×10^6	4.5×10^6	5.0×10^6	3.0×10^6	5.5×10^6
Chitinolytic microorganisms (CFU/g)	2.5×10^6	9.5×10^6	8.5×10^6	4.0×10^6	3.0×10^6	4.5×10^6	3.0×10^6	5.5×10^6
Cellulolytic microorganisms (CFU/g)	1.8×10^7	2.4×10^7	1.6×10^7	2.3×10^7	1.0×10^7	1.6×10^7	1.0×10^7	1.0×10^6
Proteolytic microorganisms (CFU/g)	8.0×10^6	3.0×10^7	1.6×10^7	1.0×10^7	1.0×10^7	1.5×10^7	7.0×10^6	1.2×10^7
Rhizobia (CFU/g soil)	3.1×10^6	2.8×10^6	3.9×10^6	3.7×10^6	1.6×10^6	2.0×10^6	2.2×10^6	3.9×10^6
Winter wheat experiment								
Fungi (CFU/g)	2.2×10^5	1.1×10^5	1.6×10^5	1.4×10^5	2.9×10^5	1.9×10^5	1.6×10^5	2.4×10^5
Cultivable bacteria (CFU/g)	3.5×10^7	3.0×10^7	1.0×10^7	4.0×10^7	4.0×10^7	5.5×10^7	2.5×10^7	1.5×10^7
Fluorescent pseudomonades (CFU/g)	1×10^3	$\leq 10^3$	1×10^3	$\leq 10^3$	1×10^3	$\leq 10^3$	1×10^3	$\leq 10^3$
Microorganisms that solubilize inorganic phosphorus (CFU/g)	9.5×10^5	1.5×10^6	9.0×10^5	1.0×10^6	3.5×10^6	8.0×10^6	2.0×10^6	1.0×10^6
Chitinolytic microorganisms (CFU/g)	8.3×10^5	2.0×10^5	6.0×10^5	1.0×10^5	9.5×10^5	9.5×10^5	1.0×10^6	1.0×10^6
Cellulolytic microorganisms (CFU/g)	6.5×10^6	5.0×10^6	6.5×10^6	6.0×10^6	7.0×10^6	9.5×10^6	8.0×10^6	7.5×10^6
Proteolytic microorganisms (CFU/g)	1.0×10^6	7.0×10^6	2.0×10^6	$<10^6$	6.0×10^6	1.1×10^6	7.5×10^6	4.0×10^6
Rhizobia (CFU/g soil)	3.6×10^6	3.4×10^6	2.3×10^6	3.4×10^6	2.9×10^6	4.0×10^6	3.6×10^6	2.7×10^6
Soybean experiment								
Fungi (CFU/g)	2.9×10^5	2.1×10^5	0.9×10^5	0.8×10^5	0.8×10^5	0.7×10^5	0.9×10^5	0.9×10^5
Cultivable bacteria (CFU/g)	2.5×10^7	2.0×10^7	2.0×10^7	1.5×10^7	3.0×10^7	3.0×10^7	3.5×10^7	2.5×10^7
Fluorescent pseudomonades (CFU/g)	1×10^3	$\leq 10^3$	1×10^4	$\leq 10^4$	$\leq 10^4$	$\leq 10^3$	$\leq 10^4$	$\leq 10^3$
Microorganisms that solubilize inorganic phosphorus (CFU/g)	2.5×10^6	1.5×10^6	1.0×10^6	0.3×10^6	1.0×10^6	0.5×10^6	1.0×10^6	1.0×10^6
Chitinolytic microorganisms (CFU/g)	5.0×10^5	1.3×10^6	1.0×10^6	5.0×10^5	3.0×10^5	1.0×10^6	6.0×10^5	1.0×10^6
Cellulolytic microorganisms (CFU/g)	6.0×10^6	5.5×10^6	5.5×10^6	4.0×10^6	4.5×10^6	6.5×10^6	4.5×10^6	2.0×10^6
Proteolytic microorganisms (CFU/g)	9.0×10^6	5.0×10^6	9.5×10^6	2.0×10^6	6.0×10^6	6.5×10^6	4.0×10^6	1.0×10^6
Rhizobia (CFU/g soil)	4.6×10^6	2.7×10^6	2.6×10^6	1.6×10^6	3.8×10^6	1.5×10^6	1.2×10^6	2.8×10^6
Maize experiment								
Fungi (CFU/g)	4.6×10^5	3.1×10^5	3.3×10^5	5.2×10^5	3.8×10^5	4.4×10^5	4.7×10^5	4.2×10^5
Cultivable bacteria (CFU/g)	6.0×10^7	4.5×10^7	3.0×10^7	3.5×10^7	7.0×10^7	3.5×10^7	7.0×10^7	4.5×10^7
Fluorescent pseudomonads (CFU/g)	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$
Microorganisms that solubilize inorganic phosphorus (CFU/g)	5.0×10^6	3.5×10^6	4.5×10^6	4.0×10^6	7.0×10^6	5.0×10^6	6.0×10^6	4.0×10^6

Table 8. *Cont.*

Microbial Composition	V1—Control	V2—NPK	V3—15 t/ha MC	V4—15 t/ha MC + NPK	V5—30 t/ha MC	V6—30 t/ha MC + NPK	V7—60 t/ha MC	V8—60 t/ha MC + NPK
Chitinolytic microorganisms (CFU/g)	4.0×10^6	5.0×10^6	4.5×10^6	2.5×10^6	4.3×10^6	4.0×10^6	2.5×10^6	3.0×10^6
Cellulolytic microorganisms (CFU/g)	2.0×10^7	2.0×10^7	2.0×10^7	1×10^7	1.1×10^7	1.3×10^7	4.3×10^7	1.1×10^7
Proteolytic microorganisms (CFU/g)	7.0×10^6	7.5×10^6	7.0×10^6	7.5×10^6	8.0×10^6	1.5×10^7	1.5×10^7	8.0×10^6
Rhizobia (CFU/g soil)	2.2×10^6	1.5×10^6	1.1×10^6	2.0×10^6	1.4×10^6	1.0×10^6	2.9×10^6	1.1×10^6

Abbreviations: colony-forming units (CFU); manure compost (MC); NPK only (NPK).

Under soybean cultivation and the application of mineral and organic amendments, the microbial population changed. Fungal activity was influenced by the MC application. Compared to the control, the number of fungal colonies was lower in the variants treated with MC and MC + NPK fertilizers, and the differences between treatments were not significant (Table 8). In the treated variants, the highest load of cultivable bacteria was found in variant V7 followed by V6 and V5. The load of fluorescent pseudomonads was low in all treatments. The microorganisms that solubilize inorganic phosphorus and the rhizobia load were negatively affected by the organic and mineral fertilization compared to the control variant, where the highest number was recorded. The abundance of chitinolytic microorganisms was somewhat low, with the highest reached in V2. A high load of cellulolytic microorganisms was found in V6 compared to the control and the load of proteolytic microorganisms was higher in V3 compared to V1.

In the maize plots, the fungal population did not vary too much between treatments over the same period, with the highest load observed in V4. However, the lowest number of cellulolytic microorganisms was found in this variant. Many cultivable bacteria were found in variants V5 and V7 and the highest number of microorganisms that solubilize the inorganic phosphorus was also found in these variants. Pseudomonads colonies were also reduced (below 10^3). In the maize plots where 30 t/ha MC + NPK and 60 t/ha MC were applied, a high abundance of proteolytic microorganisms was observed. There were no major differences in rhizobia between the variants, with the highest numbers recorded in V7.

Regarding the initial antifungal effect of the soil microorganisms, it can be seen in Table 9 that the percentage of fungal inhibition was medium to high in the initial phase of the experiment.

Table 9. Antifungal efficacy of the microbial communities in the initial soil samples.

Phytopathogenic Fungus	Measurement Unit	Antifungal Efficacy of Microorganisms (E) (Mean Values)
Fusarium graminearum	%	82.22 ± 9.55
Fusarium culmorum	%	80.12 ± 9.28
Sclerotium bataticola	%	80.50 ± 6.35
Sclerotinia sclerotiorum	%	73.95 ± 4.99

In all experiments and for all treatment variants, the antifungal activity of the soil microbial communities against different plant pathogenic fungi (*Fusarium culmorum*, *Fusarium graminearum*, *Sclerotinia sclerotiorum*, and *Sclerotium bataticola*) was expressed as a percentage of the pathogenic growth inhibition efficacy. Figure 4a shows that the highest antifungal efficacy was found for *Fusarium culmorum* in the winter wheat experiment, with values ranging from 67.44% (V2) to 90.7% in V8. For *Fusarium graminearum*, an important pathogen for winter wheat that can lead to considerable yield reductions, the antifungal efficacy ranged from 65.21% (V8) to 78.26% (V6). The inhibitory effect against *Sclerotium bataticola* was weak to medium (from 30.43% in V2 to 70.65% in V6) and was medium in all variants

against *Sclerotinia sclerotiorum*, with the lowest value in V2 (41.3%) and the highest value in V6 (61.96%).

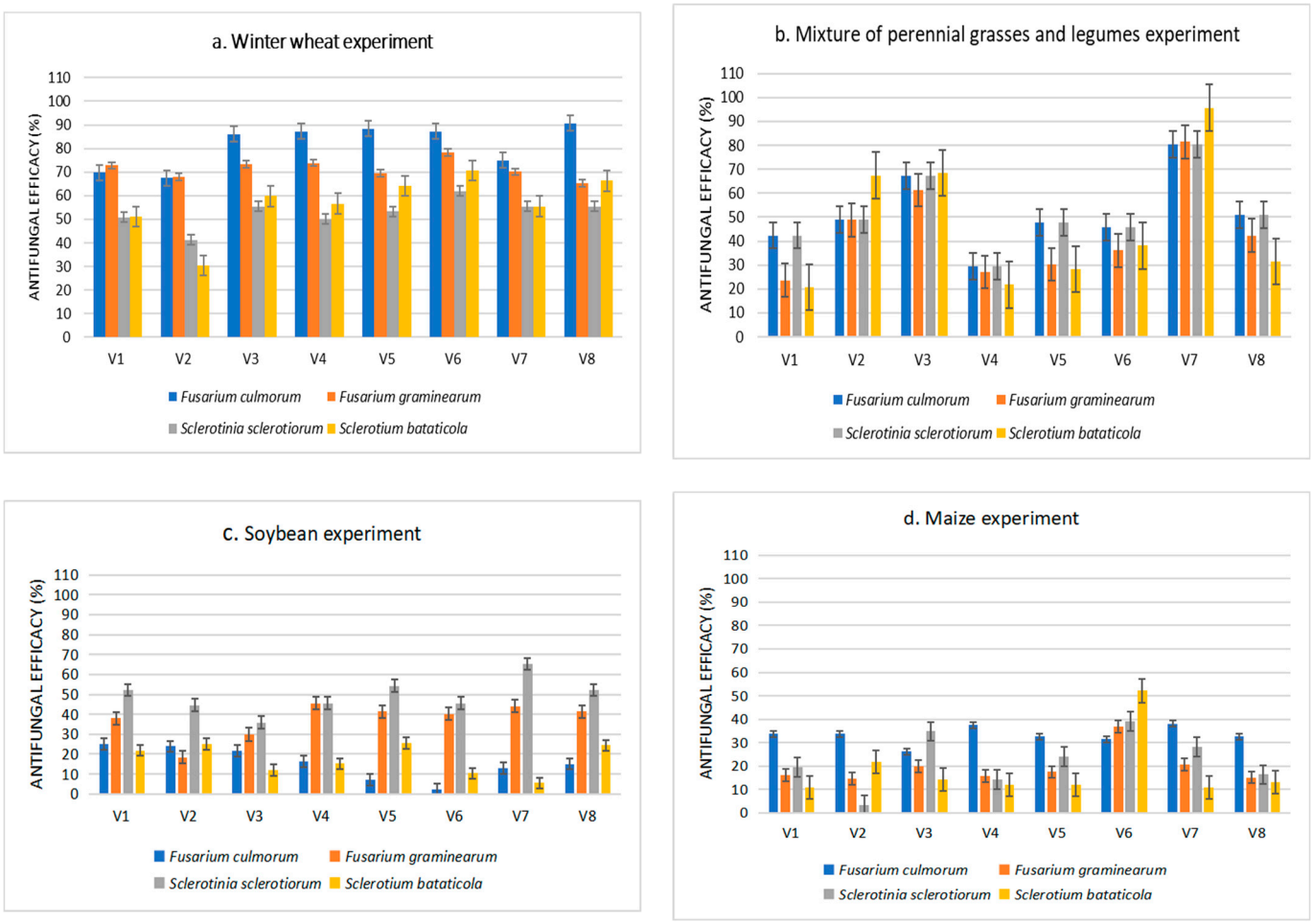

Figure 4. (**a**–**d**). Antifungal efficiency of soil microorganisms from (**a**) winter wheat, (**b**) mixture of perennial grasses and legumes, (**c**) soybean, and (**d**) maize under different fertilization treatments. Errors bars represent the standard error.

In the experiment with a mixture of perennial grasses and legumes, the soil microbial population from V7 (60 t/ha MC) had a high inhibitory effect against all pathogens tested, with values ranging from 80.43% (for *Fusarium culmorum* and *Sclerotinia sclerotiorum* pathogens) to 95.65% (for *Sclerotium bataticola*). A medium inhibitory effect was observed in V3 samples (15 t/ha MC) with minimum values of 61.34% (for *Fusarium graminearum*) and maximum values of 68.48% (for *Sclerotium bataticola*) (Figures 4b and 5A,B).

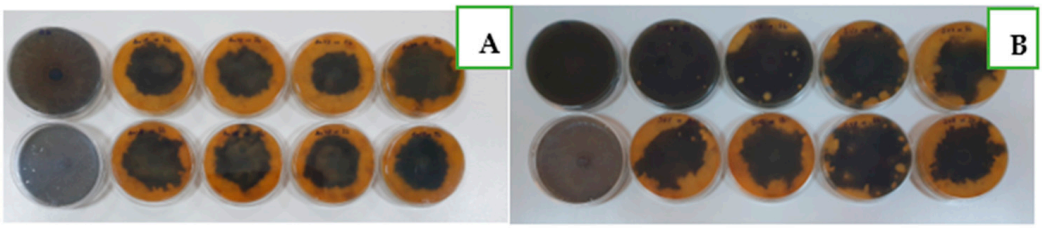

Figure 5. (**A**,**B**). Inhibitory effect of soil microorganisms from (**A**) a mixture of perennial grasses and legumes and (**B**) winter wheat plots against *Sclerotium bataticola*.

In the soil samples taken from the soybean plots in the fall, the microbial population had a low inhibitory effect against *Fusarium culmorum* (values ranged from 2.33% in V6 to 25% in the control) and against *Sclerotium bataticola* (from 5.43% in V7 to 25.54% in V5). The inhibition effect for *Sclerotinia sclerotiorum* was highest in variant V7 (65.22%) (Figure 4c).

The microorganisms counted in the soil samples from the maize plots had a low antifungal effect on the pathogens tested. As for the efficacy against *Fusarium graminearum*, a pathogen that can reduce maize yield and produce toxins that can affect human health, the values ranged from 15.12% (V8) to 36.83% (in V6). Low inhibition against *Sclerotinia sclerotiorum* and *Sclerotium bataticola* was also found in all treatment variants, except in V6 (30 t/ha MC + NPK), where efficacy against *Sclerotium bataticola* was 52.17%. The inhibitory effect on *Fusarium culmorum* was medium (Figure 4d).

4. Discussion

Knowledge of soil quality is very important for evaluating the positive or negative effects of agricultural practices, integrating the results obtained into the management process, promoting sustainable agricultural practices, and monitoring the changes in soil indicators during the growing season [68]. According to Zhang et al. [69], mineral fertilization alone has no effect on certain parameters such as Nt in the short term, but the combination of chemical fertilizers with organic amendments can increase Nt content. In addition, Liu et al. [70] indicated that soil organic matter, Nt content, and pH did not change with short-term application of mineral and organic fertilizers. Our results showed a slight variation of pH, soil C_{org}, humus, and Nt between treatments and between all experimental plots during the whole growing season, which also agrees with Deru et al. [71], who reported that chemical parameters were only weakly affected by fertilizers and their effects can only be seen after a longer period of time. The combined application of different doses of MC and NPK led to a decrease in soil pH in most cases (see Tables S3 and S4). A decrease in soil pH was also observed in almost all treatments in the autumn, with some exceptions. This decrease cannot be considered threatening to soil quality as the values are close to neutrality. Nevertheless, we must consider in the future that optimizing the N fertilizer doses to the actual crop needs may reduce the acidification potential through lower nitrate leaching and indirectly lower N deposition due to reduced NH_3 emissions [72].

Soil C_{org} is a key factor for the soil fertility of agroecosystems and therefore all agricultural practices that may lead to a change in its content should be considered to avoid dangerous environmental changes [73]. Compared to the initial state of the soil, our data showed, as expected, that the C_{org} and humus contents increased after the application of different doses of MC and MC in combination with NPK fertilization, in all treatment variants and independently of all crops, with some exceptions. This agrees with the results of Kumar et al. [74] and is particularly important because it can have a major impact on the parameters that determine the productivity and sustainability of agroecosystems [75]. Moreover, improving soil organic matter content could be an important strategy to mitigate climate change and make agroecosystems more resilient [76] and is particularly important in our case, mainly because the soil of the Moara Domnească has a generally low organic matter content. The increase in soil C_{org} content due to the addition of organic fertilizers can be caused by the higher organic matter content in the manure compost. According to Angelova et al. and Li et al. [77,78], the C_{org} and humus contents of the soil increase with the fertilizer doses. In our experiments, this can only be associated with the soil from the mixture of perennial grasses and legumes. In the autumn, the decrease in the C_{org} and humus content of the soil was low compared to the summer. Rosa and Debska [79] also mentioned the decrease in C_{org} content in autumn compared to spring, which could be explained by the migration of dissolved C_{org} into the deeper soil profile layers.

Nitrogen dynamics in soil depend on several factors such as N_2 fixation, nitrification, denitrification, N mineralization, and immobilization as well as nitrogen uptake by plants [80]. In our experiment, the Nt content changed during the growing season. In summer, the values were lower than in the autumn. These results agree with those of

Taylor et al. [81], who explained that the low nitrogen content in the soil during the summer months could be due to the decrease in ammonium and nitrate production, with uptake rates exceeding mineralization rates. The greatest amount of nitrate and ammonium nitrogen accumulates in spring and autumn due to reduced nitrification–ammonification processes and high nitrogen consumption by plants and soil microorganisms in the summer [82]. Seasonal changes in soil organic matter are one of the most controversial issues in the scientific literature. The main factors that can lead to changes in C_{org} and Nt in soils are organic and mineral fertilization, soil water regime, soil temperature regime, microbiological activity, etc. In our future studies, it is therefore important to evaluate these factors in more detail and to analyze the variation of soil parameters in the soil profile [82].

Good soil quality, characterized among other things by a diverse and rich microbial community, is also a prerequisite for plant growth and optimal yields [83]. According to He et al. [84], the abundance of microorganisms in the topsoil can be influenced by some soil chemical properties, crop species, fertilization, or seasonal variation in climatic conditions. Soil microbial composition and abundance change with the seasons, possibly due to differences in soil temperature, moisture, and soil organic matter content [85]. In addition, soil microbial abundance and diversity can be influenced by fertilizer application, especially mineral fertilization [86]. Geisseler and Scow [87] found that mineral fertilizers can significantly increase soil microbial biomass, while Lazcano et al. [88] reported that organic fertilizers stimulate soil microbial activity and increase soil microbial biomass compared to mineral fertilizers. According to our results, the soil microbial load in autumn was influenced by the fertilization regime and the cultivated species, but there were no significant differences between the variants (see Table 8).

There is intense bacterial activity in the rhizosphere, with both beneficial and phytopathogenic organisms attracted to the root exudates [89]. In general, the abundance of the bacterial population in soil is higher than that of fungi [85], which is also consistent with our results. These results could be because the bacterial population dominates in soils with lower carbon storage, in contrast to fungi [90]. Microbial populations show complex relationships in nutrient uptake and utilization, so bacteria and fungi may not respond in the same way to N and/or P addition [91]. In the soil under a mixture of perennial grasses and legumes, the lowest load of fungi, cultivable bacteria, microorganisms that solubilize inorganic phosphorus, and proteolytic microorganisms was found in the variant with 60 t/ha MC, while the highest load was recorded in the variant with exclusive NPK application (in the case of chitinolytic, cellulolytic, and proteolytic microorganisms), in the variant with 15 t/ha MC and NPK fertilizers (for fungi and microorganisms that solubilize inorganic phosphorus), and in the variant with only 15 t/ha MC (for cultivable bacteria and rhizobia). In the winter wheat plots, the high doses of manure compost (30 t/ha and 60 t/ha) applied alone or in combination with mineral fertilizers had a positive effect on the soil microbial population, with the highest load recorded in all these variants. These results are consistent with the conclusions of the study by Liu et al. [92], in which the combination of manure and chemical fertilizers showed great benefits in terms of the bacterial community.

High levels of cultivable bacteria (V7) and cellulolytic microorganisms (V6) were found when soybean was grown, and high doses of manure compost (60 t/ha) were applied alone or in combination with NPK in the autumn. These results are consistent with other studies conducted in soybean plots [93] which showed an improvement in the beneficial bacteria population when organic fertilizers were applied. Fungi, microorganisms that solubilize inorganic P and rhizobia populations were negatively affected by organic and mineral fertilization, with the highest number observed in the non-fertilized variant. This agrees with Lourenço et al. [94], who stated that the application of mineral fertilizer alone or in combination with organic fertilizer can reduce the saprotrophs fungi population by 40%.

The high MC doses (60 t/ha) for maize increased the soil load of cultivable bacteria, cellulolytic and proteolytic microorganisms, and rhizobia in the soil (except for chitinolytic microorganisms), and the MC dose of 30 t/ha was also beneficial for cultivable bacteria and

microorganisms that solubilize inorganic phosphorus. These results agree with those of another study [95], which showed that the highest abundance and diversity of bacteria and archaea was recorded in the rhizosphere of maize and under MC application. The fungal load did not vary much between treatments. As for the fluorescent pseudomonads in the soil, the levels were lower in all treatments.

The highest bacterial load was recorded in soil with a mixture of perennial grasses and legumes in the variant with only 15 t/ha MC, followed by soil cultivated with maize and fertilized with 30 t/ha and 60 t/ha MC. A higher number of fungi was observed in the mixture of perennial grasses and legumes, while in the winter wheat experiment, the number of rhizobia was higher despite the fertilization regime. In the other cultivated plots, the organic and mineral fertilization reduced the rhizobia load. Under soybean cultivation, the microbial population density was influenced by the fertilization regime, which was also found by Guo et al. [96].

Overall, we found that the soil microbial population was improved by organic and mineral fertilization, but the response to nutrient addition varied from one crop to another and from one fertilization regime to another. In most cases, organic fertilization only increased the number of cultivable bacteria and rhizobia, except for winter wheat plots, where the combination of 30 t/ha MC and NPK had a greater effect on the population. Regardless of the fertilizer dose, the application of MC increased the abundance of proteolytic microorganisms, which are responsible for organic substrate decomposition and can convert ammonium to nitrate [97]. This was also found by Liang et al. [98], who observed the effects of different fertilizer treatments on the bacterial community in the rhizosphere. The number of chitinolytic microorganisms increased especially in the soil where only NPK fertilizers were applied. In addition, the cellulolytic microorganisms were more abundant in soils treated with a combination of MC and mineral fertilizers and with high doses of MC. The microorganisms that solubilize inorganic phosphorus were positively affected by the application of MC and NPK, except in the soybean rhizosphere, where the highest number was found in the unfertilized variant. The fungal population was higher in the soil fertilized with MC in combination with mineral fertilizers and MC only. Since the results indicate that the effects of organic and mineral fertilizers on soil microorganisms depend on the type of the soil and crop, fertilization regime, etc., their effects on soil microbial properties still need to be studied in the long term and dynamically. Further research should also be carried out to identify the specific microbial communities affected by chemical or organic fertilizer applications, as the bacterial or fungal populations may not respond in the same way due to their ecological differences.

The antibiotics produced by various microorganisms isolated from the soil can suppress or inhibit the growth of some plant pathogens. For example, soil bacteria produce extracellular lytic enzymes, siderophores, salicylic acid, antibiotics, and volatile metabolites that have antifungal activity [99]. Our studies have shown that although a rich diversity of microorganisms was found in the soil both before organic and mineral fertilization and at the end of the growing season, the antifungal efficacy was medium to low, except for the winter wheat experiment, where the efficacy of the microbial population found in the soil was medium to high against *Fusarium culmorum* and medium against other pathogens. This high inhibitory effect can be explained by the presence of microorganisms in the samples that produce lytic enzymes capable of degrading the cellular components of plant pathogenic fungi. In the autumn, the antagonistic activity of the microorganisms isolated from the soil was medium to low in the other cultivated species. Thus, we can say that fertilization affects the growth of plant pathogens and their antagonists. Compared to our results, in other research, organic fertilizers suppressed fungal phytopathogens, and conversely, mineral fertilization increased the abundance of pathogens in the rhizosphere. However, organic fertilizers had, on average, a medium inhibitory effect on the fungal phytopathogens studied and may therefore be used to enhance soil suppressiveness against plant pathogens [100]. Also, the use of fungicide seed treatment or cultivar resistance is an

integrated approach to protect crops against different pathogens throughout the growing season [101].

Maintaining an active ecosystem at the soil level is crucial for improving the biological properties and agronomic value of soils such as the red preluvosoil soil of Moara Domnească and thus for maintaining its health. Therefore, healthy soil can influence agroecosystem sustainability, provide more ecosystem services (e.g., nutrient cycling, improvement of microbial diversity, etc.), improve crop yields, and also be more resilient to different factors. This could be possible through the application of different practices such as diversification of nutrient sources, especially organic sources [102]. The results obtained thus form the basis for further studies on how fertilization practices could affect soil properties, microbial diversity, and resilience in the short and long term in a preluvosoil from southeastern Romania.

5. Conclusions

The study highlights the short-term changes in the chemical properties of the soil during the growing season after different doses of organic and mineral fertilizer application, the activity of microbial populations after crop harvesting compared to the basic properties of the soil before fertilization, and determines which fertilization treatment is most suitable for improving soil fertility and for growing different crops in a red preluvosoil from a sylvosteppe area. One of the objectives was also to show the inhibitory effect of the microorganisms isolated from the soil on some plant pathogens such as *Fusarium graminearum* and *Fusarium culmorum*, which are important pathogens for cereal crops, Sclerotium bataticola, a pathogen for grain legumes, and *Sclerotinia sclerotiorum*, a soil pathogen with a diverse host range.

The results show that soil chemical properties in the topsoil, which are important for some ecosystem services, were weakly influenced by fertilizers and their effects may become apparent in the long term. Thus, a slight variation in soil pH, C_{org}, humus, and Nt between treatments and between cultivated species was observed during the growing season. In most cases, the combined application of MC at different doses and NPK led to a decrease in soil pH. In the autumn, a decrease in soil pH was observed in almost all treatments, but this cannot have a negative impact on soil quality as the values are close to neutrality. The C_{org} and humus content increased after the application of different doses of MC and MC in combination with NPK fertilization in almost all treatment variants and under different crops. Because the use of organic fertilizers only is not reasonable for sustaining plant productivity, a combination of chemical and organic fertilizers could be the right solution, especially for soils with low contents in N, P, and organic C [103].

The microbial population changed under organic and mineral fertilization, but their response was different. Fertilization can rapidly replenish nutrient elements, increasing available nitrogen and phosphorus in the soil, which could further increase microbial activity. Organic fertilizers increased nutrient enrichment in the soil and created a rich environment for microorganisms. Compared with the unfertilized treatment, the application of manure compost in combination with mineral fertilizers increased the load of fungi, cellulolytic microorganisms, or microorganisms that solubilize inorganic phosphorus, while organic fertilizers can create a suitable environment for cultivable bacteria, proteolytic microorganisms, and fungi.

Our studies have shown that although a rich diversity of microorganisms was found in the soil before both organic and mineral fertilization and at the end of the growing season, the antifungal efficacy was medium to low, except in the winter wheat experiment, where the efficacy of the microbial population found in the soil was medium to high against *Fusarium culmorum* and medium against other pathogens.

Since the results indicate that the effects of organic and mineral fertilizers on soil chemical properties and microbial behavior depend on the type of the soil and crop, fertilization regime, etc., their effects still need to be investigated dynamically and not only seasonally, but in the long term.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13112837/s1, Figure S1: Rainfall (mm) and maximum and minimum air temperatures (°C) in the Moara Domnească area during September 2021–October 2022 period; Table S1: Air temperatures and rainfall values for the Moara Domnească area during September 2021–October 2022 vegetation period; Table S2: Red preluvosoil physicochemical properties at different soil layer depths; Table S3: Soil chemical properties under mineral and organic fertilizers and different crops (June 2022); Table S4: Soil chemical properties under mineral and organic fertilizers and after crop harvesting (October 2022).

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