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Consequences of the Long-Term Fertilization System Use on Physical and Microbiological Soil Status in the Western Polissia of Ukraine

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Abstract: The response of soil microbial diversity to long-term fertilization is still not well understood in the context of different soil types. The purpose of this research was to reveal the impact of fertilization systems on soil parameters and life activity of the main taxonomic and physiological groups of microorganisms responsible for nitrogen, carbon, and phosphorus transformation. Reported results were obtained in the course of a 55-year-long experiment on fertilization of sod-podzolic soil in a grain-flax-potato crop rotation. Soil sampling was conducted within a 0–20 cm depth in five sites: without fertilizer (C); organic fertilization system, manure (O1FS); mineral fertilization system, NPK (MFS); organic-mineral fertilization system, manure + NPK (O1MFS); and organic-mineral fertilization system, siderate + NPK (O2MFS). Long-term use of various fertilization systems has led to changes in the soil properties. Bacteria dominated the microbial community in all examined areas. Soil fertilization supported bacteria development in all variants, except for MFS, and negatively affected the micromycetes content. A strong relationship between the change of the main soil indicators and the number of microorganisms from the main taxonomic groups was found between the soil pH KCl and the number of micromycetes. The O1FS option had the most beneficial effect on the development of soil nitrifiers and denitrifiers. The O1MFS fertilization system was the most favorable for the development of non-symbiotic anaerobic nitrogen-fixing, cellulose-degrading and phosphate-mobilizing microorganisms. In turn, the least favorable conditions for the development of physiological groups of microorganisms were found in cases of continuous use of mineral fertilizers.

Keywords: long-term experiment; fertilization systems; soil properties; bacteria; micromycetes; physiological groups of microorganism

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1. Introduction

Soil is one of the most important natural resources on Earth and one of the world's most valuable assets. It serves as the basis for human food production systems, for crops grown for feed, fiber, and fuel, and it plays an important role in climate control and mitigation [1,2]. Continuous growth of the human population requires a corresponding increase in food production, which means more fertilizers will need to be applied to agricultural areas to enhance productivity [3]. However, the intensification of agriculture often leads to alarming changes in ecosystems [4]. High-performance agriculture production needs the best possible conditions where biological, chemical, and physical soil properties are maintained at a certain level through appropriate management practices [5]. The use of mineral and organic fertilizers is one of the key agricultural practices for increasing yields. However systematic application of fertilizers affects soil properties as well as the structure and function of the microbial component [6,7].

The assessment of soil conditions is still carried out only by chemical indicators, despite the growing awareness of the importance of soil biodiversity [1,8]. The soil microbiome is a fundamental driver of ecosystem functioning [9]. Soil microorganisms play an important role in shaping soil structure, decomposing and converting various organic compounds, and neutralizing toxic ones. Additionally, rhizospheric microbes colonizing plant roots and surrounding soils can accumulate certain substances, promote nutrient absorption by plants, directly or indirectly accelerate overall plant growth, contribute to stress resistance, and promote protective processes against biotic and abiotic factors [10]. Various types of bacteria and fungi improve soil structure, contributing to the formation of soil aggregates and soil pores [11]. They also play a key role in increasing soil fertility [12]. Soil microbial diversity is extremely sensitive to fertilization, which is one of the main anthropogenic actions associated with global changes [9]. The use of fertilizers is one of the important issues of sustainable agriculture, so more attention is paid to the reaction of soil parameters and its microbiological component [13].

Mineral and organic fertilizers generally increase soil microbial biomass by providing nutrients and/or carbon (C) to the soil microbiome [14,15]. It was found that the response of soil microbial diversity depends on the type of applied fertilizer (i.e., inorganic or organic) [16,17]. There are reports of changes in the microbial community involved in the N-cycle after long-term fertilization with mineral and organic fertilizers of animal and plant origin [18]. Long-term use of mineral fertilizers in combination with organic fertilizers enriches and diversifies the soil microbiome and increases the activity of soil enzymes [7]. Li et al. [19] indicated that the use of mineral and organic fertilizers causes an increase in the total number of bacteria by 92.57–178.38% and an increase in the carbon of microbial biomass by 7.57–20.87 times that of the control. The application of fertilizers directly affects soil microorganisms by increasing the availability of nutrients and indirectly by changing soil pH [20]. In soils, pH is usually indicated as the most important factor for the structure of soil prokaryotic communities [16,21–26]. It is well known that soil pH affects the number and diversity of prokaryotes [27–31]. Studies report an increase in microbial diversity or changes in group composition as a result of the introduction of organic farming [32–40]. Enriching the soil with organic fertilizers increases the number of bacteria that participate in nitrogen mineralization [35,41].

The effects of agricultural management on the soil microbiome are complex and diverse [42,43], and it is currently difficult to make universal conclusions about the effects of organic and conventional farming systems [32]. Most studies have focused mainly on natural ecosystems over relatively short periods of time (less than four years). Thus, a comprehensive understanding of long-term and large-scale effects is largely lacking. The direction and scale of the response of soil microbial diversity to long-term fertilization remain uncertain in different ecological contexts (e.g., different climate and soil types). Different fertilization systems lead to changes in the main soil characteristics, which is accompanied by changes in the vital activity of both taxonomic and physiological groups of microorganisms as a result of their response to the presence of nutrient substrates in the soil.

Sustainable management of soil fertility in agricultural systems with long-term fertilization should ensure the absence of degradation processes in microbial communities.

We assumed that long-term use of different fertilization systems not only leads to changes in the main characteristics of the soil but that it also creates different conditions for the existence of microorganisms (due to the presence of different substrates). The purpose of this study was to reveal the impact of continuous use of different fertilization systems on sod-podzolic soil parameters and the vital activity of the main taxonomic and physiological groups of soil microorganisms responsible for the nitrogen, carbon, and phosphorus transformation.

2. Materials and Methods

2.1. Research Area and the Characteristics of Experimental Plots

The research was carried out as part of a long-term stationary experiment titled "The influence of the main types of fertilizers and their combinations on the productivity of crop rotation and soil properties" on the basis of the Volyn State Agricultural Research Station of the Institute of Potato Growing of the National Academy of Agrarian Sciences of Ukraine. The experimental sites were located in Bryshche (Ukraine, Volyn Region 50°51′09″ N 25°12′08″ E Figure 1).

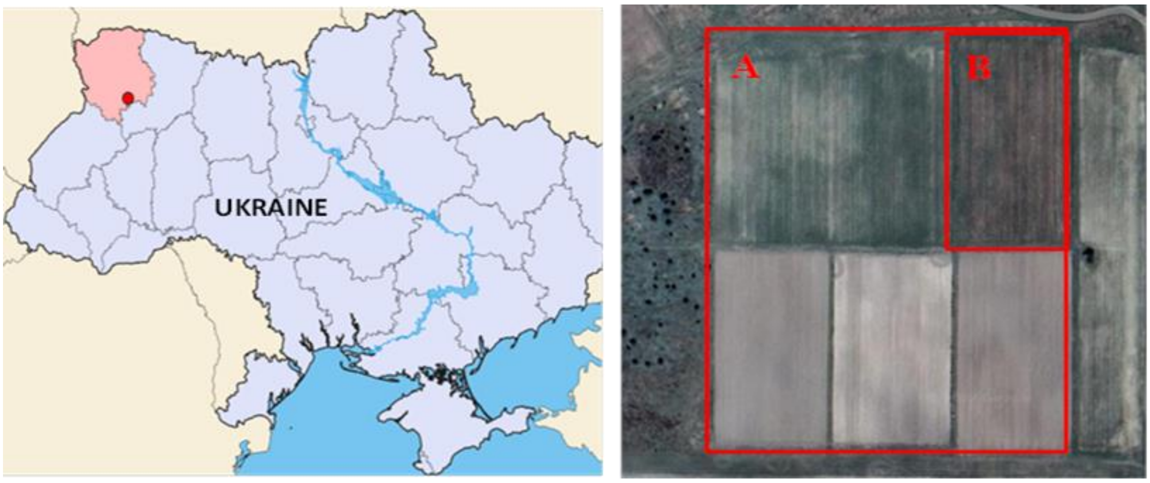

Figure 1. Location of the research area against the background of Ukraine and Volyn region; area of the experimental crop rotation outlined with red color: A—area of the stationary experiment; B—soil sampling area.

Stationary research was established in 1966 and was registered and recognized by Global Change and Terrestrial Ecosystem: Soil Organic Matter Network (GCTE EuroSOM-NET) and Core Project of the International Geosphere–Biosphere Programme (IGBP) [44]. The data on the correlation between soil fertility and fertilization systems which are reported in this work were taken from the stationary experiment database.

The soil type of the experimental site was sod-podzolic, surface-glazed, and dusty-sandy, and it was typical soil cover for the Ukrainian Polissia [45]. Soil parameters of a 0–20 cm horizon are illustrated in Table 1. The research was conducted in the eighth crop rotation of a grain-flax-potato crop rotation with the following crop sequence: potato-winter wheat-flax-barley with sub sowing of perennial grasses-perennial grasses-corn-winter rye.

An additional calculation of the amount of fertilizers was based on the initial amount of bioavailable nutrients in the soil and accounted for the particular plant needs. Semi-decomposed cattle manure (on straw litter) was used as an organic fertilizer (Organic 1—O1). The cattle manure's average nutrient content was: nitrogen (N) 13.2 g kg^{-1}, phosphorus (P) 7.1 g kg^{-1}, and potassium (K) 7.3 g kg^{-1}. Organic fertilizers were applied to the field surface in autumn before deep plowing for potatoes and corn at 40 t per hectare. In fact, we studied the aftereffects of manure long-term use on the experimental plots.

The amount of mineral fertilizers used was N—90 kg per hectare in the form of ammonium nitrate, P—80 kg per hectare in the form of simple granular superphosphate, and K—114 kg per hectare in the form of potassium chloride and potassium-magnesium (mineral fertilizer system—MFS). Mineral fertilizers were applied during pre-sowing cultivation for grain crops; phosphorus-potassium were applied for potatoes and flax in autumn. Nitrogen fertilization of winter cereals was carried out twice (early spring and at the beginning of the emergence of plants in the tube).

Table 1. Soil characteristics of the experimental site.

Property	Unit	Value
Exchange acidity, pH KCL	1 mol KCl	5.1
Hydrolytic acidity	meq 100 g	2.34
Cation exchange capacity, S	meq 100 g	2.6
Degree of bases saturation, V	%	52.6
Humus content	%	1.39
Easily hydrolyzed nitrogen	mg kg^{-1}	61
Mobile phosphorus	mg kg^{-1}	39
Exchangeable potassium	mg kg^{-1}	52
Copper, Cu	mg kg^{-1}	2.05
Zinc, Zn	mg kg^{-1}	11.7
Boron, B	mg kg^{-1}	0.08
Cobalt, Co	mg kg^{-1}	1.39
Nickel, Ni	mg kg^{-1}	5.9
Lead, Pb	mg kg^{-1}	4.7
Cadmium, Cd	mg kg^{-1}	0.06
Manganese, Mn	mg kg^{-1}	98.0

The oil radish variety Kiyanochka (*Raphanus sativus* L. var. *oleiformis Pers.*) was used as siderate (Organic 2—O2), which was sown after potato harvesting and plowed at the beginning of the bloom phase. A soil plot with no fertilizers was used as the control variant. In all areas, except for the control plots, soil liming was carried out once per rotation at 4–5.5 ton per hectare of $CaCO_3$ (99.2%) according to the need calculated using a hydrolytic acidity indicator.

Experiment scheme:

1. Soil without fertilization (C);
2. O1FS—organic fertilization system: soil + manure;
3. MFS—mineral fertilization system: soil + NPK;
4. O1MFS—organic-mineral fertilization system: soil + manure + NPK;
5. O2MFS—organic-mineral fertilization system: soil + siderate + NPK.

Variant placements were stepwise in four repetitions. The sown area in each variant was 100 m^2, and the accounting area was 50 m^2. Soil sampling for microbiological tests was carried out during 2019–2021 at one site in the potatoes–winter wheat–flax plant sequence.

Agricultural growing techniques were the same for all variants and did not differ from those generally applied in this zone.

2.2. Meteorological Conditions

Meteorological conditions during the experiment are presented in Figure 2. Data were received from the Volyn Regional Center of Hydrometeorology in Lutsk (http://meteolutsk.net.ua accessed on 22 January 2022). The data presented here illustrate the weather conditions from March to August, i.e., during the period of the greatest soil microflora activity.

The climatic conditions of 2019–2021 in terms of temperature indicators exceeded the average long-term constants, which confirmed further climate warming. Only in May 2020 was the monthly average air temperature lower than the long-term average by 2 °C. The data analysis indicated that the research period was characterized by increased humidity compared to the average long-term indicators. During the spring and summer of 2019, 2020, and 2021, precipitation levels were 384, 436, and 550 mm, respectively, while the long-term average was 362.8 mm. At the time of soil sampling, the highest precipitation of 117 mm (against the average of 57 mm) was recorded in May 2019. However, the air temperature did not differ from the long-term average. In July 2021, the lowest precipitation level of 27.1 mm was registered (against the average of 83 mm), and the air temperature was

25.4 °C, which is 6.8 °C higher than the average monthly long-term indicator. The weather conditions during the research period were favorable for soil microbiome development.

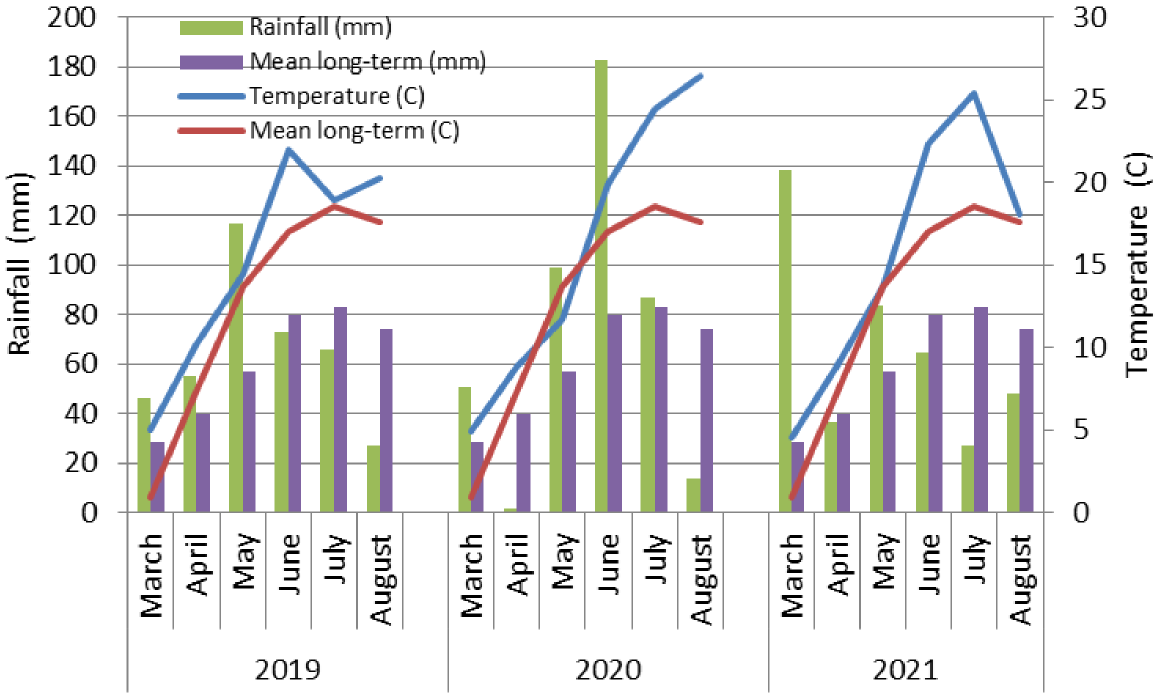

Figure 2. Mean monthly temperatures and rainfall at the experimental site during the experimental period as well as the long-term 1991–2021 trend.

2.3. Soil Sampling

The study on the soil physical and chemical parameters in the long-term experiment was conducted after the full cycle of each crop rotation in accordance with the methodology in [44]. For this purpose, soil sampling from the 0–20 cm layer was carried out from five points at each site in four repetitions using a manual gouging drill BP-25-15. The average composite sample of each variant of the experiment consisted of 20 separate samples. The soil samples were dried to an air-dry state, crushed, and sieved through a sieve with 1 mm diameter holes.

For microbiological testing and the appraisal of sod-podzolic sandy soil indicators in the stationary experiment, the main periods of plant vegetation and soil microbiome maximum activity were taken as the basis: spring (May) and summer (July). Topsoil samples (0–20 cm) were collected twice during each growing season during 2019–2021 from randomly selected points from each plot using a manual gouging drill BP-25-15 in potato-winter wheat-flax fields. During the research period, 56 soil samples were taken from each variant of the experiment twice a year at seven randomly selected points and in four repetitions. The total number of samples was 280 per year and 840 over three years. The average composite sample of each variant of the experiment was formed by mixing 28 separate samples. Fresh samples for the time of processing and analysis were stored in plastic containers at +4 °C. Before analysis, the soil samples were sieved through a sieve with 2 mm diameter holes to separate debris and waste matter.

2.4. Chemical Analyses

Exchangeable acidity of soil samples (pH KCl) was determined potentiometrically in accordance with DSTU ISO 10390:2007; soil quality. The pH was determined following ISO 10390:2005; IDT. The extraction solution consisted of 1 M KCl. The determination was carried out using a pH meter. Available hydrolysable nitrogen was determined using the Kornfild method (DSTU 7863:2015). This method is based on the hydrolysis of soil organic compounds with a 1 M NaOH solution in a Conway cup with a polished lid. Nitrogen was quantitatively determined using titration with a H_2SO_4 solution. Mobile phosphorus and mobile potassium were determined using the Kirsanov method (DSTU 4405:2005), which is the standard for podzolic soils. This method is based on the extraction of mobile phosphorus and potassium compounds from the soil with a 0.2 N HCl solution (pH 1). Phosphorus was determined by the color intensity of molybdenum blue (blue phosphoromolybdenum complex) on a spectrophotometer, and potassium was determined using a flame photometer. Humus content was determined in accordance with Tyurin (DSTU ISO 14235:2005; soil quality). Determination of organic carbon was conducted using sulfochromic oxidation (ISO 14235:1998, IDT). This method is based on the oxidation of soil organic components (humus) to CO_2 in an acidic environment by a strong oxidant ($K_2Cr_2O_7$).

2.5. Microbiological Analyses

The number of the main taxonomic and physiological groups of soil microorganisms was determined in soil samples using the method of soil suspension sowing on nutrient media, which are generally accepted in soil microbiology. These included: meat-peptone agar medium (MPA) for bacteria assimilating nitrogen from organic compounds (NORG), starch-ammonia agar medium (CAA) for bacteria assimilating mineral forms of nitrogen (NMIN), Chapek's medium for micromycetes, Giltaya's medium for nitrate respiring-microorganisms (denitrifiers), Vinohradsky's liquid medium for determining anaerobic nitrogen fixers, Hutchinson's medium for cellulose-decomposing microorganisms, and Muromtsev's medium for phosphate-dissolving bacteria [46]. The compositions of nutrient media used in the experiment are presented in Table 2. Ten grams of soil were suspended in 100 mL of sterile water, and a tenfold serial dilution was then prepared. Next, 1 mL of diluted suspensions was added to each nutrient media. Sowing on each media was carried out in triple repetitions. Incubation duration was 5–14 days at a temperature of 28 °C. Colonies grown on solid media were calculated based on the assumption that a single colony was formed from a single viable cell. The results of the calculated number of microorganisms grown on nutritious solid media were expressed in colony-forming units (CFU) per 1 g of soil.

Table 2. Media composition used for microbiological culture.

Medium	**Media Composition for 1000 mL H_2O**
Meat-peptone agar medium (MPA)	Dry fermented peptone—10.0 g; meat extract—11.0 g; NaCl—5.0 g; agar—15.0 g.
Starch-ammonia agar medium (CAA)	KH_2PO_4—1.0 g; $(NH_4)_2SO_4$—2.0 g; $MgSO_4$—1.0 g; NaCl—1.0 g; $CaCO_3$—3.0 g; soluble starch—10.0 g; agar—20.0 g
Chapek's medium	KH_2PO_4—1.0 g; $MgSO_4$—0.5 g; $NaNO_3$—3.0 g; KCl—0.5 g; sucrose—30 g; $FeSO_4$—0.01 g; agar—20 g. Before pouring into Petri dishes, streptomycin was added to a flask with a hot medium.
Giltaya's medium	Two solutions were prepared, which were later combined. Solution 1: KNO_3—2.1 g; asparagine—1.0 g; distilled water—250 mL. Solution 2: sodium citric acid—5.0 g; KH_2PO_4—2.0 g; $MgSO_4$—2.0 g; $CaCl_2$—2.0 g; $FeCl_3$—traces; distilled water—500 mL.
Hutchinson's medium	K_2HPO_4—1.0 g; $CaCl_2$—0.1 g; $MgSO_4$—0.3 g; NaCl—0.1 g; $FeCl_3$—0.01 g; $NaNO_3$—2.50 g; $CaCO_3$—10 g; agar—20 g. After solidification of the medium in Petri dishes, before sowing, sterile filter paper was placed on the surface of the medium as a source of cellulose.
Muromtsev's medium	Glucose—10.0 g; asparagine—1.0 g; K_2SO_4—0.2 g; $MgSO_4 \bullet 7H_2O$—0.4 g; yeast autolysate—0.5 g; agar—17.0 g; KH_2PO_4—1.0 g; $MgSO_4$—0.5 g; $NaNO_3$—3.0 g; KCl—0.5 g; sucrose—30 g; $FeSO_4$—0.01 g; agar—20 g. $Ca_3(PO_4)_2$ was added before pouring the medium into Petri dishes.
Vinohradsky's liquid medium	K_2HPO_4—0.5 g; $MgSO_4$—0.5 g; glucose—15 g; NaCl—traces; $FeSO_4$—traces; $MnSO_4$—traces. Before pouring the medium, $CaCO_3$ was added to each tube at the tip of the spatula.

2.6. Statistical Analysis

The data obtained in the experiment were processed by the correlation–regression method of analysis using the standard package of MS Excel 2013 software by Microsoft Corporation, Redmond, USA. The average arithmetic indicators of the soil's physical and chemical properties in the long-term stationary experiment for one rotation were taken into account. Regression equations are presented analytically (using formulas) and graphically (trend line). Statistical significance was checked by the value of the approximation reliability (R^2). Microbiological analysis results from 2019–2021 are expressed as mean values ± standard deviation (n = 18). The level of significance was selected as $p < 0.05$.

3. Results and Discussion

3.1. Soil Physical and Chemical Properties after Long-Term Fertilization Systems

Understanding the physical and chemical parameters of the microorganism habitat is essential to assess ongoing soil microbiological processes. The long-term use of different fertilization systems caused changes in the physical, chemical, and agrochemical soil properties of the experimental site. A graphical interpretation of the multi-year observations, taken from the stationary experiment database, is presented in Figure 3a–e). Additional application of mineral fertilizers on options with organic fertilizer caused an increase in exchangeable acidity index (pH KCl) (Figure 3a), which indicates a decrease in soil acidity. However, differences in the value of the acidity indicator were found depending on the fertilizer system. During the seven previous rotations, after the application of organic fertilizers in the form of manure, an increase in the pH KCl indicator was observed in the range from 5.2 to 5.8. The organic-mineral fertilization system, which involves a combination of manure and mineral fertilizers, caused a change in the soil acidity index over the duration of the experiment from 5.2 to 5.5. The cultivation of siderates simultaneously with mineral fertilization led to a slight change in the soil acidity to 5.3. The use of only mineral fertilizers that are physiologically acidic in the stationary experiment had little effect on the acidity index, which remained at the level of 4.9 during the years of the experiment. Further soil acidification was observed on the control variant.

The amount of available hydrolysable nitrogen in the soil of the experimental site is considered very low (according to DSTU 4362:2004. Soil Quality). The changes in soil fertility indicators over the years of observation are shown in Figure 3b. The mineral fertilizer system provided the accumulation of this form of soil nitrogen at 73 mg kg^{-1}. The combination of organic fertilizer in the form of manure with mineral NPK had the best effect on the content of available hydrolysable nitrogen. In this case, the indicator was 81.2 mg kg^{-1}. The introduction of NPK and the simultaneous use of siderates in crop rotation barely affected the content of easily hydrolyzed nitrogen. In the case of the organic fertilization system, this indicator was 57 mg kg^{-1}. In the control variant, where no fertilizer was applied, the content of easily hydrolyzed nitrogen during the period of the stationary experiment dropped to 43.9 mg kg^{-1}.

The content of mobile phosphorus in the soil of the experimental field varied depending on the fertilization system (Figure 3c). The use of organic fertilizer (manure) in crop rotation for seven rotations led to an increase in mobile phosphorus content of only 12%, which was 45 mg kg^{-1}. However, such an indicator is considered to be a low level (according to DSTU 4362:2004 Soil Quality. Soil Fertility Indicators). In the control variant, the lack of additional fertilizer caused a decrease in mobile phosphorus content in the 0–20 cm soil layer to 34 mg kg^{-1}. In the variant with additional introduction of mineral forms of phosphorus, its accumulation in the soil was 129 mg kg^{-1}. An increased level of mobile phosphorus was also noted in the variant with the complex use of NPK and siderates (119 mg kg^{-1}). The combination of manure and mineral fertilizer in seven rotations caused the formation of a high level of mobile phosphorus (186 mg kg^{-1}).

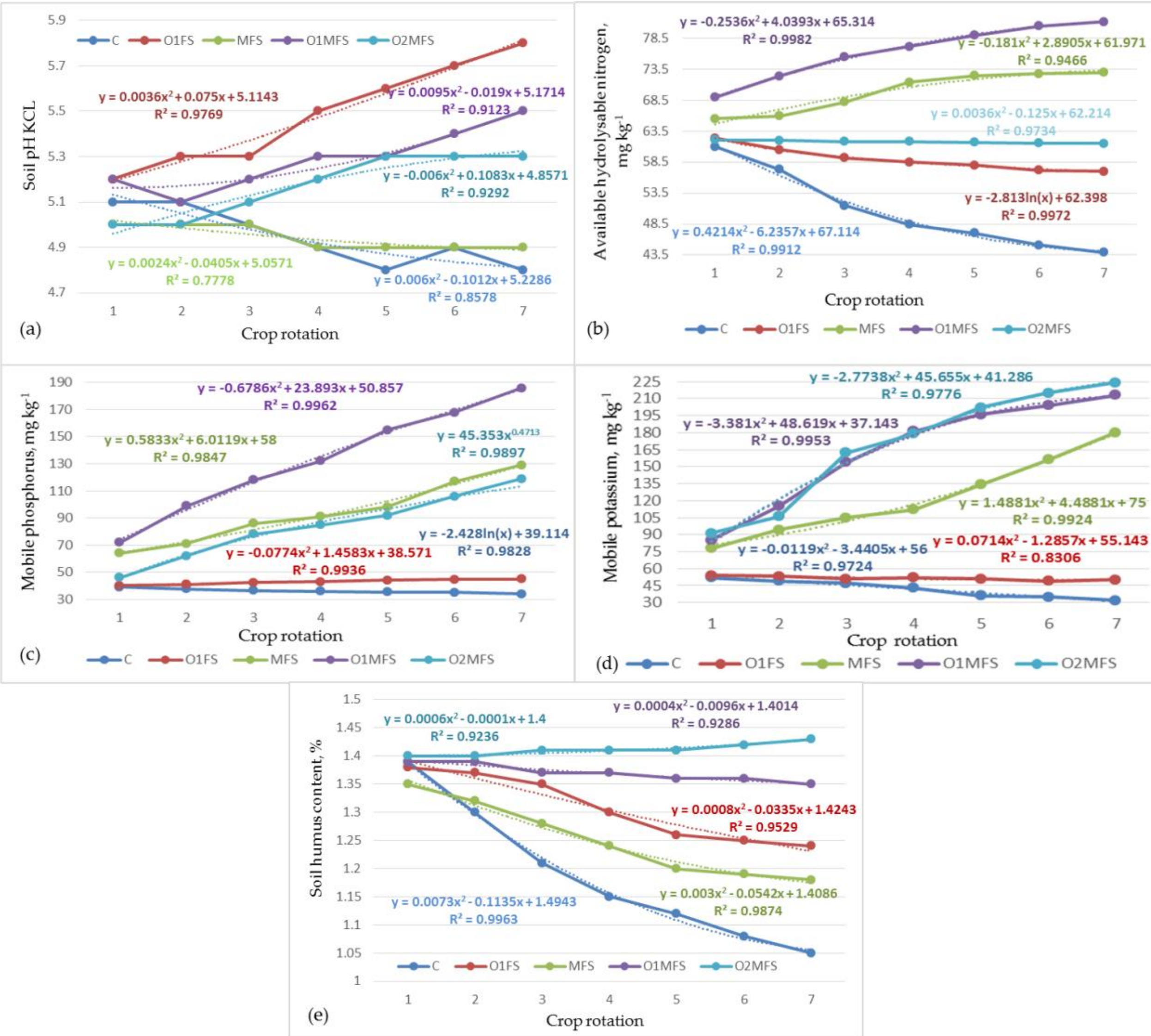

Figure 3. Changes in soil physical, chemical, and agrochemical properties depending on fertilization system and number of crop rotations. Average data at the end of crop rotation cycle: (**a**)-pH KCl; (**b**)-available hydrolysable nitrogen; (**c**)-mobile phosphorus; (**d**)-mobile potassium; (**e**)-humus content. Experiment variants: without fertilizer (C); organic fertilization system, manure (O1FS); mineral fertilization system, NPK (MFS); organic-mineral fertilization system, manure + NPK (O1MFS); and organic-mineral fertilization system, siderate + NPK (O2MFS).

During long-term observations in the stationary experiment on the control variant and under the only organic fertilization system, the 0–20 cm soil layer showed low amounts of mobile potassium (32 mg kg^{-1} and 50 mg kg^{-1}, respectively (Figure 3d)). Complete absence of fertilizer in the crop rotation led to a decrease in mobile potassium content in the soil to 39%. The mineral fertilization system and the use of combined treatments in accordance with the O1MFS and O2MFS schemes caused the accumulation of mobile potassium in the top soil layer up to 180, 213, and 224 mg kg^{-1}, respectively, which is considered to be a high amount of potassium (according to DSTU 4362:2004 Soil Quality. Soil Fertility Indicators).

Long-term use of different fertilization systems in grain-row crop rotation caused changes in soil humus content (Figure 3e). The general trend was that of a decrease in the humus content in the top soil layer in O1FS, MFS, and O1MFS variants to 1.24, 1.18, and 1.35% respectively. The lack of additional fertilizer in any form caused soil depletion and a decrease in humus content to a critically low level of –1.05%. An accumulation of humic substances in the 0–20 cm soil layer to 1.43% was noted in the variant with organic matter application in the form of a green mass of siderates and NPK mineral fertilizer.

Different fertilization systems affect soil quality, which is expressed through physico-chemical and agrochemical indicators. This ultimately determines its productive potential [5,19,47].

The soil acidity indicator is sensitive to the additional nutrients supplied and changes depending on the type of fertilizer [48–50]. Thus, an increase in soil pH was noted in fertilization systems that included various types of organic matter, which therefore contributed to a decrease in soil acidity [47]. Changes in soil acidity during mineral fertilization are determined by the nature of the fertilizers [48,50,51]. Studies have proven that the constant use of nitrogen fertilizers gradually causes soil acidification, which leads to deterioration in plant nutrients uptake and a decrease in yield [51]. Field experiments revealed a tendency of soil pH to decrease with an increased dosage of nitrogen fertilizers in the form of ammonium nitrate [48]. It was reported that nitrogen fertilizers retain hydrogen (H^+) in the soil, which decreases soil pH and causes acidification of the soil environment [50]. However, a rational use of mineral nutrients in a long-term experiment excludes the accumulation of an excessive amount of hydrogen in the soil of the experimental areas [52]. The soil pH is particularly important because of its influence on the vital activity of soil microorganisms and the soil nitrogen transformation process [48]. The pH indicator itself, among a number of physical and chemical parameters describing soil properties, greatly affects the content of nutrients available to plants in the soil and their uptake rate from applied fertilizers [53,54]. Nitrogen, phosphorus, and potassium are the basic elements required for plant growth. Their amount in the soil is an important parameter that reflects the value of agricultural land [55].

The nitrogen content in the soil changes dynamically depending on different fertilization systems [19,48,56]. The index of available hydrolysable nitrogen in the soil gives a comprehensive understanding of the effectiveness of different fertilization systems. An analysis of the obtained results did not reveal changes in the content of hydrolyzed forms of nitrogen under the organic fertilization system. Therefore, it is suggested that optimized strategies for fertilization with organic manure maintain the balance of soil nutrients with high efficiency and provide the possibility of N-management in sustainable cropping systems [56]. It is the process of decomposition of organic matter in the soil that improves soil structure and functioning and that enhances the availability of nutrients to crops [57]. Systematic application of optimized NPK rates not only meets the plant's needs but also enhances the nitrogen use efficiency. The addition of manure increases the content of organic matter in the upper soil layer, which boosts the availability of nitrogen forms [58].

Complete mineral fertilization ensures an increase in the content of hydrolysable forms of nitrogen, especially with an increase in the amount of nitrogen fertilizers, which is caused by ammonium ion retention by the soil adsorption complex. Intensification of humus mineralization processes and the release of ammonium ions also occur [48]. A system of soil fertilization combined with the use of organic manure and mineral fertilizers significantly improved the total nitrogen and NO_3^- content compared with the use of mineral fertilizers only. However, although the use of only organic manure improved the NO_3^- content significantly, it also reduced the NH_4^+ content in the soil compared with the variant of combined use of mineral and organic matter [49]. Previous results [42] showed an increase in alkaline hydrolyzed N content up to 95.32–128.34% due to organic-mineral fertilization.

Long-term fertilization of crop rotation soil with mineral fertilizers leads to accumulation of mobile phosphorus compounds in the 0–20 cm soil layer. In addition to improving the physical, chemical, and biological soil indicators, organic and mineral fertilizers in particular promote an increase in moisture content, soil pH, organic matter content, the total number of bacteria, and microbial biomass carbon. They have also been observed to contribute to an increase in available phosphorus by 338.44–491.41% [19]. An increase in soil organic matter content enhances the availability of P compounds [58]. The use of only organic fertilizer simultaneously with the increase in the organic matter content and cations exchange capacity increases the P content [59]. However, a combination of organic matter with mineral fertilizers significantly increases this indicator [5,14]. It has been shown that manure phosphorus is more mobile than inorganic fertilizer phosphorus [60]. The introduction of nitrogen fertilizers improved the current and potential state of the phosphorus system in the soil [48]. A sufficient concentration of P and a positive P balance is ensured by the introduction of inorganic fertilizers and organic manure and by improving the microbiological activity of the soil and soil health [61].

The mobile potassium content in the soil, as well as the phosphorus content, increased both after the application of mineral fertilizers (NPK) and in the case of combined fertilization [49,60]. Because ammonium ions and potassium ions compete for attachment sites on the adsorption complex, the additional application of nitrogen-containing fertilizers promotes the release of potassium, thus boosting the potassium content in the soil [48].

Soil humus is a colloidal substance. The accumulation of humus increases the soil cation-exchange capacity and, therefore, its ability to retain nutrients through chelation. Nutrient cations become available to plants, are fixed in the soil, and are not washed out easily. Humus, as a part of soil organic matter, changes dynamically with long-term fertilization as well. The study showed that a higher humus content in field crop rotation was detected when organic fertilizers were used than when treated with mineral fertilizers only, and the highest content was recorded with a combined fertilization system (organic + NPK) [62].

The total content of nitrogen and humus partly depends on soil pH due to its influence on the vital activity of the soil biome and soil nitrogen transformation. Thus, a positive soil humus balance should be supported by the use of nitrogen fertilizers [48]. We assume that the increase in soil humus driven by O2MFS fertilization may be caused by an additional amount of organic matter in the soil due to intensive crop growth and the higher content of N in plant remains caused by higher rates of nitrogen fertilizers (as this leads to an increase in soil C content) or that it is the result of the simultaneous action of both these mechanisms [63]. It is assumed that the use of natural organic fertilizers has a greater practical value for preserving the content of soil organic matter [64]. Therefore, to maintain a high level of soil fertility, it is necessary to constantly monitor the changes that occur in its chemical properties after the application of fertilizers. By understanding the trends in the physical, chemical, agrochemical, and biological soil properties, we can correctly choose the type and amount of fertilizers to properly meet the biological needs of plants and preserve the permanent fertility of the soil [48].

3.2. The Number of Microorganisms of the Main Taxonomic Groups

The research results illustrated that in the taxonomic structure of the microbial community of sod-podzolic soil, bacteria dominate in terms of the number of microorganisms (Table 3). The percentage of bacteria in the microbial groups in different variants of the experiment was within 99.6–99.8%. The obtained data point out that long-term use of different fertilization systems of sod-podzolic soil changed the number of microorganisms from the main taxonomic groups. The largest number of bacteria was found in the soil with an organic fertilizer system (14.5×10^6 CFU g^{-1} of soil). Mineral fertilizer application inhibited the development of bacteria; their number in the soil in this variant was the lowest at 9.66×10^6 CFU g^{-1} of soil. The largest number of micromycetes was registered in the soil of the control variant (44×10^3 CFU g^{-1} of soil). In the variant with an organic fertilization system, the number of micromycetes was the lowest (24×10^3 CFU g^{-1} of soil).

The dominance of fungi or bacteria in microbial communities indicates both their response to environmental changes and their impact on the function of the ecosystem [65]. The quantitative relationship between them is determined by the combined effects of a number of environmental factors (temperature, humidity, reaction of the pH medium, etc.) and anthropogenic activity [66–68]. The conducted studies indicate that the maximum range of variation in the number of microorganisms in the experimental areas was 34%

for bacteria and 45% for micromycetes. At this point, it is possible to assert a significant influence of different fertilization systems of sod-podzolic soil on the change in the number of microorganisms of the main taxonomic groups in microbial communities.

Table 3. The total number of the main taxonomic groups of microorganisms in the sod-podzolic soil of the studied crop rotation averaged from 2019–2021.

Experiment Variants	Bacteria, $\times 10^6$ CFU g^{-1} of Soil	Micromycetes, $\times 10^3$ CFU g^{-1} of Soil
C	11.0 ± 0.2	44 ± 1.2
O1FS	14.5 ± 0.3	24 ± 0.5
MFS	9.6 ± 0.3	38 ± 1.1
O1MFS	13.0 ± 0.4	32 ± 0.8
O2MFS	13.8 ± 0.3	28 ± 0.5

The data are statistically significant, $p < 0.05$, x ± SD, $n = 18$.

The long-term use of different fertilization systems in sod-podzolic soil also led to changes in exchangeable acidity indicators (Figure 3a) and humus content (Figure 3e). Soil pH and the content of organic matter are important factors influencing changes in the microbial community [21–26,32–40,69,70]. Our research confirms the relationship between the abundance of the main taxonomic groups of microorganisms, pH indicators, and humus content in the 0–20 cm layer of sod-podzolic soil (Table 4).

Table 4. Influence of the soil pH and humus content in the 0–20 cm layer of sod-podzolic soil on the number of microorganisms of the main taxonomic groups averaged from 2019–2021.

Taxonomic Groups	Regression Equation y = ax ± c	Coefficients of	
		Correlation, R	Determination, R^2
pH KCl			
Bacteria	y = 1.04x + 9.26	0.88	0.65
Micromycetes	y = −4.6x + 47	−0.91	0.84
Humus, %			
Bacteria	y = 1.04x + 9.26	0.75	0.65
Micromycetes	y = −3.8x + 44.6	−0.74	0.57

A close inverse correlation was found between the number of micromycetes and exchange acidity pH KCl at the level of R = −0.91, R^2 = 0.84, which indicates a decrease in the number of micromycetes with an increase in exchange acidity in sod-podzolic soil. Between the number of bacteria and metabolic acidity, the correlation is slightly weakened and is direct at the level of R = 0.88, R^2 = 0.65. A strong correlation between the number of bacteria and humus content was revealed at the level of R = 0.75, R^2 = 0.65, which indicates an increase in bacteria number with an increase in soil humus content. An inverse correlation was found between the number of micromycetes and humus content (%) at the level of R = -0.74, R^2 = 0.57.

The correlation analysis showed that soil pH and humus content in sod-podzolic soil are important factors influencing the number of microorganisms of the main taxonomic groups in the microbial communities. The greatest influence was found between the soil pH KCl and the micromycetes number.

3.3. The Number of Different Physiological Groups of Soil Microorganisms

3.3.1. Atmospheric Nitrogen-Fixing Bacteria (Diazotrophs)

The number of anaerobic diazotrophs in the tested soil samples ranged from 0.5 to 2.6 × 10^5 CFU g^{-1} of soil depending on the variant of fertilization system. (Figure 4a).

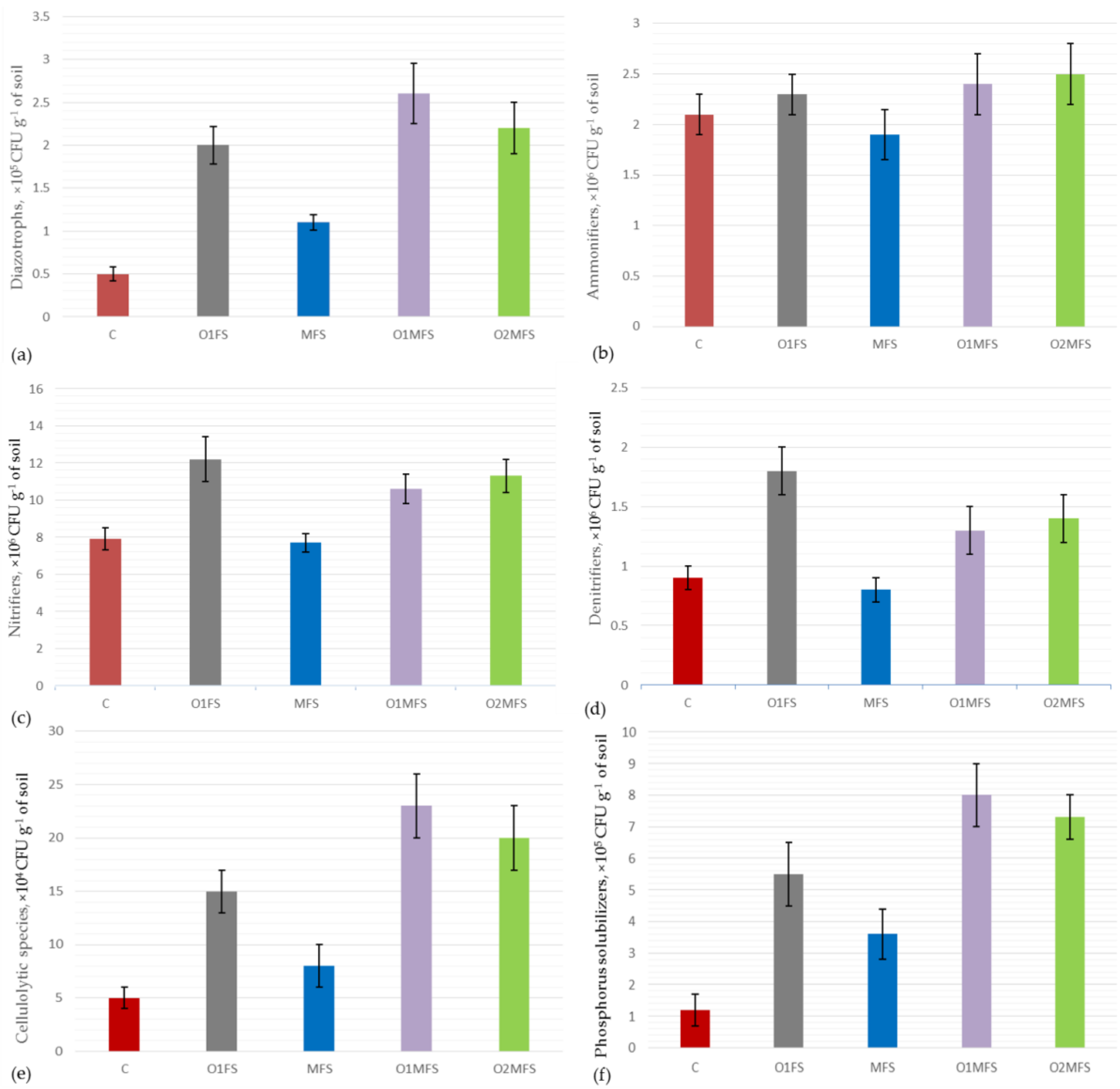

Figure 4. Number of physiological groups of soil microorganisms in sod-podzolic soil depending on fertilization system, ($p < 0.05$), average from 2019–2021: (**a**)-anaerobic nitrogen-fixing bacteria; (**b**)-organotrophic bacteria; (**c**)-soil nitrifiers; (**d**)-denitrifiers; (**e**)-cellulolytic microorganisms; (**f**)-phosphorus-solubilizing microorganisms. Experiment variants: without fertilizer (C); organic fertilization system, manure (O1FS); mineral fertilization system, NPK (MFS); organic-mineral fertilization system, manure + NPK (O1MFS); and organic-mineral fertilization system, siderate + NPK (O2MFS).

The low level of anaerobic non-symbiotic nitrogen fixer development was noted on the control variant without additional fertilization (0.5×10^5 CFU g^{-1} of soil (C)).

The long-term application of mineral fertilizers promoted their increase; in the soil, their number doubled, and in the variants with the application of organic and organic-mineral fertilizers, it grew 4–5 times. The greatest development of anaerobic nitrogen-fixing bacteria in our studies was noted in the variant with the O1MFS fertilization system. Such a significant increase in microorganism development was caused by the additional nutrients from fertilizers, particularly from manure. Other researchers have also found that long-term fertilization led to an increase not only in the number but also in the diversity of nitrogen fixers [71].

3.3.2. Ammonifiers (Organotrophic Bacteria)

The number of organotrophic bacteria in areas with different fertilization systems ranged from 1.9 to 2.5 × 10^6 CFU g^{-1} of soil (Figure 4b). The amount of organotrophic bacteria was low in the experimental plot after the use of mineral fertilizers (MFS). A general tendency towards a slight increase in the number of organotrophic bacteria was noted in variants with organic and organic-mineral fertilizer systems compared with the control variant. Similar results regarding an increase in the number of organotrophic bacteria number after manure use and their decrease following the use of mineral fertilizer system only were confirmed in other studies [72].

3.3.3. Nitrifiers (Mineral Nitrogen Assimilating Bacteria)

Soil nitrifier quantity depending on the fertilization system is shown in Figure 4c. The lowest quantity of nitrifying bacteria of 7.7 × 10^6 CFU g^{-1} of soil was noted in the experimental plot with mineral fertilizer application (MFS). Long-term use of mineral fertilizers may be toxic to this group of microorganisms. A meta-analysis [73] based on 107 data sets from 64 long-term worldwide trials showed that mineral fertilizer application caused an overall increase in soil microbial biomass by 15.1% above the levels in unfertilized standard treatments. In variants with the O1FS, O1MFS, and O2MFS fertilization systems, the number of nitrifiers was 25–35% higher compared with the control variant (C). Such results imply that plant remains (mostly straw), which get into the soil with manure, and siderates are the main substrates that foster the development of this group of organisms.

The results of another long-term study [18] on the influence of mineral and organic fertilizers on the abundance of the main microbial groups also showed that organic fertilizers of various origins had an impact on communities of nitrifying microbes that possess AOA and AOB genes. Moreover, pig manure had a more important effect than plant residues, as it led to an increase in the size of soil aggregates. In a long-term study utilizing different fertilization systems, it was found that organic soil management improved the percentage (by 21–65%) of Gram-negative bacteria (G−), which include nitrifiers, while with a chemical fertilization system, the percentage of Gram-negative bacteria (G−) was lower than the control [35]. A long-term, 16-year-long experimental study with different doses of manure application and mineral fertilizers showed that increased doses of manure application stimulated some microbial groups, particularly those involved in nitrogen mineralization [41].

3.3.4. Denitrifiers

The number of denitrifiers detected in the soil samples of the different variants of the experiment are shown in Figure 4d. The lowest number of denitrifiers (0.8 × 10^6 CFU g^{-1} of soil) was found in the soil samples taken from the site with the mineral fertilizer system (MFS). On the plots with manure application (O1FS), their number was much higher (1.8 × 10^6 CFU g^{-1} of soil). A long-term application of manure with high humidity and high content of available nitrogen and carbon changes the structure of the microbial community and stimulates an increase in the number and activity of nitrifying and denitrifying microorganisms [74]. Studies of four different fertilization systems demonstrated that organic fertilizer use increases the soil denitrification potential, while the use of only inorganic fertilizers did not lead to any significant changes. [75]. According to the results of our research, long-term fertilization of sod-podzolic soil with manure (O1FS), as opposed to the mineral fertilization system (MFS), had a significant impact on the development of nitrifying and denitrifying microorganisms.

3.3.5. Cellulolytic Microorganisms

The quantity of cellulolytic microorganisms found in soil samples in our experiment fluctuated from 5 to 20 × 10^4 CFU g^{-1} of soil (Figure 4e). The intensive development of cellulolytic organisms was found in the variants of the experiment where organic fertilizers were used (O1FS, O1MFS, and O2MFS). It is obvious that the development of these organisms depended on the amount of available substrate, which was straw in the O1FS and O1MFS options and siderate in the O2MFS option. A study [76] on the impact of different crop management systems and manure fertilization indicated that farmyard manure (FYM) was a powerful factor that stimulated the amplification of cellulolytic bacterial communities, which was most likely due to the presence of straw in FS. According to our results, greater development of cellulolytic microorganisms was found in both variants with an organic-mineral fertilization system (O1MFS and O2MFS). Other data [77] from a 34-year-long regime of fertilization experiments also showed a significant increase in the number of cellulolytic microbial communities with the use of crop remains, organic manure, and chemical fertilizers compared with unfertilized soils. Based on this fact, we conclude that in conditions of long-term use of various fertilization systems, the development of cellulolytic organisms increases in the case of a combination of mineral and organic fertilizers, which have a significant content of undecomposed plant remains (straw, siderate).

3.3.6. Phosphorus-Solubilizing Microorganisms

The quantity of phosphorus-solubilizing microorganisms found in soil samples in our experiment ranged from 1.2 to 7 × 10^5 CFU g^{-1} of soil (Figure 4f). The long-term application of organic fertilizers (O1FS) doubled the number of phosphorus-solubilizing microorganisms in the soil, and the number quadrupled after the application of mineral fertilizers (MFS) compared with the plots without a fertilizer (C). Research [78] on long-term fertilization of rice field soils that received phosphorus fertilizers (+P), compared with soils without phosphorus fertilizers (−P), demonstrated that the presence of phosphorus in the soil significantly affected the interrelation of bacterial communities. The use of low amounts of inorganic phosphorus, lime, and compost, used to improve soil for the rhizosphere, also increased the abundance of phosphate solubilizers [79]. The populations of phosphorus-dissolving bacteria were more widespread and diverse [80]. Therefore, we assume that the introduction of inorganic phosphorus to soil with a high content of organic matter will contribute to both rapid growth of microorganisms and a higher solubilization rate of microbial phosphorus. This explains such a large number of phosphorus-mobilizing organisms at the plots with combined application of mineral and organic fertilizers O1MFS and O2MFS.

4. Conclusions

Continuous application of various fertilization systems on sod-podzolic soil in a grain-flax-potato crop rotation led to changes in the main physical, chemical, and agrochemical parameters. It also affected the microbiological properties of the soil and revealed a close correlation between the number of the main taxonomic groups of microorganisms, whose quantity decreases with an increase in acidity. In the O1FS fertilization system, the numbers of bacteria that are dominant in the taxonomic structure of the microbial group as well as the numbers of microorganisms from the physiological groups that convert nitrogen (nitrifiers and denitrifiers) were the largest.

In the O1MFS fertilization system, the amount of bacteria was smaller, compared with the O1FS, but the physiological groups of microorganisms that participate in the transformation of nitrogen (diazotrophs), carbon (cellulolytics), and phosphorus (phosphate mobilizing) were more abundant. The results of the O2MFS system were in between the results of the O1FS and O1MFS variants. The lowest content of bacteria and functional groups, except for phosphate-mobilizing microorganisms, were noted in the soil samples from MFS fertilization system.

The final analysis of the results demonstrated that, in time, different fertilization systems caused changes in the main physicochemical and agrochemical parameters of the soil and affected its microbiological properties. Bacteria dominate the taxonomic structure of the microbial community of sod-podzolic soil when different fertilization systems are used. The reaction of the soil environment is the main factor affecting the number of micromycetes. The combined application of organic and mineral fertilizers created optimal conditions for the development of functional groups of microorganisms responsible for the transformation of carbon and phosphorus, and it had a positive effect on the development of diazotrophs. Long-term fertilization with mineral fertilizers suppresses the development of bacteria and functional groups of microorganisms responsible for the transformation of nitrogen and carbon. These results demonstrate that the combined application of organic and mineral fertilizers is a method of sustainable soil management with long-term fertilization and that while an exclusively mineral fertilization system should not be practiced.

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