sustainability

MDPI

Article

Distinct Changes in Abundance of Culturable Microbial Community and Respiration Activities in Response to Mineral–Organic Mixture Application in Contaminated Soil

Katarzyna Wolny-Koładka [1,2], Renata Jarosz [2,*], Michał Juda [2] and Monika Mierzwa-Hersztek [2,3,*]

[1] Department of Microbiology and Biomonitoring, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Krakow, Poland
[2] Department of Mineralogy, Petrography and Geochemistry, AGH University of Science and Technology, al. Mickiewicza 30, 30-059 Krakow, Poland
[3] Department of Agricultural and Environmental Chemistry, University of Agriculture in Krakow, al. Mickiewicza 21, 31-120 Krakow, Poland
* Correspondence: jarosz@agh.edu.pl (R.J.); monika6_mierzwa@wp.pl (M.M.-H.)

Abstract: The availability and affordability of fertilizers are the main risks currently faced by the fertilizer market. Therefore, there is a need to look for other sources of nutrient supply for plants, while taking care of soil properties. The application of fertilizers with the addition of functionalized materials could help in the efficient use of nutrients. The aim of the study was to assess the impact of the application of mixtures with the addition of zeolite–vermiculite composites (NaX–Ver) on the culturable microorganisms and selected soil properties. A two-year pot experiment was conducted on soil with elevated contents of cadmium, zinc, and lead. The test treatments included soil mixed with NPK and additives in two doses of NaX–Ver combined with leonardite (Leo) or lignite (L). The test plant used in the experiment was maize. The soil material was analyzed for the number of bacteria, mold fungi, actinomycetes, and ammonifiers. Furthermore, soil pH, EC, N total, and SOC contents, as well as soil respiration activity, were tested. The applied fertilizer mixtures had a great effect on changes in the N total and SOC contents. The N total increase was 45.5% in NaX–Ver3%L3% and 51% in NaX–Ver9%Leo6%, and the largest SOC increase (24.3%) was recorded in the NaX–Ver3%Leo3% treatment. The highest respiration activity was determined in NaX–Ver3%Leo3% and NaX–Ver9%Leo6%: 2.12 μg C-CO_2 g^{-1} DM h^{-1} and 2.14 μg C-CO_2 g^{-1} DM h^{-1}, respectively. A significant correlation between pH values and the number of culturable microorganisms was found. The number of soil microorganisms depended on the type of fertilization used. The best stimulation of the number of culturable soil microorganisms was found in treatments with the addition of 3% of L or Leo in combination with NaX–Ver. The percentage increases in the number of the analyzed culturable microorganisms after the application of leonardite-based fertilization in combination with the zeolite–vermiculite composite were, on average: bacteria, 1096%; mold fungi, 1529%; actinomycetes, 1477%; ammonifiers, 910%.

Keywords: culturable microorganisms; basal respiration; fertilization; maize; soil contamination; zeolite

Citation: Wolny-Koładka, K.; Jarosz, R.; Juda, M.; Mierzwa-Hersztek, M. Distinct Changes in Abundance of Culturable Microbial Community and Respiration Activities in Response to Mineral–Organic Mixture Application in Contaminated Soil. *Sustainability* **2022**, *14*, 15004. https://doi.org/10.3390/su142215004

Academic Editor: Chenggang Gu

Received: 13 October 2022
Accepted: 10 November 2022
Published: 13 November 2022

1. Introduction

Due to their great diversity, microorganisms perform a number of functions in agroecosystems. The most important of these functions, leading to an increase in the fertility and productive value of soils are: decomposition and mineralization of organic matter, release of nutrients from hard-to-reach sources, circulation of elements in the environment, formation of structures and humus in the soil, and support of plant growth and plant protection, as well as detoxification and bioremediation of the soil environment [1]. That great diversity of microorganisms, which synthesize numerous enzymes and have specific

genes, causes bacteria and fungi to be involved in virtually all stages of nutrient cycling in the soil environment. The abundance of soil microorganisms contributes to the formation of soil humus, as bacteria and fungi, through the production of specific metabolites, biomass, and enzymes, which participate in the synthesis and formation of humic compound precursors [2]. With this diversity of soil microorganisms and their activities, various types of soil aggregates are formed, in which bacterial cells and fungal hyphae act as binders, and polysaccharides synthesized by microorganisms ensure stability and give the appropriate structure to the soil. Benefits of improved soil structure directly related to the maintenance of biodiversity, especially in an agricultural setting, include: reduced risk of water and wind erosion, improved plant germination due to easier plant access to nutrients, reduced soil sealing, and increased infiltration, retention, and water availability resulting from improved soil porosity [1]. The soil environment biodiversity also affects plant growth and yield. During seed germination and plant growth, rhizosphere microorganisms (present in the root zone) multiply at an increased rate, thus, enabling faster development of the plant by providing nutrients. In addition, these microorganisms synthesize phytohormones, i.e., auxins, gibberellins, and cytokinins, which affect plant development and reduce ethylene levels, which have an adverse effect on plant rooting [2–4]. Soil microorganisms play a key role in the growth and development of plants [5]. Microorganisms can break down and mineralize organic matter, allowing the elements to circulate. Microorganisms are involved in the weathering of minerals by decomposing them through the production of organic and inorganic acids. With this ability, they take an active part in the creation of humic compounds, which determine soil fertility [2]. Numerous ammonifiers and fungi are involved in the ammonification process, i.e., changes of nitrogen with the participation of microorganisms. Ammonifiers include aerobic and anaerobic organisms that prefer a very wide pH range and are adapted to different temperatures and humidity. Factors that can affect the mineralization of organic nitrogen compounds in the soil are: soil physicochemical properties, moisture, pH, weather conditions, season, and nitrogen fertilization. The ammonification process is more intense when the soil is acidic, thus, increasing the amount of NH^{4+} [6]. The number of soil culturable microorganisms results from many factors. The type of soil, its pH, organic matter content, and structure are also important. Abiotic factors, such as humidity and temperature, directly affect the rate of microbial proliferation and indirectly determine plant growth and the quantity and quality of chemical compounds secreted by the roots into the soil. Man, through the agrotechnical methods used and the type of fertilization or pesticides applied, also significantly affects the culturable microorganisms in cultivated soils [7]. The soil pH value is the basic and necessary factor that significantly affects the behavior of metals in the soil and determines the toxicity of soil and plants both directly and indirectly [8]. The basic fact is that it modifies environmental conditions that are crucial for the growth and reproduction of microorganisms. pH influences the chemical activity of protons, which fundamentally determine the course of the redox reaction but also participate in the dissolution and precipitation of minerals and many other biogeochemical reactions. All this translates into changes in the salinity and composition of aqueous solutions as well as the availability of nutrients and trace elements [9]. The pH value also has a very significant influence on the activity of extracellular enzymes and the reactivity of natural organic matter. Therefore, pH is considered to be an indicator of the environmental conditions that shape the composition and activity of soil microorganisms [10]. According to a study by Shi et al. [11], optimal conditions favoring bacterial and fungal diversity in acidic and alkaline soils occur at pH values of ~5.5 and ~8.3, respectively. Changes in the pH value in soils affect the intensity of heavy metal mobilization. In strongly acidic soils, the mobility of metallic elements is much higher than in neutral and alkaline soils. The mobility of metals in soils with a low pH decreases in the following order: Cd > Ni > Zn > Mn > Cu > Pb. However, it should be remembered that the influence of pH on the mobility of metallic elements in the soil is very variable depending on the content and type of organic matter [12]. Zeolites, in general, have a high cation exchange capacity, and they attract positive-charged ions; therefore, zeolites are widely used for cationic pollutant

sequestration, such as the sequestration of heavy metals [13]. Contamination of the soil environment with various substances, organic compounds, or heavy metals fundamentally changes the diversity of soil microorganisms, activating those resistant to stress factors (selection pressure). In arable soil, microorganisms may, in particular, promote nutrient cycling, maintain soil fertility, and improve crop health [14]. Soil bacteria are sensitive to contaminants such as heavy metals and organic pollutants. The composition of soil microbial communities has a direct or indirect impact on ecosystem-level processes; therefore, it is important to identify the largest possible number of the soil microorganisms that dominate them [15]. There are two sources of heavy metals in soils; their content may be due to their natural presence in the soil or may result from anthropogenic activities. However, not just the presence but the amount is of particular importance here, as too high a content of heavy metals can have a negative impact on both plants and soil microorganisms [16,17]. Heavy metals in soil with an increased content have a negative effect on soil microorganisms (their functionality and abundance). The inhibitory effect of heavy metals on microorganisms directly translates into decline in soil fertility. However, there are also publications stating that microorganisms can adapt to elevated concentrations of heavy metals in the soil [18]. This is due to the fact that microorganisms develop resistance even to toxic concentrations of heavy metals [19]. Heavy metals in the soil affect the inactivation of enzymes that catalyze the biochemical reactions of microorganisms, damage microbial cells by perforation of membranes, or permanently bind substances and nutrients, making them inaccessible to microorganisms. In addition, metals affect the growth, morphology, and metabolism of microorganisms through functional disorders, damage the genetic material, and contribute to the denaturation of proteins and the integrity of cell membranes [20].

The use of nitrogen fertilizers can lead to the loss of nitrogen unused by plants by leaching nitrogen into water courses or by allowing ammonia to escape into the atmosphere. Due to the fact that a surplus of nitrogen fertilizers can lead to environmental pollution, it is very important that the applied nitrogen doses are properly balanced depending on the needs, which allows minimization of the losses of N [21]. Moreover, Thompson et al. [22] indicated the use of fertilizers as an important source of nitrogen oxide emissions to the atmosphere. This means that agricultural practices and fertilizer application can have an indirect effect on climate change. This indicates a great need to retain nitrogen in the soil in order to reduce its negative impact on the environment. In view of the issue of restoring the use value of degraded land and soils with elevated heavy metal content, solutions are constantly being sought to stabilize and remove soil contaminants. One such solution is the application of mineral–organic mixtures containing zeolite composites and lignite or leonardite. It is believed that the addition of these materials, on the one hand, increases the efficiency of applied fertilizers by slowing down the release of nutrients while reducing their loss through leaching and, on the other hand, supports the process of absorbing inorganic contaminants. According to Mondal et al. [23], the use of zeolite-enriched mineral fertilizer significantly reduces the leaching of nutrients from the soil, allowing the plant to use them throughout the growing season. The ability of zeolites to retain, store, and slowly release nutrients slows down their mineralization and is an important feature from an agricultural point of view [17]. Due to their molecular sieve structure and high cation exchange capacity (CEC), zeolites enable the exchange of ions with the environment, which generally decreases soil EC [24]. In their study, Lahori et al. [25] showed that the use of zeolites reduced the mobility of lead, cadmium, zinc, and copper. Their results revealed a reduced translocation of heavy metals in shoots and roots both in cabbage and maize crops. Głąb et al. [26] also recommend the use of zeolites for soil remediation purposes. Their results showed that the application of zeolite to soil contaminated with cadmium, zinc, and lead increased the yield and improved the root morphological parameters of cultivated grass. As stated by Mühlbachová and Šimon [27], depending on the soil type, zeolites can have an ambiguous effect on microbial populations, especially in contaminated soils. As a result, the authors emphasized the need for further research to better understand how zeolites influence the microbial properties of different soil types. Basal respiration (BR)

provides information about the current state of microbial activity in the soil and indicates the ability of the microbial community to mineralize soil organic matter. This parameter depends on the physiological state of the microorganisms as well as on the conditions in the soil environment [28,29]. Basal respiration activity in soil reflects the availability of carbon to soil microorganisms [30]. As reported by Eisentraeger et al. [31], the respiratory activation quotient can be used as an indicator of bioremediation efficiency, as it reflects the amount of biodegradable carbon source in the soil. QR values between 0.1 and 0.3 indicate a slow remediation rate. On the other hand, higher BR values, pointing to greater basal respiration activity, are considered a favorable indicator of microbial activity.

The aim of the present study was to evaluate the effects of mineral–organic mixtures containing various additions of lignite or leonardite and zeolite–vermiculite composite on changes in the culturable microorganisms of soil with elevated cadmium, zinc, and lead contents.

2. Material and Methods

2.1. Soil Material Properties

The pot experiment was conducted on soil with a granulometric composition of slightly loamy sand [32] in the growing hall of the University of Agriculture in Krakow in the years 2020–2021. The soil had an acidic pH and elevated concentrations of cadmium, lead, and zinc (Table 1).

Table 1. Selected soil properties before setting up the pot experiment.

Determinant	Value	Determinant	Value
Fraction 2–0.5 mm	85%	N total	0.40 $g \cdot kg^{-1}$ DM
Fraction 0.05–0.002 mm	12%	C total	5.74 $g \cdot kg^{-1}$ DM
Fraction < 0.002	3%	S total	0.118 $g \cdot kg^{-1}$ DM
pH H_2O	5.24	Pb total	188 ± 15 $mg \cdot kg^{-1}$ DM
pH KCl	5.03	Cd total	1.15 ± 0.08 $mg \cdot kg^{-1}$ DM
EC	273 $\mu S \cdot cm^{-1}$	Zn total	267 ± 18 $mg \cdot kg^{-1}$ DM
		Cr total	5.32 ± 0.73 $mg \cdot kg^{-1}$ DM
		Cu total	5.14 ± 1.33 $mg \cdot kg^{-1}$ DM
		Ni total	2.22 ± 0.18 $mg \cdot kg^{-1}$ DM

2.2. Design and Conduct of the Pot Experiment

The experiment included 6 treatments (Table 2) carried out in 4 replications.

Table 2. The objects amendments description.

Symbol	Mineral Salt	Zeolite–Vermiculite Composite	Lignite	Leonardite
C	-	-	-	-
MF	NPK	-	-	-
NaX–Ver3%L3%	NPK	3%	3%	-
NaX–Ver9%L6%	NPK	9%	6%	-
NaX–Ver3%Leo3%	NPK	3%	-	3%
NaX–Ver9%Leo6%	NPK	9%	-	6%

The reference was the control treatment without fertilization and with mineral fertilization (chemically pure salts, N–NH_4NO_3; P–$Ca(H_2PO_4)_2 \cdot H_2O$; K–KCl). The experiment

was set up in PVC pots holding 9 kg of air-dry soil mass. The vases used for the experiment were 25 cm high and 22 cm in diameter. The doses of nutrients introduced into the treatment soils were: nitrogen 0.20 g·kg^{-1} DM soil, phosphorus 0.10 g·kg^{-1} DM soil, and potassium 0.25 g·kg^{-1} DM soil, respectively. The nutrients, together with the mineral–organic mixture, were properly mixed with the soil before setting up the experiment. During the growing season, in the presence of nitrogen deficiency symptoms, additional doses of this nutrient in the form of NH_4NO_3 solution were applied (top dressing). The supplemental nitrogen dose was 0.02 g N·kg^{-1} DM soil for the maize. After applying mineral salts and mineral–organic mixtures and mixing them with the soil, maize grains of the Kosynier variety were sown. Soil moisture during plant growth was maintained at 40% to 60% of the maximum water capacity of soil (depending on the plant development stage). After the plants were harvested, soil samples were taken from each pot for chemical and biological analyses. Soil for biological analyses was 1 mm sieved and stored at 4 °C. Soil for physical and chemical analyses was air-dried, 1 mm sieved, and stored at room temperature.

2.3. Chemical Analyses in Soil Material

The following parameters were determined in the soil: pH KCl and pH H_2O potentiometrically, electrical conductivity (EC) conductometrically, total nitrogen content by a CNS analyzer (Vario MAX Cube, Elementar Analysensysteme GmbH, Langenselbold, Germany). Soil organic carbon content was determined by the Turin's oxidation and titration method. The total trace element content of the soil was determined by the ICP-OES method on a Perkin Elmer Optima 7300DV apparatus [33].

2.4. Microbiological Analyses of Soil

In order to evaluate the effect of mineral–organic mixtures on the number of selected soil microorganisms, 10 g of each soil sample was analyzed by Koch's serial dilution method using a range of microbial media. The following microorganisms were determined: total bacteria, mold fungi, actinomycetes, ammonifiers. The number of individual groups of microorganisms was determined using the method described previously [34]. Abundance analysis and identification of microorganisms were performed with the use of microbiological media and macroscopic and microscopic observations. The following microorganisms were determined: total bacteria (Trypticasein Soy Lab Agar, BTL, Warszawa, Poland, grown at 37 °C for 24 h), mold fungi (Malt Extract Agar, BTL, Warszawa, Poland, grown at 28 °C for 5 days), actinomycetes (Actinomycete Isolation Lab Agar, Biocorp, Warszawa, Poland, grown at 28 °C for 7 days), ammonifiers (medium according to Rougieux, Lanfroicourt, France, grown at 28 °C for 7 days) [34]. The number of colony-forming units (CFU) of microorganisms was determined by the dilution culture method, and the result was converted into 1 g DM of soil.

2.5. Respiration Activity

Soil respiration activity was determined according to the methodology published by the International Organization for Standardization 16072 [35]. For determination of basal respiration (BR), field-moist soil subsamples of 25 g, together with beakers with NaOH, were placed in hermetic jars and incubated at 20 ± 2 °C for 24 h. The released CO_2 was absorbed in 0.05 M solution of NaOH and precipitated as barium carbonate by adding 0.5 M solution of $Bacl_2$. Non-consumed sodium hydroxide was titrated with 0.1 M HCl in the presence of phenolphthalein as an indicator and then the amount of CO_2 was calculated according to ISO 14240-1 [36]. The next step was to add glucose to the soil samples and place additional NaOH beakers in jars for another 6 h to assess substrate-induced respiration activity (SIR). Respiratory activation quotient (QR) was calculated by dividing the BR rate by the SIR rate according to ISO 17155 [37].

2.6. Statistical Analyses

Differences between each treatment and control as well as between individual treatments were evaluated using one-way analysis of variance (ANOVA, Duncan's test, $p \leq 0.05$). Variation within treatments was determined by calculating standard deviation values (±SD). Correlation coefficient values were calculated using Spearman's non-parametric test for chemical and biological properties. All statistical analyses were performed using Statistica PL 13 software (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. Changes in Soil Physicochemical Properties

The determined pH H_2O (Table 3) in soil with fertilizer additions after both the first and the second years of the study was lower than the values determined in the control treatment, by 10.9% in the first year and 12.6% in the second year on average. A similar trend was found for pH measured in KCl (Table 3).

Table 3. Value of pH and EC in soil after the 1st year and 2nd year of the experiment.

	pH H_2O		**pH KCl**		**EC (µS·cm^{-1})**	
Treatment	1st year	2nd year	1st year	2nd year	1st year	2nd year
C	5.91 c ± 0.10	5.97 c ± 0.12	5.24 d ± 0.05	5.34 d ± 0.08	269 b ± 23.3	341 c ± 9.18
MF	5.28 ab ± 0.06	5.34 b ± 0.16	4.81 bc ± 0.09	4.79 ab ± 0.15	351 c ± 7.59	763 h ± 20.8
NaX–Ver3%L3%	5.22 ab ± 0.12	5.17 ab ± 0.13	4.90 bc ± 0.06	4.65 a ± 0.05	400 d ± 2.05	710 g ± 15.1
NaX–Ver6%L6%	5.30 ab ± 0.07	5.25 ab ± 0.18	4.89 c ± 0.06	4.74 a ± 0.16	231 a ± 5.88	641 f ± 49.1
NaX–Ver3%Leo3%	5.31 b ± 0.16	5.24 ab ± 0.13	4.84 c ± 0.07	4.67 a ± 0.12	434 d ± 4.92	699 g ± 52.9
NaX–Ver6%Leo6%	5.23 ab ± 0.05	5.09 a ± 0.14	4.89 c ± 0.12	4.61 a ± 0.17	559 e ± 6.94	1112 i ± 33.0

Means marked with the same letters do not differ significantly according to Duncan's test at $p \leq 0.05 \pm$ SD; factors: treatment, year; $n = 4$.

The values of soil pH H_2O determined after the second year of the study were not significantly different from those obtained after the first year. On the other hand, values measured in KCl indicated a reduction in pH (by 5.0% on average) in treatments with the addition of fertilizer composites. These results agree with the findings of Doni et al. [24] and contradict the findings of Moeen et al. [38].

The obtained values of electrical conductivity (EC) (Table 3) increased after the second year of the study in all treatments amended with fertilizers compared to the values obtained after the first year. After the first year of testing, EC values ranged from 231 to 559 µS cm^{-1}. On the other hand, the values obtained after the second year of the study were in the range between 341 and 1112 µS cm^{-1}. The highest increase in EC (increase of 177.5%) was determined in the soil of the NaX–Ver9%L6% treatment and in the soil with the addition of NaX–Ver9%Leo6% (increase of 98.9%). However, in our study, the results obtained for EC showed that the addition of all fertilizer composites increased electrical conductivity, and the most significant increase was caused by the addition of mixtures containing a 9% addition of NaX–Ver composite and a 6% addition of lignite or leonardite.

The soil organic carbon (SOC) content after the first year of the study ranged from 5.18 g kg^{-1} DM in the soil with NaX–Ver3%L3% to 6.27 g kg^{-1} DM in the soil of the NaX–Ver9%Leo6% treatment (Figure 1). After the second year of the experiment, an increase in SOC content was observed in all treatments. The largest increases, i.e., of 24.3% and 19.7%, were recorded in the NaX–Ver3%Leo3% treatment and in the MF treatment, respectively.

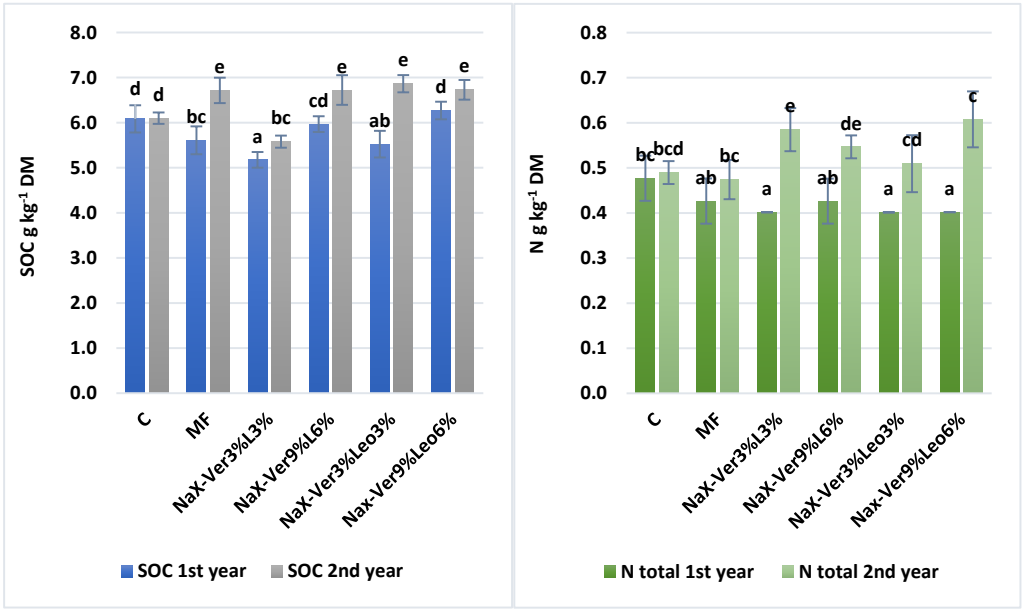

Figure 1. Organic carbon content and nitrogen content in soil after the 1st and 2nd year of the experiment; means marked with the same letters do not differ significantly according to Duncan's test at $p \leq 0.05$; factors: treatment, year; $n = 4$.

The total nitrogen (N total) content in the soil after the first and the second year of the study is shown in Figure 1. Comparing the obtained results of total nitrogen content after the first year and after the second year of the experiment with the addition of mixtures containing zeolite composites, an increase in N total content was determined in all treatments; however, the greatest parameter increase was found in NaX–Ver3%L3% (45.5%) and in NaX–Ver9%Leo6% (51%). Based on the results of total nitrogen content after the second year of the study, it was concluded that the application of mineral–organic mixtures containing zeolite composites increased the N total content compared to the reference treatments (C and MF).

3.2. Abundance of Culturable Microorganisms in Soil

After the second year of the experiment, the number of bacteria in all samples increased relative to the first year. This trend was particularly evident in soils with mixtures containing lignite (L3%, L6%) and leonardite (Leo3%, Leo6%) combined with a zeolite–vermiculite composite (NaX–Ver) where the difference in the number of bacteria between the first and second year was significant. The results of the number of bacteria in the soil after the first year ranged from 96,500 CFU·g^{-1} DM in the control to 1,213,333 CFU·g^{-1} DM in the soil with the addition of NaX–Ver3%Leo3%. However, the results obtained after the second year of research ranged from 328,000 CFU·g^{-1} DM in the control object to 3,722,167 CFU·g^{-1} DM in the soil with the addition of NaX–Ver3%Leo3%. Based on the analysis of the averages from the two years of the experiment, this fertilization variant with the addition of 3% leonardite combined with 3% zeolite–vermiculite composite was considered the most effective (Figure 2). The effectiveness of applied fertilizer mixtures on the number of soil microorganisms may vary depending on the type of soil to which the mixtures are applied and the plant grown in the given soil.

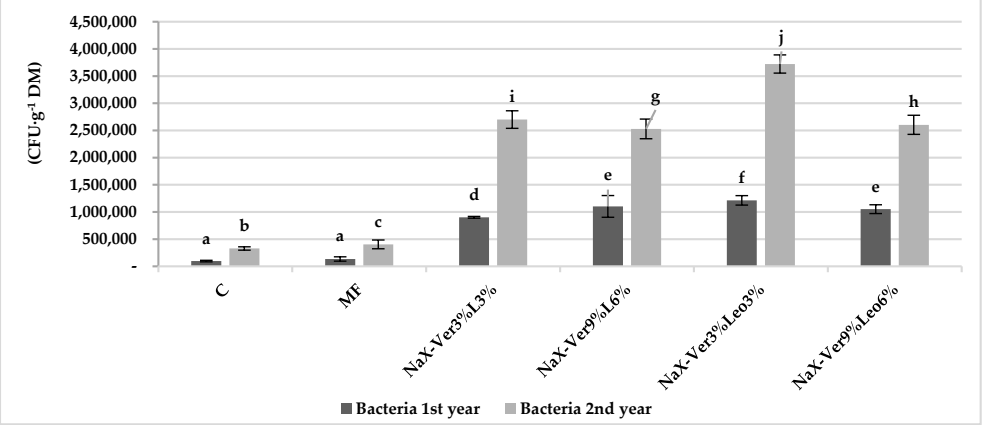

Figure 2. Average number (CFU g^{-1} DM) of bacteria after 1st and 2nd year of the experiment in soil samples; means marked with the same letters do not differ significantly according to Duncan's test at $p \leq 0.05$; factors: treatment, year; $n = 4$.

Similarly, in the case of mold fungi (Figure 3), the NaX–Ver3%Leo3% variant created the most favorable conditions for the growth of this group of microorganisms. Their average number from the two years was 852,500 (CFU·g^{-1} DM). In the case of the analysis of the number of mold fungi in the soil, a decrease was observed in the control object (from 108,000 CFU·g^{-1} DM after the first year to 44,000 CFU·g^{-1} DM after the second year) and in the MF object (from 117,000 CFU·g^{-1} DM after the first year to 73,000 CFU·g^{-1} DM after the second year). In soils with an addition of fertilizer mixtures, the number of mold fungi increased after the second year. The largest increase in the number of mold fungi after the second year was observed in the soil with the addition of NaX–Ver3%Leo3% and NaX–Ver9%Leo6%. The increasing number of total bacteria and mold fungi in fertilized soil indicates favorable soil conditions for these microorganisms, the presence of nutrients, and an optimal pH for them [39]. Bacteria grow best at a pH between 6.5 and 7.5, and fungi at a pH between 4.5 and 8.3; therefore, the pH of the studied soils was considered fully favorable for mold fungi and close to optimal for bacteria [40].

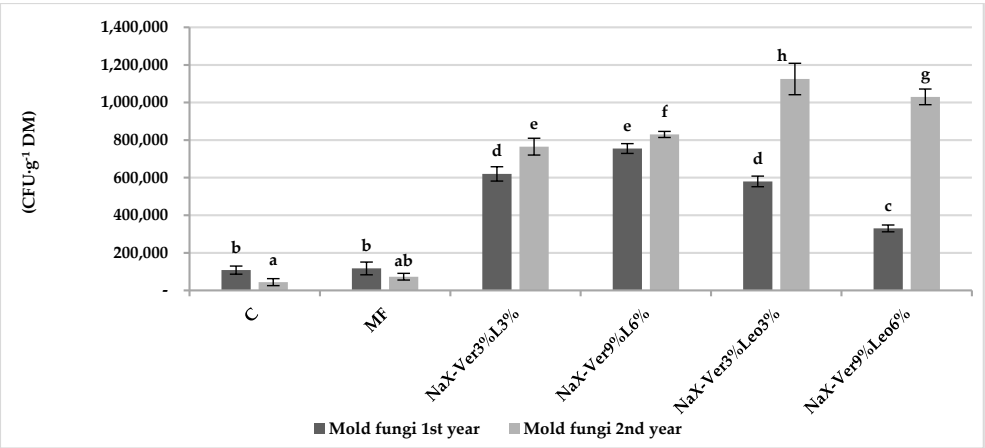

Figure 3. Average number (CFU g^{-1} DM) of mold fungi after 1st and 2nd year of the experiment in soil samples; means marked with the same letters do not differ significantly according to Duncan's test at $p \leq 0.05$; factors: treatment, year; $n = 4$.

Actinomycetes were the microorganisms whose growth was the most affected by NPK fertilization (MF) compared to the control (C). The number of actinomycetes increased from 203 CFU·g^{-1} DM after the first year to 1100 CFU·g^{-1} DM after the second year in soil without fertilization (C). After applying only mineral fertilization (MF), the

number of actinomycetes in the soil decreased from 4000 CFU·g^{-1} DM after the first year to 2350 CFU·g^{-1} DM after the second year. The greatest increase in the number of actinomycetes was observed in the soil with the addition of NaX–Ver3%Leo3% (from 3800 CFU·g^{-1} DM after the first year to 6500 CFU·g^{-1} DM after the second year. Analysis of the average abundances from the two years showed the greatest increase in actinomycete counts (5700 CFU·g^{-1} DM) in soil with a mixture of 6% leonardite addition combined with a zeolite–vermiculite composite (NaX–Ver9%Leo6%) (Figure 4).

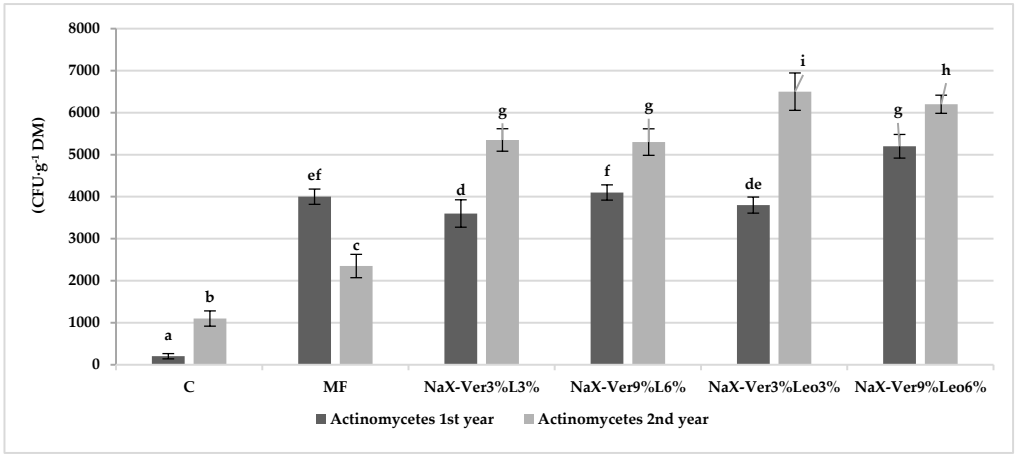

Figure 4. Average number (CFU g^{-1} DM) of actinomycetes after 1st and 2nd year of the experiment in soil samples; means marked with the same letters do not differ significantly according to Duncan's test at $p \leq 0.05$; factors: treatment, year; n = 4.

In our research, the smallest number of ammonifying bacteria, after both the first and the second year of research, was determined in the control soil. After the first year of research, the greatest number of ammonifiers was determined for soil with the addition of NaX–Ver9%Leo6%, while, after the second year of research, the highest number of ammonifiers was found in soil with the addition of NaX–Ver3%Leo3%. Interestingly, a decrease in the number of ammonifiers was observed in the second year of the experiment (with addition of NaX–Ver3%L3%); nevertheless, they still remained at several times higher levels than in soil without fertilization (C). Analysis of the average abundances from the two years of the pot experiment showed that ammonifiers reached the highest abundance in the NaX–Ver3%Leo3% soil (Figure 5). There was no proportional increase in the number of culturable microorganisms with a doubling of the percentage of lignite or leonardite in the mixture for the analyzed microbial groups. These relationships were much more complex, as discussed in detail above.

3.3. Changes in Soil Respiration Activity

The results of basal respiration (Figure 6a) obtained after the first year of the study ranged from 1.58 µg C–CO_2 g^{-1} DM h^{-1} to 2.99. The highest C–CO_2 content was determined in the control treatment, while the lowest respiration activity was found for soil from the NaX–Ver9%Leo6% treatment. The respiration activity determined after the second year changed. The lowest respiration activity was determined in MF, 1.32 µg C–CO_2 g^{-1} DM h^{-1}, while the highest was determined in NaX–Ver3%Leo3% and NaX–Ver9%Leo6%: 2.12 µg C–CO_2 g^{-1} DM h^{-1} and 2.14 µg C–CO_2 g^{-1} DM h^{-1}, respectively. An increase in BR values after the second year was observed in the treatments amended with mineral–organic mixtures containing the NaX–Ver composite in combination with leonardite, which may indicate the positive effect of these materials on microbial growth and activity.

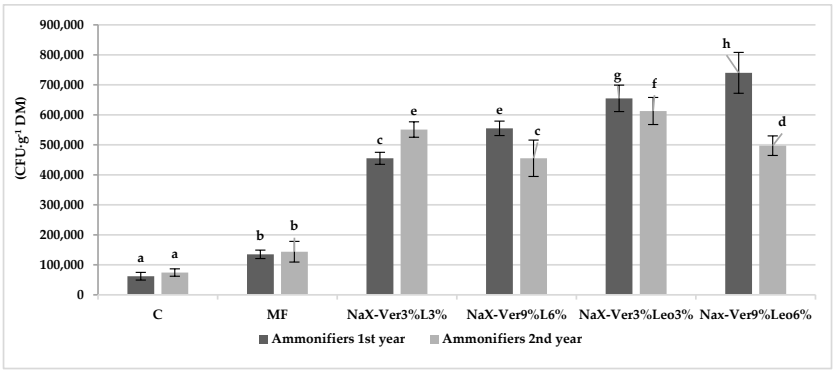

Figure 5. Average number (CFU g^{-1} DM) of ammonifiers after 1st and 2nd year of the experiment in soil samples; means marked with the same letters do not differ significantly according to Duncan's test at $p \leq 0.05$; factors: treatment, year; $n = 4$.

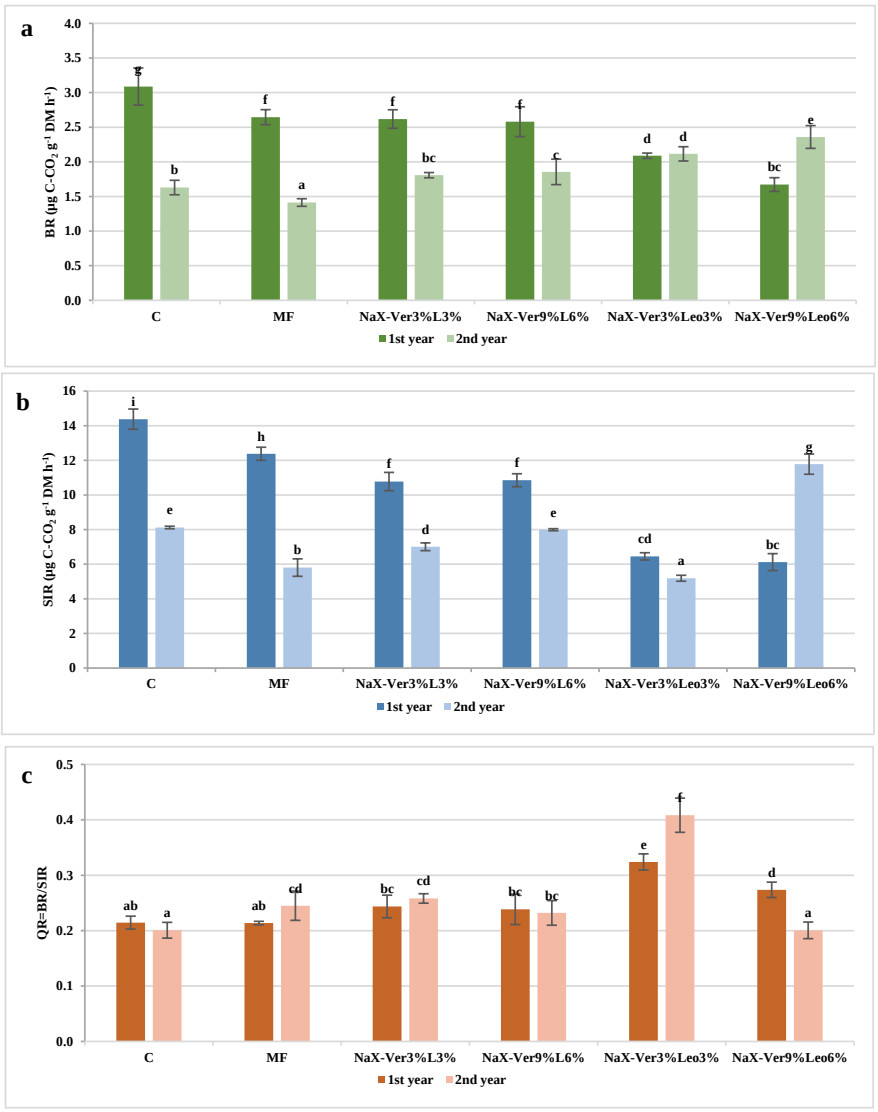

Figure 6. (**a**)—Basal respiration (BR) in soil after the 1st and 2nd year of the experiment (µg C–CO_2 g^{-1} DM h^{-1}); (**b**)—Substrate-induced respiration (SIR) in soil after the 1st and 2nd year of the experiment (µg C–CO_2 g^{-1} DM h^{-1}); (**c**)—Respiratory activation quotient (QR) in soil after the 1st and 2nd year of the experiment (µg C-CO_2 g^{-1} DM h^{-1}); means marked with the same letters do not differ significantly according to Duncan's test at $p \leq 0.05$; factors: treatment, year; $n = 4$.

The highest substrate-induced respiration activity, SIR (Figure 6b), in the analyzed samples after the first year of the study was determined in the control treatment, while the values obtained in the treatments amended with mineral–organic mixtures were significantly lower. In contrast, respiration activity determined after the second year of the experiment showed a different trend, as the highest SIR activity was found in the treatment with NaX–Ver9%Leo6%. The respiratory activation quotient (QR) values obtained after the first year ranged from 0.207 in the control treatment to 0.310 in the treatment with NaX–Ver3%Leo3%. On the other hand, QR values obtained after the second year ranged from 0.192 to 0.445, with the highest value determined in the treatment with the NaX–Ver3%Leo3% addition (Figure 6c).

Statistical analysis using Spearman's non-parametric test to evaluate the relationship between the number of microorganisms and basic parameters such as pH, EC, SOC content, and total nitrogen content in the soil showed varying correlations between the analyzed data (Figure 7).

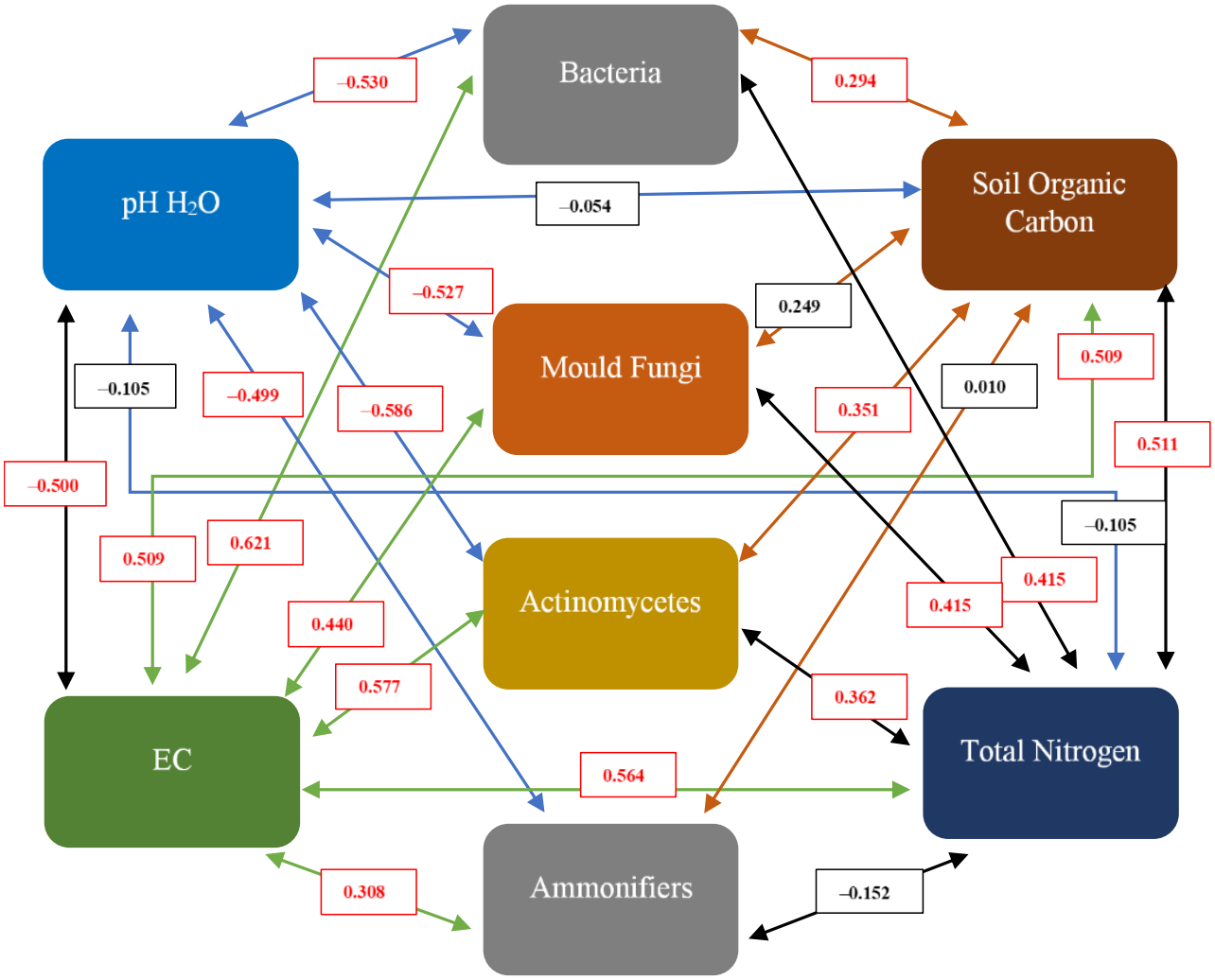

Figure 7. Spearman's correlation coefficients between the selected soil chemical and abundances of culturable microbes ($n = 4$); $p < 0.05$; significant values are marked in red.

The study revealed that the total number of bacteria in the soil was significantly positively correlated with the content of SOC, N total, and EC, but negatively correlated with the obtained pH values. The same correlation was observed between the analyzed parameters and the number of actinomycetes. In the case of the number of fungi, a similar trend was observed with respect to pH, EC, and N total, while no significant relationship was found between the number of fungi and SOC. The number of ammonifiers was

significantly positively correlated with the obtained EC values and significantly negatively correlated with pH values, while the relationship between the contents of SOC and N total and the number of ammonifiers was not statistically significant.

4. Discussion

4.1. Mineral–Organic Mixture Application Affects Soil Physicochemical Properties

Soil pH is a very important parameter that determines the number of different groups of microorganisms in the soil. Results obtained by Zheng et al. [41] indicated that the application of zeolites increased soil pH by 0.59 compared to control soil. Our own results revealed that the combination of mineral fertilization with a synthetic zeolite–vermiculite composite and organic materials in the form of lignite or leonardite reduced soil pH compared to the treatment without fertilization. Moeen et al. [38] showed an increase in soil pH from 3% to 5% depending on the incubation time and zeolite dose. In contrast, Doni et al. [24] demonstrated that the soil application of zeolite in grapevine cultivation did not significantly change soil pH values. In a study by Badora [42], zeolites applied in a higher dose (600 mg kg^{-1} soil) increased the pH of soil contaminated with lead compounds the most. A study presented by Ravali et al. [43] showed a substantial increase in soil electrical conductivity after zeolite application. Significantly higher pH and EC values were recorded when zeolite was applied in doses of 7.5 t ha^{-1} and 5 t ha^{-1}. The high exchange capacity of zeolites affects electrical conductivity because zeolites can introduce cations to the water being used to measure EC. Similar results were obtained by Ramesh et al. [44] and Li et al. [45], who observed an increase in EC with the application of zeolite.

Ravali et al. [43] showed no significant effect on the organic carbon content in red soil after the application of a mixture of zeolite and nitrogen fertilizer. On the other hand, a study by Zaidun et al. [46] investigated the effect of soil application of zeolite (clinoptilolite) and a combination of zeolite and biochar on soil organic carbon (SOC) content. The cited authors showed that the addition of 2.5 t ha^{-1} of zeolite increased the SOC content by 2.28%, while the combination of zeolite and biochar (2.5 t ha^{-1} + 20 t ha^{-1}) increased the SOC content by 3.98%. The study of Yousefian et al. [47] showed different results, as the authors found no significant effect of zeolite application in different doses on total nitrogen content in semiarid soils. In a study by Doni et al. [24], there was no clear effect on the contents of total organic carbon and total nitrogen in the soil six months after applying zeolite. Santoso et al. [48] studied the effect of zeolite and manure application on pH values and total nitrogen and organic carbon contents in sandy soil. The values of pH, N total, and SOC increased with increasing doses of manure and zeolite. The soil analysis results showed that the addition of zeolite in a dose of 4 t ha^{-1} increased soil pH by 0.66 84 days after its application. The study conducted by the cited authors also showed a 0.15% and 1.13% increase in N total and SOC contents, respectively.

4.2. Mineral–Organic Mixture Application Impact on Microorganism Abundance

Soil fertilization is an important factor that influences the culturable microorganisms in soil [49]. Analyzing changes in soil microorganisms after the application of different fertilizers is one of the necessary measures that can help the assessment of the direct impact of fertilizers on the soil environment [50]. A study by Qin et al. [51] showed that the addition of nitrogen changed the organic carbon content in the soil, which proves that the nitrogen content indirectly affects soil physicochemical properties and bacterial community structure. According to Tian et al. [52], the addition of external organic matter to the soil changes the abundance, species composition, and activity of soil microorganisms. Soil microorganisms, such as bacteria, mold fungi, actinomycetes, and ammonifiers, have a significant impact on soil fertility, because they are able to convert elements into plant-accessible forms and can also take part in the decomposition of toxic substances [49]. Based on many years of research, it has been shown that low doses of mineral fertilizers have a positive effect on the productivity of plants as well as on the agrochemical and microbiological properties of the soil. Importantly, mineral fertilizers intensively affect

the development of microorganisms involved in the transformation of nitrogen in the soil; they promote the multiplication of bacteria and fungi carrying out numerous biochemical processes in the soil [53]. Due to the above, they indirectly influence the mineralization of crop residues. However, the excessive use of mineral fertilizers to increase fertility leads to complete soil degradation and disturbance of biological connections, leading to loss of homeostasis. The presence of mineral–organic additives in the soil is a factor influencing the population of microorganisms. Properly balanced fertilization not only contributes to the increase in plant yield, but also affects soil microorganisms, which additionally stimulate plant growth [54]. Unfortunately, there is a risk associated with too much fertilizer addition to the soil. Incorrectly selected and excessive addition of mineral–organic materials to the soil has a negative effect on microorganisms, as the species diversity of microorganisms decreases. Such a phenomenon leads to the multiplication of pathogenic and crop-infecting microorganisms. That is why it is so important to conduct research aimed at determining the effect of fertilization on soil microorganisms because the greater diversity of microorganism communities has a decisive impact on the quality of the soil [55].

4.3. Changes in Respiration Activity in Soil

The respiration activity in soil is determined by the overall diversity of microorganisms, including unculturable microbes. Hence, despite the fact that the abundance of culturable microorganisms was estimated here, the respiration activity in soil is an indirect function of complete microbial diversity present in the soil, and its changes may be attributed to variation in microbial populations. As stated by Ramsey et al. [56], soil contamination with trace elements has a negative effect on the respiration activity of soil microorganisms. Adding substrate in the form of glucose to soil samples generally significantly increases respiration rates, and this is associated with access to readily biodegradable carbon for microorganisms [29,57]. In a study by Bikkinia et al. [58], the application of mechanically activated zeolite and nanostructured zeolite increased the respiration activity of the soil, and the obtained results ranged from 27.3 to 57.9 mg CO_2 100 g^{-1} 24 h^{-1} of soil depending on the mixture used. Increasing the QR value may indicate an available source of carbon in the soil for microorganisms; on the other hand, QR may be an indicator of non-biodegradable pollutants, e.g., heavy metals [57]. If the basal respiration is high in contaminated soil, this may indicate that the contaminants may provide a suitable substrate for the growth of certain groups of microorganisms in soil. However, more detailed studies are needed to verify the exact effect of soil contaminants on respiration activity [31].

4.4. Correlation between Physicochemical Properties and Culturable Microorganisms' Population

The literature data show that the soil organic carbon content is related to the soil physicochemical properties, as well as to the abundance and diversity of microorganisms [51]. As demonstrated by Xiao et al. [59], the addition of exogenous matter affects the abundance and diversity of bacteria and fungi. Moreover, the soil pH determines the variety of fungi; the authors showed that an increase in pH leads to a decrease in the variety of fungi. This correlation is consistent with the results obtained in this study. An interesting approach to the presence of soil microorganisms in polluted/post-industrial areas was presented in the study of Feketeova et al. [60]. The quoted authors concluded that, in soils contaminated with heavy metals, microorganisms can utilize available nutrients very efficiently without unnecessary energy losses and without difficulties related to survival. Undoubtedly, contaminated ecosystems are capable of self-settlement over time, even in polluted areas.

5. Conclusions

In this work, very low abundance of culturable ammonifying bacteria, after both the first and the second year of research, was observed in the control soil. This indicates the stimulating effect of the applied mixtures on selected groups of soil microorganisms. At the same time, it is clear that fertilization based on mineral and organic additions in the

form of lignite and leonardite combined with zeolite–vermiculite composite is much more effective in increasing the number of microorganisms than NPK supplementation alone. The stimulation of soil microbes to grow and develop was similar for both organic additives to the mixtures, i.e., lignite and leonardite stimulated soil microbes to grow and develop at similarly high levels, while leonardite was marginally better in this respect. A double dose of organic materials (6% instead of 3%) did not increase the number of microorganisms in the soil; therefore, the lower addition (3%) was considered fully sufficient from a microbiological point of view. The applied fertilizer mixtures had a positive effect on increasing the organic carbon and total nitrogen contents in the analyzed soils. The correlation analysis confirmed that the number of soil culturable microorganisms significantly depends on basic soil properties, such as pH, EC, and SOC and N total contents, as confirmed by correlation analysis. The obtained QR results allowed us to conclude that remediation of contaminated soil was most effective in soil with NaX–Ver3%Leo3%. The demonstrated cumulative effect of mineral–organic mixtures on the development and abundance of soil microorganisms is desirable from an agricultural point of view. In general, respiration activity results obtained after the second year differed from those obtained after the first year, indicating the need to monitor and analyze the long-term effects of the applied fertilizer mixtures on respiration activity in fertilized soils.

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

Funding: The POIR.04.04.00-00-14E6/18-00 project was carried out within the TEAM-NET program of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The "Fly ash as the precursors of functionalized materials for applications in environmental engineering, civil engineering and agriculture" no. POIR.04.04.00-00-14E6/18-00 project is carried out within the TEAM-NET programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.

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

References

1. Jeffery, S.; Gardi, C.; Jones, A.; Montanarella, L.; Marmo, L.; Miko, L.; Ritz, K.; Pérès, G.; Römbke, J.; van der Putten, W.H. European Atlas of Soil Biodiversity. *Eur. Commision. Publ. Off.* **2010**, *128*, 94222. [CrossRef]
2. Paul, E.A.; Clark, F.E. *Soil Microbiology and Biochemistry*; UMCS: Lublin, Poland, 2000. (In Polish)
3. Torsvik, V.; Øvreås, L. Microbial Diversity and Function in Soil: From Genes to Ecosystems. *Curr. Opin. Microbiol.* **2002**, *5*, 240–245. [CrossRef]
4. Nannipieri, P.; Ascher, J.; Ceccherini, M.T.; Landi, L.; Pietramellara, G.; Renella, G. Microbial Diversity and Soil Functions. *Eur. J. Soil Sci.* **2003**, *54*, 655–670. [CrossRef]
5. Mendes, R.; Garbeva, P.; Raaijmakers, J.M. The Rhizosphere Microbiome: Significance of Plant Beneficial, Plant Pathogenic, and Human Pathogenic Microorganisms. *FEMS Microbiol. Rev.* **2013**, *37*, 634–663. [CrossRef]
6. Jones, D.L.; Magthab, E.A.; Gleeson, D.B.; Hill, P.W.; Sánchez-Rodríguez, A.R.; Roberts, P.; Ge, T.; Murphy, D.V. Microbial Competition for Nitrogen and Carbon Is as Intense in the Subsoil as in the Topsoil. *Soil Biol. Biochem.* **2018**, *117*, 72–82. [CrossRef]
7. Ishaq, S.L.; Ishaq, S.L. Plant-Microbial Interactions in Agriculture and the Use of Farming Systems to Improve Diversity and Productivity. *AIMS Microbiol.* **2017**, *3*, 335–353. [CrossRef]
8. Radziemska, M. Study of Applying Naturally Occurring Mineral Sorbents of Poland (Dolomite Halloysite, Chalcedonite) for Aided Phytostabilization of Soil Polluted with Heavy Metals. *Catena* **2018**, *163*, 123–129. [CrossRef]

9. Jin, Q.; Kirk, M.F. PH as a Primary Control in Environmental Microbiology: 2. Kinetic Perspective. *Front. Environ. Sci.* **2018**, *6*, 101. [CrossRef]
10. Thompson, L.R.; Sanders, J.G.; McDonald, D.; Amir, A.; Ladau, J.; Locey, K.J.; Prill, R.J.; Tripathi, A.; Gibbons, S.M.; Ackermann, G.; et al. A Communal Catalogue Reveals Earth's Multiscale Microbial Diversity. *Nature* **2017**, *551*, 457–463. [CrossRef]
11. Shi, Y.; Li, Y.; Yang, T.; Chu, H. Threshold Effects of Soil PH on Microbial Co-Occurrence Structure in Acidic and Alkaline Arable Lands. *Sci. Total Environ.* **2021**, *800*, 149592. [CrossRef]
12. Fijałkowski, K.; Kacprzak, M.; Grobelak, A.; Placek, A. The Influence of Selected Soil Parameters on the Mobility of Heavy Metals in Soils. *Inżynieria Ekol.* **2012**, *15*, 81–92.
13. Cataldo, E.; Salvi, L.; Paoli, F.; Fucile, M.; Masciandaro, G.; Manzi, D.; Masini, C.M.; Mattii, G.B. Application of Zeolites in Agriculture and Other Potential Uses: A Review. *Agronomy* **2021**, *11*, 1547. [CrossRef]
14. Fierer, N. Embracing the Unknown: Disentangling the Complexities of the Soil Microbiome. *Nat. Rev. Microbiol.* **2017**, *15*, 579–590. [CrossRef]
15. Liu, H.; Wang, C.; Xie, Y.; Luo, Y.; Sheng, M.; Xu, F.; Xu, H. Ecological Responses of Soil Microbial Abundance and Diversity to Cadmium and Soil Properties in Farmland around an Enterprise-Intensive Region. *J. Hazard. Mater.* **2020**, *392*, 122478. [CrossRef]
16. Belviso, C. Zeolite for Potential Toxic Metal Uptake from Contaminated Soil: A Brief Review. *Processes* **2020**, *8*, 820. [CrossRef]
17. Jarosz, R.; Szerement, J.; Gondek, K.; Mierzwa-Hersztek, M. The Use of Zeolites as an Addition to Fertilisers—A Review. *Catena* **2022**, *213*, 106125. [CrossRef]
18. Lenart, A.; Wolny-Koładka, K. The Effect of Heavy Metal Concentration and Soil Ph on the Abundance of Selected Microbial Groups within Arcelormittal Poland Steelworks in Cracow. Bull. Environ. *Contam. Toxicol.* **2013**, *90*, 85–90. [CrossRef] [PubMed]
19. Rathnayake, I.V.N.; Megharaj, M.; Bolan, N.; Naidu, R. Tolerance of Heavy Metals by Gram Positive Soil Bacteria. *Int. J. Bioeng. Life Sci.* **2009**, *3*, 270–274. [CrossRef]
20. Xie, Y.; Fan, J.; Zhu, W.; Amombo, E.; Lou, Y.; Chen, L.; Fu, J. Effect of Heavy Metals Pollution on Soil Microbial Diversity and Bermudagrass Genetic Variation. *Front. Plant Sci.* **2016**, *7*, 755. [CrossRef]
21. Good, A.G.; Beatty, P.H. Fertilizing Nature: A Tragedy of Excess in the Commons. *PLoS Biol.* **2011**, *9*, 1001124. [CrossRef]
22. Thompson, R.L.; Lassaletta, L.; Patra, P.K.; Wilson, C.; Wells, K.C.; Gressent, A.; Koffi, E.N.; Chipperfield, M.P.; Winiwarter, W.; Davidson, E.A.; et al. Acceleration of Global N2O Emissions Seen from Two Decades of Atmospheric Inversion. *Nat. Clim. Change* **2019**, *9*, 993–998. [CrossRef]
23. Mondal, M.; Biswas, B.; Garai, S.; Sarkar, S.; Banerjee, H.; Brahmachari, K.; Bandyopadhyay, P.K.; Maitra, S.; Brestic, M.; Skalicky, M.; et al. Zeolites Enhance Soil Health, Crop Productivity and Environmental Safety. *Agronomy* **2021**, *11*, 448. [CrossRef]
24. Doni, S.; Gispert, M.; Peruzzi, E.; Macci, C.; Mattii, G.B.; Manzi, D.; Masini, C.M.; Grazia, M. Impact of Natural Zeolite on Chemical and Biochemical Properties of Vineyard Soils. *Soil Use Manag.* **2020**, *2*, 12665. [CrossRef]
25. Lahori, A.H.; Mierzwa-Hersztek, M.; Demiraj, E.; Sajjad, R.U.; Ali, I.; Shehnaz, H.; Aziz, A.; Zuberi, M.H.; Pirzada, A.M.; Hassan, K.; et al. Direct and Residual Impacts of Zeolite on the Remediation of Harmful Elements in Multiple Contaminated Soils Using Cabbage in Rotation with Corn. *Chemosphere* **2020**, *250*, 126317. [CrossRef]
26. Głąb, T.; Gondek, K.; Mierzwa–Hersztek, M. Biological Effects of Biochar and Zeolite Used for Remediation of Soil Contaminated with Toxic Heavy Metals. *Sci. Rep.* **2021**, *11*, 6998. [CrossRef]
27. Mühlbachová, G.; Šimon, T. Effects of Zeolite Amendment on Microbial Biomass and Respiratory Activity in Heavy Metal Contaminated Soils. *Plant Soil Environ.* **2003**, *49*, 536–541. [CrossRef]
28. Han, G.; Zhou, G.; Xu, Z.; Yang, Y.; Liu, J.; Shi, K. Soil Temperature and Biotic Factors Drive the Seasonal Variation of Soil Respiration in a Maize (*Zea Mays* L.) Agricultural Ecosystem. *Plant Soil* **2007**, *291*, 15–26. [CrossRef]
29. Mierzwa-Hersztek, M.; Klimkowicz-Pawlas, A.; Gondek, K. Influence of Poultry Litter and Poultry Litter Biochar on Soil Microbial Respiration and Nitrifying Bacteria Activity. *Waste Biomass Valorization* **2018**, *9*, 379–389. [CrossRef]
30. Álvarez-Ayuso, E.; Abad-Valle, P. Application of Different Alkaline Materials as Polluted Soil Amendments: A Comparative Assessment of Their Impact on Trace Element Mobility and Microbial Functions. *Ecotoxicol. Environ. Saf.* **2021**, *227*, 112927. [CrossRef]
31. Eisentraeger, A.; Maxam, G.; Rila, J.P.; Dott, W. A Stepwise Procedure for Assessment of the Microbial Respiratory Activity of Soil Samples Contaminated with Organic Compounds. *Ecotoxicol. Environ. Saf.* **2000**, *47*, 65–73. [CrossRef]
32. WRB. 2015 World Reference Base for Soil Resources. Available online: https://www.fao.org/3/i3794en/I3794en.pdf (accessed on 11 October 2022).
33. Oleszczuk, N.; Castro, J.T.; da Silva, M.M.; Korn, M.; Das, G.A.; Welz, B.; Vale, M.G.R. Method Development for the Determination of Manganese, Cobalt and Copper in Green Coffee Comparing Direct Solid Sampling Electrothermal Atomic Absorption Spectrometry and Inductively Coupled Plasma Optical Emission Spectrometry. *Talanta* **2007**, *73*, 862–869. [CrossRef] [PubMed]
34. Wolny-Koładka, K.; Jarosz, R.; Marcińska-Mazur, L.; Lošák, T.; Mierzwa-Hersztek, M. Effect of Mineral and Organic Additions on Soil Microbial Composition. *Int. Agrophysics* **2022**, *36*, 131–138. [CrossRef]
35. ISO. 16072 International Organization for Standardization 16072 (ISO 16072 2002—Szukaj w Google). Available online: https://www.google.com/search?q=International+Organization+for+Standardization+16072+(ISO+16072+2002&rlz=1C1AVFC_enPL885PL885&oq=International+Organization+for+Standardization+16072+(ISO+16072+2002&aqs=chrome..69i57.744j0j4&sourceid=chrome&ie=UTF-8 (accessed on 11 October 2022).

36. Singer, S.; Ewing, M. *ISO 14240-1:1997*; Soil Quality—Determination of Soil Microbial Biomass. Part 1: Substrate-Induced Respiration Method. ISO: Geneva, Switzerland, 2000; pp. G271–G278.
37. *ISO 17155:2012*; Soil Quality—Determination of Abundance and Activity of Soil Microflora Using Respiration Curves. ISO: Geneva, Switzerland, 2012.
38. Moeen, M.; Qi, T.; Hussain, Z.; Ge, Q.; Maqbool, Z.; Jianjie, X.; Kaiqing, F. Use of Zeolite to Reduce the Bioavailability of Heavy Metals in a Contaminated Soil. *J. Ecol. Eng.* **2020**, *21*, 186–196. [CrossRef]
39. Malinowski, M.; Wolny-Koładka, K.; Vaverková, M.D. Effect of Biochar Addition on the OFMSW Composting Process under Real Conditions. *Waste Manag.* **2019**, *84*, 364–372. [CrossRef]
40. Rousk, J.; Brookes, P.C.; Bååth, E. Contrasting Soil PH Effects on Fungal and Bacterial Growth Suggest Functional Redundancy in Carbon Mineralization. *Appl. Environ. Microbiol.* **2009**, *75*, 1589–1596. [CrossRef] [PubMed]
41. Zheng, X.J.; Chen, M.; Wang, J.F.; Liu, Y.; Liao, Y.Q.; Liu, Y.C. Assessment of Zeolite, Biochar, and Their Combination for Stabilization of Multimetal-Contaminated Soil. *ACS Omega* **2020**, *5*, 27374–27382. [CrossRef] [PubMed]
42. Badora, A. The Influence of Zeolites on Quality Indicators of Soil-Plant Connection and Food Safety. In *Zeolites—Useful Miner*; IntechOpen: London, UK, 2016; p. 64590. [CrossRef]
43. Ravali, C.; Rao, J.K.; Anjaiah, T.; Suresh, K. Effect of Zeolite on Soil Physical and Physico-Chemical Properties. *J. Sci. Agric. Eng.* **2020**, *10*, 776–781.
44. Ramesh, K.; Biswas, A.K.; Patra, A.K. Zeolitic Farming. *Indian J. Agron.* **2015**, *60*, 185–191.
45. Feng, W.; Wan, Z.; Daniels, J.; Li, Z.; Xiao, G.; Yu, J.; Xu, D.; Guo, H.; Zhang, D.; May, E.F.; et al. Synthesis of High Quality Zeolites from Coal Fly Ash: Mobility of Hazardous Elements and Environmental Applications. *J. Clean. Prod.* **2018**, *202*, 390–400. [CrossRef]
46. Zaidun, S.W.; Jalloh, M.B.; Awang, A.; Sam, L.M.; Besar, N.A.; Musta, B.; Ahmed, O.H.; Omar, L. Biochar and Clinoptilolite Zeolite on Selected Chemical Properties of Soil Cultivated with Maize (*Zea Mays* L.). *Eurasian J. Soil Sci.* **2019**, *8*, 468100. [CrossRef]
47. Yousefian, M.; Jafari, M.; Tavili, A.; Arzani, H.; Jafarian, Z. The Effects of Superabsorbent Polymer on Atriplex Lentiformis Growth and Soil Characteristics under Drought Stress (Case Study: Desert Research Station, Semnan, Iran). *J. Rangel. Sci.* **2018**, *8*, 65–76.
48. Santoso, B.; Cholid, M.; Wijayanto, R.A. Effect of Zeolite and Cow Manure Application on Soil Nitrogen Content in Ramie (Boehmeria Nivea) Plant Growth. *IOP Conf. Ser. Earth Environ. Sci.* **2022**, *974*, 012073. [CrossRef]
49. Mierzwa-Hersztek, M.; Wolny-Koładka, K.; Gondek, K.; Gałązka, A.; Gawryjołek, K. Effect of Coapplication of Biochar and Nutrients on Microbiocenotic Composition, Dehydrogenase Activity Index and Chemical Properties of Sandy Soil. *Waste Biomass Valorization* **2020**, *11*, 3911–3923. [CrossRef]
50. Wolny-Koładka, K.; Żukowski, W. Mixed Municipal Solid Waste Hygienisation for Refuse-Derived Fuel Production by Ozonation in the Novel Configuration Using Fluidized Bed and Horizontal Reactor. *Waste Biomass Valorization* **2019**, *10*, 575–583. [CrossRef]
51. Qin, J.; Liu, H.; Zhao, J.; Wang, H.; Zhang, H.; Yang, D.; Zhang, N. The Roles of Bacteria in Soil Organic Carbon Accumulation under Nitrogen Deposition in Stipa Baicalensis Steppe. *Microorganisms* **2020**, *8*, 326. [CrossRef]
52. Tian, J.; Wang, J.; Dippold, M.; Gao, Y.; Blagodatskaya, E.; Kuzyakov, Y. Biochar Affects Soil Organic Matter Cycling and Microbial Functions but Does Not Alter Microbial Community Structure in a Paddy Soil. *Sci. Total Environ.* **2016**, *556*, 89–97. [CrossRef]
53. Sivojiene, D.; Kacergius, A.; Baksiene, E.; Maseviciene, A.; Zickiene, L. The Influence of Organic Fertilizers on the Abundance of Soil Microorganism Communities, Agrochemical Indicators, and Yield in East Lithuanian Light Soils. *Plants* **2021**, *10*, 2648. [CrossRef]
54. Triberti, L.; Nastri, A.; Baldoni, G. Long-Term Effects of Crop Rotation, Manure and Mineral Fertilisation on Carbon Sequestration and Soil Fertility. *Eur. J. Agron.* **2016**, *74*, 47–55. [CrossRef]
55. Qu, Y.; Tang, J.; Li, Z.; Zhou, Z.; Wang, J.; Wang, S.; Cao, Y. Soil Enzyme Activity and Microbial Metabolic Function Diversity in Soda Saline–Alkali Rice Paddy Fields of Northeast China. *Sustainability* **2020**, *12*, 10095. [CrossRef]
56. Ramsey, P.W.; Rillig, M.C.; Feris, K.P.; Gordon, N.S.; Moore, J.N.; Holben, W.E.; Gannon, J.E. Relationship between Communities and Processes; New Insights from a Field Study of a Contaminated Ecosystem. *Ecol. Lett.* **2005**, *8*, 1201–1210. [CrossRef]
57. Eisentraeger, A.; Hund-Rinke, K.; Roembke, J. Assessment of Ecotoxicity of Contaminated Soil Using Bioassays. *Monit. Assess. Soil Bioremed.* **2005**, *5*, 321–360. [CrossRef]
58. Bikkinina, L.M.-H.; Ezhkov, V.O.; Faizrakhmanov, R.N.; Gazizov, R.R.; Ezhkova, A.M. Effect of Zeolites on Soil Modification and Productivity. *BIO Web Conf.* **2020**, *17*, 00117. [CrossRef]
59. Xiao, D.; Huang, Y.; Feng, S.; Ge, Y.; Zhang, W.; He, X.; Wang, K. Soil Organic Carbon Mineralization with Fresh Organic Substrate and Inorganic Carbon Additions in a Red Soil Is Controlled by Fungal Diversity along a PH Gradient. *Geoderma* **2018**, *321*, 79–89. [CrossRef]
60. Feketeová, Z.; Hrabovský, A.; Šimkovic, I. Microbial Features Indicating the Recovery of Soil Ecosystem Strongly Affected by Mining and Ore Processing. *Int. J. Environ. Res. Public Health* **2021**, *18*, 3240. [CrossRef]