agriculture

MDPI

Article

The Effect of Fertilization with Antibiotic-Contaminated Manure on Microbial Processes in Soil

Jadwiga Wyszkowska [1,*], Dariusz Mikulski [2], Agata Borowik [1], Magdalena Zaborowska [1], Jan Kucharski [1], Krzysztof Kozłowski [2], Magdalena Bilecka [3], Anna Gajda [3], Konrad Pietruk [3], Piotr Jedziniak [3], Katarzyna Ognik [4] and Jan Jankowski [2]

[1] Department of Soil Science and Microbiology, Faculty of Agriculture and Forestry, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland; agata.borowik@uwm.edu.pl (A.B.); m.zaborowska@uwm.edu.pl (M.Z.); jan.kucharski@uwm.edu.pl (J.K.)
[2] Department of Poultry Science and Apiculture, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland; dariusz.mikulski@uwm.edu.pl (D.M.); kristof@uwm.edu.pl (K.K.); janj@uwm.edu.pl (J.J.)
[3] Department of Pharmacology and Toxicology, National Veterinary Research Institute, Partyzantów 57, 24-100 Puławy, Poland; magdalena.bilecka@piwet.pulawy.pl (M.B.); anna.gajda@piwet.pulawy.pl (A.G.); konrad.pietruk@piwet.pulawy.pl (K.P.); piotr.jedziniak@piwet.pulawy.pl (P.J.)
[4] Department of Biochemistry and Toxicology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland; katarzyna.ognik@up.lublin.pl
* Correspondence: jadwiga.wyszkowska@uwm.edu.pl

Abstract: Antibiotics are a great blessing for humanity, and they have saved millions of human lives. Antimicrobials have enabled humans to produce animal-based foods that are free of pathogens. However, antibiotics also have a number of weaknesses. The use of antimicrobials in livestock production can have adverse consequences for the natural environment. The aim of this study is to evaluate the applicability of manure from turkeys administered monensin (M), enrofloxacin (E), and doxycycline (D) as soil fertilizer and to determine the impact of these antibiotics on the physicochemical, microbiological, and biochemical properties of soil in a pot experiment. The following treatments were established: unfertilized soil (S), soil fertilized with turkey manure free of antibiotics (C), soil fertilized with turkey manure containing only M (M), soil fertilized with turkey manure containing M and E (ME), and soil fertilized with turkey manure containing M, E, and D (MED). The experimental plant was *Zea mays*. The study demonstrated that the soil application of turkey manure containing all three antibiotics (MED) did not inhibit the growth of *Zea mays*, did not lead to adverse changes in the physicochemical properties of soil, and did not disrupt the abundance or diversity of culturable microorganisms, despite the fact that these antibiotics were identified in both the soil and *Zea mays* roots. The application of manure containing M, E, and D in the cultivation of *Zea mays* contributed to the transfer and presence of E and D in soil and maize roots. Antibiotics were not detected in above-ground plant parts. Monensin was not identified in soil or plant samples. The tested manure induced significant changes in the biochemical index of soil quality and in the microbiome of non-culturable bacteria and fungi at both phylum and genus levels. These results indicate that manure from turkeys administered M, E, and D should be used with caution to avoid permanent changes in the microbiome and biochemical properties of soil. Manure contaminated with antimicrobials can be used in the production of fodder crops that do not accumulate antibiotics in above-ground parts.

Keywords: antibiotics in soil; antibiotics in plants; soil microbiome; soil enzymes

check for updates

Academic Editor: Cristina Abbate

Received: 15 April 2025
Revised: 26 April 2025
Accepted: 28 April 2025
Published: 30 April 2025

Citation: Wyszkowska, J.; Mikulski, D.; Borowik, A.; Zaborowska, M.; Kucharski, J.; Kozłowski, K.; Bilecka, M.; Gajda, A.; Pietruk, K.; Jedziniak, P.; et al. The Effect of Fertilization with Antibiotic-Contaminated Manure on Microbial Processes in Soil. *Agriculture* **2025**, *15*, 979. https://doi.org/10.3390/agriculture15090979

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Soil microorganisms and enzymes are important predictors of disturbances in soil homeostasis, and they are widely used to interpret ecosystem processes [1]. This is due to their participation in the biochemical cycling of nutrients and in the stabilization of soil aggregates by modifying the structural and hydrological properties of soil [2]. Soil enzymes are also used to allocate microbial resources and determine the severity of disruptions in the biochemical transformation of soil nutrients such as carbon, nitrogen, phosphorus, and sulfur. The activity of dehydrogenases, catalase, and β-glucosidase reflects changes in carbon conversion; urease activity reflects changes in nitrogen conversion, whereas the activity of hydrolases such as acid phosphatase/alkaline phosphatase and arylsulfatase is measured to analyze changes in the transformation of phosphorus and sulfur, respectively [3,4].

Given the projected 67% increase in antibiotic consumption between 2010 (63,151 Mg) and 2030 associated with the growth of the global livestock and poultry industries [5], the use of manure will become increasingly important in moderating the structural diversity of the soil microbiome. While the high availability of carbon and nutrients contributes to an increase in microbial biomass, it also promotes the proliferation and spread of antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs) in agricultural soils [6]. The abundance of ARGs in manure can be as high as 10^{-1} 16srRNA genes [7]. It has been proven that enrofloxacin induces qnrS, qnrA, aac(6′)-Ib-cr, gyrA, and gnrB genes [8], whereas doxycycline (antibiotic of the tetracycline class) increases the abundance of tetG, tetA, and tetC efflux pump genes, as well as the tetX gene [9].

Exogenous antibiotics introduced into the soil with fertilizers allow ARB to exert selective pressure on the autochthonous microbiome, resulting in various negative effects. Antibiotics inhibit ribosome functions, disrupt the processes associated with the replication and transcription of genetic material, and disturb the synthesis of cell wall components, mainly peptidoglycan [10]. In the same ecosystem, microorganisms also develop resistance to antibiotics through mutations in the chromosomal gene or through the acquisition of exogenous ARGs in the diversification process, including via horizontal gene transfer (HGT) [11]. This process is facilitated by five classes of mobile integrons, transposons [12], plasmids on which genes are exchanged during conjugation [13], and gene cassettes [14]. Conjugation is the most important HGT mechanism responsible for the rapid spread of antibiotic resistance as well as microbial proliferation and evolution [15]. One of the conjugation plasmids abundant in soils exposed to poultry manure is a LowGC-type plasmid that ensures the spread of ARGs between closely related microorganisms [16]. In addition to natural transformation and transduction processes that do not involve direct contact between donor and recipient cells [17], a novel HGT mechanism mediated by membrane vesicles (MVs) has been recently identified. Membrane vesicles mediate the transfer of ARGs because these biologically active structures carry chromosomal and plasmid DNA and RNA, including rRNA, tRNA, mRNA, and sRNA [18]. The transfer of ARGs is a complex process that includes migration between ARB and the soil matrix, as explained by the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory [19]. This theory postulates that attractive van der Waals forces and electrostatic forces play an important role in the interactions between the solid phase of soil and microorganisms [20].

The abundance and diversity of plant-associated microbiomes, which correspond to their antibiotic sensitivity, are influenced by their phenotype, genotype, and the interactions between microorganisms [21]. It is also important to have a deep understanding of the mechanisms underlying the spread of ARGs in soil/plant systems that provide antibiotic resistance to plants. These processes involve mainly bacteria of the phylum Proteobacteria that migrate between root surfaces and internal plant structures and carry large numbers of plasmids [22]. A total of 144 ARGs have been identified in endophytic and epiphytic

microorganisms colonizing plant roots and leaves in soil fertilized with poultry manure [17]. In addition to HGT, the proliferation and transmission of ARGs can also take place via vertical gene transfer (VGT) [23]. Moreover, plants harbor both endogenous and exogenous ARGs. Endogenous ARGs are defined and determined by bacterial chromosomal genes and selective pressure from antibiotics produced by plants. These include phytoalexins that inhibit the growth of pathogenic microorganisms [24]. Exogenous ARGs, such as tetA, tetAP, and tetC, have been identified as those whose proliferation is induced by external environmental factors, including manure fertilization [25,26]. According to Xu et al. [27], exposure to high antibiotic doses can negatively affect plant metabolism, mainly by inhibiting root development, which reduces the abundance of ARGs in vegetative plant parts, including roots, stems, and leaves.

Zea mays was selected for the study not only because it is the third most important food crop in the world [28] but also because it has a complex root system [29], which facilitates thorough analysis of the interactions between plants and the microbiome of soil fertilized with manure from antibiotic-treated turkeys. It should be noted that reports dedicated to its effects on the growth and development of *Zea mays* are unique to date.

The aim of this study is to evaluate the applicability of manure from turkeys administered monensin (M), enrofloxacin (E), and doxycycline (D) as soil fertilizer and to determine the impact of these antibiotics on the physicochemical, microbiological, and biochemical properties of soil.

2. Materials and Methods

2.1. Soil Parameters

A pot experiment was conducted in the greenhouse of the University of Warmia and Mazury in Olsztyn, Poland (53°45′36″ N, 20°27′15″ E). Pots were filled with brown soil (Eutric Cambisols) with the granulometric composition of silt loam. Soil was obtained from Olsztyn Lakeland in the western part of the Masurian Lake District, Poland, in the East European Plain. The soil was sampled from the arable layer of a field sown with oats at a depth of 0–25 cm. Soil samples had the following composition: sand—44.80% (0.05–2 mm), silt—54.44% (0.002–0.05 mm), and clay—0.76% (<0.002 mm). Soil pH (in 1 mol KCl) was 4.71 at the beginning of the experiment; soil redox potential (Eh) was 417.25 mV kg^{-1}; organic carbon content was 6.89 g kg^{-1}; total nitrogen content was 0.98 g kg^{-1}; the sum of exchangeable base cations (EBC) was 38.51 mmol (+) kg^{-1}; hydrolytic acidity (HAC) was 31.05 mmol (+) kg^{-1}; and cation exchange capacity (CEC) was 69.56 mmol (+) kg^{-1}. In 2024, the mean annual temperature in Olsztyn (Poland) was 8.6 °C [30]. The freeze-free period lasted 180 days [31]. Yearly sunshine duration was 1600 h [32]. The mean daily temperature was 18.8 °C in July 2024 and 18.3 °C in August 2024 [30,33]. The experiment was conducted in pots to control environmental conditions and to guarantee the reliability and replicability of the results. The experiment was designed to accurately simulate real-world soil processes and eliminate the influence of external factors.

2.2. Experimental Design

Manure was obtained from four groups of turkeys that (1) were not administered any antibiotics (C), (2) were administered monensin (M), (3) were administered monensin and enrofloxacin (ME), and (4) were administered monensin, enrofloxacin, and doxycycline (MED). The average dry matter content of turkey manure was determined at 44.23%, nitrogen content—at 26.40 g, phosphorus content—at 5.91 g, potassium content—at 16.01 g, and calcium content—at 9.45 g kg^{-1} on a fresh weight (FW) basis. The concentrations (mg kg^{-1} FW) of the tested antibiotics in manure were as follows: monensin—4.67 in group M, 4.49 in group ME, and 5.27 in group MED; enrofloxacin—7.83 in group ME and

5.11 in group MED; doxycycline—3.39 in group MED. The experiment was conducted in polyethylene pots with a volume of 10 dm^3. Each pot was filled with 9 kg of soil that was passed through a sieve with a 1 cm mesh size. Before pot filling, soil was combined with 90 g of each manure variant. Soil not fertilized with manure was the control. The following treatments were established: soil not fertilized with manure (S) and soil fertilized with the manure of group C, M, ME, and MED turkeys. Each of the five treatments was established in four replicates. Soil moisture content was maintained at 60% maximum water holding capacity with the use of demineralized water.

The experimental plant was the DS1897B hybrid variety of *Zea mays* (produced by Pioneer, Warsaw, Poland). Eight maize plants were sown per pot. Five plants were left per pot after seedling emergence. The growing season lasted 50 days. The leaf greenness index (SPAD) was measured three times in the growing season (on experimental days 20, 35, and 50) with a Spectrum Technologies chlorophyll meter (Konica Minolta Inc., Chiyoda, Japan). *Zea mays* was harvested at the end of heading (BBCH 59, Biological Bundesanstalt, Bundessortenamt and Chemical). Biomass yield (above-ground parts and roots) and antibiotic concentrations were determined in maize plants after harvest. Soil samples were passed through a sieve with a 2 mm mesh size and analyzed for microbiological, biochemical, and physicochemical properties, as well as antibiotic concentrations. Microbiological and biochemical parameters were determined in fresh soil samples, whereas chemical and physicochemical parameters were determined in air-dried samples.

2.3. Determination of Antibiotic Concentrations in Soil and Plants

2.3.1. Quantitative Determination of Doxycycline and Enrofloxacin in Above-Ground Parts and Roots of Zea mays

Chemicals and Reagents

The analytical standards of doxycycline and enrofloxacin were obtained from Dr. Ehrenstorfer (Augsburg, Germany). The internal standards (IS) of demeclocycline and ciprofloxacin-d_8 were purchased from Sigma–Aldrich (St. Louis, MO, USA). The analytical reagents, including acetonitrile and methanol, were of LC-MS grade (J.T. Baker, Deventer, The Netherlands). Citric acid and acetic acid were purchased from POCH (Gliwice, Poland), and heptafluorobutyric acid (HFBA) was obtained from Sigma–Aldrich (St. Louis, MO, USA). Oasis HLB Cartridges (3CC, 60 mg sorbent) were supplied by Waters (Milford, MA, USA). Hydrophilic polyvinylidene fluoride (PVDF) syringe membrane filters (0.22 μm) were purchased from Restek (Bellefonte, PA, USA). Ultra-pure water was generated by a Millipore Milli-Q System (Millipore, Molsheim, France).

Sample Preparation

The sample preparation method for the antibiotic analysis had been previously developed, validated, and described by the authors [34]. Briefly, a sample of 2 ± 0.01 g was weighed into a 50 cm^3 polypropylene tube, and IS solutions were added before the extraction. Next, 1 cm^3 of ultrapure water was added, and the sample was stirred and left to incubate at room temperature in the dark for 10 min. Then, 8 cm^3 of acetonitrile and 0.5 cm^3 of 10% citric acid with pH 4.0 were added, and the sample was agitated with a rotary stirrer for one hour, ultrasonicated for 30 min at room temperature, and centrifuged at 3060 × RFC for 10 min at 4 °C. The supernatant was filtered through Oasis HLB cartridges. The filtered supernatant was collected in glass tubes and evaporated to dryness under a stream of nitrogen at 45 ± 5 °C. In the last step, the dry residue was dissolved in 500 μL of 0.025% HFBA and filtered through 0.22 μm PVDF syringe filters into LC vials.

UHPLC-MS/MS

The UHPLC–MS/MS assay was conducted using a Shimadzu Nexera X2 (Shimadzu, Kyoto, Japan) system connected to a QTRAP®4500 triple quadrupole mass spectrometer (Sciex, Framingham, MA, USA). The instruments were controlled, and data were processed in Analyst Software v. 1.6.3.

The analytes were separated on a Luna® Omega 1.6 µm Polar C18 10 column, 100 × 2.1 mm (Phenomenex, Torrance, CA, USA), integrated with a guard column of the same type. The column oven was set to a temperature of 35 °C with a flow rate of 0.4 cm^3 min^{-1}. The gradient was started with 92% mobile phase B (0.025% HFBA) and 8% mobile phase A (acetonitrile), held for 30 s, decreased to 20% after 2.30 min, and then decreased to 10% after 1 min. This mobile phase composition was held for 1 min and then increased again to 92% mobile phase B and held for 2 min. Total run time was 7 min, and injection volume was 5 µL. The mass spectrometer was operated in positive electrospray ionization (ESI+) mode, and fragmentation occurred in multiple reaction monitoring (MRM) mode. The ion transitions and parameters for doxycycline and enrofloxacin were as follows: 445 → 428/154 and 360 → 342/286, respectively. The transitions for demeclocycline and ciprofloxacin-d_8 as IS were 465 → 448 and 340 → 322, respectively. The MS/MS parameters for doxycycline and enrofloxacin were set at declustering potential (DP)—60 and 100 V, respectively; cell exit potential (CXP)—20 V and 23 V, respectively; collision energy (CE) for ion 1 and ion 2—23 and 42 V and 33 and 48 V, respectively. The values of DP, CE, and CXP were set at 60 V, 17 V, and 13 V, respectively, for demeclocycline and at 60 V, 29 V, and 13 V, respectively, for ciprofloxacin-d_8 DP.

Validation of the Method

The method used in the analysis of doxycycline and enrofloxacin was validated for linearity, precision (repeatability and within-laboratory reproducibility), selectivity, recovery, and the matrix effect according to the Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 [35]. The limit of quantification (LOQ) was measured based on the Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food, EUR 28099 [36]. The method was linear, with an r^2 value of 0.997 for doxycycline and 0.995 for enrofloxacin. In assessments of repeatability and within-laboratory reproducibility, the CV reached 7.3 ± 0.7 and 10.0 ± 0.9, respectively, for doxycycline, and 10.1 ± 1.1 and 14.1 ± 1.5, respectively, for enrofloxacin. The LOQs of the method were set as the lowest point on the calibration curve (5.0 µg kg^{-1} for doxycycline and 10 µg kg^{-1} for enrofloxacin). In the specificity analysis, there were no significant interfering peaks in the blank at the corresponding retention times of the target analytes and the IS (internal standards). The obtained criteria met the recovery requirements and were determined at 101% for doxycycline and 102% for enrofloxacin. The matrix effect (ME, %) was below ±20%, and it reached 93.5% for doxycycline and 93.6% for enrofloxacin. The matrix effect was considered negligible for values between 85% to 115%. Therefore, the signals of the target compounds were not enhanced or suppressed.

2.3.2. Quantitative Determination of Monensin in the Above-Ground Parts and Roots of *Zea mays*

Chemicals and Reagents

Monensin was purchased from Sigma-Aldrich (Taufkirchen, Germany). LC-MS grade acetonitrile, dimethyl sulfoxide, and methanol were supplied by J.T. Baker (Karlsruhe, Germany); ammonium solution (25%) was purchased from POCh (Poland); acetic acid and ammonium formate were purchased from Merck (Darmstadt, Germany); formic acid was

supplied by Sigma-Aldrich (Taufkirchen, Germany); and ultrapure water was generated by a Milli-Q purification system (Millipore, France).

Sample Preparation

The samples (2.5 g ± 0.01 g each) were weighed into 50 cm^3 polypropylene centrifuge tubes and left to stand for one hour to promote the dispersal of the standards.

An aliquot of 12.5 cm^{-3} of 1% ammonium solution in methanol (prepared by diluting 1 cm^3 of 25% ammonium solution in 100 cm^3 methanol) was added to each sample. The sample was then transferred to an ultrasonic bath for 15 min. After the bath, 12.5 cm^3 of 2% acetic acid solution in methanol was added. The sample was shaken (30 min, 200 cycles min^{-1}) and centrifuged (4500× *g*, 20 °C, 15 min). In the last step, 1 cm^{-3} of the supernatant was transferred to the vial for instrumental analysis.

UHPLC-MS/MS

Chromatography was performed using a Shimadzu Nexera X2 HPLC system (Shimadzu, Kyoto, Japan). The analytes were separated on a Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm, chromatographic column (Agilent, Santa Clara, CA, USA), in gradient mode. Mobile phase A consisted of 0.01 M ammonium formate (pH 4.0), and mobile phase B was composed of acetonitrile, methanol, and mobile phase A at a ratio of 60:35:5 (*v*:*v*:*v*). The initial conditions were as follows: 90% mobile phase A and 10% mobile phase B at a flow rate of 0.25 mL/min. A linear gradient program was applied: 90% A at 1:00 min, 95% B at 8:00 to 15:00, followed by a return to the initial settings at 16:00 until the end of the run at 24:00 min. The oven temperature was set to 55 °C, and injection volume was 2 µL. The LCMS-8050 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) was used for detection. The LC and MS systems were controlled by LabSolution 5.60 SP2 software. The analysis was conducted in electrospray ionization mode (ESI), with both negative and positive ionization. The target compounds were quantified in selected reaction monitoring (SRM) mode, and dwell time was set at 5 ms for all examined coccidiostats.

The ion-source parameters were as follows: the nebulizer gas flow rate was 3 dm^{-3} min^{-1}, and heating gas and drying gas flow rates were set at 10 dm^3 min^{-1}. Interface temperature was 300 °C, desolvation temperature was 250 °C, and heat block temperature was 400 °C. Capillary voltage was −3 kV and 4 kV for negative and positive ionization mode, respectively.

Validation of the Method

The method's fitness for purpose was assessed in a validation experiment. The validation parameters were calculated according to Commission Regulation (EU) 2021/808 [35]. Linearity and working range were calculated by preparing five series of matrix-matched calibration curves. The recovery of the method was assessed by adding known amounts of monensin to a blank matrix. Specificity was determined by analyzing 20 blank samples and monitoring the retention times of peaks of interest and any interferences. The results of the validation experiment were as follows: the matrix-matched calibration curve for linearity was determined in the range of 0.25–12.5 mg kg^{-1} with a correlation coefficient (R^2) of 0.995. The repeatability standard deviation was 10.2%, and the reproducibility standard deviation was 14.6%. The calculated recovery rate was 96.7%. The limit of quantification was set at the lowest point of the calibration curve—0.25 mg kg^{-1}.

2.4. Chemical and Physicochemical Analyses of Soil

The chemical and physicochemical properties of soil were determined in four replicates. Particle size distribution in soil samples was measured with a Mastersizer 3000 laser diffraction analyzer with a Hydro MU sample dispersion unit (Malvern Instruments Ltd.,

Worcestershire, UK). The content of C_{org} and N_{Total} was determined with a TOC-5000 analyzer (Shimadzu, Kyoto, Japan) with an SSM 5000 solid sample combustion unit. The sum of exchangeable base cations (EBC) and hydrolytic acidity (HAC) were measured by the methods described by Carter [37]. The cation exchange capacity (CEC) of soil was calculated as the sum of HAC and EBC [38]. Soil redox potential (Eh) and soil pH were measured with a CP-505 pH meter. Soil pH was determined based on ISO 10390:2021 [39].

2.5. Biochemical Analyses of Soil

The activity of the following soil enzymes was measured in biochemical analyses: oxidoreductases, including dehydrogenases (Deh) and catalase (Cat), and hydrolases, including β-glucosidase (Glu), acid phosphatase (AcP), alkaline phosphatase (AlP), urease (Ure), and arylsulfatase (Aryl). Dehydrogenase activity was measured by reducing 2,3,5-triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF), and Cat activity was determined by reacting the enzyme solution with hydrogen peroxide and measuring the amount of produced oxygen. The activity of Glu, AcP, AlP, and Aryl was determined with a spectrophotometer by measuring the amount of 4-nitrophenol (PNP) produced during the enzymatic cleavage of the respective substrates. The following substrates were used: 4-nitrophenyl-β-D-glucopyranoside (PNG) for Glu, 4-nitrophenyl phosphate disodium salt 6-hydrate (PNPNa) for AcP and AlP, and potassium 4-nitrophenyl sulfate (PNS) for Aryl. Urease activity was determined based on the amount of NH_4^+ released from 10% aqueous solution of urea. Before the analysis, soil samples were incubated in a Memmert Ine 550 laboratory incubator (Schwabach, Germany) at a temperature of 37 °C for 1 h (for AcP, AlP, Glu, and Aryl) or 24 h (for Deh and Ure). The analyses were conducted in four replicates using the previously described methods [38,40]. Enzyme activity was measured with a Perkin–Elmer Lambda 25 spectrophotometer (Waltham, MA, USA). The results were used to calculate the biochemical index of soil quality (BA) using methods and the formula described in a previous study [40].

2.6. Microbiological Analyses of Soil

The abundance and diversity of culturable bacteria and fungi were determined by serial dilutions. The composition of microbial culture media and the enumeration procedure were described previously by Wyszkowska et al. [41]. Microbial analyses were conducted in four replicates. Microbial counts were used to calculate the colony development (CD) index according to the method proposed by Sarathchandra et al. [42] and the ecophysiological diversity (EP) index according to the method described by De Leij et al. [43]. The following formula was used to calculate the CD index: CD = [N1/1 + N2/2 + N3/3. . . N10/10] × 100, where N1, N2, N3, . . . N10 is the number of microbial colonies identified on day 1, 2, 3 . . . 10 relative to the total number of colonies. The EP index was calculated with the following formula: EP = −Σ(pi·log10 pi), where pi is the total number of microbial colonies after one day of incubation relative to the total number of microbial colonies after 10 days of incubation.

Non-culturable bacteria were identified using the Illumina MiniSeq platform. DNA was isolated with the MagnifiQ™ 1 Genomic DNA instant kit (A&A Biotechnology, Gdańsk, Poland). Bacteria were identified by amplifying the V3–V4 region with the use of the 341F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′) primer pair. Fungi were identified by sequencing the ITS1 region with the use of the (5′-GAACCWGCGGARGGATCA-3′) and 5.8S (5′-CGCTGCGTTCTTCATCG-3′) primer pair. Identification, genomic DNA isolation, and genetic sequencing procedures were described previously [44]. The results of metagenomic analyses were used to calculate the Shannon diversity index (H), Simpson's diversity index (D), Margalef's diversity index

(Dm), richness index (R), and Pielou's evenness index (J) for bacteria and fungi. Microbial sequences were submitted to the National Center for Biotechnology Information under GenBank accession numbers: prokaryotic 16S rRNA: https://www.ncbi.nlm.nih.gov/nuccore/?term=PV094906:PV096975[accn] (accessed on 17 February 2025) and Eukaryotic Nuclear rRNA/ITS: https://www.ncbi.nlm.nih.gov/nuccore/?term=PV104740:PV105077[accn] (accessed on 18 February 2025).

2.7. Statistical Analysis

The results were processed statistically in Statistica 13.3 [45], STAMP 2.1.3 [46], RStudio v1.2.5033 [47] with a gplots library [48], and R core [49]. Homogeneous groups were identified using Tukey's HSD test at $p < 0.050$. Metagenomic data were presented after ASV and ITS reads containing less than 1% of total ASVs or OTUs had been removed from the dataset. The results were subjected to principal component analysis (PCA) and presented as arithmetic means ± standard deviation (SD).

3. Results

3.1. Response of Zea mays to Fertilization with Manure Containing Antibiotics

Manure fertilization significantly increased the yield of aerial biomass and roots in *Zea mays* (Table 1). Soil fertilization with turkey manure free of antibiotics led to a 1.98- and 1.84-fold increase in the average yield of aerial biomass and root biomass, respectively. The length of aerial plant parts also increased 1.30-fold in the above treatment.

Table 1. Biomass yield (g pot^{-1}) and height (cm) of *Zea mays*.

Treatment	Aerial Parts	Roots	Height
S	70.56 ± 5.55 ^{d}	8.43 ± 0.50 ^{c}	143.90 ± 5.91 ^{b}
C	139.43 ± 4.32 ^{bc}	15.52 ± 1.29 ^{a}	187.65 ± 6.76 ^{a}
M	146.95 ± 3.36 ^{ab}	14.99 ± 1.76 ^{a}	195.00 ± 9.25 ^{a}
ME	151.61 ± 2.29 ^{a}	13.13 ± 0.87 ^{ab}	201.05 ± 3.21 ^{a}
MED	138.01 ± 0.32 ^{c}	11.99 ± 0.71 ^{b}	191.35 ± 4.06 ^{a}

S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline. Values in columns marked with the same letters form homogeneous groups, $p < 0.050$, N = 4 for each standard deviation, and N = 16 for each tested property = 16.

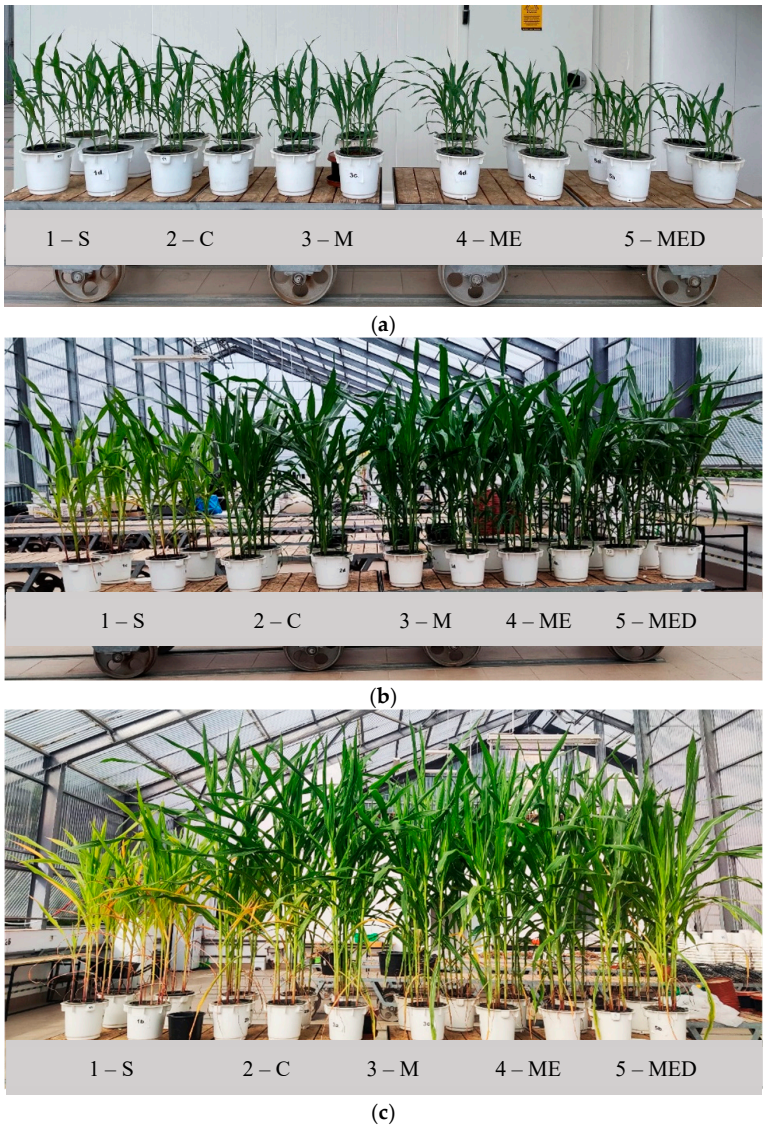
The application of turkey manure containing antibiotics also stimulated the development of maize plants. Depending on the treatment, aerial biomass yield increased 2.15-(ME) to 1.78-fold (MED), whereas root biomass yield increased 1.78-(M) to 1.42-fold (MED). However, in comparison with plants supplied with antibiotic-free manure, root biomass decreased significantly with the rise in the number of antibiotics. Unlike root growth, the development of above-ground plant parts was not inhibited by antibiotics, as shown by aerial biomass yield, plant height, and the values of the leaf greenness index (SPAD) (Table 2). The observed decrease in *Zea mays* root biomass may be a consequence of a disruption in the structure of the soil microbiome, particularly a reduction in the abundance of rhizosphere microorganisms responsible for mobilizing plant nutrients. The leaf greenness index was influenced by manure fertilization and the phenological stage of *Zea mays*. The lowest SPAD values were observed in the leaves of unfertilized maize plants, and this parameter continued to decline over time. Manure fertilization led to a significant increase in SPAD values. On average, this parameter increased by 43% in the treatment fertilized with antibiotic-free manure, by 51% in treatment M, by 52% in treatment ME, and by 37% in treatment MED. In treatments fertilized with manure, regardless of its origin,

SPAD values were highest on day 35 in the 8 leaves unfolded stage and lowest on day 50 in the 11 leaves unfolded stage (Table 2, Scheme 1).

Table 2. Leaf greenness index (SPAD) in *Zea mays*.

Treatment	5th Leaf (Day 20)	8th Leaf (Day 35)	11th Leaf (Day 50)	Average
S	34.55 ± 1.41^{e}	24.13 ± 2.09^{g}	17.48 ± 2.24^{h}	25.38^{D}
C	37.01 ± 1.44^{d}	43.62 ± 3.08^{a}	28.56 ± 2.57^{f}	36.36^{B}
M	39.68 ± 1.53^{bc}	45.05 ± 2.07^{a}	30.56 ± 2.68^{f}	38.32^{A}
ME	39.76 ± 2.38^{bc}	45.05 ± 3.02^{a}	30.79 ± 2.45^{f}	38.53^{A}
MED	37.50 ± 1.36^{cd}	40.88 ± 2.24^{b}	26.01 ± 2.66^{g}	34.80^{C}

S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline. Parameter values marked with the same lowercase letters in columns form homogeneous groups, and values marked with uppercase letters denote relevance to treatments, $p < 0.050$, N = 4 for each standard deviation, and N = 16 for each tested property.



Scheme 1. Growth of *Zea mays* on day 20 (**a**), day 35 (**b**), and day 50 (**c**). S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline.

3.2. Antibiotic Residues in Soil and in the Above-Ground Parts and Roots of Zea mays Grown on Soil Fertilized with Manure

Antibiotics were not detected in soil that was not supplied with manure (S) and in treatments fertilized with antibiotic-free manure (C). Monensin was not identified in soil supplied with turkey manure containing this coccidiostat. The concentrations of the analyzed antibiotics in manure were as follows: monensin—4670 μg kg^{-1} (M); monensin—4490 μg kg^{-1} and enrofloxacin—7830 μg kg^{-1} (ME); monensin—5270 μg kg^{-1}, enrofloxacin—5110 μg kg^{-1}, and doxycycline—3390 μg kg^{-1} (MED).

In treatment ME, E was identified in soil and maize roots (Table 3). In turn, in treatment MED, both E and D residues were detected in soil and roots. None of the tested antibiotics was detected in above-ground plant parts. Antibiotic concentrations were higher in soil than in roots. Enrofloxacin concentration was also higher in soil and roots in the treatment fertilized with manure containing both E and D. Monensin was not identified in soil or plant samples. The results are presented in Table 3.

Table 3. Antibiotic concentrations in soil and in different parts of *Zea mays* grown in soil not fertilized with manure, fertilized with antibiotic-free manure, and fertilized with manure containing antibiotics.

Treatment	Antibiotic Concentrations (μg kg^{-1})								
	Soil			Roots			Above-Ground Parts		
	M*	E*	D*	M*	E*	D*	M*	E*	D*
S	nd	nd	nd	nd	nd	nd	nd	nd	nd
C	nd	nd	nd	nd	nd	nd	nd	nd	nd
M	nd	nd	nd	nd	nd	nd	nd	nd	nd
ME	nd	125	nd	nd	14.8	nd	nd	nd	nd
MED	nd	193	117	nd	33.8	36.2	nd	nd	nd

S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline. M*—monensin, E*—enrofloxacin, D*—doxycycline, nd—not detected < LOQ of the method.

3.3. Physicochemical Properties of Soil Fertilized with Manure Containing Antibiotics

Manure fertilization increased the content of organic carbon and nitrogen in soil (Table 4). In these treatments, the sum of exchangeable base cations (EBC) in soil increased by 15% (C) to 21% (M and MED), and cation exchange capacity (CEC) increased by 11% (C and MED), 14% (ME), and 15% (M) (Table 5). These results clearly indicate that turkey manure containing antibiotics had no negative effect on the physicochemical properties of soil.

Table 4. Chemical properties of soil (kg^{-1} soil DM).

Treatment	C_{org}	SOM	N_{Total}	C:N
	g			
S	6.97 ± 0.04 ^{c}	12.02 ± 0.07 ^{c}	1.11 ± 0.03 ^{b}	6.31 ± 0.13 ^{b}
C	7.85 ± 0.07 ^{a}	13.53 ± 0.11 ^{a}	1.23 ± 0.08 ^{a}	6.40 ± 0.37 ^{ab}
M	7.48 ± 0.08 ^{b}	12.90 ± 0.14 ^{b}	1.19 ± 0.04 ^{ab}	6.29 ± 0.28 ^{b}
ME	7.38 ± 0.14 ^{b}	12.72 ± 0.24 ^{b}	1.18 ± 0.02 ^{ab}	6.25 ± 0.01 ^{b}
MED	8.06 ± 0.20 ^{a}	13.90 ± 0.34 ^{a}	1.17 ± 0.02 ^{ab}	6.89 ± 0.26 ^{a}

S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline, SOM—soil organic carbon. Values marked with the same letters in columns form homogeneous groups, $p < 0.050$, N = 4 for each standard deviation, and N = 16 for each tested property.

Table 5. Physicochemical properties of soil (kg^{-1} soil DM).

Treatment	pH_{KCl}	Eh	HAC	EBC	CEC
		mV	mmol		
S	4.73 ± 0.01 ^{e}	418.30 ± 0.08 ^{a}	31.13 ± 0.31 ^{c}	39.00 ± 4.08 ^{b}	70.13 ± 4.39 ^{b}
C	4.82 ± 0.01 ^{b}	412.45 ± 0.53 ^{b}	32.63 ± 0.31 ^{b}	45.00 ± 0.82 ^{a}	77.63 ± 1.12 ^{a}
M	4.78 ± 0.01 ^{c}	413.15 ± 0.78 ^{b}	33.38 ± 0.31 ^{a}	47.00 ± 0.82 ^{a}	80.38 ± 0.51 ^{a}
ME	4.76 ± 0.01 ^{d}	412.95 ± 0.61 ^{b}	33.75 ± 0.01 ^{a}	46.00 ± 1.63 ^{a}	79.75 ± 1.63 ^{a}
MED	4.90 ± 0.01 ^{a}	408.45 ± 0.12 ^{c}	31.13 ± 0.31 ^{c}	47.00 ± 0.82 ^{a}	78.13 ± 1.12 ^{a}

S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline, Eh—redox potential, HAC—hydrolytic acidity, EBC—sum of exchangeable base cations, CEC—cation exchange capacity. Values marked with these small letters (a–e) form homogeneous groups. These were calculated separately for each of the soil properties.

3.4. Response of Soil Enzymes to Fertilization with Manure Containing Antibiotics

Fertilization with antibiotic-free manure (C) enhanced the activity of soil enzymes (Table 6). Manure had the most stimulating effect on dehydrogenase activity (136% increase) and the least stimulating effect on arylsulfatase activity (13% increase). It also improved soil fertility, as shown by an increase in the fertility index (BA) value of up to 87%. Fertilization with manure containing antibiotics also led to a significant increase in BA values, ranging from 38% (MED) to 74% (ME), but the noted increase was 8% (M) to 26% lower than in the treatment supplied with antibiotic-free manure (C). These results were influenced mainly by lower dehydrogenase activity in treatments fertilized with turkey manure containing antibiotics, in particular, manure contaminated with all three antimicrobials (MED). Other enzymes responded differently to the tested manure variants. For example, catalase and *β*-glucosidase activity was higher in treatments fertilized with manure containing all three antibiotics than with antibiotic-free manure; arylsulfatase activity was higher in treatments ME and MED, whereas urease activity was higher in treatment ME.

Table 6. Enzyme activity in 1 kg soil DM h^{-1}.

Treatment	Deh	Cat	Ure	AcP	AlP	Aryl	Glu	BA
	µmol TFF	mol O_2	mmol N-NH_4	mmol PN				
S	6.27 ± 0.02 ^{d}	0.25 ± 0.01 ^{c}	0.51 ± 0.03 ^{c}	3.35 ± 0.01 ^{d}	0.79 ± 0.01 ^{b}	0.16 ± 0.01 ^{c}	0.77 ± 0.01 ^{c}	12.09 ± 0.01 ^{d}
C	14.78 ± 0.54 ^{a}	0.31 ± 0.01 ^{b}	0.69 ± 0.03 ^{b}	4.48 ± 0.06 ^{b}	1.16 ± 0.09 ^{a}	0.18 ± 0.01 ^{b}	1.02 ± 0.03 ^{b}	22.62 ± 0.38 ^{a}
M	13.00 ± 0.17 ^{b}	0.33 ± 0.01 ^{a}	0.71 ± 0.01 ^{ab}	4.40 ± 0.01 ^{bc}	1.14 ± 0.01 ^{a}	0.18 ± 0.01 ^{b}	1.09 ± 0.01 ^{b}	20.86 ± 0.16 ^{b}
ME	12.83 ± 0.35 ^{b}	0.34 ± 0.01 ^{a}	0.77 ± 0.03 ^{a}	4.68 ± 0.01 ^{a}	1.18 ± 0.05 ^{a}	0.21 ± 0.01 ^{a}	1.09 ± 0.07 ^{b}	21.09 ± 0.31 ^{b}
MED	8.75 ± 0.33 ^{c}	0.34 ± 0.01 ^{a}	0.69 ± 0.03 ^{b}	4.26 ± 0.17 ^{c}	1.20 ± 0.02 ^{a}	0.21 ± 0.01 ^{a}	1.18 ± 0.02 ^{a}	16.63 ± 0.45 ^{c}

S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline, Deh—dehydrogenases, Cat—catalase, Ure—urease, AcP—acid phosphatase, AlP—alkaline phosphatase, Glu—*β*-glucosidase, Aryl—arylsulfatase, BA—biochemical index of soil quality. Values marked with the same letters in columns form homogeneous groups, $p < 0.050$, N = 4 for each standard deviation, and N = 16 for each tested property.

3.5. Response of the Soil Microbiome to Fertilization with Manure Containing Antibiotics

3.5.1. Culturable Microorganisms

Manure fertilization stimulated the proliferation of culturable organotrophic bacteria and Actinobacteria and inhibited fungal proliferation (Table 7).

The counts of organotrophic bacteria increased by 296% (C) to 262% (MED), and Actinobacteria counts increased by 210% to 184%, respectively. A much smaller decrease was noted in fungal counts, ranging from 18% in treatment C to 25% in treatment MED. Fungal abundance decreased by 15% in treatment M and by only 2% in treatment ME. The values of the colony development (CD) index indicate that manure also modified

the structure of soil microbial communities by increasing the abundance of slow-growing organotrophic bacteria and Actinobacteria as well as fast-growing fungi. Manure containing antibiotics induced greater changes in microbial composition than antibiotic-free manure. Manure fertilization also increased the ecological diversity of microorganisms. The diversity of organotrophic bacteria was highest in treatments ME and MED, whereas the diversity of Actinobacteria and fungi was highest in treatment C.

Table 7. Abundance (CFU kg^{-1}), colony development index (CD), and the ecophysiological diversity index of soil microorganisms after *Zea mays* harvest.

Treatment	CFU	CD	EP
	Org		
S	2.74 ± 0.18^{b}	40.38 ± 1.83^{a}	0.65 ± 0.10^{b}
C	10.85 ± 0.42^{a}	34.43 ± 0.14^{b}	0.79 ± 0.01^{a}
M	10.28 ± 0.78^{a}	32.51 ± 1.59^{b}	0.79 ± 0.04^{a}
ME	10.54 ± 0.27^{a}	27.13 ± 0.48^{c}	0.88 ± 0.02^{a}
MED	9.92 ± 0.76^{a}	26.69 ± 0.91^{c}	0.87 ± 0.02^{a}
	Act		
S	1.60 ± 0.22^{b}	22.25 ± 2.90^{a}	0.76 ± 0.09^{b}
C	4.96 ± 0.17^{a}	22.02 ± 0.74^{a}	0.90 ± 0.03^{a}
M	4.85 ± 0.24^{a}	21.87 ± 0.26^{a}	0.86 ± 0.01^{ab}
ME	4.70 ± 0.32^{a}	20.54 ± 2.19^{ab}	0.85 ± 0.05^{ab}
MED	4.54 ± 0.12^{a}	17.96 ± 0.92^{b}	0.78 ± 0.06^{b}
	Fun		
S	4.08 ± 0.30^{a}	32.43 ± 1.28^{b}	0.62 ± 0.04^{b}
C	3.36 ± 0.25^{ab}	36.17 ± 3.45^{ab}	0.82 ± 0.05^{a}
M	3.46 ± 0.13^{ab}	38.59 ± 1.57^{a}	0.63 ± 0.03^{b}
ME	3.98 ± 0.89^{ab}	40.17 ± 0.80^{a}	0.72 ± 0.12^{ab}
MED	3.05 ± 0.18^{b}	40.02 ± 1.67^{a}	0.73 ± 0.06^{ab}

S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline, Org—organotrophic bacteria 10^{11} CFU, Act—actinomyces 10^{11} CFU, Fun—fungi 10^{8} CFU. Values marked with the same letters in columns form homogeneous groups, $p < 0.050$, N = 4 for each standard deviation, and N = 16 for each tested property.

3.5.2. Non-Culturable Microorganisms

Soil samples were colonized mainly by bacteria of the phylum Proteobacteria (Figure 1). These bacteria accounted for 32.91% (S) to 41.10% (MED) of the total number of identified microorganisms. Actinobacteriota were the second most abundant microbial phylum, and their relative abundance ranged from 26.69% (S) to 43.78% (ME). The relative abundance of Gemmatimonadota ranged from 8.71% (ME) to 10.80% (C), Acidobacteriota—from 3.56% (ME) to 12.42% (S), and Chloroflexi—from 2.97% (ME) to 9.3% (S). The remaining bacterial phyla were less abundant. Fertilization with antibiotic-free manure (C) stimulated the growth of Proteobacteria, Actinobacteriota, Bacteroidota, and Firmicutes but partly inhibited the proliferation of Acidobacteriota, Chloroflexi, Planctomycetota, Verrucomicrobiota, and Myxococcota. A comparison of the effects of antibiotic-free manure (C) and manure containing antibiotics (M, ME, MED) clearly indicates that the tested antimicrobials affected bacterial growth. For example, the growth of Proteobacteria was significantly inhibited by ME but not by MED. In turn, the abundance of Actinobacteriota decreased in response to MED but increased under the influence of M and ME. All antibiotics exacerbated the negative effect of manure fertilization on Acidobacteriota and Chloroflexi but enhanced its

beneficial influence on Firmicutes, particularly in treatments M and ME. The combination of all three antibiotics (MED) also increased the abundance of Bacteroidota.

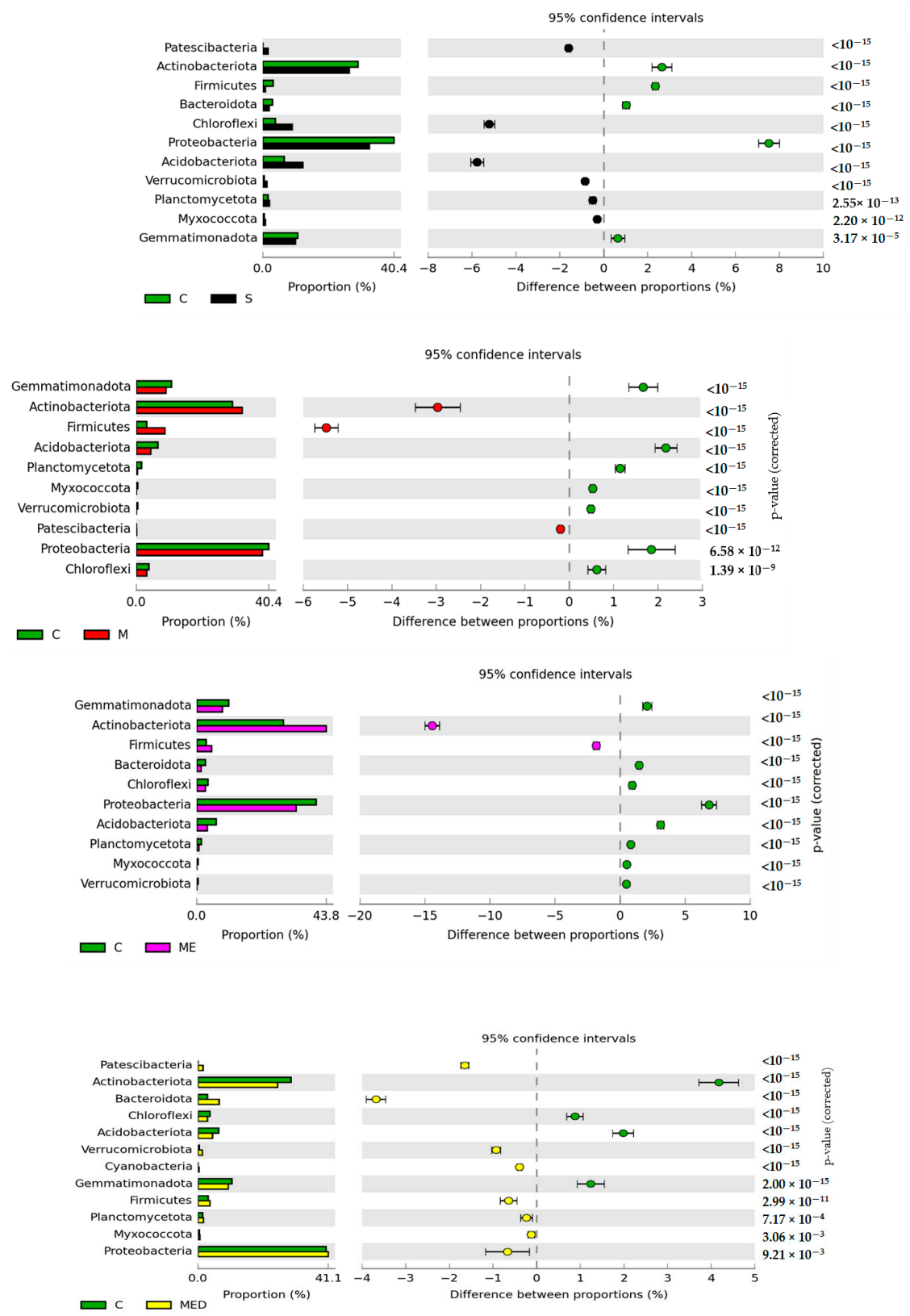


Figure 1. Relative abundance of bacterial phyla (ASV ≥ 1%) in soil. S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline.

The core microbiome of soil fertilized and not fertilized with manure was composed of the following bacterial genera: *Sphingomonas* (p_Proteobacteria), *Gemmatimonas* (p_Gemmatimonadota), *Nocardioides* (p_Actinobacteriota), and *Bryobacter* (p_Acidobacteriota) (Figure 2). Soil not supplied with manure (S) was characterized by the highest number of unique bacterial genera: *Conexibacter* (p_Actinobacteriota), *Lysobacter* and *Pseudolabrys* (p_Proteobacteria), *Candidatus_Udaeobacter* (p_Verrucomicrobiota), and *Candidatus_Solibacter* (p_Acidobacteriota). Manure fertilization contributed to a decrease in the counts of unique bacterial genera, including *Ramlibacter* (p_Proteobacteria) in treatment C, *Rummeliibacillus* (p_Firmicutes) and *Burkholderia-Caballeronia-Paraburkholderia* (p_Proteobacteria) in treatment M, *Jatrophihabitans* (p_Actinobacteriota) in treatment ME, and *Chitinophaga* (p_Bacteroidota) in treatment MED.

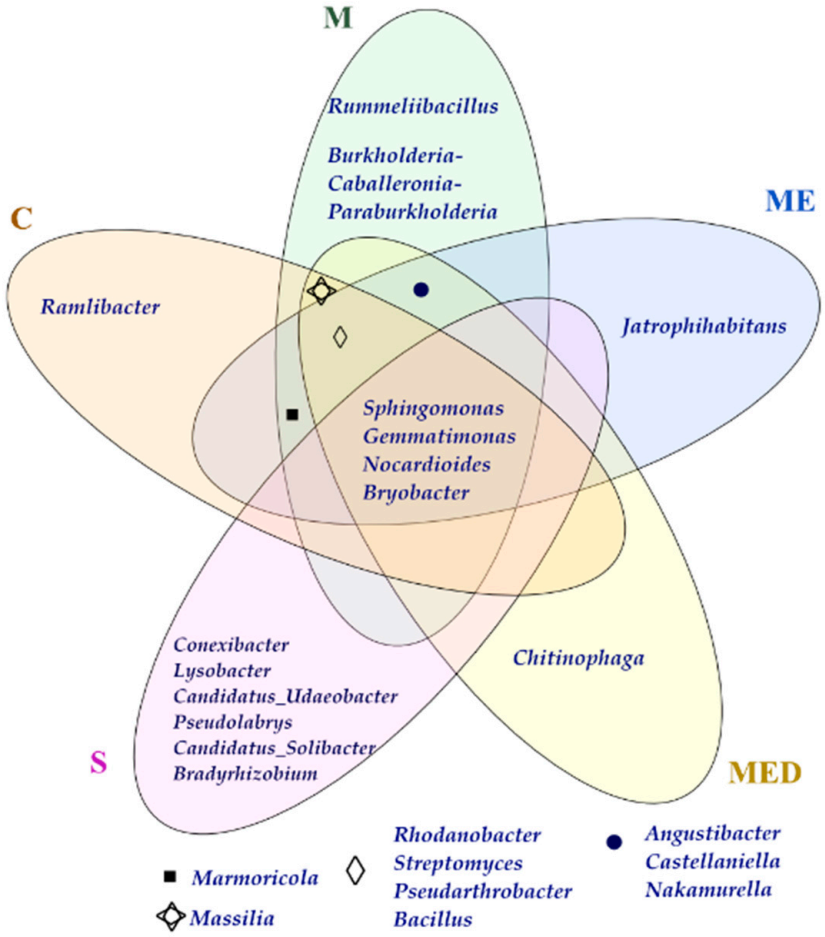


Figure 2. Venn diagram of soil bacteria genera (ASV $\geq$ 1%). S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline.

In the group of core genera, *Sphingomonas* were most abundant (from 19.32% (M) to 52.10% (S)), and *Bryobacter* were least abundant (from 1.27% (M) to 5.99% (S)) (Figure 3). Fertilization with antibiotic-free manure decreased the counts of *Sphingomonas* by 17.17%, *Bryobacter*—by 3.08%, *Gemmatimonas*—by 2.43%, *Nocardioides*—by 1.27%, whereas manure containing antibiotics decreased the proportions of *Sphingomonas* and *Gemmatimonas* in the structure of bacterial communities. The relative abundance of *Nocardioides* in soil increased in response to M and ME.

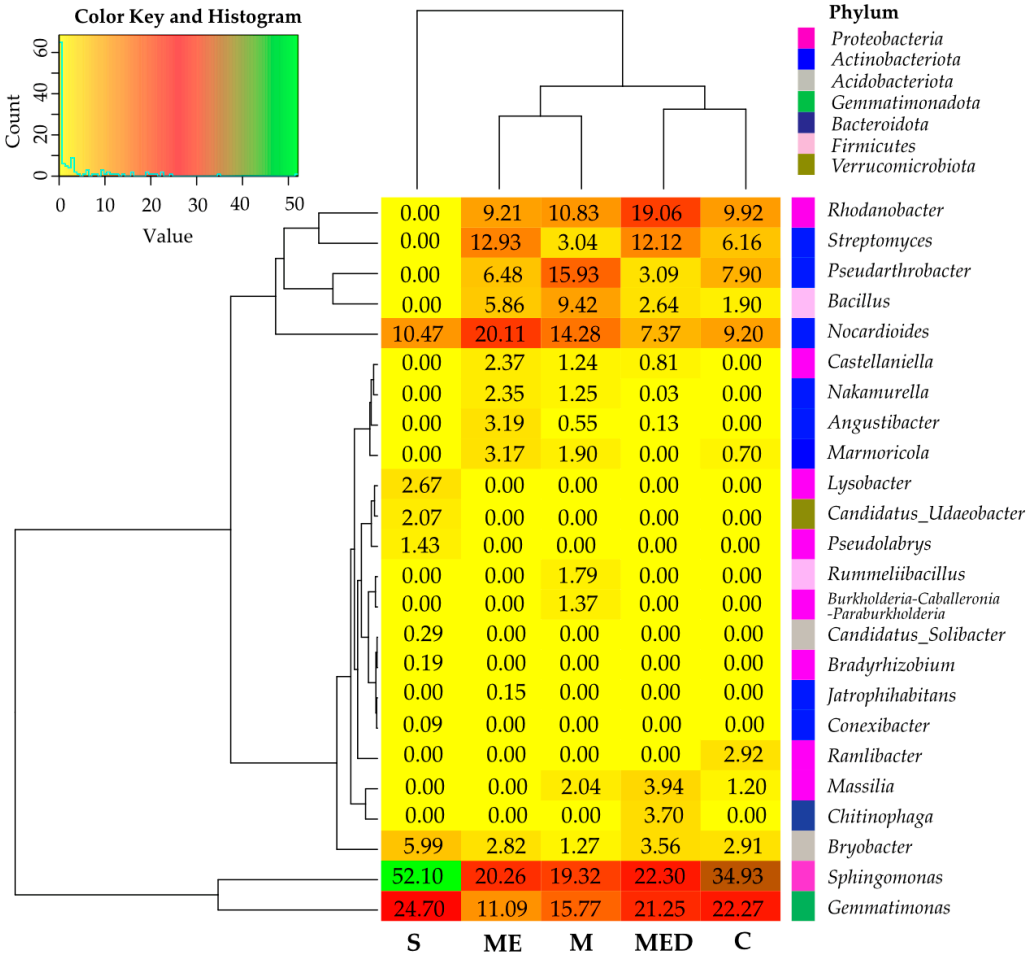


Figure 3. Relative abundance of bacterial genera (ASV ≥ 1%) in soil. S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline.

Manure fertilization decreased the values of Margalef's diversity index (Dm) and bacterial richness (R) but had no effect on Simpson's diversity index (D) or Pielou's evenness index (J) (Figure 4). Simpson's diversity index was close to the maximum value (0.99) in all treatments, whereas the Shannon diversity index was nearly identical in treatments S, C, M, and MED and only somewhat lower in treatment ME.

Fungi of the phylum Ascomycota were most abundant in all treatments (Figure 5). Their relative abundance ranged from 33.05% (S) to 99.46% (ME). Fertilization with antibiotic-free manure (C) increased the relative abundance of fungal phyla Ascomycota (by 42.52%) and Mucoromycota (by 5.10%) but led to a significant decrease in the relative abundance of Mortierellomycota (by 47.56%). Antibiotics induced significant changes in the structure of fungal communities at the phylum level. In comparison with the antibiotic-free treatment (C), the relative abundance of Ascomycota increased by 3.75% in response to M and by 23.89% in response to ME but decreased by 3.87% under the influence of MED. Fungi of the phylum Mortierellomycota responded differently to the application of manure containing antibiotics. The abundance of Mortierellomycota decreased by 2.35% in treatment M and increased by 4.26% in treatment MED. The relative abundance of Mucoromycota decreased in response to M, ME, and MED.

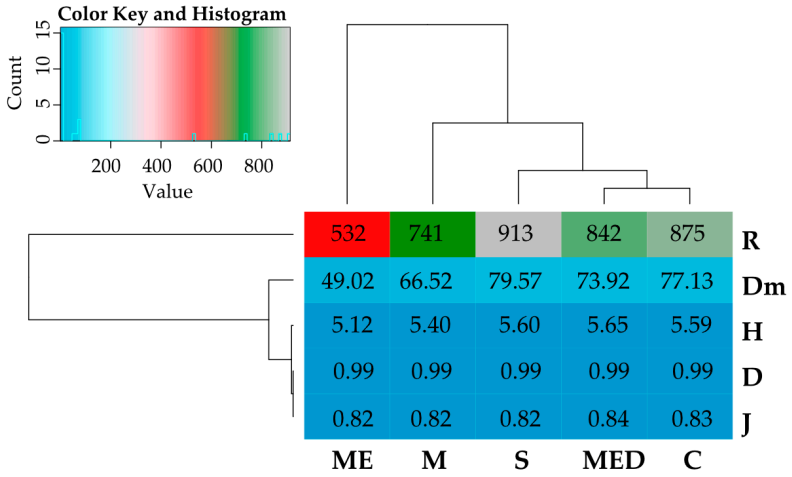


Figure 4. Indicators of bacterial diversity in soil. S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin and doxycycline, H—Shannon diversity index, D—Simpson's diversity index, Dm—Margalef's diversity index, R—richness, J—Pielou's evenness index.

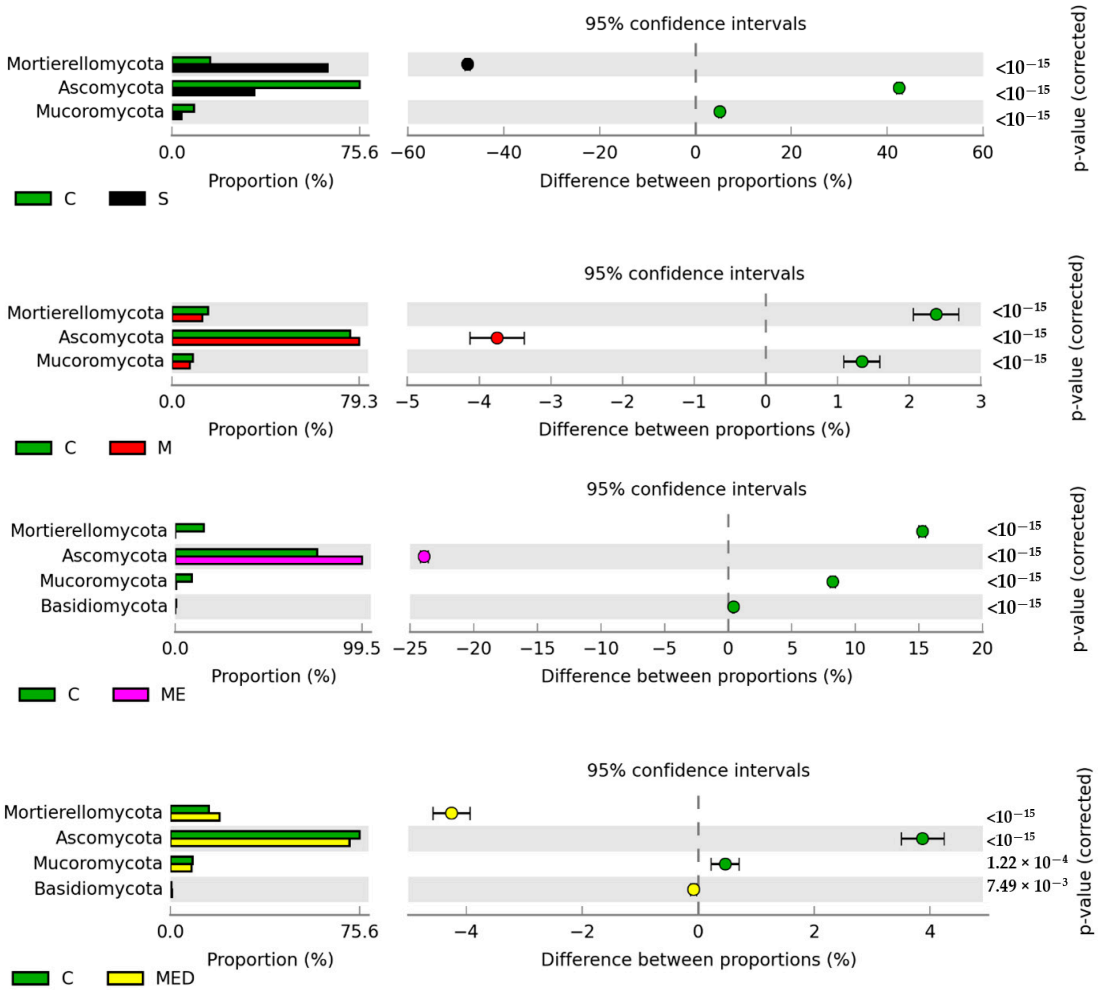


Figure 5. Relative abundance of fungal phyla (OTU ≥ 1%) in soil. S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline.

Two genera of the phylum Ascomycota, *Humicola* and *Penicillium*, belonged to the core microbiome in all treatments (Figure 6). In this phylum, *Sagenomella* was a unique genus in treatment M, and *Chaetomium* was a unique genus in treatment MED. *Rhizopus* (S) and *Mucor* (M) were the unique genera of the phylum Mucoromycota. Unique fungal genera were not identified in treatments C and ME.

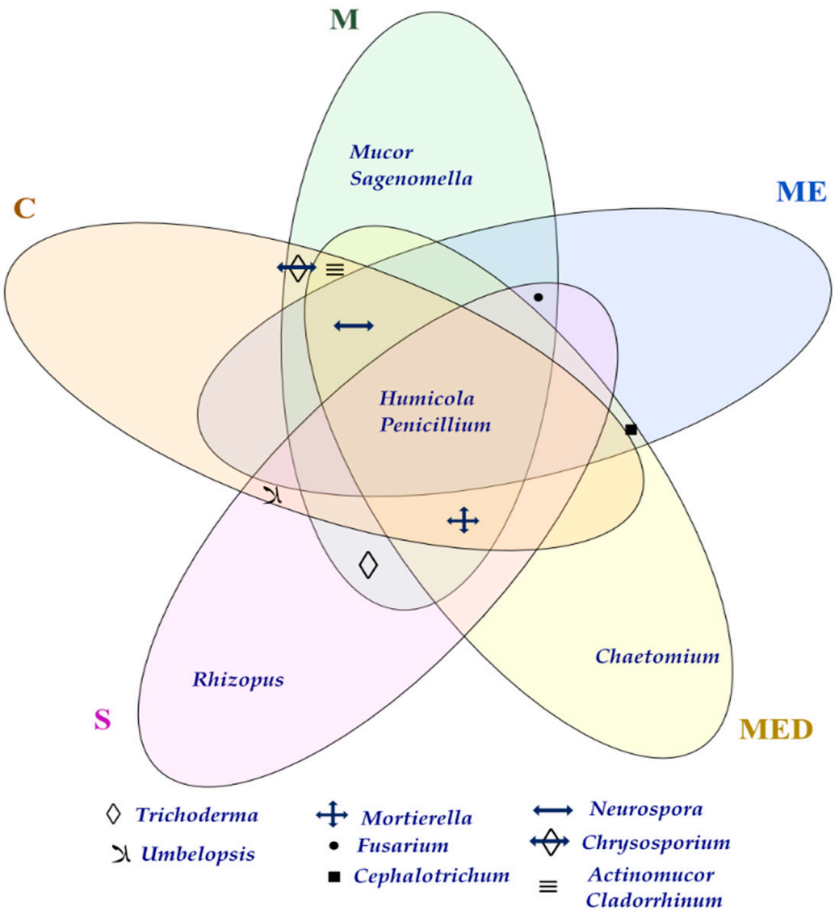


Figure 6. Venn diagram of soil fungi genera (OTU ≥ 1%). S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline.

Manure fertilization (C) increased the relative abundance of *Humicola* (from 18.66% to 35.01%) and *Penicillium* (from 12.43% to 27.78) and decreased the proportion of *Mortierella* (from 54.68% to 18.49%) in the taxonomic structure of fungal communities (Figure 7). In turn, the abundance of fungal genera *Rhizopus*, *Fusarium*, and *Trichoderma* decreased below 1%. The application of manure containing antibiotics had a more stimulating effect on *Penicillium* spp. than antibiotic-free manure. The effect of all antibiotic combinations (M, ME, MED) on *Humicola* fungi was similar to that noted in treatments supplied with antibiotic-free manure. The growth of *Mortierella* was strongly inhibited by all tested antibiotics.

As indicated in Figure 8, the greatest decrease in fungal diversity was observed in treatment ME, which was characterized by the lowest values of the Shannon diversity index (H), Simpson's diversity index (D), Margalef's diversity index (Dm), richness (R), and Pielou's evenness index (J). A lower value of Margalef's diversity index and a higher value of the Shannon diversity index were noted in treatment MED.

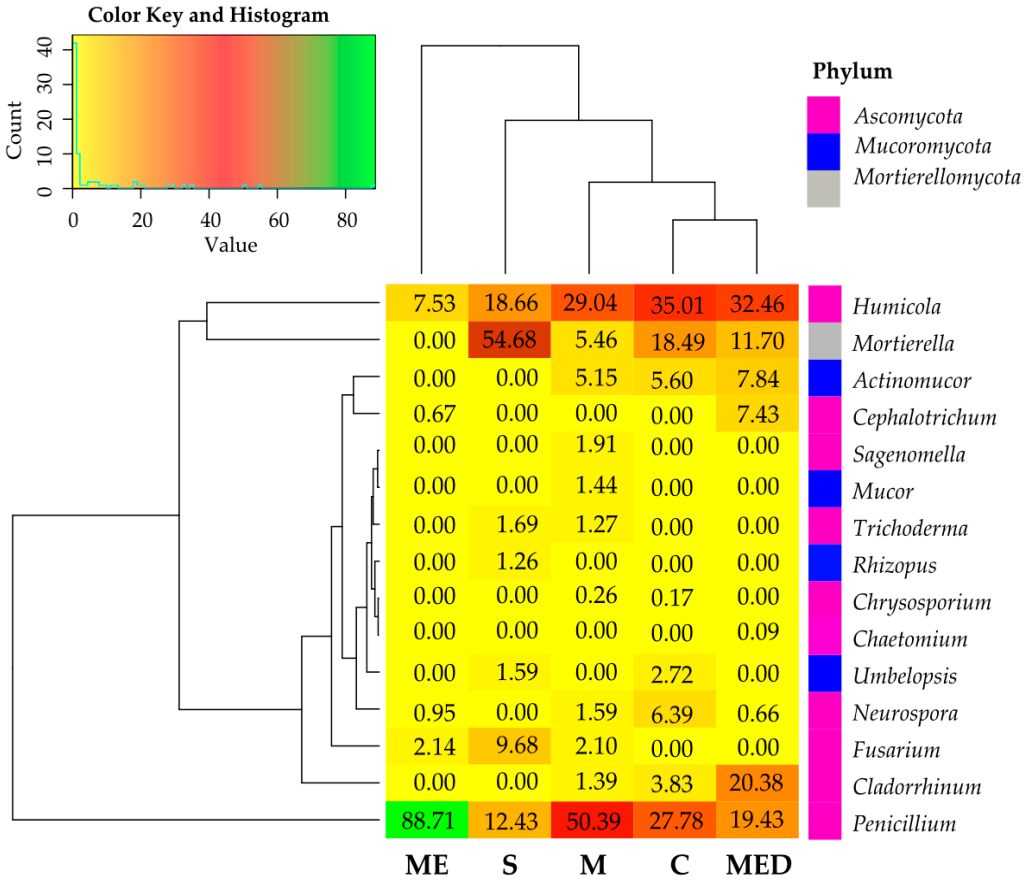


Figure 7. Relative abundance of fungal genera (ITS ≥ 1%) in soil. S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline.

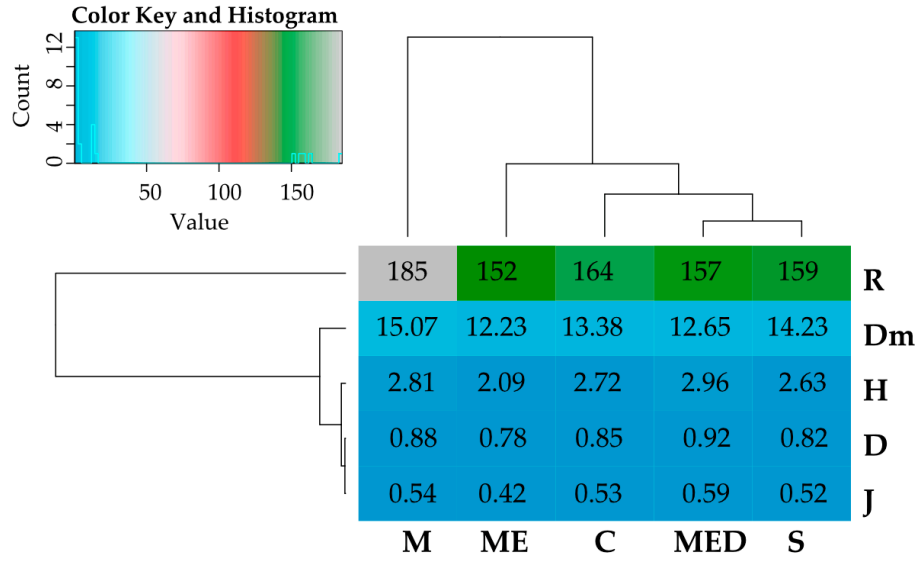
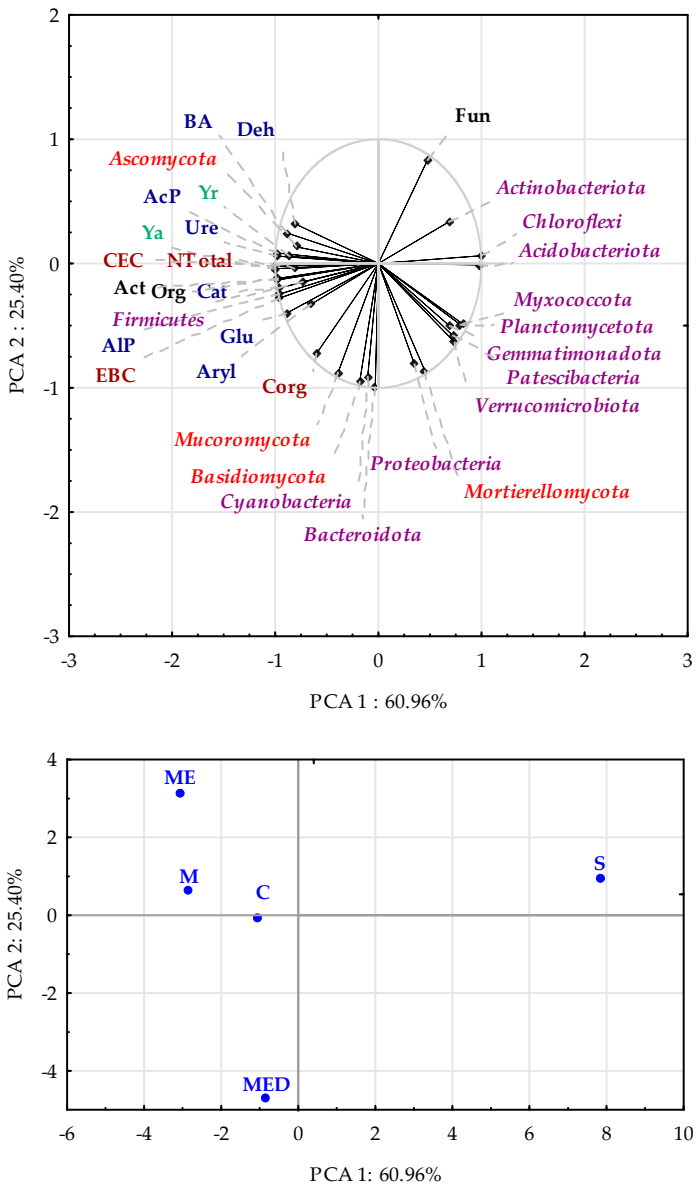


Figure 8. Indicators of fungal diversity in soil. S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline, H—Shannon diversity index, D—Simpson's diversity index, Dm—Margalef's diversity index, R—richness, J—Pielou's evenness index.

Using PCA principal component analysis, three groups of dependent variables were identified (Figure 9). The first group was composed of Deh, BA, AcP, Ure, Ya, Ap, Org, Act, Cat, AlP, EBC, Glu, Aryl, N_{Total}, CEC, and Firmicutes; the second group consisted of C_{org}, Mucoromycota, Basidiomycota, Bacteroidota, Proteobacteria, Mortierellomycota, Verrucomicrobiota, Patescibacteria, Gemmatimonadota, Planctomycetota, and Myxococcota, whereas the third group contained Actinobacteriota, Chloroflexi, Acidobacteriota, and Fungi. Manure fertilization significantly influenced the analyzed parameters. The distribution of variables indicates that manure exerted similar effects on the studied parameters in treatments C, M, and ME but somewhat different effects in treatment MED. However, contrary observations were made in unfertilized soil (S), which was more abundant in fungi of the phylum Mortierellomycota and bacteria of the phyla Chloroflexi, Acidobacteriota, Planctomycetota, Patescibacteria, Myxococcota, and Verrucomicrobiota.



Variables	PCA 1	PCA 2
Ya	−1.00	−0.04
Yr	−0.86	0.06
C_{org}	−0.60	−0.72
N_{Total}	−0.81	−0.04
EBC	−0.95	−0.28
CEC	−0.99	−0.02
Deh	−0.80	0.32
Cat	−0.96	−0.20
Ure	−0.98	0.06
Pac	−0.97	0.08
Pal	−0.96	−0.25
Aryl	−0.65	−0.33
Glu	−0.89	−0.40
BA	−0.88	0.24
Org	−0.97	−0.12
Act	−0.97	−0.13
Fun	0.48	0.83
Proteobacteria	0.35	−0.81
Actinobacteriota	0.68	0.33
Gemmatimonadota	0.69	−0.50
Bacteroidota	−0.03	−0.99
Acidobacteriota	0.98	−0.01
Firmicutes	−0.73	−0.15
Chloroflexi	0.99	0.06
Planctomycetota	0.79	−0.49
Patescibacteria	0.72	−0.58
Verrucomicrobiota	0.74	−0.63
Myxococcota	0.82	−0.49
Cyanobacteria	−0.10	−0.91
Ascomycota	−0.80	0.13
Basidiomycota	−0.18	−0.95
Mortierellomycota	0.45	−0.86
Mucoromycota	−0.39	−0.88

Cases	PCA 1	PCA 2
S	7.82	0.97
C	−1.08	−0.05
M	−2.84	0.64
ME	−3.07	3.16
MED	−0.83	−4.71

Figure 9. Correlations between variables in principal component analysis (PCA). S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin, and doxycycline.

These results indicate that the yield of *Zea mays* was significantly correlated with the activity of oxidoreductases and hydrolases, the biochemical index of soil quality (BA), and the abundance of culturable bacteria and Actinobacteria. In the group of non-culturable microorganisms, bacteria of the phylum Firmicutes and fungi of the phylum Ascomycota were significantly correlated with the biomass yield of *Zea mays*. The yield of *Zea mays* was also significantly correlated with the content of C_{org} and N_{Total}, sum of exchangeable base cations (EBC), and cation exchange capacity (CEC).

4. Discussion

4.1. Response of Zea mays to Fertilization with Manure Containing Antibiotics

In the current study, turkey manure stimulated the growth and development of *Zea mays* (Table 1). These observations corroborate the scientific consensus that organic fertilizers have a strong and positive influence on soil fertility. As the stability of the aggregates is mainly influenced by organic matter, of which manure is an indisputable source, this fertilizer contributes to the supply of nutrients to plants by intervening in their stable growth and development [50,51]. Even manure containing antibiotic residues and their combinations (MED) induced a 1.96-fold increase in aerial biomass yield and a 1.42-fold increase in root biomass yield, compared with the unfertilized treatment (S) (Table 1), most likely due to the potential of plant/soil interactions. According to Zhao et al. [25], manure is the main pathway for the transfer of ARGs, such as tet and qnr genes encoding resistance to tetracyclines and quinolones, respectively, to the soil/plant system. These ARGs have been identified in soil even 120 days after manure application [52]. Horizontal gene transfer has also been implicated in the resistance of *Zea mays* plants [53]. Plants can be a reservoir of ARGs because transposase genes tnpA and tp614 localized in roots and leaves correspond to the genes encoding resistance to tetracyclines [54]. Integrase genes intl1 and intl2 detected on the surface of leaves also play an important role by conferring resistance to quinolones, including enrofloxacin [55]. Underlying antibiotic resistance is the encoding by plant genes of ATP-binding cassette (ABC) transporter proteins. The most important ABC transport proteins include homologs of multidrug resistance proteins MRP/ABCC and MDR/ABCB and homologs of pleiotropic drug resistance proteins PDR/ABCG [21,56]. Interestingly, plants exposed to antibiotic stress may recruit soil-dwelling microorganisms that are more tolerant of these xenobiotics [57]. Under antibiotic pressure, the plant microbiome can also induce the activity of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) to activate the expression of factors responsible for cell protection against oxidative stress [27]. In the present study, *Zea mays* responded positively to manure application, but root biomass decreased under exposure to antibiotics (Table 1). In the study by Han et al. [53], tetracyclines also contributed to reducing root length by 41% and root biomass by 46%. It is worth noting that the researchers also observed the emergence of new roots in the plant, which could be considered as one way for the plant to avoid the consequences of the toxicity of this pool of antibiotics. The results obtained demonstrate that antibiotics can induce the overproduction of reactive oxygen species (ROS). Elevated ROS levels trigger plant defense mechanisms, but they also carry information about root cell damage. This phenomenon may be determined by the toxic, synergistic interactions between various classes of antibiotics [27]. Wu et al. [58] found that enrofloxacin inhibited oxidative phosphorylation and disrupted hormonal regulation by increasing the production of reduced glutathione (GSH) and decreasing the synthesis of ascorbic acid (AsA). This antibiotic is also an activator of the proteasome degradation pathway. However, knowledge of a wide range of antibiotics such as phenazine, pyoluteorin, or pyrrolnitrin, which, as root exudates, enhance mutation or recombination of genes, thereby inducing antibiotic resistance, should not be overlooked [59,60]. In addition, induced systemic resistance (ISR)

can be modulated by two phytohormones—salicylic acid and jasmonic acid, as well as by abscisic acid, auxins, and cytokinins [61,62]. In maize, a protective role is played by benzoxazinoids (BX), heteroaromatic secondary metabolites of plants [63]. The beneficial effect of manure, irrespective of its origin, is demonstrated not only by the amount of biomass obtained and the height of *Zea mays* but also by the values of the greenness index (SPAD) (Table 2). Wyszkowska et al. [41] also reported an increase in the SPAD values of maize leaves exposed to tetracyclines. According to Xu et al. [27], the increase in SPAD values can be attributed to the stimulation of photosystem II (PSII), which is responsible for electron transfer to PSI and chlorophyll biosynthesis under exposure to lower tetracycline doses.

4.2. Physicochemical Properties of Soil Fertilized with Manure Containing Antibiotics

Fertilization with turkey manure containing M, E, and D had no negative effect on the physicochemical properties of soil (Tables 4 and 5). It should be noted that antibiotics can be adsorbed to soil particles by electrostatic attraction, which reduces their toxicity by decreasing their bioavailability to plants. Soil organic matter can exert dual effects on this parameter: it can decrease antibiotic adsorption by offering competitive binding sites, or it can increase antibiotic adsorption through deprotonation of functional groups that ultimately assume ionic form [64]. Furthermore, once penetrated into the soil, antibiotics can be adsorbed onto clay minerals, and the intensity of this process is determined by low pH. Ultimately, cationic forms of antibiotics can easily enter into electrostatic interactions with negatively charged surfaces of clay minerals [65].

4.3. Antibiotic Concentrations in Zea mays and in Soil Fertilized with Manure Containing Antibiotics

The use of antibiotics in livestock production can lead to the presence of drug residues in manure, which may contribute to soil and groundwater contamination. Tetracyclines and quinolones are among the most popular classes of antibiotics that are used around the world as first-line treatments for common infections in veterinary medicine [66]. In the European Union (EU), approximately 84% of broiler starter, grower, and finisher diets contain an anticoccidial drug such as monensin. Monensin has been found to be extremely effective in preventing coccidiosis outbreaks under field conditions [67]. Up to 90% of the ingested antibiotics and their metabolites can be excreted with urine and feces. As a result, manure or organic fertilizers can be contaminated with antibiotics or their metabolites. In a study by Berendsen et al. [68], the time required for a drug to degrade to 10% of its initial concentration (DT90) in manure was determined at up to 422 days for doxycycline and 2000 days for enrofloxacin. Most quinolones are very persistent in manure, with more than 10% of the native compound remaining in most types of manure after one year.

The uptake of selected antibiotics from soil and wastewater by plants or vegetables has been described in the literature [69–71]. In a study investigating the transfer of doxycycline from the substrate to white button mushrooms (*Agaricus bisporus*), the concentration of doxycycline reached 3.93–0.87 μg kg^{-1} in *Agaricus bisporus* grown on a substrate contaminated with 500 μg kg^{-1} of this antibiotic [34]. However, such comprehensive studies investigating the use of manure contaminated with monensin, doxycycline, and enrofloxacin in maize cultivation have not been conducted to date. In the present study, soil was contaminated with doxycycline at 117 μg kg^{-1}, and the concentration of this antibiotic in *Zea mays* roots was 36.2 μg kg^{-1}. Doxycycline was more effectively transferred from soil to plants (30.9%) than enrofloxacin (11.8% and 17.5%). The results indicate that the class of antibiotics is one of the key determinants of a compound's stability and persistence in manure and the environment, as well as its ability to migrate to different components of the ecosystem.

Broekaert et al. [72] analyzed the transfer of monensin from contaminated manure to plants. They found that various vegetables (carrots, potatoes, lettuce, zucchini, and

tomatoes) were able to absorb coccidiostats from soil. When the studied vegetables were fertilized with manure containing monensin, this antibiotic was detected only in lettuce at a concentration of 0.94 ± 0.72 μg kg^{-1}. Higher concentrations of this antibiotic were observed when soil was spiked with a monensin premix. Monensin residues were detected in potatoes (47.1 ± 25.3 μg kg^{-1}) and lettuce (2.06 ± 0.41 μg kg^{-1}), which suggests that some vegetables are more capable of incorporating coccidiostats when grown on soil spiked with premixes rather than contaminated manure. The transfer rate can also be influenced by the placement of edible plant parts and the content of lipids. However, the cited study was performed on vegetables, and its findings may have limited relevance to *Zea mays*.

4.4. Response of Soil Enzymes to Fertilization with Manure Containing Antibiotics

When undertaking an assessment of the effect of manure on soil enzyme activity, the ability of enzymes to decompose organic matter should be considered Indeed, they are recognized as an important factor involved in its synthesis and degradation [40]. There is no controversy over the response of dehydrogenases, considered to be a particularly sensitive indicator of changes in the soil environment [73]. In the present study, as expected, dehydrogenase activity decreased under the influence of the tested antibiotics (Table 6), although manure containing antibiotic residues increased soil dehydrogenase activity compared with unfertilized soil. This was probably due to the fact that manure is a rich source of carbon, which acts as an energy substrate for microorganisms, enhancing microbial metabolism and ultimately improving soil fertility [1]. The observed increase in dehydrogenase activity can be attributed to the fact that these intracellular enzymes are present in the structure of both bacterial and fungal cells [74]. The advantage of urease, whose activity increased after the application of manure, is its ability to form stable enzyme systems with soil particles outside the cells of the microorganisms. In addition, manure, due to its amino acid and protein content, is a reservoir of nitrogen, the metabolism of which in the soil is catalyzed by urease. This enzyme hydrolyzes urea to NH_3 and CO_2 [75].

Admittedly, even a compilation of antibiotics (MED) in turkey manure enhanced soil enzyme activity, and BA increased by 38% relative to unfertilized soil (Table 5). However, this parameter was lower than in soil fertilized with antibiotic-free manure, which could be explained by the fact that dehydrogenases are sensitive to the tested antibiotics. Dehydrogenase activity decreased by 41% under the influence of MED. Dehydrogenases responded similarly to tetracyclines in the study by Unger et al. [76] and Chen et al. [77]. Wyszkowska et al. [41] reported a 24% decrease in dehydrogenase activity after the application of 100 mg of tetracycline kg^{-1} soil DM. In the current experiment, the observed decrease in enzyme activity in treatment MED can probably be attributed to doxycycline, a tetracycline-class antibiotic, that exacerbated the inhibitory effects of the remaining antibiotics. The increase in urease activity in treatment ME confirms the toxic impact of doxycycline. In a study by Santás-Miguel et al. [78], urease activity in soil with low organic matter content decreased in the presence of chlortetracycline and oxytetracycline. The correlations obtained also lent credence to the study by Molaei et al. [79]. The catalase response also deserves discussion. Regardless of the composition of the antibiotics, it was positive. Dehydrogenase and catalase are both classified as oxidoreductases; therefore, they could be expected to respond similarly to antibiotic pressure. However, the observed increase in catalase activity could be indicative of stress caused by this group of xenobiotics. The difference in the responses of both enzymes may be attributed to the fact that catalase degrades hydrogen peroxide to protect microbial cells against oxidative stress and death [80]. The present results are consistent with those reported by Wyszkowska et al. [41], who found that tetracyclines also decreased dehydrogenase activity but had no negative effect on catalase.

4.5. Response of the Soil Microbiome to Fertilization with Manure Containing Antibiotics

4.5.1. Culturable Microorganisms

Awareness that the antibiotics tested are potent inhibitors of protein synthesis invokes the hypothesis that assumes the negative effects of antibiotics in the soil against its microbiome [81]. This belief is also reinforced by the fact that quinolones and tetracyclines inhibit nitrification processes in the soil. They also reduce microbial biomass as determined by PFLAs (Phospholid Fatty Acids) [24,82]. Our own research (Table 6) showed that the residues of the tested antibiotics in manure did not affect the status of organotrophic bacteria in the soil (Table 7). However, it should be noted that, depending on the study conditions and the type of antibiotics tested, the response of these bacteria may vary [44]. This is probably also related to the potential of microorganisms to degrade antibiotics and the wide range of mechanisms that ensure their resistance to this group of pollutants. Męcik et al. [83] include antibiotic deactivation among the most significant, attributing its causality at 41.74%.In turn, Wang et al. [84] emphasize the importance of activating the efflux mechanisms of antibiotics involving efflux pumps, reporting a 33% efficiency. These mechanisms should also be expanded to include reduced antibiotic uptake through modification of cell membranes and mutations in genes encoding three important proteins: DNA/RNA polymerase, DNA gyrase, and topoisomerase [66]. According to Guo et al. [85], the antibiotic degradation pathway involves demethylation, deamination, and hydroxylation, followed by oxidation that leads to ring cleavage. The substances secreted by roots in the rhizosphere, including threonine and citronellic acids, facilitate these processes [86]. In addition, the rhizosphere of *Zea mays* is colonized by plant growth-promoting bacteria (PGPB) that act as a reservoir of ARGs harboring numerous mobile genetic elements (MGEs) [26]. The neutral response of Actinobacteria to manure, regardless of its origin (Table 7), was more predictable, especially since these bacteria are a source of antibiotics themselves. Monensin is synthesized by *Streptomyces cinnamonensis*. Transcription factor DasR, a member of the GntR protein family, is responsible for the activation and synthesis of monensin [87,88]. In addition, the abundance of *Streptomyces* sp. increased by 4.5% in soil amended with manure containing enrofloxacin relative to the treatment fertilized with antibiotic-free manure. This bacterial genus was represented by two species: *Streptomyces* sp. RPA4_2 and *Streptomyces* sp. CC008 [83]. An intriguing relationship was also reported in the literature, where *Streptomyces* sp. exposed to a sub-inhibitory dose of tetracyclines induced a spectacular increase in bacterial resistance to quinolones [16]. The inhibitory effect of MED on mold fungi, found in our study, may also become a subject of scientific discourse. It may be due to their interference with fungal sterol biosynthesis. This process involves disruption of cytochrome P450 enzyme activity. There may also be interference with the integrity of the fungal cell wall, leading to the lysis of their cells [89]. The adverse effects of antimicrobials on molds could have been exacerbated by phenoxazinones, the degradation products of benzoxazinoids (BX), secondary plant metabolites with antifungal properties that are secreted by maize roots [90].

4.5.2. Non-Culturable Microorganisms

The moderating effect of antibiotics on the diversity of bacterial communities in the soil has been supported by numerous research studies [27,41,83,91]. In the present study, the core soil microbiome is composed of the three most abundant bacterial phyla: Proteobacteria, followed by Actinobacteriota and Gemmatimonadota (Figure 1). The application of antibiotic-free manure induced an increase in the abundance of Proteobacteria and Actinobacteriota but also stimulated the proliferation of bacteria belonging to the phyla Bacteroidota and Firmicutes. Similar trends were observed by Iltchenco et al. [92], who found that soil fertilized with turkey manure was colonized mainly by bacteria of the phyla

Bacteroidota (52%), Firmicutes (29%), and Actinobacteriota (11%). According to Dyksma et al. [93], the widespread distribution of Bacteroidota and Firmicutes can be attributed to their ability to degrade protein and carbonate complexes in soil. Syntrophic bacteria of the phylum Firmicutes also synthesize volatile fatty acids (VFAs). Yang et al. [94] concluded that the proportions of major phyla in manure significantly affect the distribution of ARGs. Bacteroidota are specialist degraders of organic matter, and their presence is positively correlated with the prevalence of tetG and tetX genes [95,96]. In turn, Proteobacteria harbor mefA, mdtA, and ermQ genes [97]. For this reason, antibiotic stress closely corresponds to organic matter transformation, including absorption, degradation, and metabolism [27]. In the current experiment, the application of manure containing antibiotics induced changes in the soil microbiome. The abundance of Proteobacteria decreased in treatment ME. The presence of ME promoted the proliferation of Actinobacteriota, but the combination of all three antibiotics (MED) exerted pressure not only on this phylum but also on Acidobacteriota and Chloroflexi. These effects could have been exacerbated by the presence of a negative correlation between the total content of organic carbon and nitrogen in soil and Acidobacteriota, which was reported by Zhou and Yao [98]. It should also be noted that MED stimulated the growth of Firmicutes (Figure 1). In the soil environment, resistance to MED residues is associated with the antibiotic degradation pathway involving epimerization and hydrolysis [91]. In the study by Beyi et al. [99], the abundance of Actinobacteriota decreased under pressure from enrofloxacin. The cited authors identified 26 bacterial phyla in soil contaminated with enrofloxacin, where Firmicutes, Bacteroidota, and Proteobacteria were most abundant. At the genus level, MED had the most stimulating effect on *Chitinophaga*, a representative of the phylum Bacteroida (Figure 3). In turn, after manure application, M and ME promoted the proliferation of the genus *Nocardioides* of the phylum Actinobacteriota. In *Nocardioides*, antibiotic resistance could also be triggered by acetyltransferase and phosphotransferase enzymes that are capable of inactivating antimicrobial substances [83]. Resistance to quinolones, including enrofloxacin, can also be initiated by plasmid-mediated mechanisms, including antibiotic modification with a piperazinyl substituent that is catalyzed by acetyltransferase Aac(6′)-Ib-cr. OqxAB and QepA transporters encoded by ARGs such as qnrB and qnrD also play an important role in this process [100].

The response of mold fungi to manure without antibiotics was an increase in phyla Ascomycota and Mucoromycota and inhibition of Mortierellomycota proliferation (Figure 5). In the study by Frąc et al. [101], some of the dominant phyla of fungi representing soil fertilized with chicken manure were Ascomycota and Mortierellomycota, in addition to Basidiomycota. Mucoromycota were classified as subordinate phyla. Manure samples were colonized mainly by *Mortierella indohi*, *Mortierella sargyensis*, and *Mortierella hyaline*. Fertilization with turkey manure containing antibiotics exerted both stimulatory and inhibitory effects on the proliferation of soil fungi. The greatest increase in the abundance of Ascomycota (23.89%) was noted in treatment ME. In turn, Ascomycota counts decreased by 3.87% under exposure to MED (Figure 5). All of the tested antibiotic combinations reduced the abundance of Mucoromycota. Thus, it is mainly the toxicity of doxycycline that needs to be addressed. However, in a study by Wen et al. [102], tetracyclines were effectively eliminated by lignin and manganese peroxidases, enzymes secreted by the *Phanerochaete chrysosporium* fungus. Laccases, extracellular fungal enzymes, also catalyzed the degradation of tetracyclines by oxidizing their A- and C-rings in the last step of the process. Despite the above, laccases did not lead to the cleavage of the four-ring structure [103]. This demonstrates the stability of tetracyclines against mold. The positive response of *Chaetomium globosum* to MED should also be discussed (Figure 6). Rajalakshmi et al. [104] demonstrated that this fungus not only produces more than 200 bioactive compounds

but it also is a source of antibiotics such as chaetoglobosin A, chaetomin, and chaetocin. According to Goda et al. [105], *Chaetomium globosum* may offer a solution to the problem of multidrug resistance in bacteria because its metabolites exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria.

5. Conclusions

Soil fertilization with turkey manure containing monensin, enrofloxacin, and doxycycline residues has no negative effect on the aerial biomass yield of *Zea mays*, but manure contaminated with all three antibiotics (MED) decreases root biomass and the leaf greenness index. The analyzed antibiotics are relatively persistent, and they were detected in both soil and maize roots after harvest. These results suggest that for safety reasons, manure contaminated with the tested antimicrobials can be used in the production of fodder crops that do not accumulate antibiotics in above-ground parts, such as *Zea mays* and *Helianthus annuus*. However, further research is needed on other forage species, which differ in their potential to accumulate these chemicals. The application of turkey manure containing antibiotics does not compromise the physicochemical properties of soil or the abundance and diversity of culturable microorganisms but induces significant changes in the structure of non-culturable microbiota, particularly in the dominant bacterial phyla of Actinobacteria, Proteobacteria, and Gemmatimonadota, and the most ubiquitous fungal phyla of Ascomycota and Mortierellomycota. The above can be attributed mainly to the stimulating effects of M and ME on bacteria of the genus *Nocardioides* and fungi of the genus *Penicillium* and the inhibitory effects of all antibiotics on bacteria of the genera *Sphingomonas* and *Gemmatimonas* and fungi of the genus *Humicola*. These differences, as well as the antibiotic-induced decrease in the activity of soil dehydrogenases, indicate that manure from turkeys administered monensin, enrofloxacin, and doxycycline should be used with caution to avoid permanent changes in the microbiome and biochemical properties of soil, and ultimately long-term consequences for soil health. This determines the direction of future research, which should focus on analyzing long-term effects on soil health, changes in microbiome structure, and the impact of antibiotic residues on the efficiency and safety of plants.

Author Contributions: Conceptualization, J.W., J.J., J.K., A.B., and M.Z.; experimental part, J.W., J.K., A.B., M.Z., and D.M.; methodology, J.W., J.J., J.K., A.B., M.Z, M.B., A.G., K.P., K.K., and K.O.; formal analysis, J.W., A.B., M.Z., J.J., J.K., M.B., and K.P.; investigation, J.W., J.J., A.B., M.Z, J.K., M.B., and K.P.; writing—original draft preparation, J.W., JK., J.J., M.Z., A.B., A.G., K.P., and P.J.; writing—review and editing J.W., A.B., J.J., M.Z., J.K., and P.J.; visualization, J.W., A.B., M.Z., and J.K.; supervision, J.W., J.K., and J.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of Warmia and Mazury in Olsztyn, Faculty of Agriculture and Forestry, Department of Soil Science and Microbiology (grant No. 30.610.006-110) and the National Science Center in Poland (grant No.2020/39//B/NZ9/00765).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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

Abbreviations

S—soil without manure, C—control soil (soil fertilized with manure free of antibiotics), M—soil fertilized with manure containing monensin, ME—soil fertilized with manure containing monensin and enrofloxacin, MED—soil fertilized with manure containing monensin, enrofloxacin,

and doxycycline, M*—monensin, E*—enrofloxacin, D*—doxycycline, Deh—dehydrogenases, Cat—catalase, Ure—urease, AcP—acid phosphatase, AlP—alkaline phosphatase, Glu—*β*-glucosidase, Aryl—arylsulfatase, BA—biochemical index of soil quality, Org—organotrophic bacteria, Act—Actinomyces, Fun—fungi, CD—colony development index; EP—ecophysiological diversity index, H—Shannon diversity index, D—Simpson's diversity index, Dm—Margalef's diversity index, R—richness, J—Pielou's evenness index, SPAD—leaf greenness index, Ya—aerial parts, Yr—root yield, SOM—soil organic matter.

References

1. McBride, S.G.; Wepking, C.; Hedin, M.L.; Thompson, R.C.; Barrett, J.E.; Strickland, M.S. Livestock manure and antibiotics alter extracellular enzyme activity. *Appl. Soil Ecol.* **2020**, *155*, 103667. [CrossRef]
2. Liu, B.; Li, Y.; Zhang, X.; Wang, J.; Gao, M. Effects of chlortetracycline on soil microbial communities: Comparisons of enzyme activities to the functional diversity via Biolog EcoPlates™. *Eur. J. Soil Biol.* **2015**, *68*, 69–76. [CrossRef]
3. Wyszkowska, J.; Borowik, A.; Zaborowska, M.; Kucharski, J. The potential for restoring the activity of oxidoreductases and hydrolases in soil contaminated with petroleum products using perlite and dolomite. *Appl. Sci.* **2024**, *14*, 3591. [CrossRef]
4. Zaborowska, M.; Wyszkowska, J.; Borowik, A.; Kucharski, J. Evaluation of the effectiveness of innovative sorbents in restoring enzymatic activity of soil contaminated with bisphenol A (BPA). *Molecules* **2024**, *29*, 3113. [CrossRef]
5. Tiseo, K.; Huber, L.; Gilbert, M.; Robinson, T.P.; Van Boeckel, T.P. Global trends in antimicrobial use in food animals from 2017 to 2030. *Antibiotics* **2020**, *9*, 918. [CrossRef] [PubMed]
6. Shu, Y.; Li, D.; Xie, T.; Zhao, K.; Zhou, L.; Li, F. Antibiotics-heavy metals combined pollution in agricultural soils: Sources, fate, risks, and countermeasures. *Green Energy Environ.* 2024; *in press*. [CrossRef]
7. Xie, W.-Y.; Shen, Q.; Zhao, F.J. Antibiotics and antibiotic resistance from animal manures to soil: A review. *Eur. J. Soil Sci.* **2017**, *69*, 181–195. [CrossRef]
8. Seo, K.W.; Lee, Y.J. Molecular characterization of fluoroquinolone-resistant *Escherichia coli* from broiler breeder farms. *Poult. Sci.* **2021**, *100*, 101250. [CrossRef]
9. Gao, P.; Mao, D.; Luo, Y.; Wang, L.; Bingjie Xu, B.; Xu, L. Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment. *Water Res.* **2012**, *46*, 2355–2364. [CrossRef]
10. Crofts, T.S.; Gasparrini, A.J.; Dantas, G. Next-generation approaches to understand and combat the antibiotic resistome. *Nat. Rev. Microbiol.* **2017**, *15*, 422–434. [CrossRef]
11. Hu, Y.; Gao, G.F.; Zhu, B. The antibiotic resistome: Gene flow in environments, animals and human beings. *Front. Med.* **2017**, *11*, 161–168. [CrossRef]
12. Gillings, M.R.; Gaze, W.H.; Pruden, A.; Smalla, K.; Tiedje, J.M.; Zhu, Y.G. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *ISME J.* **2014**, *9*, 1269–1279. [CrossRef]
13. Cabezón, E.; Ripoll-Rozada, J.; Peña, A.; de la Cruz, F.; Arechaga, I. Towards an integrated model of bacterial conjugation. *FEMS Microbiol. Rev.* **2014**, *39*, 81–89. [CrossRef]
14. Ling, B.; Feng, W.; Yang, N.; Fan, L.; Guo, G.; Li, X.; Zheng, J. High incidence of multiple intI1 genomic gene cassettes in *Aeromonas* strains. *Aquaculture* **2024**, *579*, 740171. [CrossRef]
15. Pornsukarom, S.; Thakur, S. Horizontal dissemination of antimicrobial resistance determinants in multiple salmonella serotypes following isolation from the commercial swine operation environment after manure. *Appl. Environ. Microbiol.* **2017**, *83*, e01503-17. [CrossRef] [PubMed]
16. Kyselkova, M.; Chrudimsky, T.; Husnik, F.; Chronakova, A.; Heuer, H.; Smalla, K.; Elhottova, D. Characterization of tet(Y)-carrying LowGC plasmids exogenously captured from cow manure at a conventional dairy farm. *FEMS Microbiol. Ecol.* **2016**, *92*, fiw075. [CrossRef] [PubMed]
17. Wu, R.; Fang, J.; Yang, Y.; Liu, L.; Liu, H.; Du, S. Transmission pathways and intrinsic mechanisms of antibiotic resistance genes in soil-plant systems: A review. *Environ. Technol. Inno.* **2025**, *37*, 103985. [CrossRef]
18. Domingues, S.; Nielsen, K.M. Membrane vesicles and horizontal gene transfer in prokaryotes. *Curr. Opin. Microbiol.* **2017**, *38*, 16–21. [CrossRef]
19. Deng, J.; Zhang, W.; Zhang, L.; Qin, C.H.; Wang, H.; Ling, W. Micro-interfacial behavior of antibiotic-resistant bacteria and antibiotic resistance genes in the soil environment: A review. *Environ. Int.* **2024**, *191*, 108972. [CrossRef]
20. Zheng, Z.J.; Wang, X.L.; Zhang, W.Z.; Wang, L.; Lyu, H.H.; Tang, J.C. Regulation of ARGs abundance by biofilm colonization on microplastics under selective pressure of antibiotics in river water environment. *J. Environ. Manag.* **2024**, *355*, 120402. [CrossRef]
21. Trivedi, P.; Leach, J.; Tringe, S.; Sa, T.; Singh, B. Plant-microbiome interactions: From community assembly to plant health. *Nat. Rev. Microbiol.* **2020**, *18*, 607–621. [CrossRef]

22. Xu, H.; Chen, Z.; Huang, R.; Cui, Y.; Li, Q.; Zhao, Y.; Wang, X.; Mao, D.; Luo, Y.; Ren, H. Antibiotic Resistance Gene-Carrying Plasmid Spreads into the Plant Endophytic Bacteria using Soil Bacteria as Carriers. *Environ. Sci. Technol.* **2021**, *55*, 10462–10470. [CrossRef]
23. Wei, Z.; Feng, K.; Wang, Z.; Zhang, Y.; Yang, M.; Zhu, Y.G.; Virta, M.P.J. High-Throughput single-cell technology reveals the contribution of horizontal gene transfer to typical antibiotic resistance gene dissemination in wastewater treatment plants. *Environ. Sci. Technol.* **2021**, *55*, 11824–11834. [CrossRef]
24. Chen, Y.; Sun, L.; Hong, Y.; Chen, M.; Zhang, H.; Peng, Y.; Liako, K.; Wang, H.; Zhu, F. Exploring the third-generation tetracycline resistance of multidrug-resistant livestock-associated methicillin-resistant *Staphylococcus aureus* ST9 across healthcare settings in China. *J. Antimicrob. Chemoth.* **2023**, *78*, 1871–1881. [CrossRef]
25. Zhao, X.; Wang, J.; Zhu, L.; Wang, J. Field-based evidence for enrichment of antibiotic resistance genes and mobile genetic elements in manure-amended vegetable soils. *Sci. Total Environ.* **2019**, *654*, 906–913. [CrossRef]
26. Ramakrishna, W.; Yadav, R.; Li, K. Plant growth promoting bacteria in agriculture: Two sides of a coin. *Appl. Soil Ecol.* **2019**, *138*, 10–18. [CrossRef]
27. Xu, J.; Liu, X.; Lv, Y.; Guo, X.; Lu, S. Response of *Cyperus involucratus* to sulfamethoxazole and ofloxacin-contaminated environments: Growth physiology, transportation, and microbial community. *Ecotoxicol. Environ. Saf.* **2020**, *206*, 111332. [CrossRef]
28. Cheng, Y.; Cao, M.; Shi, X.; Chen, X.; Li, Z.; Ma, Y. Mitigating salt stress in *Zea mays*: Harnessing *Serratia nematodiphila*-biochar-based seed coating for plant growth promotion and rhizosphere microecology regulation. *Ind. Crop Prod.* **2025**, *223*, 120164. [CrossRef]
29. Bonkowski, M.; Tarkka, M.; Razavi, B.S.; Schmidt, H.; Blagodatskaya, E.; Koller, R.; Yu, P.; Knief, C.; Hochholdinger, F.; Vetterlein, D. Spatiotemporal dynamics of maize (*Zea mays* L.) root growth and its potential consequences for the assembly of the rhizosphere microbiota. *Front. Microbiol.* **2021**, *12*, 619499. [CrossRef]
30. Available online: https://pl.climate-data.org/europa/polska/warmian-masurian-voivodeship/olsztyn-758/ (accessed on 5 December 2024).
31. Available online: https://olsztyn.stat.gov.pl/files/gfx/olsztyn/pl/defaultstronaopisowa/1414/1/1/2024_dzial_01_warunki_naturalne_i_ochrona_srodowiska.pdf (accessed on 5 December 2024).
32. Available online: https://polska-energia.com/nasloneczenienie-i-liczba-slonecznych-dni-w-polsce-co-warto-wiedziec/ (accessed on 5 December 2024).
33. Available online: https://pl.weatherspark.com/y/85075/%C5%9Arednie-warunki-pogodowe-w:-Olsztyn-Polska-w-ci%C4%85gu-roku (accessed on 5 December 2024).
34. Gbylik-Sikorska, M.; Gajda, A.; Felipe-Sotelo, M.; Caniça, M.; Cabal-Rosel, A.; Tenson, T.; Korínková, M.; Arbo, K.; Kisand, V.; Rab, G.; et al. Investigation of 29 Antimicrobial Compounds in Soil Using Newly Developed UHPLC-MS/MS Method. *Molecules* **2023**, *28*, 649. [CrossRef]
35. Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to. *Off. J. Eur. Union.* **2021**, *180*, 84–109.
36. Wenzl, T.; Haedrich, J.; Schaechtele, A.; Robouch, P.; Stroka, J. *Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food*; EUR 28099 EN; Publications office of the EU: Luxembourg, 2016; ISBN 978-92-79-61768-3.
37. Carter, M.R. *Soil Sampling and Methods of Analysis*; Canadian Society of Soil Science; Lewis Publishers: London, UK, 1993.
38. Borowik, A.; Wyszkowska, J.; Wyszkowski, M. Resistance of aerobic microorganisms and soil enzyme response to soil contamination with Ekodiesel Ultra fuel. *Environ. Sci. Pollut. Res.* **2017**, *24*, 24346–24363. [CrossRef]
39. *ISO 10390:2021*; Soil, Treated Biowaste and Sludge—Determination of pH. The International Organization for Standardization: Geneva, Switzerland, 2021.
40. Wyszkowska, J.; Borowik, A.; Zaborowska, M.; Kucharski, J. The usability of sorbents in restoring enzymatic activity in soils polluted with petroleum-derived products. *Materials* **2023**, *16*, 3738. [CrossRef]
41. Wyszkowska, J.; Borowik, A.; Kucharski, J. The role of grass compost and *Zea Mays* in alleviating toxic effects of tetracycline on the soil bacteria community. *Int. J. Environ. Res. Public Health* **2022**, *19*, 7357. [CrossRef]
42. Sarathchandra, S.U.; Burch, G.; Cox, N.R. Growth patterns of bacterial communites in the rhizoplane and rhizosphere of with clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) in long-term pasture. *Appl. Soil Ecol.* **1997**, *6*, 293–299.
43. De Leij, F.A.A.M.; Whipps, J.M.; Lynch, J.M. The use of colony development for the characterization of bacterial communities in soil and on roots. *Microb. Ecol.* **1993**, *27*, 81–97. [CrossRef]
44. Ferris, M.J.; Muyzer, G.; Ward, D.M. Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring microbial mat community. *Appl. Environ. Microbiol.* **1996**, *62*, 340–346.
45. Data Analysis Software System, version 13; The R Foundation for Statistical Computing: Vienna, Austria. 2017. Available online: https://www.statistica.com (accessed on 5 December 2024).

46. Parks, D.H.; Tyson, G.W.; Hugenholtz, P.; Beiko, R.G. STAMP: Statistical analysis of taxonomic and functional profiles. *Bioinformatics* **2014**, *30*, 3123–3124. [CrossRef]
47. RStudio Team. *RStudio: Integrated Development for R*; RStudio, Inc.: Boston, MA, USA, 2019. Available online: http://www.rstudio.com/ (accessed on 5 December 2024).
48. Warnes, G.R.; Bolker, B.; Bonebakker, L.; Gentleman, R.; Huber, W.; Liaw, A.; Lumley, T.; Maechler, M.; Magnusson, M.; Moeller, S.; et al. Gplots: Various R Programming Tools for Plotting Data, version 3.20.0.; R Package; R Foundation for Statistical Computing, Vienna, Austria. 2020. Available online: https://rdrr.io/cran/gplots/ (accessed on 5 December 2024).
49. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019. Available online: https://www.R-project.org/ (accessed on 5 December 2024).
50. Teressa, D.; Kibret, K.; Dechasa, N.; Wogi, L. Soil properties and nutrient uptake of maize (*Zea mays*) as influenced by mixed manure and blended inorganic fertilizer in Haramaya district, eastern Ethiopia. *Heliyon* **2024**, *10*, e35784. [CrossRef]
51. Stehlíková, I.; Kodešová, R.; Kunzová, E.; Czakó, A.; Mayerová, M.; Madaras, M. Sixty-year impact of manure and NPK on soil aggregate stability. *Geoderma Reg.* **2024**, *39*, e00858. [CrossRef]
52. Han, X.M.; Hu, H.W.; Chen, Q.L.; Yang, L.Y.; Li, H.L.; Zhu, Y.G.; Li, X.Z.; Ma, Y.B. Antibiotic resistance genes and associated bacterial communities in agricultural soils amended with different sources of animal manures. *Soil Biol. Biochem.* **2018**, *126*, 91–102. [CrossRef]
53. Han, T.; Liang, Y.; Wu, Z.; Zhang, L.; Liu, Z.; Li, Q.; Chen, X.; Guo, W.; Jiang, L.; Pan, F.; et al. Effects of tetracycline on growth, oxidative stress response, and metabolite pattern of ryegrass. *J. Hazard. Mater.* **2019**, *380*, 12885. [CrossRef] [PubMed]
54. Song, M.; Peng, K.; Jiang, L.; Zhang, D.; Song, D.; Chen, G.; Xu, H.; Li, Y.; Luo, C. Alleviated antibiotic-resistant genes in the rhizosphere of agricultural soils with low antibiotic concentration. *Agric. Food Chem.* **2020**, *68*, 2457–2466. [CrossRef]
55. Wen, X.; Xu, J.; Xiang, G.; Cao, Z.; Yan, Q.; Mi, J.; Ma, B.; Zou, Y.; Zhang, N.; Liao, X.; et al. Multiple driving factors contribute to the variations of typical antibiotic resistance genes in different parts of soil-lettuce system. *Ecotoxicol. Environ. Saf.* **2021**, *225*, 112815. [CrossRef]
56. Conte, S.; Lloyd, A. Exploring multiple drug and herbicide resistance in plants-Spotlight on transporter proteins. *Plant Sci.* **2011**, *180*, 196–203. [CrossRef]
57. Zhalnina, K.; Louie, K.; Hao, Z.; Mansoori, N.; da Rocha, U.; Shi, S.; Cho, H.; Karaoz, U.; Loque, D.; Bowen, B.; et al. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nat. Microbiol.* **2018**, *3*, 470. [CrossRef]
58. Wu, X.; Chen, X.; Zhang, D.; Hu, X.; Ding, W.; Wang, Y.; Li, G.; Dong, N.; Hu, H.; Hu, T.; et al. Integrative multi-omics analysis reveals the underlying toxicological mechanisms of enrofloxacin on the growth of wheat seedling roots. *J. Hazard. Mater.* **2024**, *477*, 135303. [CrossRef]
59. Doornbos, R.; van Loon, L.; Bakker, P. Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. *Agron. Sustain. Dev.* **2012**, *32*, 227–243. [CrossRef]
60. Zheng, F.; Zhou, G.; Zhu, D.; Neilson, R.; Zhu, Y.; Chen, B.; Yang, X. Does plant identity affect the dispersal of resistomes above and below ground? *Environ. Sci. Technol.* **2022**, *56*, 14904–14912. [CrossRef]
61. Lebeis, S.; Paredes, S.; Lundberg, D.; Breakfield, N.; Gehring, J.; McDonald, M.; Malfatti, S.; del Rio, T.; Jones, C.; Tringe, S.; et al. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* **2015**, *349*, 860–864. [CrossRef]
62. Carvalhais, L.; Dennis, P.; Badri, D.; Kidd, B.; Vivanco, J.; Schenk, P. Linking Jasmonic Acid Signaling, Root Exudates, and Rhizosphere Microbiomes. *Mol. Plant Microbe Interact.* **2015**, *28*, 1049–1058. [CrossRef]
63. Neal, A.L.; Ahmad, S.; Gordon-Weeks, R.; Ton, J. Benzoxazinoids in root exudates of maize attract *Pseudomonas* putida to the rhizosphere. *PLoS ONE* **2012**, *7*, e35498. [CrossRef]
64. Rath, S.; Fostier, A.; Pereira, L.; Dioniso, A.; Ferreira, F.; Doretto, K.; Peruchi, L.; Viera, A.; Neto, O.; Bosco, S.; et al. Sorption behaviors of antimicrobial and antiparasitic veterinary drugs on subtropical soils. *Chemosphere* **2019**, *214*, 111–122. [CrossRef]
65. Du, H.; Du, J.; Liu, F.; Zhang, Y.; Guo, H.; Wan, D. Binding of tetracycline on soil phyllosilicates with Cd(II) as affected by pH and mineral type. *J. Soil Sediment.* **2021**, *21*, 775–783. [CrossRef]
66. Van Boeckel, T.P.; Brower, C.; Gilbert, M.; Grenfell, B.T.; Levin, S.A.; Robinson, T.P.; Teillant, A.; Laxminarayan, R. Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 5649–5654. [CrossRef]
67. Chapman, H.D.; Jeffers, T.K.; Williams, R.B. Forty years of monensin for the control of coccidiosis in poultry. *Poult. Sci.* **2010**, *89*, 1788–1801. [CrossRef]
68. Berendsen, B.J.A.; Lahr, J.; Nibbeling, C.; Jansen, L.J.M.; Bongers, I.E.A.; Wipfler, E.L.; Van de Shans, M.G.M. The persistence of a broad range of antibiotics during calve, pig and broiler manure storage. *Chemosphere* **2018**, *204*, 267–276. [CrossRef]
69. Hussain, S.; Naeem, M.; Chaudhry, M.N.; Iqbal, M.A. Accumulation of residual antibiotics in the vegetables irrigated by pharmaceutical wastewater. *Expo. Health* **2016**, *8*, 107–115. [CrossRef]
70. Pan, M.; Chu, L.M. Transfer of antibiotics from wastewater or animal manure to soil and edible crops. *Environ. Pollut.* **2017**, *231*, 829–836. [CrossRef]

71. Pan, M.; Chu, L.M. Fate of antibiotics in soil and their uptake by edible crops. *Sci. Total Environ.* **2017**, *599–600*, 500–512. [CrossRef]
72. Broekaert, N.; Daeseleire, E.; Delezie, E.; Vandecasteele, B.; De Beer, T.; Van Poucke, C. Can the Use of Coccidiostats in Poultry Breeding Lead to Residues in Vegetables? An Experimental Study. *J. Agric. Food Chem.* **2012**, *60*, 12411–12418. [CrossRef]
73. Borowik, A.; Wyszkowska, J.; Zaborowska, M.; Kucharski, J. Microbial diversity and enzyme activity as indicators of permethrin-exposed soil health. *Molecules* **2023**, *28*, 4756. [CrossRef] [PubMed]
74. Wallenstein, M.D.; Burns, R.G. Ecology of extracellular enzyme activities and organic matter degradation in soil: A complex community-driven process. In *Methods of Soil Enzymology*; Dick, R.P., Ed.; Soil Science Society of America; Wiley: Madison, WI, USA, 2011; pp. 35–55.
75. Miśkowiec, P.; Olech, Z. Searching for the correlation between the activity of urease and the content of nickel in the soil Samples: The role of metal speciation. *J. Soil Sci. Plant Nutr.* **2020**, *20*, 1904–1911. [CrossRef]
76. Unger, I.M.; Goyne, K.W.; Kennedy, A.C.; Kremer, R.J.; Mclain, J.E.T.; Williams, C.F. Antibiotic Effects on Microbial Community Characteristics in Soils under Conservation Management Practices. *Soil Sci. Soc. Am. J.* **2013**, *77*, 100–112. [CrossRef]
77. Chen, W.; Liu, W.; Pan, N.; Jiao, W.; Wang, M. Oxytetracycline on functions and structure of soil microbial community. *J. Soil Sci. Plant Nutr.* **2013**, *13*, 967–975. [CrossRef]
78. Santás-Miguel, V.; Díaz-Raviña, M.; Martín, A.; García-Campos, E.; Barreiro, A.; Núñez-Delgado, A.; Álvarez-Rodríguez, E.; Arias-Estévez, M.; Fernández-Calviño, D. Soil enzymatic activities and microbial community structure in soils polluted with tetracycline antibiotics. *Agronomy* **2021**, *11*, 906. [CrossRef]
79. Molaei, A.; Lakzian, A.; Datta, R.; Haghnia, G.; Astaraei, A.; Rassouli-Sadaghiani, M.; Ceccherini, M.T. Impact of chlortetracycline and sulfapyridine antibiotics on soil enzyme activities. *Int. Agrophys.* **2017**, *31*, 499–505. [CrossRef]
80. Guangming, L.; Xuechen, Z.; Xiuping, W.; Hongbo, S.; Jingsong, Y.; Xiangping, W. Soil enzymes as indicators of saline soil fertility under various soil amendments. *Agric. Ecosyst. Environ.* **2017**, *237*, 274–279. [CrossRef]
81. Janse, I. Tetracycline amide antibiotics. In *Bioactive Carboxylic Compound Classes: Pharmaceuticals and Agrochemicals*, 1st ed.; Part III: Infectious Diseases, Chapter 9; Lamberth, C., Dinges, J., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2016; pp. 115–132.
82. Bloor, M.C.; Kiryishina, A.; Kydralieva, K. Divergent effects of antibiotics on plants and microbiota in soils with contrasting humus content. *Water Air Soil Pollut.* **2021**, *232*, 518. [CrossRef]
83. Męcik, M.; Buta-Hubeny, M.; Paukszto, Ł.; Maździarz, M.; Wolak, I.; Harnisz, M.; Korzeniewska, E. Poultry manure-derived microorganisms as a reservoir and source of antibiotic resistance genes transferred to soil autochthonous microorganisms. *J. Environ. Manag.* **2023**, *348*, 119303. [CrossRef]
84. Wang, Y.; Yang, L.; Liu, W.; Zhuang, J. The effect of manure application rates on the vertical distribution of antibiotic resistance genes in farmland soil. *Soil Syst.* **2024**, *8*, 89. [CrossRef]
85. Guo, X.; Weining Qi, W.; Yao Feng, Y.; Zhaojun Li, Z. Degradation of oxytetracycline in soil by a *Pseudomonas* strain. *J. Integr. Agric.* **2024**, *24*, 2002–2014. [CrossRef]
86. Chen, J.; Xu, H.; Sun, Y.; Huang, L.; Zhang, P.; Zou, C.; Zhao, C. Interspecific differences in growth response and tolerance to the antibiotic sulfadiazine in ten clonal wetland plants in South China. *Sci. Total Environ.* **2016**, *543*, 197–205. [CrossRef] [PubMed]
87. Zhang, S.; Hou, Q.; Wang, Z.; Tian, D.; Zhang, X.; Zhang, Y.; Wu, Q.; Sun, F. Fatty acid addition strategy redirected the metabolic flux towards an ultra-high monensin productivity of *Streptomyces cinnamonensis*. *Synth. Syst. Biotechnol.* **2025**, *10*, 532–542. [CrossRef]
88. Zhang, Y.; Lin, C.Y.; Li, X.M.; Tang, Z.K.; Qiao, J.; Zhao, G.R. DasR positively controls monensin production at two-level regulation in *Streptomyces cinnamonensis*. *J. Ind. Microbiol. Biotechnol.* **2016**, *43*, 1681–1692. [CrossRef] [PubMed]
89. Barabote, R.D.; Thekkiniath, J.; Strauss, R.E.; Vediyappan, G.; Fralick, J.A.; San Francisco, M.J. Xenobiotic efflux in bacteria and fungi: A genomics update. *Adv. Enzymol. Relat. Areas Mol. Biol.* **2011**, *77*, 237–306. [CrossRef]
90. Kondratowicz-Maciejewska, K.; Lemanowicz, J.; Jaskulska, I. Effects of Long-Term Multi-Treatment Experiments on Organic Matter and Enzymatic Activity in Sandy Soil. *Sustainability* **2025**, *17*, 3252. [CrossRef]
91. Gong, P.; Liu, H.; Xin, Y.; Wang, G.; Dai, X.J.; Yao, J. Composting of oxytetracycline fermentation residue in combination with hydrothermal pretreatment for reducing antibiotic resistance genes enrichment. *Bioresour. Technol.* **2020**, *318*, 124271. [CrossRef]
92. Iltchenco, J.; Smiderle, M.D.; Gaio, J.; Magrini, F.E.; Paesi, S. Metataxonomic characterization of the microbial present in the anaerobic digestion of turkey litter waste with the addition of two inocula: Allochthonous and commercial. *Int. Microbiol.* **2025**, *28*, 539–551. [CrossRef]
93. Dyksma, S.; Jansen, L.; Gallert, C. Syntrophic acetate oxidation replaces acetoclastic methanogenesis during thermophilic digestion of biowaste. *Microbiome* **2020**, *8*, 1–14. [CrossRef]
94. Yang, J.; Xiang, J.; Gin, K.; Xie, Y.; Yu, K.; Li, P.; He, Y. Antibiotic resistome associated with influencing factors in industrial-scale organic waste aerobic composting plants. *Bioresour. Technol.* **2023**, *385*, 129354. [CrossRef]
95. Fang, D.; Zhao, G.; Xu, X.; Zhang, Q.; Shen, Q.; Fang, Z.; Huang, L.; Ji, F. Microbial community structures and functions of wastewater treatment systems in plateau and cold regions. *Bioresour. Technol.* **2018**, *249*, 684–693. [CrossRef] [PubMed]

96. Gong, P.; Liu, H.; Wang, G.; Yao, J.; Dai, X. Enhanced depletion of antibiotics and accelerated estabilization of dissolved organic matter by hydrothermal pretreatment during composting of oxytetracycline fermentation residue. *Bioresour. Technol.* **2021**, *339*, 125618. [CrossRef]
97. Feng, M.; Liu, Y.; Yang, L.; Li, Z. Antibiotics and antibiotic resistance gene dynamics in the composting of antibiotic fermentation waste—A review. *Biores. Technol.* **2023**, *390*, 129861. [CrossRef] [PubMed]
98. Zhou, Z.; Yao, H. Effects of composting different types of organic fertilizer on the microbial community structure and antibiotic resistance genes. *Microorganisms* **2020**, *8*, 268. [CrossRef]
99. Beyi, A.F.; Brito-Goulart, D.; Hawbecker, T.; Ruddell, B.; Hassall, A.; Dewell, R.; Dewell, G.; Sahin, O.; Zhang, Q.; Plummer, P.J. Enrofloxacin alters fecal microbiota and resistome irrespective of its dose in calves. *Microorganisms* **2021**, *9*, 2162. [CrossRef]
100. Rodríguez-Martínez, J.M.; Cano, M.E.; Velasco, C.; Martínez-Martínez, L.; Pascual, A. Plasmid-mediated quinolone resistance: An update. *J. Infect. Chemother.* **2011**, *17*, 149–182. [CrossRef]
101. Frąc, M.; Pertile, G.; Panek, J.; Gryta, A.; Oszust, K.; Lipiec, J.; Usowicz, B. Mycobiome composition and diversity under the long-term application of spent mushroom substrate and chicken manure. *Agronomy* **2021**, *11*, 410. [CrossRef]
102. Wen, X.; Jia, Y.; Li, J. Enzymatic degradation of tetracycline and oxytetracycline by crude manganese peroxidase prepared from *Phanerochaete chrysosporium*. *J. Hazard Mater.* **2010**, *177*, 924–928. [CrossRef]
103. Llorca, M.; Rodríguez-Mozaz, S.; Couillerot, O.; Panigoni, K.; de Gunzburg, J.; Bayer, S.; Czaja, R.; Barceló, D. Identification of new transformation products during enzymatic treatment of tetracycline and erythromycin antibiotics at laboratory scale by an on-line turbulent flow liquid-chromatography coupled to a high resolution mass spectrometer LTQ-Orbitrap. *Chemosphere* **2015**, *119*, 90–98. [CrossRef]
104. Rajalakshmi, J.; Harish, S.; Rajendran, L.; Parthasarathy, S.; Saravanakumari, K.; Raguchander, T. Crude antibiotics and antifungal metabolites from *Chaetomium globosum* Cg6 suppress pythium aphanidermatum, causal agent of rhizome rot of turmeric. *Curr. Microbiol.* **2025**, *82*, 105. [CrossRef] [PubMed]
105. Goda, M.S.; El-Kattan, N.; Abdel-Azeem, M.A.; Allam, K.A.M.; Badr, J.M.; Nassar, N.A.; Almalki, A.J.; Alharbi, M.; Elhady, S.S.; Eltamany, E.E. Antimicrobial potential of different isolates of *Chaetomium globosum* combined with liquid chromatography tandem mass spectrometry chemical profiling. *Biomolecules* **2023**, *13*, 1683. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.