

Article

The Spreading of Antibiotic-Resistant Bacteria in Terrestrial Ecosystems and the Formation of Soil Resistome

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Abstract: Terrestrial ecosystems play a crucial role in the formation of soil resistome and the spread of antibiotic-resistant bacteria. Comprehensive studies of soil microbial communities, their structure, integrity, and level of antibiotic resistance (AR) in various terrestrial ecosystems were conducted. In total, 389 strains of dominant bacteria were isolated from the studied ecosystems, 57 of which were resistant to antibiotics, with levels of antibiotic resistance exceeding 70%. The soil microbiome of primeval forests was characterized by a low content of bacteria resistant to antibiotics; only two species, *Bacillus cereus* and *Pantoea agglomerans*, showed a high resistance to antibiotics. In the soil of agroecosystems of medicinal plants, among 106 strains of bacteria, a high level of resistance to antibiotics was found in 13 species. It was established that the number of antibiotic-resistant bacteria is highest in the soil of agroecosystems contaminated by enrofloxacin. Among 190 tested bacterial strains, 42 (22%) were characterized by a high level of antibiotic resistance. Therefore, the soil ecosystem is a key link in the formation and spread of antibiotic-resistant bacteria, which is a potential danger to humans. To reduce the risk of AR for humans, it is necessary to take appropriate measures to manage the soil microbiome and avoid soil contamination with antibiotics.

Keywords: ecosystem; resistome; soil; microbiome; antibiotic resistance



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1. Introduction

Soil plays an important role in ecosystems; it is the habitat of animals, plants, and microorganisms. Many biological processes take place in the soil, including the mineralization of organic remains, the accumulation of nutrients and their migration, and the degradation of xenobiotics and pollutants. The soil environment is a reservoir for the accumulation and storage of antibiotics that enter with waste from pharmaceutical, medical, and animal husbandry enterprises, household waste, and through the use of animal husbandry waste as fertilizer [1]. At the same time, the environment contains a significant number of microorganisms, and there is a constant horizontal transfer of genes, including antibiotic-resistant genes, in the soil environment. This should be taken into account since soils and soil biodiversity, as key components of terrestrial ecosystems, play an important role in achieving Sustainable Development Goals. Soil biodiversity is a sensitive indicator of the action of various factors, including anthropogenic changes in the structure and qualitative and quantitative composition of the soil microbiome [2]. Microorganisms serve as a convenient object of monitoring due to their high sensitivity to changes in the environment. Microorganisms in the soil are characterized by a high rate of reproduction and growth and are in constant interaction with the surrounding environment. This property of microorganisms, which is key in soil formation and affects the course of basic biological processes, is important

in ensuring ecosystem health and providing ecosystem services. At the same time, the presence of various ecological-trophic and physiological groups of microorganisms in the soil, their significant biodiversity, and their abundance can have a positive effect on genetic exchange and, accordingly, contribute to the formation of antibiotic resistance in bacteria in the presence of antibiotics in the soil [3]. The species diversity of microorganisms in the soil is quite significant, including pathogenic bacteria that are dangerous for humans and animals and cause infectious diseases, for example, *Clostridium perfringens*, *Escherichia coli*, *Citrobacter* spp., *Enterobacter* spp., *P. vulgaris*, *E. faecalis*, *Staphylococcus aureus*, etc. Antibiotic-producing microorganisms were also isolated from the environment, in particular the soil, which was effective against the causative agents of bacterial infections [4]. Therefore, it is important to understand the importance of soil as an environment for the accumulation of antibiotics and the location of antibiotic-resistant bacteria, the formation of resistance in other species, and their distribution in various ecosystems. This determines the relevance and importance of considering terrestrial ecosystems as a key link in the formation and spread of antibiotic resistance and their management in mitigating potential risks to the environment, humans, and animals [5].

Environmental contamination by antimicrobial compounds and antibiotic residues is an important factor in the development of antimicrobial resistance in non-target species of bacteria. Bacteria can have different functions and perform ecosystem services but, at the same time, they pose a health threat as pathogens or have incompletely understood functions in nature. The development of antibiotic resistance and its spread in the environment due to human activities is an extremely complex process that requires additional attention. The sensitivity of microbes can be changed by connecting genetic information that codes resistance or by mutating their deoxyribonucleic acid (DNA). Antibiotic-resistant genes are recognized as important environmental contaminants [6,7]. It is now accepted that resistance is a natural property of all bacteria [8,9]. The term “resistome” is used to describe the framework that encompasses all forms of resistance and precursor elements [10]. The environment and its various components (water sources, soil, biodiversity) act as a depository for the accumulation, formation, and spread of antibiotic resistance. One of the ways of spreading antibiotic resistance is through the horizontal and vertical transfer of genes in bacterial populations. However, bacteria are characterized by a high rate of adaptation to reduce the negative impact on them of new types of antibiotics that are introduced into the medical or veterinary field. Antibiotic-resistant bacteria, resistance genes, and biologically active substances (for example, enzymes) are always present in the environment, destroy antibiotics and thereby hinder and inhibit the processes of AR formation and spread. Therefore, it is quite difficult to distinguish between natural and acquired AR [11].

Antibiotics are commonly used in animal husbandry and agriculture for therapeutic and prophylactic purposes. As animal waste from livestock complexes is increasingly utilized as fertilizer for crop cultivation, antibiotics can be released into the environment. Studies have shown that even low concentrations of antibiotics introduced into soil or water sources via animal waste can contribute to the development and spread of antibiotic-resistant bacteria [12–16]. Therefore, antibiotics are considered a powerful environmental factor that causes environmental pollution and disturbances in the microbial population which can affect the evolutionary processes of the structure of biological communities [17–20]. As a result, the change in the structure of the soil microbiome affects the health and quality of the soil, the provision of ecosystem services, the course of basic biological processes, etc. Future environmental consequences are also uncertain. For the treatment and prevention of bacterial diseases in veterinary medicine, enrofloxacin from the fluoroquinolone antibiotic class is most often used. The degradation of enrofloxacin in the environment occurs in various ways, but most often it is the result of hydrolysis processes. Enrofloxacin has long time degradation in the environment. Such properties affect the structure and activity of soil microbiomes, higher plants, and biodiversity in general. Enrofloxacin and its decomposition products (in particular, ciprofloxacin) enter agroecosystems with

waste from animal husbandry enterprises, sewage, and livestock manure, which is used as organic fertilizer. In addition, the manure of agricultural animals contains other types of antibiotics and their derivative compounds, as well as antibiotic-resistant microorganisms, which increases the problem of soil pollution and the spread of AR [21,22]. Improper use of antimicrobial drugs, even in small concentrations, has a significant number of dangerous side effects for humans and animals, including allergic reactions, the formation of resistance to antibiotics, etc. At the same time, there is little information in the scientific literature on the results of research on the impact of soil contamination with antibiotics in the agroecosystem due to the use of manure on the soil microbiome, changes in the structure and functional activity of soil bacteria, etc. [23–26]. A promising direction in modern microbiology, ecology, and soil science is the study of terrestrial ecosystems as a habitat for antibiotic-resistant microorganisms and the screening of antibiotic-resistant bacteria as potentially dangerous bioagents for human and animal health. The alimentary route of transmission of antibiotic resistance to humans and animals, in particular through fresh vegetables and berries grown in agroecosystems, also requires additional research taking soil contamination with antibiotics and manure into account.

The aim of this study is to investigate the soil microbiome (structure and integrity) of terrestrial ecosystems, as well as screen for antibiotic-resistant bacteria and the formation of soil resistomes. The characterization of soil microbiomes and resistomes is of great importance for understanding the dynamics of antibiotic resistance in natural environments. Our research findings will provide a better understanding of the mechanisms underlying the emergence and dissemination of antibiotic resistance and inform the development of effective strategies to prevent and control its spread. Furthermore, this study can contribute to the preservation of soil health and the maintenance of a sustainable environment.

2. Materials and Methods

2.1. Soil Sample Collection

Soil samples for scientific research were collected in ecosystems of two types: anthropogenic ecosystems and non-anthropogenic ecosystems. Collections were made in the years 2017–2020. Sampling was carried out seasonally at different altitudes from 450 m to 650 m in forest ecosystems and agroecosystems at a depth of 0–25 cm. Following the standard protocols, all soil samples were analyzed within 24 h [27,28].

2.2. Description of Localities

Uzhanskyi National Nature Park (49.0233° N; 22.6000° E, Ukraine) is primarily made up of primeval beech forests which were recognized by UNESCO and added to the World Heritage List in 2007 under the name “Primeval Beech Forests of the Carpathians”. The park is located in the western part of Transcarpathia, within the Uzh river basin, and stretches from the southwest of Zabrod village (at an altitude of 226 m) to the northeast of Uzhotskyi Pass (at an altitude of 852 m). The average annual rainfall is 856–909 mm. The climatic conditions in the region of soil sample collection are as follows. Absolute temperature maximums are most common in July and August and range from 34 to 37 °C; however, they can occur in April and May in some years. The absolute minimum temperatures in January and February range from –28 °C to –32 °C. The total vegetation period in the region lasts 195 days. The soil type is characterized as mainly brown mountain-forest soils (brown soils) with two subtypes: dark brown and light brown mountain-forest soils.

Anthropogenic ecosystems presented agroecosystems where different crops were grown. There were fields with *Lactuca sativa* var. *crispa*, *Anethum graveolens*, *Thymus serpyllum*, *Mentha piperita*, and *Calendula officinalis* contaminated by an antibiotic (enrofloxacin), and in agroecosystems of medicinal plants without manure application and antimicrobial contamination.

2.3. Microbial Assays

The study of the presence of microbiota in soil samples was performed in sterile conditions. Endos agar, Meat peptone agar, Strepto agar, Entero agar, Agar-Agar, Eshbi agar, Soil agar, Chapek agar, and Starch agar (HiMedia Laboratories, Mumbai, India) were used as the media (4 repetitions). The serial dilutions of the samples were provided until the suspension contained a microorganism titre within the range of 10^{-3} – 10^{-5} CFU/mL. Then, a sterile spatula was used to distribute 100 μ L of the soil suspension evenly on the surface of the medium in a Petri dish. Soil samples in different media were incubated at 29–37 °C for 48–72 h in the thermostat under aerobic conditions.

ENTEROtest 24 N, OFtest, NEFERMtest 24, STREPTOtest 24, and ANAEROtest 23 (Erba, Lachema, Brno, Czech Republic) biochemical test systems were used for the identification of isolated microorganisms. Antimicrobial susceptibility testing was performed using the Kirby Bauer disk diffusion method according to EUCAST. Standard discs (μ g/disc) (bioMérieux, Marcy-l’Etoile, France) of 12 antibiotics from the main pharmacological groups: AK30 Amikacin; AMP10 Ampicillin; AMX10 Amoxicillin; CIP5 Ciprofloxacin; CXM30 Cefuroxime; DO30 Doxycycline; GEN10 Gentamicin; E15 Erythromycin L10 Lincomycin; OL15 Oleandomycin; TE30 Tetracycline; and VA30 Vancomycin were used to detect and measure induced inhibition for specified antibiotic concentrations. Discs were placed on Mueller–Hinton agar seeded with 0.5 McFarland of bacteria. Anaerobic microbiota was additionally tested for Clindamycin CD2, Metronidazole MT5, and Rifampicin RIF5. The plates were incubated for 24 h. After incubation was complete, the zone inhibition diameter, in mm, was measured.

2.4. HPLC Analysis of Enrofloxacin

The quantitative analysis of enrofloxacin in the soil samples was carried out in triplicate on each sample using high-performance liquid chromatography (HPLC) on an Agilent 1290 Infinity system (Agilent Technologies, Waldbronn, Germany) and Waters Xselect HSS T3 (150 \times 2.1 mm) C18 column (Waters Corp., Milford, MA, USA). The fluorometric detector’s isocratic mode was used with the wavelengths of excitation and emission at 280 and 458 nm, respectively. A 0.01 M oxalic acid solution in aqueous methanol (80:20) with a pH of 2.2 was used as the mobile phase. The oxalic acid buffer solution was prepared daily by dissolving 1.26 g of oxalic acid dihydrate in one liter of distilled water. The pH was adjusted by adding NaOH and filtered through a 0.22 μ m membrane before use. A flow rate of 1.5 mL per minute and an injection volume of 50 μ L were applied. Quantification was carried out by external calibration (a measure of the peak area).

2.5. Statistics

Statistica 10.0 (Stat Soft Inc., Tulsa, OK, USA) was used to evaluate the data from the bioassays. The results are expressed as mean values (\pm) standard deviation (SD) and (SSD05) smallest significant difference of experiments conducted four times. The level of significance was set at $p < 0.05$. An assessment of the integrability of the soil microbial community in different edaphotope ecotypes was carried out using correlation regression analysis.

3. Results

The structure and abundance of the soil microbiome in natural ecosystems are complex and influenced by a range of environmental factors, including the vertical placement of edaphotopes and factors such as humidity, temperature, and plant cover. The vertical placement of edaphotopes can affect the distribution of nutrients, moisture, and microbial populations in the soil, which in turn can influence the structure and abundance of the soil microbiome.

The highest abundance of various ecological-trophic and functional groups of microorganisms were in the soil at altitudes of 450 m. A significant difference in the number of different groups of microorganisms was recorded depending on the vertical placement of

edaphotopes and, accordingly, the enrichment of the soil with organic matter. The highest number of ammonifying microorganisms was in the soil at an altitude of 450 m, which is 1.8 times more than at an altitude of 650 m (Figure 1). These data correlate with the content of organic matter in the soil, which depends on the vegetation cover. It is known that the higher the altitude, the smaller the number of plants there are, and thus less organic matter enters the soil. Under such conditions, the number of organotrophic and pedotrophic microbiota decreases with altitude and the number of oligotrophs increases. The same fluctuation was seen with the percentage of aerobic nitrogen-fixing bacteria (Figure 2). The content of this functional group of microorganisms decreased with altitude by 32%.

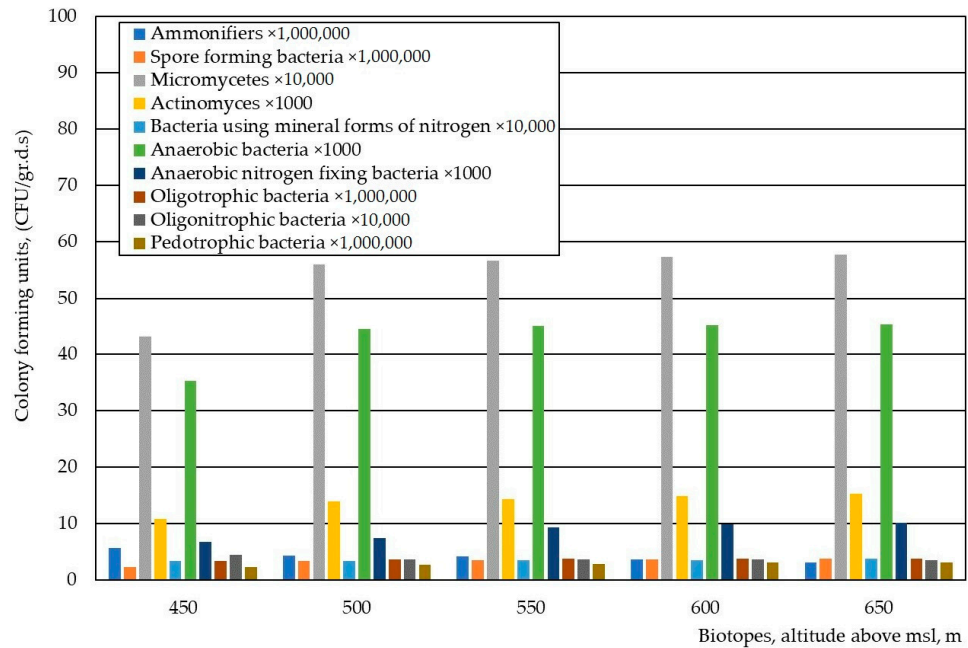


Figure 1. Microbial community composition (CFU/gr.d.s.) in the soils of the forest ecosystem. The data are statistically significant, $p < 0.05$, $x \pm SD$, $n = 4$.

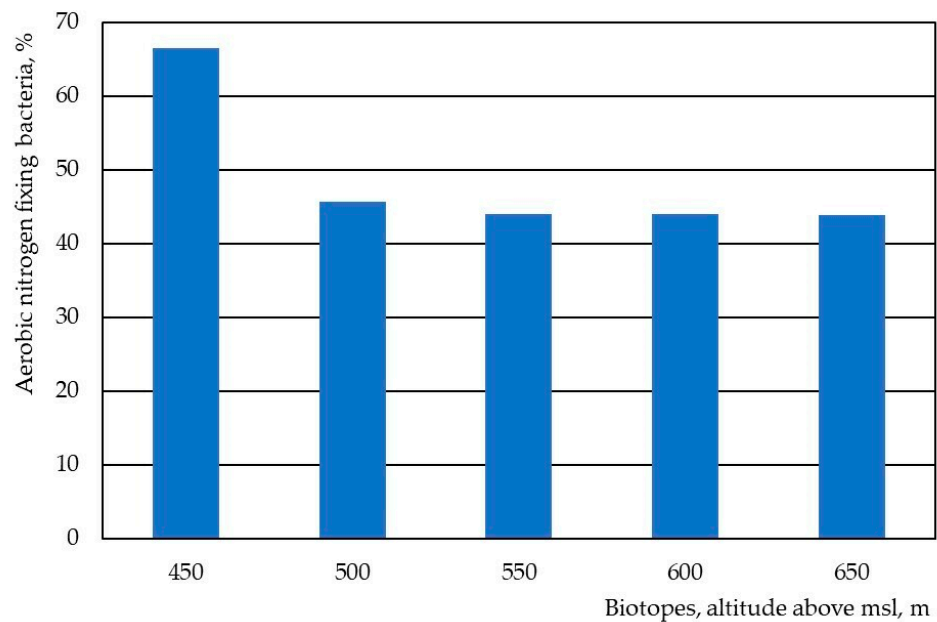


Figure 2. Aerobic nitrogen-fixing bacteria (%) in the soils of forest ecosystems. The data are statistically significant, $p < 0.05$, $x \pm SD$, $n = 4$.

The soil microbiome is a complex and dynamic system that is influenced by a range of biotic and abiotic factors, and changes in the environment can have significant effects on the composition and function of soil microbial communities. Under favorable environmental conditions, the soil microbiome can shift in favor of more competitive microorganisms that are better adapted to the new conditions.

Thus, with the rise in altitude, a high number of oligotrophic microorganisms was found. In terrestrial ecosystems, the role of soil microbiota is decisive in the formation of soils, their health and provision of ecosystem services, the mitigation of climate change, etc. Soil microorganisms participate in important biogeochemical transformations, in particular the carbon and nitrogen cycles, providing plants with nutrients and regulating the coexistence between neighboring species in the communities [29]. The study of soil microbiomes, their functioning, and their stability depending on the action of various factors remains relevant in modern science [30–33]. Since the microbiological component of the soil is a sensitive indicator of the habitat and the action of various environmental and anthropogenic factors, the changes in the relationship between soil microorganisms and plants during climate change will also have serious unforeseen consequences for the composition of phytocenoses and the functioning of ecosystems in general. Our results indicate the high stability of the studied soil systems and suggest the existence of functional redundancy among soil microorganisms, leading to ecosystem resistance and resilience. A high level of functional redundancy within a functional community, that is, a high number of species performing the same function, might act as a buffer against the effect of biodiversity loss on functioning. The results of our studies have shown that there are strong correlations between the majority of functional groups of soil microorganisms in non-disturbed edaphotopes (Table 1).

Table 1. The integrity of the soil microbial community in forest ecosystems.

Microorganisms	Ammonifiers	Spore Forming Bacteria	Micromycetes	Actinomycetes	Bacteria Using Mineral Forms of Nitrogen	Anaerobic Bacteria	Anaerobic Nitrogen Fixing Bacteria	Oligotrophic Bacteria	Oligonitrophic Bacteria	Pedotrophic Bacteria	Aerobic Nitrogen Fixing Bacteria
Ammonifiers	1										
Spore-forming bacteria	−0.96	1									
Micromycetes	−0.92	0.99	1								
Actinomycetes	−0.97	1.00	0.98	1							
Bacteria using mineral forms of nitrogen	−0.85	0.71	0.61	0.73	1						
Anaerobic bacteria	−0.90	0.99	1.00	0.98	0.58	1					
Anaerobic nitrogen-fixing bacteria	−0.90	0.85	0.79	0.87	0.82	0.77	1				
Oligotrophic bacteria	−0.97	0.92	0.87	0.94	0.85	0.86	0.98	1			
Oligonitrophic bacteria	0.95	−1.00	−1.00	−1.00	−0.67	−0.99	−0.82	−0.90	1		
Pedotrophic bacteria	−0.98	0.97	0.94	0.98	0.79	0.92	0.93	0.98	−0.96	1	
Aerobic nitrogen-fixing bacteria	0.89	−0.98	−1.00	−0.97	−0.57	−1.00	−0.77	−0.85	0.99	−0.92	1

A high level of correlation ($R = 0.87$; 0.94 and 0.94 ; 0.98) was established for oligotrophs and pedotrophs of bacteria with mycelial organisms (micromycetes and actinomycetes), which is quite natural because these species are adapted to conditions with limited readily available organic matter. A close correlation has been established between microorganisms in the nitrogen cycle. Thus, a high correlation (at the level of $R = 0.89$) was established between aerobic nitrogen-fixing bacteria and ammonifiers and a less close one ($R = 0.57$) with bacteria that use mineral forms of nitrogen. A close relationship was found between anaerobic bacteria and mycelial organisms (with micromycetes $R = 0.79$, actinomycetes $R = 0.87$) and spore-forming bacteria ($R = 0.85$).

Microbiological monitoring and the control of antibiotic resistance in environmental settings remain crucial for public health and ecological safety. This approach allows for a timely detection, management, and the prediction of risks associated with the emergence and dissemination of antibiotic-resistant microorganisms. In general, it should be noted that the soil microbiome of the forest ecosystem has a high level of integrity; 49 bonds were identified between different groups of microorganisms, which indicates a high level of correlation ($R > 0.75$).

A comparative study of the soil microbiome in natural ecosystems and agroecosystems showed that the former was characterized by a low content of AR microorganisms (Figures 3 and 4). The results showed that 23% of isolated bacteria had resistance to antibiotics at the level of 30%, 3% of bacteria showed resistance to antibiotics at the level of 70%, and two species of bacteria (*Pantoea agglomerans* and *Bacillus cereus*) showed a high level of resistance to antibiotics. *Pantoea agglomerans* and *Bacillus cereus* demonstrated a high level of resistance to the tested antibiotics.

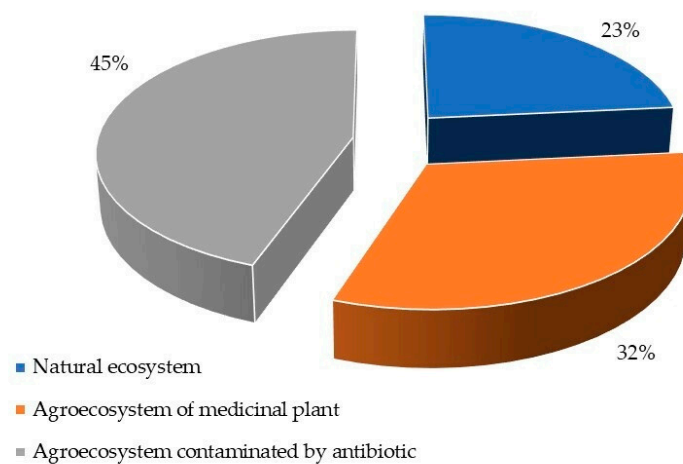


Figure 3. Percentage of bacteria in the different ecosystems with levels of AR for tested antibiotics greater than 30%, $p < 0.05$.

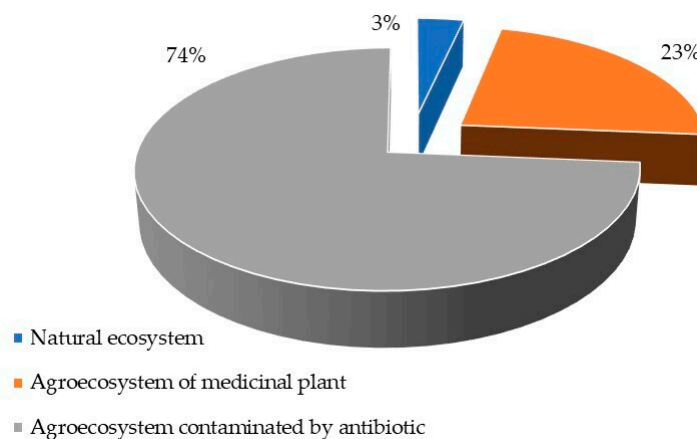


Figure 4. Percentage of bacteria in the different ecosystems with levels of AR for tested antibiotics greater than 70%, $p < 0.05$.

The highest percentage of antibiotic-resistant bacteria (74% with a level of AR for tested antibiotics greater than 70%) was determined in the soil of agroecosystems contaminated by enrofloxacin. Enrofloxacin belongs to the class of fluoroquinolone antibiotics that have been intensively used for the treatment of bacterial infections in veterinary medicine. The effect of enrofloxacin on the function and structure of soil microbial communities was evaluated (Figure 3). Among AR microorganisms, there were aerobic bacteria such as *Enterococcus faecalis*, *Yersinia enterocolitica*, *Enterobacter cloacae* and anaerobic bacteria, such as *Clostridium*

difficile and *Clostridium perfringens*. Other dominant bacteria were characterized by a high or moderate level of antibiotic resistance.

In the soil, there were isolated bacteria resistant to the majority of tested antibiotics. They were representatives of aerobic microbiota: *Bacillus licheniformis*, *Serratia fonticola*, *Hafnia alvei*, *Bacillus cereus*, *Pantoea agglomerans*, *Bacillus megaterium*, and one anaerobic bacterium, *Clostridium difficile*. In natural conditions, from the soil of model ecosystems, mostly bacteria of the genus *Bacillus* were isolated. All of them are antibiotic-resistant, are the causative agents of foodborne infections, and pose a threat not only to the environment but also to human health.

It should be noted that soil with a high concentration of antibiotics is characterized by a high number of oligotrophic and spore-forming microbiota (Figure 5). The highest concentration of enrofloxacin was in the agroecosystem of *Mentha piperita* (681.9 mg kg⁻¹) and the lowest in the agroecosystem of *Lactuca sativa* (248.6 mg kg⁻¹).

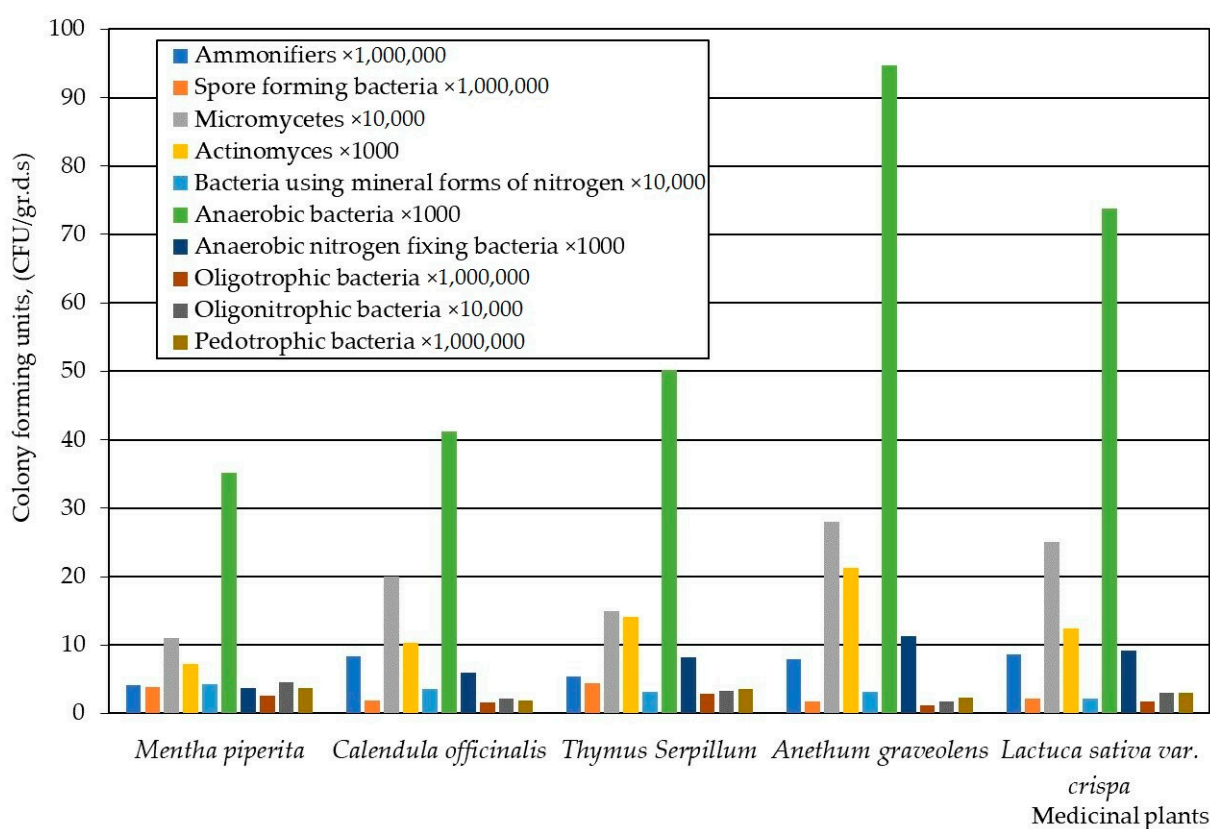


Figure 5. Microbial community composition (CFU/gr.d.s.) in soils of agroecosystems contaminated by antibiotics. The data are statistically significant, $p < 0.05$, $\bar{x} \pm SD$, $n = 4$.

In the conditions of soil contamination with an antibiotic, high values of anaerobic bacteria and mycelial organisms (micromycetes and streptomycetes) were recorded in the rhizosphere of all studied medicinal plants, which, in our opinion, reacted more tolerantly to the presence of enrofloxacin in the soil. The highest content of micromycetes was found in the rhizosphere of *Calendula officinalis*, *Anethum graveolens*, and *Lactuca sativa var. crispa*, which almost doubled, and more than three times exceeded their number in the rhizosphere of *Mentha piperita*. The dominance of micromycetes in soil contaminated with antibiotics can be attributed to their unique physiological characteristics. Micromycetes have a more efficient metabolism coupled with higher biochemical activity, making them the most competitive and adaptable microorganisms in such environments. However, it is important to consider that these microorganisms may also possess a high degree of toxicity, which could impact the phytopathogenic background of soil in agroecosystems.

The high content of enrofloxacin in the *Mentha piperita* agroecosystem did not have a negative effect on pedotrophic bacteria or bacteria that use nitrogen from mineral compounds. The number of these bacteria was higher than in the agroecosystems of other medicinal crops.

Integration of microbiomes in contaminated soil was lower than in the forest ecosystem (Table 2). Between different ecological functional groups of soil microorganisms, there were 22 correlation connections with a high level of correlation ($R > 0.75$). Anaerobic bacteria were highly correlated with actinomycetes ($R = 0.89$) and micromycetes ($R = 0.89$).

Table 2. The integrity of the soil microbial community in edaphotopes of agroecosystem contaminated by an antibiotic.

Microorganisms	Ammonifiers	Spore Forming Bacteria	Micromycetes	Actinomycetes	Bacteria Using Mineral Forms of Nitrogen	Anaerobic Bacteria	Anaerobic Nitrogen Fixing Bacteria	Oligotrophic Bacteria	Oligonitrophic Bacteria	Pedotrophic Bacteria	Aerobic Nitrogen Fixing Bacteria
Ammonifiers	1										
Spore-forming bacteria	−0.88	1									
Micromycetes	0.88	−0.74	1								
Actinomycetes	0.44	−0.40	0.75	1							
Bacteria using mineral forms of nitrogen	−0.70	0.38	−0.72	−0.46	1						
Anaerobic bacteria	0.59	−0.57	0.89	0.89	−0.67	1					
Anaerobic nitrogen-fixing bacteria	0.61	−0.45	0.85	0.93	−0.76	0.94	1				
Oligotrophic bacteria	−0.87	0.99	−0.90	−0.52	0.41	−0.68	−0.56	1			
Oligonitrophic bacteria	−0.83	0.79	−0.85	−0.75	0.45	−0.66	−0.73	0.82	1		
Pedotrophic bacteria	−0.79	0.82	−0.55	−0.15	0.16	−0.14	−0.17	0.75	0.71	1	
Aerobic nitrogen-fixing bacteria	0.80	−0.85	0.96	0.82	−0.51	0.88	0.82	−0.92	−0.90	−0.58	1

Aerobic nitrogen-fixing bacteria correlated with ammonifiers ($R = 0.80$) and micromycetes ($R = 0.96$). Oligotrophic bacteria correlated with spore-forming bacteria; the correlation coefficients R were 0.99. The contamination of agroecosystems by enrofloxacin changed the integrity of the soil microbiome and its structure.

The presence of antibiotics in agroecosystems has been found to have a negative impact on the abundance of aerobic nitrogen-fixing microorganisms. These microorganisms play a critical role in maintaining soil health and productivity by facilitating nitrogen fixation, a key process for plant growth. The decline in their numbers can have long-term implications for the sustainability of agricultural practices (Figure 6).

The lowest content of aerobic nitrogen-fixing bacteria was in agroecosystems with *Mentha piperita* (19.22%) and *Thymus serpyllum* (22.34%), and the highest was in the agroecosystem of *Anethum graveolens* (38.23%). Intermediate values of the content of aerobic nitrogen-fixing bacteria were recorded in the soil of the *Lactuca sativa* var. *crispa* and *Calendula officinalis* agroecosystems. The presence of the antibiotic enrofloxacin in the soil was found to have a detrimental effect on the soil microbiome associated with medicinal plants, leading to a significant decrease in the abundance and development of agronomically valuable nitrogen-fixing bacteria by 1.5–2.6 times. These findings highlight the potential negative impact of antibiotics on soil microbiota and the need for more sustainable agricultural practices that prioritize the preservation and restoration of soil health (Figure 6). The greatest inhibition of nitrogen-fixing bacteria was recorded in the agroecosystems of *Mentha piperita* and *Calendula officinalis* (2.6 and 2.3 times, respectively), and the least (1.5 times) in the agroecosystem of *Anethum graveolens*.

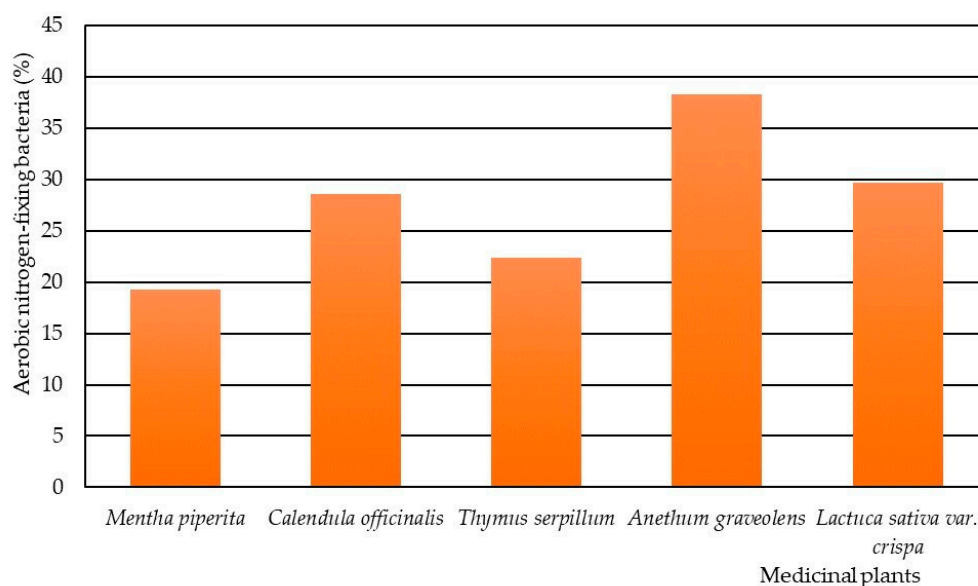


Figure 6. Aerobic nitrogen-fixing bacteria (%) in soils of agroecosystems contaminated by antibiotics. The data are statistically significant, $p < 0.05$, $x \pm SD$, $n = 4$.

Antimicrobials present in the soil can have detrimental effects on the microbial community by increasing the number of antibiotic-resistant bacteria and compromising the stability and integrity of the soil microbiome. These negative changes can have significant implications for soil health, including reductions in nutrient cycling and other key ecological functions. Therefore, it is crucial to better understand the impacts of antimicrobials on soil microbiota and develop strategies to mitigate their harmful effects, particularly in agricultural settings where the use of antimicrobials is widespread. Environmental contamination by antimicrobials is one of the important factors in the formation of soil resistome. The microbial composition of soil resistome revealed differences in the structure and integration of microbial communities. Antibiotic contamination had a negative impact on the sustainability of microbial communities.

Analysis of soil from agroecosystems of medicinal plants not contaminated with antibiotics showed that under these conditions more stable communities were formed, with 26 strong correlation connections ($R > 0.75$) (Table 3).

Thus, spore-forming bacteria were correlated with oligotrophs; the correlation coefficient was 0.98. A high correlation was found between actinomycetes and anaerobic bacteria ($R = 0.96$) and anaerobic nitrogen-fixing bacteria ($R = 0.93$).

It is well-known that plants have a profound impact on the microbial communities that inhabit the surrounding soil, leading to the development of a distinct set of microorganisms at the genus and species level. This process is driven by a variety of factors, including root exudates, plant–microbe signaling, and soil chemistry, and has important implications for plant growth and ecosystem functioning. As shown in Figure 7, there is a high content of spore-forming bacteria (2.67×10^6 CFU/gr.d.s.), bacteria using nitrogen from mineral compounds (5.27×10^4 CFU/gr.d.s.), pedotrophs (2.34×10^6 CFU/gr.d.s.), and oligotrophs (1.66×10^6 CFU/gr). At the same time, the content of mycelial organisms was the lowest (micromycetes: 13×10^3 CFU/gr.d.s.; actinomycetes: 8.34×10^3 CFU/gr.d.s.) compared to soil microbiocenoses formed under other medicinal crops.

Table 3. The integrity of the soil microbial community in edaphotopes of agroecosystem of medicinal plants without antibiotic contamination.

Microorganisms	Ammonifiers	Spore Forming Bacteria	Micromycetes	Actinomyces	Bacteria Using Mineral Forms of Nitrogen	Anaerobic Bacteria	Anaerobic Nitrogen Fixing Bacteria	Oligotrophic Bacteria	Oligonitrophic Bacteria	Pedotrophic Bacteria	Aerobic Nitrogen Fixing Bacteria
Ammonifiers	1										
Spore-forming bacteria	-0.79	1									
Micromycetes	0.77	-0.83	1								
Actinomyces	0.30	-0.47	0.83	1							
Bacteria using mineral forms of nitrogen	-0.87	0.67	-0.79	-0.43	1						
Anaerobic bacteria	0.46	-0.79	0.91	0.96	-0.64	1					
Anaerobic nitrogen-fixing bacteria	0.42	-0.36	0.82	0.93	-0.61	0.94	1				
Oligotrophic bacteria	-0.72	0.98	-0.78	-0.38	0.51	-0.47	-0.22	1			
Oligonitrophic bacteria	-0.64	0.88	-0.82	-0.64	0.42	-0.63	-0.46	0.91	1		
Pedotrophic bacteria	-0.75	0.98	-0.81	-0.31	0.56	-0.42	-0.17	0.99	0.85	1	
Aerobic nitrogen-fixing bacteria	0.45	-0.80	0.43	0.15	-0.13	0.16	-0.11	-0.91	-0.83	-0.89	1

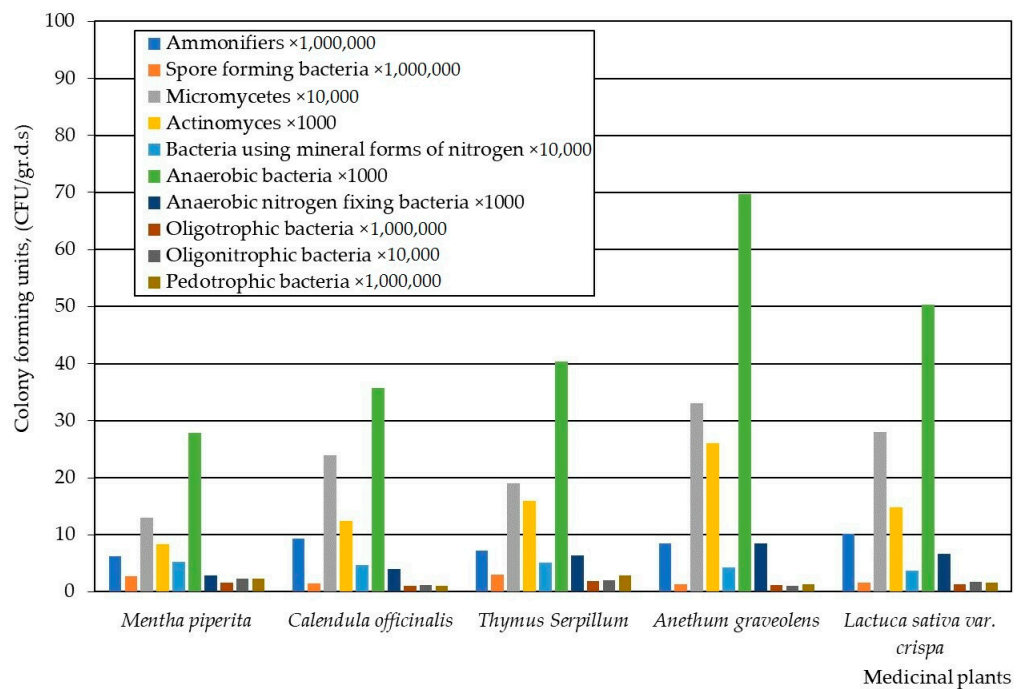


Figure 7. Microbial community composition (CFU/gr.d.s.) in soils of agroecosystems with medicinal plants without antibiotics. The data are statistically significant, $p < 0.05$, $x \pm SD$, $n = 4$.

There is also a high concentration of such microorganisms as spore-forming bacteria (3.02×10^6 CFU/gr.d.s.), bacteria that use nitrogen from mineral compounds (5.07×10^4 CFU/gr.d.s.), pedotrophs (2.88×10^6 CFU/gr.d.s.), oligotrophs (1.84×10^6 CFU/gr.d.s.), and oligonitrophils (2.06×10^4 CFU/gr.).

The soil microbiome of the agroecosystem *Lactuca sativa* var. *crispa* is characterized by a high content of ammonifiers (10.2×10^6 CFU/gr.d.s.) and micromycetes (28×10^3 CFU/gr.d.s.). *Calendula officinalis* has a high content of ammonifiers (9.34×10^6 CFU/gr.d.s.) and the lowest content of pedotrophs (1.05×10^6 CFU/gr.d.s.), oligotrophs (1.04×10^6 CFU/gr.d.s.), and oligonitrophils (1.12×10^4 CFU/gr.d.s.).

The highest content of mycelial organisms (micromycetes: 33×10^3 CFU/gr.d.s., actinomycetes: 25.98×10^3 CFU/gr.d.s.) was found in the *Anethum graveolens* agroecosystem, which was almost 3 times more than in the *Mentha piperita* agroecosystem and 1.5–2 times more than in the agroecosystem of *Calendula officinalis*.

The content of aerobic nitrogen-fixing bacteria in the soil directly depended on the type of medicinal crop cultivated (Figure 8).

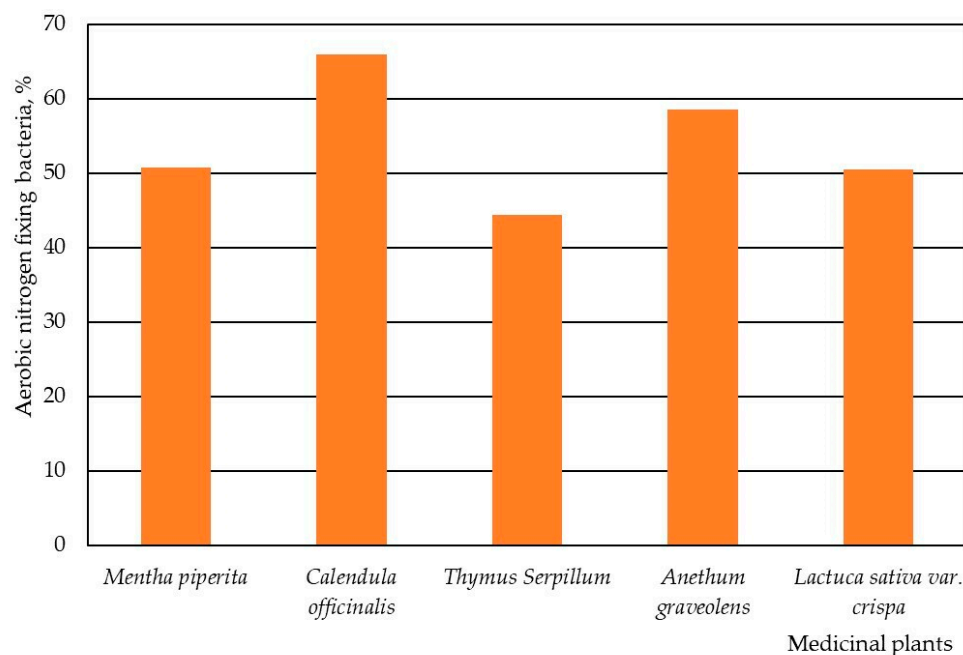


Figure 8. Aerobic nitrogen-fixing bacteria (%) soils of agroecosystems with medicinal plants without antibiotics. The data are statistically significant, $p < 0.05$, $\bar{x} \pm SD$, $n = 4$.

The highest rates of nitrogen-fixing bacteria in the soil were characteristic of the rhizospheres of *Calendula officinalis* (65.90%) and *Anethum graveolens* (58.56%). In the agroecosystem, *Mentha piperita* and *Lactuca sativa var. crispa* recorded the content of nitrogen-fixing bacteria at almost the same level (50.67% and 50.43%). Conversely, 1.5 times fewer nitrogen fixers (44.35%) were found in the rhizosphere of *Thymus serpillum*.

4. Discussion

Antibiotic resistance is an issue that has garnered significant attention in recent years, not only in healthcare but also in the domains of biosafety and ecology. The “One Health” concept, which recognizes the interconnectedness of human health, animal health, and the natural environment, highlights the need for a comprehensive approach to addressing the problem of antibiotic resistance, emphasizing intersectoral cooperation [34,35]. The focus on the natural environment as a vector for the spread of antibiotic-resistant pathogenic strains motivates researchers to investigate the mechanisms of antibiotic migration and the formation of resistant microorganisms. An important place is given to the issue of water and soil contamination with antibiotics and the formation of a resistant microbiome. They can contaminate soil, crops, and water sources and promote the development of resistant microbiomes [36]. In addition to the formation of a resistome, soil contamination with antibiotics can also lead to changes in the functional structure of the microbiome, which was confirmed by our investigations. This means that the types and functions of bacteria present in the soil may be altered, which can have important implications for soil health and nutrient cycling.

Several studies have confirmed these findings, showing that soil contamination with antibiotics can alter the abundance and diversity of bacterial species in the soil, as well as the functions they perform. For example, some studies have shown that antibiotic

contamination can lead to a decrease in the abundance of beneficial soil bacteria, such as those involved in nutrient cycling and plant growth promotion [37,38].

To minimize the risk of harm to human health, it is essential to monitor opportunistic and pathogenic microorganisms as part of ecological and sanitary soil diagnostics. Of particular concern is the need to control the spread of antibiotic-resistant microorganisms, which can enter the bodies of humans and animals in various ways. Effective monitoring and management of these microorganisms are crucial for maintaining soil health and preventing the transmission of infections. By identifying and addressing the sources of these microorganisms in the soil, we can reduce the potential risks to human and animal health [39,40].

Certain dependencies have been established in the structure of the microbiome in the presence of antibiotic-resistant pathogenic microorganisms. In particular, a connection was established between the increase in the number of oligotrophs and pedotrophs and AR microorganisms, such as: *Clostridium difficile*, *Clostridium oedematiens*, and *Clostridium perfringens*. A high content of oligotrophic microbiota indicates a bad ecological status of the ecosystem. Soil oligotrophic microbiota associated with oligotrophic environments are characterized by a limited supply of nutrients (carbon, nitrogen, and phosphorus) and are distributed [41,42].

A potential danger for humans and animals is the increase in the number of *Clostridium* bacteria in the soil of agroecosystems, which is fertilized with manure. These bacteria are phylogenetically related to human pathogens. Under such conditions, there is a high risk of horizontal transfer of antibiotic-resistant genes to human pathogenic microorganisms. It is known that horizontal gene transfer is more common between closely related organisms [43–46]. The dispersal of antibiotic-resistant organisms over long distances can occur by air through attachment to dust particles or by soil transport. This is especially dangerous in the case of medicinal plants that use the ground part, leaves, flowers, etc. The risks of contamination due to air pollution by antibiotic residues and antibiotic-resistant bacteria, which can be transported by air masses together with soil nanoparticles, are increasing. A significant number of microorganisms that showed resistance to various antibiotics were isolated from the agroecosystems. It has been determined that these microorganisms are pathogenic for humans and can pose a health hazard through the alimentary tract. Therefore, the soil is considered a reservoir for the accumulation of antibiotics and microorganisms resistant to them [47–50] and as a hotspot for the formation and spread of AR, which is a threat to human and animal health.

5. Conclusions

The microbiome and resistome of soil in terrestrial ecosystems are profoundly influenced by human activities. The anthropogenic introduction of antibiotics and their metabolites can significantly alter the composition and function of the soil microbiome. This can result in disruptions to the stability and integration of microbial communities, especially in heavily contaminated agroecosystems. Moreover, these ecosystems are hotspots for the emergence and spread of soil resistance. It is crucial to consider the long-term impacts of these disturbances on soil health and the provision of ecosystem services. In contaminated agroecosystems, not only the number of antibiotic-resistant bacteria increased but also the level of their resistance to existing antibiotics. Forest ecosystems are characterized by high functional biodiversity, stability, and a small number of antibiotic-resistant bacteria and human pathogens. The structure of soil microbiome and resistome depends on the level of transformation and contamination of ecosystems. Antibiotic-resistant bacteria also exist in natural (forest) ecosystems that are not directly affected by humans, but their number is much smaller, and their antibiotic resistance is lower. In such ecosystems, the oligotrophicity of the microbiome is much lower and the stability is higher.

The screening of conditionally pathogenic and pathogenic microorganisms in the soil has proved that terrestrial ecosystems are the source of spreading pathogenic and opportunistic antibiotic-resistant microorganisms. Effective management strategies are

needed to minimize the risk of antibiotic-resistant exposure through produce consumption. Addressing this issue requires a multi-faceted approach, including the elimination of antibiotics in agriculture, particularly on farms that use moderate doses of antibiotics as growth promoters. Moreover, the development of new technologies to detect and monitor the presence of antibiotic-resistant microorganisms in soil microbiomes is essential to preventing the spread of resistance. Such measures are critical in reducing the risk of antibiotic resistance transmission from soil to produce, ultimately safeguarding public health. Another important aspect of solving this problem is the implementation of international standards in agriculture regarding the use of antibiotics. This is a way to protect the environment and bioproduction from contamination with pathogenic microorganisms.

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