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Functional diversity of soil microbiomes in forest ecosystems and the spread of ESKAPE pathogens

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Abstract
The article presents original research results focused on the long-term investigation of the soil microbiome in forest ecosystems, particularly examining microbial community structure, the abundance of major ecological-functional groups, and the spread of ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species)*. The study aims to analyze the impacts of both endogenous and exogenous factors on soil microbial communities and succession processes. Monitoring of soil microbiome in the forest ecosystems of the Carpathian Biosphere Reserve showed changes in microbial communities over a 12-year period. These changes included an increase in the number of spore-forming, pedotrophic and oligotrophic bacteria. Furthermore, an increased presence of ESKAPE pathogens in the soil was observed. The Antibiotic Resistance Profile (ARP) of ESKAPE pathogens in unmodified forest ecosystems was determined for the first time. Long-term studies investigating changes in soil microbial communities in natural ecosystems revealed that the soil microbiome in such environments is impacted by external factors and can act as a reservoir for pathogenic bacteria, posing risks to both human and ecosystem health.

Keywords: *soil, microbiome, ESKAPE pathogens, antibiotic resistance, forest, ecosystem, monitoring*

Introduction

Forests represent one of the Earth's largest and most essential ecosystems, covering over 40 million km^2 and constituting about 30% of the total global land area (Forzieri et al., 2022). Primeval forests serve as ideal environments for investigating the intricate interactions among bacteria, fungi, and archaea within their abiotic surroundings. These ancient forests play a crucial role in preserving biological and genetic diversity, hosting relict and endemic species of flora and fauna. Exploring primeval forests provides a unique opportunity to study the natural structure, diversity, and genetic composition of unaltered ecosystems, as well as the dynamic processes and relationships shaped by ecological factors. Despite centuries of intensive forest exploitation, forested areas have declined by 3.5 times, highlighting the special value of virgin forest ecosystems, particularly in the Carpathian Mountains. Moreover, in Europe, where many forest stands have been managed for centuries, our understanding of the diversity, ecology,

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and distribution of soil microorganisms in undisturbed forest ecosystems remains limited (Symochko et al., 2015; Symochko et al., 2020). Soil microorganisms, though often overlooked in conservation efforts, play crucial roles in biogeochemical processes and possess immense diversity, abundance, and potential for harboring valuable genetic information and metabolic products. Understanding authentic soil microbiota is essential for conserving microbial diversity and establishing the foundation for eco-microbiological monitoring (Patyka & Symochko, 2013). Recognizing their significance parallels the importance of animals and plants in sustaining the biosphere and human welfare. Forests represent complex ecosystems characterized by diverse microbial habitats with distinct properties. These habitats include foliage, living tree wood, bark surfaces, ground vegetation, roots and the rhizosphere, litter, soil, deadwood, rock surfaces, invertebrates, wetlands, and the atmosphere. These components exhibit dynamic changes across various temporal scales, encompassing short-term events, seasonal fluctuations, and long-term stand development processes. Within this intricate web of ecological dynamics, fungi, bacteria, and other microorganisms comprising the forest microbiome play pivotal roles in sustaining ecosystem functions and health. Soil is the fundamental cornerstone of forest ecosystems, vital for their ongoing health and vitality. It serves as a crucial medium for the cycling of essential elements within ecosystems, enabling the intricate interplay among soil microorganisms, plants, and other organisms. This dynamic interaction ensures the resilience and productivity of forest ecosystems, sustaining their delicate balance and biodiversity. (Gomeiro, 2016; Demyanyuk et al., 2019; Demyanyuk et al., 2020; Gomes et al., 2023; Symochko et al., 2023). Primeval forests offer an ideal setting for investigating the intricate interactions among bacteria, fungi, and archaea within their abiotic surroundings (Grayston & Rennenberg, 2006). These untouched ecosystems are crucial for preserving biological and genetic diversity, harboring relict and endemic species of flora and fauna. Exploring primeval forests presents a unique opportunity to delve into the natural structure, diversity, and genetic makeup of unaltered forests, along with the dynamic ecosystem processes influenced by ecological factors.In Europe, where forests have been managed for generations, there remains a significant gap in understanding the diversity, ecology, and distribution of soil microorganisms in natural, undisturbed forest ecosystems (Symochko et al., 2021). The exploration of authentic soil microbiota lays the groundwork for conserving microbial diversity and establishing the foundation for microbiological monitoring (Patyka V., Symochko L., 2013). Since biological indicators of soil quickly respond to natural and anthropogenic factors, they are widely used in monitoring studies, to assess ecosystems health (Symochko et al., 2021; Bhaduri et al., 2022; Symochko et al., 2024). The objective of this research is to investigate the soil microbiome, functional and structural successions, and assess the spread of ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species)* in forest ecosystems. Hosts of ESKAPE Pathogens could be different: 1. Humans (Primary Host): The primary hosts for ESKAPE pathogens are humans, particularly those who are immunocompromised, critically ill, or undergoing invasive medical procedures. 2. Animals: Some ESKAPE pathogens, such as *Staphylococcus aureus* and *Enterococcus faecium*, are also found in animals and livestock, contributing to zoonotic infections and the spread of resistance. 3. Environment: ESKAPE pathogens can survive in various environmental reservoirs, including soil, water, and hospital surfaces, where they may interact with humans and animals, acting as sources of infection. ESKAPE pathogens is a group of hazardous bacteria characterized by high levels of AR. (Venkateswaran et al, 2023; Symochko et al., 2024; Khasapane et al., 2024). Antibiotic resistance (AR) presents a global challenge for humanity (Miller et al., 2022; Larsson & Flach, 2022). AR is defined as the capacity of microorganisms to withstand the effects of an antibiotic to which they were previously susceptible. This ability allows microorganisms to survive. Microorganisms can acquire AR through diverse mechanisms like mutation, horizontal gene transfer, or efflux pumps. This poses a significant threat to public health, as it can result in treatment ineffectiveness and foster the dissemination of infections, consequently elevating morbidity and mortality rates (Uddin et al., 2021). The environment, including soil, could be considered as a reservoir of antibiotic-resistant bacteria, including ESKAPE pathogens. These pathogens are renowned for their capacity to develop resistance to multiple antibiotics, rendering them challenging to treat. Two types of natural resistance mechanisms exist in bacteria: intrinsic resistance and induced resistance. Intrinsic

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resistance is consistently expressed across an entire bacterial species and is independent of horizontal gene transfer or prior exposure to antibiotics. A defining feature of intrinsic resistance is that it is uniformly present in all members of the species. Common mechanisms underlying intrinsic resistance include reduced outer membrane permeability—particularly in Gram-negative bacteria due to the presence of lipopolysaccharide (LPS)—and the activity of inherent efflux pumps, which expel antibiotics from the bacterial cell (Cox&Wright, 2013; Symochko et al., 2024). In contrast, induced resistance is expressed at higher levels only after exposure to specific antibiotics, demonstrating an adaptive, environment-dependent response.They are frequently linked to nosocomial (hospital-acquired) infections and present substantial challenges to healthcare systems globally. ESKAPE pathogens exhibit resilience to various antimicrobial agents, leading to a rise in multidrug-resistant infections and prompting concerns about the effectiveness of antibiotic treatments and its very important to estimate they spreading in natural ecosystems. The study focuses on utilizing the forests of the Carpathian Biosphere Reserve as a representative ecosystem model. These primeval forests are exemplary due to their exceptional blend of resilience, stability, and high biomass productivity (Symochko et al., 2020). Situated in the Transcarpathian region of Ukraine, the Carpathian Biosphere Reserve offers a unique opportunity to explore the biodiversity and natural processes of primeval forest ecosystems. These forests have been minimally impacted by human activity, providing valuable insights into undisturbed ecological dynamics and patterns of biodiversity.

Materials and Methods

Experimental site and investigation design

The soil samples collected during 2008-2020 years from natural ecosystems, specifically the virgin forests of the Shyrokoluzhansky massif within the Carpathian Biosphere Reserve, from depths of 0-25cm. The Carpathian Biosphere Reserve (CBR) covers an area of approximately 53.650 hectares, this region became part of the UNESCO World Network in 1992. The soils in the area are predominantly stony, primarily composed of mid-loam texture. The climate undergoes a transition from mild-warm to cold, reflecting the massif's location across three distinct climatic zones. Annual average temperatures range from 0 to +7°C, while annual average precipitation varies between 1.000 mm and 1.500 mm. In July, temperatures rise from +12°C to +17°C, whereas in January, they drop from -3°C to -10°C. The sum of active temperatures varies with altitude, ranging from 2.300 °C to 800 °C. Soil sampling was conducted at various altitudes, ranging from 555m to 1040m (Table 1).

Table 1. *Characterization of soil sampling points*

N°	Vegetation	Latitude	Longitude	Altitude, m asl
1	Fagetum (silvaticae)	48°17.663´	23°44.389´	800
2	Fagetum (silvaticae)	48°17.659´	23°45.523´	910
3	Fagetum (silvaticae)	48°19.349´	23°45.628´	1010
4	Fagetum (silvaticae)	48°20.126´	23°45.390´	655
5	Fagetum (silvaticae)	48°20.069´	23°44.026´	650
6	Fagetum (silvaticae)	48°20.595´	23°45.115´	1020
7	Fagetum (silvaticae)	48°21.292´	23°44.595´	700
8	Fageto (sylvaticae) Abietum (albae)	48°21.817´	23°45.557´	885
9	Fagetum (silvaticae)	48°21.805´	23°44.529´	1040
10	Fagetum (silvaticae)	48°18.454´	23°43.223´	773
11	Abieto (albae) Piceeto (abietis) Fagetum (silvaticae)	48°19.203´	23°43.658´	776
12	Fagetum (silvaticae)	48°20.226´	23°43.498´	683
13	Fagetum (silvaticae)	48°19.928´	23°42.879´	800
14	Fagetum (silvaticae)	48°19.832´	23°42.119´	844
15	Fagetum (silvaticae)	48°20.980´	23°41.826´	970
16	Fagetum (silvaticae)	48°21.089´	23°43.399´	645
17	Fagetum (silvaticae)	48°18.673´	23°44.389´	555
18	Fagetum (silvaticae)	48°21.568´	23°43.422´	925
19	Fagetum (silvaticae)	48°21.730´	23°41.997´	890
20	Fagetum (silvaticae)	48°19.455´	23°44.547´	770

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All samples underwent a unified preparation process: air drying and grinding to a size < 3 mm, with visible plant and mesofauna residues meticulously removed. Experiments were conducted with fivefold repetition (five soil samples from each point) for accuracy and reliability.

Microbiological analyses of soil

Soil samples were analyzed in sterile conditions according standart microbiological protocols (Shyrobokov, 2011; Goldman & Green, 2015). The method of serial dilution was used to obtain the suspension where microorganisms titre were 10^{-3} CFU/ml. - 10^{-5} CFU/ml (CFU-Colony Forming Units) 100 µl of the soil suspension was distributed on the surface of the medium. Four types of media were used to determine different functional groups of bacteria, namely Meat peptone agar, Agar-Agar, Soil agar, and Starch agar, each replicated four times for our study. Petri dishes containing the study material were then placed in a thermostat set at 29-37°C for 48-72 hours under aerobic conditions. The quantification of microorganisms grown on the nutrient media was expressed as CFU per 1 gram of dry soil.

Isolation and identification ESKAPE pathogens

Soil suspensions were carefully inoculated onto Blood agar (BA) and MacConkey agar (MCA), after which they were incubated at 37°C for 24 hours. Subsequent to incubation, isolated bacteria underwent a comprehensive series of tests for their identification. These tests included evaluation of colony morphology, gram-staining, and conventional biochemical tests. For Gram-positive bacteria, catalase and coagulase tests were performed to discern their characteristics. Conversely, for Gram-negative bacteria, the assessment encompassed tests for glucose fermentation, hydrogen sulfide production, indole production, urease production, citrate utilization, and motility.

Antibiotic resistance of ESKAPE pathogens

Antimicrobial susceptibility testing was conducted utilizing the Kirby Bauer disk diffusion method following the guidelines established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Standard antimicrobial discs (µg/disc) sourced from bioMérieux, Marcy-l'Etoile, France, representing antibiotics across the primary pharmacological groups, were employed for the testing process. Antibiotic categories tested encompassed carbapenems (meropenem/imipenem), β-lactam antibiotics, 3rd generation cephalosporins (ceftazidime/cefotaxime/ceftriaxone), aminoglycosides (amikacin/kanamycin), sulfonamides (cotrimoxazole), fluoroquinolones (ciprofloxacin), and penicillin. Identification of methicillin-resistant *Staphylococcus aureus* (MRSA) was based on resistance to cefoxitin.

Statistical analysis

The statistical analysis was conducted using Statistica 10.0 (Stat Soft Inc., USA) software to evaluate the data obtained from the bioassays. Each analysis was performed with 3-5 replicates to ensure robustness and reliability. Furthermore, mean values (x) along with their corresponding standard deviations (SD) were determined to provide insights into the variation within the data.A significance level of $P < 0.05$ was selected for the study, ensuring that observed differences were considered statistically significant.

Results and Discussion

Each microbial niche harbors distinct properties, thus hosting specific bacterial communities. Biocenotic relationships, both trophic and topical, exert decisive influences on the shaping of edaphotopes across various ecosystems. Through studies conducted in primeval ecosystems, general patterns have emerged regarding the distribution of key ecological-functional groups of microorganisms and their population dynamics within different habitats. Optimal conditions for microbial development and functioning were found in edaphotopes situated at altitudes ranging from 555 to 776 meters above sea level. These conditions are closely linked to local temperature and water regimes, as well as the organic nutrient reserves present in the soil (Figure 1). At altitude of 555 meters, the number of ammonifiers surged sixfold, what signifies about enrichment of soil organic matter, predominantly derived from plant sources. Similarly, there was a notable increase in bacterial populations reliant on mineral nitrogen. At 555 meters was the highest number of these microorganisms (Figure 1). Conversely, at the highest sampling point of 1040 meters, their abundance diminished. The succession and dynamic fluctuations within soil microbial communities primarily stem from abiotic factors such as temperature and humidity. The restructuring of the functional composition of soil microbial communities, driven by exogenous factors, is evident not only in the oscillation of specific ecological-trophic groups of soil microorganisms but also in the modification of microbiological processes in ecosystems. Notably, discernible shifts in microbial

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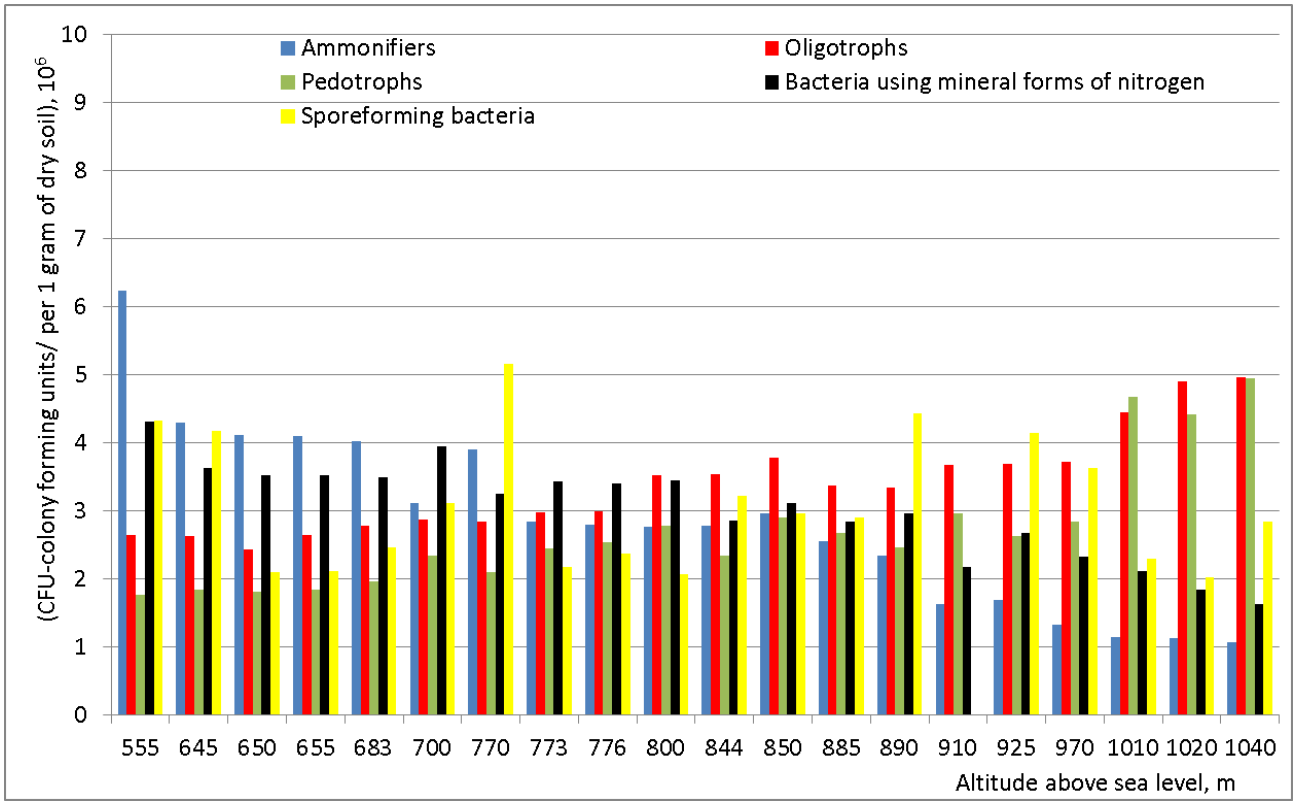

Figure 1. *Functional diversity of soil microbiome (CFU/gr.d.s.) in the forest ecosystems. The data are statistically significant, $p < 0.05$, $x \pm SD$, $n = 4$. (2008)*

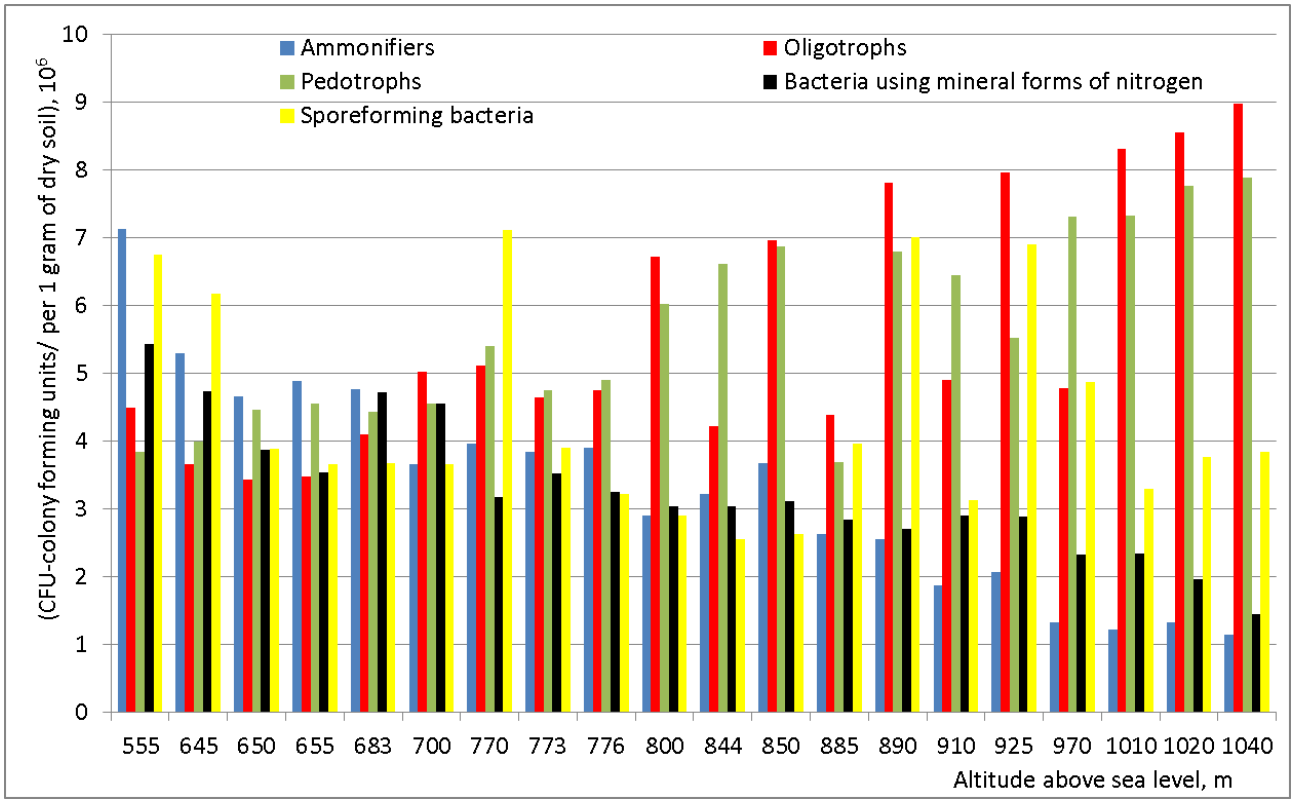

Figure 2. *Functional diversity of soil microbiome (CFU/gr.d.s.) in the forest ecosystems. The data are statistically significant, $p < 0.05$, $x \pm SD$, $n = 4$. (2020)*

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community structure manifest at 776 meters above sea level. The prevalence of pedotrophs and oligotrophs within the soil microbiome escalates notably at this altitude, indicating structural and functional successions and the emergence of localized hotspots. Given the microscale habitat preferences of bacteria, the attributes of their immediate milieu exert a disproportionate influence on local bacterial communities compared to average soil properties. These findings underscore the intricate interplay between altitude, soil microbial communities, and environmental factors in shaping ecosystem dynamics. Long term microbiological monitoring (2008–2020 years) showed significant changes in the structure of soil microbiome, increased in twice the number of oligotrophic and pedotrophic bacteria (Figure 2), but number of ammonifiers wasn't changed significantly.

Changes in the structure of the soil microbiome can stem from two primary factors: the influence of external factors and the availability of resources. Resource availability is indeed a fundamental driver of microbial succession, with the constraints and environmental influences regulating succession being notably complex due to the vast physiological diversity inherent in microbial communities and the diverse range of environments in which succession occurs. In autotrophic succession, nutrients and light are likely the principal resources limiting biomass accumulation. Microbial communities are pivotal components in maintaining soil health, fulfilling crucial roles in processes like the turnover of organic matter and nutrient cycling (Naylor et al., 2022), but at the same time soil could be a source of spreading antibiotic resistant bacteria, including pathogens (Xiao et al., 2023; Symochko et al., 2023a). In 2017, the European Union instituted the "One Health" approach as a strategic measure to combat antibiotic resistance (Dafale et al., 2022). This approach acknowledges the imperative of safeguarding human health by concurrently preserving animal and environmental health, along with related fields ESKAPE bacteria, which exhibit antimicrobial resistance (AMR), are extensively disseminated throughout the environment and various ecosystems (Savin et al., 2020; Symochko et al., 2023b). Notably, within the spectrum of ESKAPE pathogens, *Enterobacter spp.* and *Acinetobacter* stand out as enteric bacteria and soil commensals, ubiquitous in their distribution. Soil screening in forest ecosystems has revealed the presence of ESKAPE pathogens. Table 2 presents the antibiotic resistance profile (ARP) of ESKAPE pathogens isolated from forest ecosystem soils. In 2008, 28 isolates were obtained from forest ecosystem soil, while in 2020, 103 isolates were retrieved, which is 3.6 times higher than in 2008. In our opinion, the increase in the quantity of ESKAPE pathogens may also be attributed to the alteration in the functional structure of the soil microbiome. Over 12 years, there has been a significant increase in the population of sporulating microbiota and the representation of oligotrophic and pedotrophic microbiota (Figure 2). The Antimicrobial Resistance Profile (ARP) analysis reveals that the isolated strains exhibit varying degrees of resistance to the tested antibiotics in accordance with EUCAST recommendations. Specifically, the strains demonstrate notable levels of both high and moderate resistance across the antibiotic spectrum. These findings underscore the urgency of addressing antimicrobial resistance as a multifaceted challenge. However, it should be noted that over 12 years, the number of isolates *Escherichia coli* increased by 4.4 times, *Enterobacter cloacae* by 3 times, *Enterococcus faecium* by 2.2 times, and *Acinetobacter baumannii* by 4 times. Significant attention should be given to *Enterococcus faecium*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* which pose potential threats to human health and the environment. Populations of *Enterococcus faecium* represent only a small fraction of soil microbiota and often constitute autochthonous populations in soil environments. While the primary source of *Enterococcus* populations in soil is debated in some cases, potential sources include human and animal (including wildlife) waste, and over time, a subset of the primary population may adapt to the soil environment. However, along with this, this bacterium can cause various diseases in both humans and animals and is often characterized by a high level of antibiotic resistance. *Enterococci*, unlike *staphylococci* and *streptococci*, do not produce toxins. However, their virulence stems from other properties such as durability, structure, and antibiotic resistance. They are capable of causing serious infections such as endocarditis and urinary tract infections (Byappanahalli et al., 2012; Symochko et al, 2023a). *Pseudomonas aeruginosa* is a Gram-negative, rod-shaped bacteria, facultative anaerobe commonly found in the normal gut flora. While its prevalence in the general population is relatively low, it tends to be more prevalent among hospital inpatients, particularly those with compromised immune systems. Infections are typically acquired from external sources, including direct or indirect contact with the environment, although internal sources cannot be ruled out. Main

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percentage of *Pseudomonas aeruginosa* (82.3%) was isolated from the soil at the altitudes 770-910 m. Many strains of *Pseudomonas aeruginosa* inherently exhibit reduced susceptibility to various antibacterial agents and have a tendency to develop resistance during treatment, particularly in the case of carbapenem-resistant strains, notably imipenem (Chuang et al., 2017; Hoff et al., 2020). The development of imipenem resistance in *Pseudomonas aeruginosa* primarily stems from a dual mechanism involving chromosomal AmpC production and structural changes in porins. While the presence of low levels of AmpC enzyme production alone does not confer substantial carbapenem resistance due to its limited capacity to hydrolyze carbapenem drugs, the combination of heightened AmpC production alongside diminished outer membrane porin permeability and/or increased efflux pump expression significantly contributes to the emergence of carbapenem resistance in this pathogen. *Pseudomonas aeruginosa* also produces Extended-Spectrum Beta-Lactamases (ESBLs) and can harbor other antibiotic resistance enzymes such as Klebsiella pneumoniae carbapene-mases (KPC) and Verona Integron-encoded Metallo-β-lactamases (VIM) encoded by blaVIM, leading to high rates of carbapenem resistance among *Pseudomonas aeruginosa* isolates. Additionally, *Pseudomonas aeruginosa* may give rise to fluoro-quinolone-resistant strains, as the corresponding mechanisms of resi-stance may be carried by the same plasmid. This intricate interplay underscores the multifaceted nature of antimicrobial resistance mechanisms and emphasizes the importance of understanding the underlying molecular pathways to combat resistant strains effectively.

Table 2. *Profile of ESKAPE pathogens in the soil of forest ecosystems*

ESKAPE pathogen	Environmental reservoir of isolation	Number of isolates (2008) Total 28	Number of isolates (2020) Total 103	Antibiotic Resistance Profile ARP
Enterococcus faecium	forest ecosystem	7	16	Resistance to gentamicin (77.3%), penicillin (84.5%), erythromycin (74.4%), amoxicillin (68.9%), nitrofurantoin (65.2%), ampicillin (59.8%), and tetracycline (49.8.%), meropenem (44.7%),
Staphylococcus aureus	forest ecosystem	0	3	Methicillin resistant isolates (2), resistance to clindamycin (94.1%), erythromycin (85.6%), rifampin (68.7%), and vancomycin (57.3%), cefoxitin (69.3%).
Klebsiella pneumoniae	forest ecosystem	0	0	-
Acinetobacter baumannii	forest ecosystem	3	12	Resistance to cefepime (96.6%), ceftazidime (82.1%), amikacin (84.3%), ciprofloxacin (77.2%), gentamicin (75.6%), levofloxacin (74.3%), ampicillin (66.7%), meropenem (77.3%).
Pseudomonas aeruginosa	forest ecosystem	7	32	Resistance to β-lactam antibiotics (89.4%), amikacin (83.7%), levofloxacin (79.2%), gentamicin (77.3%) quinolones (88.6%), meropenem (67.3%).
Enterobacter spp. Escherichia coli	forest ecosystem	5	22	Resistance to β-lactam antibiotics (85.4%), amikacin (77.1%), levofloxacin (73.5%), gentamicin (68.3%) quinolones (61.6%). All isolates (100%) susceptible to imipenem and meropenem.
Enterobacter cloacea	forest ecosystem	6	18	Resistance to β-lactam antibiotics (84.5%), amikacin (72.4%), levofloxacin (78.1%), gentamicin (65.7%) quinolones (60.4). All isolates (100%) susceptible to imipenem and meropenem.

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percentage of *Pseudomonas aeruginosa* (82.3%) was isolated from the soil at the altitudes 770-910 m. Many strains of *Pseudomonas aeruginosa* inherently exhibit reduced susceptibility to various antibacterial agents and have a tendency to develop resistance during treatment, particularly in the case of carbapenem-resistant strains, notably imipenem (Chuang et al., 2017; Hoff et al., 2020). The development of imipenem resistance in *Pseudomonas aeruginosa* primarily stems from a dual mechanism involving chromosomal AmpC production and structural changes in porins. While the presence of low levels of AmpC enzyme production alone does not confer substantial carbapenem resistance due to its limited capacity to hydrolyze carbapenem drugs, the combination of heightened AmpC production alongside diminished outer membrane porin permea-*Acinetobacter baumannii*, an opportunistic Gram-negative coccobacillus, exhibits remarkable resilience across diverse environmental settings and possesses inherent resistance to commonly prescribed antibiotics (Peleg et al., 2008). The prevalence of multidrug-resistant strains of *A. baumannii* is now widespread globally, with the Mediterranean region reporting the highest rates of carbapenem resistance, exceeding 90%. This trend imposes significant challenges on healthcare systems. The World Health Organization (WHO) has designated carbapenem-resistant *A. baumannii* as a critical priority, highlighting the urgent need for new antibiotics. Moreover, once nosocomial outbreaks of *A. baumannii* occur, eradicating it from the environment proves challenging due to its extraordinary resistance to disinfectants and its ability to rapidly develop tolerance to antibacterial agents. This phenomenon contributes to prolonged colonization and transmission (Tacconelli et al., 2018). *Acinetobacter spp.* are incredibly versatile organisms, occupying a diverse range of natural habitats including water bodies, soil, sewage, sludge, solid surfaces, cheese, milk, unprocessed vegetables, human skin, wild animals, and plants. However, the ecological niche of *A. baumannii* remains elusive, largely due to its predominant isolation from hospital settings and communities with close interpersonal interactions. The natural habitats of *A. baumannii* remain poorly defined, as it is predominantly isolated from hospital environments and communities with close interpersonal contact. *Acinetobacter baumannii* has long been acknowledged as a significant opportunistic pathogen responsible for nosocomial infections, or healthcare-associated infections. It manifests across a broad spectrum of infections, encompassing respiratory infections, bloodstream infections, urinary tract infections, and meningitis (Antunes et al., 2014; Atrouni et al., 2016). *Acinetobacter baumannii* was isolated from the soil at the altitude 650- 850 meters above sea level and isolates had high resistance to cefepime (96.6%), ceftazidime (82.1%), amikacin (84.3%). Thus, the soil microbiome plays a vital role in the functioning of forest ecosystems. However, forest soil ecosystems can also be considered as reservoirs of antibiotic-resistant microorganisms, including ESKAPE pathogens.

Conclusions

Long-term microbiological monitoring of soil microbiomes has revealed significant changes in the functional structure of soil microbiomes over a period of 12 years. There has been an increase in the population of sporulating microbiota, as well as an increase in oligotrophy and pedotrophy in soils. It should be noted that number of these micro-organisms increased with altitude above sea level. For the first time, screening and monitoring of the spread of ESKAPE pathogens in soils of forest ecosystems that have not undergone significant anthropogenic modification have been conducted, establishing that natural ecosystems can serve as reservoirs for ESKAPE pathogens. For the first time, the ARP profile was analyzed, and the sensitivity levels of ESKAPE pathogens to antibiotics recommended by EUCAST were determined. The conducted research has practical and theoretical significance, as it can be used as a basis for monitoring studies on the spread of ESKAPE pathogens in the natural environment.

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