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Soil microbiome along the carpathian mountains

KEYWORDS: soil microbiome, next-generation sequencing, climate change, soil, fungi, bacteria

Introduction

The Carpathian Mountains represent one of the largest mountain forest chains in Central Europe, characterized by diverse forest types predominantly composed of beech (*Fagus sylvatica* L.) and mixed conifer stands (*Picea abies* Karst and/or *Abies alba* Mill.) at altitudes ranging from 500 to 1450 meters (Dinca et al., 2022; Knorn et al., 2013). This forest zone has been significantly impacted by human activities and climate change over centuries. Numerous studies have focused on the adverse effects of climate change on the radial increment of beech stems (Levanič et al., 2023) and the regeneration potential of silver fir (Čater and Levanič, 2019). Despite these studies, the Carpathian arc, like many other European mountain chains, remains underexplored, particularly in terms of soil-related parameters such as soil biodiversity, ecosystem services, and functions (Guerra et al., 2020). Recent large-scale soil sampling initiatives, such as the LUCAS survey, have largely excluded this region as well (Labouyrie et al., 2023). Motivated by the study of Darenova et al. (2024), which investigated topographical, biological, and climatic drivers of soil CO_2 efflux in beech-silver fir forests, we aimed to analyse the alpha diversity of bacterial and fungal communities along the Carpathian arc. Using the same experimental setup as Darenova et al. (2024), we employed highly informative metabarcoding of soil environmental DNA to estimate the diversity of selected taxonomic groups (Vasar et al., 2023).

Methods

Following the sampling scheme of Darenova et al. (2024), we collected soil samples from eight locations along the Carpathian arc. At each location, samples were taken from "open canopy", "forest edge," and "closed canopy" positions, including bulk soil and samples from the vicinity of roots. Soil samples were collected using a standardized soil probe (Grebenc et al., 2007). Molecular barcoding of bacteria was performed using the primer pair 341f/805r and fungi using the primer pair gITS7/ITS4, as described in Unuk et al. (2019). Bioinformatics and statistical analyses were conducted in R (v. 4.3.2) using RStudio (v. 2024.04.2) with the following libraries: phyloseq, vegan, ggplot2, data.table, parallel, multcomp, devtools, ggpubr, stringr, tibble, tidyverse, dplyr, indicspecies, and pairwiseAdonis.

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Results

ANOVA and subsequent Tukey HSD tests revealed a statistically significant effect of sampling location on the alpha diversity indexes (richness, evenness, and Shannon index) of bacterial communities. For fungal communities, sampling location significantly affected species richness. The alpha diversity of fungal communities was significantly influenced by soil sample type (bulk soil vs. rhizosphere soil). Species richness of fungal communities was significantly different between locations Buzau and Gorj versus Neamt. Conversely, bacterial diversity indexes showed consistent differences between Beskidy and Neamt, Suceava, and Vrancea.

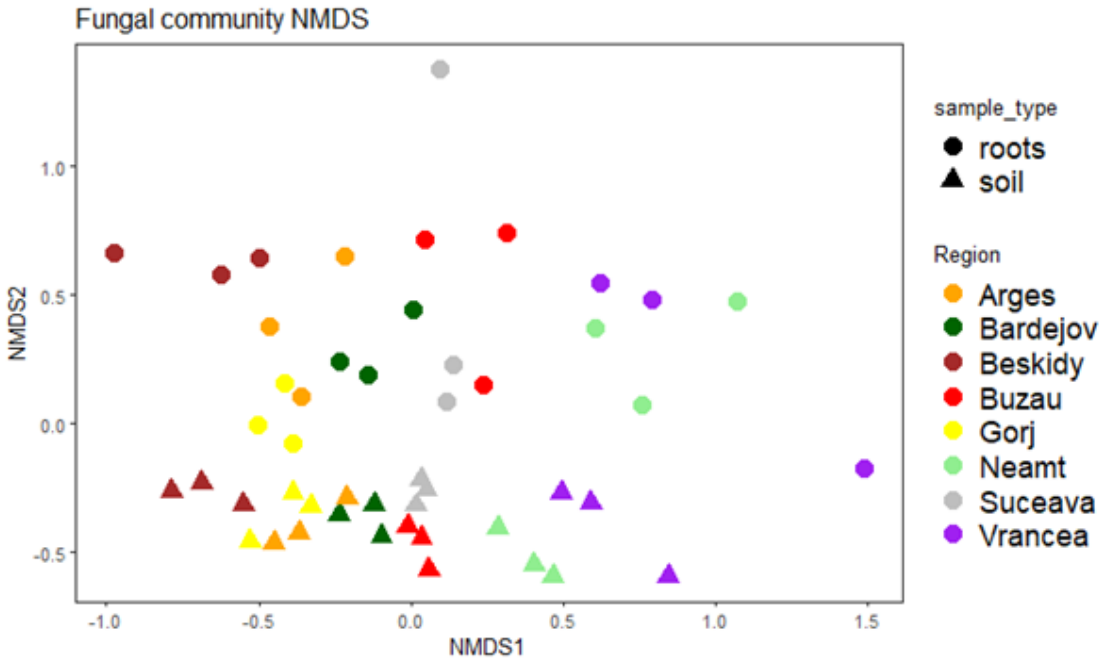

Fig. 1. Fungal community for roots and soil collected along the Carpathian Mountains analysis and analysed by the non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity.

We visualized differences in bacterial and fungal communities using non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity. Community data were standardized using Hellinger transformation. Pairwise PERMANOVA with Bonferroni correction was used to test community differences between locations and environmental factors. NMDS stress values indicated reliable dissimilarity matrix representation. PERMANOVA analysis showed that 31.29% of bacterial community variation was explained by sampling location, while sample type explained 5.24%. Similarly, 28.6% of fungal community variance was significantly influenced by sampling location, followed by sample type (11.7%) (Figure 1).

The Figure 2 summarizes the relative abundance plot of bacterial taxonomic groups revealed *Actinobacteriota* as the predominant phylum, followed by *Proteobacteria*, except at Agres, where *Planctomycetota* were slightly more abundant. Other represented phyla included *Verrucomicrobiota*, *Firmicutes*, *Chloroflexi*, *Acidobacteriota*, *Myxococcota*, *Gemmatimonadota*, and *Bacteroidota*.

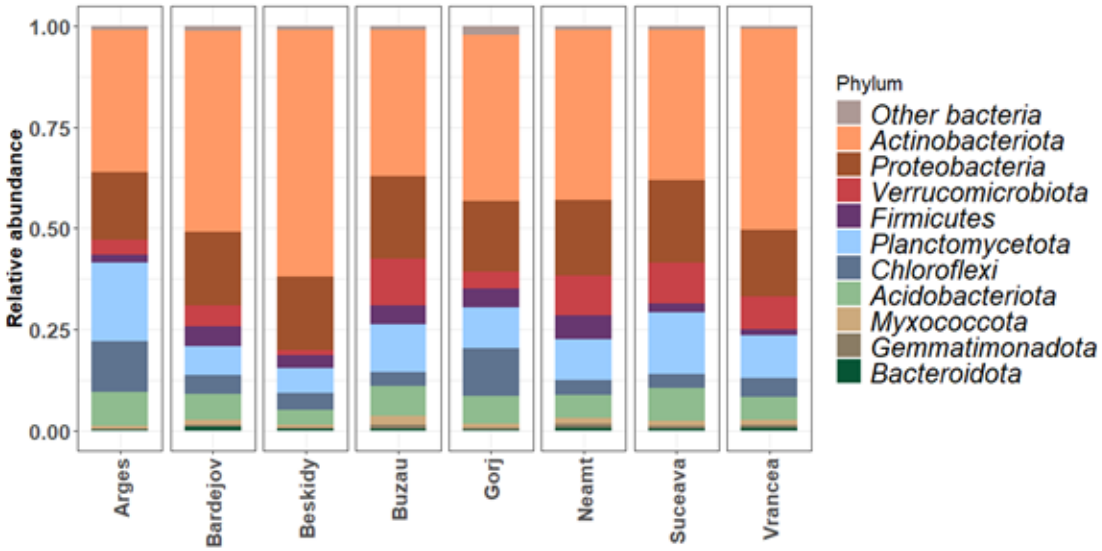

Fig. 2. Relative abundance of the dominant bacterial phyla identified from roots and soils collected along the Carpathian Mountains analysis. Communities were assessed by a metabarcoding of the hypervariable region of the 16S rRNA gene.

Fungal taxonomic group abundance, identified to the genus level with a 92% similarity threshold, showed diverse compositions at each location. Some genera were unique to rhizosphere samples at specific locations (e.g., *Tomentella* and *Ganoderma*) or predominantly present at few locations (e.g., *Cladophialophora*, *Sistotrema*, *Podila*) (Figure 3). Ectomycorrhizal fungi (Figure 4) were mainly attributed to 15 genera, with all but *Tuber* (Ascomycota) belonging to Basidiomycota. *Tuber* and *Melanogaster* were notably present at Neamt. Other genera, such as *Laccaria*, were widespread, while some, like *Amphinema*, *Tylospora*, and *Xerocomellus*, were location specific.

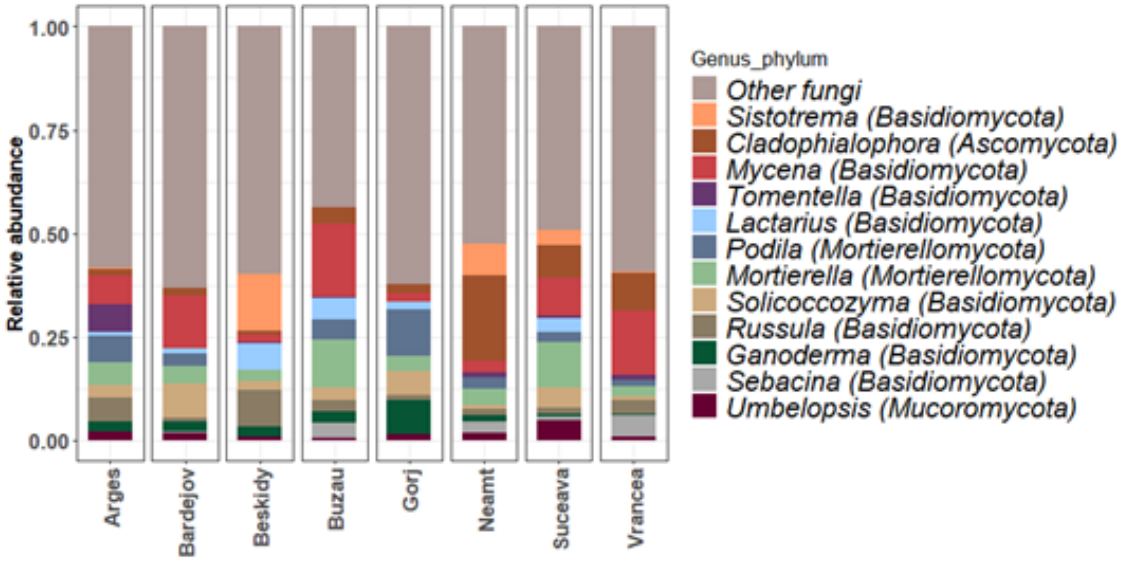

Fig. 3. Fungal community composition at analysed locations along the Carpathian Mountains. Rhizosphere communities were identified based on a 92% similarity threshold for an identification at the genus level.

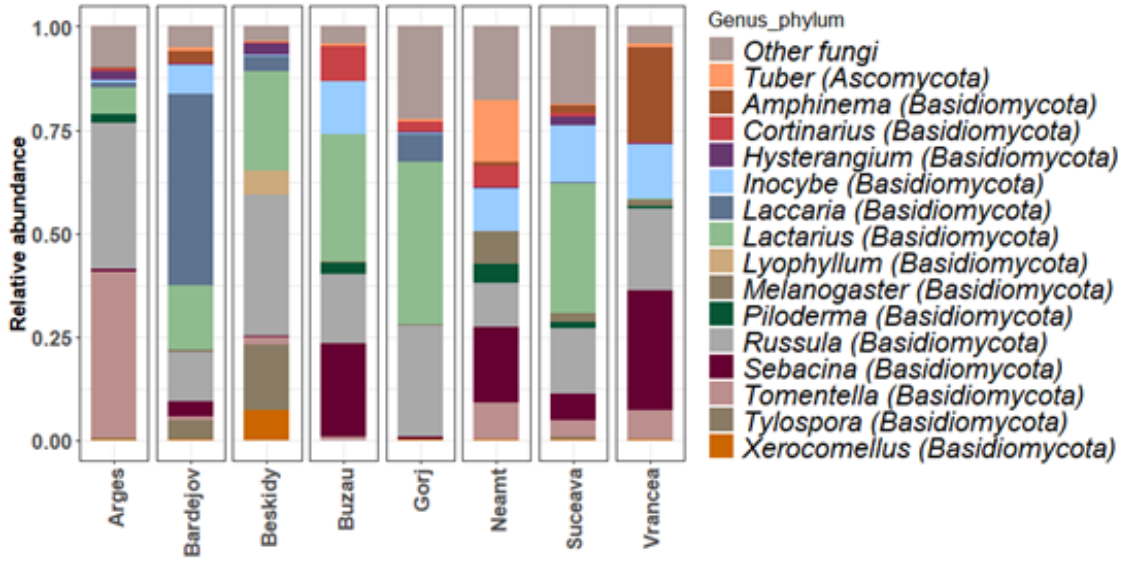

Fig. 4. Fungal community composition at analysed locations along the Carpathian Mountains. Ectomycorrhizal fine roots associated communities were identified based on a 92% similarity threshold for an identification at the genus level.

Functional group analysis based on the total rhizosphere samples revealed higher abundances of soil saprotroph fungi in bulk soil samples (29.5%) compared to rhizosphere samples (14.3%) (Figure 5). Conversely, rhizosphere samples had higher abundances of litter saprotrophs (27.7%) compared to bulk soil (4.9%). Bulk soil also showed a higher presence of plant pathogen fungi (6.72%) compared to rhizosphere samples (1.6%).

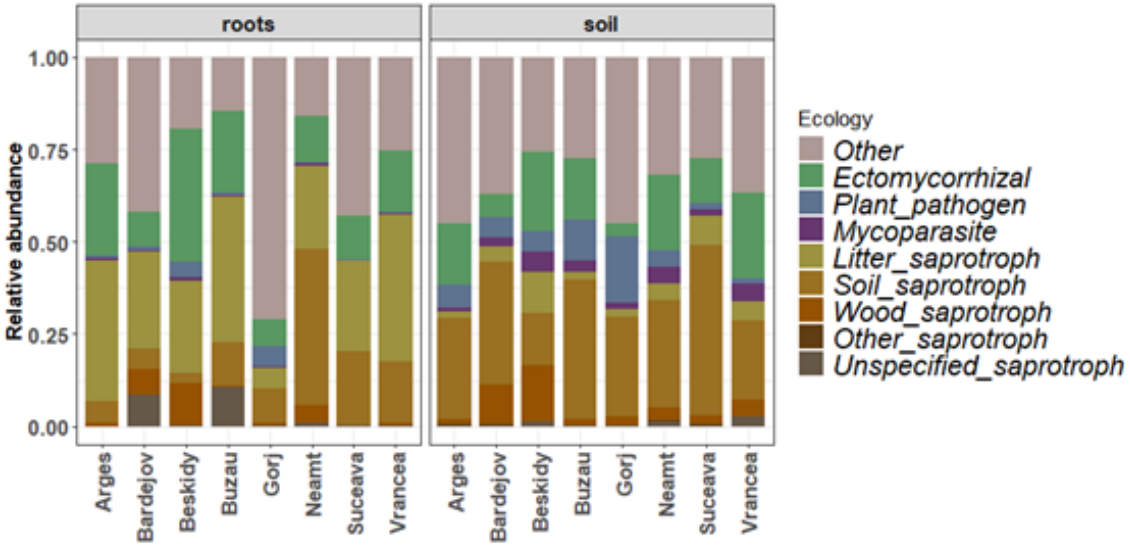

Fig. 5. Relative abundance of the fungal community functional groups based on the total rhizosphere samples composition analysed in soil samples collected along the Carpathian Mountains.

Conclusions

The analysis of soil microbial communities along the Carpathian Mountains revealed variations in diversity indexes and taxonomic compositions across different sampling locations and soil types (bulk soil vs. rhizosphere). However, no significant effects were found related to the sampling point's position relative to tree canopies. The composition of fungal communities indicated some widely present groups, with some taxa showing specific location preferences, likely due to soil properties, vegetation, or environmental conditions. The differing compositions of saprotrophs in bulk soil and rhizosphere samples align with substrate availability.

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