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THE EFFECT OF FOREST GAPS ON THE DIVERSITY AND COMPOSITION OF BACTERIAL COMMUNITIES IN MIXED-TYPE FOREST SOILS ACROSS THE CARPATHIAN MOUNTAINS

VPLIV GOZDNIH VRZELI NA PESTROST IN SESTAVO BAKTERIJSKIH ZDRUŽB V TLEH MEŠANIH GOZDOV KARPATOV

Nejc SUBAN[1], Olivera MAKSIMOVIĆ[2], Nataša ŠIBANC[3], Tijana MARTINOVIĆ[4,5], Eva DARENOVA[6], Matjaž ČATER[7,8], Tine GREBENC[9]

(1) Slovenian Forestry Institute, Department of forest physiology and genetics, nejc.suban@gozdis.si
(2) Slovenian Forestry Institute, Department of forest physiology and genetics, olivera.maksimovic@gozdis.si
(3) Slovenian Forestry Institute, Department of forest physiology and genetics, natasa.sibanc@gozdis.si
(4) Slovenian Forestry Institute, Department of forest physiology and genetics, tijana.martinovic@gozdis.si
(5) Laboratory of Environmental Microbiology, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic, tijana.martinovic@biomed.cas.cz
(6) Global Change Research Institute CAS, Czech Republic, darenova.e@czechglobe.cz
(7) Department of Silviculture, Faculty of Forestry and Wood Technology, Mendel University, Czech Republic, matjaz.cater@mendelu.cz
(8) Slovenian Forestry Institute, Department of forest physiology and genetics, matjaz.cater@gozdis.si
(9) Slovenian Forestry Institute, Department of forest physiology and genetics, tine.grebenc@gozdis.si

ABSTRACT

Mixed forests of European beech (*Fagus sylvatica* L.) and silver fir (*Abies alba* Mill.) play a vital ecological role in Central and South-Eastern Europe. This study investigates the diversity and composition of soil bacterial communities in these forests, focusing on rhizosphere and bulk soils under varying canopy structures. Soil samples were collected from eight sites along the Carpathian Mountains, including managed forests and the remnants of old growth. Metabarcoding of bacterial communities revealed that alpha diversity (species richness, Shannon index, and evenness) was significantly affected by sampling location but not by forest canopy structure or soil type (rhizosphere and bulk soil). The lowest bacterial diversity was found in the old-growth forest of the Beskidy region, while the highest was recorded in managed forest in Vrancea. Beta diversity analyses showed minimal variation between rhizosphere and bulk soil bacterial communities, with geographic distance being the strongest predictor of community composition. *Actinobacteriota* and *Proteobacteria* were the dominant phyla across all sites, with higher relative abundance of *Actinobacteriota* in all rhizosphere samples compared to bulk soil. Complex combinations of various environmental conditions at each sampling location, including soil parameters (mainly pH and C:N ratio), the age of forest gaps, the type and intensity of disturbances, and species composition of above-ground vegetation, can strongly affect soil bacterial communities. A closer examination of additional environmental variables would be necessary to better explain the observed differences in the diversity and composition of bacterial communities.

Key words: forest gaps, forest management, soil microbiome, soil, rhizosphere, Carpathians, temperate forest

IZVLEČEK

Mešani gozdovi navadne bukve (*Fagus sylvatica* L.) in bele jelke (*Abies alba* Mill.) so eden najpomembnejših ekosistemov na območju srednje in jugovzhodne Evrope. S pristopom molekularne identifikacije smo analizirali diverziteto in sestavo talnih bakterijskih združb na osmih lokacijah vzdolž Karpatov. Metagenomska analiza bakterijskih združb je pokazala, da je na alfa diverziteto (bogastvo vrst, Shannonov indeks in enakomernost) pomembno vplivala le lokacija vzorčenja, ne pa tudi struktura gozdne krošnje ali vrsta tal (rizosfera in zemlja, oddaljena od vpliva korenin). Najmanjšo bakterijsko vrstno pestrost smo potrdili v pragozdnem rezervatu Beskidy, največjo pa v gospodarskem gozdu v Vranceji. Analiza beta diverzitete je pokazala minimalne razlike med bakterijskimi združbami rizosfere in zemlje zunaj rizosfere, pri čemer je bila geografska oddaljenost glavni dejavnik vpliva na sestavo bakterijske združbe. *Actinobacteriota* in *Proteobacteria* sta bila prevladujoča rodova bakterij na vseh analiziranih lokacijah, pri čemer je bila relativna pojavnost rodu *Actinobacteriota* v vseh vzorcih rizosfere višja kot v zemlji zunaj območja rizosfere. Kombinacije različnih okoljskih razmer, predvsem pH tal in razmerje med C in N, ter drugih dejavnikov, kot so starost gozdne vrzeli, intenziteta in vrsta motnje, ki je povzročila njen nastanek, ter vrstna sestava vegetacije na vzorčenih lokacijah, lahko pomembno vplivajo na mikroorganizme v tleh. Da bi bolje pojasnili razlike v pestrosti in sestavi bakterijskih združb, bi bilo treba v analize vključiti dodatne spremenljivke.

Ključne besede: gozdne vrzeli, gospodarjenje z gozdom, mikrobna združba tal, tla, rizosfera, Karpati, gozdovi zmernega klimatskega pasu

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1 INTRODUCTION

1 UVOD

European beech (*Fagus sylvatica* L.) and silver fir (*Abies alba* Mill.) are crucial components of Central and South-Eastern European forest ecosystems and are expected to remain key species in mid- and high-altitude European forests (Leuscher, 2009; Dobrowolska et al., 2017). European beech exhibits high adaptability and ecological plasticity, thriving primarily in temperate regions due to its sensitivity to drought and high temperatures (Jump et al., 2006; Colin et al., 2017; Levanič et al., 2023). It plays a vital role in providing ecosystem services such as timber production, carbon sequestration, biodiversity preservation, and maintaining soil fertility, soil stability, and water resources (Duncker et al., 2012). In contrast, silver fir is predominantly found in colder temperate regions, particularly in the Alps and Carpathians, where lower elevations have recently seen declines in growth due to climatic extremes, air pollution, and subsequent pest and pathogen attacks (Bošela et al., 2018; Čater and Levanič, 2019; Čater et al., 2024).

While trees are key drivers of atmospheric carbon uptake, soil microbial communities are fundamental to ecosystem function, stability, and productivity (van der Heijden et al., 2008; Baldrian et al., 2017a, 2017b; Mercado-Blanco et al., 2018). Microbes, as pathogens, symbionts, mutualists, and decomposers, regulate carbon cycling, as well as nutrient cycling and availability through processes such as litter decomposition, mineral weathering, nitrogen fixation, and nutrient uptake (Uroz et al., 2013; Lladó et al., 2017). Fungi, particularly in temperate and boreal forests, are the primary decomposers of recalcitrant organic matter (Voříšková et al., 2013; Kohout et al., 2021). The role of bacteria, however, is less clear, varying between taxa that rely on low molecular weight carbon compounds and those capable of decomposing complex biopolymers (Lladó et al., 2017; Algora et al., 2022) since complexity of lignocellulose demands that no single enzyme or bacterial strain is able to fully degrade plant biomass (López-Mondéjar et al., 2019). Forest tree species influence soil microbial communities through root exudates, litter deposition, and fine root turnover, affecting community composition and related ecosystem services (Martinović et al., 2022).

The Carpathian Mountains, one of the largest forested mountain chains in Central Europe, feature diverse forest types dominated by beech and mixed conifer stands at elevations between 500 and 1450 meters (Dinca et al., 2022; Knorn et al., 2013). This region, underexplored in terms of soil biodiversity and ecosystem functions (Guerra et al., 2020), has been largely excluded from large-scale soil sampling efforts, such as the LUCAS survey (Labouyrie et al., 2023). Complementary to Dařenova et al. (2024), who studied the drivers of soil CO_2 efflux in beech-silver fir forests, this study analyzes bacterial community diversity in the rhizosphere and bulk soil under varying forest canopy structures in mixed forests of European beech and silver fir along the Carpathian Mountains. Using the same sampling design, we employed metabarcoding of soil environmental DNA to assess the diversity of selected bacterial taxa (Vasar et al., 2022).

Trees under the open and closed canopy show differential growth rates (Orman et al., 2021; Čater et al.,

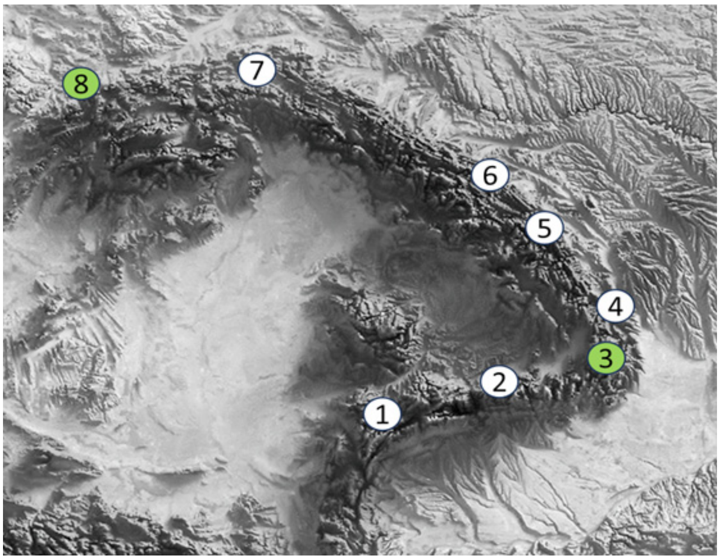


Fig. 1: Sampling locations along the Carpathian arc (adapted from Čater et al., 2024)

Slika 1: Lokacije vzorčenj vzdolž karpatske gorske verige (prilagojeno po Čater et al., 2024)

2024), and the understorey vegetation community is different (Dařenova et al., 2024). A higher amount of solar radiation and precipitation reaching the ground under open forest canopy, as opposed to a closed one, presents more favourable conditions for soil microbial activity (Jianxin et al., 2016; Chen and Yang, 2015). However, the area under open canopy receives a smaller input of organic matter due to lower root density and litterfall from trees (Griffiths et al., 2010; Kohout et al., 2018) while receiving more organic matter from herbaceous plants that usually develop abundantly under canopy openings. We hypothesized that bacterial beta diversity would differ significantly between soils under open and closed canopies, as well as between bulk and rhizosphere samples. In a study across European countries (Labouyrie et al., 2023), microbial richness and diversity were found to increase from less disturbed (i.e. woodlands) to more managed areas (i.e. grasslands and croplands). We hypothesized that bacterial alpha diversity would be significantly lower in the protected old-growth forests (Buzau and Beskidy) compared to managed sites.

2 METHODS

2 METODE

2.1 Study sites and research design

2.1 Območja raziskave in zasnova raziskave

A total of 48 soil samples was collected from eight sites along the Carpathian arc in Romania, Slovakia, and the Czech Republic (Adamič et al., 2023; Čater et al., 2024) (Fig. 1). All sites were located above 800 m on Cambisols and were selected for their favorable climate and soil conditions for beech and fir regeneration (Dařenova et al., 2024). Following the approach of Han et al. (2020) and Lyu et al. (2022), three plots of different light intensities were selected per site, categorized as closed canopy, forest edge, and open gap (Dařenova et al., 2024; Čater et al., 2024). Two sites (Buzau and Beskidy) were unmanaged old-growth forest remnants, while the others had been managed with low-intensity silvicultural systems for over a century (Adamič et al., 2023). In each plot, three bulk soil and three rhizosphere samples (top 10 cm) were collected using standardized probes (Grebenc and Kraigher, 2007). The soil samples were kept at 4°C during transport to the institute's laboratory. Bulk soil and root samples were then separated under a binocular microscope, and roots were washed under tap water to remove soil particles (Mrak et al., 2019). Samples were freeze-dried and stored at -20°C until further analyses.

Mean monthly temperature and precipitation data from 1901 to 2020 were obtained via kriging from the Royal Netherlands Meteorological Institute's Climate Explorer web page (http://climexp.knmi.nl) to calculate mean annual temperature and precipitation (Dařenova et al., 2024).

2.2 Molecular analysis

2.2 Molekularna analiza

DNA was extracted from 250 mg of soil adjacent to roots (rhizosphere soil) and bulk soil using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Bacterial metabarcoding was conducted using the 341f/805r primer pair, with PCR conditions as follows: 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s, with a final extension of 7 min at 72°C. DNA was purified, quantified, and sequenced on an Illumina MiSeq platform (350 bp paired-end) (Unuk Nahberger et al., 2019).

2.3 Bioinformatics and statistical analysis

2.3 Bioinformatika in statistična analiza

Raw sequences were processed with SEED v2.1.2 (Větrovský et al., 2013), with minimum quality thresholds set at 30 for sequences and 12 for base pairs. Chimeric sequences were removed using USEARCH

Table 1: Regions of each sampling location, with corresponding geographical coordinates, altitude, average annual air temperature, and total annual precipitation

Preglednica 1: Območja vzorčnih lokacij s pripadajočimi zemljepisnimi koordinatami, nadmorsko višino, povprečno letno temperature in povprečno skupno letno količino padavin

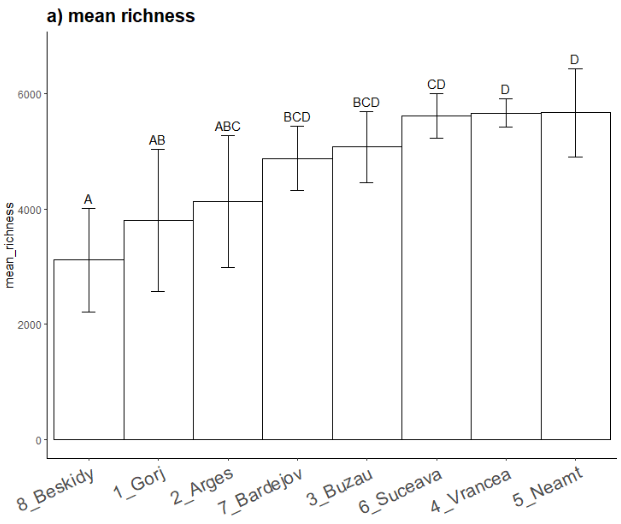
Plot number	Region	Altitude (m)	Longitude	Latititude	Average annual air temperature (°C)	Total annual precipitation (mm)
1	Gorj	985	22.916944°	45.169444°	4.7	1073
2	Arges	995	24.651111°	45.460278°	7.4	812
3	Buzau	1038	26.228889°	45.614167°	6.8	744
4	Vrancea	830	26.603889°	46.001389°	8.3	603
5	Neamt	950	26.168333°	46.854167°	5.8	704
6	Suceava	850	25.683333°	47.468333°	5.4	738
7	Bardejov	880	21.016562°	49.254738°	7.2	758
8	Beskidy	820	18.416805°	49.402483°	7.1	744

(Edgar, 2010), and sequences were clustered at a 97% similarity threshold using VSEARCH (Rognes et al., 2016). BLAST analysis was performed against UNITE (Kõljalg et al., 2013) and NCBI databases, with an e-value cutoff of 1e-50 and 92% similarity. The sequences are deposited in the data repository of the Slovenian forestry institute.

Statistical analyses were conducted in R (v. 4.3.2) using the phyloseq, vegan, ggplot2, multcomp, indicspecies, and pairwiseAdonis packages. Alpha diversity indices (species richness, Shannon index, and evenness) were compared between regions, forest stand types, and soil sample types using one-way ANOVA and Tukey HSD tests. Homogeneity of variance was assessed with Levene's test. Beta diversity was assessed using pairwise PERMANOVA on Bray-Curtis distance matrices (number of permutation = 999), with Hellinger transformation for standardization. Community composition was visualized via NMDS ordinations. We used a statistical approach developed by De Cáceres et al. (2012) to identify indicator species at the given locations. The relative abundances of the OTUs at each sampling site were calculated from the phloseq object by dividing the number of each respective OTU by the sum of all OTU counts at the location as described in McMurdie and Holmes (2013). The soil parameter values are extracted from the study by Dařenova et al. (2024) since the analyses were preformed on the same soil samples used in our metagenomic study.

3 RESULTS
3 REZULTATI

ANOVA and subsequent Tukey HSD tests indicated that only the sampling location (region) had a statistically significant effect (p<0.05) on bacterial richness, evenness, and Shannon diversity index (results are presented in Fig. 2; the p-values from the ANOVA test are presented in Table 1 in Supplementary Data). Levene's test confirmed the homogeneity assumption of variance across the groups of the region variable (p=0.031). A total of 73,038 OTUs were identified with sequence similarity greater than 82%, and 52,987 OTUs with similarity greater than 92%. The lowest diversity was observed in the Beskidy region, where the mean species richness (a) and its standard deviation was 3,117 ± 888.6, the mean Shannon index (b) was 6.5 ± 1, and the mean evenness index (c) was 0.81 ± 0.1. In contrast, the highest diversity was recorded in Vrancea, with a mean species richness (a) of 5,656 ±



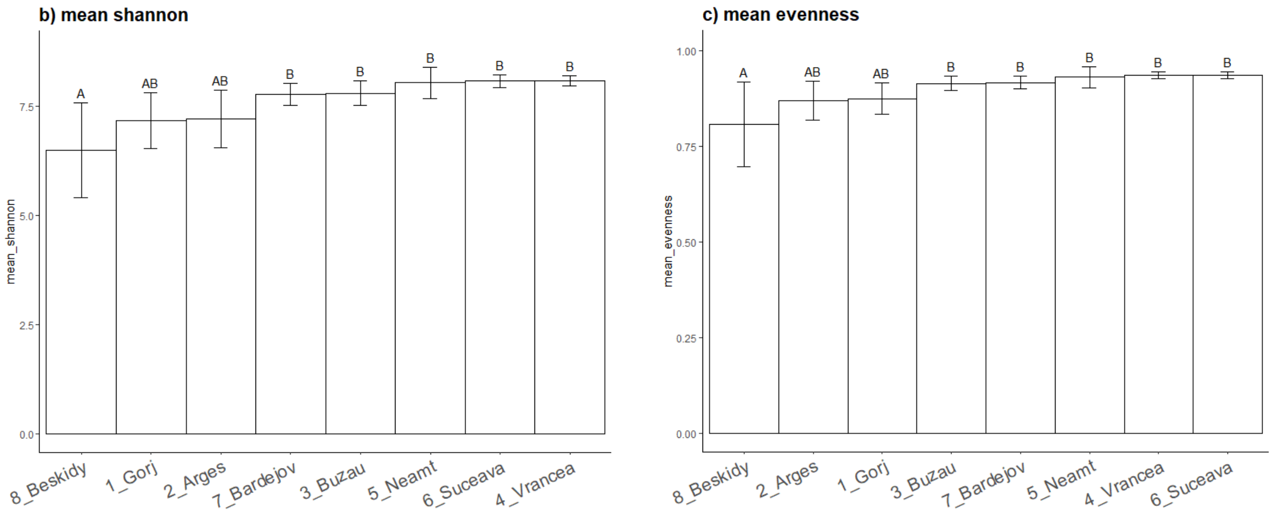


Fig. 2: Species richness (a), Shannon index (b), and evenness (c) values, along with grouping of these values by Tukey's HSD test of statistical significance for bacterial communities in soil samples collected from eight sites along the Carpathian arc

Slika 2: Ocene vrstnega bogastva (a), Shannonovega indeksa (b) ter indeksa enakomernosti (c) za bakterijske združbe v tleh. Vrednosti so združene v skupine glede na statistično značilnost razlik med posameznimi lokacijami, ocenjeno s HSD Tukeyevim testom variance.

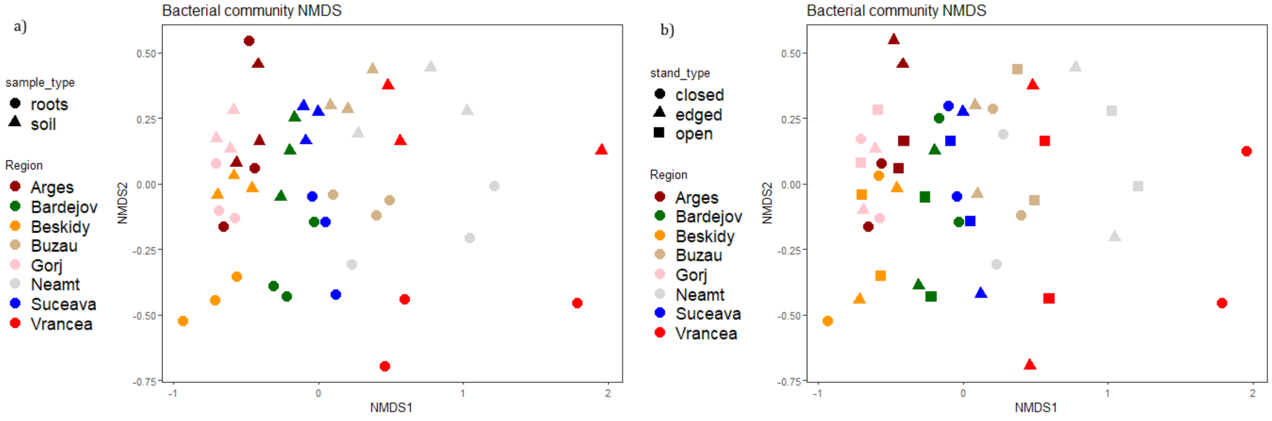


Fig. 3: NMDS graphs of bacterial community composition at each sampling location. a) bacterial community composition under different forest canopy types; b) bacterial community composition from different soil sample types (rhizosphere soil and bulk soil).

Slika 3: Grafi NMDS ordinacije vrstne sestave bakterijskih združb na posameznih vzorčnih lokacijah. a) sestava bakterijskih združb v tleh pod različno pokrovnostjo drevesnih kroženj; b) sestava bakterijskih združb glede na tip tal (rizosferna tla in zemlja, oddaljena od korenin).

234.3, a mean Shannon index (b) of 8.1 ± 0.1, and a mean evenness index (c) of 0.83 ± 0.01. Tukey's HSD test revealed statistically significant differences (adjusted p-value<0.05) in alpha diversity indices between Beskidy and the other regions (Bardejov, Buzau, Suceava, Neamt, and Vrancea), while Gorj and Arges were only found to be statistically different from Suceava, Vrancea, and Neamt in terms of species richness (Fig. 2; p-values from Tukey's HSD test are presented in Table 2 in the Supplementary Data).

The NMDS stress values (0.104) suggested a reliable representation of the NMDS dissimilarity matrix. The first NMDS plot (Fig. 3a), depicting communities under different forest canopy types, showed no discernible clustering along the axes. However, the second plot (Fig. 3b) exhibited a slight clustering of rhizosphere samples along the NMDS2 axis within the more widely scattered bulk soil data points. PERMANOVA results revealed that sample type had a statistically significant effect (p<0.001; see Table 3 in Supplementary Data) although it explained only 4.4% of the variance, whereas geographic distance, expressed through a space variable consisting of three significant PCNM vectors, explained the largest proportion of variation (13.2%) (Table 3 in Supplementary Data).

Bar plots of OTU relative abundance at each sampling location (Fig. 4) showed that *Actinobacteriota* was the dominant phylum across all sampling locations, followed by *Proteobacteria*. Other important phyla included *Planctomycetota*, *Verrucomicrobiota*, *Firmicutes*, *Chloroflexi*, *Acidobacteriota*, *Myxococcota*, *Gemmatimonadota*, and *Bacteroidota*. Notably, *Actino-*

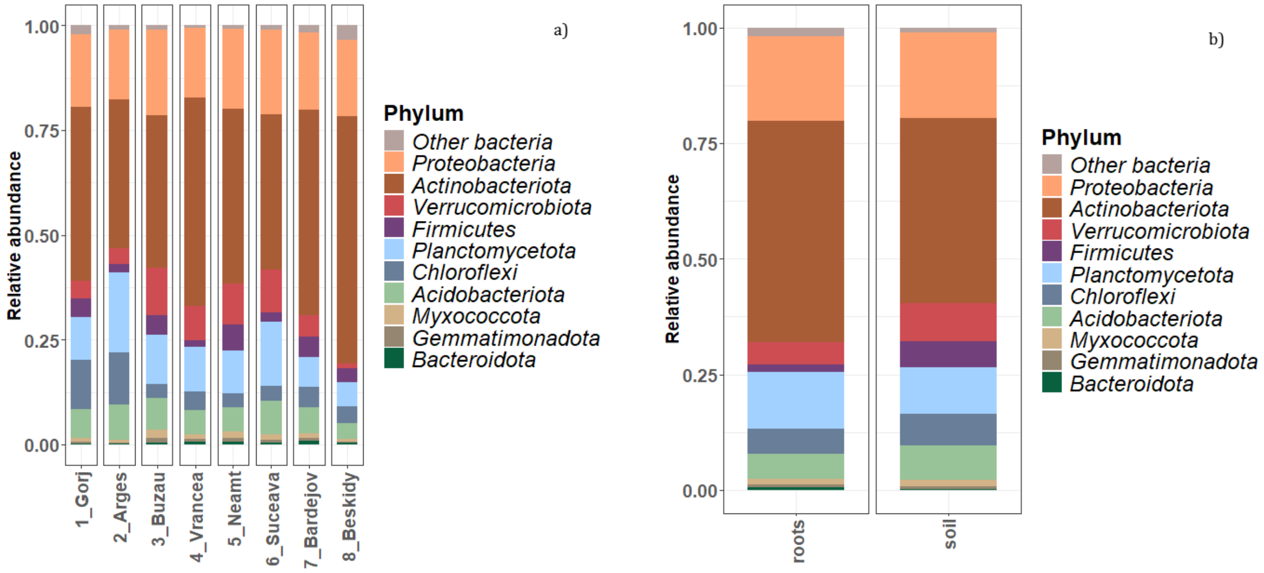


Fig. 4: Relative abundance of bacterial phyla at each sampling location (a) and sample type (b)

Slika 4: Relativna pojavnost bakterijskih filogenetskih debel na posameznih lokaciji (a) ter v obeh tipih tal (b) *bacteriota* exhibited higher relative abundance in the rhizosphere compared to bulk soil. However, the relative abundance of bacterial indicator genera in both the rhizosphere and bulk soil was extremely low (0.08% and 0.015%, respectively).

4 DISCUSSION

4 RAZPRAVA

Our findings suggest that soil bacterial community composition does not differ significantly between closed and open forest canopies. This may be due to the limited variation in environmental conditions observed at the sampling sites, as reported by Dařenova et al. (2024). For example, soil temperature increases from closed canopy plots to gap edges and open canopy areas, but statistically significant differences were only observed at site 1 (Gorj) in 2022 and site 5 (Neamt) in 2023, with the highest temperatures recorded at forest edges. Soil water content was generally higher under open canopies, though the pattern varied among plots. Furthermore, the content of carbon (C) and nitrogen (N), as well as the C:N ratio, showed no significant trends across plots or sites, and pH values also lacked consistent patterns (Dařenova, 2024). Given the importance of soil properties, particularly pH and the C:N ratio, in predicting bacterial richness, diversity, and composition (Lauber et al., 2008, 2009; Wang et al., 2022; Labouyrie et al., 2023), these results likely explain the lack of pronounced differences in bacterial community structure between forest canopy types. Recent studies have shown that higher soil pH increases both bacterial richness and Shannon index values, while higher calcium carbonate content and C:N ratios have the opposite effect (Labouyrie et al., 2023). Bacterial beta diversity is similarly influenced by pH, followed by the C:N ratio (Labouyrie et al., 2023).

Our hypothesis that alpha diversity indices would be lowest in unmanaged old-growth forests (Buzau and Beskidy) was not fully supported by the data. While alpha diversity was significantly lower in the Beskidy region, the Buzau site grouped with regions exhibiting the highest diversity. This incongruence may be attributed to factors such as above-ground species composition, forest gap age and size, and the intensity of disturbance events, which can influence microbial diversity (Urbanová et al., 2015; Yang et al., 2017; Chen et al., 2022; Labouyrie et al., 2023). Variation in these factors across sites may also explain the lack of significant differences in bacterial community composition between closed and open forest canopies. Post-disturbance forest development, characterized by increasing tree biomass and shifts in ground vegetation composition, can alter microbial activity, biomass, and community composition (Wang, 2022; Chauvat et al., 2003; Lucas-Borja and Delgado-Baquerizo, 2019).

Our results indicate no significant differences in bacterial alpha diversity between bulk soil and rhizosphere samples. While some studies on herbaceous plants have reported higher bacterial diversity in rhizosphere soils compared to bulk soils (Laurent and Aragno, 1999; Wei et al., 2023), our findings are more consistent with a study conducted in mountain forest ecosystems, which found no significant differences in alpha diversity between rhizosphere and bulk soils (Cui et al., 2019). However, PERMANOVA did reveal a significant effect of soil sample type on bacterial community composition, aligning with results from temperate mixed forests in Europe. For instance, Uroz et al. (2016) found that bacterial communities differed between rhizosphere and bulk soils regardless of tree species, while other studies have noted shifts in Acidobacterial taxa linked to preferences for leaf or needle litter from beech, spruce, or fir trees (Urbanová et al., 2015; Nacke et al., 2016). Consistent with European soil studies (Labouyrie et al., 2023), including soils in mixed-forests dominated by European beech *Proteobacteria*, *Actinobacteria*, and *Acidobacteria* were among the most abundant phyla in our samples. However, the unusually high abundance of *Planctomycetota* in our samples, compared to previous studies where this phylum was found in much lower abundances (Siles and Margesin, 2016; Bárta et al., 2017; Baćmaga et al., 2022), may indicate a methodological or technical bias in our data.

5 SUMMARY

5 POVZETEK

Mešani gozdovi zmerno toplega podnebnega pasu, v katerih prevladujeta navadna bukev (*Fagus sylvatica* L) in bela jelka (*Abies alba* Mill.), so na območju srednje in jugovzhodne Evrope ekonomsko najpomembnejši in geografsko najbolj razširjeni gozdni ekosistemi. Vse bolj pogosta sušna obdobja in visoke temperature negativno vplivajo na naravno obnovo in uspešnost preživetja omenjenih vrst, predvsem jelke in smreke, na nižjih nadmorskih višinah. Poleg dreves, ki imajo ključno vlogo pri kroženju ogljika v naravi, vplivajo mikrobne združbe v tleh na vse bistvene biogeokemijske procese, omogočajo kroženja hranil in energije ter delovanje ekosistemov. Drevesa, z izločanjem produktov fotosinteze prek koreninskega sistema in opada odmrlega rastlinskega materiala vplivajo na aktivnost mikrobnih združb in njihovo raznovrstnost. Biodiverziteta tal in delovanje mikrobnih združb sta na območju Karpatov pomanjkljivo raziskana. V raziskavi smo s pomočjo

metagenomskih pristopov opisali bakterijske združbe v tleh ter s Shannonovim indeksom, indeksom vrstne pestrosti in enakomernosti ocenili njihovo diverziteto. Primerjali smo bakterijske združbe v rizosferi in zemlje zunaj območja korenin (bulk soil) ter bakterijske združbe odvzetih na območjih različne intenzitete zastiranja matičnega sestoja.

Vzorčenje smo opravili na osmih lokacijah vzdolž Karpatskega gorovja v Romuniji, na Slovaškem in Češkem. Izbrane vzorčne lokacije so na nadmorskih višinah od 800 do 1100 metrov v mešanih gozdovih bukve in jelke. Iz pridobljenih podatkov sekvenciranja vzorcev zemlje smo identificirali 73.038 delovnih operacijskih enot (OTU) s podobnostjo sekvence (similarity), večje od 82 % glede na referenčno sekvenco, in 52.987 delovnih operacijskih enot s podobnostjo, večjo od 92 % baznih parov. S testom ANOVA smo pokazali, da le prostorska razporeditev vzorcev statistično značilno vpliva na vrednosti indeksov alfa diverzitete (vrstno bogastvo, Shannonov indeks in enakomernost združbe). Največ bakterijskih vrst smo zaznali v gospodarskem gozdu na vzorčni lokaciji Vrancea, najmanj vrst pa na najbolj severni lokaciji Beskidy. Z analizo beta diverzitete združb smo ocenili, da tip talnega vzorca statistično značilno pojasni le manjši del variance v vrstni sestavi bakterijske združbe (4,4 %), medtem ko je vpliv zemljepisne širine med lokacijami veliko večji (13,2 %). V vseh vzorcih so bili najpogosteje zastopane vrste iz rodu *Aktinobacteria,* njihova pogostost pa je večja v rizosfernih tleh kot v prostih tleh. Drugi prevladujoči taksoni so vključevali *Proteobacterie, Planctomycetota, Verrucomicrobiota* in *Acidobacteriota.*

V raziskavi mikrobnih združb na območju Karpatov nismo odkrili značilnih razlik med bakterijskimi združbami v tleh pod zastorom krošenj ali na odprtem, čemur je morebiti botroval zelo majhen razpon vrednosti okoljskih spremenljivk med različnimi lokacijami. Razlike v povprečnih letnih temperaturah in skupnih količinah padavin niso statistično značilno vplivale na indekse diverzitete ter tudi talni parametri (pH in razmerje C:N), izmerjeni in testirani v sklopu študije Dařenove et al. (2024), niso sledili trendom geografske širine med vzorčnimi lokacijami. Predvidevali smo, da bo vrstno bogastvo najmanjše na lokacijah pragozdnih rezervatov, kar smo potrdili na eni izmed lokaciji (Beskidy), medtem ko je bila bakterijska združba druge lokacije (Buzau) pestra in vrstno bogata. Vrstna sestava rastlin, način gospodarjenja z gozdom ter razlike v intenziteti in vrsti motenj, ki so botrovale nastanku vrzeli, so nekateri od dejavnikov, ki lahko vplivajo na nekatere neskladnosti izsledkov naših analiz. Tudi primerjave alfa diverzitete med rizosfero in prostimi tlemi niso pokazale pomembnih razlik med združbami, čeprav lahko rastline s svojimi koreninskimi eksudati ustvarjajo razmere za specifično bakterijsko združbo, ki se razlikuje od tiste v prostih tleh. Z analizo teksonomske pestrosti in pojavnosti smo ocenili, da največji delež zastopanosti v celotni združbi ponazarjata skupini *Actinobacteriota* in *Proteobacteriota*. Zaznali smo tudi velik deležih skupine *Planctomycetota*, ki v drugih primerljivih študijah nikoli ni bila prepoznana s tolikšno pojavnostjo, kar pripisujemo bodisi specifičnim rastiščnim razmeram bodisi morebitni pristranskosti uporabljene metode.

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SUPPLEMENTARY DATA

DODATNI PODATKI

Table 1: Adjusted p-values from Tukey's HSD test for pairwise comparisons of the alpha diversity index values at the sampling locations

Preglednica 1: Prilagojene p-vrednosti HSD Tukeyevega testa za primerjavo vrednosti indeksov alfa diverzitete za pare vzorčnih lokacij

	Richness	Shannon	Evenness
2_Arges-1_Gorj	0.997	1	1
3_Buzau-1_Gorj	0.137	0.497	0.822
4_Vrancea-1_Gorj	*0.006	0.097	0.358
5_Neamt-1_Gorj	*0.005	0.134	0.465
6_Suceava-1_Gorj	*0.008	0.103	0.353
7_Bardejov-1_Gorj	0.310	0.540	0.787
8_Beskidy-1_Gorj	0.807	0.380	0.261
3_Buzau-2_Arges	0.459	0.590	0.719
4_Vrancea-2_Arges	*0.037	0.133	0.264
5_Neamt-2_Arges	*0.036	0.180	0.356
6_Suceava-2_Arges	*0.049	0.140	0.259
7_Bardejov-2_Arges	0.734	0.633	0.678
8_Beskidy-2_Arges	0.381	0.302	0.355
4_Vrancea-3_Buzau	0.906	0.983	0.994
5_Neamt-3_Buzau	0.903	0.994	0.999
6_Suceava-3_Buzau	0.942	0.985	0.994
7_Bardejov-3_Buzau	1	1	1
8_Beskidy-3_Buzau	*0.003	*0.003	*0.008
5_Neamt-4_Vrancea	1	1	1
6_Suceava-4_Vrancea	1	1	1
7_Bardejov-4_Vrancea	0.687	0.975	0.997
8_Beskidy-4_Vrancea	*<0.001	*<0.001	*<0.001
6_Suceava-5_Neamt	1	1	1
7_Bardejov-5_Neamt	0.682	0.990	1
8_Beskidy-5_Neamt	*<0.001	*<0.001	*0.001
7_Bardejov-6_Suceava	0.758	0.978	0.996
8_Beskidy-6_Suceava	*<0.001	*<0.001	*<0.001
8_Beskidy-7_Bardejov	*0.010	*0.004	*0.007

Table 2: Results of the one-way ANOVA test for statistical significance (expressed as p-values) of selected variables (region, sample, type, and stand type) in explaining alpha diversity index values

Preglednica 2: Rezultati enosmernega testa ANOVA za preverjanje statistične značilnosti učinka izbranih spremenljivk (regije vzorčenja, tipa vzorčenja in vrste sestoja) na vrednosti indeksov alfa diverzitete

	Richness	Shannon	Evenness
Region	*<0.001	*<0.001	*<0.001
Sample type	0.876	0.701	0.579
Stand type	0.146	0.096	0.095

Table 3: Results of PERMANOVA test on the explanatory effect of the selected variables on bacterial species composition at the given locations. The tested variables were region, sample, type, stand type, and a space variable consisting of three PCNM vectors.

Preglednica 3: Rezultati testa PERMANOVA za ocenjevanje učinka izbranih spremenljivk pri pojasnjevanju sestave bakterijske združbe na vzorčnih lokacijah. Testirane spremenljivke vključujejo: regije vzorčenja, tipa vzorčenja, vrste sestoja ter prostorsko spremenljivko, sestavljeno iz treh PCNM-vektorjev.

		Degrees of freedom	Sum of squares	R^2	F-statistic	p-value
Region	Model	7	4.6856	0.27008	2.1144	*0.001
	Residual	40	12.6631	0.72992		
	Total	47	17.3487	1		
Sample type	Model	1	0.7694	0.04435	2.1348	*0.001
	Residual	46	16.5793	0.95565		
	Total	47	17.3487	1		
Stand type	Model	2	0.6464	0.03726	0.8707	0.925
	Residual	45	16.7023	0.96274		
	Total	47	17.3487	1		
Space	Model	3	2.2928	0.13216	2.2336	*0.001
	Residual	44	15.0559	0.86784		
	Total	47	17.3487	1		