

Original scientific article

Early view

This articles have been accepted for publication in Acta Silvae et Ligni and undergone full peer review but have not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record.

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 Suban¹, Olivera Maksimović¹, Nataša Šibanc¹, Tijana Martinović^{1,2}, Eva Darenova³, Matjaž Čater^{1,4}, Tine Grebenc¹

(1) Slovenian Forestry Institute, Večna pot 2, SI-1000 Ljubljana, Slovenia

(2) Laboratory of Environmental Microbiology, Institute of Microbiology of the Czech Academy of Sciences, Praha 4, Czech Republic

(3) Global Change Research Institute CAS, v.v.i., Belidla 4a, 60300 Brno, Czech Republic

(4) Department of Silviculture, Faculty of Forestry and Wood Technology, Mendel University, Zemedelska 3, 61300 Brno, Czech Republic

DOI 10.20315/ASetL.135.5

UDC 181.351(045)=111

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 diverzitetu in sestavo talnih bakterijskih združb na osmih lokacijah vzdolž Karpatov. Metagenomska analiza bakterijskih združb je pokazala, da je na alfa diverzitetu (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 diverzitet 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

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, 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 (Voríšková 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). 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 CO₂ 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., 2024), and the understory 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, 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.

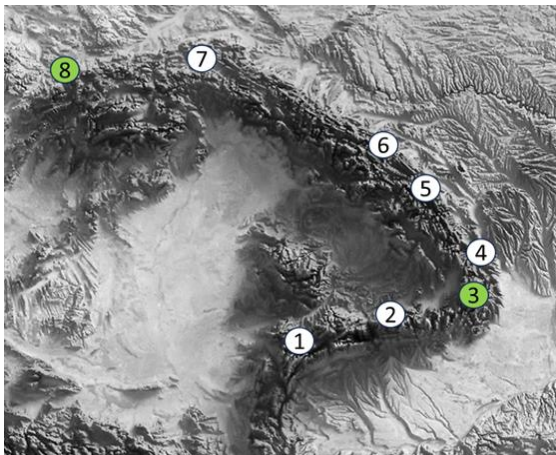


Fig. 1: Sampling locations along the Carpathian arc (adapted from Čater et al., 2024)
Slika 1: Lokacije vzorčenj vzdolž karpatske gorske verige (prilagojeno po Čater et al., 2024)

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, 2023). In each plot, three bulk soil and three rhizosphere samples (top 10 cm) were collected using standardized probes (Grebenc et al., 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.

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 | Latitude | 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 |

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

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

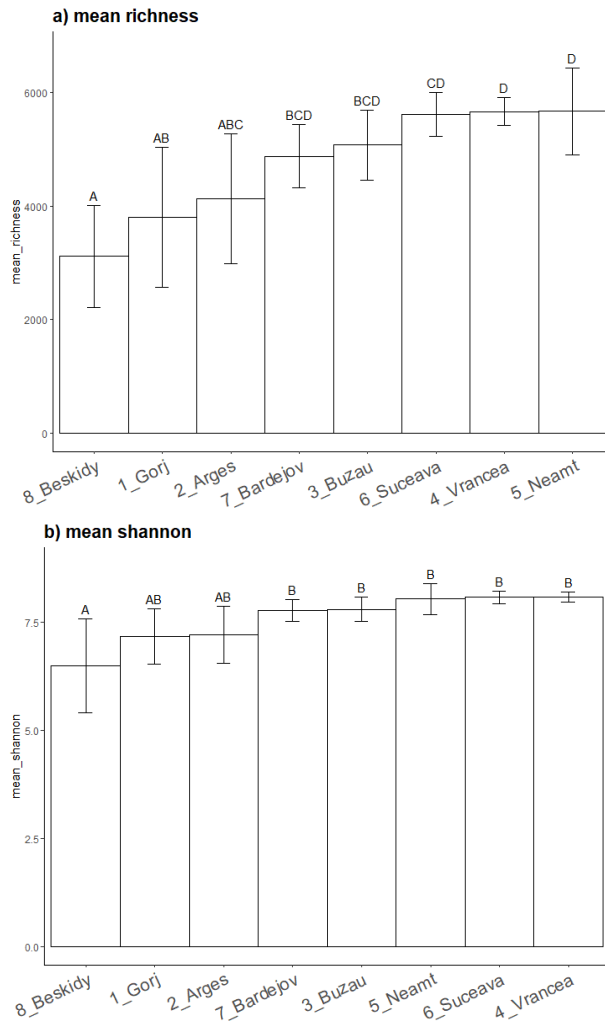
Raw sequences were processed with SEED v2.1.2 (Větrovský et al., 2013), with minimum quality thresholds set at 30 for sequences and 12 for base pairs. Chimeric sequences were removed using USEARCH

(Edgar, 2016), 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 pairwise Adonis 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 performed on the same soil samples used in our metagenomic study.



3 RESULTS

3 REZULTATI

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.

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 199,845 OTUs with similarity greater than 92%. The lowest diversity was observed in the Beskidy region, where the mean species richness (a) and its

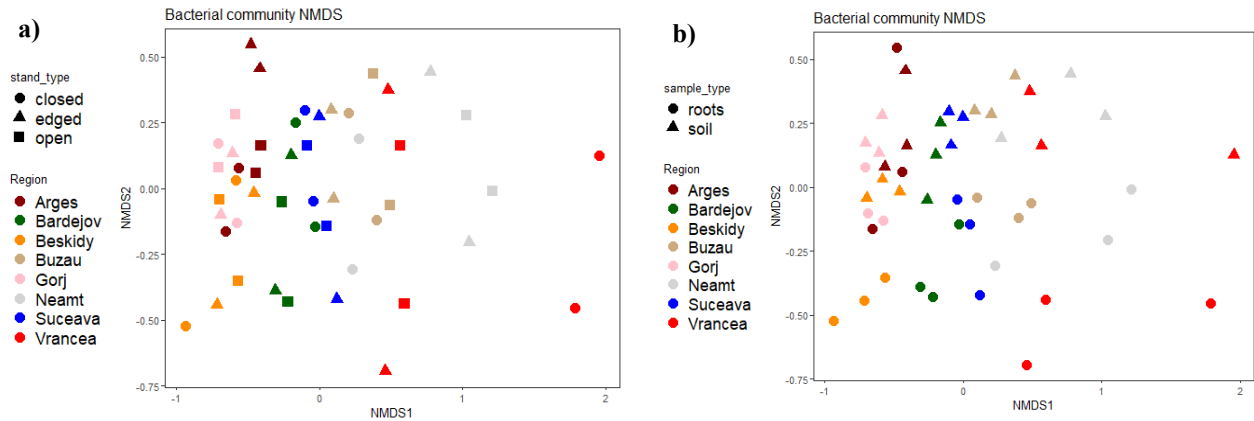


Fig. 3: NMDS graphs of bacterial community composition at each sampling location. a) bacterial community composition under different forest canopy types; b) bacterial community composition from different soil sample types (rhizosphere soil and bulk soil).

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).

standard deviation was $3,117 \pm 888.6$), 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 $5,656 \pm 234.3$, 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, *Actinobacteriota* 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).

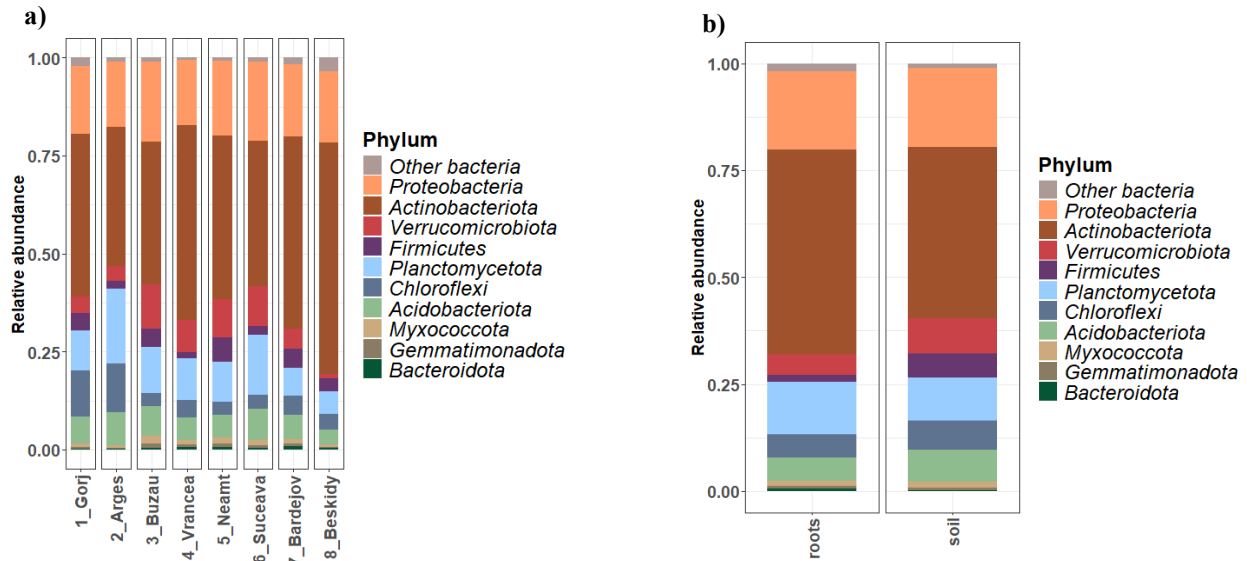


Fig. 4: Relative abundance of bacterial phyla at each sampling location (a) and sample type (b)

Slika 4: Relativna pojavnost bakterijskih filogenetskih debel na posameznih lokaciji (a) ter v obeh tipih tal (b)

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; Labouyrie, 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, 2023). Bacterial beta diversity is similarly influenced by pH, followed by the C:N ratio (Labouyrie, 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, 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 & 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 (Marilley 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, 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, 2016; Bárta et al., 2017; Baćmaga et al., 2022), may indicate a methodological or technical bias in our data.

6 SUMMARY

6 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. Biodiverzitetata 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 diverzitetu. 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 199.845 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 najpogostejše zastopane vrste iz rodu *Actinobacteria*, 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.

ACKNOWLEDGEMENTS

ZAHVALE

This work was supported by Slovenian Research and Innovation Agency research projects J4-4547, J4-3086, Research Core funding P4-0107 at the Slovenian Forestry Institute and the Czech Science Foundation (GAČR 21-47163L). NS was supported by a scholarship from the The Public Scholarship, Development, Disability, and Maintenance Fund of the Republic of Slovenia through call no. 327. No animals, plants, or fungi were damaged during this study.

REFERENCES

VIRI

- Adamič P., Levanič T., Hanzu M. & Čater M. 2023. Growth response of European Beech (*Fagus sylvatica* L.) and Silver Fir (*Abies alba* Mill.) to climate factors along the Carpathian Massive. *Forests*, 14. <https://doi.org/10.3390/f14071318>
- Algora, C., Odriozola, I., Human Z.R., Hollá S.A., Baldrian P., & López-Mondéjar R. 2022. Specific utilization of biopolymers of plant and fungal origin reveals the existence of substrate-specific guilds for bacteria in

temperate forest soils. *Soil Biology and Biochemistry*, 171, 108696. <https://doi.org/10.1016/j.soilbio.2022.108696>

Baldrian P. 2017a. Microbial activity and the dynamics of ecosystem processes in forest soils. *Current Opinion in Microbiology*, 37: 128–134.

Baldrian P. 2017b. Forest microbiome: diversity, complexity, and dynamics. *FEMS Microbiology Reviews*, 41, 1:109–130. <https://doi.org/10.1093/femsre/fuw040>

Bárta J., Tahovská K., Šantrůčková H., Oulehle F. 2017. Microbial communities with distinct denitrification potential in spruce and beech soils differing in nitrate leaching. *Scientific Reports*, 7, 9738. <https://doi.org/10.1038/s41598-017-08554-1>

Baćmaga M., Wyszowska J., Borowik A., Kucharski J., Paprocki Ł. 2022. Role of forest site type in determining bacterial and biochemical properties of soil. *Ecological Indicators*, 135, 108557. <https://doi.org/10.1016/j.ecolind.2022.108557>

Bošela M., Lukac M., Castagneri D., Sedmák, R., Biber P., Carrer M., Konôpka B., Nola P., Nagel T.A., Popa I., Roibu C.C., Svoboda M., Trotsiuk V., Büntgen U. 2018. Contrasting effects of environmental change on the radial growth of co-occurring beech and fir trees across Europe. *Science of the Total Environment*, 615: 1460–1469. <https://doi.org/10.1016/j.scitotenv.2017.09.092>

Chen S., Jiang C., Bai Y., Wang H., Jiang C., Huang K., Guo L., Zeng S., Wang S. 2022. Effects of forest gap on soil microbial communities in an evergreen broad-leaved secondary forest. *Forests*, 13, 12: 2015. <https://doi.org/10.3390/f13122015>

Chen S., Yang Y. 2015. Effects of gaps in the forest canopy on soil microbial communities and enzyme activity in a Chinese pine forest. *Pedobiologia*, 61: 51–60. <https://doi.org/10.1016/j.pedobi.2017.03.001>

Chauvat M., Zaitsev A.S., Wolters V. 2003. Successional changes of Collembola and soil microbiota during forest rotation. *Oecologia*, 137: 269–276

Cui Y.X., Bing H.J., Fang L.C., Wu Y.H., Yu J.L., Shen G.T., Jiang M., Wang X., Zhang X. 2019. Diversity patterns of the rhizosphere and bulk soil microbial communities along an altitudinal gradient in an alpine ecosystem of the eastern Tibetan Plateau. *Geoderma*, 338: 118–127. <https://doi.org/10.1016/j.geoderma.2018.11.047>

Čater M., Levanič T. 2019. Beech and silver fir's response along the Balkan's latitudinal gradient. *Scientific Reports*, 9, 16269. <https://doi.org/10.1038/s41598-019-52670-z>

Čater M., Adamič P.C., Dařenova E. 2024. Response of beech and fir to different light intensities along the Carpathian and Dinaric Mountains. *Frontiers in Plant Science*, 15. <https://doi.org/10.3389/fpls.2024.1380275>

Dařenova E., Adamič P., & Čater M. 2024. Effect of temperature, water availability, and soil properties on soil CO₂ efflux in beech-fir forests along the Carpathian Mts. *Catena*, 240, 107974. <https://doi.org/10.1016/j.catena.2024.107974>

De Cáceres M., Legendre P., Wiser S.K., Brotons L. 2012. Using species combinations in indicator value analyses. *Methods in Ecology and Evolution*, 3, 6: 973–982.

Delgado-Baquerizo M., Maestre F.T., Reich P.B., Jeffries T.C., Gaitan J.J., Encinar D., Berdugo M., Campbell C.D., Singh B.K.

2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications*, 7, 10541. <https://doi.org/10.1038/ncomms10541>
- Dinca L., Marin M., Radu V., Murariu G., Drasovean R., Cretu R., Georgescu L., Timiș-Gânsac V. 2022. Which are the best site and stand conditions for silver fir (*Abies alba* Mill.) located in the Carpathian Mountains? *Diversity*, 14, 7, 547. <https://doi.org/10.3390/d14070547>
- Dobrowolska D., Bončina A., Klumpp R. 2017. Ecology and silviculture of silver fir (*Abies alba* Mill.): a review. *Journal of Forest Research*, 22, 6: 326–335. <https://doi.org/10.1080/13416979.2017.1386021>
- Duncker P., Barreiro S., Hengeveld G., Lind T., Mason W., Ambroży S., Spiecker H. 2012. Classification of forest management approaches: a new conceptual framework and its applicability to European forestry. *Ecology and Society*, 17, 4, 51. <https://doi.org/10.5751/ES-05262-170451>
- Edgar R.C. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26, 19: 2460–2461. <https://doi.org/10.1093/bioinformatics/btq461>
- Grebenc T., Kraigher H. 2007. Changes in the community of ectomycorrhizal fungi and increased fine root number under adult beech trees chronically fumigated with double ambient ozone concentration. *Plant Biology*, 9: 279–287. <https://doi.org/10.1055/s-2006-924489>
- Griffiths R.P., Gray A.N., Spies T.A. 2010. Soil properties in old-growth Douglas-fir forest gaps in the Western Cascade Mountains of Oregon. *Northwest Science*, 84, 1: 33–45. <https://doi.org/10.3955/046.084.0104>
- Guerra C.A., Heintz-Buschart A., Sikorski J. et al. 2020. Blind spots in global soil biodiversity and ecosystem function research. *Nature Communications*, 11, 3870. <https://doi.org/10.1038/s41467-020-17688-2>
- Han M., Tang M., Shi B., Jin G. 2020. Effect of canopy gap size on soil respiration in a mixed broadleaved-Korean pine forest: evidence from biotic and abiotic factors. *European Journal of Soil Biology*, 99, 103194. <https://doi.org/10.1016/j.ejsobi.2020.103194>
- Jianxin X., Li X., Zhiyao S. 2016. Impacts of forest gaps on soil properties after a severe ice storm in a *Cunninghamia lanceolata* stand. *Pedosphere*, 26: 408–416. [https://doi.org/10.1016/S1002-0160\(15\)60053-4](https://doi.org/10.1016/S1002-0160(15)60053-4)
- Jump A.S., Hunt J.M., Peñuelas J. 2006. Rapid climate change-related growth decline at the southern range edge of *Fagus sylvatica*. *Global Change Biology*, 12: 2163–2174. <https://doi.org/10.1111/j.1365-2486.2006.01250.x>
- Knorn J., Kuemmerle T., Radeloff V.C., Keeton W.S., Gancz V., Biriş I.A., Svoboda M., Griffiths P., Hagatis A., Hostert P. 2013. Continued loss of temperate old-growth forests in the Romanian Carpathians despite an increasing protected area network. *Environmental Conservation*, 40, 2: 182–193. <https://doi.org/10.1017/S0376892912000355>
- Köljalg U., Nilsson R.H., Abarenkov K. et al. 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, 22, 21: 5271–5277. <https://doi.org/10.1111/mec.12481>
- Kohout P., Charvátová M., Štursová M., Mašínová T., Tomšovský M., Baldrian P. 2018. Clearcutting alters decomposition processes and initiates complex restructuring of fungal communities in soil and tree roots. *ISME Journal*, 12, 3: 692–703. <https://doi.org/10.1038/s41396-017-0027-3>

- Kohout P., Charvátová M., Štursová M., Mašínová T., Tomšovský M., Baldrian P., Tedersoo L. 2021. Fungal communities in small forest gaps respond variably to tree species, soil chemistry and their interactions. *Fungal Ecology*, 54, 101107. <https://doi.org/10.1016/j.funeco.2021.101107>
- Leuschner C., Lenzion J. 2009. Air humidity, soil moisture and soil chemistry as determinants of the herb layer composition in European beech forests. *Journal of Vegetation Science*, 20: 288–298. <https://doi.org/10.1111/j.1654-1103.2009.05641.x>
- Levanič T., Ugarković D., Seletković I., Ognjenović M., Marušić M., Bogdanić R., Potočić N. 2023. Radial increment of beech (*Fagus sylvatica* L.) is under a strong impact of climate in the continental biogeographical region of Croatia. *Plants*, 12, 13, 2427. <https://doi.org/10.3390/plants12132427>
- Lladó S., Lopez-Mondejar R., Baldrian P. 2017. Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. *Microbiology and Molecular Biology Reviews*, 81, 2. <https://doi.org/10.1128/MMBR.00063-16>
- López-Mondéjar R., Algora C., Baldrian P. 2019. Lignocellulolytic systems of soil bacteria: comparative omics analysis reveals unique and shared features across taxa. *Applied and Environmental Microbiology*, 85, 3, e02495-18. <https://doi.org/10.1128/AEM.02495-18>
- Labouyrie M., Ballabio C., Romero F., Panagos P., Jones A., Schmid M.W., Mikryukov V., Dulya O., Tedersoo L., Bahram M., Lugato E., van der Heijden M.G.A., Orgiazzi A. 2023. Patterns in soil microbial diversity across Europe. *Nature Communications*, 14, 3311. <https://doi.org/10.1038/s41467-023-37937-4>
- Laurent M., Aragno M. 1999. Phylogenetic diversity of bacterial communities differing in degree of proximity of *Lolium perenne* and *Trifolium repens* roots. *Applied Soil Ecology*, 13, 2: 127–136. [https://doi.org/10.1016/S0929-1393\(99\)00028-1](https://doi.org/10.1016/S0929-1393(99)00028-1)
- Lucas-Borja M.E., Delgado-Baquerizo M. 2019. Plant diversity and soil stoichiometry regulates the changes in multifunctionality during pine temperate forest secondary succession. *Science of The Total Environment*, 697, 134204. <https://doi.org/10.1016/j.scitotenv.2019.134204>
- Lyu Q., Luo Y., Dong Y., Xiang Y., Zhao K., Chen G., Chen Y., Fan C., Li X. 2022. Effects of forest gaps on the structure and diversity of soil bacterial communities in Weeping Cypress Forest Plantations. *Frontiers in Microbiology*, 13, 882949. <https://doi.org/10.3389/fmicb.2022.882949>
- Martinović T., Mašínová T., López-Mondéjar R., Jansa J., Štursová M., Starke R., Baldrian P. 2022. Microbial utilization of simple and complex carbon compounds in a temperate forest soil. *Soil Biology and Biochemistry*, 173, 108786. <https://doi.org/10.1016/j.soilbio.2022.108786>
- McMurdie P.J., Holmes S. 2013. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS one*, 8, 4, e61217. <https://doi.org/10.1371/journal.pone.0061217>
- Mercado-Blanco J., Abrantes I., Barra Caracciolo A., Bevivino A., Ciancio A., Grenni P., Hryniewicz K., Kredics L., Proença D.N. 2018. Belowground microbiota and the health of tree crops. *Frontiers in Microbiology*, 9, 1006. <https://doi.org/10.3389/fmicb.2018.01006>

- Mrak T., Štraus I., Grebenc T., Gričar J., Hoshika Y., Carreiro G., Paoletti E., Kraigher H. 2019. Different belowground responses to elevated ozone and soil water deficit in three European oak species (*Quercus ilex*, *Q. pubescens* and *Q. robur*). *Science of The Total Environment*, 651, part 1: 1310–1320. <https://doi.org/10.1016/j.scitotenv.2018.09.246>
- Nacke H., Goldmann K., Schöning I., Pfeiffer B., Kaiser K., Villamizar G.A.C., et al. 2016. Fine spatial scale variation of soil microbial communities under European beech and Norway spruce. *Frontiers in Microbiology*, 7: 2067. <https://doi.org/10.3389/fmicb.2016.02067>
- Orman O., Wrzesiński P., Dobrowolska D., et al. 2021. Regeneration growth and crown architecture of European beech and silver fir depend on gap characteristics and light gradient in the mixed montane old-growth stands. *Forest Ecology and Management*, 482, 118866. <https://doi.org/10.1016/j.foreco.2020.118866>
- Rahman S.A., Jacobsen J.B., Healey J.R., Roshetko J.M., Sunderland T. 2017. Finding alternatives to swidden agriculture: does agroforestry improve livelihood options and reduce pressure on existing forest? *Agroforestry Systems*, 91, 1: 185–199. <https://doi.org/10.1007/s10457-016-9930-5>
- Rognes T., Flouri T., Nichols B., Quince C., Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, 4, e2584. <https://doi.org/10.7717/peerj.2584>
- Rosenstock T.S., Dawson I.K., Aynekulu E., Chomba S., Degrande A., Fornace K., et al. 2019a. A planetary health perspective on agroforestry in Sub-Saharan Africa. *One Earth*, 1, 3, 330–344. <https://doi.org/10.1016/j.oneear.2019.10.017>
- Siles J.A., Cajthaml T., Filipová A., Minerbi S., et al. 2017. Succession of bacterial and fungal communities during initial decomposition of Norway spruce deadwood. *Scientific Reports*, 7: 7339. <https://doi.org/10.1038/s41598-017-07351-x>
- Siles J.A., Margesin R. 2016. Abundance and diversity of bacterial, archaeal, and fungal communities along an altitudinal gradient in Alpine forest soils: What are the driving factors? *Microbial Ecology*, 72: 207–220. <https://doi.org/10.1007/s00248-016-0748-2>
- Urbanová M., Šnajdr J., Baldrian P. 2015. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biology and Biochemistry*, 84: 53–64. <https://doi.org/10.1016/j.soilbio.2015.02.011>
- Unuk Nahberger T., Martinović T., Finžgar D., Šibanc N., Grebenc T., Kraigher H. 2019. Root-associated fungal communities from two phenologically contrasting Silver Fir (*Abies alba* Mill.) groups of trees. *Frontiers in Plant Science*, 10, 214. <https://doi.org/10.3389/fpls.2019.00214>
- Uroz S., Ioannidis P., Lengelle J., Cébron A., Morin E., Buée M., et al. 2013. Functional assays and metagenomic analyses reveal differences between the microbial communities inhabiting the soil horizons of a Norway spruce plantation. *PLoS one*, 8, 2, e55929. <https://doi.org/10.1371/journal.pone.0055929>
- Uroz S., Oger P., Tisserand E., et al. 2016. Specific impacts of beech and Norway spruce on the structure and diversity of the rhizosphere and soil microbial communities. *Scientific Reports*, 6, 27756. <https://doi.org/10.1038/srep27756>
- Van Der Heijden M.G.A., Bardgett R.D., Van Straalen N.M. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11, 296–310.

<https://doi.org/10.1111/j.1461-0248.2007.01139.x>

Vasar M., Davison J., Sepp S.-K., Mucina L., Oja J., Al-Quraishy S., Anslan S., Bahram M., Bueno G., Cantero J., Decocq G., Fraser L., Hiiesalu I., Hodge A., Kohout P., Kukkurainen T., McKenzie S., Miettinen A., van der Heijden M.G.A., Tedersoo L. 2022. Global factors driving soil fungal diversity. *Journal of Biogeography*, 49, 4: 749–768. <https://doi.org/10.1111/jbi.14395>

Wang Q., Garrity G.M., Tiedje J.M., Cole J.R. 2007. Naïve Bayesian classifier for rapid

assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, 73: 5261–5267. <https://doi.org/10.1128/AEM.00062-07>

Yuan Z., Liu J., Wang Y., Li M., Wen Z., Li W., et al. 2021. Relationships between the structural diversity and functional diversity of trees in a subtropical forest of China. *Ecological Indicators*, 131, 108169. <https://doi.org/10.1016/j.ecolind.2021.108169>

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 | 0.31 | 0.54 | 0.787 |
| 8_Beskidy-1_Gorj | 0.807 | 0.38 | 0.261 |
| 3_Buzau-2_Arges | 0.459 | 0.59 | 0.719 |
| 4_Vrancea-2_Arges | *0.037 | 0.133 | 0.264 |
| 5_Neamt-2_Arges | *0.036 | 0.18 | 0.356 |
| 6_Suceava-2_Arges | *0.049 | 0.14 | 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 | 0.99 | 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 | *0.01 | *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 enosmernege 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 | 0.0958 | 0.0946 |

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 | R2 | 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 | | |