

Assessing soil biodiversity in different land uses of the Nevesinje Field

Vid NAGLIČ

ABSTRACT

This study investigates soil biodiversity in agricultural landscapes and focuses on the Nevesinje field in Bosnia and Herzegovina, which was sampled as part of the Neretva Science Week 2023. Soil microarthropods, including mites and springtails, play an important role in ecosystem health through nutrient cycling and organic matter decomposition. Using separate approaches — morphological techniques with the QBS-ar index and metabarcoding of DNA extracted from soil samples — we investigated the diversity and abundance of soil microarthropods at different sites. Our results showed that the arable field had the highest abundance of soil microarthropods, especially Acarina and Collembola, while the orchard had the lowest abundance but the highest QBS-ar value, which reflects higher soil quality according to the QBS-ar index. Insects (Insecta) dominate with 68.6% of identified taxa, followed by arachnids (Arachnida) with 20.2%. The arable field next to the Zalomka River showed the highest taxonomic richness. The area faces imminent threats from the proposed dam construction, which could alter the hydrological dynamics and integrity of the habitat. Continued monitoring and further sampling are important to assess the ecological impact and develop conservation strategies to maintain soil biodiversity amid developmental pressures.

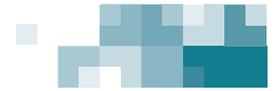
IZVLEČEK

Ocenjevanje biodiverzitete različnih tipov rabe tal na Nevesinjskem polju

Ta študija raziskuje biotsko raznovrstnost tal v kmetijski krajini Nevesinjskega polja v Bosni in Hercegovini, ki je bilo vzorčeno v okviru Neretva Science Week 2023. Talni mikronevretenčarji, vključno s pršicami in skakači, imajo pomembno vlogo pri zdravju ekosistema z vplivom na kroženje hranil in razgradnjo organske snovi. Z dvema različnima pristopoma, morfološkimi tehnikami z indeksom QBS-ar in metabarkodiranjem DNK iz talnih vzorcev, smo raziskali raznolikost in številčnost talnih organizmov na različnih lokacijah. Naši rezultati so pokazali, da je imela njiva največjo številčnost talnih mikronevretenčarjev, zlasti Acarina in Collembola, medtem ko je imel sadovnjak najmanjšo številčnost, vendar najvišjo vrednost QBS-ar, kar kaže na visoko kakovost tal glede na QBS. Taksonomska analiza DNK je pokazala prevlado žuželk (Insecta) s 68,6 %, z 20,2 % so pomembno prispevali še pajkovci (Arachnida). Njiva ob reki Zalomki je bila najbolj taksonomsko pestra od vseh zbranih vzorcev. To območje se sooča z neposrednimi grožnjami zaradi predlagane gradnje jezusa, ki bi lahko spremenila hidrološko dinamiko in celovitost habitata. Stalno spremljanje in nadaljnje vzorčenje je pomembno za oceno ekološkega vpliva in razvoj strategij za ohranjanje biotske raznovrstnosti tal v luči razvojnih pritiskov.

Agricultural institute of Slovenia,
Hacquetova ulica 17, SI-1000 Ljubljana,
Slovenia; E-mail: vid.naglic@kis.si





APSTRAKT

Procjena biodiverziteta različitih tipova korištenja zemljišta u Nevesinjskom polju

Ova studija istražuje biodiverzitet zemljišta u poljoprivrednom području Nevesinjskog polja u Bosni i Hercegovini, koji je proučavan u okviru Sedmice nauke Neretva 2023. Zemljišni mikroartropodi, uključujući grinje i skokune, igraju važnu ulogu u održanju zdravih ekosistema utičući na kruženje hranjivih materija i razgradnju organske materije. Koristeći dva različita pristupa, morfološke tehnike s QBS-ar indeksom i metabarkodiranje DNK iz uzoraka zemljišta, istražili smo raznolikost i brojnost organizama u zemljištu na različitim lokacijama. Naši rezultati su pokazali da je obradivo zemljište imalo najveću zastupljenost mikroinvertebrata, posebno Acarina i Collembola, dok je voćnjak imao najmanju zastupljenost, ali najvišu QBS-ar vrijednost, što ukazuje na visok kvalitet zemljišta prema QBS indeksu. Insekti (Insecta) dominiraju sa 68,6 % identifikovanih taksona, a slijede ih paukoliki zglavkari (Arachnida) sa 20,2 %. Obradivo zemljište uz rijeku Zalomku bilo je taksonomski najraznovrsnije od svih prikupljenih uzoraka. Ovo područje se suočava sa neposrednim prijetnjama zbog predložene izgradnje brane, koja bi mogla promijeniti hidrološku dinamiku i integritet staništa. Kontinuirano praćenje i dalje uzorkovanje su važni za procenu ekološkog uticaja i razvoj strategija zaštite kako bi se održao biodiverzitet zemljišta usled razvojnih pritisaka.

KEY WORDS:

soil microarthropods, DNA metabarcoding, QBS-ar, mesofauna, Upper Horizons project

KLJUČNE BESEDE:

talni mikronevretenčarji, DNA metabarkodiranje, QBS-ar, mezofavna, projekt Gornji Horizonti

KLJUČNE RIJEČI:

mikroartropodi u zemljištu, DNK metabarkodiranje, QBS-ar, mezofauna, projekat Gornji Horizonti

INTRODUCTION

Soil microarthropods are crucial for maintaining soil health and play a key role in ecosystem stability through processes such as nutrient cycling and organic matter decomposition (Wall & Lynch 2000; Coleman et al. 2004). Despite their importance, these small soil organisms are often overlooked when assessing soil quality (Parisi et al. 2005). In agricultural landscapes, especially those exposed to anthropogenic pressures, the diversity and abundance of soil microarthropods can serve as important indicators of ecosystem health and resilience (Menta 2012; Postma-Blaauw et al. 2010). The Nevesinje field in Bosnia and Herzegovina is an agriculturally important region that mostly features traditional extensive type of agriculture. That area could soon experience significant environmental changes due to the planned construction of a dam (Gallop 2023). Such developments may lead to changes in land use and potentially affect soil biodiversity (Foley et al. 2005). In order to assess the potential ecological impact of such infrastructure projects, it is important to understand the current biodiversity of soil organisms in this region. The aim of our research was to assess the biodiversity of soil microarthropods in the Nevesinje field using two complementary approaches: morphological analysis with the QBS-ar index and DNA metabarcoding (defined here as DNA extracted from homogenized bulk soil samples without prior removal of soil organisms, also referred to as eDNA). The former provides a qualitative assessment of soil biological quality based on the presence of soil-adapted microarthropods (Parisi et al. 2005), while the latter provides a detailed genetic insight into the soil community and identifies organisms that may be missed by conventional microscopy (Taberlet et al. 2012, 2018). Morphological analysis is highly time-consuming, requir-

ing careful extraction and identification of microarthropods, which limited the number of samples that could be processed. Due to time constraints, we were only able to collect morphological samples from three sites, while DNA metabarcoding was applied to samples from six sites. We hypothesized that the agricultural land of the Nevesinje field would harbor a rich and diverse soil fauna due to its habitat diversity (Wardle et al. 2004). By combining traditional morphological techniques with modern genetic tools, this study aims to provide a basic understanding of soil biodiversity in this little-studied region.

MATERIAL AND METHODS

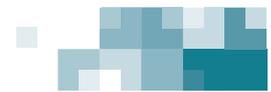
STUDY AREA AND SAMPLING

Soil samples were collected from six sites within the Nevesinje field, Bosnia and Herzegovina, representing different types of agricultural use. They included a meadow, an orchard, an arable field, a riparian habitat, an arable field near Zalomka river, and an oak forest. These locations were chosen on the basis of land use and proximity to the proposed dam construction site.

GPS coordinates were recorded for each sampling location:

- Meadow: N 43.184816, E 18.160509 (QBS, DNA)
- Orchard: N 43.179056, E 18.158028 (QBS, DNA)
- Arable field: N 43.178778, E 18.157995 (QBS, DNA)
- Riparian habitat near Zalomka river: N 43.210843, E 18.221617 (DNA)
- Arable field near Zalomka river: N 43.211345, E 18.222782 (DNA)
- Oak forest: N 43.185420, E 18.121981 (DNA)





Sampling was conducted over two days (5. and 6. 6. 2023), approximately at the same time of the day. Descriptions of sampling locations are included in the supplementary material. Samples for morphological analysis (QBS-ar) were taken at three sites (arable field, meadow, orchard). At each, three subsamples of soil were taken from the top 10 cm of the soil profile using a soil corer with a diameter of 11.3 cm. Samples for DNA analysis were collected at six sites. On each site, three subsamples for DNA analysis were collected, using the same sampling technique as for the QBS. Subsamples were joined and manually homogenized in the field. Both the QBS and DNA samples were stored in a cool box immediately after collection to preserve their integrity. Three days after sampling, the QBS samples were processed using a Kempson extractor with the organisms heat-extracted and stored in ethanol, while the DNA samples were frozen until subsequent analysis.

MORPHOLOGICAL ANALYSIS

Morphological analysis of soil samples was performed using the QBS-ar (Biological Soil Quality Index) method (Parisi et al. 2005). This index assesses soil biological quality by examining the presence of soil microarthropods with morphological adaptations to life in the soil. The organisms were identified to class or order level under a stereomicroscope, and a combined value of QBS-ar was calculated for each site based on three collected samples. The QBS-ar index is calculated as the sum of the Ecomorphological Index (EMI) values assigned to taxa identified in the sample. EMI values range from 1 to 20, with higher values assigned to taxa with greater adaptation to soil life. In addition, the abundance of animals was recorded in all subsamples.

DNA EXTRACTIONS FROM SOIL, LIBRARY PREPARATIONS, SEQUENCING AND BIOINFORMATIC ANALYSIS

Six composite samples underwent DNA analysis using the DNeasy PowerLyzer PowerSoil isolation kit (Qiagen, Denmark). Approximately 40 ml of soil per sample was used. Soil samples were freeze-dried for 72 hours and homogenized with a Bead Ruptor Elite (Omni International) at 4 m s^{-1} for 30 seconds, the process was repeated three times. For the PowerSoil extractions, 0.25 g of homogenized soil was used, following the manufacturer's instructions. DNA concentrations were quantified using a Qubit 4.0 Fluorometer (Invitrogen, USA). The COI region was amplified with mCOLintF/jgHCO2198 primers (Geller et al. 2013) using a two-step PCR and dual indexing for Illumina MiSeq sequencing. Final PCR products were cleaned with HighPrep™ magnetic beads (MagBio Genomics Inc.) and pooled for sequencing. Illumina reads were processed in QIIME2 (v2020.10.0) (Bolyen et al. 2019), where primer sequences were trimmed, and reads truncated after 230 base pairs. Reads were filtered, denoised, merged, and dereplicated using DADA2 (Callahan et al. 2016), producing Amplicon Sequence Variants (ASVs). Details about the number of reads before and after processing are included in the supplementary material. Taxonomic classification was performed via BLAST against the

NCBI database (Sayers et al. 2022). ASVs associated with the thirty-three taxonomic groups used for QBS-ar index identification were retained, including soil microarthropods (e.g. Acari, Collembola), myriapods (Diplopoda, Chilopoda, Pauropoda, Symphyla), isopods, insects (Coleoptera, Hymenoptera, Diptera), and other soil-dwelling arthropods. Data analysis and visualization were conducted in Python, version 3.13.

RESULTS AND DISCUSSION

We morphologically identified individuals in the soil samples to the level of morphospecies and counted cumulative abundance of important classes, number of EMI groups and QBS-ar index, for three sites. The arable field and orchard had QBS-ar values higher than 93.7 (Tab. 1), the tentative threshold value to distinguish high-quality soils (Menta et al. 2018), while the meadow had a lower QBS-ar value (Tab. 1). Based on morphological identification, the arable field had the most EMI groups, followed by the meadow and the orchard (Tab. 1). The arable field had the highest cumulative abundance (absolute counts of individuals) of soil microarthropods, especially mites (Acarina) and springtails (Collembola) (Fig. 1), while meadow and orchard had lower cumulative abundances.

Table 1. QBS-ar index and number of ecomorphological (EMI) groups for each site.

Tabela 1. Indeks QBS-ar in število ekomorfoloških (EMI) skupin za vsako lokacijo.

Tabela 1. QBS-ar indeks i broj ekomorfoloških (EMI) grupa za svaki lokalitet.

Site	QBS-ar	Number of EMI groups
Arable Field	102	14
Orchard	106	11
Meadow	86	12

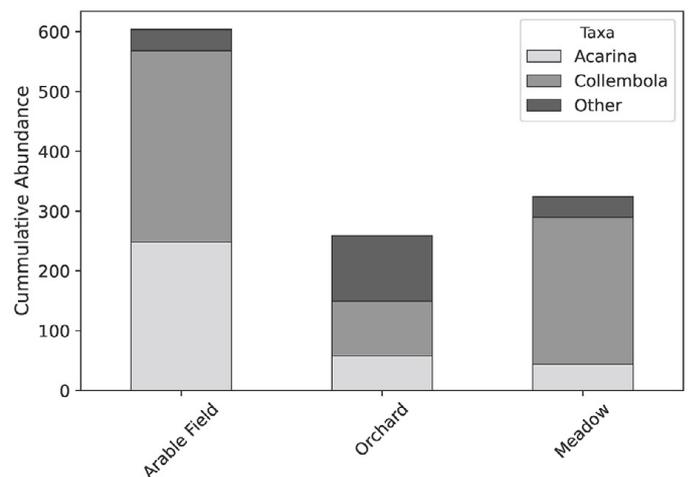
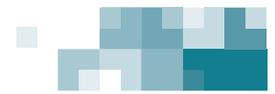


Figure 1. Cumulative abundance of soil microarthropods for each site.

Slika 1. Kumulativna številčnost talnih mikronevretenčarjev za vsako lokacijo.

Slika 1. Kumulativna brojnost mikroartropoda u tlu za svaki lokalitet.





We acknowledge that statistical comparison between different localities cannot be made, since we do not have biological replicates. However, the first results are in congruence with our expectations for all analysed types of soil. For example, low QBS-ar value of the meadow can be explained with lower plant diversity and soil compaction due to grazing or use of machinery, which can have a negative effect on the microarthropod communities in the soil, resulting in lower diversity and abundance of soil microarthropods (Wardle et al. 2004; Beylich et al. 2010). The reason could also be the low organic matter content in the soil, which may limit the availability of food resources necessary to sustain a diverse and abundant microarthropod community, although this was not directly tested as no physico-chemical analysis of the soil was conducted. Overall, first insights based on QBS-ar index point to the fact that agricultural land of the Nevesinje field indeed harbors a rich and diverse soil fauna.

Next, we employed the DNA metabarcoding approach to analyse the taxonomic composition of soil microarthropods at six different sites. We filtered out ASVs associated with fauna groups used in QBS-ar index and analysed taxon richness (Fig. 2) and relative abundance of most represented classes and orders (Fig. 3).

Taxon richness for each site was calculated as the number of unique ASVs identified and assigned to analysed taxonomic groups, for each site (Fig. 2). The agricultural lands had higher taxon richness of soil related taxa than natural habitats. These results are preliminary and based on a single sampling event, yet they align with several studies, that showed that agricultural practices, particularly in arable fields, may promote soil biodiversity by increasing organic matter and creating diverse microhabitats, which are known to increase the diversity of soil microarthropods (Mäder et al. 2002; Wardle et al. 2004). In contrast, the riparian habitat and the meadow had lower taxon richness, probably due to specific habitat characteristics that limit the occurrence of certain soil taxa. Riparian zones, although dynamic, can also have fluctuating moisture levels that may limit the diversity of certain soil fauna (Naiman & Décamps 1997). Meadows, on the other hand, may have lower taxon richness due to factors already described in the first part of the results chapter: we obtained similar results by means of morphological identification with the meadow having the lowest QBS-ar score out of the three sampled sites. These patterns we observe may also reflect the fact that certain habitats (i.e. those used for QBS assessment) inherently contain fewer taxa that were included in our analysis. Additionally, limitations in

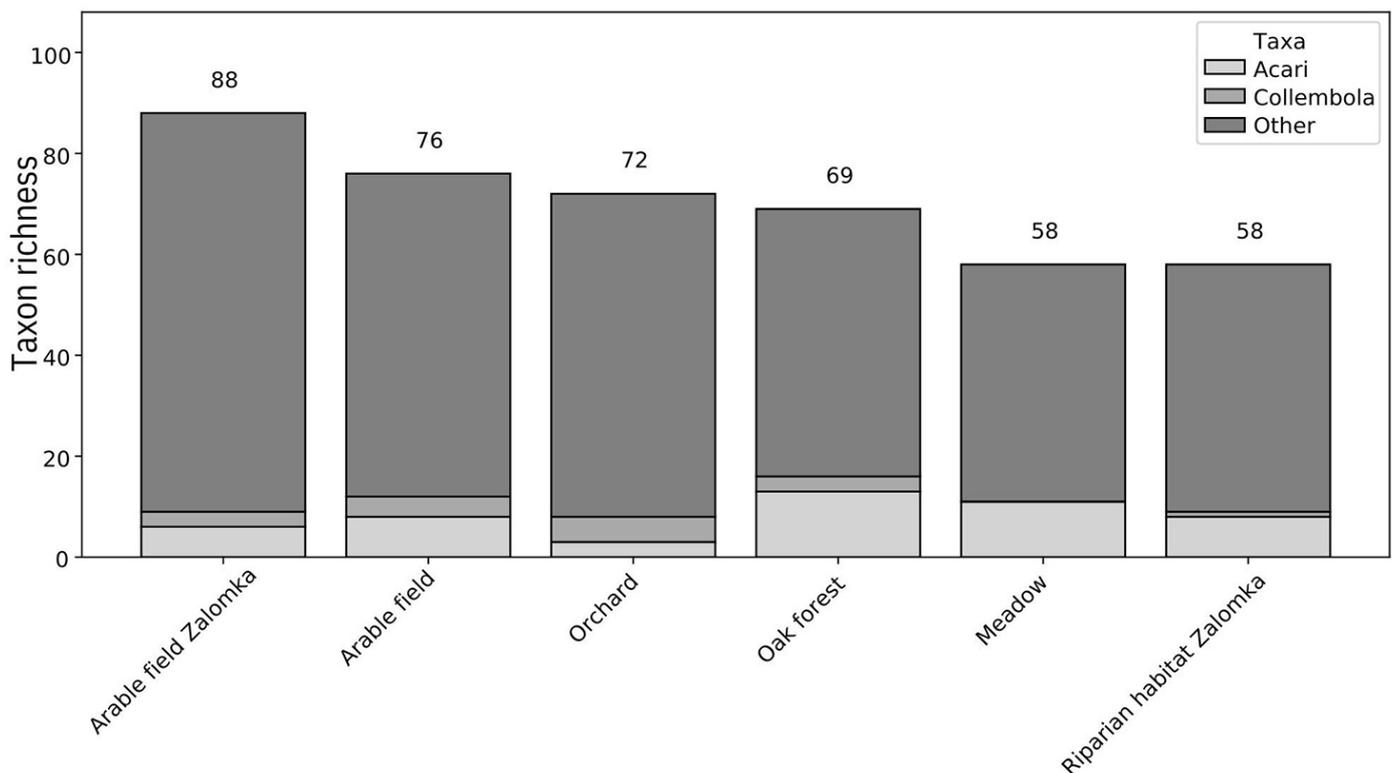


Figure 2. Taxon richness across various habitats. The numbers above each bar represent the total taxon richness (calculated as the number of unique ASVs identified) for each habitat. The bar colors indicate the proportional contributions of Acari (light gray), Collembola (dark gray), and other taxa (black) to the total richness.

Slika 2. Taksonomska pestrost v različnih habitatih. Številke nad posameznimi stolpci predstavljajo skupno taksonomsko pestrost (izraženo kot število unikatnih ASV-jev, ki so bili identificirani) za vsak habitat. Barve stolpcev označujejo proporcionalni prispevek pršic (Acari) (svetlo siva), skakačev (Collembola) (temno siva) in drugih taksonov (črna) k skupni pestrosti.

Slika 2. Taksonomsko bogastvo vrstama u različitim staništima. Brojevi iznad svake kolone predstavljaju ukupnu taksonomsko bogastvo (izraženo kao broj jedinstvenih ASV-ova koji su identifikovani) za svako stanište. Boje u stupcima označavaju proporcionalni doprinos grinja (Acari) (svijetlosiva), skokuna (Collembola) (tamnosiva) i ostalih taksona (crna) ukupnoj raznolikosti.



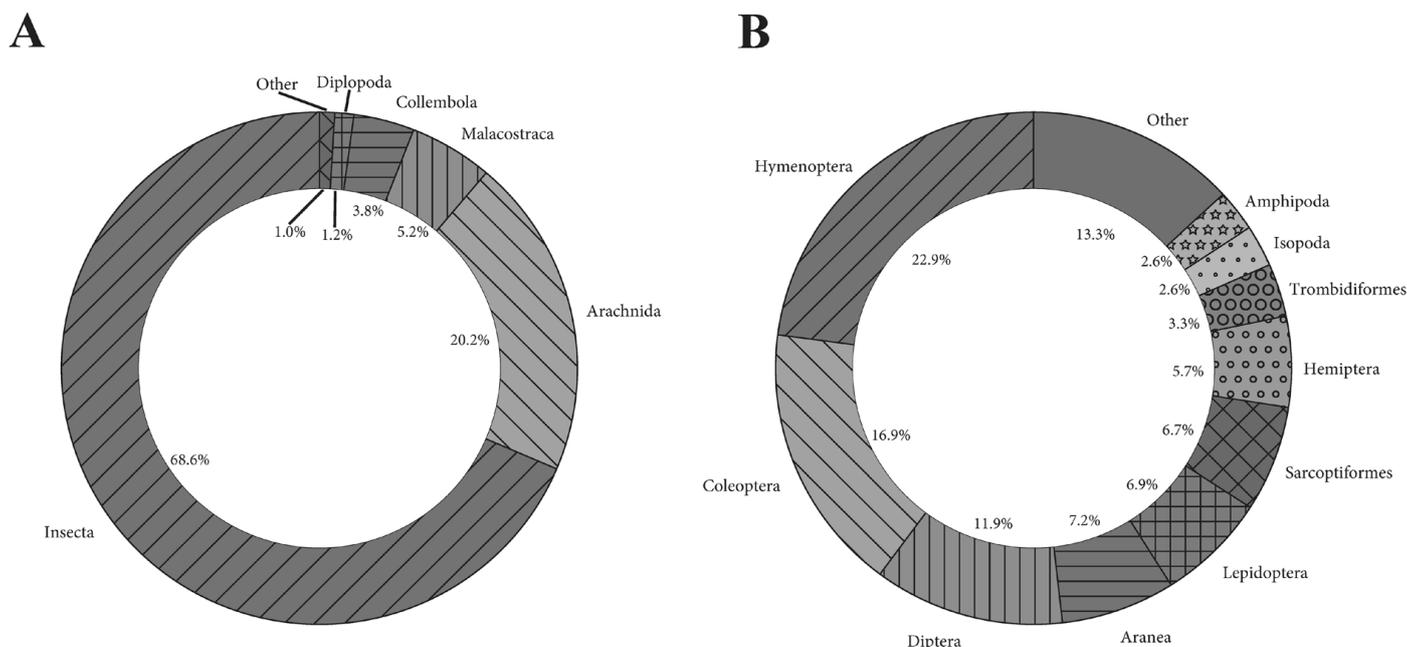


Figure 3. Relative abundance of taxonomic classes (A) and orders (B) of soil microarthropods across all sampling sites.

Slika 3. Relativna številčnost taksonomskih razredov (A) in redov (B) talnih mikronevretenčarjev na vseh vzorčnih mestih.

Slika 3. Relativna brojnost taksonomskih klasa (A) i redova (B) mikroartropoda u zemljištu na svim mjestima uzorkovanja.

taxonomic databases, particularly for some microarthropod groups, or the relatively low number of our samples, combined with the rapid, non-replicated sampling approach, could have influenced the observed richness patterns. With the given time constraints, this was the most comprehensive assessment we could achieve.

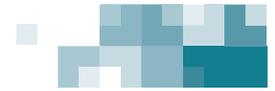
The taxonomic composition of soil invertebrates derived from the DNA metabarcoding analysis for different habitats is shown in the pie charts in Fig. 3. The left-hand diagram (Fig. 3A) shows the distribution of ASVs across the five most represented classes. Insects (Insecta) account for 68.6% of the ASVs identified, followed by arachnids (Arachnida) with 20.2%. Other classes, such as Malacostraca, Collembola, and Diplopoda, were represented at lower percentages. The diagram on the right (Fig. 3B) shows the distribution of ASVs assigned to the ten most represented orders in our dataset. Hymenoptera accounted for 22.9% of the identified ASVs, followed by Coleoptera with 16.9%. It is important to note that these percentages represent the proportion of ASVs classified within each order, not the actual abundance of organisms in the soil community. The observed percentages reflect the taxonomic resolution achieved through DNA metabarcoding, which depends on reference databases and the biases associated with PCR amplification.

When comparing these results with morphological identification, it is important to note that the two different approaches – morphology-based abundance and DNA-based diversity of soil microarthropods – do not necessarily correlate. Nevertheless, we find that two orders of mites (Sarcotiformes and Trombidiformes) are among the most represented orders in our DNA analysis, and they were also abundant in morphological analysis. In contrast, Collembola were among the most abundant taxa in the morphological results but was only represented at the class level in the DNA data. Different Collembola orders

were included in the »Other« category in Fig. 3B because their relative representation among the identified ASVs was insufficient to rank them among the top ten orders. We also found that many Collembola sequences could not be identified at a higher taxonomic resolution, possibly due to the limited capabilities of the reference databases for Collembola in the soil. This highlights the challenges in metabarcoding studies associated with incomplete reference libraries, which can hinder accurate taxonomic assignment (Porco et al. 2013). Improving these databases is crucial to increase the resolution and reliability of metabarcoding in soil biodiversity assessment.

The results should be interpreted as the first qualitative observations in the area rather than definitive conclusions about soil diversity. This is a snapshot study based on a single sampling event, which does not account for biological replicates, temporal variability or the full extent of soil biodiversity. However, it offers the first insight into soil diversity of this remote, understudied and critically endangered area. The results highlight the ecological significance of the study area, suggesting that the relatively high soil quality in agricultural lands may be attributed to a combination of extensive farming and traditional agricultural practices. This biodiversity is threatened by the planned dam construction, which could disrupt hydrological patterns, destroy and fragment habitats and ultimately reduce the richness and abundance of the soil microarthropods. Fast changes in water flow and habitat structure could lead to the loss of important microhabitats. Given the potential impact on this fragile ecosystem, continuous sampling is essential to monitor these changes and develop strategies to protect soil biodiversity in the face of development pressure. This study represents the first attempt to assess the quality of soil, using a combination of traditional and DNA techniques in this area. We hope that it will shed some light on this hidden yet important ecosystem, which is tightly connected with local traditions and human use of the landscape.





POVZETEK

V raziskavi smo preučevali biotsko raznovrstnost talnih organizmov na območju Nevesinjskega polja v Bosni in Hercegovini, kjer načrtovana gradnja jezua predstavlja potencialno grožnjo za tamkajšnji ekosistem. Zbrali smo vzorce tal na šestih različnih lokacijah, ki vključujejo dve njivi, sadovnjak, travnik, obrežni habitat ob reki Zalomki in hrastov gozd. Raziskava je temeljila na dveh pristopih: morfološki analizi preko QBS-ar indeksa ter DNA metabarkodiranju, kar nam je omogočilo vpogled v genetsko pestrost talnih organizmov. Morfološki rezultati kažejo, da je bila največja številčnost talnih mikronevretenčarjev, predvsem pršic (Acarina) in skakačev (Collembola), zaznana na njivi, medtem ko je imel sadovnjak najmanjšo številčnost, a najvišji QBS-ar indeks (106), ki nakazuje na visoko biološko kakovost tal. Nasprotno je bil QBS-ar indeks na njivi 102, na travniku pa 86. Z metabarkodiranjem smo največjo taksonomsko pestrost mikronevretenčarjev zaznali na njivi ob reki Zalomki, kar kaže na bogato biotsko raznovrstnost tega območja,

SAŽETAK

Tokom istraživanja smo proučavali biodiverzitet organizama u zemljištu na području Nevesinjskog polja u Bosni i Hercegovini, gdje planirana izgradnja brane predstavlja potencijalnu prijetnju lokalnim ekosistemima. Uzorke zemljišta prikupili smo na šest različitih lokacija, uključujući dva obradiva polja, voćnjak, livadu, priobalno stanište uz rijeku Zalomku i hrastovu šumu. Istraživanje je zasnovano na dva pristupa: morfološkoj analizi putem QBS-ar indeksa i DNK metabarkodiranju, što nam je omogućilo da steknemo uvid u genetsku raznolikost organizama u zemljištu. Morfološki rezultati pokazuju da je najveća brojnost mikroartropoda u zemljištu, uglavnom grinja (Acarina) i skokuna (Collembola), otkrivena na polju, dok je voćnjak imao najmanju brojnost, ali najviši QBS-ar indeks (106), što ukazuje na visok biološki kvalitet tla. Nasuprot tome, QBS-ar indeks na polju je bio 102, a na livadi 86. Korištenjem metabarkodiranja, detektovali smo najveću taksonomsku raznolikost mikroinvertebrata na polju uz rijeku Zalomku, što ukazuje

medtem ko sta travnik in obrežni habitat pokazala nižjo pestrost. Taksonomska analiza DNA je razkrila prevlado žuželk (Insecta), ki so predstavljale 68,6 % vseh prepoznanih taksonov, z znatno prisotnostjo pajkovcev (Arachnida) (20,2 %). Med najbolj pogostimi redovi so bili kožekrilci (Hymenoptera) in hrošči (Coleoptera). Red Collembola je bil vključen v analizo, vendar njihova relativna številčnost taksonomskih skupin ni dosegla prve deseterice kljub temu, da so bile glede na morfološke rezultate vsaj v nekaterih vzorcih najštevilčnejše, kar nakazuje na potrebo po izboljšanih podatkovnih bazah za njihovo natančnejšo identifikacijo. Ti rezultati kažejo na pomembno biotsko raznovrstnost Nevesinjskega polja, ki pa je zaradi načrtovane gradnje jezua resno ogrožena. Nadaljnje spremljanje stanja in dodatno vzorčenje sta ključnega pomena za oceno dolgoročnih ekoloških posledic in za razvoj strategij, ki bodo zagotovile ohranitev talne biotske raznovrstnosti v tem občutljivem okolju.

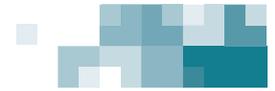
na bogatu biodiverzitet ovog područja, dok su livadsko i priobalno stanište pokazali nižu raznolikost. Taksonomska analiza DNK otkrila je dominaciju insekata (Insecta), koji su predstavljali 68,6% svih prepoznatih taksona, sa značajnim prisustvom paučnjaka (Arachnida) (20,2%). Među najčešćim redovima bili su Hymenoptera i Coleoptera. Red Collembola je uključen u analizu, ali njihova relativna brojnost taksonomskih grupa nije dostigla prvih deset uprkos tome što su bili najbrojniji prema morfološkim rezultatima u barem nekim uzorcima, što ukazuje na potrebu za poboljšanim bazama podataka za njihovu precizniju identifikaciju. Ovi rezultati ukazuju na važan biodiverzitet Nevesinjskog polja, koji je ozbiljno ugrožen planiranom izgradnjom brane. Daljnje praćenje i dodatno uzorkovanje ključni su za procjenu dugoročnih ekoloških posljedica i razvoj strategija koje će osigurati očuvanje biodiverziteta tla u ovom osjetljivom okruženju.

ACKNOWLEDGEMENTS

I would like to thank Špela Di Batista Borko for inviting me to participate in the Neretva Science Week, Marija Kravanja for her invaluable assistance during the fieldwork in Bosnia, Irena Bertonec for the introduction to the field of soil biodiversity and the Agricultural Institute of Slovenia for funding this research. I also extend my gratitude to the Environmental Micro-

biology section at the Department of Environmental Science, Aarhus University, for their support and collaboration. I would also like to acknowledge the Research Infrastructure ELIXIR-SI (<https://elixir-slovenia.org>), funded by the European Regional Development Fund, the Ministry of Science, Education and Sports, and the Slovenian Research and Innovation Agency, which provided me with the computing resources needed for the bioinformatics analysis.

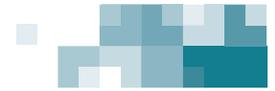




REFERENCES

- Beylich A, Oberholzer H-R, Schrader S, Höper H, Wilke B-M. 2010. Evaluation of soil compaction effects on soil biota and soil biological processes in soils. *Soil Tillage Research*. 109(2): 133-143. <https://doi.org/10.1016/j.still.2010.05.010>
- Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*. 37(8): 852-857. <https://doi.org/10.1038/s41587-019-0209-9>
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*. 13(7): 581-583. <https://doi.org/10.1038/nmeth.3869>
- Coleman DC, Crossley Jr. DA, Hendrix PF. 2004. *Fundamentals of Soil Ecology*. 2nd ed. Burlington, MA: Elsevier Academic Press.
- Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR, Chapin FS, Coe MT, Daily GC, Gibbs HK, et al. 2005. Global consequences of land use. *Science*. 309(5734): 570-574. <https://doi.org/10.1126/science.1111772>
- Gallop P. 2023. The Upper Horizons complex, Bosnia and Herzegovina. Prague (CZ): Bankwatch Network; [accessed 10. 9. 2024]. <https://bankwatch.org/publication/the-upper-horizons-complex-bosnia-and-herzegovina>
- Geller J, Meyer C, Parker M, Hawk H. 2013. Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*. 13(5): 851-861. <https://doi.org/10.1111/1755-0998.12138>
- Mäder P, Fliessbach A, Dubois D, Gunst L, Fried P, Niggli U. 2002. Soil fertility and biodiversity in organic farming. *Science*. 296(5573): 1694-1697. <https://doi.org/10.1126/science.1071148>
- Menta C. 2012. Soil fauna diversity—Function, soil degradation, biological indices, soil restoration. In: Larramendy ML, Soloneski S, editors. *Biodiversity Conservation and Utilization in a Diverse World*. Rijeka (HR): InTech. p. 59-94.
- Menta C, Conti FD, Pinto S, Bodini A. 2018. Soil Biological Quality index (QBS-ar): 15 years of application at global scale. *Ecological Indicators*. 85: 773-780. <https://doi.org/10.1016/j.ecolind.2017.11.030>
- Naiman RJ, Décamps H. 1997. The ecology of interfaces: Riparian zones. *Annual Review of Ecology and Systematics*. 28: 621-658. <https://doi.org/10.1146/annurev.ecolsys.28.1.621>
- Parisi V, Menta C, Gardi C, Jacomini C, Mozzanica E. 2005. Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy. *Agriculture, Ecosystems & Environment*. 105(1-2): 323-333. <https://doi.org/10.1016/j.agee.2004.02.002>
- Porco D, Skarżyński D, Decaëns T, Hebert PDN, Deharveng L. 2013. Barcoding the Collembola of Churchill: A molecular taxonomic reassessment of species diversity in a sub-Arctic area. *Molecular Ecology Resources*. 13(6): 1132-1137. <https://doi.org/10.1111/1755-0998.12172>
- Postma-Blaauw MB, de Goede RGM, Bloem J, Faber JH, Brussaard L. 2010. Soil biota community structure and abundance under agricultural intensification and extensification. *Ecology*. 91(2): 460-473.
- Sayers EW, Bolton EE, Brister JR, Canese K, Chan J, Comeau DC, Connor R, Funk K, Kelly C, Kim S, et al. 2022. Database resources of the national center for biotechnology information. *Nucleic Acids Research*. 50(D1): D20-D26. <https://doi.org/10.1093/nar/gkab1112>
- Taberlet P, Coissac E, Pompanon F, Brochmann C, Willerslev E. 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*. 21(8): 2045-2050. <https://doi.org/10.1111/j.1365-294X.2012.05470.x>
- Taberlet P, Bonin A, Zinger L, Coissac E. 2018. *Environmental DNA: For Biodiversity Research and Monitoring*. Oxford (UK): Oxford University Press.
- Wall D, Lynch JM. 2000. Soil biodiversity and ecosystem functioning. In: Balázs E, Galante E, Lynch JM, Schepers JS, Toutant J-P, Werner D, Werry PATHJ, editors. *Biological resource management connecting science and policy*. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-662-04033-1_24
- Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH. 2004. Ecological linkages between aboveground and belowground biota. *Science*. 304(5677): 1629-1633. <https://doi.org/10.1126/science.1094875>





SUPPLEMENTARY MATERIAL

DESCRIPTION OF LOCATIONS

The meadow was a hay meadow with extensive management, showing no visible signs of recent fertilization or heavy grazing. The arable field had been recently ploughed, with no crops present at the time of sampling. The orchard consisted of *Prunus* trees, with a high undergrowth of various grasses and other vegetation, suggesting a less intensively managed system. The riparian habitat near the Zalomka River had a rocky soil structure with scarce vegetation, likely due to occasional flooding that limits plant establishment. The arable field near the Zalomka River was similarly ploughed at the time of sampling, with no visible crops. The oak forest consisted primarily of deciduous oak trees, with a well-developed litter layer and relatively sparse undergrowth, indicating a stable, less-disturbed environment.

Table S1. Sequence processing summary for DNA metabarcoding analysis. Details of the sequence processing pipeline, including the number of raw sequences, sequences retained after quality filtering, denoising, merging, and chimera checking, as well as their respective percentages, for each sample.

Site	Input	Filtered	% Input Passed Filter	Denoised	Merged	% Input Merged	Non-chimeric	% Input Non-chimeric
Oak forest	52599	47691	90.67	44200	36983	70.31	35578	67.64
Meadow	90556	82478	91.08	79486	70565	77.92	60280	66.57
Arable field near Zalomka river	68107	58259	85.54	55795	49413	72.41	47350	69.52
Riparian habitat near Zalomka river	36063	30273	83.94	28275	25423	70.5	24761	68.66
Arable field	76867	65772	85.57	62432	56006	72.86	54729	71.2
Orchard	41986	35864	85.42	33882	30662	73.03	30132	71.77



Vsebinska je dostopna pod pogoji licence Creative Commons Priznanje avtorstva 4.0 Mednarodna (CC BY 4.0), ki omogoča neomejeno uporabo, razširjanje in reproduciranje v kateremkoli mediju ali obliki, pod pogojem, da sta ustrezno navedena izvorni avtor in vir.

The content is distributed under the terms of the Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

