Does the substrate on which cryptogams grow matter for limno-terrestrial meiofauna?

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Abstract: Cryptogam habitats support a wide range of limno-terrestrial meiofauna, but the factors that shape their communities are still not well understood. The physical substrate that cryptogams grow on (e.g., soil, the base of a tree, or its trunk) can influence local moisture, temperature, and nutrient conditions, yet its role in structuring meiofaunal assemblages has rarely been tested systematically. We examined this question in montane forests of the Ukrainian Carpathians, using DNA metabarcoding to compare the diversity and composition of tardigrade and rotifer communities across substrates. We expected that rotifers, with shorter life cycles and often higher population densities, would respond more strongly to environmental differences among substrates, whereas tardigrades would be less affected and more influenced by chance colonization. Our results supported these expectations. Rotifer richness and community composition varied across substrates, while tardigrade assemblages showed weaker and more variable responses. Analysis of phylogenetic diversity indicated phylogenetic clustering in rotifers, suggesting that closely related species have similar ecological preferences. Despite these contrasts, both groups showed signs of largely stochastic community assembly, with species turnover dominating differences among samples. Overall, our findings suggest that meiofaunal communities in cryptogam-associated habitats are shaped mainly by random colonization events, modulated by subtle and taxon-specific effects of microhabitat structure and vertical positioning.

Keywords: DNA metabarcoding; limno-terrestrial meiofauna; microhabitat filtering; phylogenetic diversity; Rotifera; Tardigrada;

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1. Introduction

Meiofauna is often defined as small-sized organisms that inhabit benthic substrates, being associated with sediment particles. In practice, this term is often used to refer to organisms that can pass through a 1 mm sieve but are retained on a 42 µm mesh (Fenchel, 1978; Higgins and Thiel, 1988). Meiofauna represent an ecologically important but often overlooked component of biodiversity (Martínez et al., 2025). The term typically refers to marine and freshwater animals, but such microscopic invertebrates inhabiting moist terrestrial substrates are also often directly referred to as limnoterrestrial meiofauna or hygrophilous mesofauna in soils. This group includes taxa such as tardigrades, rotifers, nematodes, and gastrotrichs that live in habitats that experience at least transient moisture availability, such as mosses, lichens, leaf litter, and soil surfaces (Majdi et al., 2024). Despite their small size, they play key roles in nutrient cycling, microbial regulation, and microfood webs (Maboreke et al., 2018; Majdi et al., 2020; Pausch et al., 2016; Schratzberger and Ingels, 2018), while some of them exhibit remarkable adaptations to environmental stress, including desiccation and freezing survival (Ingels et al., 2023; Møbjerg and Neves, 2021; Rebecchi et al., 2020).

Among terrestrial microhabitats, cryptogams (bryophytes and lichens) are particularly significant as they provide structurally complex, moisture-retaining substrates that support diverse and often specialized meiofaunal assemblages (Asplund and Wardle, 2017; Bokhorst et al., 2015; Gerson, 1973; Klarenberg et al., 2025). Although cryptogams often grow above ground level, their role in moisture retention, nutrient cycling, and soil–biota interactions makes them integral to the soil ecological context (Cornelissen et al., 2007; Durán et al., 2021; Sun et al., 2017; Xiao et al., 2016). These cryptogam-dwelling communities offer tractable models for studying fine-scale biodiversity patterns, species turnover, and community assembly in terrestrial ecosystems. Their high microhabitat heterogeneity, discrete spatial boundaries, and compatibility with DNA metabarcoding approaches make them ideal systems for testing ecological theory and exploring spatial drivers of community structure. This is especially true in the study of neglected meiofauna groups inhabiting cryptogams (Asplund and Wardle, 2017; Fontaneto and Ricci, 2006; Surmacz et al., 2025).

In studies on limnoterrestrial meiofauna dwelling in cryptogams, researchers often report the type of surface on which examined cryptogams grow. Among the most common substrates from which the samples are usually collected are soil, the base of a tree or the tree trunk, or rocks. Because these discrete microhabitat types differ in their position relative to the ground, they are likely to present distinct microclimatic and physico-chemical conditions – factors known to strongly influence poikilohydric organisms (Lakatos, 2011). Therefore, the distribution of cryptogam species and life forms in different microhabitats reflects the vertical variation of humidity and light regimes (Holz et al., 2002). Soil-based cryptogams generally retain moisture longer and experience buffered temperatures, whereas those on tree trunks are more exposed to drying and temperature fluctuations, with tree bases representing an intermediate microhabitat influenced by moisture runoff and organic matter accumulation (Hauck, 2011; Kaufmann et al., 2019). As a result, the composition of meiofaunal communities may vary depending on the substrate, with some taxa favoring more stable, humid conditions and others tolerating or even preferring more variable ones.

However, apart from the niche-based effects, the meiofauna communities are also shaped by neutral processes (Brown et al., 2022; Fontaneto, 2019; Meyer, 2006), like dispersal limitation and random fluctuations of populations, which may be especially visible in small, passively-dispersed taxa in ephemeral cryptogam microhabitats. Therefore, the community composition may not reflect the environmental heterogeneity, but may be masked by stochastic spatiotemporal dynamics.

For tardigrades, studies investigating whether the substrate on which cryptogams grow influences community composition have produced mixed and difficult-to-interpret results, mostly due to different approaches and methodologies. However, several studies suggest (either explicitly or implicitly) that tardigrade assemblages in leaf litter or cryptogams growing on the ground tend to be compositionally different and more species-rich, than those inhabiting epiphytic cryptogams (Bartels and Nelson, 2006; Guidetti et al., 2024; Guil and Sanchez-Moreno, 2013; Ito, 1999; Jönsson, 2003; Nelson, 1975; Nelson et al., 2020). Rotifers are known for their resilience to desiccation and are found in diverse cryptogam-associated habitats, being frequent and often dominant components of limno-terrestrial communities (Fontaneto et al., 2011; Ricci and Fontaneto, 2009). In contrast to tardigrades, rotifers (especially bdelloid rotifers) have received comparatively more focused attention regarding microhabitat filtering across terrestrial substrates, with several studies emphasizing that the substrate on which cryptogams grow affects their communities (Hamil et al., 2024; Kaya and Erdoğan, 2015;

Pokpongmongkol et al., 2025; Wang et al., 2023). However, tardigrades and rotifers are rarely examined together, leaving open questions about the consistency and specificity of substrate-related filtering effects across vertical gradients.

Given the inconsistent and scattered findings to date, a more systematic analysis of substrate-level variation, encompassing vertical stratification and microclimatic differences, is needed to clarify the ecological role of substrate in shaping limno-terrestrial meiofaunal communities. Here, we used DNA metabarcoding and phylogenetic diversity metrics to assess how meiofaunal communities (tardigrades and rotifers) vary across cryptogam sampled from different substrates (soil, tree bases, and tree trunks) in montane forests of the Ukrainian Carpathians. We tested whether substrate type influences richness, phylogenetic structure, and community composition, and whether these patterns differ between taxa. Since there is evidence that life history traits can affect the spatial scale of habitat–organism interactions (Fisher et al., 2011; Holland et al., 2005; Thornton and Fletcher Jr, 2014), we expected to observe differences in the substrate effects between the two analyzed groups. We hypothesized that rotifers, being smaller, having shorter life cycles, and typically forming more abundant populations, would be less limited by dispersal and more influenced by environmental filtering than tardigrades, which are generally larger, slower-reproducing, and often occupy higher trophic levels. We also hypothesized that the epiphytic communities are more likely to reflect stochastic colonization patterns (due to less frequent dispersal to aboveground microhabitats), while tree bases may receive more colonists from epiphytic communities (e.g., via stemflow), but also soil-related communities, due to the high abundance and temporal stability of on soil bryophyte microhabitats, are less likely to be limited by dispersal.

2. Material and Methods

2.1. Sampling

Cryptogam samples were collected in the Ukrainian Carpathians in July 2021. Sampling was conducted at four sites, each covering approximately 100 m² (Figure 1). Two sites were positioned at lower elevation (SLL1, SLL2; 671 and 767 m a.s.l., respectively; distance between sites ca. 500 m) while two others were positioned in higher elevation (SLH1, SLH2; 1201 and 1211 m a.s.l., respectively; distance between sites ca. 100 m). The distance between the lower and higher elevation sites were about 2.5 km. At each site, 25 samples were collected across three visually distinguished substrate types: soil (10), tree base (10), and tree trunk (5). Samples collected from soil included ground-level cryptogams with no contact with trees; tree base samples came from cryptogams within 20 cm of the tree base; and tree trunk samples were taken from 100–150 cm above ground. Because not all samples consisted solely of mosses, sample type (moss only vs. mixed moss-lichen) was also recorded. Sterile forceps were used to collect each sample, comprising approximately 10–15 cm in diameter of moss or moss-lichen cover, while taking care to avoid cross-contamination. In total, 100 samples were collected. Samples were placed into paper envelopes, transported to the laboratory, and stored at room temperature at the V. N. Karazin Kharkiv National University. In March 2022, a 1g subsample was taken from each original sample using sterile forceps, transferred to clean zip-lock plastic bags, and transported to the Institute of Systematics and Evolution of Animals PAS (ISEA-PAS, Poland) for further processing. During transport, seven samples from the SLL1 site were lost.

2.2. Sample processing

In order to obtain samples for metabarcoding analyses, the cryptogam samples were pooled in groups of five (where possible) by substrate category within each sampling site. Due to the loss of some samples, it was possible to prepare a total of 19 pooled samples. Detailed information about the pooled samples is provided in Supplementary Materials (SM.01). The samples to be pooled were placed together in a 0.5-liter plastic beaker and soaked overnight. The recently described metabarcoding protocol for moss-associated meiofauna was then followed (Surmacz et al., 2025). This protocol includes sieving the soaked cryptogam material, isolating DNA from the sediment using a soil-specific extraction kit, amplifying a fragment of the Cytochrome C Oxidase I (COI) gene using highly degenerate primers, and performing deep sequencing of the libraries to mitigate low primer specificity. A portion of 0.3 g of sediment collected in the finest sieve from each sample was transferred into 1.5 ml Eppendorf tubes using a sterile metal spatula, labeled, and stored in a freezer until further processing. The remaining sediment was rinsed into a 15 ml Falcon tube using a wash bottle and stored frozen as a backup. Between the processing of different samples, all equipment was sterilized with 20% bleach.

2.3. DNA extraction and library preparation

DNA was extracted from a portion of the frozen sediment using the DNeasy® PowerSoil® Pro Kit (Qiagen). To transfer the sediment from the Eppendorf tube, the first kit solution was added to the tube, mixed with an automatic pipette, and transferred to the bead-beating tube. Then the manufacturer's protocol was followed with a modification involving preincubation with proteinase K, as described in (Surmacz et al., 2025). Extraction blank sample was included to monitor for potential contamination. The final DNA was eluted in 100 µl of elution buffer. The entire portion of each DNA extract was additionally purified with Syngen DNA clean-up Kit according to the manufacturer's instructions, and used for subsequent analyses. For metabarcoding, a fragment of the COI gene was used as the target, amplified using primers optimized for tardigrades (BF2_TardF_2 and BR2; Surmacz et al., 2025). Library preparation was performed using a two-step PCR method. The first PCR amplified the target region using region-specific primers with Illumina overhangs. This reaction involved an initial denaturation step of 5 minutes at 95°C, followed by 30 cycles of 30 seconds of denaturation at 95°C, 90 seconds of annealing at 55°C, and 20 seconds of elongation at 72°C, with a final elongation step of 10 minutes at 72°C. The product of the first reaction was then used in a second, indexing PCR to produce uniquely barcoded libraries. This step employed primer sets containing flow-cell binding domains and unique indices from the Nextera XT Index Kit (FC-131-1001/FC-131-1002) and followed the Illumina protocol (Illumina, 2013). The resulting libraries were sequenced on an AVITI instrument (Element Biosciences, San Diego, CA) using 300-bp paired-end mode. Both library preparation and sequencing were conducted by a commercial provider, IGA Technology, based in Udine, Italy. The raw sequence reads are deposited in NCBI SRA under accession number PRJNA1346949.

2.4. Metabarcoding data analysis

The metabarcoding analysis pipeline followed the one used by (Surmacz et al., 2025), including pair-read merging, primer-trimming, denoising, and chimera-filtering using PEAR v0.9.11 (Zhang et al., 2014), Cutadapt 4.6 (Martin, 2011), vsearch (Rognes et al., 2016), and USEARCH- UCHIME (Edgar et al., 2011). The resulting denoised zero-radius Operational Taxonomic Units were clustered into operational taxonomic units (OTUs) with a 97% similarity threshold. We applied post-clustering curation of the OTU table implemented in the R package ‘lulu’ (Frøslev et al., 2017) with default parameters. We discarded all OTUs of sequence length outside the range of 418-424 base pairs. The OTUs’ representative sequences were translated into amino acids using the R package ‘Biostrings’ (Pagès et al., 2025) using translation table 5, in order to check against stop codons. To classify the OTUs, we used the ‘MIDORI2’ database (v. GB265; Leray et al., 2022). To improve the taxonomic assignment of tardigrade sequences, we replaced the reference sequences from MIDORI2 with a curated tardigrade-specific database ‘Tardi-COI’ (v. 02;Surmacz et al., 2025). The taxonomy was assigned to each OTU by classifying its representative sequences by a local BLAST search (Altschul et al., 1990; Camacho et al., 2009) using a threshold of 85% sequence identity pident = 85 (Clarke et al., 2021; Surmacz et al., 2025) to confidently recover only truly tardigrade and rotifer OTUs. Only such OTUs were used in the analyses. When the similarity to the known (described, formally named) tardigrade or rotifer sequences in the BLAST search was ≥ 97%, we assigned the taxonomy to the species level. In cases when the highly similar reference sequence belongs to unidentified species or similarity to the reference sequence is < 97%, we considered such OTUs confidently assigned only to the genus level (Surmacz et al., 2025). The complete pipeline is provided in Supplementary Materials (SM.02). To investigate phylogenetic diversity patterns, the ultrametric trees were constructed based on OTUs’ representative sequences separately for tardigrade and rotifer OTUs. We selected an appropriate model of sequence evolution and the best partitioning scheme using PartitionFinder (Lanfear et al., 2017) under the Bayesian Information Criterion. Ultrametric trees were inferred in BEAST v2.6 (Bouckaert et al., 2019) via the CIPRES Science Gateway (Miller et al., 2010), based on COI sequences of rotifer and tardigrade OTUs. For each dataset, we performed three independent BEAST runs, each for 100 million generations with a 10% burn-in, using a relaxed log-normal clock and default priors except for models and partitions defined by PartitionFinder2. The runs were combined using LogCombiner, and the resulting tree files were summarized using TreeAnnotator to produce a Maximum Clade Credibility (MCC) tree for each taxon.

2.5. Statistical analysis

All statistical analyses were performed in R v4.3.3 (R Core Team, 2024). OTUs with fewer than 10 reads per sample were removed from the OTU tables. All analyses were conducted separately for Tardigrada and Rotifera, based on taxonomic annotations assigned during sequence processing. Alpha diversity was calculated as observed OTU richness, Faith's phylogenetic diversity (PD), and its standardized effect size (SES.PD). Richness was computed as the number of OTUs present per sample. Faith's PD and SES.PD) were estimated using the *picante* package (Kembel et al., 2010), based on 999 null models generated using the "taxa.labels" algorithm. Differences in alpha diversity (OTU richness, PD, SES.PD) across substrate types and sampling sites were tested using Kruskal–Wallis tests followed by Dunn's post hoc comparisons using *FSA* package (Ogle et al., 2025).

The effect of substrate and sampling site on beta diversity was tested using two approaches: using multivariate modelling, as well as in a more simplistic distance-based approach. In a multivariate modelling approach, we tested the effect of substrate and site on the OTUs' presence using multivariate generalized linear models using *manyglm* function from *mvabund* package (Wang et al., 2012) with a binomial error distribution, followed by resampling-based analysis of deviance (999 permutations of PIT-trap resampling). In the distance-based approach, we calculated taxonomic and phylogenetic beta diversity matrices, using Sørensen dissimilarities and UniFrac distances computed using *vegan* (Oksanen et al., 2025) and *phyloseq* (McMurdie and Holmes, 2013) packages, respectively. To test for the effects of site and substrate, we applied PERMANOVA (999 permutations), and assessed homogeneity of multivariate dispersion with the betadisper function from the *vegan* package, reporting ANOVA results alongside PERMANOVA. The beta diversity patterns were visualized using non-metric multidimensional scaling (NMDS) ordination of taxonomic and phylogenetic beta diversity matrices. To evaluate the relative contribution of stochastic versus deterministic processes to community assembly, we calculated the Dispersal–Niche Continuum Index (DNCI) using the DNCI_multigroup() function from the DNCImper R package (Gibert and Escarguel, 2019; Vilmi et al., 2021). Analyses were performed separately for tardigrades and rotifers using binary presence–absence OTU matrices. Instead of conducting individual pairwise comparisons, we used the multigroup implementation of DNCI, which computes all pairwise contrasts among substrate types (soil, tree base, tree trunk) within a single model.

For each analysis, we applied 1,000 permutations to generate null distributions and calculate standardized effect sizes and 95% confidence intervals for the ΔDN index. Negative ΔDN values indicate that stochastic processes dominate assembly, whereas positive values suggest deterministic, niche-based filtering. Additionally, we partitioned taxonomic beta diversity into turnover and nestedness components using the *betapart* package (Baselga and Orme, 2012) to estimate the role of both processes in shaping beta diversity. Correlations between (i) total number of reads classified to all OTUs per each sample and rotifer as well as tardigrade OTU richness within these samples, (ii) diversity metrics (e.g., richness vs. SES.PD) and (iii) between tardigrade and rotifer diversity were assessed using Spearman's correlation tests. Binary heatmaps of OTU occurrence were generated using the *ComplexHeatmap* package (Gu, 2022; Gu et al., 2016) to visualize the distribution of tardigrade and rotifer OTUs across samples, annotated by substrate type, sampling site, and elevation, whereas the OTUs' names were provided with the % similarity to the reference sequence from which their name was taken. Figures were created using *ggplot2* (Wickham, 2016) and arranged with *patchwork* (Pedersen, 2025). The R code, input data (OTU table and samples metadata, tardigrade and rotifer phylogenetic trees) are provided as Supplementary Materials (SM.03) while tardigrade and rotifer heatmaps are provided as Supplementary Materials (SM.04-05).

3. Results

3.1. Sample overview and OTU summary

A total of 19 pooled samples were analyzed in this study, including seven from soil, eight from tree base, and four from tree trunk substrates. At one sampling site, only a single pooled sample from the soil substrate could be prepared, while one pooled sample from the tree base consisted of only two original samples. The latter was sequenced but excluded from subsequent analyses. The sequencing resulted in, on average, 668,247 reads per sample. After all the filtering steps in the bioinformatic pipeline, a total of 3,420,875 reads were retained (26.94%). Of these, 13.9% and 0.93% were assigned to classified tardigrade and rotifer OTUs, respectively. Following the removal of low-abundance occurrences (<10 reads), 33 tardigrade OTUs and 83 rotifer OTUs were retained for analysis. Tardigrade OTUs were better matched to the reference sequences, with 26 OTUs (79%) showing ≥ 97% similarity, compared to rotifers, where only 6 OTUs (7%) met this threshold (SM.04 and SM.05).

3.2. Taxonomic and phylogenetic alpha diversity

OTU richness was not significantly correlated with the total number of reads for either taxon (Tardigrades: $\rho = 0.28, p = 0.268$; Rotifers: $\rho = 0.45, p = 0.061$), suggesting that richness estimates were not strongly influenced by sequencing depth. However, OTU richness was significantly positively correlated between tardigrades and rotifers across samples ($\rho = 0.61, p = 0.0066$), indicating that local richness patterns of the two taxa were partly congruent. OTU richness varied across sampling sites and substrates for both tardigrades and rotifers (Figures 2 and 3). Tardigrade richness ranged from 4 to 16 OTUs per sample, while rotifer richness ranged from 2 to 27. Higher richness was observed at sampling sites located at higher elevations – (SLH1 and SLH2, Figure 2). Among substrates, the lowest richness was recorded in samples collected from tree trunks (Figure 3). However, no statistically significant differences in richness were found among sampling sites for either taxon (Tardigrades: $\chi^2 = 6.32$, df = 3, $p = 0.097$; Rotifers: $\chi^2 = 2.97$, df = 3, $p = 0.396$). Similarly, richness did not differ significantly across substrate types, although a marginal trend was observed for rotifers ($\chi^2 = 5.08$, df = 2, $p = 0.079$).

Observed phylogenetic diversity (PD) differed significantly among sampling sites for tardigrades (Kruskal–Wallis $\chi^2 = 8.67$, df = 3, p = 0.034), but not for rotifers ($\chi^2 = 1.66$, df = 3, p = 0.645). However, post hoc pairwise comparisons for tardigrades did not remain significant after Benjamini–Hochberg correction (e.g., SLH2–SLL1: unadjusted p = 0.053; adjusted p = 0.107; SLH2–SLL2: unadjusted p = 0.018; adjusted p = 0.107). Substrate type had no significant effect on observed PD in either group (tardigrades: $\chi^2 = 1.13$, df = 2, p = 0.568; rotifers: $\chi^2 = 4.81$, df = 2, p = 0.090; Figures 2 and 3).

In case of tardigrades, the standardized phylogenetic diversity (SES.PD) values were not correlated with OTU richness (Spearman's $\rho = 0.03$, p = 0.90), indicating that phylogenetic diversity was not simply a function of species richness. In contrast, rotifers showed a significant negative correlation between SES.PD and richness ($\rho = -0.54$, p = 0.021), suggesting that phylogenetic clustering tended to increase with community richness in this group. The values of SES.PD of tardigrade communities did not significantly differ among sampling sites (Kruskal–Wallis $\chi^2 = 3.87$, df = 3, p = 0.276; Figure 2), but did vary significantly across substrates ($\chi^2 = 6.67$, df = 2, p = 0.036; Figure 3). Post hoc comparisons revealed significantly higher SES.PD in tree trunk samples than in soil (adjusted p = 0.030). In contrast, rotifer SES.PD was almost consistently negative across sites and substrates (Figures 2 and 3) and differed significantly among sites ($\chi^2 = 13.65$, df = 3, p = 0.003; Figure 2), but not among substrates ($\chi^2 = 1.13$, df = 2, p = 0.568; Figure 3). Pairwise comparisons indicated significantly higher SES.PD in SLL2 compared to SLH1 (adjusted p = 0.010), and a significant difference between SLH1 and SLL1 (adjusted p = 0.016).

3.3. Taxonomic and phylogenetic beta diversity

Partitioning of multisite beta diversity revealed that species turnover dominated overall dissimilarity across samples for both taxa. For tardigrades, the total Sørensen dissimilarity (β_{SOR}) was 0.852, with turnover (β_{SIM}) accounting for 91% and nestedness (β_{SNE}) for 9%. For rotifers, β_{SOR} was even higher at 0.907, with turnover comprising 94% (β_{SIM}) and nestedness just 6% (β_{SNE}). These results indicate that community differences among samples were primarily driven by species replacement rather than nested species loss.

Multivariate modeling indicated factors shaping the distributions of meiofauna species. For tardigrades, community composition was significantly affected by sampling site (LRT deviance = 365.9, $p = 0.001$), but not by substrate (LRT deviance = 105.4, $p = 0.107$). In contrast, rotifer community composition was significantly influenced by both site (LRT deviance = 643.3, $p = 0.001$) and substrate (LRT deviance = 274.3, $p = 0.039$). Wald tests from the fitted models confirmed that for rotifers, both 'tree base' and 'tree trunk' substrates significantly influenced community composition in comparison to the reference level (soil), $p = 0.016$ and $p = 0.048$, respectively. PERMANOVA on Sørensen dissimilarities broadly corroborated these patterns. For tardigrades, both site (F = 5.64, $R^2 = 0.466$, p = 0.001) and substrate (F = 3.72, $R^2 = 0.205$, p = 0.001) were significant; however, multivariate dispersion differed among sites (F = 4.67, p = 0.018) but not among substrates (F = 0.18, p = 0.833), so the site effect should be interpreted with caution. For rotifers, both site (F = 3.43, $R^2 = 0.378$, p = 0.001) and substrate (F = 2.40, $R^2 = 0.176$, p = 0.006) were significant, with no evidence of unequal dispersion by site (F = 1.76, p = 0.200) or substrate (F = 0.12, p = 0.891). Notably, while the multivariate model indicated a nonsignificant substrate effect for tardigrades, the distance-based PERMANOVA detected a significant substrate term; given the significant among-site differences only for tardigrades, we emphasize caution for site effects but note consistent substrate homogeneity. Importantly, the distance-based beta diversity analysis using phylogenetic dissimilarities instead of compositional dissimilarities also revealed a strong influence of predictor variables. PERMANOVA on unweighted UniFrac distances indicated significant differences in phylogenetic community composition among sites and substrates for both taxa: tardigrades: site ($F = 4.54$, $R^2 = 0.427$, $p = 0.001$) and substrate ($F = 3.24$, $R^2 = 0.203$, $p = 0.003$); rotifers: site ($F = 3.17$, $R^2 = 0.353$, $p = 0.001$) and substrate ($F = 2.63$, $R^2 = 0.195$, $p = 0.004$). The abovementioned beta diversity patterns were also visible on NMDS plots. The ordination of Sørensen dissimilarities revealed a clear structure in community composition. For tardigrades, NMDS ordination (stress = 0.154) showed partial separation of samples by site and by substrate, though with notable overlap (Figure 4). For rotifers, NMDS ordination (stress = 0.087) indicated stronger clustering by site, with less distinct separation by substrate (Figure 4). The ordination of unweighted UniFrac distances yielded stress values of 0.142 (tardigrades) and 0.106 (rotifers), and patterns largely mirrored the taxonomic NMDS: tardigrades showed partial separation by site and substrate with notable overlap, whereas rotifers clustered more clearly by site with less distinct separation by substrate (Figure 5).

The Dispersal–Niche Continuum Index (DNCI) indicated that community assembly was predominantly stochastic in both studied animal groups. In rotifers, all pairwise substrate contrasts showed negative ΔDN values (range: –1.80 to –3.05), with confidence intervals overlapping zero, indicating no significant deterministic filtering. In tardigrades, two contrasts (soil vs. tree base: $\Delta DN = -2.89$; soil vs. tree trunk: $\Delta DN = -0.60$) also suggested stochasticity, but the tree base vs. tree trunk comparison yielded a weakly positive index value ($\Delta DN = 1.01$), indicating limited niche-based assembly between these substrates. However, none of the ΔDN values significantly exceeded the confidence bounds (approximately ±2.5 to ±3.2), emphasizing the dominant role of stochastic processes in shaping meiofaunal communities across studied substrates.

4. Discussion

We tested whether the type of substrate supporting cryptogams (soil, tree base, or tree trunk) affects the composition and diversity of limno-terrestrial meiofauna in montane forests of the Ukrainian Carpathians. Using DNA metabarcoding and phylogenetic diversity metrics, we found that geographic site exerted a consistent effect on community structure in both tardigrades and rotifers, whereas the influence of substrate was weaker and more variable. Our results indicate that meiofaunal communities are shaped mainly by neutral processes, with substrate-related effects emerging secondarily and more clearly for rotifers than for tardigrades.

In both tardigrades and rotifers, we observed only a moderate effect of substrate on the diversity patterns, which suggests that community assembly is primarily driven by stochastic processes, as revealed by the DNCI analysis. However, taking the phylogenetic information into account, we found that many of the communities show patterns of non-random community assembly (phylogenetic clustering), indicating that groups of related taxa show similar substrate preferences. This was especially visible among rotifers, where the standardized phylogenetic diversity was consistently negative. In contrast, for tardigrades, the phylogenetic clustering was only sporadically visible in samples from soil. The analyses of beta diversity (multivariate models and distance-based tests) also supported the effect of substrate for rotifers, and only limited support for its effect for tardigrades. Similarly, here, the differences in OTU richness between different substrates also suggest that rotifer communities are more affected by substrate than tardigrades. These results clearly show that substrate affects rotifer communities more strongly than tardigrades. Interestingly, the direction of the substrate effect was comparable in both taxa: samples from soil substrate had high taxonomic richness and the strongest phylogenetic clustering, while the tree samples had the lowest richness and weaker phylogenetic clustering, even with the trend of overdispersion in tardigrade communities. However, such patterns may not indicate the presence of especially divergent communities in epiphytic cryptogams but may instead reflect the higher environmental heterogeneity of above-ground microhabitats (samples from distinct microhabitats could be pooled into one bulk sample, artificially increasing sample diversity).

The contrasting patterns observed in rotifers and tardigrades likely reflect differences in their responsiveness to microenvironmental variation caused by their different life histories. Bdelloid rotifers respond strongly to fine-scale differences in humidity, surface structure, and microbial food availability - factors that vary among cryptogam substrates and influence hydration regimes and microbial abundance (Ricci and Fontaneto, 2009; Ricci, 1987; Xiang et al., 2024). Consequently, their communities tend to show higher richness and stronger phylogenetic clustering in soil and tree base microhabitats, which provide more stable moisture and microbial resources. Tardigrades, in contrast, showed weaker substrate-related variation in α- and β-diversity, suggesting greater dependence on other factors, which may include cryptogam architecture, microhabitat conditions or biotic interactions, rather than the supporting substrate (Guidetti et al., 2024; Ramsay et al., 2021; Stec et al., 2025; Tůmová et al., 2024). Previous studies similarly found that tardigrade richness and composition are often weakly related to substrate or height above ground, although some report subtle vertical effects or associations with epiphyte type (Bartels and Nelson, 2006; Nelson et al., 2020; Young et al., 2018). In rotifers, however, several studies demonstrated substrate-linked filtering, with richness and composition varying among mosses on different supports (Kaya and Erdoğan, 2015; Pokpongmongkol et al., 2025; Wang et al., 2023). These consistent taxon-specific patterns support the view that rotifer communities are more responsive to environmental heterogeneity, whereas tardigrade assemblages are structured mainly by stochastic processes and microhabitat variation. Importantly, our results align with evidence from other meiofaunal taxa, such as free-living nematodes, where canopy or epiphytic assemblages show reduced local richness but higher turnover, reflecting the joint influence of spatial heterogeneity and neutral dynamics (Kudrin et al., 2024).

Beyond the effects of site and substrate, several unmeasured factors may have contributed to the community patterns observed in our study. One potential influence is the taxonomic identity of the host cryptogam, as some tree-trunk samples included mixed moss–lichen assemblages. Previous studies indicate that mosses and lichens can differ in their capacity to retain water and support meiofaunal communities, with mosses often hosting higher tardigrade richness and abundance (Kaczmarek et al., 2011; Zawierucha et al., 2017, 2016), although not all surveys detect consistent differences (Nelson et al., 2020). Similar trends have been reported for rotifers, where mosses usually support richer but more homogeneous communities than lichens (Fontaneto and Ricci, 2006). Structural and physiological traits of the cryptogam matrix (i.e., water-holding capacity and surface texture) may therefore underlie some of the differences seen in trunk samples. Another possible source of variation involves trophic interactions between these two but maybe also other microscopic taxa. Several tardigrade genera identified here are known to feed facultatively or obligatorily on rotifers (Bryndová et al., 2020; Guidetti et al., 2012; Tůmová et al., 2022), raising the possibility that local rotifer abundance could influence tardigrade composition. While such interactions may contribute to spatial covariation between the groups, these relationships should be explored with targeted experimental or quantitative approaches in future studies.

Several methodological factors may have influenced the strength or detectability of the observed patterns. Pooling samples by site and substrate reduced the number of independent replicates, especially for tree-trunk microhabitats, and may have limited the resolution of fine-scale effects. Also, due to the pooling strategy, the observed patterns of alpha diversity were, in fact, the joint richness of multiple cryptogam samples, which may not directly correspond to the individual community richness and may be biased by heterogeneity of microhabitats. Additionally, rotifer richness was likely underestimated because of the limited representation of limno-terrestrial lineages in COI reference databases. Although GenBank contains more COI sequences for rotifers than for tardigrades, most derive from aquatic species (Fontaneto et al., 2019; Lin et al., 2025; Zhang et al., 2021). In contrast, the curated tardigrade database included many moss- and lichen-associated taxa (Surmacz et al., 2025), resulting in higher match quality and confidence. This discrepancy underscores the broader gap in molecular reference coverage for terrestrial meiofauna and highlights the need for more ecologically representative barcode datasets to improve metabarcoding-based diversity assessments.

Despite the strong influence of stochastic processes on the assembly of limno-terrestrial meiofaunal communities, our results indicate that vertical and structural variation among cryptogam substrates also contributes to shaping their composition. The contrasting responses of rotifers and tardigrades suggest that life-history traits mediate the balance between environmental filtering and neutral dynamics, with smaller, fast-reproducing taxa being more responsive to microhabitat-scale variation. The findings presented in our study highlight that even under predominantly stochastic assembly, microhabitat heterogeneity and organismal traits interact to generate subtle but consistent community differences. Future work should integrate more detailed cryptogam characterization, increased replication, and improved molecular reference databases to better disentangle how deterministic and stochastic forces jointly structure meiofaunal diversity.

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CRediT authorship contribution statement:

Yelyzaveta Matsko: Investigation, Conceptualization, Writing – review & editing; Methodology, Data curation; **Bartłomiej Surmacz**: Investigation, Conceptualization, Writing – review & editin, Methodology, Data curation; **Yevgen Kiosya**: Investigation, Conceptualization, Writing – review & editing, Methodology, Resources; **Daniel Stec**: Investigation, Conceptualization, Writing – original draft, Writing – review & editing, Formal analysis, Visualization, Supervision, Resources, Validation, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The nucleotide sequence data reported are available in the Sequence Reads Archive [http://www.ncbi.nlm.nih.gov/sra] under the project number PRJNA1346949; the dataset underling the analyses in this study is in the FigShare repository: https://doi.org/10.6084/m9.figshare.30499346.

Declaration of competing interests

We have nothing to declare

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Asplund, J., Wardle, D.A., 2017. How lichens impact on terrestrial community and ecosystem properties. Biol. Rev. 92, 1720–1738. https://doi.org/10.1111/brv.12305

Bartels, P.J., Nelson, D.R., 2006. A Large-scale, Multihabitat Inventory of the Phylum Tardigrada in the Great Smoky Mountains National Park, USA: A Preliminary Report. Hydrobiologia 558, 111–118. https://doi.org/10.1007/s10750-005-1405-9

Baselga, A., Orme, C.D.L., 2012. betapart: an R package for the study of beta diversity. Methods Ecol. Evol. 3, 808–812. https://doi.org/10.1111/j.2041-210X.2012.00224.x

Bokhorst, S., Asplund, J., Kardol, P., Wardle, D.A., 2015. Lichen physiological traits and growth forms affect communities of associated invertebrates. Ecology 96, 2394–2407. https://doi.org/10.1890/14-1030.1

Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., Maio, N.D., Matschiner, M., Mendes, F.K., Müller, N.F., Ogilvie, H.A., Plessis, L. du, Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., Suchard, M.A., Wu, C.-H., Xie, D., Zhang, C., Stadler, T., Drummond, A.J., 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLOS Comput. Biol. 15, e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Brown, P.D., Schröder, T., Ríos-Arana, J.V., Rico-Martinez, R., Silva-Briano, M., Wallace, R.L., Walsh, E.J., 2022. Processes contributing to rotifer community assembly in shallow temporary aridland waters. Hydrobiologia 849, 3719–3735. https://doi.org/10.1007/s10750-022-04842-8

Bryndová, M., Stec, D., Schill, R.O., Michalczyk, Ł., Devetter, M., 2020. Dietary preferences and diet effects on life-history traits of tardigrades. Zool. J. Linn. Soc. 188, 865–877. https://doi.org/10.1093/zoolinnean/zlz146

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. BMC Bioinformatics 10, 421. https://doi.org/10.1186/1471-2105-10-421

Clarke, L.J., Suter, L., Deagle, B.E., Polanowski, A.M., Terauds, A., Johnstone, G.J., Stark, J.S., 2021. Environmental DNA metabarcoding for monitoring metazoan biodiversity in Antarctic nearshore ecosystems. PeerJ 9, e12458. https://doi.org/10.7717/peerj.12458

Cornelissen, J.H.C., Lang, S.I., Soudzilovskaia, N.A., During, H.J., 2007. Comparative Cryptogam Ecology: A Review of Bryophyte and Lichen Traits that Drive Biogeochemistry. Ann. Bot. 99, 987–1001. https://doi.org/10.1093/aob/mcm030

Durán, J., Rodríguez, A., Heiðmarsson, S., Lehmann, J.R.K., del Moral, Á., Garrido-Benavent, I., De los Ríos, A., 2021. Cryptogamic cover determines soil attributes and functioning in polar terrestrial ecosystems. Sci. Total Environ. 762, 143169. https://doi.org/10.1016/j.scitotenv.2020.143169

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Fenchel, T.M., 1978. The Ecology of Micro-and Meiobenthos. Annu. Rev. Ecol. Evol. Syst. 9, 99–121. https://doi.org/10.1146/annurev.es.09.110178.000531

Fisher, J.T., Anholt, B., Volpe, J.P., 2011. Body mass explains characteristic scales of habitat selection in terrestrial mammals. Ecol. Evol. 1, 517–528. https://doi.org/10.1002/ece3.45

Fontaneto, D., 2019. Long-distance passive dispersal in microscopic aquatic animals. Mov. Ecol. 7, 10. https://doi.org/10.1186/s40462-019-0155-7

Fontaneto, D., Eckert, E.M., Anicic, N., Lara, E., Mitchell, E.A.D., 2019. We are ready for faunistic surveys of bdelloid rotifers through DNA barcoding: the example of Sphagnum bogs of the Swiss Jura Mountains. Limnetica 38, 213–225. https://doi.org/10.23818/limn.38.02

Fontaneto, D., Ricci, C., 2006. Spatial gradients in species diversity of microscopic animals: the case of bdelloid rotifers at high altitude. J. Biogeogr. 33, 1305–1313. https://doi.org/10.1111/j.1365-2699.2006.01502.x

Fontaneto, D., Westberg, M., Hortal, J., 2011. Evidence of Weak Habitat Specialisation in Microscopic Animals. PLOS ONE 6, e23969. https://doi.org/10.1371/journal.pone.0023969

Frøslev, T.G., Kjøller, R., Bruun, H.H., Ejrnæs, R., Brunbjerg, A.K., Pietroni, C., Hansen, A.J., 2017. Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. Nat. Commun. 8, 1188. https://doi.org/10.1038/s41467-017-01312-x

Gerson, U., 1973. Lichen-Arthropod Associations. The Lichenologist 5, 434–443. https://doi.org/10.1017/s0024282973000484

Gibert, C., Escarguel, G., 2019. PER-SIMPER—A new tool for inferring community assembly processes from taxon occurrences. Glob. Ecol. Biogeogr. 28, 374–385. https://doi.org/10.1111/geb.12859

Gu, Z., 2022. Complex heatmap visualization. iMeta 1, e43. https://doi.org/10.1002/imt2.43

Gu, Z., Eils, R., Schlesner, M., 2016. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics 32, 2847–2849. https://doi.org/10.1093/bioinformatics/btw313

Guidetti, R., Altiero, T., Marchioro, T., Sarzi Amadè, L., Avdonina, A.M., Bertolani, R., Rebecchi, L., 2012. Form and function of the feeding apparatus in Eutardigrada (Tardigrada). Zoomorphology 131, 127–148. https://doi.org/10.1007/s00435-012-0149-0

Guidetti, R., Ingemar Jönsson, K., Kaczmarek, Ł., Meier, T., Speed, J.D.M., Prestø, T., Stur, E., Topstad, L., Cesari, M., Roszkowska, M., Zawierucha, K., Hassel, K., Ekrem, T., 2024. Tardigrade diversity and community composition across Norwegian boreal forests. Zool. J. Linn. Soc. 200, 156–171. https://doi.org/10.1093/zoolinnean/zlad136

Guil, N., Sanchez-Moreno, S., 2013. Fine-scale patterns in micrometazoans: tardigrade diversity, community composition and trophic dynamics in leaf litter. Syst. Biodivers. 11, 181–193. https://doi.org/10.1080/14772000.2013.798370

Hamil, S., Chikha, C., Alili, M., Arab, S., Essahran, W., Baha, M., Arab, A., 2024. Testing the Habitat Selectivity of Bdelloid Rotifers in a Humid Area; National Park of Chrea (Algeria), in: Ksibi, M., Negm, A., Hentati, O., Ghorbal, A., Sousa, A., Rodrigo-Comino, J., Panda, S., Lopes Velho, J., El-Kenawy, A.M., Perilli, N. (Eds.), Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions (3rd Edition). Springer Nature Switzerland, Cham, pp. 715–718. https://doi.org/10.1007/978-3-031-43922-3_160

Hauck, M., 2011. Site factors controlling epiphytic lichen abundance in northern coniferous forests. Flora - Morphol. Distrib. Funct. Ecol. Plants 206, 81–90. https://doi.org/10.1016/j.flora.2010.02.001

Higgins, R., Thiel, H., 1988. Introduction to the study of meiofauna. Smithsonian Institution Press, Washington D.C.

Holland, J.D., Fahrig, L., Cappuccino, N., 2005. Body size affects the spatial scale of habitat–beetle interactions. Oikos 110, 101–108. https://doi.org/10.1111/j.0030-1299.2005.13638.x

Holz, I., Gradstein, S.R., Heinrichs, J., Kappelle, M., 2002. Bryophyte Diversity, Microhabitat Differentiation, and Distribution of Life Forms in Costa Rican Upper Montane Quercus Forest. The Bryologist 105, 334–348. https://doi.org/10.1639/0007-2745(2002)105%255B0334:BDMDAD%255D2.0.CO;2

Ingels, J., Zeppilli, D., Giere, O., 2023. Meiofauna—Adapted to Life at the Limits, in: Giere, O., Schratzberger, M. (Eds.), New Horizons in Meiobenthos Research: Profiles, Patterns and Potentials. Springer International Publishing, Cham, pp. 363–400. https://doi.org/10.1007/978-3-031-21622-0_11

Ito, M., 1999. Ecological distribution, abundance and habitat preference of terrestrial tardigrades in various forests on the northern slope of Mt. Fuji, central Japan. Zool. Anz. 238, 225–234.

Jönsson, K.I., 2003. Population density and species composition of moss-living tardigrades in a boreo-nemoral forest. Ecography 26, 356–364. https://doi.org/10.1034/j.1600-0587.2003.03458.x

Kaczmarek, Ł., Gołdyn, B., Wełnicz, W., Michalczyk, Ł., 2011. Ecological factors determining Tardigrada distribution in Costa Rica. J. Zool. Syst. Evol. Res. 49, 78–83. https://doi.org/10.1111/j.1439-0469.2010.00603.x

Kaufmann, S., Weinrich, T., Hauck, M., Leuschner, C., 2019. Vertical variation in epiphytic cryptogam species richness and composition in a primeval Fagus sylvatica forest. J. Veg. Sci. 30, 881–892. https://doi.org/10.1111/jvs.12775

Kaya, M., Erdoğan, S., 2015. Testing the habitat selectivity of bdelloid rotifers in a restricted area. Turk. J. Zool. 39, 1132–1141. https://doi.org/10.3906/zoo-1410-46

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P., Webb, C.O., 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26, 1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Klarenberg, I.J., Liu, R., Convey, P., Cornelissen, J., Bokhorst, S., 2025. How the small host the small: Cryptogam trait-mediated structuring of Antarctic microarthropod communities. https://doi.org/10.22541/au.174678850.01347224/v1

Kudrin, A.A., Salavatulin, V.M., Mikryukov, V.S., Sushchuk, A.A., Kudrina, S.E., Konakova, T.N., Nguyen, T.D., Tiunov, A.V., 2024. Metabarcoding reveals that arboreal habitats contribute significantly to nematode diversity in different forest ecosystems. Biodivers. Conserv. 33, 4371–4386. https://doi.org/10.1007/s10531-024-02960-4

Lakatos, M., 2011. Lichens and Bryophytes: Habitats and Species, in: Lüttge, U., Beck, E., Bartels, D. (Eds.), Plant Desiccation Tolerance. Springer, Berlin, Heidelberg, pp. 65–87. https://doi.org/10.1007/978-3-642-19106-0_5

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B., 2017. PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. Mol. Biol. Evol. 34, 772–773. https://doi.org/10.1093/molbev/msw260

Leray, M., Knowlton, N., Machida, R.J., 2022. MIDORI2: A collection of quality controlled, preformatted, and regularly updated reference databases for taxonomic assignment of eukaryotic mitochondrial sequences. Environ. DNA 4, 894–907. https://doi.org/10.1002/edn3.303

Lin, G., Plewka, M., Brekke, E., Kielland, Ø.N., Dunshea, G., Fontaneto, D., 2025. A survey of bdelloid rotifers from coastal ponds in Southern Norway. J. Limnol. 84. https://doi.org/10.4081/jlimnol.2025.2206

Maboreke, H.R., Bartel, V., Seiml-Buchinger, R., Ruess, L., 2018. Micro-Food Web Structure Shapes Rhizosphere Microbial Communities and Growth in Oak. Diversity 10, 15. https://doi.org/10.3390/d10010015

Majdi, N., Araujo, T.Q., Bekkouche, N., Fontaneto, D., Garrigue, J., Larrieu, L., Kamburska, L., Kieneke, A., Minowa, A.K., Laumer, C., Sabatino, R., Sorel, D., Stec, D., Traunspurger, W., 2024. Freshwater and limno-terrestrial meiofauna of the Massane Forest Reserve in the Eastern French Pyrenees. Biogeogr. – J. Integr. Biogeogr. 39. https://doi.org/10.21426/B639162226

Majdi, N., Schmid-Araya, J.M., Traunspurger, W., 2020. Examining the diet of meiofauna: a critical review of methodologies. Hydrobiologia 847, 2737–2754. https://doi.org/10.1007/s10750-019-04150-8

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10–12. https://doi.org/10.14806/ej.17.1.200

Martínez, A., Bonaglia, S., Di Domenico, M., Fonseca, G., Ingels, J., Jörger, K.M., Laumer, C., Leasi, F., Zeppilli, D., Baldrighi, E., Bik, H., Cepeda, D., Curini-Galletti, M., Cutter, A.D., dos Santos, G., Fattorini, S., Frisch, D., Gollner, S., Jondelius, U., Kerbl, A., Kocot, K.M., Majdi, N., Mammola, S., Martín-Durán, J.M., Menegotto, A., Montagna, P.A., Nascimento, F.J.A., Puillandre, N., Rognant, A., Sánchez, N., Santos, I.R., Schmidt-Rhaesa, A., Schratzberger, M., Semprucci, F., Shimabukuro, M., Sommerfield, P.J., Struck, T.H., Sørensen, M.V., Wallberg, A., Worsaae, K., Yamasaki, H., Fontaneto, D., 2025. Fundamental questions in meiofauna research highlight how small but ubiquitous animals can improve our understanding of Nature. Commun. Biol. 8, 1–17. https://doi.org/10.1038/s42003-025-07888-1

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE 8, e61217. https://doi.org/10.1371/journal.pone.0061217

Meyer, H.A., 2006. Small-scale Spatial Distribution Variability in Terrestrial Tardigrade Populations. Hydrobiologia 558, 133–139. https://doi.org/10.1007/s10750-005-1412-x

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, in: 2010 Gateway Computing Environments Workshop (GCE). Presented at the 2010 Gateway Computing Environments Workshop (GCE), pp. 1–8. https://doi.org/10.1109/GCE.2010.5676129

Møbjerg, N., Neves, R.C., 2021. New insights into survival strategies of tardigrades. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 254, 110890. https://doi.org/10.1016/j.cbpa.2020.110890

Nelson, D.R., 1975. Ecological distribution of tardigrades on Roan Mountain, Tennessee – North Carolina, in: International Symposium on Tardigrades., Memorie Dell'Instituto Italiano Di Idrobiologia. Pallanza, Italy, pp. 225–276.

Nelson, D.R., Bartels, P.J., Fegley, S.R., 2020. Environmental correlates of tardigrade community structure in mosses and lichens in the Great Smoky Mountains National Park (Tennessee and North Carolina, USA). Zool. J. Linn. Soc. 188, 913–924. https://doi.org/10.1093/zoolinnean/zlz043

Ogle, D.H., Doll, J.C., Wheeler, A.P., dunnTest()), A.D. (Provided base functionality of, 2025. FSA: Simple Fisheries Stock Assessment Methods.

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M.D., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.-H., Cunha, E.R., Smith, T., Stier, A., Braak, C.J.F.T., Weedon, J., Borman, T., 2025. vegan: Community Ecology Package.

Pagès, H., Aboyoun, P., Gentleman, R., DebRoy, S., 2025. Biostrings.

Pausch, J., Hofmann, S., Scharroba, A., Kuzyakov, Y., Ruess, L., 2016. Fluxes of root-derived carbon into the nematode micro-food web of an arable soil. Food Webs 9, 32–38. https://doi.org/10.1016/j.fooweb.2016.05.001

Pedersen, T.L., 2025. patchwork: The Composer of Plots.

Pokpongmongkol, P., Jaturapruek, R., Sa-ardrit, P., Maiphae, S., 2025. Substrate Preference and Seasonal Distribution of Bdelloid Rotifers in Mosses in a Primary Forest in Thailand. Diversity 17, 171. https://doi.org/10.3390/d17030171

Ramsay, B.P.L., Marley, N.J., Bilton, D.T., Rundle, S.D., Ramsay, P.M., 2021. The structure of tardigrade communities at fine spatial scales in an Andean *Polylepis* forest. Neotropical Biodivers. 7, 443–454. https://doi.org/10.1080/23766808.2021.1943216

Rebecchi, L., Boschetti, C., Nelson, D.R., 2020. Extreme-tolerance mechanisms in meiofaunal organisms: a case study with tardigrades, rotifers and nematodes. Hydrobiologia 847, 2779–2799. https://doi.org/10.1007/s10750-019-04144-6

Ricci, C., Fontaneto, D., 2009. The importance of being a bdelloid: Ecological and evolutionary consequences of dormancy. Ital. J. Zool. 76, 240–249. https://doi.org/10.1080/11250000902773484

Ricci, C.N., 1987. Ecology of bdelloids: how to be successful. Hydrobiologia 147, 117–127. https://doi.org/10.1007/BF00025734

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

Schratzberger, M., Ingels, J., 2018. Meiofauna matters: The roles of meiofauna in benthic ecosystems. J. Exp. Mar. Biol. Ecol. 502, 12–25. https://doi.org/10.1016/j.jembe.2017.01.007

Stec, D., Vecchi, M., Budzik, K., Matsko, Y., Miler, K., 2025. Distribution of tardigrade cryptobiotic abilities across a fine-scale habitat gradient. Org. Divers. Evol. 25, 43–54. https://doi.org/10.1007/s13127-024-00660-z

Sun, S.-Q., Liu, T., Wu, Y.-H., Wang, G.-X., Zhu, B., DeLuca, T.H., Wang, Y.-Q., Luo, J., 2017. Ground bryophytes regulate net soil carbon efflux: evidence from two subalpine ecosystems on the east edge of the Tibet Plateau. Plant Soil 417, 363–375. https://doi.org/10.1007/s11104-017-3264-3

Surmacz, B., Vecchi, M., Fontaneto, D., Budzik, K., Godziek, J., Matsko, Y., Stec, D., 2025. COI Metabarcoding With a Curated Reference Database and Optimized Protocol Provides a Reliable Species-Level Diversity Assessment of Tardigrades. Integr. Zool. https://doi.org/10.1111/1749-4877.12972

Thornton, D.H., Fletcher Jr, R.J., 2014. Body size and spatial scales in avian response to landscapes: a meta-analysis. Ecography 37, 454–463. https://doi.org/10.1111/j.1600-0587.2013.00540.x

Tůmová, M., Jílková, V., Macek, P., Devetter, M., 2024. Tardigrade distribution in soils of high Arctic habitats. Ecol. Evol. 14, e11386. https://doi.org/10.1002/ece3.11386

Tůmová, M., Stec, D., Michalczyk, Ł., Devetter, M., 2022. Buccal tube dimensions and prey preferences in predatory tardigrades. Appl. Soil Ecol. 170, 104303. https://doi.org/10.1016/j.apsoil.2021.104303

Vilmi, A., Gibert, C., Escarguel, G., Happonen, K., Heino, J., Jamoneau, A., Passy, S.I., Picazo, F., Soininen, J., Tison-Rosebery, J., Wang, J., 2021. Dispersal–niche continuum index: a new quantitative metric for assessing the relative importance of dispersal versus niche processes in community assembly. Ecography 44, 370–379. https://doi.org/10.1111/ecog.05356

Wang, W., Yang, Y., Cui, Z., Chen, M., Ma, X., Wang, Q., 2023. High diversity and strong habitat preference of bdelloid rotifers in the moss and leaf litter from a small area of urban plain and adjacent hill in China. Biodivers. Conserv. 32, 2769–2789. https://doi.org/10.1007/s10531-023-02630-x

Wang, Y., Naumann, U., Wright, S.T., Warton, D.I., 2012. mvabund– an R package for model-based analysis of multivariate abundance data. Methods Ecol. Evol. 3, 471–474. https://doi.org/10.1111/j.2041-210X.2012.00190.x

Wickham, H., 2016. ggplot2, Use R! Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-24277-4

Xiang, X., Wang, C., Huang, L., Zhu, L., Xu, J., Wen, X., Xi, Y., 2024. The resistance to short-term dehydration in the bdelloid rotifer Rotaria rotatoria from different climate regions. Hydrobiologia 851, 3185–3198. https://doi.org/10.1007/s10750-023-05375-4

Xiao, B., Hu, K., Ren, T., Li, B., 2016. Moss-dominated biological soil crusts significantly influence soil moisture and temperature regimes in semiarid ecosystems. Geoderma 263, 35–46. https://doi.org/10.1016/j.geoderma.2015.09.012

Young, A.R., Miller, J.E.D., Villella, J., Carey, G., Miller, W.R., 2018. Epiphyte type and sampling height impact mesofauna communities in Douglas-fir trees. PeerJ 6, e5699. https://doi.org/10.7717/peerj.5699

Zawierucha, K., Węgrzyn, M., Ostrowska, M., Wietrzyk, P., 2017. Tardigrada in Svalbard lichens: diversity, densities and habitat heterogeneity. Polar Biol. 40, 1385–1392. https://doi.org/10.1007/s00300-016-2063-2

Zawierucha, K., Zmudczyńska-Skarbek, K., Kaczmarek, Ł., Wojczulanis-Jakubas, K., 2016. The influence of a seabird colony on abundance and species composition of water bears (Tardigrada) in Hornsund (Spitsbergen, Arctic). Polar Biol. 39, 713–723. https://doi.org/10.1007/s00300-015-1827-4

Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30, 614–620. https://doi.org/10.1093/bioinformatics/btt593

Zhang, Y.-N., Xu, S.-L., Huang, Q., Liu, P., Han, B.-P., 2021. Application of COI Primers 30F/885R in Rotifers to Regional Species Diversity in (Sub)Tropical China. Diversity 13, 390. https://doi.org/10.3390/d13080390

Figures captions

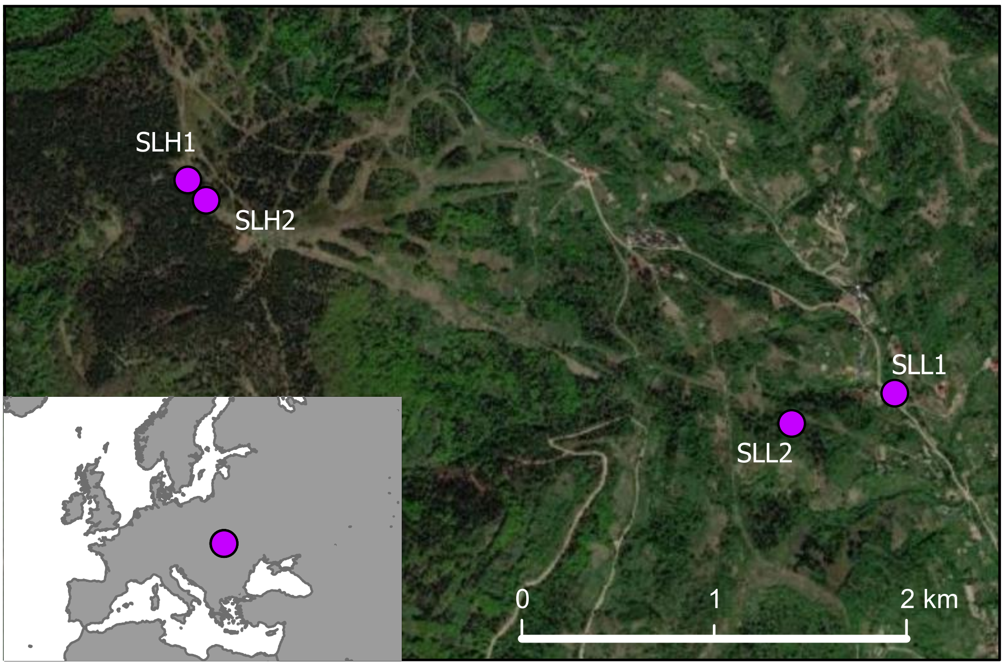

Figure 1. Map showing distribution of the four sampling sites. Basemap source: Esri, Earthstar Geographics.

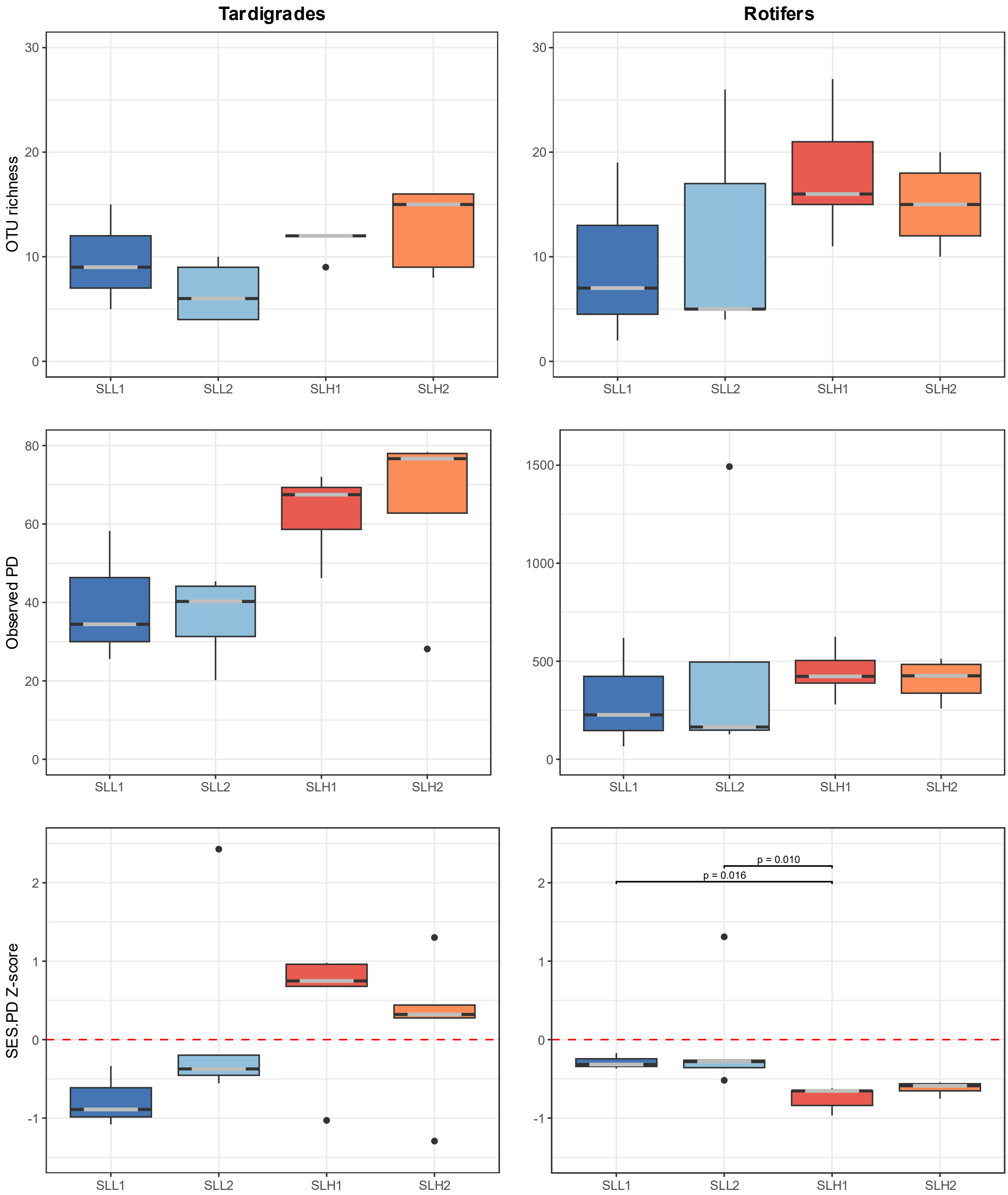

Figure 2. OTU richness, observed phylogenetic diversity (PD), and standardized effect size of PD (SES.PD) for tardigrades and rotifers across the four sampling sites. Boxplots show the median and interquartile range; whiskers indicate 1.5× the interquartile range. A red dashed line at SES.PD = 0 indicates the expectation under random assembly.

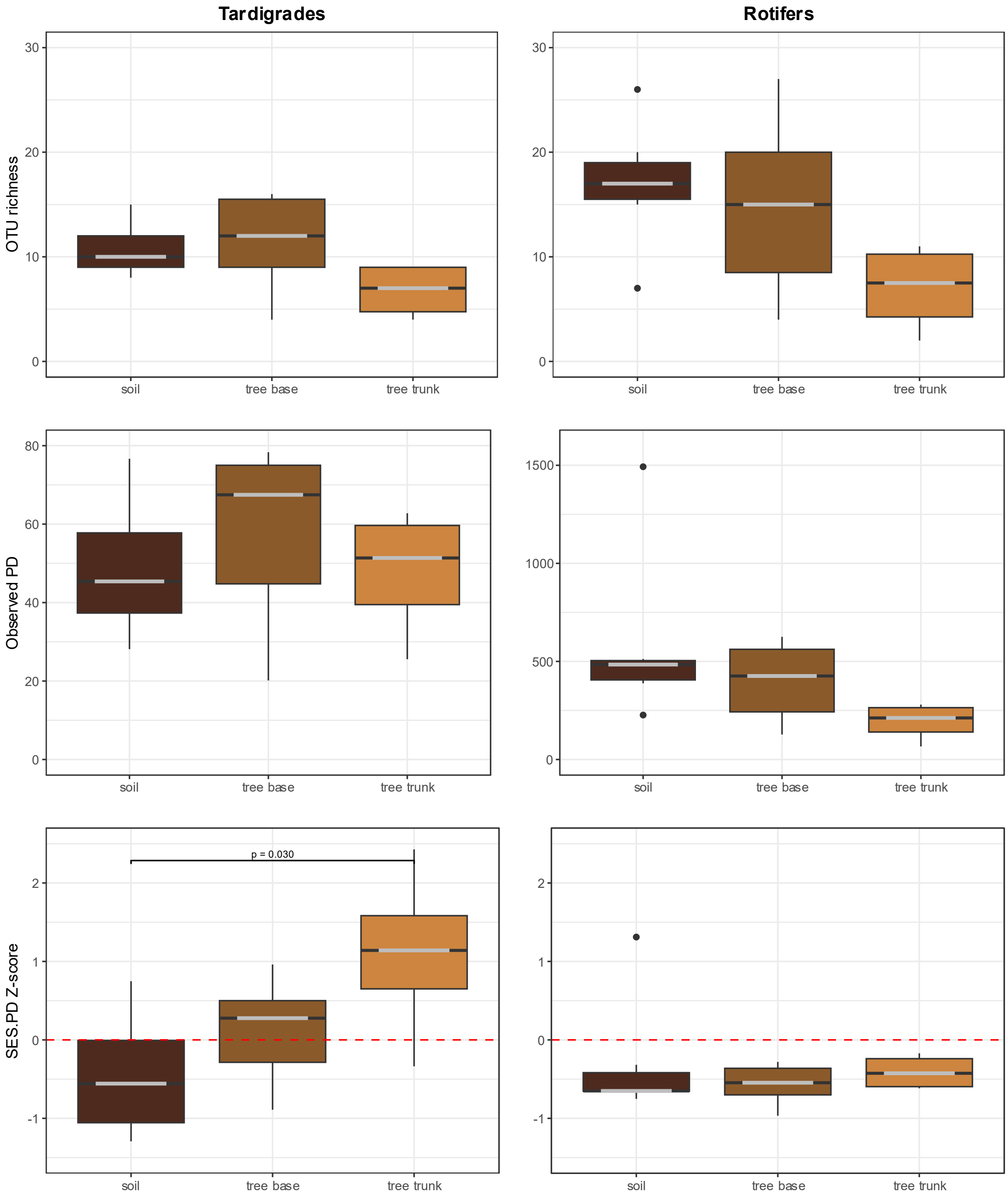

Figure 3. OTU richness, observed phylogenetic diversity (PD), and standardized effect size of PD (SES.PD) for tardigrades and rotifers across the three substrate types. Boxplots show the median and interquartile range; whiskers indicate 1.5× the interquartile range. A red dashed line at SES.PD = 0 indicates the expectation under random assembly.

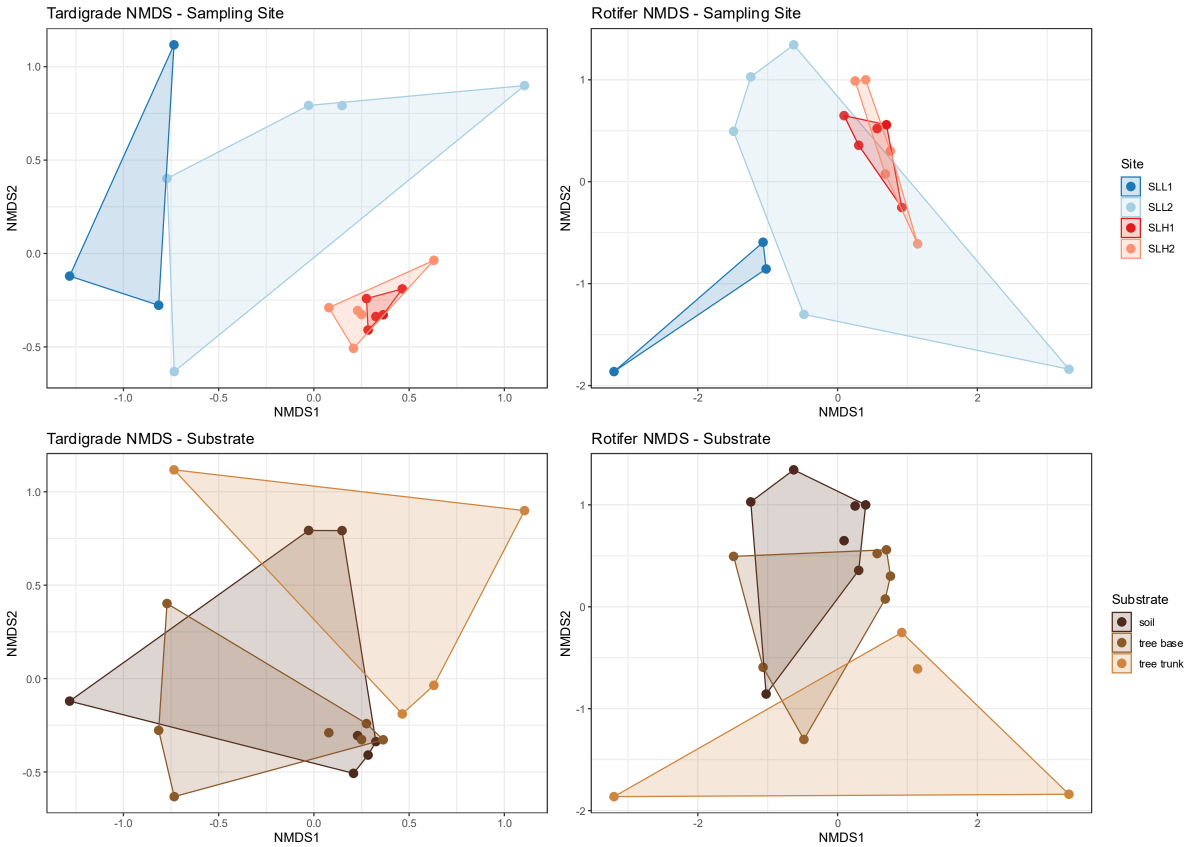

Figure 4. Non-metric multidimensional scaling (NMDS) ordinations of tardigrade and rotifer communities based on binary Bray–Curtis dissimilarity (Sørensen index). Each point represents a sample; shaded polygons indicate convex hulls enclosing groups.

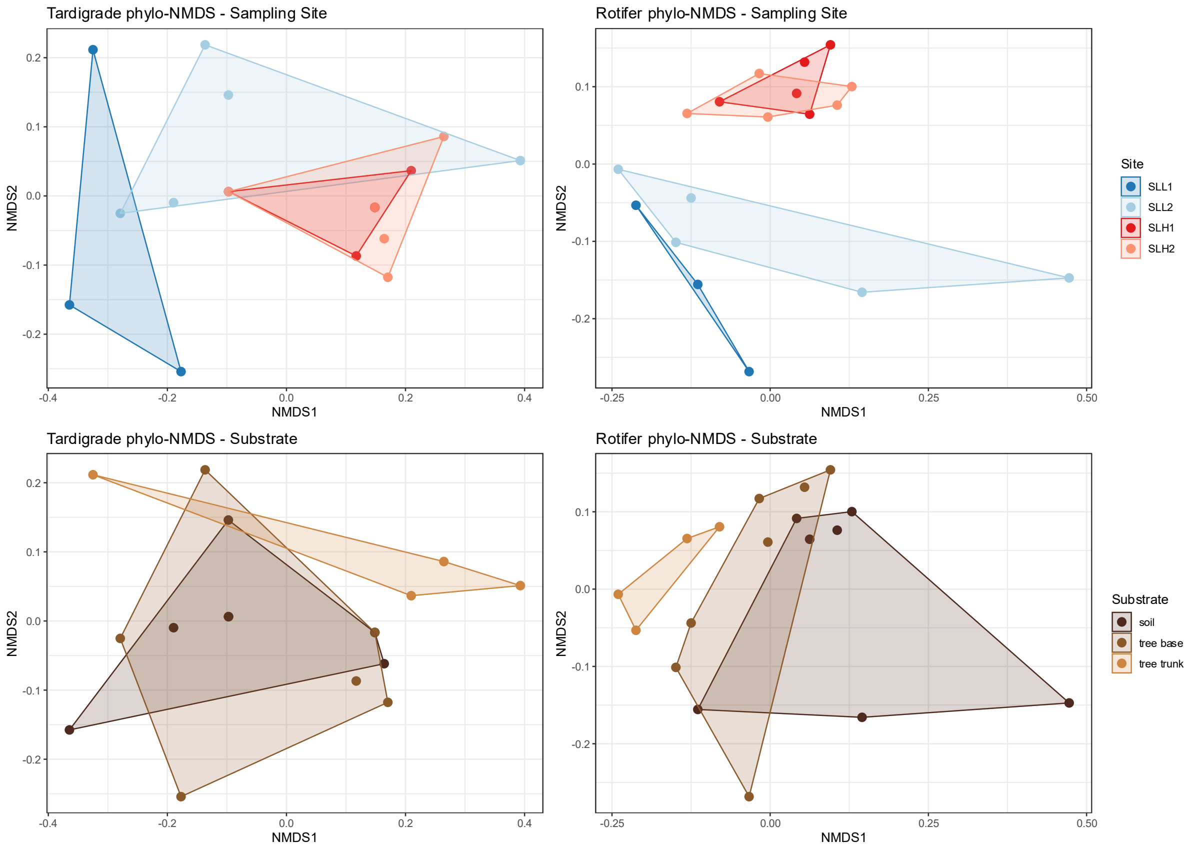

Figure 5. Non-metric multidimensional scaling (NMDS) ordinations of tardigrade and rotifer communities based on unweighted UniFrac (phylogenetic) distances. Each point represents a sample; shaded polygons indicate convex hulls enclosing groups.

Supplementary Materials

SM.01 – detailed information about the samples used in this study for metabarcoding analysis.

SM.02 – bioinformatic pipeline

SM.03 – Archive comprising: ReadMe file, R code, OTU table; samples data for statistical analysis; tardigrade Maximum Clade Credibility tree in Newick format; rotifer Maximum Clade Credibility tree in Newick format;

SM.04 – heatmap of tardigrade OTU occurrences.

SM.05 – heatmap of rotifer OTU occurrences.