Soil compartments (bulk soil, litter, root and rhizosphere) as main drivers of soil protistan communities distribution in forests with different nitrogen deposition

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Abstract

Protists, in particular bacterivores, are essential players in the rhizosphere; thus, how their interactions with bacteria and fungi affect plant productivity and soil nutrient cycles warrants more attention. Using next-generation sequencing of the 18S rRNA gene, we investigated the distribution of two major protistan phyla, Cercozoa and Endomyxa, across four seasons, and four soil compartments - rhizosphere, root, soil and litter. The sampling was replicated in two forests in Norway and the Czech Republic, in order to test our results across biogeographic scales. Compartment had a major influence in shaping protistan communities, over and above

spatial distance and seasonal variation. Protistan diversity was highest in the bulk soil while lowest in the roots, suggesting that the plants select for restricted assemblages of protists. Accordingly, only the root compartment harboured a subset of the bulk soil protistan diversity. In addition, protistan communities showed markedly different distributions according to their feeding modes, with opposite patterns for bacterivores versus omnivores and eukaryvores. The small bacterivorous flagellates (mostly Glissomonadida) were more abundant in roots, while the larger amoeboid eukaryvores (e.g. some of the Cryomonadida and vampyrellids) dominated in soil and in the rhizosphere, and the omnivores (e.g. Euglyphida and part of the Cercomonadida), also large and mostly amoeboid, were more abundant in litter. The current view of the soil microbiome is mostly focused on bacteria and fungi: this detailed study on the community distribution of protists according to their feeding modes reveals the essential role they play in each of the soil compartments, an essential precondition for a detailed understanding of the soil food web and nutrient cycling in forest.

1 Introduction

Recently, it has been shown that the distribution of soil protists at large scales is limited spatially and is non-random, with habitat being the major environmental filter (Grossmann et al., 2016). At regional scales the influence of diverse habitats has been found preponderant: forest versus grassland explained most of the protistan community composition changes in temperate climate (Fiore-Donno et al., 2020; Fiore-Donno and Bonkowski, 2021), and taxon richness increased from grasslands towards the tree-dominated climax vegetation in Brazil (Ferreira de Araujo et al., 2018). Within single habitats, however, protistan communities also respond to soil properties, the most commonly cited being pH (Öztoprak et al., 2020; Seppey et al., 2020), soil moisture (Fiore-Donno et al., 2019; Öztoprak et al., 2020), soil texture (Dequiedt et al., 2011; Fiore-Donno et al., 2019; Fiore-Donno and Bonkowski, 2021), C/N ratio and/or soil organic matter (Dequiedt et al., 2011; Zhao et al., 2018) and plant biomass

(Öztoprak et al., 2020). Yet, it would be naïve to expect unequivocal responses to environmental factors from the heterogeneous assemblage named "protists", since they feature a vast array of functions - much wider than that of multicellular plants, animals, fungi and brown algae (Bonkowski et al., 2019).

It is not until recently that functional traits - such as feeding preference or lifestyles - have favoured a deeper understanding of protistan functional distribution, a necessary step for linking biodiversity to ecosystem processes. Globally in soils protistan consumers dominate over parasites and phototrophs (Singer et al., 2021). This view was supported by a large-scale study in the Swiss Alps (Seppey et al., 2020); however, each of these three functional groups responded differently to the considered environmental variables (Seppey et al., 2020). A detailed functional trait database is available for a major protistan phylum, Cercozoa, which has been shown to be very abundant in soils (Grossmann et al., 2016; Ferreira de Araujo et al., 2018): it assigns nutrition, morphology, locomotion traits and habitat to each taxon, down to the genus (Dumack et al., 2020). This traits database allows to explore in detail the different biogeographies of each nutritional group (bacterivores, omnivores, eukaryvores and plant parasites of Cercozoa and of the related phylum Endomyxa), and how they respond to environmental drivers. In one study, bacterivores and omnivores plus eukaryvores showed opposite responses to edaphic properties: for example, low organic C, clay content and pH in soil increased the relative abundances of the bacterivores while decreasing that of the eukaryvores (Fiore-Donno et al., 2020). When considering aboveground habitats in forest - a vertical stratigraphy from tree crowns to the litter and soil on the ground, bacterivores and omnivores were more abundant in the canopy than in the litter, while eukaryvores were more abundant in litter (Walden et al., 2021). It seems legitimate to postulate that different functional groups will show distinct distributions among different soil habitats, and to question to which abiotic and biotic factors they will respond.

Most bacteria and fungi occur in the topsoil of coniferous forests (Baldrian, 2017), but their communities clearly differ across the four main compartments (i.e. bulk soil, litter, rhizosphere and root), both in diversity and functions (Baldrian, 2017; Starke et al., 2021). These compartments harbour distinct prokaryotic and eukaryotic communities, exceeding the influence of plant species or soil type (Maciá-Vicente et al., 2020; Tkacz et al., 2020). The network built by the roots, their ectomycorrhizal sheath and the fungal hyphae extending from the mycorrhizae into the soil (Baldrian, 2017), strongly influence the microbial communities of conifer forest soils. In turn, the ectomycorrhizae of the conifers are surrounded by bacterial and saprotrophic fungal communities (Ballhausen and de Boer, 2016), with specific bacteria modulating the mycorrhizal symbiosis (Lladó et al., 2017). Roots and rhizosphere clearly represent a large and unique habitat where bacteria and fungi interact (Baldrian, 2017): ectomycorrhizal fungi favour bacterial communities that are particularly effective at releasing inorganic nutrients, while saprotrophic fungi from the surrounding soil are more effective in organic matter degradation (Uroz et al., 2013). Litter decomposition is mainly driven by saprotrophic fungi, which comprise most of the biomass, although bacteria dominate in abundance (Purahong et al., 2016). Protists, and notably heterotrophic ones, have been shown to represent a notable fraction of the litter eukaryotic diversity (Voss et al., 2019), and they contribute, as consumers of bacteria and fungi, to C mineralization (Schröter et al., 2003).

The current understanding of the rhizosphere microbiota in forests is unfortunately largely limited to ectomycorrhizal fungi and the specific and diverse populations of bacteria and microfungi (Baldrian, 2017). Protists are strikingly neglected, although their role in the "microbial loop" - releasing ammonia available for plants through consumption of bacteria - has been established in cultivated herbaceous plants (Bonkowski and Clarholm, 2012), and interactions with ectomycorrhizae have been suggested (Bonkowski et al., 2001). Accordingly protists, especially bacterivores, are more abundant in the rhizosphere of wheat with respect

to the bulk soil (Turner et al., 2013). Thus we addressed the distribution of each of these nutrition guilds, in each compartment (litter, soil, rhizosphere and root) in two coniferous forests, located in Norway and in the Czech Republic, that are characterized by the legacy of low and high nitrogen deposition, respectively. Sampling was carried out four times, in spring, summer, early winter and late winter, and .fungal biomass and bacterial/fungal ratio were estimated. To survey the protistan communities, with emphasis on Cercozoa and Endomyxa, we conducted metabarcoding of the V4 region of the rRNA 18S gene, using Illumina sequencing (Fiore-Donno et al., 2018; Fiore-Donno et al., 2020). Our main questions were: i) which are the main drivers of the protistan communities composition: forest type, season or compartment? ii) how are bacterivores, omnivores and eukaryvores distributed among compartments? We hypothesize that: i) compartment, as already shown in agricultural fields, will exert the major influence on beta-diversity of Cercozoa and Endomyxa; ii) eukaryvores (among them fungivores) will be more abundant in the fungus-dominated litter; iii) temporal shifts of the microbial community will not be important, since seasonality was not the main driver in several natural environments (Fiore-Donno et al., 2019; Fournier et al., 2020; Shen et al., 2021; Walden et al., 2021), and in particular in a coniferous forest topsoil (Žifčáková et al., 2017); iv) the selective force exerted by the plant would be more evident in the root and the rhizosphere, resulting in highly nested communities. Our observations allowed assessing the relative distribution of each protistan nutritional guild with respect to soil habitat, spatial distance and seasonality in two distinct forests.

2 Materials and Methods

2.1 Study area and sample collection

The study was performed in two areas with different history of past nitrogen deposition, one in the Bohemian Forest mountain range (Central Europe; 49°02 N, 13°37 E) in an unmanaged Norway spruce (*Picea abies*) forest with a long-term legacy of high rates of atmospheric N

deposition (c. 500 mg.m^{-2}) (Fagerli et al., 2020). It was located at an altitude of 1170-1200 m, with a mean annual temperature of 5 °C, and a mean annual precipitation 1000 mm in the year of sampling. Understory vegetation was sparse and consisted of grasses (*Avenella*, *Calamagrostis*), blueberries (*Vaccinium myrtillus*) and mosses. Detailed studies already exist on the composition of soil microbial communities and their contribution to ecosystem processes in this area (Baldrian et al., 2012; Žifčáková et al., 2016; Starke et al., 2021). The other study area was located in the Maridalen valley near Oslo (Norway; 59° 59' 56.04" N, 10° 47' 25.08" E), covered predominantly with *Picea abies* and with low levels of past nitrogen deposition (c. 200 mg.m^{-2}) (Fagerli et al., 2020). The understory was denser compared to the area in the Czech Republic, but was likewise dominated by grasses (*Avenella*, *Calamagrostis*), ericoid shrubs (*Vaccinium* spp.) and mosses. The area is located at an elevation of 170-180 m, with a mean annual temperature of 5.8 °C and mean annual precipitation of 680 mm.

Sampling was performed at five sites in each area that were located approximately 250 m from each other. Samples were collected every 3 months to represent early summer (June), late summer (September), early winter (December) and late winter (March-April). The sampling in the Czech Republic ran from June 2016 to March 2017, in Norway from April 2017 to December 2017. Sixteen soil cores (4 cm diameter) were collected per site from a defined area of 3 x 3 m. Litter (2-4 cm thickness), soil (organic horizon, 3-6 cm of depth), rhizosphere soil (soil adhering to plant roots that had to be manually detached) and plant roots (< 2 mm in diameter) were separated from each other. Litter was cut into 0.5 cm pieces and mixed, soil was passed through a 5-mm sterile mesh and mixed, and the same was done for rhizosphere soil. Due to the need for immediate freezing in liquid nitrogen, roots were washed on site in sterile deionised water until all visible soil material was removed. All root material was pooled per site and at each sampling time. All samples were immediately frozen in liquid

nitrogen and stored on dry ice. Samples for nucleic acid extraction were subsequently stored at -80°C. In total, 160 samples were collected (2 study areas × 5 sites × four habitats × four seasons). To quantify fungal biomass in rhizosphere soil, bulk soil and litter samples, ergosterol was extracted from freeze-dried material using 10% KOH in methanol (Šnajdr et al., 2008) and quantified by high-performance liquid chromatography (HPLC).

2.2 Nucleic acid extraction and amplification

DNA was extracted as previously described (Žifčáková et al., 2016; Žifčáková et al., 2017) except for the spruce roots for which DNA was extracted using a DNeasy Plant Maxi Kit according to the manufacturers protocol (Qiagen GmbH, Hilden, Germany). The V4 region of 18S rRNA gene was amplified using primers specific for the phyla Cercozoa and Endomyxa in a two-steps semi-nested PCR (Fiore-Donno et al., 2018; Fiore-Donno et al., 2020). The first reaction consisted of 1 µl of a 1:10 dilution of template DNA and a 1:1 mixture of forward primers S615F_Cerco (GTTAAAAAGCTCGTAGTTG) and S615F_Phyt (GTTAAAARGCTCGTAGTCG) and a single reverse primer S963R_Phyt (CAACTTTCGTTCTTGATYAAA); the second reaction consisted of 1 µl of the resulting amplicon mixture and primers S615F_Cer (GTTAAAARGCTCGTAGTYG) and reverse S947R_Cer (AAGARGAYATCCTTGGTG) with unique barcodes on both the forward and reverse primer. Both reactions contained 5 µl Q5 reaction buffer (New England Biolabs, USA), 0.75 µl BSA (20 mg ml−1), 1 µl of each primer (0.01 mM), 0.5 µl of PCR nucleotide mix (10 mM each), 0.75 µl polymerase (2 $U.\mu l^{-1}$ Q5 High Fidelity DNA polymerase, New England Biolabs, USA), 5 µl of Q5 High GC Enhancer (New England Biolabs, USA). Cycling conditions were 95°C for 2 min, followed by 24 cycles at 95°C for 30 s, 50°C for 30 s, 72°C for 30 s and a final elongation step at 72°C for 5 min. Both reactions were performed in triplicate to reduce the possibility of artificial dominance of few amplicons. Pooled PCR products were purified using a Qiagen MinElute Purification Kit (Qiagen GmbH, Hilden,

Germany). Equimolar concentrations of amplicons were used for library preparation using a TruSeq DNA PCR-Free LT Kit (Illumina Inc., San Diego, CA, USA) and sequenced in-house on an Illumina MiSeq with 2 x 250 reads (MiSeq v2 Reagent Kit, Illumina Inc., San Diego, CA, USA).

Bacterial and fungal rRNA gene copies were quantified by quantitative PCR (qPCR) using the 1108f and 1132r primers for bacteria (Amman et al., 1995) and the FR1 and FF390 primers for fungi as described previously (Žifčáková et al., 2016). The bacterial/fungal rRNA gene ratio was calculated by dividing the respective rRNA gene copy numbers.

2.3 Bioinformatic analyses

Raw forward and reverse fastq files were merged using fastq-join (Aronesty, 2011). Sequences containing ambiguous nucleotides, sequences without barcodes or primers and joined sequences with an overlap shorter than 100 bp were discarded. Sequences were clustered into Operational Taxonomic Units (OTUs) using VSEARCH implemented in mothur v39.5 (Schloss et al., 2009) with abundance-based greedy clustering (agc) and a similarity threshold of 97%. To reduce amplification or sequencing noise, clusters represented by less than 50 reads (0.005% of the reads) were removed (Fiore-Donno et al., 2018). Using BLAST+ (Camacho et al., 2008) with an e-value of 1^{e-50} and keeping only the best hit, sequences were identified in the PR2 4.11.1 database (Guillou et al., 2013) and non cercozoan sequences were removed. Sequences were aligned with the provided template (Fiore-Donno et al., 2018) with a penalty for opening gaps of -5. Chimeras were identified using UCHIME as implemented in mothur and chimeric sequences were removed. The results are provided in Table S1 with the OTUs abundance/site, their taxonomic assignment according to the best hit by Blast and their functional assignment according to Dumack et al. (2020), the updated reference is accessible at https://github.com/Kenneth-Dumack/Functional-Traits-Cercozoa-

198 Endomyxa (last accessed Oct. 2021). The relative abundance of each cercozoan taxonomic
199 level was illustrated using the open source SankeyMatic (http://sankeymatic.com/, last
200 accessed Oct. 2021) and refined with the open-source vector graphic editor Inkscape
201 (https://inkscape.org/en/, last accessed Oct. 2021).

202 **2.4 Statistical analyses**

203 All statistical analyses were carried out within the R environment (R v. 3.5.1) (R
204 Development Core Team, 2014) on the OTU abundance/sites table; biotic parameters were
205 the bacterial/fungal ribosomal copy ratio and the ergosterol content of all compartments but
206 roots. Unless otherwise specified, community analyses were performed with the package
207 vegan (Oksanen et al., 2013). **Alpha diversity**: To evaluate if more sampling and sequencing
208 effort would have revealed more OTU richness, we carried out an analysis based on OTUs'
209 accumulation curves, function specaccum, method rarefaction with 1 000 random
210 permutations; species richness was extrapolated using the function specpool. Alpha diversity
211 estimates were based on relative abundances of OTUs (function decostand, method "total");
212 Shannon diversity and Pielou's evenness were obtained with the function diversity. ***Beta-***
213 ***diversity****:* Variation partitioning (function varpart applied to the Hellinger-transformed OTUs
214 dataset and using RDA, function rda) was applied to assess the amount of explained beta
215 diversity by the factors country ("country" refers to the two study sites, in Norway and the
216 Czech Republic), season, or compartment. Beta diversity between countries and
217 compartments was inferred by Principal Coordinate Analysis (PCoA, function cmdscale),
218 using Bray-Curtis dissimilarities (function vegdist, method "bray") on the relative abundances
219 of OTUs, then plotted with the package ggplot2. The same distance matrix was used to assess
220 significant differences in beta diversity, with Tukey's Honest Significant Differences
221 calculated on the output of the function betadisper (method "median"), applied on the distance
222 dissimilarity matrix as before. Because the relative abundance is an important component of

the beta diversity, the effects of compartments and countries on the **relative abundances** of the trophic guilds were tested with general linear models (function glm, core package). They were then subjected to the general linear hypothesis test (function glht, package multcomp) with Tukey's test for multiple comparisons of means and a heteroskedasticity-consistent covariance matrix estimation (function vcovHC, package sandwich). Since it could be argued that with relative abundances, one group will decrease when the other is increasing, we also compared absolute abundances, obtaining exactly the same trends as with the relative abundances (results not shown).

Nestedness: To infer to which extent the taxonomic assemblages in each compartments were subsets of the main assemblage, nestedness analyses were conducted following the procedure described in (Strona and Stefani, 2013) using nestednodf and oecosimu (package vegan), ignoring empty rows and columns, z-values calculated from 100 iterations with the model r00, which maintains the number of presences (fill). A group of species assemblage is said to be nested if the species composing its smaller subsets are only a fraction of the larger subsets. Briefly, the NODF metric and the z-score were calculated from the complete binary matrix (160 sites as columns and 1308 OTUs as rows) and from the four matrices of each compartment (40 sites and a variable number of OTUs, since there are empty rows). Then 50 submatrices of different sizes, from very small to submatrices approaching the number of cells of the complete matrix (from 500 rows and ten columns to 1300 rows and 100 columns) were created by random extraction of rows and columns from the complete matrix. The assemblages of each compartment were considered nested if their z-scores were significantly different (outside the 95% confidence intervals, p=0.05) from that obtained from random matrices of the same size (i.e. the total occurrences or fill). The distances of the observed z-scores from the regression line was used to establish which compartments were more nested (Strona and Stefani, 2013).

3 Results

3.1 Taxonomic and functional diversity of Cercozoa and Endomyxa

We obtained circa 3 million reads, of which 2 135 264 passed the quality checks. Clustering at 97% similarity resulted in 2 397 OTUs (representing 1 729 157 sequences), after eliminating the rare ones. After removing non-cercozoan, badly aligned and chimeric sequences, we obtained 1 308 OTUs representing 1 164 680 sequences, on average 7 279 reads/sample (min = 3 082, max = 18 288, SE= 2 325). A table with the OTU abundance per sample, their taxonomic assignment and the functional traits is provided (Supplementary Table S1). Our sequencing and sampling efforts were sufficient to cover the overall (gamma) diversity, since the actual OTU richness was reached after only c. 80 000 sequences (Fig. S1A) and c. 30 samples (Fig. S1B). The 1 308 OTUs represented 248 unique Blast best hits, of which 90% were assigned to the phylum Cercozoa and only 10% to Endomyxa (Fig. 1). Within Cercozoa, the class Sarcomonadea dominated (66%), composed of Glissomonadida (53%) and Cercomonadida (13%). Glissomonadida is mostly composed of small flagellates while Cercomonadida mainly comprise amoeboflagellates. Other main lineages were represented by Euglyphida, which are testate amoebae with silica shells (6%) and Cryomonadida (9%), amoebae with mainly organic tests. Endomyxa was composed of the amoeboid algal and fungal-feeder Vampyrellida (9%), which seemed to prefer the litter (Fig. 1B). The plant parasitic Plasmodiophorida accounted only for 1% (Fig. 1). The five most abundant OTUs (>30 000 sequences, accounting for 29% of all sequences) belonged to the Glissomonadida (families Allapsidae and Sandonidae), that is, small bacterivorous gliding flagellates (Table S2). Among trophic guilds, bacterivores represented more than half of the OTUs, (53%), but omnivores (18%) and eukaryvores (12 %) also contributed sizeable proportions (Table S1). The plant parasites (0.6%) and hyperparasites of Oomycota (0.1%) were only marginally present. No trophic guild could be assigned to 17% of the OTUs. Naked

cells (flagellates 46%, amoeboflagellates 14%, and amoebae 9%) together constituted the predominant morphotypes (69%), whereas testate cells (18%) were less frequent, with organic/agglutinated and siliceous tests in equal proportions; no morphology could be attributed to 12% of the OTUs. The overwhelmingly dominant locomotive mode was creeping/gliding on the substrate (88%), the remaining OTUs were non-motile endoparasites (1%) or with unknown locomotion mode (11%) - no freely-swimming taxa were recorded.

3.2 Differences in alpha and beta diversity between compartment, season and country

We found a significantly higher alpha diversity, estimated by the Shannon index (Fig. S2) in Norway than in the Czech Republic; in the compartments, the diversity was higher in soil, litter and rhizosphere than in the root; and no significant difference was found between seasons. The OTUs tended to be evenly distributed, as shown by evenness (average of all samples 0.66) (Fig. S2). The beta diversity was low, since most OTUs were shared between countries, compartments and seasons. Only root showed significant differences with both litter and soil ($p < 0.001$), while no significant differences were found between the other compartments as well as between seasons or countries. Variance partitioning showed that the combined effects of the compartment (21.5%), country (13.8%), and season (0.13%) explained 36.5% (adjusted R^2) of the total variation in beta diversity. Since the seasonal variability in alpha and beta diversity was only marginal, we did not consider it in the subsequent analyses. PCoA revealed different communities between compartments and countries (Fig. 2), with the first and second axis explaining 27% and 14% of the variation of the Bray-Curtis distances, respectively. The litter communities appeared as most distinct, from the other compartments as well as between countries. For each compartment, there was little overlap between countries. The rhizosphere and soil communities shared many taxa in both countries. The compartments differed in bacterial-fungal ratio and fungal biomass (estimated by ergosterol content), also between countries, with generally higher bacterial-

298 fungal ratios in the Czech Republic (Fig. S3 A & B). Despite this, bacterial-fungal ratios
299 followed the same trend in both countries, the highest in soil and the lowest in the litter (Fig.
300 S3 A). The estimated fungal biomass was higher in Norway than in the Czech Republic, and
301 in both countries higher in litter, while lower in soil and intermediate in the rhizosphere (Fig.
302 S3 B).

303 Marked differences were present in the distribution of functional groups across countries and
304 compartments. The relative proportion of bacterivores was significantly higher in the Czech
305 Republic, whereas that of omnivores and eukaryvores was significantly higher in Norway
306 (Fig. 3A). Across compartments, the bacterivores dominated in root, the omnivores in litter
307 and the eukaryvores in rhizosphere and soil (Fig. 3B).

308 All compartments, separately and together, showed nestedness, both in taxon composition and
309 in site occupancy (Table S2). The compartments appear more nested than expected for a
310 matrix of equal size extracted at random from the respective entire matrix (Fig. 4) - i.e.,
311 outside the confidence interval. Differences between observed and predicted z scores
312 decreased in the following order: soil>root>litter>rhizosphere, root having the highest
313 nestedness and being the only compartment more nested than expected (Fig.4A and Table
314 S2). Similarly, when comparing soil (the largest matrix) with the other three compartments,
315 only the z-score of the root was outside the 95% confidence interval, with a large, positive
316 difference from the expected z-score, indicating that the root communities were nested subsets
317 of the soil community (Fig. 4B).

318 # 4 Discussion

319 Our study revealed that the communities of major protistan components of the soil biota,
320 Cercozoa and Endomyxa, were not homogeneously distributed in spruce forest soils, but
321 strongly differed between environmental niches shaped by living plants (root, rhizosphere),

plant remains (litter) and the characteristics of the bulk soil. This was mainly due to differences in community composition, since most taxa were nearly ubiquitous. Such a pattern of high alpha- and low beta-diversity of protists is consistently observed in different environments and at different scales (Lentendu et al., 2018; Fiore-Donno et al., 2019; Fiore-Donno et al., 2020; Walden et al., 2021). The local influence of the soil compartment was almost twice as important as the geographical location (21.5% and 13.8%, respectively) in structuring the protistan communities, despite marked differences in the legacy of nitrogen deposition between forests of the two countries. This is in keeping with recent studies, showing the major effect of compartments in forest soils, compared to other drivers, e.g. plant species and edaphic parameters (Uroz et al., 2016; Tkacz et al., 2020). It seems therefore important to consider compartments for a precise understanding of protistan diversity in soils and its drivers at different scales.

In the two compartments where the influence of plants was the strongest (root and rhizosphere), we also observed strikingly different distributions of protists according to their feeding habits. The bacterivores, predominantly small flagellates belonging to the order Glissomonadida (family Allapsidae in particular), were more abundant in the root compartment (especially in the Czech forest where the bacterial/fungal ratio was higher), thus probably benefitting from the bacteria associated to the mycorrhizosphere surrounding spruce roots. This suggests an essential role for phagotrophic protists in the plant-associated soil food web, under the indirect structuring influence of the plant, since the relative abundance of bacterivores decreased in the other compartments. A similar trend was also observed in grasses, e.g. in a study involving switchgrass, where the families Sandonidae and Allapsidae were more abundant in the rhizosphere than in bulk soil (Ceja-Navarro et al., 2021) and the rhizosphere of wheat, oat and pea was enriched in bacterivorous protists as well (Turner et al., 2013) (these studies did not consider the root). Glissomonadida usually dominate the

cercozoan assemblages, in diverse environments (Bugge Harder et al., 2016; Fiore-Donno et al., 2020; Walden et al., 2021), so that they should be recognized as key players in the soil food web in general, but also in the plant-associated interactions of the microbial loop. A recent study added insight in the dynamics of the root colonization of maize by Cercozoa: colonization starts randomly at the root tips, probably related to fast-growing bacteria benefitting from the root exudates, becoming more stable in older parts of the root. Concomitantly, the interactions between protists and bacteria become more intense in the older parts of the root (Rüger et al., 2021). We found that the communities associated with the roots were non-random subsets of the larger soil communities, probably shaped by the selection exerted by the plant roots and their associated microbiome. By comparing nestedness z -scores, we showed that the root compartment was highly nested as well as more nested than expected, while the influence of the plant decreased already in the rhizosphere (Fig. 4).

As the litter of spruce forests is usually quite uniform, it was surprising how much the communities in the litter compartment differed between countries. The same was observed by Nuccio et al. (2021), who showed that the prokaryotic and eukaryotic communities were most distinct in the litter (in this case, dead roots) compared to bulk soil and rhizosphere. It has been suggested that the litter inherits the microbiome already present in the foliage - a process called "founder" or " priority effect" (Debray et al., 2022). A clear founder effect was observed in wood disks colonized with specific fungal isolates, which developed different microbiomes 12 months after deposition in leaf litter (Hiscox et al., 2015). Observation of communities in situ revealed a stochastic founder effect from foliage to litter for fungal (Guerreiro et al., 2018) and protistan communities (Walden et al., 2021). In our study, the drivers of the protistan litter communities appeared to be at least partially different from those

371 of the bulk soil and plant-associated communities, maybe reflecting the aforementioned
372 stochastic founder effect.

373 Very sensitively, the litter protists reflected the changes in their food sources: In Norway, the
374 fungal proportion (as estimated by the ergosterol content) in litter was nearly twice as that in
375 the Czech Republic (Fig. S3 B). Accordingly, we found in the Norwegian litter a higher
376 relative proportion of potential fungivores (i.e. omnivores and eukaryvores), compared to the
377 litter of the Czech forest where bacterivores dominated (Fig. 3A). Cercozoan eukaryvores
378 were also more abundant in the litter than in tree crowns of a temperate forest (Walden et al.,
379 2021).

380 The general trend observed for prokaryotes and fungi, whose diversity decreases from the
381 bulk soil to the rhizosphere (Nannipieri, 2020), was also valid for protists (Fig. S2). In the
382 investigated forests, both the diversity and the evenness decreased from soil, rhizosphere,
383 litter and the root. A higher alpha protistan diversity in agricultural bulk soil versus
384 rhizosphere was also observed in potato and lettuce (Dumack et al., 2021) and switchgrass
385 (Ceja-Navarro et al., 2021); in the latter, the plant structuring effect was demonstrated, since
386 the community differences increased during plant growth. In our study, however, the
387 communities of the litter and rhizosphere were not subsets of the soil communities (as shown
388 by nestedness analyses); only in the root could the strong selecting influence of the plant be
389 observed.

390 5 Conclusions

391 Despite an increasing interest in soil protists, there is no consensus on the main drivers
392 shaping their distribution. In this study, we show a clear niche partitioning across
393 compartments, surpassing the influence of drivers exerting community selection at larger
394 scales. In addition, we show that protists, here exemplified by two phyla, should not be

treated as a homogeneous ensemble, because they show opposite distribution patterns according to their feeding preferences. We hypothesize that two distinct selection processes could be at work: firstly in the root communities, where the plant indirectly selects a subset of the soil community, and secondly in the litter communities, where founder effect of protists already present in the foliage may influence community assembly on the forest floor.

6 Availability of data and material

Raw sequences have been deposited in Sequence Read Archive (NCBI), Bioproject PRJNA776131. All other data generated or analysed during this study are included in this published article and its supplementary information files.

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587

588 # 9 Figure legends

589 **Figure 1**. Diversity of cercozoan and endomyxan OTUs in 160 samples from spruce forests
590 in Norway and the Czech Republic. Taxonomical assignment is based on the best hit by
591 BLAST, taxa representing <1% are not shown. **A**. Sankey diagram showing the relative
592 contribution of the cercozoan and endomyxan OTUs to diversity. From left to right, names
593 refer to phylum (Cercozoa, Endomyxa), class (ending -ea) and orders (ending -ida). Numbers
594 are percentages of OTUs abundance. Drawings (not to scale): Glissomonadida are mostly
595 small flagellates; Cercomonadida are mostly amoeboflagellates; Euglyphida are mostly
596 amoebae with a shell made of silica; Cryomonadida are mostly amoebae with an organic
597 shell; Limnofilida are amoeboflagellate with filose pseudopodia; Vampyrellida are amoebae;
598 Plasmodiophorida are plant endoparasites. **B**. Comparison between compartments of the
599 relative contribution of the main orders to the taxonomic diversity.

600 **Figure 2**. Principal Component Analysis of the Bray-Curtis dissimilarity indices between
601 cercozoan and endomyxan OTUs, showing different communities between compartments
602 (litter, root, soil and rhizosphere) and countries.

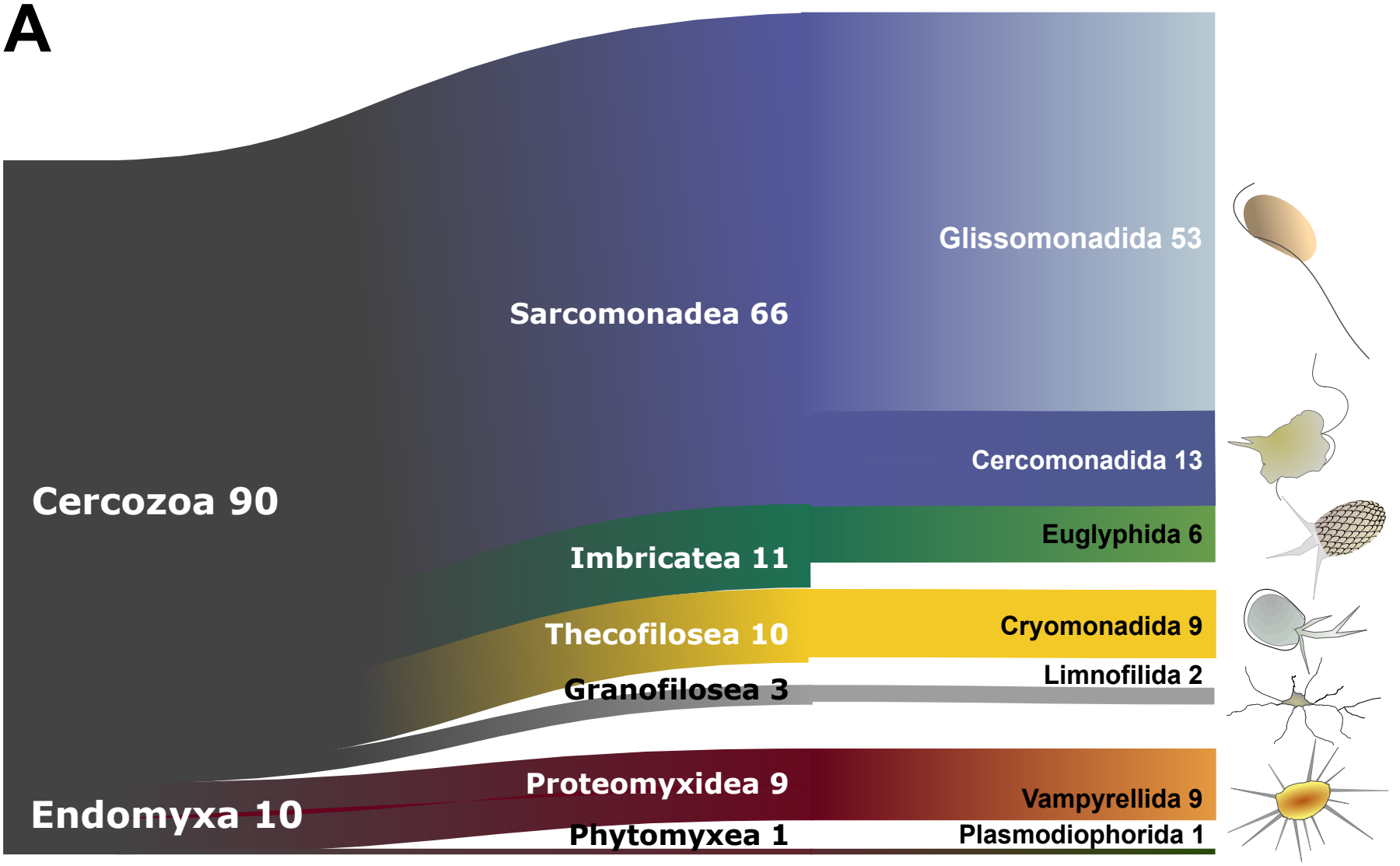
603 **Figure 3.** Boxplots of the variation of the relative abundances of the three main nutrition
604 modes of Cercozoa and Endomyxa. **A.** By country; **B.** By compartments. The y-scale varies
605 between graphs. Red letters: a change from “a” to “b” indicates a significant difference
606 (multiple comparison of means, Tukey's test); two letters (“ab”) indicate non-significant
607 differences between plots sharing those letters. Red dots indicate the mean.

608 **Figure 4**. Nestedness. **A**. Regression line showing the relationship between the matrix size
609 (number of occurrences) and their corresponding z-scores. The shaded area indicate the 95%
610 confidence interval. Red dots indicate the four compartments, which are all outside the
611 confidence interval, thus nested. **B**. Same as in A for the three compartments compared to a

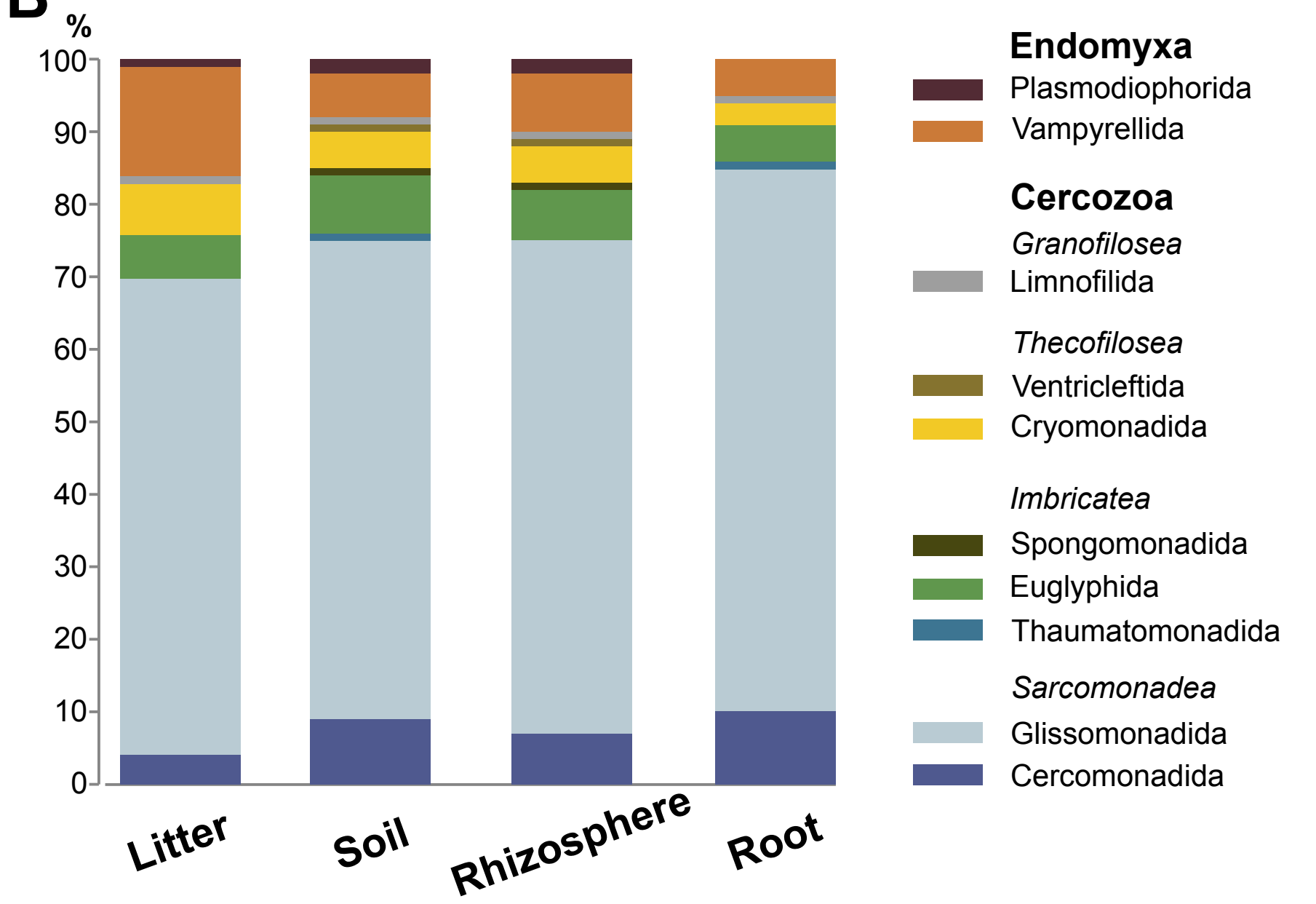
612 regression line of the z-scores of 90 submatrices of the soil matrix. Only the root is a
613 subsample of the soil compartment. Detailed statistics in Table S2.

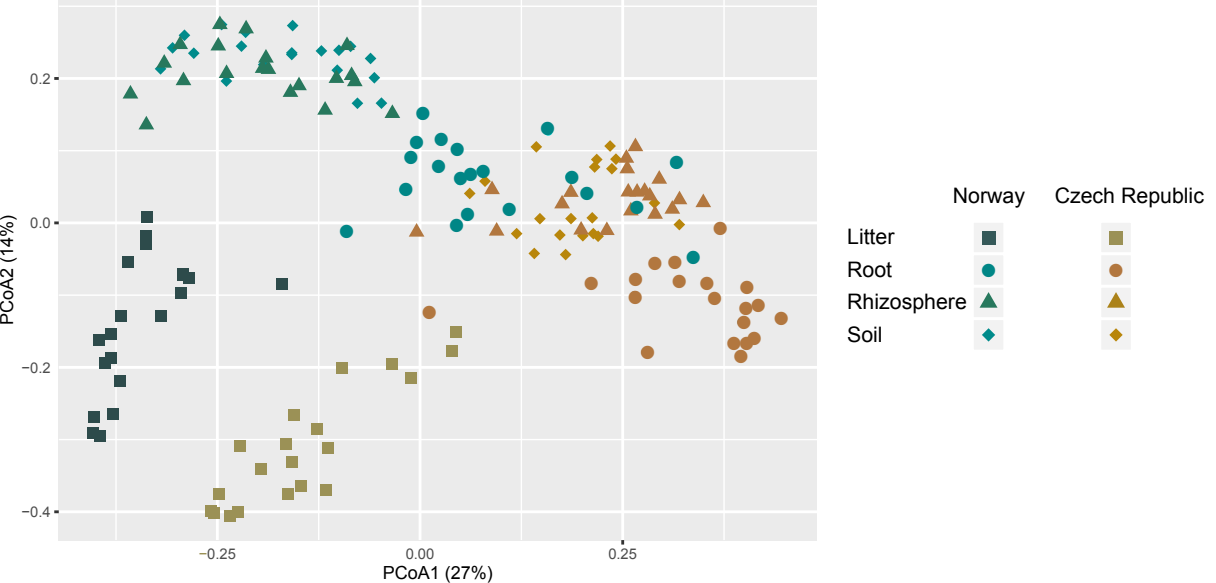
614

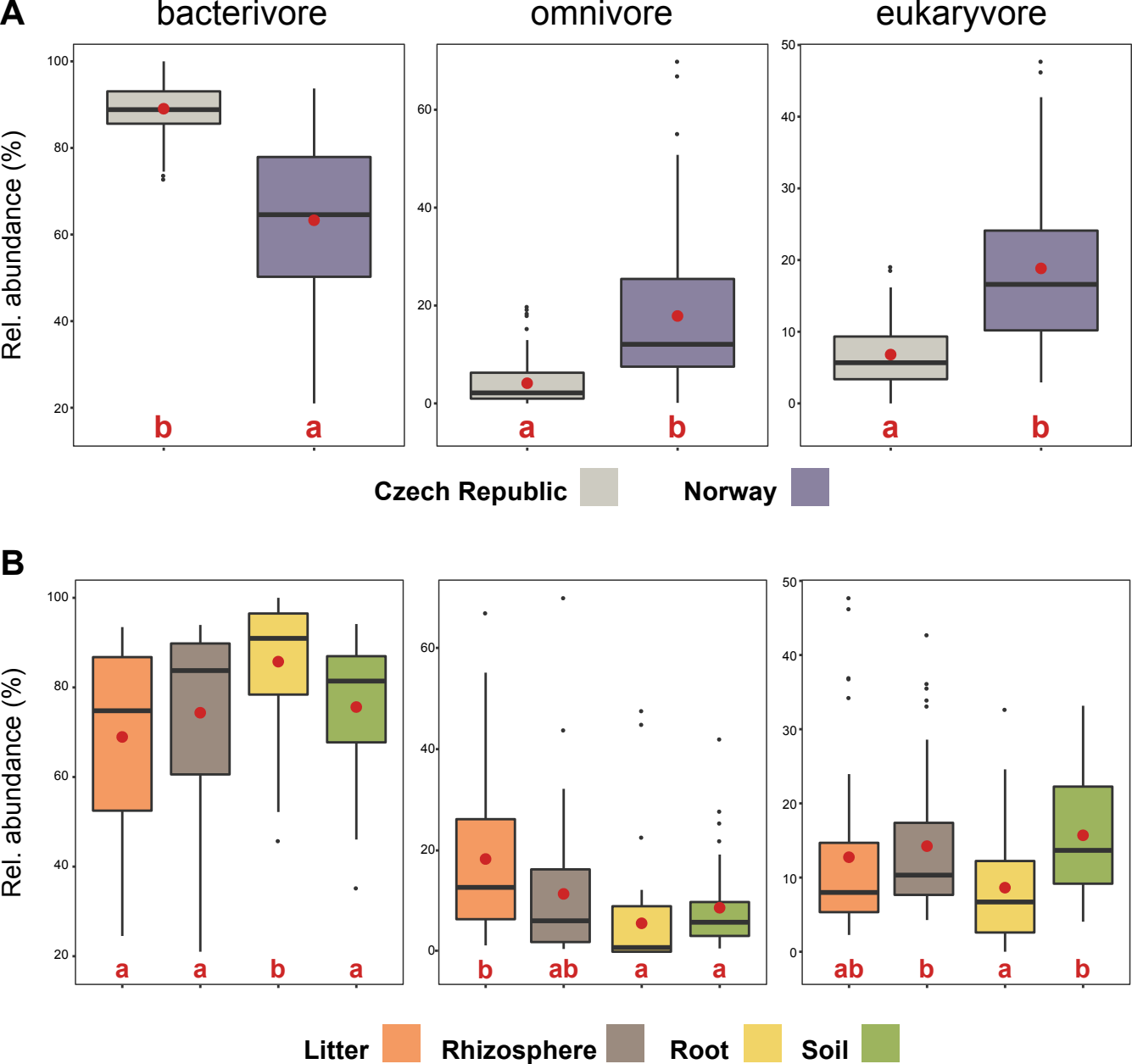
A

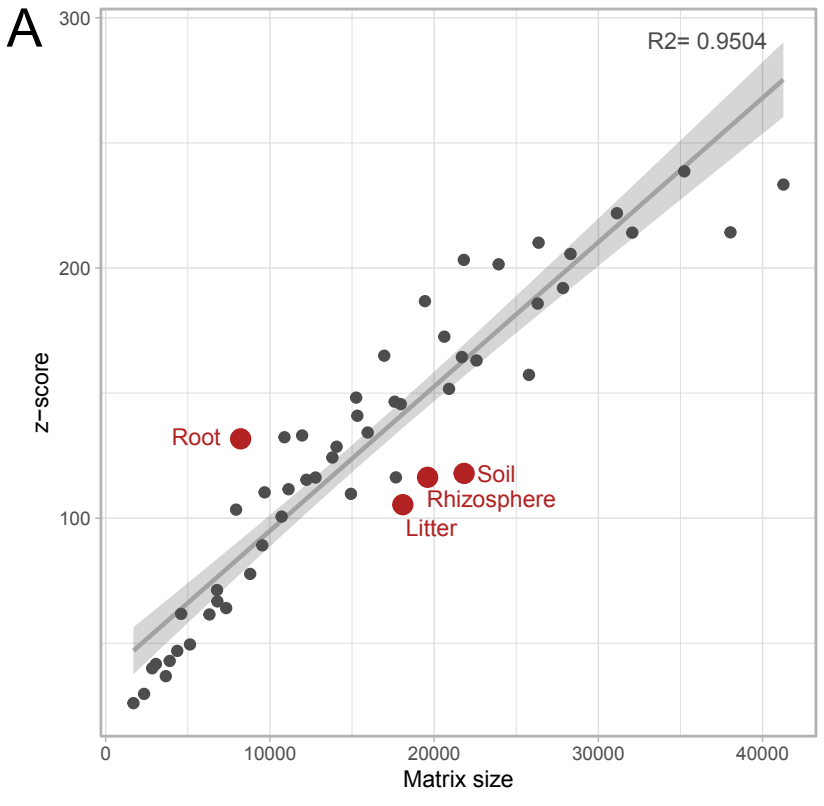

Cercozoa 90
Endomyxa 10
Sarcomonadea 66
Imbricatea 11
Thecofilosea 10
Granofilosea 3
Proteomyxidea 9
Phytomyxea 1
Glissomonadida 53
Cercomonadida 13
Euglyphida 6
Cryomonadida 9
Limnofilida 2
Vampyrellida 9
Plasmodiophorida 1

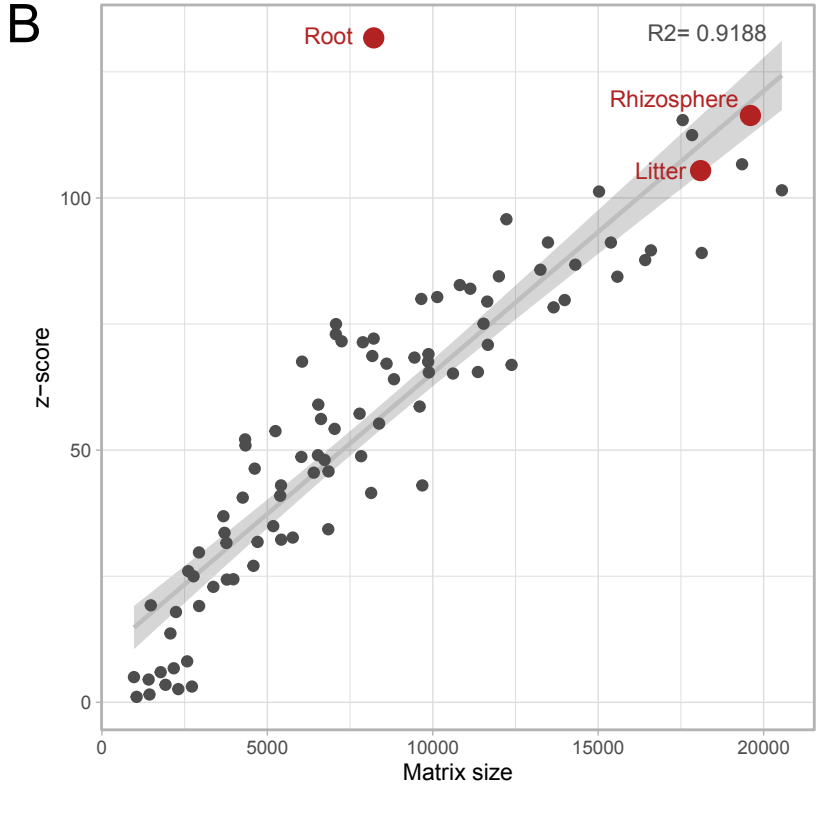
B


%
100
90
80
70
60
50
40
30
20
10
0
Litter
Soil
Rhizosphere
Root
Endomyxa
Plasmodiophorida
Vampyrellida
Cercozoa
Granofilosea
Limnofilida
Thecofilosea
Ventricleftida
Cryomonadida
Imbricatea
Spongomonadida
Euglyphida
Thaumatomonadida
Sarcomonadea
Glissomonadida
Cercomonadida


0.2
0.0
−0.2
−0.4
PCoA2 (14%)
−0.25
0.00
0.25
PCoA1 (27%)
Norway
Czech Republic
Litter
Root
Rhizosphere
Soil


A
bacterivore
omnivore
eukaryvore
Rel. abundance (%)
b
a
a
b
a
b
Czech Republic
Norway
B
Rel. abundance (%)
a
a
b
a
b
ab
a
a
ab
b
a
b
Litter
Rhizosphere
Root
Soil


A
R2= 0.9504
z−score
Matrix size
Root
Soil
Rhizosphere
Litter
300
200
100
0
10000
20000
30000
40000


B
R2= 0.9188
z−score
Matrix size
Root
Rhizosphere
Litter
100
50
0
5000
10000
15000
20000

Soil compartments as main drivers of protistan communities