

Additive partitioning of testate amoeba species diversity across habitat hierarchy within the pristine southern taiga landscape (Pechora-Ilych Biosphere Reserve, Russia)

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Abstract

In order to better understand the distribution patterns of terrestrial eukaryotic microbes and the factors governing them, we studied the diversity partitioning of soil testate amoebae across levels of spatially nested habitat hierarchy in the largest European old-growth dark coniferous forest (Pechora-Ilych Biosphere Reserve; Komi Republic, Russia). The variation in testate amoeba species richness and assemblage structure was analysed in 87 samples from six biotopes in six vegetation types using an additive partitioning procedure and principal component analyses. The 80 taxa recorded represent the highest value of species richness for soil testate amoebae reported for taiga soils so far. Our results indicate that testate amoeba assemblages were highly aggregated at all levels and were mostly controlled by environmental factors rather than dispersal processes. The variation in species diversity of testate amoebae increased from the lowest to the highest hierarchical level. We conclude that, similarly to macroscopic organisms, testate amoeba species richness and community structure are primarily controlled by environmental conditions within the landscape and suggest that metacommunity dynamics of free-living microorganisms are driven by species sorting and/or mass effect processes.

Keywords: Biodiversity; Community ecology; Metacommunity; Micro-habitat; Soil protozoa; Vegetation type

Introduction

What are the diversity patterns of different groups of living organisms? How is this diversity generated and maintained? Answering these questions is a prerequisite for effective biodiversity preservation and assessment and mitigation of the

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effects of natural and human-induced environmental perturbations on biodiversity. However, the diversity of many groups of organisms is poorly documented, and this is especially true for soil microorganisms (Decaens 2010). Extensive data sets on soil microorganisms documenting their diversity but also allowing to assess the factors potentially controlling their diversity and community patterns remain rare; existing data are mostly patchy, making it difficult to test ecological theories and to develop sound management policies for a major component of Earth's biodiversity.

The theoretical framework of metacommunity ecology (Leibold et al. 2004) considers that local communities are linked by dispersal of multiple interacting species and that patterns in species diversity are regulated by dispersal processes as well as environmental factors. Depending on the relative importance of these processes, four types of metacommunity dynamics can be distinguished: species sorting, mass effects, patch dynamics and the neutral model (reviewed by Holyoak et al. 2005). The species sorting dynamics is mostly related to niche processes, which assume that variation in community composition should be more strongly associated with environmental factors in regional contexts with high environmental variation than in those with low environmental variation (Heino 2011). Mass effect occurs when increasing dispersal in heterogeneous environments results in local populations being quantitatively affected by dispersal through source-sink dynamics, independent of environmental gradients. Dispersal limitation is more important in patch dynamics and the neutral model. The patch metacommunity dynamics implies a trade-off between dispersal capacity and competitive strength among species, whereas species are ecologically equivalent in the neutral model. Thus, an estimation of the contribution of dispersal processes and environmental factors to the species diversity of a particular area or habitat is necessary to assess the drivers of species diversity, which ultimately has practical implications for biodiversity management.

One of the ways to quantify the contribution of dispersal and local environmental conditions to the general species diversity of an area (or a region, or a habitat) (γ) is to compare components of diversity that occur within (α) and among sampling units (β) at hierarchical sampling scales or at the levels of a habitat hierarchy (Crist et al. 2003; Wagner et al. 2000). If the α component contributes most to species diversity then variations in broad-scale processes are more important than local process and the number of species in each fine-scale area is only a predictable fraction of the broad-scale diversity. On the contrary, in cases where the β component is more important, then local processes strongly regulate local diversity, which will vary considerably from one site to another. This approach has been successfully applied to describe species diversity patterns in agricultural landscapes (Wagner et al. 2000), tropical forests (DeVries et al. 1997), temperate forests (Gering et al. 2003) and aquatic ecosystems (Schmera and Podani 2013). However, it has rarely been used for describing diversity patterns

of protists and other microorganisms (Cadotte 2006; Mazei 2008).

Biogeographical and macroecological patterns of microorganisms might differ fundamentally from those of larger organisms because of the smaller size, the presence of resting stages and quick reproductive rates which can considerably increase dispersal and colonisation abilities of microorganisms (Foissner and Hawksworth 2009; Fontaneto and Hortal 2012). Earlier views considered that these features could allow many microscopic organisms to attain cosmopolitan and ubiquitous distribution (De Wit and Bouvier 2006; Finlay 2002). In contrast, recent studies indicated that biogeographical and macroecological patterns of microorganisms might be similar to those observed for macroorganisms (Azovsky and Mazei 2013; Foissner 2006; Fontaneto and Hortal 2012). Our focus here is on testate amoebae, a common group of free-living amoeboid protozoa, which are characterised by the presence of a shell. Testate amoebae have a worldwide distribution and inhabit soils, aquatic ecosystems, mosses, etc. In soils, testate amoebae constitute a considerable part of biota in terms of biomass and biodiversity (Schröter et al. 2003) and they are considered as reliable indicators of micro-environmental characteristics (soil type, moisture, pH, etc.) (Mitchell et al. 2008). They prey on a wide range of organisms, including bacteria, protozoa, microalgae, fungi, and micro-metazoa, and may also consume dead organic matter (Gilbert et al. 2003; Wilkinson and Mitchell 2010).

The diversity of testate amoebae has been intensively studied in many regions including the taiga (boreal dark coniferous forest) zone (Bobrov et al. 1994; Schönborn 1986; Schröter et al. 2003). They also have been used as model organisms for studying spatial patterns of diversity distribution (Finlay et al. 2001; Mazei 2008). Although these studies provide useful information on the diversity of testate amoebae and its spatial patterns, most were conducted in strongly disturbed areas (but see Mazei et al. 2012) where the species diversity patterns might be biased, e.g. as a result of reduced biotope diversity and introduced species. Our objective was therefore to characterise the diversity patterns of testate amoebae in a very pristine old-growth dark coniferous forest in the Pechora-Ilych Biosphere Reserve (Smirnov 2013; Smirnova et al. 2006) and to estimate how the diversity is distributed across nested levels of habitat hierarchy. To this purpose we collected samples to cover the entire diversity of terrestrial vegetation types and biotopes existing in the area, using a hierarchically nested sample design (Gering et al. 2003). We analysed the variation in species richness and species structure of testate amoeba assemblages using an additive partitioning procedure and principal component analyses. We hypothesised that (i) the pristine forest will be characterised by greater species richness of testate amoebae than similar but less pristine taiga regions due to higher habitat diversity; (ii) species richness of testate amoebae will be strongly regulated by local environmental conditions (vegetation and habitat type) within the studied taiga landscape.

Material and Methods

Study site

The study was performed in a taiga area located in the Pechora-Ilych Biosphere Reserve (western slopes of the Ural Mountains; 62°34'N, 58°15'E) (Fig. 1). The region is characterised by humid continental climate with a strong influence of cold arctic air masses (Bobretsov and Teplov 2000). The mean annual air temperature is $-0.4\text{ }^{\circ}\text{C}$ and the mean annual precipitation is 700 mm year^{-1} (weather station in Ust'-Un'ya). The growing season (when average daily temperature is above $+10\text{ }^{\circ}\text{C}$) lasts for 80–85 days. The typical soils in the region are podzolic and brown soils (Bovkunov et al. 2010). The vegetation in the area represents an old-growth (pristine) forest with the tree layer dominated by *Picea obovata* Ledeb., 1833, *Abies sibirica* Ledeb., 1833, *Pinus sibirica* Du Tour, 1803. Based on the composition of the tree layer and undergrowth, the vegetation could be classified into four main forest types: large fern, green moss, *Sphagnum* and nitrophilous tall herb forest (Cherepanov 1995; Ignatov et al. 2006; Lugovaya et al. 2013; Zaugol'nova et al. 2010). The green moss and *Sphagnum* forests are further divided into two subtypes based on the composition of the field layer resulting in a total of six vegetation types included in this study (Fig. 2).

Large fern spruce (*P. obovata*) and fir (*A. sibirica*) forests with *P. sibirica* (*Piceeta Abieta magnofilico-sum*), (hereafter referred to as large fern taiga; Fig. 2A; 62°03'13.62"N, 59°03'12.54"E) occupy the upper part of the slopes. The overstorey is dominated by *P. obovata* and *A. sibirica* with *P. sibirica* and less frequent *Betula pubescens* Ehrh.,

1791 (total crown cover: 30–60%). Undergrowth mainly consists of *P. obovata* and *A. sibirica*, rarer *B. pubescens*, and isolated individuals of *P. sibirica*. The shrub layer is poorly developed and consists of *Rosa acicularis* Lindl., 1820 together with *Juniperus communis* L. (1753) (total cover: 10–20%). The large fern *Dryopteris dilatata* (Hoffm.) A. Gray, 1848 dominates in the field layer (total cover: 90–100%). Under *D. dilatata*, small boreal ferns and herbs (*Gymnocarpium dryopteris* (L.) Newm., 1851, *Maianthemum bifolium* (L.) F.W. Schmidt (1794), *Oxalis acetosella* L. (1753), *Linnaea borealis* L. (1753)), the dwarf shrub *Vaccinium myrtillus* L., 1753 and others occur in very low abundance. The bottom layer is poorly developed and mainly formed by boreal green mosses (*Pleurozium schreberi* (Brid.) Mitt., 1869 and *Hylocomium splendens* (Hedw.) Schimp., 1852).

Green moss spruce (*P. obovata*) and fir (*A. sibirica*) forests (*Piceeta Abieta hylocomiosa*) occupy the middle parts and bottoms of the slopes. *P. obovata* and *A. sibirica* prevail in the overstorey; *B. pubescens* is frequent and *P. sibirica* occurs sporadically (total crown cover: 40–60%). The undergrowth mainly consists of *P. obovata* and *A. sibirica*. The shrub layer is poorly developed (cover no more than 15%) and formed by *Sorbus aucuparia* L., 1753, *J. communis* and very rarely *Ribes rubrum* L. (1753). The field layer is formed by boreal dwarf shrubs *V. myrtillus*, *L. borealis* and boreal small ferns and herbs (*G. dryopteris*, *M. bifolium*, *Avenella flexuosa* (L.) Drejer (1837), *O. acetosella*) with total cover of 70–90%. Boreal green mosses *P. schreberi*, *H. splendens* and *Ptilium crista-castrensis* (Hedw.) De Not. dominate on fallen logs, under-crown and inter-crown microsites. This type is subdivided into two subtypes: **fruticoso-hylocomiosa** with *V.*



Fig. 1. Study site location and topographic map of the Pechora-Ilych biosphere reserve (Russia) showing the position of the sampled vegetation types (LF – large fern taiga, VM – *Vaccinium*-moss taiga, GM – *Gymnocarpium*-moss taiga, ES – *Equisetum*-*Sphagnum* taiga, VS – *Vaccinium*-*Sphagnum* taiga, TH – tall herb taiga).



Fig. 2. Images of the main vegetation types studied in the area. (A) Large fern taiga, (B) *Vaccinium*-moss taiga, (C) *Gymnocarpium*-moss taiga, (D) *Equisetum*-*Sphagnum* taiga, (E) *Vaccinium*-*Sphagnum* taiga, (F) tall herb taiga.

myrtilus as a dominant component of the field layer (further referred to as *Vaccinium*-moss taiga; Fig. 2B; 62°02'42.06"N, 59°00'51.84"E) and *parviherbosohylocomiosa* with *G. dryopteris* as a dominant component of the field layer (further referred to as *Gymnocarpium*-moss taiga; Fig. 2C; 62°02'39.42"N, 59°01'10.50"E).

***Sphagnum* spruce (*P. obovata*) forests with *B. pubescens* and *P. sibirica* (*Piceeta sphagnosa*)** occupy weakly drained bottom of slopes with constant moisture. *P. obovata* dominates in the overstorey with a lower density of *B. pubescens* and *P. sibirica*; *A. sibirica* occurs sporadically (total crown cover: 30–50%). The shrub layer is dominated by *S. aucuparia* with rare *J. communis* (total cover: 10–40%). Cover of the field layer is 20–60% and the bottom layer is 90–95%. Oligotrophic species *Carex globularis* L., *Carex loliacea* L., *Comarum palustre* L. (1753), *Rubus chamaemorus* L., 1753, *Sphagnum* sp. are most frequently in flat areas of the inter-crown and under-crown microsites and in small depressions. Boreal dwarf shrubs (*V. myrtilus*, *Rubus arcticus* L. 1753),

small herbs (*Orthilia secunda* (L.) House, 1921, *Equisetum sylvaticum* L., 1753, *Trientalis europaea* L. (1753)) and green mosses occur mostly on decaying logs and on higher ground around the base of living trees. This type is subdivided into two subtypes: ***fruticoso-sphagnosa*** with a field layer dominated by *V. myrtilus* (further referred to as *Vaccinium-Sphagnum* taiga; Fig. 2D; 62°02'34.80"N, 58°58'00.72"E) and ***parviherbososphagnosa*** with a field layer dominated by *E. sylvaticum* (further referred to as *Equisetum-Sphagnum* taiga; Fig. 2E; 62°03'07.80"N, 58°57'58.62"E).

Nitrophilous tall herb spruce (*P. obovata*) forests (*Piceeta nitrophilo-magnoherbosa*) further referred to as tall herb taiga; Fig. 2F; 62°03'37.68"N, 59°03'54.60"E) grow along streams with running water on rocky substrate. *P. obovata* dominates the overstorey, *B. pubescens* is frequent (total crown cover: 40–60%). The understory is dominated by *P. obovata*, *A. sibirica*, *Alnus incana* (L.) Moench, 1794 and *Salix* spp. with rare *Lonicera pallasii* (Ledeb.) Browicz, *S. aucuparia* and *Ribes nigrum* L., 1753 (total cover: 20–40%).

The field layer can consist of two or three sublayers with total cover of 80–100%. The upper sublayer is formed by boreal and nitrophilous tall herbs *Filipendula ulmaria* (L.) Maxim. (1879), *Calamagrostis langsdorffii* (Link) Trin., 1824, *Cirsium oleraceum* (L.) Scop., etc. Nitrophilous and water-marsh herbs *Crepis paludosa*, *Bistorta major*, *Carex* spp. grow in the second field sublayer. The third sublayer can be formed by low herbs *Chrysosplenium alternifolium* L., 1753, *Stellaria nemorum* L. (1753), *Adoxa moschatellina* L. (1753), etc. Boreal dwarf shrubs and boreal small herbs *V. myrtillus*, *G. dryopteris*, *O. acetosella*, etc. can be found on the fallen logs and on the elevations around trees' trunks. The bottom layer is formed by green hemiboreal mosses of genera *Mnium*, *Plagiomnium*, *Brachythecium*, *Rhodobryum*, *Aulaacomnium* together with green boreal mosses such as *P. schreberi*, *H. splendens* and *Climacium dendroides* (total cover: 20–60%).

Each vegetation type is characterised by well-pronounced spatial heterogeneity, which is related to local variations in vegetation cover (inter-crown sites which are free of mature trees, under-crown sites of *P. obovata*, and *Sphagnum*-dominated biotopes in local depressions) and pit-mound topography (pits, mounds and decomposing trunks of uprooted trees) formed as a result of old tree uprooting. These components of vegetation and topography were treated as internally homogeneous biotopes for testate amoebae.

Sampling design

Samples were collected in order to cover the entire habitat diversity of the studied area, as described above, using a hierarchically nested sampling design (Gering et al. 2003). Habitat hierarchy included the following levels (starting from the highest one): landscape, vegetation type, biotope and sample (Table 1). The whole diversity of biotopes was found only in three vegetation types: large fern, *Gymnocarpium*-moss and tall herb taiga. The other vegetation types included fewer biotopes (*Vaccinium*-moss taiga consisted of mound, pit, tree trunk and spruce stand; *Equisetum-Sphagnum* taiga included mound, tree trunk, spruce stand and inter-crown locations, and *Vaccinium-Sphagnum* taiga included mound, pit and spruce stand). Sampling was performed on 23–26 July 2010. In each biotope samples (~10 cm³) were taken in three replicates. Samples represented the most typical substrate in the biotope (spruce litter and humus in spruce stands; *Sphagnum* spp. stems in *Sphagnum*-dominated biotopes; decomposing wood and mosses in decomposing tree trunks; mosses and soil in other biotopes). The samples were kept in plastic bags and stored in a cool dry place until analysis. In total, 87 samples were collected.

Laboratory analysis

The samples were prepared for counting of testate amoebae following a modified version of the water-based technique (Hendon and Charman 1997). Five grams of the substrate

were mixed with an arbitrary amount of water and were left soaking for 24 h. After that, the mixture was thoroughly shaken for 10 min for extraction of testate amoebae. The suspension was passed through a wide-mesh sieve (0.5–1.0 mm) in order to remove large particles. The sieving residue was gently washed with water and the filtrate was left for sedimentation for several hours. Then the supernatant was carefully poured off to concentrate the sample to 10 mL volume. Two millilitres of the concentrated sample were placed in a Petri dish (5 cm diameter) and were left to settle down for several minutes. After that, testate amoebae were identified and counted at the magnification of $\times 160$ using a dissecting microscope Olympus SZX16 (Olympus Corporation, Japan). In order to avoid counting the same individual twice the Petri dish was moved using the “lawnmower method” so that each next row begun right at the side of the previous one. Two subsamples were analysed for each sample. A minimum number of 150 individuals of testate amoebae was counted in each sample. The obtained counts were expressed as density (number of individuals per gram of absolutely dry substrate).

Data analyses

All calculations and statistical analyses were performed in the R software (R Core Team 2012). The variation in species diversity was estimated using both species richness (the number of taxa per sampling unit) and species structure of testate amoeba assemblages. In order to compare species richness of testate amoebae among the studied vegetation types and biotopes the analysis of sample-based rarefaction curves was performed in the package ‘BiodiversityR’ (Kindt and Coe 2005). The function ‘diversityresults’ in the package was used to calculate the total species richness of testate amoebae in the survey area using the 1st order Jackknife, 2nd order Jackknife, Chao and bootstrap total gamma diversity estimators. The total species richness observed in the study area (γ) was partitioned into within-unit richness (α -diversity) and between-unit richness (β -diversity) separately for each of the three hierarchical levels (Table 1) using additive partitioning (Gering et al. 2003). For each level, α -diversity was estimated as the mean number of taxa found within samples (α_{sp}), biotopes (α_{bt}) or vegetation types (α_{vt}). The β -diversity for the biotope and sample level was calculated as $\beta_{bt} = \alpha_{vt} - \alpha_{bt}$ and $\beta_{sp} = \alpha_{bt} - \alpha_{sp}$, respectively, whereas for the highest level, vegetation type level, the following formula was used $\beta_{vt} = \gamma - \alpha_{vt}$. The additive partition was performed and tested against expected values predicted by the individual-based null model (999 permutations) using ‘adipart’ function in ‘vegan’ package (Oksanen et al. 2012). This function implements a statistical approach developed by Crist et al. (2003) for testing null hypotheses that observed partitions of species richness differed from those expected by chance. A rejection of the null hypothesis indicates the non-random distribution of individuals due to intraspecific aggregation, resource partitioning and community saturation.

Table 1. Hierarchy of habitats studied in the old-grown dark coniferous forest of the Pechora-Ilych biosphere reserve (Russia) with a description of hypothetical factors and processes that may influence the species diversity of testate amoebae. In parentheses is the total number of samples at each level of analysis.

Level	Ecological meaning	Units	Spatial scale (m)	Diversity type	Hypothetic factors of species diversity
Landscape (1)	A set of ecosystems connected by fluxes of materials (nutrients, dead organic matter, etc.) and organisms (following the meta-ecosystem concept of Massol et al. 2011)	Taiga (dark coniferous boreal forest)	10^5	Total richness (γ)	<ul style="list-style-type: none"> • Geophysical properties of the region (climate, geology, size, age, etc.). • Evolutionary and large scale ecological processes (speciation, extinctions, dispersal, etc.)
Vegetation type (6)	A vegetation type is an area with homogeneous vegetation cover and which is assumed to have relatively closed cycling of nutrients because of the presence of all functional groups (producers, consumers and decomposers)	Large fern taiga <i>Vaccinium</i> -moss taiga <i>Gymnocarpium</i> -moss taiga <i>Vaccinium-Sphagnum</i> taiga <i>Equisetum-Sphagnum</i> taiga Tall herb taiga	10^3	Among vegetation type richness (β_{vt}) Within vegetation type richness (α_{vt})	<ul style="list-style-type: none"> • Environmental conditions related to variation in topography and soil characteristics. • Differences in the number, shape and spatial arrangement of habitat patches
Patch (biotope) (29) (local variations in vegetation cover due to natural heterogeneity)	A patch (biotope) is an area which is assumed (for the purposes of this study) to be internally homogeneous in terms of physical conditions for testate amoebae and which contains a specific set of interacting testate amoeba species i.e. assemblage	Inter-crowns sites Under-crown sites of <i>Picea obovata</i> <i>Sphagnum</i> -dominated sites Pit Mound Tree trunk	10^1	Among biotope richness (β_{bt}) Within biotope richness (α_{bt})	<ul style="list-style-type: none"> • Variation in environmental conditions related to plant distribution, pit-mound topography, etc. • Species interactions (competition, predation, mutualism, etc.)
Sample (87)	A random part of a patch (a part of testate amoeba assemblage)	Samples	10^{-1}	Among sample richness (β_{sp}) Within sample richness (α_{sp})	<ul style="list-style-type: none"> • Organism movements, autocorrelation. • Stochastic processes and sampling errors

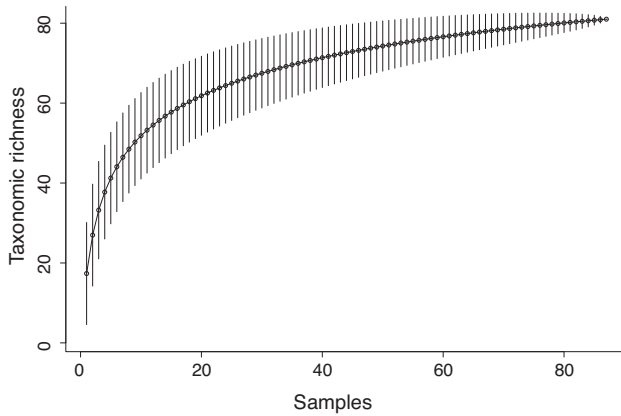


Fig. 3. Sample-based testate amoeba species accumulation curve for all samples collected in the old-grown dark coniferous forest of the Pechora-Ilych biosphere reserve (Russia). The bars are standard deviations.

The variation in species composition of testate amoeba assemblages was estimated using principal component analysis (PCA). The taxa abundances were Hellinger-transformed so that the Hellinger distance, which is a more appropriate measure for community composition data than Euclidian distance, was preserved in the PCA (Legendre and Gallagher 2001). In order to quantify relationships between the assemblage structure of testate amoebae, on the one hand, and vegetation type and biotopes, on the other, we calculated R^2 values of the latter as categorical variables for the first two components separately (function 'envfit', package 'vegan'). The significance of variables was tested with 999 permutations. A hierarchical cluster analysis with the Ward method was performed on the testate amoeba abundance data (function 'hclust', package 'vegan') in order to classify samples. The resulted hierarchical tree was projected on the ordination plane of the first two principal components (function 'ordi-cluster', package 'vegan'). The clusters were characterised by the categories of the categorical variables (vegetation type and biotope) by calculating the proportion of the samples of a particular category to the total number of samples in a cluster and the proportion of the samples of a particular category in a cluster to the total number of samples in this category (Lê et al. 2008). Indicator species for each cluster were identified following the IndVal approach (Dufrene and Legendre 1997) with the 'indval' command in the package 'labdsv' (Roberts 2010).

Results

General observations

The analysis of the 86 samples revealed 80 testate amoeba taxa belonging to 21 genera (Supplementary Table A1). The total sample-based species accumulation curve (Fig. 3) flat-tened off for large numbers of samples, suggesting that the

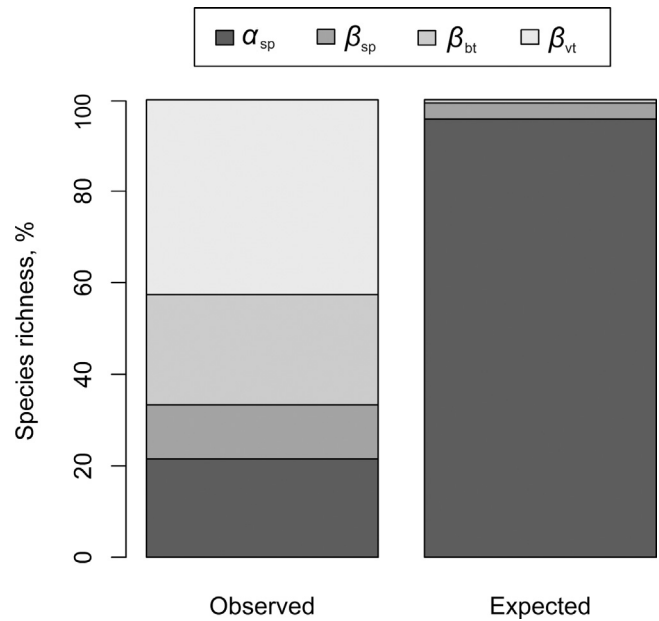


Fig. 4. Additive partitioning of total species richness of testate amoeba assemblages from the old-grown dark coniferous forest of the Pechora-Ilych biosphere reserve (Russia) at the landscape scale. Contribution of the average α -diversity at the sample level and three β -diversity components: β_{sp} – beta diversity among samples within biotope, β_{bt} – among biotopes within a vegetation type, β_{vt} – among vegetation types within the landscape to γ -diversity. The observed partitions are compared with the expected values as predicted by the null model based on 999 randomisations.

sampling had captured nearly all the species in the study area. Estimates of total species richness in the survey area varied from 87 to 94 taxa (Jack 1 = 91.9; Jack 2 = 93.9; Chao = 87.7, bootstrap estimator = 86.5). The most abundant taxa were *Centropyxis aerophila* Deflandre, 1929 (13% of the total counts), *Nebela tinctoria* sensu lato (8%), *Cyclopyxis eurystoma* Deflandre, 1929 (7%), *Trinema lineare* Penard, 1890 (6%), *Cyclopyxis kahli* Deflandre, 1929 (6%), *Assulina muscorum* Greef, 1888 (6%), *Trinema complanatum* Penard, 1890 (5%). These taxa were also characterised by high occurrence frequency (>60% of all samples). Twenty taxa were rare and were found in two or less samples. The number of taxa per sampled varied from 3 to 27 with a mean value of 17.4 ± 0.7 (SE; $n = 87$). Total abundance of testate amoebae varied from 0.3 to 59.1×10^3 ind. g^{-1} of dry substrate weight with a mean value of $15.3 \pm 1.3 \times 10^3$ (SE; $n = 87$).

Species richness patterns

Additive partition of species richness showed that the total diversity (γ) was mainly attributed to β -diversity. α -Diversity at the sample level (α_{sp}) was lower than expected by chance ($p < 0.001$; Fig. 4) and comprised 21.3% of the total species richness observed at landscape scale (ca. 17 species from the total γ -diversity of 80 species). β -Components together explained 78.5% of γ -diversity, 43.8% (ca. 35 species) at the

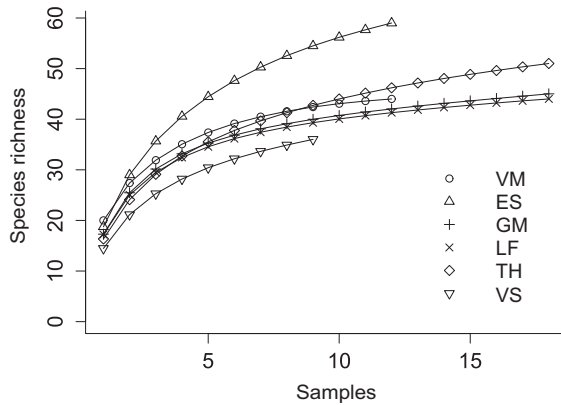


Fig. 5. Sample-based accumulation curves of testate amoeba species richness from the old-grown dark coniferous forest of the Pechora-Ilych biosphere reserve (Russia) for large fern taiga (LF), *Vaccinium*-moss taiga (VM), *Gymnocarpium*-moss taiga (GM), *Vaccinium-Sphagnum* taiga (VS), *Equisetum-Sphagnum* taiga (ES), tall herb taiga (TH) vegetation types.

vegetation type level (β_{vt}), 25.0% (ca. 20 species) among-biotopes (β_{bt}) and 12.5% (ca. 10 species) among-sample (β_{sp}). Beta-diversity at all hierarchical levels was significantly higher than expected by chance ($p < 0.001$; Fig. 4).

At the level of the vegetation type, species richness was highest in *Equisetum-Sphagnum* taiga (59 species), followed by tall herb (51 species), *Gymnocarpium*-moss (45 species), *Vaccinium*-moss (44 species) and large fern taiga (44 species). Species richness was lowest in the *Vaccinium-Sphagnum* taiga (36 species). These patterns in species richness are preserved when compared at the minimal sampling effort of nine samples (Fig. 5). The contribution of α -diversity (sample level) to γ -diversity at the landscape level varied between 18 and 25% and was significantly lower than expected for all vegetation types ($p < 0.001$). Total species richness was highest in the *Equisetum-Sphagnum* and tall herb taiga due to the important contribution of the β -component ($\beta_{bt} + \beta_{sp}$) (43–50% as compared to 27–34% in other vegetation types). In all vegetation types the contribution of β -diversity was significantly greater than expected by chance ($p < 0.001$).

Testate amoeba species richness patterns differed among biotope types (Fig. 6). Highest total species richness was observed under the crown of *Picea obovata* trees (62 species) and in the decomposing trunks of uprooted trees (61 species). However, the accumulation curve for the tree trunk biotopes was steeper than the one for the under-crown sites. Pit, mound and inter-crown biotopes had medium species richness (51–54 species) with relatively flat accumulation curves. Species richness was lowest in *Sphagnum*-dominated biotopes (34 species). These patterns are generally preserved when compared at minimal sampling effort of nine samples except for decomposing tree trunks, which fall in the category with the medium species richness. Separate partitioning analyses for each biotope showed that alpha diversity

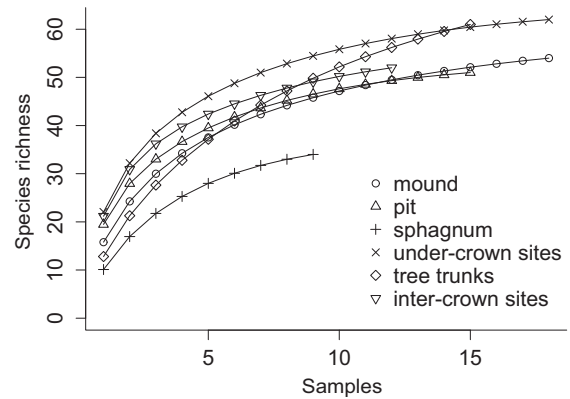


Fig. 6. Sample-based accumulation curves of testate amoeba species richness the old-grown dark coniferous forest of the Pechora-Ilych biosphere reserve (Russia) for inter-crown sites, under-crown sites of *Picea obovata*, *Sphagnum*-dominated biotopes, pits, mounds and decomposing trunks of uprooted trees.

varied between 13% and 27% being minimal in *Sphagnum*-dominated biotopes (13%) and in decomposing tree trunks (16%) and maximal in inter-crown (26%) and under-crown (27%) sites. β -Diversity contributed most to total species richness per biotope in decomposing trunks of uprooted trees (60%, c. 48 species).

Community patterns

The first two PCA axes explained 37.1% of the total variation in testate amoeba community data (Fig. 7A–C). The first principal component was characterised by vegetation type only ($R^2 = 0.73$, $p < 0.001$; Fig. 7A). The second principal component was mostly related to biotope ($R^2 = 0.49$, $p < 0.001$; Fig. 7B) and to a lesser degree, but still significantly, to vegetation types ($R^2 = 0.19$, $p < 0.01$). The shape of the cluster dendrogram (Fig. 7C, Supplementary Fig. A1) suggested partitioning the samples into three clusters, which were mostly characterised by differences among vegetation types (Pearson's Chi-squared test $\chi^2_{10} = 103.3$, $p < 0.001$) and related to the first principal component. Cluster 1, located in the left part of the ordination diagram, was the most homogeneous. It included only samples belonging to the *Vaccinium-Sphagnum* and *Equisetum-Sphagnum* taiga (all samples from the *Vaccinium-Sphagnum* taiga and 92% of the samples from the *Equisetum-Sphagnum* taiga). Cluster 3, located in the right part of the ordination diagram (Fig. 7C), included all samples from the tall herb taiga. These samples constituted 43% of all samples in that cluster. Cluster 2, located in the centre of the ordination diagram (Fig. 7C), included 67% of the samples from the *Vaccinium*-moss taiga, which constituted only 32% of all samples in that cluster. The indicator testate amoeba taxa for each cluster are presented in Table 2. The best indicators for the first cluster were *Trinema lineare*, *Phryganella hemisphaerica* and *Nebela bohemica*. The second cluster was

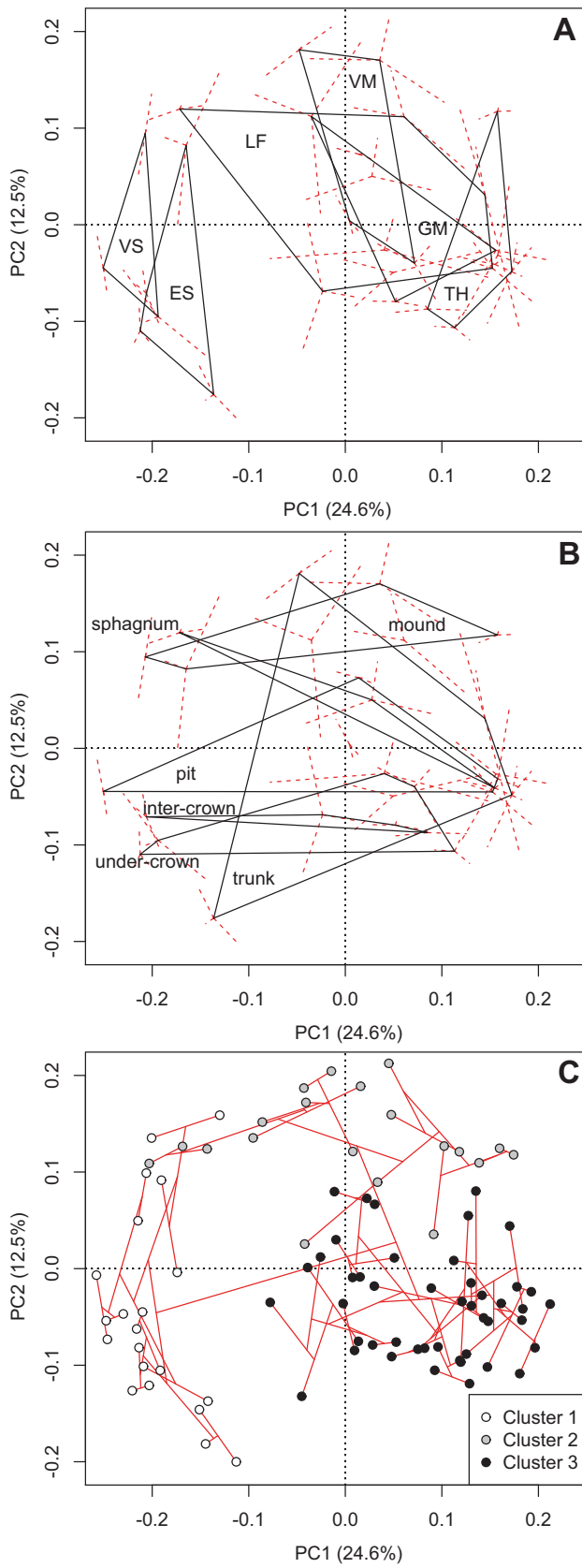


Fig. 7. PCA ordination diagrams showing patterns of variation in assemblage structure (β -diversity) of testate amoebae from the old-growth dark coniferous forest of the Pechora-Ilych biosphere reserve

characterised by species *Argygnia dentistoma*, *N. tincta* sensu lato and *Corythion dubium*. *Cyclopyxis kahli*, *Centropyxis aerophila* and *C. aerophila sphagnicola* were indicative for the third cluster. The second ordination axis was related to the gradual changes in assemblage structure in the following sequence of biotopes: mound–*Sphagnum*–trunk–pit–under-crown sites–inter-crown sites (Fig. 7B). The wide spread of sample points for pits and decomposing trunks of uprooted trees with the position of the polygon centroids near the centre of the diagram indicated that these biotopes are characterised by high variation in species composition (β -diversity) and lack specific indicator species.

Discussion

General observations

We observed a high overall species diversity of testate amoebae in the old-growth dark coniferous forest. Previous studies on soil testate amoeba assemblages in dark coniferous forests reported lower values of species diversity, which varied from 25 to 65 taxa (Aescht and Foissner 1994; Bobrov et al. 1994; Lousier 1975; Rakhleeva 2002; Schönborn 1986). Differences among studies may be due to several causes including range of habitat heterogeneity, human impact, climate, number of examined samples and taxonomic resolution. As a result, despite the high number of species recorded, it is unclear if the Pechora-Ilych Biosphere Reserve represents a hotspot of testate amoeba diversity. Determining this would require performing comparable studies with the same sampling design and using the same taxonomy in other regions. To this date, this study describes testate amoeba assemblages covering the broadest diversity of natural biotopes in old-growth dark coniferous forest and using the greatest number of samples and can therefore be used as a reference for future work. The studied assemblage of testate amoebae was dominated by typical soil, moss, and ubiquitous taxa, which have been previously

(Russia). (A) distance plot of sites scores (scaling 1); the polygons encircle the biotopes, which belong to the same vegetation type (see Fig. 1 for abbreviations). β -Diversity can be estimated as the average distance to the centroid (the geometric centre or the averaged species composition) of the points belonging to the same class (the larger the polygons the greater the variation in assemblage structure among the points they encircle). The dotted lines connect sample points to the vegetation type centroids (the averaged species composition of vegetation type based of three samples) showing the β -diversity among samples within a particular biotope. (B) Same ordination as A, but the polygons encircle the biotopes (inter-crown sites, under-crown sites of *Picea obovata*, *Sphagnum*-dominated biotopes, pits, mounds, decomposing trunks of uprooted trees). (C) Same ordination with projection of the hierarchical tree resulting from the cluster analysis on the ordination plane of the first two principal components.

Table 2. Indicator species for the cluster selected by the hierarchical cluster analysis (Fig. 7A) showing taxa with IndVal > 40, significant at $p < 0.01$ (999 permutations).

Cluster 1	Cluster 2	Cluster 3
<i>Trinema lineare</i> (64.5)	<i>Argynnia dentistoma</i> (72.7)	<i>Cyclopyxis kahli</i> (76.1)
<i>Phryganella hemisphaerica</i> (55.0)	<i>Nebela tinctoria</i> (60.5)	<i>Centropyxis aerophila</i> (64.2)
<i>Nebela bohémica</i> (45.2)	<i>Corythion dubium</i> (54.8)	<i>Centropyxis aerophila sphagnicola</i> (62.1)
<i>Euglypha laevis</i> (44.0)	<i>Nebela collaris</i> (54.5)	<i>Centropyxis orbicularis</i> (54.6)
<i>Nebela parvula</i> (43.2)	<i>Assulina muscorum</i> (53.7)	<i>Phryganella acropodia</i> (42.7)

reported to be typical components of such ecosystems (see above mentioned references). It should however be noted that our species diversity is a conservative estimate. Indeed ongoing taxonomical studies are revealing a high diversity of cryptic and pseudo-cryptic species among testate amoebae, including taxa such as *Nebela tinctoria* (in which extensive cryptic diversity was discovered and a new species described from the same region; Kosakyan et al. 2013) and *Hyalosphenia papilio* (for which twelve genetically distinct clades were observed; Heger et al. 2013). Another new species in the *Nebela collaris* complex, *N. gimlii*, was recently described from a Swiss bog (Singer et al. 2014). However, the effects of cryptic species on the observed diversity patterns remain basically unknown, so that future studies on how cryptic speciation depends on taxonomic group and environmental conditions would help to shed the light on this problem.

Species richness patterns

On average α -diversity was lower and β -diversity was higher than expected by chance in all analyses. This pattern was consistent for all vegetation types and biotopes, suggesting that testate amoeba assemblages are highly aggregated at all levels at the landscape scale (Crist et al. 2003; Shorrocks and Sevenster 1995). These results are in line with the findings of Mazei (2008) who reported that the diversity of *Sphagnum*-dwelling testate amoebae was β -dominated at all levels except for the broadest level of the forest-steppe, southern taiga and northern taiga ecoregions located on the densely populated Russian Plain. The species aggregations at the level of vegetation types (within the landscape) and biotopes (within vegetation types) can be rather attributed to variations in environmental conditions, as there seems to be no known spatial restrictions on the distribution of testate amoebae at this scale (Booth and Zygmunt 2005; Krashevskaya et al. 2010; Wilkinson et al. 2012). This observation contradicts recent empirical evidence for low habitat specialisation for lichen-dwelling rotifers (Fontaneto et al. 2011). These contradicting results may be due to the fact that the lichens studied by Fontaneto et al. (2011) were more similar to each other (i.e. foliose lichens of similar size and collected from rock or tree substrates) than the biotopes we studied. These differences highlight the effect sampling design can have on the results and interpretation of diversity and community patterns of microorganisms. A similar case of discrepancy can

be found in studies of altitudinal or latitudinal diversity patterns where studies focusing on a single habitat type showed less clear patterns (Mitchell et al. 2004) than studies in which diverse or different habitats were sampled (Krashevskaya et al. 2007; Smith and Wilkinson 1987; Wilkinson 1994).

The aggregations of testate amoeba species richness at the sample level can be attributed both to the presence of environmental heterogeneity in the macroscopically homogeneous patches (biotopes) or to spatially-dependent patterns (autocorrelations) of species distribution as shown in previous studies on distribution of testate amoebae in the scale of decimetre to several metres within macroscopically homogeneous biotopes (Balik 1996a,b; Mazei et al. 2011; Mitchell et al. 2000; Tsyganov et al., 2012). The role of species movements and biological interactions in testate amoeba community assembly at this scale has not been studied yet. Altogether these results indicate that environmental conditions play an important role in regulation of species richness patterns of testate amoeba assemblages at the spatial scales below the landscape level with the major contribution at the level of vegetation type followed by among-biotope and among-sample variation.

Vegetation types and biotopes differ in terms of internal heterogeneity and the species richness they can harbour. Species richness was highest in the tall herb and in the *Equisetum-Sphagnum* taiga. This can be related not only to the more favourable hydrological regime for testate amoebae (as indicated by the presence of hydrophilous plants and by the distribution of these vegetation types at the bottom of slopes with constant moisture and along streams with running water) but also to the higher internal heterogeneity of these vegetation types as shown by the additive partitioning analysis. This result might indicate an important role of landscape heterogeneity in terms of vegetation types in structuring species richness of microorganisms as previously shown for macroorganisms (Veech and Crist 2007). Among biotopes, highest total species richness was observed in under-crown sites of spruce and in decomposing trunks of uprooted trees. High total species richness in the under-crown sites is mostly associated with high species richness in each biotope taken separately. This can be explained by the fact that the under-crown sites of spruce are the most spacious and mature in the area so that they contain well developed microbial communities characterised by multiple trophic linkages. In contrast, in the decomposing tree trunks, high species richness is mostly

explained by turnover among vegetation types. This can be attributed to colonisation dynamics of newly created biotopes (Burkovsky et al. 2011), which might be stochastic (Wanner et al. 2007).

Community patterns

The results of the study show that the higher level of environmental hierarchy, i.e. vegetation types, explains a greater part of the variation in species structure of testate amoeba assemblages than the lower level, i.e. biotopes. A similar pattern of increasing variation among units from lowest to highest levels of sampling hierarchy was also detected for species richness. This points to the fact that the dynamics and patterns of species structure of testate amoeba assemblages in moss and soil biotopes are predominantly influenced by factors at the scale of vegetation types (Wiens 1989). Indeed, the variation in vegetation type is related to changes in a number of ecological variables, which are also relevant for testate amoebae, such as hydrological regime, acidity, quality and quantity of litter, etc. (Ledeganck et al. 2003; Tsyganov et al. 2013). Moreover, plants can affect species composition of testate amoeba assemblages by producing exudates (Bonkowski 2004; Jassey et al. 2013). By contrast, differences in environmental conditions among various biotopes within a particular vegetation type normally represent shorter gradients or just a fraction of the whole range of the variation related to vegetation types.

Testate amoeba community composition in the newly appeared biotopes, especially trunks of uprooted trees and pits, were characterised by high variation in species composition (β -diversity) and by the absences of specific species. A possible explanation for these observations could be the fact that the testate amoeba assemblages in these biotopes included different successional stages (Lousier 1982; Wanner and Xylander, 2005). Taking into account the stochastic (variable) beginning of community assembly (Wanner et al. 2007), this may indicate the important role of old tree uprooting in setting the stage for the strong influence of regional species pools on local assemblages of testate amoebae in taiga.

Our main conclusion is that species diversity patterns of microorganisms such as testate amoebae are strongly regulated by environmental conditions at spatial scales below the landscape level. The contribution of environmental variables is greatest at the level of the vegetation type decreasing at the biotope and sample levels. These results support the idea that metacommunity dynamics of free-living soil microorganisms within a region might be considerably driven by species sorting and mass effect processes, which, however, might be difficult to disentangle. We suggest that the patterns observed in this study might be detected only by experimental design, which covers the entire diversity of biotopes in a region, whereas designs with partial coverage will underestimate the effect of environmental variables. Overall, the studied old-grown dark coniferous forest was characterised

by the highest diversity of soil and moss testate amoebae reported so far for this type of biomes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ejop.2014.11.003>.

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