LOMONOSOV MOSCOW STATE UNIVERSITY

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Structural and functional characteristics of testate amoeba assemblages in terrestrial habitats of the Northern Eurasia

Specialty 1.5.15. Ecology

AUTHORIZED REVIEW

of dissertation for the degree of Candidate of Biological Sciences

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The dissertation defense will be held «___» _________ 20__ in ___ hours ___ minutes at the meeting of the Dissertation Council MSU.015.3 of Lomonosov Moscow State University at the address: Moscow, Leninskie Gory, 1 building 12, Faculty of Biology, room M-1.

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The dissertation is available for review in the dissertation department of the Library of Lomonosov Moscow State University (Lomonosovsky Prospect, 27) and on the website: https://dissovet.msu.ru/dissertation/3616/.

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Academic Secretary
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Candidate of Biological Sciences T.A. Paramonova

GENERAL DESCRIPTION OF THE WORK

Belowground communities are essential components of terrestrial ecosystems, playing pivotal roles in nutrient cycling, carbon storage, and overall ecosystem functionality (Fitter, 2005; Dubey et al., 2019). Microbial organisms, particularly protists, are central to these communities, driving biogeochemical processes and shaping ecosystem dynamics (Geisen et al., 2017; Crowther et al., 2019; Sokol et al., 2022). Among belowground microorganisms, testate amoebae occupy a prominent position within mineral soil and peatland habitats (Lamentowicz, Mitchell, 2005; Mitchell et al., 2008; Coleman et al., 2024). Functioning as consumers and mixotrophs, they make critical contributions to belowground food web dynamics, carbon and nitrogen cycling (Potapov et al., 2021; Qin et al., 2022). Key environmental factors that strongly influence the composition and diversity of testate amoeba assemblages include moisture regime, organic matter content, and soil pH (Fournier et al., 2012; Arrieira et al., 2015; Fournier et al., 2016; Jassey et al., 2016; Koenig et al., 2018; Lamentowicz et al., 2020; Krashevska et al., 2020; Marcisz et al., 2020; Tsyganov et al., 2022). Despite their high ecological importance, testate amoeba assemblages in different terrestrial habitats and geographic regions remain insufficiently studied.

Existing knowledge of testate amoeba ecology has largely been based on traditional morphological taxonomic classifications (Bonnet, 1975; Todorov, Golemansky, 1995), which fail to fully capture the ecological roles and adaptive strategies of these organisms. A functional trait-based approach offers a promising alternative by linking organismal traits with environmental conditions and ecosystem functions, thereby providing new insights into the ecological strategies of testate amoebae (Fong et al., 2023). Recent studies have demonstrated the potential of this approach to reveal ecological patterns and adaptive strategies. For instance, trait-based analyses have uncovered important relationships between the morphological and physiological characteristics of testate amoebae and their adaptation to peatland and mineral soil habitats (Krashevska et al., 2020; Marcisz et al., 2020). These findings underscore the need to shift from traditional morphological classifications toward the study of functional diversity and ecological roles of testate amoebae.

The significant dependence of testate amoebae assemblages on environmental conditions is manifested at different scales from local variability within a habitat to continental-level heterogeneity, reflecting the influence of climatic and orographic gradients.

However, comprehensive studies of various aspects of biodiversity (species composition, taxonomic α- and β-diversity, functional diversity), as well as mechanisms of community assembly and trait patterns across different terrestrial habitats along extensive geographical gradients have not yet been conducted.

Objective of the study: To perform a comparative assessment of the structural and functional organization of testate amoeba assemblages in terrestrial habitats of some regions of the Northern Eurasia – Middle Volga region, Western Siberia and Baikalia.

Specific tasks of the study:

1. Identify the main differences in the species composition and taxonomic diversity of sphagnum- and soil-dwelling testate amoeba assemblages taking into account regional specifics.
2. Develop a system of functional traits to describe the functional diversity of testate amoebae, develop a functional classification of testate amoebae.
3. Identify the main differences in the functional composition and diversity of sphagnum- and soil-dwelling testate amoeba assemblages taking into account regional specifics.
4. Perform a comparative analysis of assembly rules in sphagnum- and soil-dwelling testate amoeba assemblages.
5. Identify latitudinal distribution patterns of sphagnum- and soil-dwelling testate amoeba assemblages.

Scientific novelty. This study introduces several novel approaches and examines new objects in the field of testate amoebae ecology. By integrating both taxonomic and functional perspectives, it pioneers the development of a functional trait system and classification tailored to describe the ecological roles and diversity of testate amoebae. This functional framework advances over traditional morphology-based classifications, enabling a more detailed understanding of testate amoebae ecological strategies. Additionally, the comparative analysis of community assembly rules in sphagnum- and soil-dwelling testate amoeba assemblages, considering regional and latitudinal variation, offers a fresh perspective on the factors shaping microbial communities across diverse environments. The focus on latitudinal distribution patterns and regional specificity further highlights previously unexplored dimensions of testate amoebae ecology, providing a comprehensive and context-sensitive understanding of their diversity and function. Collectively, this research contributes novel methodologies, frameworks, and insights that enhance our ability to study and interpret testate amoeba assemblages in contrasting terrestrial habitats.

Theoretical and practical importance. This study holds significant theoretical and practical value by advancing our understanding of the ecological strategies and community assembly mechanisms of testate amoebae in contrasting terrestrial habitats. The identification of taxonomic and functional diversity patterns provides a foundation for understanding how testate amoebae adapt to different ecological conditions, offering insights into their role in soil ecosystem processes. The development of a functional trait system and classification represents a novel framework for studying microbial communities, enabling researchers to link functional traits to environmental drivers more effectively. Practically, the findings contribute to improving bioindicator applications, as testate amoebae can serve as sensitive markers of environmental changes, such as shifts in moisture regimes or climate conditions. Additionally, the exploration of latitudinal diversity gradients and regional variations enhances our ability to predict microbial responses to global environmental changes, informing conservation strategies and sustainable soil management practices. This research bridges knowledge gaps in microbial ecology and provides tools for both theoretical exploration and practical application in environmental monitoring and ecosystem management.

Object and subject of research. The object of research was sphagnum- and soil-dwelling testate amoeba assemblages. The subject of research was the dependence of the structural and functional characteristics of testate amoeba communities on the habitat type and environmental variables.

Methodology and methods. The methodology of this study is based on a comprehensive approach to the analysis of testate amoeba assemblages using metrics of taxonomic and functional diversity, functional traits, which were analysed using conventional statistical analysis methods, as well as null-model analysis.

Statements to defend:

1. The primary factor driving the structural and functional differences between soil- and sphagnum-dwelling testate amoeba assemblages is the characteristic moisture regime of mineral soils within the ecoregion. In forest-steppe ecoregions with insufficient soil moisture, sphagnum-dwelling assemblages are more diverse compared to soil-dwelling assemblages. In

contrast, in taiga and tundra ecoregions with adequate or excessive soil moisture, the diversity of soil-dwelling assemblages is higher.

2. Soil- and sphagnum-dwelling testate amoeba assemblages differ in their predominant functional traits. Sphagnum-dwelling assemblages are characterized by amoebae with larger shells, mostly of elongated shape, completely organic or covered with endogenous covering materials (idiosomes), with a straight, terminally located aperture. The soil communities are dominated by amoebae with smaller, shortened shells covered with exogenous covering materials (xenosomes) with the aperture located in the center of the ventral surface.

3. The primary assembly rule shaping the structure of soil-dwelling testate amoeba assemblages is environmental filtering, whereas in sphagnum-dwelling assemblages, biotic interactions play a more significant role.

4. The α- and β-diversity of sphagnum-dwelling testate amoebae is influenced by climatic factors such as annual mean temperature and the seasonality of precipitation. For hollow assemblages, there is an inverse latitudinal diversity gradient, whereas for assemblages on hummocks, no latitudinal gradient is observed, reflecting the overall stressful conditions of this microhabitat.

Personal impact into results. During the course of this study, author reasoned the relevance of the thesis topic, set the aim and objectives of the research, performed the analysis, made generalizations and conclusions. Specifically, author developed a comprehensive database to catalog 18 functional traits for 372 testate amoebae species and constructed the abundance database from multiple source datasets, ensuring it was robust and suitable for detailed analysis. Second, author conducted the statistical analyses including writing scripts for analysis and visualization. Third, author described the results and provided the interpretation of these results, linking them to ecological theories and highlighting their significance in understanding testate amoebae community structure and function.

Confidence and testing the results. The findings and interpretations presented in this PhD thesis have been rigorously tested and validated through multiple channels of academic dissemination and peer review. The results have been shared and discussed at esteemed international conferences, allowing for feedback and validation from global experts in the field: 10th International Symposium on Testate Amoebae (2023), Madrid, Spain; International

Scientific Online Seminar on Arctic and Subarctic Ecosystems (2023), online, Russia; International Conference “Current Trends and Achievements in Life Sciences” (2024), Shenzhen, China. These presentations have provided an opportunity for active engagement with the international research community, ensuring the results are tested against diverse perspectives.

Publications. The results have been reported in 5 publications in peer-reviewed scientific journals which are recommended for publication of results suitable for defense in the council MSU.015.3 for Specialty 1.5.15 – Ecology (biological sciences). In work [1], the author's contribution was 1.2 printed sheets (p.s.) out of 2.05 p.s.; in work [2], 1 p.s. out of 1.88 p.s.; in work [3], 0.45 p.s. out of 0.79 p.s.; in work [4] 1 p.s. out of 1.78 p.s.; in work [5] 0.15 p.s. out of 0.77 p.s.

Thesis structure and volume. This PhD thesis comprises a total of 8 chapters, spanning 157 pages. It includes 11 tables and 29 figures, which collectively illustrate and support the main findings of the study. The reference list consists of 196 sources, providing a comprehensive foundation for the research conducted.

Acknowledgements. I would like to express my deepest gratitude to my supervisor Dr. Basil Yakimov for his invaluable guidance throughout the preparation of this thesis. My heartfelt thanks also go to Dr. Yuri Mazei, Dr. Andrey Tsyganov, Dr. Natalia Mazei, Dr. Viktor Chernyshov, Dr. Alexander Komarov, Dr. Kirill Babeshko, Dr. Elena Malysheva, and Dr. Damir Saldaev for their crucial role in conducting fieldwork and collecting samples, with all data generously provided by Dr. Yuri Mazei. I am profoundly grateful to my parents and my girlfriend for their support and encouragement throughout my academic journey. Lastly, I extend my sincere appreciation to everyone who has supported and helped me along the way.

SUMMARY OF THE WORK

Chapter 1. Literature Review

This chapter provides basic overview of the biology and functional role of testate amoebae in ecosystems. It examines key environmental factors that influence their distribution and diversity. It summarises information about the functional traits of testate amoebae and their applications in ecological research.

Research gaps in the field of studying the functional diversity and structure of testate amoeba communities have been identified, which determined the aim and objectives of the dissertation study. First, while previous studies have demonstrated the influence of habitat type on testate amoebae diversity and community structure, a comprehensive comparison integrating taxonomic composition, functional traits, and community assembly processes in different ecoregions across diverse habitats is still lacking. Second, the effects of longitudinal and latitudinal gradients on diversity patterns of testate amoebae across different regions have received limited attention. Addressing this gap is essential for understanding broader biogeographic patterns and predicting community responses to environmental change. Third, existing research has predominantly relied on morphology-based classifications, which may overlook the functional roles and adaptive strategies of testate amoebae. The application of a trait-based framework provides a promising avenue to bridge this gap, yet studies systematically linking specific traits to environmental gradients and ecosystem processes are still in their early stages.

Chapter 2. Material and Methods

This study integrates three primary datasets: functional trait data, testate amoeba abundance data, and climate data.

Functional trait data. The species list was compiled from Eurasian peatland, lake, and mineral soil datasets, as well as pan-European (Amesbury et al., 2016) and North American (Amesbury et al., 2018) peatland hydrology databases. Traits were sourced from literature, online databases, and taxonomic keys. The traits involved shell dimensions, including shell length, width, and depth, shell width to length ratio R1, shell depth to width ratio R2, overall shell shape, aperture characteristics (length, width, length to width ratio R3, width to length ratio R4, aperture position, invagination, rim type, and the presence or absence of a collar), structural features (existence of internal partitions, spines, horns, or shell coverings), and feeding behavior.

Abundance data. Testate amoeba abundance data were collected from peatlands, mineral soils, and lakes across Eurasia (50–70°N, 28–158°E). Full dataset includes 1635 samples: 1115 from peatlands, 429 from mineral soils, and 91 from lakes (Fig. 1). Data were provided by Yuri Mazei.

For the comparison of sphagnum- and soil-dwelling assemblages, 170 peatland samples and 150 mineral soil samples were analyzed. In peatlands, microhabitats included hummocks, lawns, and hollows, representing moisture gradients. In mineral soils, samples were collected from six microhabitats, including leaf litter, inter-crown spaces, and moss-dominated areas. In the analysis of latitudinal gradient for α-diversity in sphagnum-dwelling testate amoeba assemblages, the dataset includes a total of 816 samples from 75 peatlands. To assess *β*-diversity latitudinal gradients in in soil-dwelling testate amoeba assemblages, 111 mineral soil samples were analyzed across multiple nested scales (sub-zone, ecosystem type, microhabitat, sample).

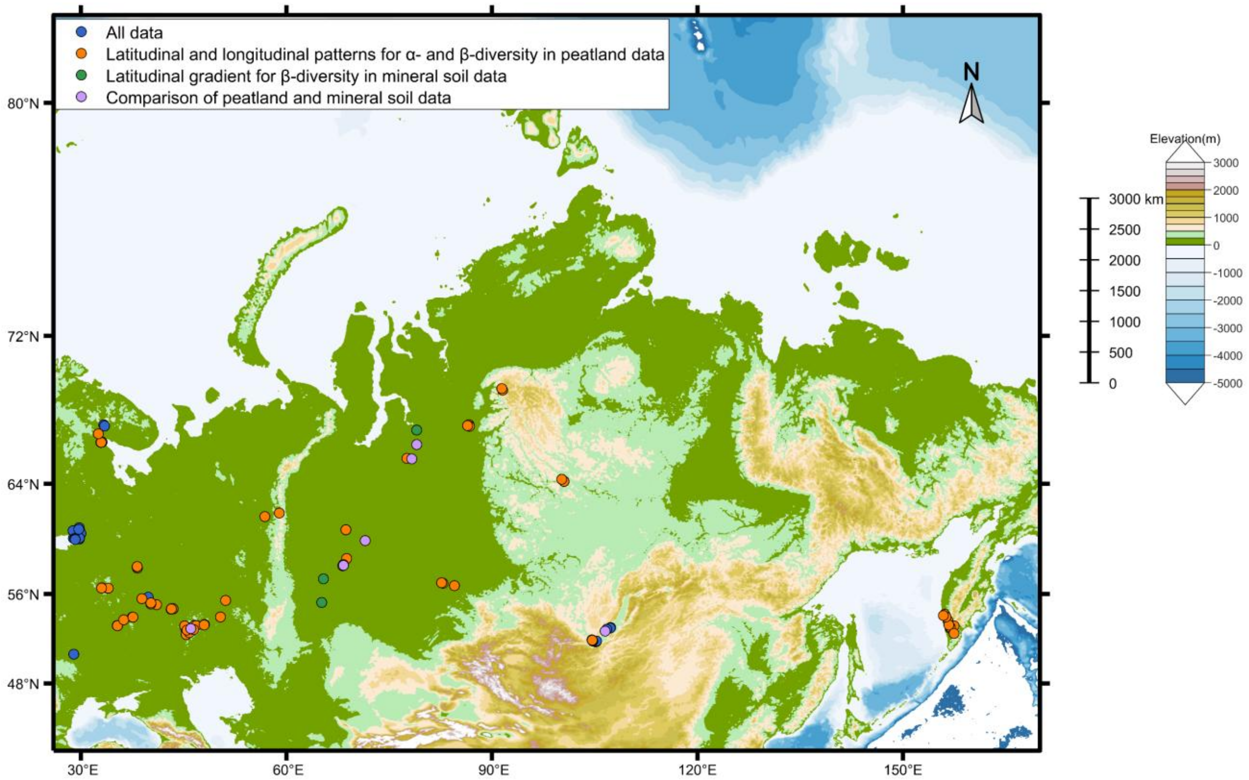


Fig 1. Location of sampling regions.

Climate data. Climate data were sourced from WorldClim v2.0 (10-min resolution) (https://www.worldclim.org) and included 19 bioclimatic variables. Additional variables, such as potential evapotranspiration and aridity index, were obtained from the Global Aridity Index database (Zomer et al., 2022).

Community structure metrics. Taxonomic diversity was quantified using Hill numbers (S, D_1, D_2) and Pielou's evenness (E). *β*-diversity was assessed via multiplicative partitioning (sub-zone, ecosystem, microhabitat) and the 1-C_{qN} framework (Chao et al., 2014). Functional diversity indices (FRic, FEve, FDiv) were derived from functional Gower distances (Laliberté et al., 2014). Community-weighted means (CWMs) were calculated for quantitative traits (e.g. shell length) using abundance weights and as proportions for categorical traits (e.g. shell shape). Null models (999 permutations) generated standardized effect sizes (SES.MPD) to infer assembly mechanisms: clustering (SES.MPD < 0) indicated environmental filtering, while overdispersion (SES.MPD > 0) suggested competitive exclusion (Kembel et al., 2010).

Statistical analyses. All statistical analyses were performed in R. To develop a trait-based classification system, hierarchical clustering using Ward's method (Murtagh, Legendre, 2014) was applied, with the optimal number of functional groups determined using the silhouette method (Rousseeuw, 1987). Principal Coordinate Analysis (PCoA) based on Gower distances was conducted to visualize group differences, with trait relationships examined via regression analyses.

For the comparison of sphagnum- and soil-dwelling testate amoeba assemblages, the Principal Component Analysis (PCA) and permutational ANOVA (PERMANOVA) based on Bray-Curtis dissimilarities were used to assess community differences across different habitats and regions. The Scheirer-Ray-Hare rank test evaluated habitat and regional effects on community structure metrics, with pairwise Wilcoxon tests used for comparisons. Standardized effect sizes of Mean Pairwise Distance (SES.MPD) were tested against zero null expectations.

The latitudinal gradient of α- and β-diversity in sphagnum communities was analysed using linear mixed-effects models, where peatland ID was a random factor, habitat type was a categorical factor with a fixed effect, and latitude, longitude, and climatic variables acted as quantitative predictors with a fixed effect. The interaction between habitat type and quantitative predictors was also considered, implying the possibility of habitat specificity.

For latitudinal gradient analysis of β-diversity in mineral soils, we calculated the normalized β-diversity ($1\text{-}C_{qN}$) at three scale levels. Null model analyses of $1\text{-}C_{qN}$ metrics ($q = 0, 1, 2, 3$) were conducted with individual-based randomization (9999 permutations) to test deviation from expected values.

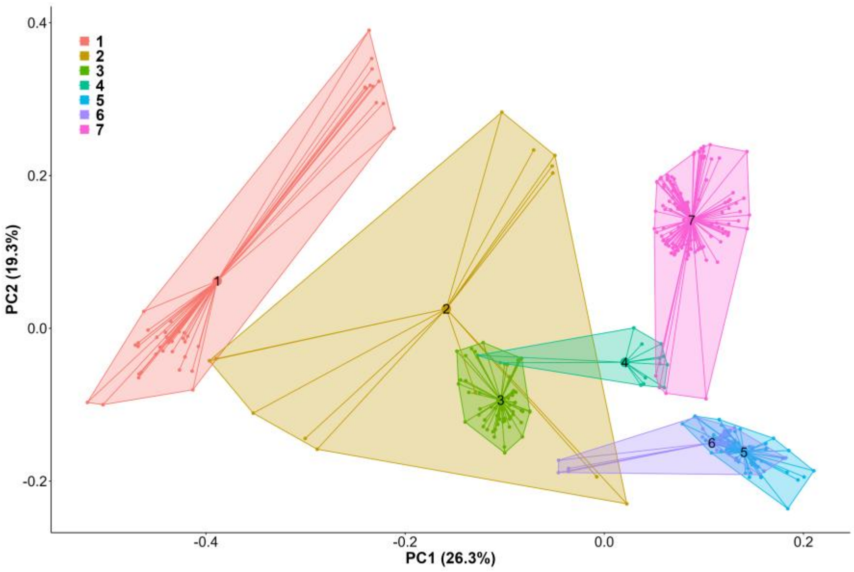
Chapter 3. Functional groups of testate amoebae

The trait data have been collected from the primary literature for 372 species of testate amoebae, including the most frequently observed species within the Northern Holarctic realm. The database includes a complete set of 18 traits for all species.

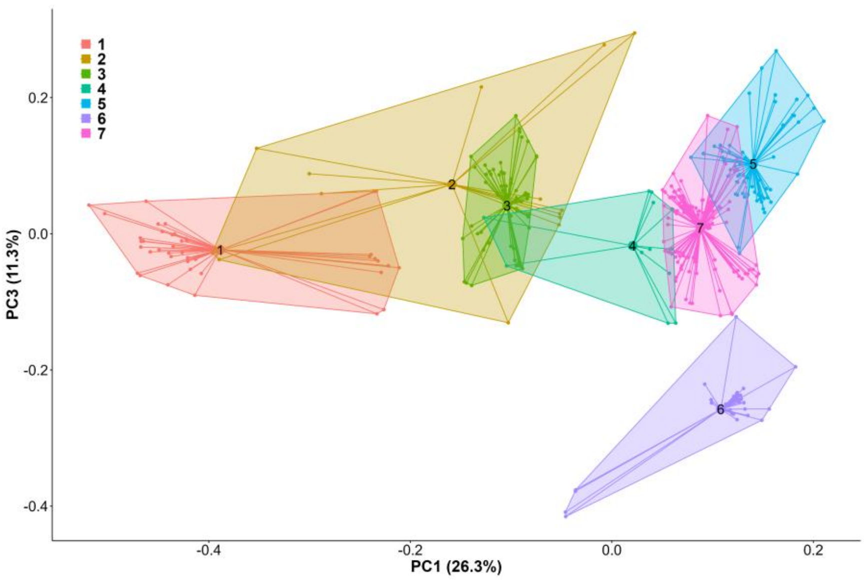
Cluster analysis identified seven functional groups representing distinct ecological adaptations of testate amoebae to various environmental conditions. Groups 1 and 2 consist mainly of species with large shells and complex structural adaptations (e.g. straight terminal apertures without invagination, collars or cleptostomes covering), many species in these two groups prefer wetter environments (e.g. fresh water, wet *sphagnum* and organic sediment). Groups 3-5 are represented by medium-sized species. Group 6 is characterized by patelliform shell shape with organic alveolar covering and central ventral aperture, its members represent adaptations to dry conditions and allow to occupy micropores within the soil matrix, particularly in fine-textured mineral soils. Group 7 contains the largest number of species, which have the smallest body size and aperture size (straight terminal position). These characteristics enable the group to occupy a variety of soil microhabitats, making them versatile contributors to different soil processes.

The distribution of species and their corresponding functional groups within the functional space, as generated by PCoA, is illustrated in Figs 2a and 2b. The first three principal coordinates account for a significant portion (56.9%) of the total variation within this functional space. The first principal coordinate serves as the primary axis of variation, explaining 26.3% of the variance, and differentiates between Groups 1, 4, and the combination of Groups 5-6. The second axis, accounting for 19.3% of the variation, distinguishes Group 7 from Groups 5 and 6, while the third axis, explaining 11.3% of the variation, differentiates Groups 3, 4, and 6. Therefore, a three-dimensional representation is necessary to adequately visualize the functional groups of testate amoebae.

To interpret the structure of the functional trait space, an analysis was conducted to examine the relationships between trait distributions and the principal coordinates. Low values along PC1 were associated with larger shell dimensions, pyriform shape, straight terminal aperture, cleptostomes covering, presence of spines, and predation as a feeding type. Low values along PC2 were associated with wide and thick shells, large and elongated apertures, and hemispherical shapes. Low values along PC3 were linked to small shell size, invaginated apertures, absence of spines, and relatively small aperture size (R3).



(a)



(b)

Fig 2. Functional groups of testate amoebae in a functional space constructed by the principal coordinate analysis based on Gower functional distances. (a) PC1 vs PC2, (b) PC1 vs PC3. The functional groups are indicated by numbers from 1 to 7 and are highlighted in color.

Chapter 4. Ecoregional patterns in sphagnum- and soil-dwelling testate amoeba assemblages

To compare sphagnum and soil-dwelling testate amoeba assemblages, we selected 320 samples across six temperate-zone regions. A total of 177 testate amoeba species and subspecies were recorded, with 110 species recorded in mineral soils and 157 in peatland environments. Species richness ranged from 1 to 60 per sample (mean ± standard deviation: 14.3 ± 8.9).

Community composition. PERMANOVA based on Bray-Curtis dissimilarities confirmed significant effects of habitat type, region, and their interaction on species composition ($p < 0.001$) (Fig 4). Regional differences had the strongest influence (R^2 = 13.6 %), reflecting the broad geographic scope of the study. Habitat type also played a significant role (R^2 = 9.4 %), while the interaction (R^2 = 10 %) indicated that habitat effects varied across regions.

PCA ordination (Fig 3) demonstrated clear separation between soil- and sphagnum-dwelling testate amoeba assemblages. The first principal component distinguished mineral soil communities, dominated by *Centropyxis aerophila sphagnicola* and *Centropyxis aerophila* (17.4 % and 16.6 %, respectively), from peatland communities, where *Trinema lineare* (17.2 %) and *Hyalosphenia papilio* (7.7 %) were prevalent. The second principal component captured regional contrasts, with *Hyalosphenia papilio* dominant in Middle Volga and Baikalia peatlands (11.8 %) and *Trinema lineare* more abundant in Western Siberia (28.3 %).

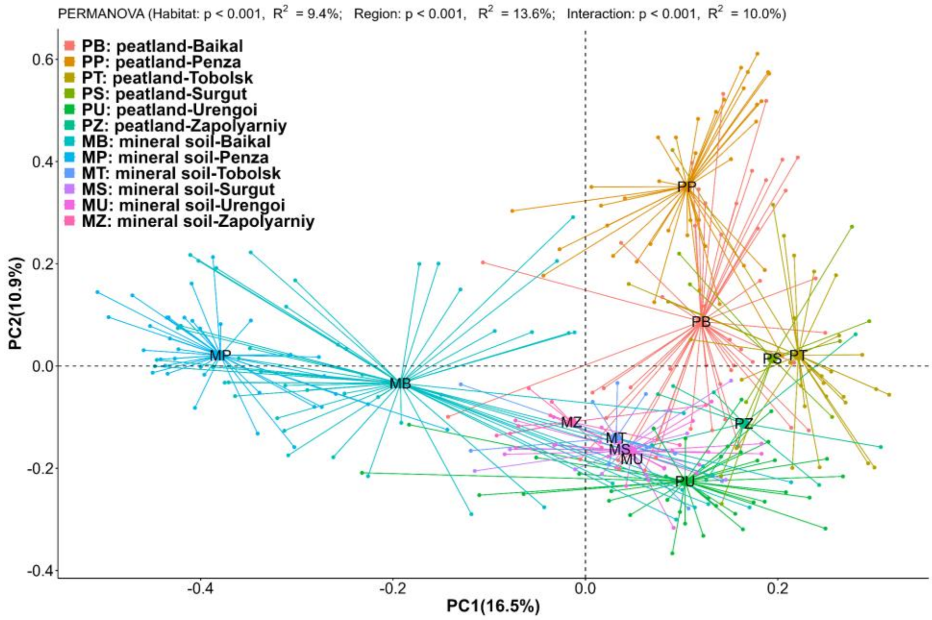


Fig 3. Ordination biplot for the principal component analysis (PCA) based on Hellinger-transformed relative abundances of testate amoebae.

Species diversity. The Scheirer-Ray-Hare test revealed a significant effect of region and habitat type on all four diversity metrics (Fig 4). Interaction effects were significant for species richness, Shannon and Simpson indices (but not for Pielou evenness), indicating regional variability in habitat influence. Patterns of taxonomic diversity varied across regions. In Penza and Baikal, sphagnum-dwelling testate amoeba assemblages exhibited higher Shannon and Simpson indices than soil-dwelling assemblages, whereas the opposite trend was observed in Western Siberia, with significant differences in two of the four regions (Figs 4a-4b). In contrast, Pielou evenness was consistently higher in soil-dwelling assemblages across all regions, with significant differences in four regions (Fig 4c).

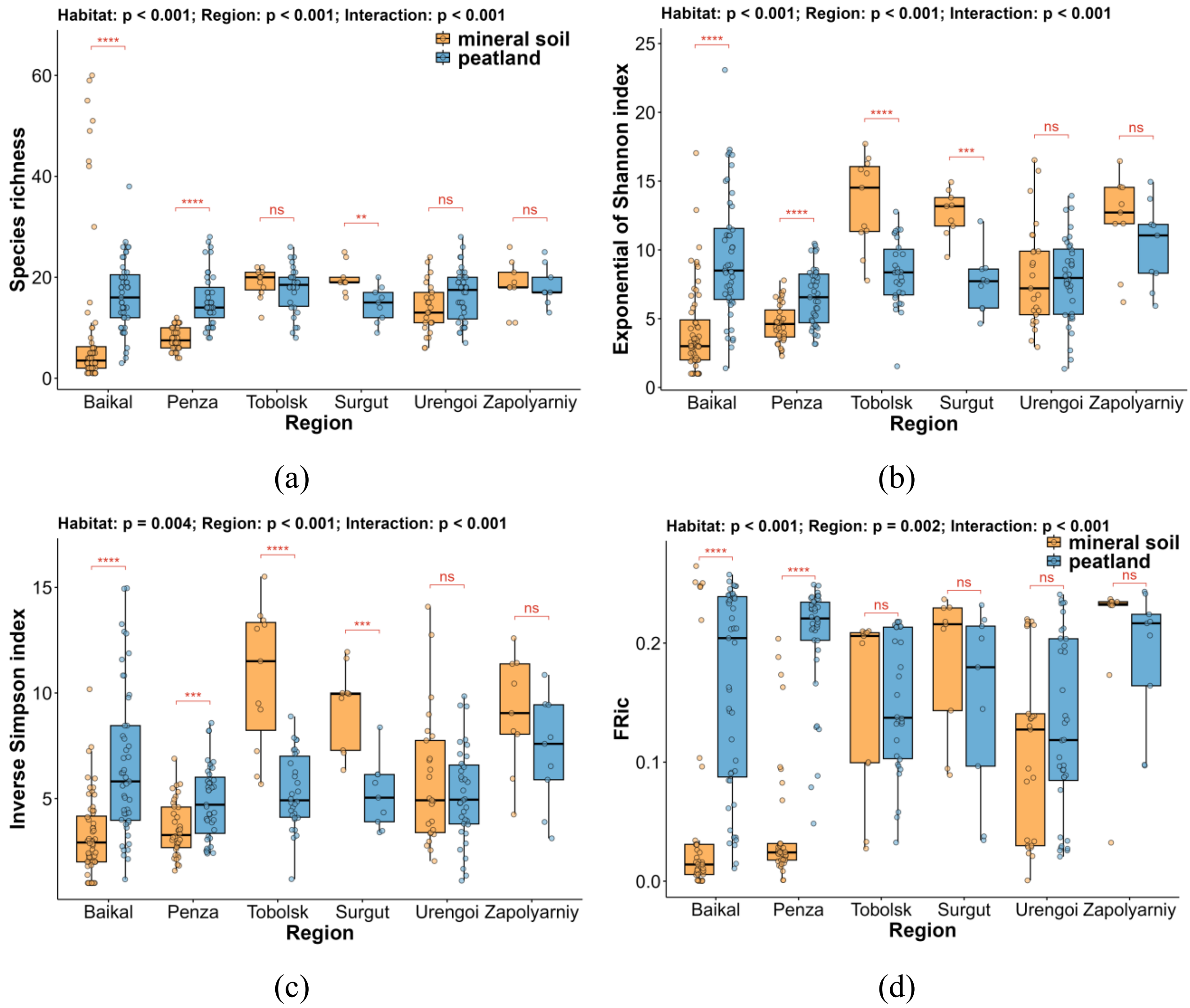


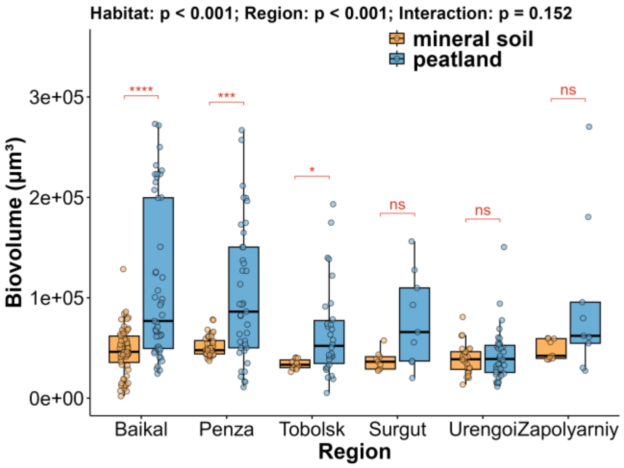
Fig 4. Boxplots of taxonomic and functional diversity metrics for sphagnum- and soil-dwelling testate amoeba assemblages across different regions. (a) Species richness. (b) The first-order diversity index. (c) The Pielou evenness index. (d) Functional richness Index. The symbols display the significance of the pairwise difference between two habitat types within regions. (****) - $p < 0.0001$, (***) - $0.0001 < p < 0.001$, (**) - $0.001 < p < 0.01$, (*) - $0.01 < p < 0.05$, (ns) - $p > 0.05$.

Functional diversity. Among functional diversity metrics, only functional richness was significantly influenced by habitat type (Fig 4d): it was higher in sphagnum-dwelling assemblages in Penza and Baikal but greater in soil-dwelling assemblages in Western Siberia, though insignificant in all four regions.

These findings highlight the role of regional environmental conditions in shaping diversity. Wet mineral soils in tundra and taiga regions provide moisture and habitat heterogeneity comparable to peatlands, supporting higher diversity. Conversely, drier forest-steppe soils impose harsher conditions, limiting diversity. The consistently higher evenness in soil communities suggests more stable conditions and reduced competitive exclusion.

Functional traits. The functional traits of testate amoebae are significantly influenced by habitat type, though patterns vary across regions. In general, sphagnum-dwelling testate amoeba assemblages exhibit larger biovolumes than those in soil-dwelling assemblages (Fig 5a), likely as an adaptation to water-saturated, anoxic environments. Larger shells may enhance buoyancy and oxygen storage, supporting survival under low-oxygen conditions. In contrast, smaller biovolumes in mineral soils suggest adaptations to better-aerated habitats.

Shell dimensions show regional inconsistencies. In Baikalia and Penza region, sphagnum-dwelling testate amoeba assemblages tend to have wider shells (Fig 5b), while wider shells are characteristic of soil-dwelling testate amoeba assemblages in Western Siberia. The ratio of shell width to length (R1) is consistently smaller in sphagnum-dwelling testate amoeba assemblages (Fig 5c), suggesting more elongated shapes. Similarly, the relative aperture size (R3) is smaller in Baikalia and Penza region in sphagnum-dwelling testate amoeba assemblages but larger in Western Siberia. These variations likely reflect differences in moisture, nutrient availability, and environmental stability.



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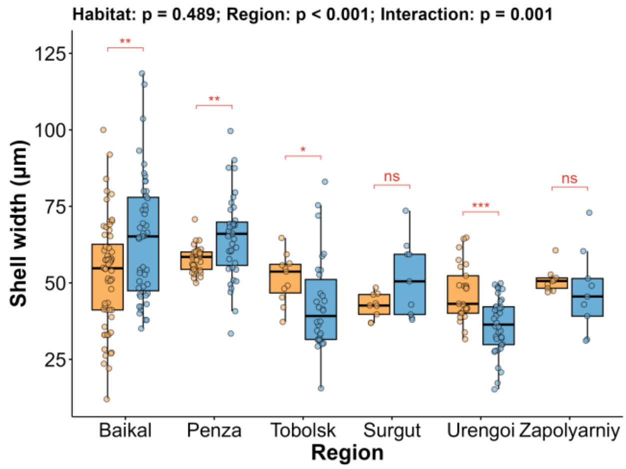

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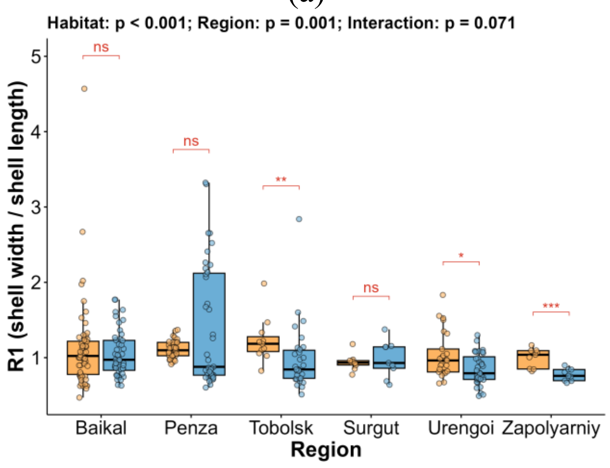


(c)

Fig 5. Boxplots of functional traits (community-weighted means) for sphagnum- and soil-dwelling testate amoeba assemblages across different regions. (a) Biovolume. (b) Shell width. (c) R1 index. The symbols display the significance of the pairwise difference between two habitat types within regions. (****) - $p < 0.0001$, (***) - $0.0001 < p < 0.001$, (**) - $0.001 < p < 0.01$, (*) - $0.01 < p < 0.05$, (ns) - $p > 0.05$.

Categorical traits display more consistent trends. Sphagnum-dwelling testate amoeba assemblages are characterized by higher proportion of patelliform and oval shells, whereas hemispherical shapes are more common in soil-dwelling testate amoeba assemblages. Straight terminal apertures dominate in sphagnum-dwelling testate amoeba assemblages, while soil-dwelling testate amoeba assemblages favor central ventral apertures. Sphagnum-dwelling testate amoeba assemblages also exhibit more denticulate aperture rims and idiosome-based shell coverings, reflecting adaptations to high organic matter content and stable water conditions. In contrast, xenosome-based coverings prevail in soil-dwelling testate amoeba assemblages, likely due to greater mineral particle availability. Bacterivory is the dominant feeding strategy in both habitat types. Mixotrophs are rare and almost exclusively restricted to sphagnum-dwelling testate amoeba assemblages. The prevalence of predatory testate amoebae varies among regions, suggesting local influences on food availability.

Overall, functional traits reflect habitat filtering and regional environmental conditions. The larger biovolumes and organic shell coverings in peatlands highlight adaptations to waterlogged, nutrient-rich environments. Meanwhile, mineral soil amoebae exhibit structural traits suited to drier, mineral-rich substrates. These patterns underscore the role of local and regional factors in shaping microbial functional traits and ecological strategies.

Community assembly rules. Null model analysis of standardized effect sizes SES.MPD revealed distinct assembly mechanisms between peatland and mineral soil communities. Non-weighted SES.MPD was consistently higher in sphagnum-dwelling communities (Fig 6). Soil-dwelling assemblages exhibited significant functional clustering (median SES.MPD < 0), indicating strong environmental filtering across all regions. In contrast, peatland assemblages generally showed functional overdispersion (median SES.MPD > 0), suggesting biotic interactions as a key structuring force, though significance was observed only in Penza.

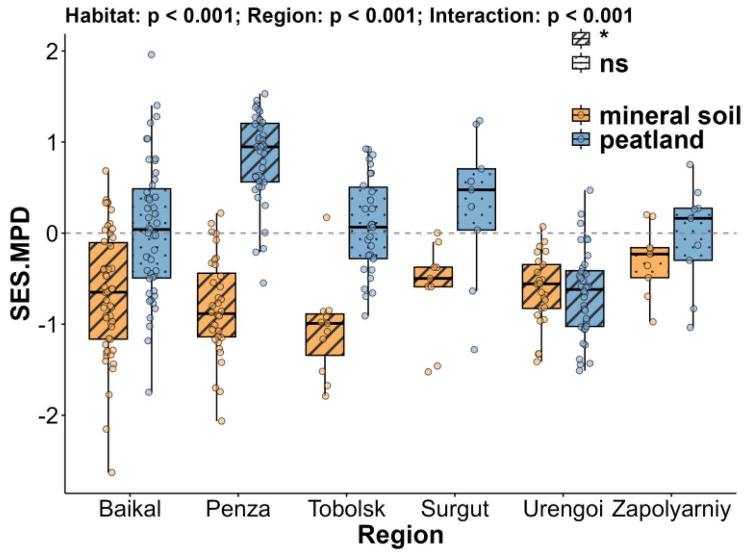
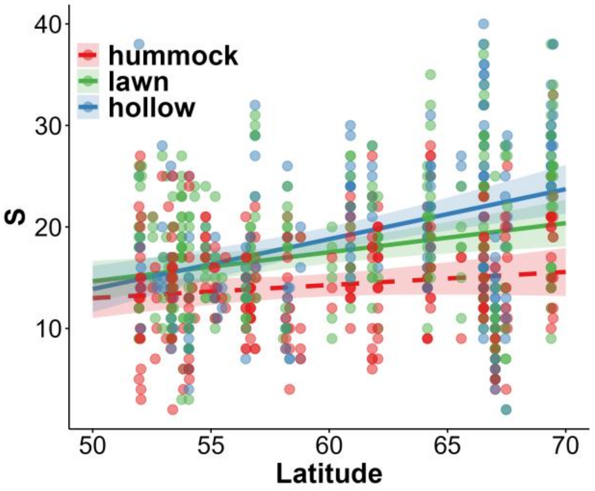


Fig 6. Boxplots of null model analysis metrics (SES.MPD) for sphagnum- and soil-dwelling testate amoeba assemblages across different regions. Box hatching displays the significance of the difference from zero.

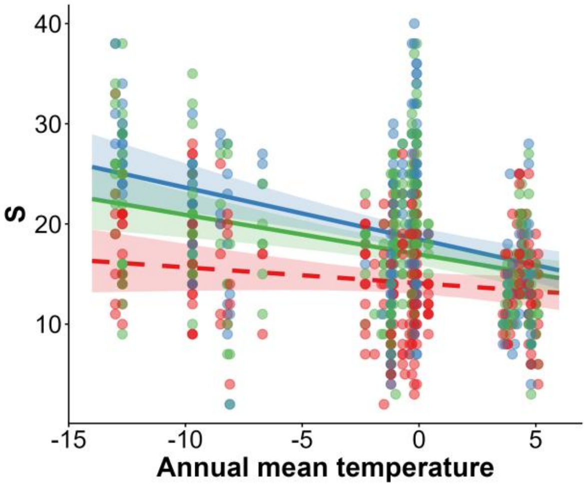
These findings indicate that environmental filtering is the dominant assembly process in mineral soils, constraining community composition. In contrast, biotic interactions play a greater role in peatland communities, promoting functional divergence. However, regional differences, such as the prevalence of environmental filtering in Urengoi peatlands, highlight the importance of local conditions in shaping microbial community structures.

Chapter 5. Latitudinal and longitudinal gradient patterns in species diversity in testate amoebae *α*- and *β*-diversity

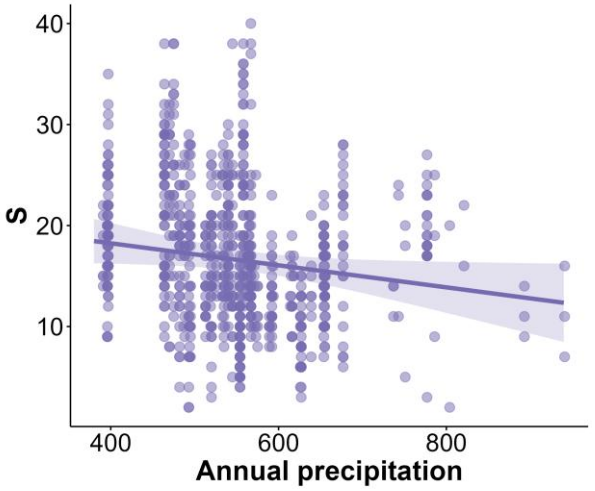
Latitudinal and longitudinal patterns in sphagnum-dwelling testate amoeba assemblages. Biogeographic patterns in peatland communities were analyzed based on 816 samples collected from 75 peatlands, encompassing a total of 240 testate amoeba taxa. Comparison across microhabitats revealed that species richness (S) was highest in hollows and lowest in hummocks. Linear mixed-effects model indicated a significant increase in species richness with increasing latitude, particularly in hollows and lawns, whereas the relationship with longitude was insignificant (Fig. 7a). In addition, species richness negatively correlated with mean annual temperature and precipitation (Figs 7b-7c), with temperature having the highest predictive power ($R^2 = 0.179$).



(a)



(b)



(c)

Fig 7. Relationship of species richness to latitude (a), annual mean temperature (b), and annual precipitation (c) in different microhabitats in sphagnum-dwelling testate amoeba communities across Russia (only significant effects with $p < 0.05$ are shown). The solid line indicates that the slope is significantly different from 0, the dashed line indicates the opposite situation.

The Shannon index (D_1) increased with latitude in hollows but remained constant in hummocks. Longitudinally, D_1 increased in lawns. Mean annual temperature was the only significant climatic predictor. Evenness (E) positively correlated with longitude in lawns and was influenced by temperature seasonality and annual precipitation.

β-diversity showed limited climatic correlations. Zeroth-order β-diversity ($1\text{-}C_{0N}$) positively depended on precipitation seasonality in hollows. First-order β-diversity ($1\text{-}C_{1N}$) increased with longitude in hollows, with contrasting effects in hummocks and hollows.

Based on the above results, in sphagnum-dwelling testate amoeba assemblages, our analysis revealed a notable inverse latitudinal gradient in α-diversity, which was negatively associated with mean annual temperature. This pattern was strongly evident in lawn and hollow microhabitats but absent in hummocks. Longitude, reflecting a gradient in precipitation seasonality, emerged as a significant factor influencing β-diversity, particularly within hollow habitats. These results highlight the role of climatic variables in shaping large-scale *α*- and *β*-diversity patterns of testate amoebae. The lack of a discernible pattern in hummocks likely reflects the dominant influence of microhabitat stressors (including low moisture and pH).

Latitudinal gradient of *β*-diversity in soil-dwelling testate amoeba assemblages. We analyzed *β*-diversity patterns of soil-dwelling testate amoebae across four latitudinal sub-zones, using 121 samples and identifying 74 taxa. *β*-diversity (1-C_{qN}) was calculated for sub-zones, ecosystems, and microhabitats using multiplicative partitioning.

In the forest-tundra, taiga, and sub-taiga sub-zones, *β*-diversity was high among ecosystems but low among microhabitats. In the northern forest-tundra, among-replicate *β*-diversity dominated, with among-ecosystem *β*-diversity being lowest. A latitudinal gradient was evident for among-ecosystem *β*-diversity, decreasing northward, while among-microhabitat and among-replicate β-diversity showed no clear trends.

Concluding remarks

This study presents a comparative analysis of sphagnum- and soil-dwelling testate amoeba assemblages across different regions of Northern Eurasia.

Our results revealed substantial taxonomic and functional differences between sphagnum- and soil-dwelling testate amoeba assemblages, largely driven by regional environmental conditions, particularly soil moisture and climate. Differences in average trait values, such as shell size and aperture characteristics, reflect adaptations to distinct moisture regimes. The contrasting patterns of diversity between peatland and mineral soil habitats across ecoregions: higher diversity in peatlands in drier forest-steppe zones versus higher diversity in mineral soils in the wetter taiga and tundra, which highlight the central role of moisture availability in shaping testate amoeba assemblages. These findings illustrate how microeukaryotic communities respond to macroecological gradients and emphasize water availability as a key driver of community structure.

Our analysis also revealed the presence of latitudinal diversity gradients, including a decline in β-diversity with increasing latitude in soil-dwelling testate amoeba assemblages and a negative α-diversity gradient in sphagnum-dwelling testate amoeba assemblages in microtopographic depressions (hollows). These large-scale biogeographic patterns shed light on the combined influence of historical biogeography, climatic variability, and habitat specialization on microbial distributions.

Conclusions

1. A comparison of the taxonomic structure and diversity of sphagnum- and soil-dwelling testate amoeba assemblages in the Middle Volga region, Western Siberia, and Baikalia revealed pronounced differences. Sphagnum-dwelling testate amoeba assemblages were dominated by *Trinema lineare* and *Hyalosphenia papilio*, whereas *Centropyxis aerophila sphagnicola* and *Centropyxis aerophila* prevailed in soil-dwelling testate amoeba assemblages. The taxonomic diversity between habitat types varied significantly across regions: sphagnum-dwelling testate amoeba assemblages exhibited higher diversity in forest-steppe ecoregions, while soil-dwelling testate amoeba assemblages were more diverse in the taiga and tundra.

2. A classification system for testate amoebae of the Northern Holarctic was developed, dividing species and subspecies into seven functional groups based on 18 traits. Compared to traditional morphology-based classifications, this trait-based system provides a more informative framework for understanding ecological roles and strategies.

3. Community-weighted mean trait values of testate amoebae differed significantly between peatland and mineral soil habitats. Sphagnum-dwelling testate amoebae were characterized by larger shell size, more elongated shell shape, narrower apertures, and a predominance of organic shells with endogenous siliceous plates (idiosomes).

4. The assembly of sphagnum- and soil-dwelling testate amoeba assemblages is driven by a combination of abiotic and biotic mechanisms. Abiotic environmental filtering plays a dominant role in soil-dwelling testate amoeba assemblages, whereas biotic interactions are more influential in sphagnum-dwelling testate amoeba assemblages.

5. The moisture regime of mineral soils across different ecoregions is a key factor underlying the structural and functional differentiation between sphagnum- and soil-dwelling testate amoeba assemblages. In forest-steppe regions, where soil moisture is limited, sphagnum-dwelling testate amoeba assemblages display higher diversity. In contrast, the sufficient soil moisture of taiga and tundra supports greater diversity in soil-dwelling testate amoeba assemblages.

6. The α- and β-diversity of sphagnum-dwelling testate amoeba assemblages is influenced by climatic variables such as mean annual temperature and precipitation seasonality. Communities in moist hollow microhabitats exhibit a positive latitudinal diversity gradient, while such a gradient is absent in drier hummock microhabitat, likely reflecting the stressful conditions of the latter. Soil-dwelling testate amoeba assemblages show a negative latitudinal gradient in β-diversity at the scale of ecosystems, but this pattern does not hold at the scale of microhabitat or within microhabitat variation.

List of publications on the dissertation topic published in peer-reviewed scientific journals which are recommended for publication of results suitable for defense in the council MSU.015.3 for Specialty 1.5.15 – Ecology (biological sciences).

1. **Su J.**, Mazei Y.A., Tsyganov A.N., Mazei N.G., Chernyshov V.A., Komarov A.A., Babeshko K.V., Mitchell E.A.D., Shimano S., Krasilnikov P., Saldaev D.A., Yakimov B.N. Ecoregional patterns of protist communities in mineral and organic soils: assembly processes, functional traits and diversity of testate amoebae in Northern Eurasia // Soil Biology and Biochemistry. 2025. V. 208. 109841. https://doi.org/10.1016/j.soilbio.2025.109841 (Q1, IF 3.982 (SRJ), EDN: не доступен)

2. **Su J.**, Mazei Y.A., Tsyganov A.N., Mazei N.G., Chernyshov V.A., Komarov A.A., Babeshko K.V., Mitchell E.A.D., Shimano S., Saldaev D.A., Yakimov B.N. Continental-Scale α– and β–Diversity Patterns of Terrestrial Eukaryotic Microbes: Effect of Climate and Microhabitat on Testate Amoeba Assemblages in Eurasian Peatlands // Journal of Biogeography. 2025. V. 52. e15082. https://doi.org/10.1111/jbi.15082 (Q1, IF 1.438 (SRJ), EDN: SJTXLM)

3. **Su J.**, Mazei Y.A., Tsyganov A.N., Chernyshov V.A., Komarov A.A., Malysheva E.A., Babeshko K.V., Mazei N.G., Saldaev D.A., Levin B., Yakimov B.N. Functional traits data for testate amoebae of Northern Holarctic realm // Scientific Data. 2024. V. 11. P. 1028. https://doi.org/10.1038/s41597-024-03874-0 (Q1, IF 1.867 (SRJ), EDN: WZJVLK)

4. **Su J.**, Mazei Y.A., Tsyganov A.N., Chernyshov V.A., Mazei N.G., Saldaev D.A., Yakimov B.N. Multi-scale beta-diversity patterns in testate amoeba communities: species turnover and nestedness along a latitudinal gradient // Oecologia. 2024. V. 205. P. 691-707. https://doi.org/10.1007/s00442-024-05602-2 (Q1, IF 0.966 (SRJ), EDN: RZNXWB)

5. Saldaev D., Babeshko K., Chernyshov V., Esaulov A., Gu X., Kriuchkov N., Mazei N., Saldaeva N., **Su J.**, Tsyganov A., Yakimov B., Yushkovets S., Mazei Y.A. Biodiversity of Terrestrial Testate Amoebae in Western Siberia Lowland Peatlands // Data. 2023. V. 8. P. 173. https://doi.org/10.3390/data8110173 (Q2, IF 0.480 (SRJ), EDN: FCUMAC)