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Abstract

Global urbanization has resulted in local habitat fragmentation, influencing ecological processes and biodiversity conservation. However, little is known about the diversity and distribution of microbial communities across urban biotopes. Here, we compared testate amoeba communities in Moscow (63 samples) and Xiamen (69 samples) urban parks across four biotopes (tree hole, moss, soil, and sediment) to better understand microbial diversity and ecological processes shaping microbial communities. A total of 116 morphospecies (31 genera), corresponding to 90 in Moscow and 84 in Xiamen, were identified using light microscopy. The species richness per sample (mean ± standard error) was higher in Moscow parks (14 ± 1 species, $n = 63$) than Xiamen parks. The mean species richness per biotope was highest in the mosses (13 ± 1 species, $n = 33$). 13–26% of the total species richness was shared by all biotopes, indicating the ubiquitous distribution of testate amoeba morphospecies. The community composition in different biotopes markedly differed in both Moscow and Xiamen regions. Community connectivity varied among biotopes, and community complexity and dynamics were substantially stronger in soil and sediment. The stochastic processes explained a significantly high percentage of community composition in all biotopes (57–81%). The standardized effect size for C-score in all biotopes changed from 1.48 to 6.92, indicating the enhanced significance of deterministic processes for the testate amoeba communities. The different relative importance of stochastic or deterministic processes in four studied biotopes suggests that factors influencing the testate amoeba communities greatly vary across heterogeneous urban environments.

Keywords (separated by '-') Microbial diversity - Urban parks - Biotopes - Testate amoebae - Ecological processes

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Stochastic and deterministic processes shaping the testate amoeba communities across different biotopes of urban parks in Moscow and Xiamen cities

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Abstract

Global urbanization has resulted in local habitat fragmentation, influencing ecological processes and biodiversity conservation. However, little is known about the diversity and distribution of microbial communities across urban biotopes. Here, we compared testate amoeba communities in Moscow (63 samples) and Xiamen (69 samples) urban parks across four biotopes (tree hole, moss, soil, and sediment) to better understand microbial diversity and ecological processes shaping microbial communities. A total of 116 morphospecies (31 genera), corresponding to 90 in Moscow and 84 in Xiamen, were identified using light microscopy. The species richness per sample (mean ± standard error) was higher in Moscow parks (14 ± 1 species, $n = 63$) than Xiamen parks. The mean species richness per biotope was highest in the mosses (13 ± 1 species, $n = 33$). 13–26% of the total species richness was shared by all biotopes, indicating the ubiquitous distribution of testate amoeba morphospecies. The community composition in different biotopes markedly differed in both Moscow and Xiamen regions. Community connectivity varied among biotopes, and community complexity and dynamics were substantially stronger in soil and sediment. The stochastic processes explained a significantly high percentage of community composition in all biotopes (57–81%). The standardized effect size for C-score in all biotopes changed from 1.48 to 6.92, indicating the enhanced significance of deterministic processes for the testate amoeba communities. The different relative importance of stochastic or deterministic processes in four studied biotopes suggests that factors influencing the testate amoeba communities greatly vary across heterogeneous urban environments.

Keywords Microbial diversity · Urban parks · Biotopes · Testate amoebae · Ecological processes

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Introduction

Urban development is vastly associated with the fragmentation, disturbance, and loss of habitats existing within cityscapes (Elmqvist et al. 2013; Alberti et al. 2020). Research on biota in urban parks has become important due to the growing understanding of the considerable role of such parks in conservation and promotion of biodiversity (Uchida et al. 2020). Despite the importance of microbial components in various types of ecosystems, our knowledge about the urban microbial biodiversity remains limited, especially in microbial eukaryotes (Nielsen et al. 2014; Hill et al. 2016; Alberti and Wang 2022). This especially concerns large cities, like Moscow and Xiamen, which have successfully promoted both the economic growth and the urban landscape development under urbanization process (Tang et al. 2013; Ivlev et al. 2017). A better understanding of microbial biodiversity in urban ecosystems of large cities will provide positive impact on the quality of urban life and further promote biodiversity study and conservation.

Testate amoebae are single-celled organisms inhabiting a wide range of environments, including soils, mosses, wetlands, and freshwaters (Ogden and Hedley 1980; Beyens and Meisterfeld 2001; Yang et al. 2006; Mitchell et al. 2008; Tran et al. 2021). They play an important role in a variety of ecological processes, such as the decomposition of organic matter and the silica cycle (Puppe 2020). Many types of these natural biotopes (e.g., soils, wetlands and mosses) have been investigated for testate amoebae (Ogden and Hedley 1980; Mazei and Tsyganov 2006; Ju et al. 2014; Todorov and Bankov 2019; Tran et al. 2021, 2022). However, some biotopes remain markedly unexplored, even though the few available studies reported undescribed testate amoeba communities (Yang et al. 2006; Bobrov et al. 2010; 2012; Ndayishimiye et al. 2020b; Wang et al. 2022). Understudied biotopes include certain freshwater environments (e.g., mosses in streams and suspended sediment), subterranean deposits from caves, buildings (e.g., wet walls and roofs), and urban parks (e.g., suspended soil of the aerial roots and tree hollows) (Mazei and Belyakova 2011; Mazei et al. 2012; Shimano et al. 2017; Bobrov et al. 2020; Ndayishimiye et al. 2020b; Bobrov and Mazei 2021). Therefore, investigation on these biotopes could considerably contribute to our understanding of testate amoeba diversity especially in urban environments.

The processes shaping the microbial community, its spatial patterns and temporal dynamics, are the central, yet poorly understood topics in the ecology of testate amoebae (Yang et al. 2010; Ndayishimiye et al. 2020a; 2021). Due to the interaction of several factors acting on numerous spatial and temporal scales, the observed patterns are often inconsistent and greatly depend on the sampling design or scales (Davidova and Vasilev 2013; Arrieira et al. 2017; Schwind et al. 2017, 2018; Ren et al. 2018; Wang et al. 2020). For that reason, many researchers have emphasized the need to implement a multi-scale approach in order to understand the link between the testate amoebae and the environment across various types of biotopes (Beyens and Meisterfeld 2001; Charman 2001; Mitchell et al. 2008). The results obtained with this approach might be integrated using the neutral and niche-based theories, which provide important and complementary mechanisms for understanding microbial community assembly (Zhou and Ning 2017). The neutral theory considers birth, death, immigration, speciation, and dispersal as stochastic processes and emphasizes that community structure results from an equilibrium between the loss and gain of species (Chen et al. 2019; Mo et al. 2021). The niche-based theory explains the community composition primarily using deterministic effects of biotic (e.g., species interactions such as competition, predation, parasitism, mutualism, and commensalism) and abiotic (e.g., environmental variables such as temperature and nutrients) factors as well as species habitat preference and fitness (Dini-Andreote et al. 2015; Zhou and Ning 2017). The relative importance of these processes in shaping microbial communities is still poorly understood due to the difficulties related to the approaches used to describe stochasticity and deterministicity in ecosystems (Gotelli and McGill 2006; Chen et al. 2019; Mo et al. 2021). Hence, extensive studies and analysis of microbial communities in different urban environments would help to improve our understanding of the comparative roles of these processes.

In this study, we analyse community composition of testate amoebae in different biotopes (tree hole, moss, soil, and sediment) from two climate zones (Moscow with continental temperate climate and Xiamen with subtropical climate). We aim to answer the following questions: (1) Is there a significant difference in testate amoeba diversity across different biotopes in urban parks? (2) What is the relative importance of stochastic or deterministic processes in shaping the testate amoeba communities across different biotopes? We expect a higher species richness in the mosses, more ubiquitous morphospecies across all biotopes, and a greater significance of both stochastic and deterministic processes for the testate amoeba communities in the four studied biotopes. Our understanding of testate amoeba ecology will be improved by the responses to these questions, especially in light of urban development and environmental conservation. It would be easier to plan cities with microbes and their importance for cities and citizen well-being in mind if there was a better understanding of large-scale spatial patterns in microbial diversity and function together with information on factors regulating microbial community (Alberti et al. 2020).

Material and methods

Site description

This study was conducted in urban parks in Moscow and Xiamen cities in June and July 2020 (Fig. 1 and Supplementary Table S1). Moscow (55°45′N, 37°37′E) is characterized by a continental temperate climate with long, cold winters that typically last from mid-November to the end of March and warm summers. The mean annual temperature is 5.8 ℃, and the total annual precipitation is 707 mm. Snow, which is present for about five months of the year, often begins to fall in mid-October, while snow cover lies in November and melts at the beginning of April (Isayev and Sherstyukov 2008). Xiamen (24°29′N, 118°05′E) is characterised by a monsoonal, humid subtropical climate with relatively long, warm, and humid summers and short, mild, and dry winters. The mean annual temperature and the total annual precipitation are 20.7 ℃ and 1,335.8 mm, respectively (Isabwe et al. 2022). The precipitation mainly occurs from April to August. From September to February, the wind is generally from the northeast and changes to a southeast wind between March and August. Snowfall is extremely rare throughout the winter season (Tang et al. 2013; Yang et al. 2017).

Sampling

Samples were collected at the territory of five urban parks in Moscow ($n = 63$) and six parks in Xiamen ($n = 69$) (Fig. 1b and Supplementary Table S1). Four types of biotopes were sampled in each park: natural hollows in trunks or branches of trees (shortened as "tree hole"), moss-dominated biotope ("moss"), top layers (0–5 cm) of soil ("soil"), and surface sediment of ponds ("sediment") (Fig. 2). The samples from tree holes ($n = 34$) represented naturally formed decomposing substrates in the trunks and branches (at the height of 0.5−1.5 m). Moss was collected from the ground beneath trees and on tree trunks (at the height of 0−0.5 m) and evenly mixed to form samples ($n = 33$). Soil samples ($n = 32$) represented unconsolidated mineral and organic substrates on the surface of the ground (distance from trees: 1−20 m). Sediments ($n = 33$) were fine and coarse organic materials with sand and clay near the shore (water depth: 0.2−0.5 m). The samples from tree hole and moss, soil, and sediment were taken using a laboratory spoon, a hand shovel, and a 3L Van Veen grab sampler, respectively. After the fieldwork, all samples were immediately taken into the laboratory and kept in the refrigerator at 4 ℃ until further processing (Mazei et al. 2015).

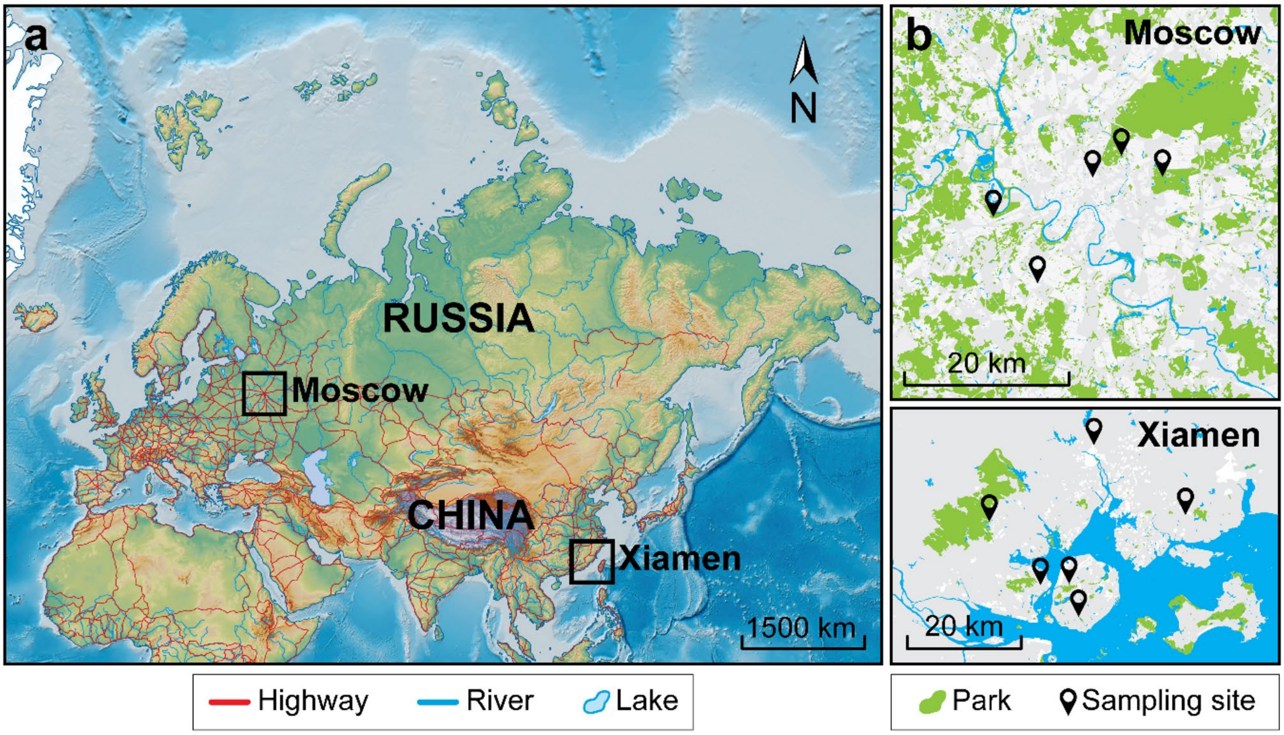

Fig. 1 Location of the study sites. **a** The map shows Moscow as an inland city in the east of Russia and Xiamen as a typical coastal city in southeast China. **b** City maps showing eleven sampled urban parks (five Moscow parks and six Xiamen parks). For simplicity, geographic coordinates, together with altitude and sea depth contours, and urban facilities, are not shown. The map was created by QGIS version 3.24.1 (QGIS Development Team 2022)

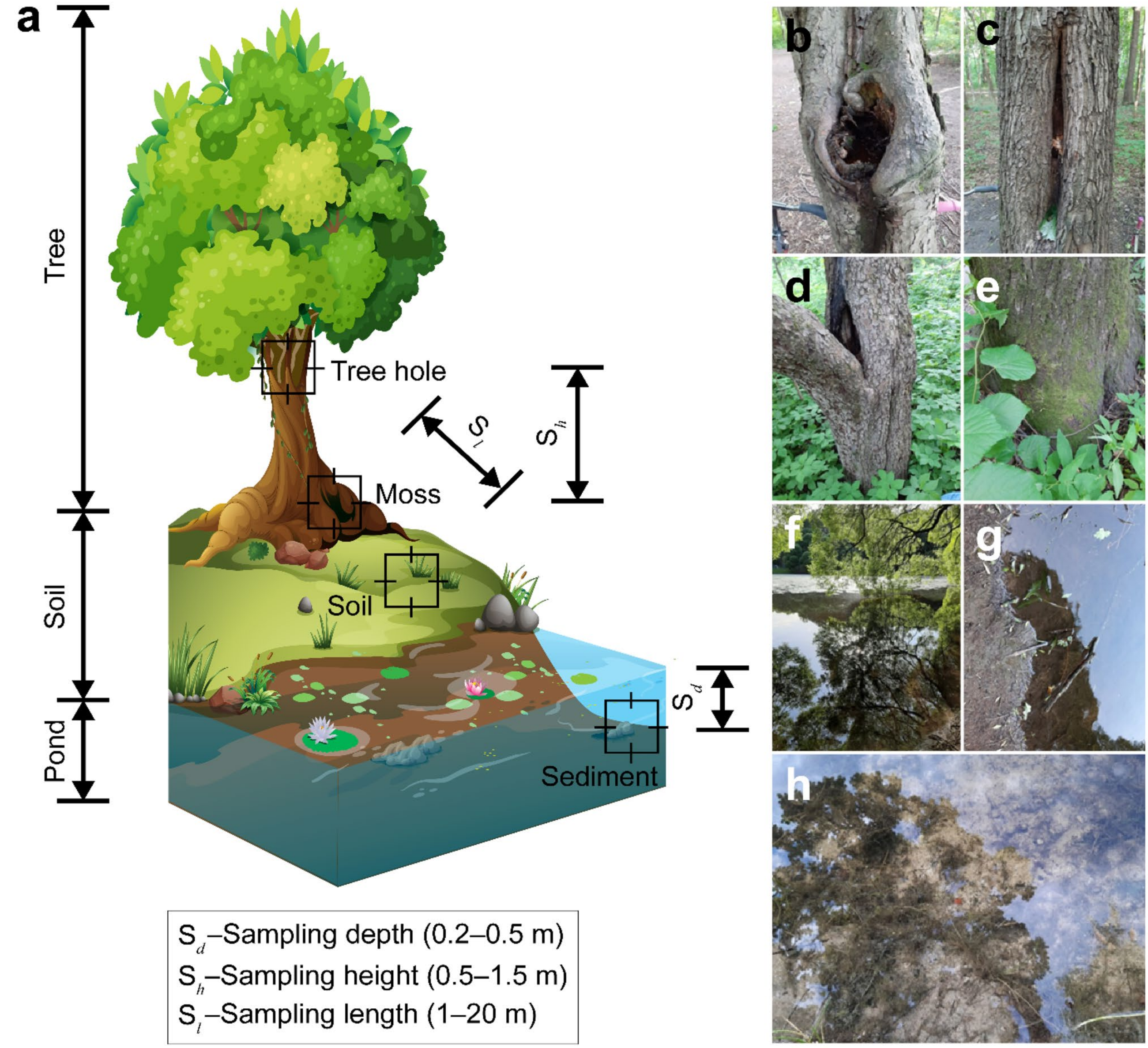

Fig. 2 Four types of biotopes that were sampled in each urban park. **a** Sampling locations (black squares) are semi-enclosed hollows that have naturally formed in the trunk or branch of a tree (tree holes), moss-covered substrates (moss), top soil layers (soil), and surface bottom sediments from ponds near the shore (sediment). **b** *Aesculus hippocastanum* tree hole, **c** *Tilia cordata* tree hole; **d** *Malus domestica* tree hole; **e** moss at *Tiliia cordata*; **f** Soldatenkovsky pond; **g** Soldatenkovsky pond bottom sediment; **h** bottom sediment of Sobachiy pond in Izmaylovsky Park

Testate amoeba analysis

In total, 132 samples (i.e., 63 samples in Moscow and 69 in Xiamen) were collected for testate amoeba analysis. Five grams were placed in a 150 ml flask and soaked in deionized water for 24 h. The resulting suspension was stirred for 10 min, filtered through a 0.5 mm mesh screen into a 0.8 l beaker. Coarse particles remaining in the screen were washed with a jet of water and permitted to settle for 24 h. The supernatant liquid was decanted, and the remaining part was transferred to a graduated container and allowed to settle. Further, the supernatant liquid was decanted so that the remaining volume was 10 ml. The resulting material was stained with an erythrosine solution for 24 h (Mazei and Chernyshov 2011). The 2 ml aliquot was then placed in a Petri dish, diluted with deionized water to facilitate even scattering of particles over the bottom, and observed using a Zeiss Axio Lab.A1 microscope (Carl Zeiss AG, Germany) at 200X or 400X magnification. Although living specimens may have been present at the time of sampling, the samples were not stained, so the microscopy analysis was performed on living plus dead specimens. Testate amoebae were identified to morphospecies using high resolution approach (Mitchell et al. 2014). A full list of morphospecies and their frequency of occurrences are shown in Supplementary Table S2.

Statistical analyses

Species accumulation curves were used to standardise samples among sites, to predict site species richness, and to estimate the minimum sampling effort required for adequate completeness of inventories (Supplementary Fig. S1). To estimate the species pool size, a range of species richness estimators (Chao 2, Jackknife 1, Jackknife 2, and bootstrap) was used (Cao et al. 2004). The number of morphospecies belonging to different genera was visualized as a heatmap using the package "pheatmap" (Kolde 2022) and R version 4.1.3 (R Core Team 2022). The segregation of morphospecies data into biotope-specific and ubiquitous morphospecies was performed using a Venn diagram. The biotope-specific morphospecies were defined as those that were only present in the samples from one type of biotope and were absent in the others. The ubiquitous morphospecies were those that were present in samples from at least two different types of biotopes. The alpha-diversity of testate amoeba community was assessed using three parameters: species richness (i.e., mean number of morphospecies per sample), the Shannon–Wiener diversity index, and the dominance index. The dominance was calculated as one minus Simpson index with the values ranging from 0 to 1, where 0 indicates equal distribution of all morphospecies abundances and 1 corresponds to the complete dominance of a single species. The pairwise comparison of the mean values was performed by Mann–Whitney U tests with a significance $P < 0.05$. The contribution (%) of each morphospecies to the overall Bray–Curtis dissimilarity among communities of all samples was determined by the similarity percentages (SIMPER test) (Clarke 1993).

Normality of the morphospecies count data of testate amoeba was tested with the Anderson–Darling test with the package "nortest" (Gross and Ligges 2015) and R version 4.1.3 (R Core Team 2022). Since most of the data did not fit the normal distribution, logarithmic transformations (log (x + 1)) were applied to the full morphospecies count data set to improve normality (McDonald 2014). Resemblance patterns among the samples were observed using non-metric multidimensional scaling (NMDS). This ordination technique is appropriate for ecological datasets, which often have numerous zeroes (i.e., the absence of a morphospecies). We employed the Bray–Curtis similarity to quantify the similarity between all pairs of samples. Then, we performed an analysis of similarities (ANOSIM) with a one-way test to assess differences among biotopes. We used the comparative measure of biotope separation, the statistical parameter for the analysis of similarity tests, also known as the global R (Clarke and Warwick 2001). $R = 1$ indicates that all replicates within a biotope are more similar to each other than any replicates from other biotopes, while $R = 0$ indicates no differences in species composition among biotopes. All calculations were made with the PRIMER 7 (PRIMER-E, Plymouth, UK).

The testate amoeba community complexity, which refers to the number and size of populations and their interactions, was investigated using an algorithm "cohesion" (Herren and McMahon 2017) in the R environment (R Core Team 2022). Scores for community complexity vary between –1 and 0, while scores for the degree of complexity, which informs how the members and their interactions are able to change over time (community dynamics), vary between 0 and 1 (Herren and McMahon 2017). The pairwise comparison of the mean values was carried out by Mann–Whitney U tests with a significance $P < 0.05$.

To evaluate the potential importance of stochastic processes on community assembly, we used a neutral community model to predict the relationship between morphospecies detection frequency and their relative abundance across the wider metacommunity (Sloan et al. 2006). This model predicts that morphospecies that are abundant in the metacommunity will be widespread, since they are more likely to disperse by chance among different sampling sites, whereas rare morphospecies are more likely to be lost in different sites due to ecological drift or the stochastic loss and replacement of individuals. In this model, the parameter Nm, which is an estimate of dispersal between communities, determines the correlation between occurrence frequency and regional relative abundance, with N describing the metacommunity size and m being the immigration rate (i.e., dispersal). The parameter R^2 represents the overall fit (i.e., goodness-of-fit) to the neutral model. When R^2 is close to 1, the community assembly is entirely consistent with stochastic processes. When it does not describe the community composition, R^2 is typically less than or equal to 0 (Sloan et al. 2006). The best-fit distribution curves of the models were determined using the non-linear least-square method with the package "Minpack.lm" (Elzhov et al. 2015) in the R environment (R Core Team 2022).

To test actual distribution of testate amoebae morphospecies for randomness (i.e., whether it is not greatly different from what it would be if morphospecies did not interact) we used the "checkerboard score" (C-score) calculated following the null model suggested by Stone and Roberts (1990). The C-scores were calculated based on incidence (presence-absence) data of testate amoeba morphospecies (Gotelli and Mccabe 2002) using the sequential swap randomization algorithm (30,000 simulations) with the package "EcoSimR" (Gotelli et al. 2015) and R version 4.1.3 (R Core Team 2022). The obtained values of C-scores were standardized to allow comparisons among communities by calculating standardized effect size (SES). The SES was calculated as the difference between the observed and the mean of the stimulated C-scores divided by the standard deviation of the stimulated C-scores. The value of SES itself can be interpreted as the strength of deterministic processes affecting communities, and the greater value indicates the stronger influence of deterministic process (Ning et al. 2019).

Results

Community composition, alpha-, beta- and gamma-diversity

In total, 116 morphospecies of testate amoebae were identified. Among them, 90 and 84 morphospecies were found in Moscow and Xiamen parks, respectively, while 58 morphospecies were found in both these places (Supplementary Table S2). The species-accumulation curves did not reach an asymptote, and the Jackknife 2 estimate was in general higher than the other estimates (Supplementary Fig. S1). In total, 31 genera of testate amoebae were recovered, with the genus *Difflugia* containing most of the morphotypes both in Moscow and Xiamen (Supplementary Fig. S2). In pond sediment, the second most species-rich genus was *Centropyxis*, and the total species richness of these two genera (*Difflugia* and *Centropyxis*) varied from 10 to 25 species (Fig. 3a). The number of biotope-specific morphospecies ranged from 3 to 22 in Moscow and from 0 to 34 in Xiamen (Fig. 3b). Pond sediments in urban parks of Xiamen harboured the greatest number (34) of biotope-specific morphospecies. In contrast, the soil habitat exhibited the lowest number of biotope-specific morphospecies in both studied areas (three in Moscow and zero in Xiamen). The number of ubiquitous morphospecies was greater in Moscow parks

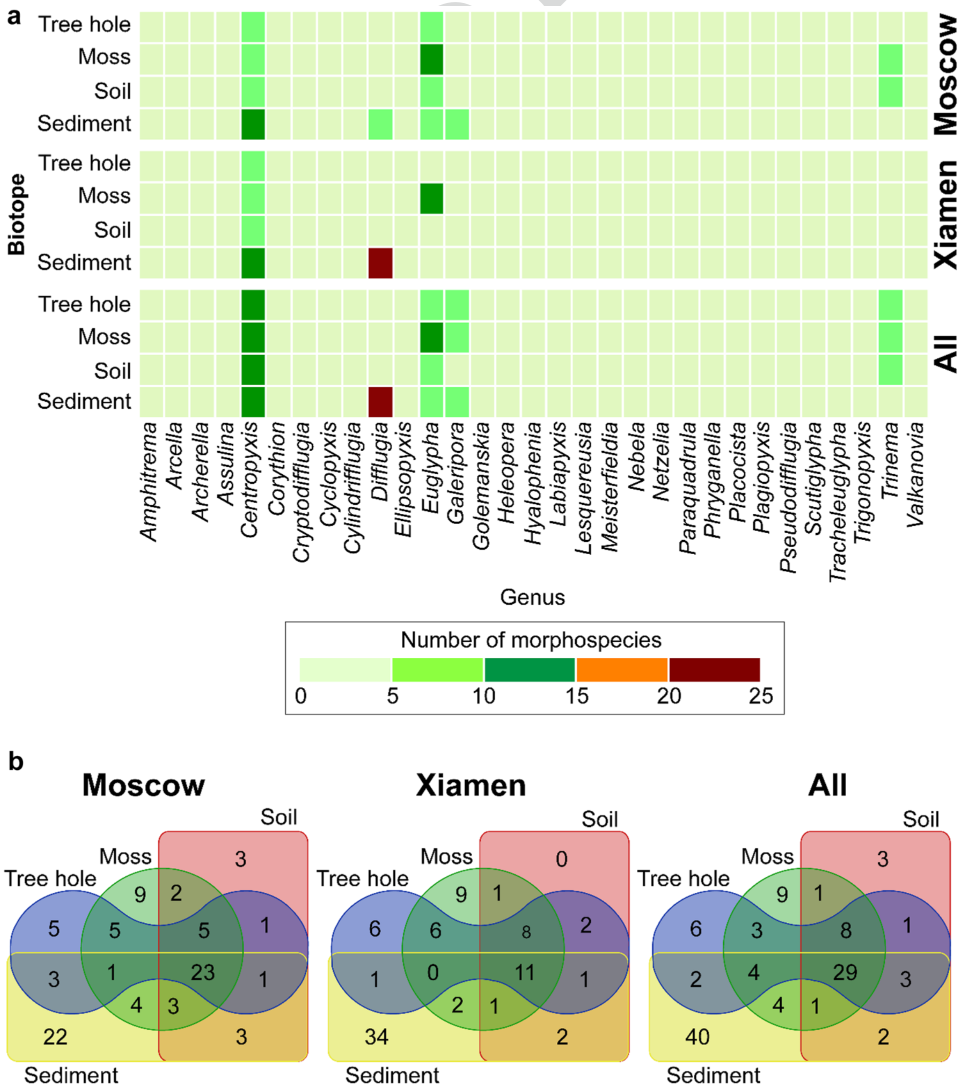

Fig. 3 Testate amoebae in four studied biotopes of urban parks. **a** Heatmap of morphospecies number of testate amoebae by biotope in each of 31 genera at the genus level. **b** Venn diagram of biotope-specific and ubiquitous morphospecies. The total number of morphospecies in Moscow parks, Xiamen parks, and in the combined data set is 90, 84, and 116, respectively

(23 morphospecies or 26% of the total species richness) as compared to Xiamen (11 morphospecies or 13% of the total species richness) (Fig. 3b). The most widely distributed testate amoebae were members of the genera *Centropyxis*, *Euglypha*, *Plagiopyxis*, and *Trinema* (Supplementary Table S2).

The alpha-diversity of testate amoeba community changed substantially across the four biotope types (Fig. 4). Mean species richness per sample was higher in Moscow parks (14 ± 1 species, $n = 63$), especially in the pond sediments (16 ± 2 species, $n = 15$). The mean species richness per biotope was highest in the moss (13 ± 1 species, $n = 33$). The dominance index was low and varied from 0.20 ± 0.01 ($n = 63$) in Moscow to 0.27 ± 0.03 ($n = 69$) in Xiamen, that indicates relatively equal distribution of morphospecies in the biotopes. The Shannon–Wiener index significantly differed between all studied biotopes in Moscow, except for soil and sediment (Mann–Whitney U tests, $P < 0.05$). However, in Xiamen, significant differences in Shannon–Wiener diversity were detected only between moss and soil samples (Mann–Whitney U tests, $P < 0.05$). Morphospecies differently contributed to the community compositions among biotopes (Supplementary Table S2). The top ten contributors to dissimilarity in community composition in Moscow parks were *Centropyxis aerophila*, *Centropyxis sphagnicola*, *Cryptodifflugia oviformis*, *Euglypha denticulata*, *Euglypha rotunda*, *Phryganella acropodia*, *Plagiopyxis callida*, *Plagiopyxis declivis*, *Trinema enchelys*, and *Trinema lineare*. The top 10 contributors in Xiamen parks were as follows: *Centropyxis aerophila*, *Centropyxis cassis*, *Difflugia sphincta*, *Euglypha rotunda*, *Phryganella nidulus*, *Plagiopyxis callida*, *Plagiopyxis declivis*, *Pseudodifflugia compressa*, *Trigonopyxis arcula*, and *Trinema lineare*.

Community variation and the role of stochastic and deterministic processes

The difference in testate amoeba community composition between the four studied biotopes was significant (Supplementary Fig. S3; Global R = 0.229–0.266, $P = 0.001$), although they shared some common morphospecies (Table 1). The cohesion metrics further revealed that the testate amoeba community varied among four biotopes (Fig. 5). Interactions among morphospecies in urban parks were much stronger in soils (-0.23 ± 0.01) than in tree holes (-0.15 ± 0.00), moss biotopes (-0.09 ± 0.01), and pond sediments (-0.11 ± 0.02). In comparison to moss biotopes (0.16 ± 0.01) and tree hole (0.15 ± 0.01), the degree of complexity, which informs community dynamics, was much stronger in soil (0.24 ± 0.01) and pond sediment (0.57 ± 0.08) (Fig. 5). Both stochastic and deterministic processes shaped the community composition of testate amoeba (Fig. 6). 42–82% of variation in the community composition can be attributed to stochastic processes within the frameworks of the neutral model (Fig. 6a). The C-score results revealed that SES changed from 1.48 to 6.92, indicating the enhanced importance of deterministic processes for the testate amoeba community, with highest values in moss and soil habitats (Fig. 6b).

Discussion

Testate amoeba diversity across four biotopes

A lack of information on microbial diversity in parks has always been a very important issue in the fundamental

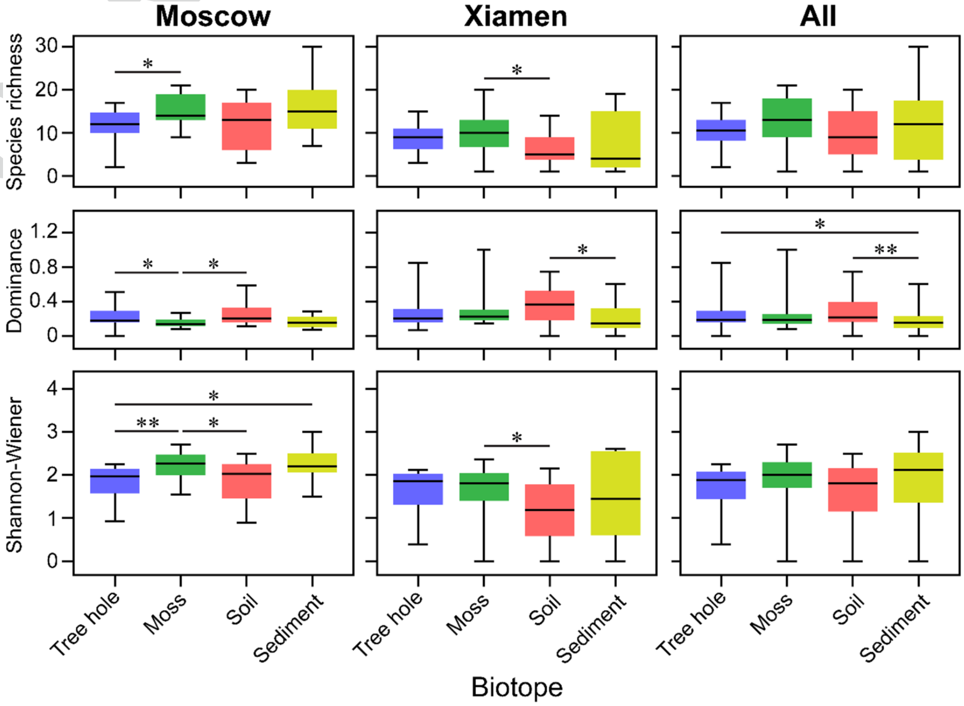

Fig. 4 Species richness (mean species number per sample), dominance (the values vary from 0 to 1, where 0 indicates equal distribution of taxon abundances and 1 indicates a complete dominance of a single taxon), and Shannon–Wiener index of testate amoeba communities in four biotopes of urban parks of Moscow and Xiamen. Significant levels: *$P < 0.05$; **$P < 0.01$. The number of total samples in Moscow and Xiamen parks is 63 and 69, respectively

Table 1 Analysis of similarities for testate amoeba communities across four biotopes of Moscow and Xiamen parks

Biotope	Moscow			Xiamen			All (Moscow and Xiamen)		
	n	R	*P*-value	*n*	R	*P*-value	*n*	R	*P*-value
Tree hole vs. moss	31	0.332	0.001	36	0.152	0.001	67	0.144	0.001
Tree hole vs. soil	33	0.087	0.010	33	0.171	0.006	66	0.076	0.001
Tree hole vs. sediment	31	0.295	0.001	36	0.290	0.001	67	0.184	0.001
Moss vs. soil	32	0.232	0.001	33	0.275	0.001	65	0.149	0.001
Moss vs. sediment	30	0.559	0.001	36	0.410	0.001	66	0.288	0.001
Soil vs. sediment	32	0.249	0.001	33	0.121	0.019	65	0.103	0.001

The total samples of Moscow parks and Xiamen parks are 63 and 69, respectively

n, number of samples; R, the degree of separation between groups in analysis of similarities (ANOSIM test)

knowledge and environmental tools, used for decision-making in urbanization management (Elmqvist et al. 2013; Nielsen et al. 2014). A total of 116 morphospecies were identified in Moscow and Xiamen parks (Supplementary Table S2), demonstrating the significance of testate amoeba in the microbial communities of the four studied urban park biotopes (tree hole, moss, soil, and sediment). The recovered diversity is comparable to that in undisturbed environments of various climatic areas (i.e., Ju et al. 2014). There are several potential reasons, causing widespread presence of testate amoebae in urban parks. First, most testate amoebae are ubiquitous in environments such as moss, soil, and pond sediment (Ogden and Hedley 1980; Mazei and Tsyganov 2006; Ju et al. 2014; Todorov and Bankov 2019); as a result, the communities revealed in both Moscow and Xiamen parks were dominated by ubiquitous testate amoebae, despite the fact that each individual morphospecies has preferred niches (Yang et al. 2010). Second, urban soils basically show the inherent and dynamic properties of natural soils (Dovletyarova et al. 2017; Huang et al. 2018). As an example, increased vegetation in urban parks provides a rich input of organic matter in soils (Setälä et al. 2016). This can favour the development of bacteria, which are the basic prey for many testate amoebae; hence, the latter will also grow and reproduce (Shi et al. 2021). Changes in the cityscape through the creation of ponds can contribute to the development of appropriate substrate for many aquatic testate amoeba species. Garden ponds, for example, allow for the formation of rocky outcrops, wetland flora, and the accumulation of various materials such as tree branches and leaves. This makes them ecologically close to the waterbodies, formed under the natural conditions (Hassall 2014). The presence of ponds in parks facilitates the growth of aquatic bacteria, algae, fungi, and rotifers, which may further serve as food for aquatic testate amoebae, e.g., those of the genera

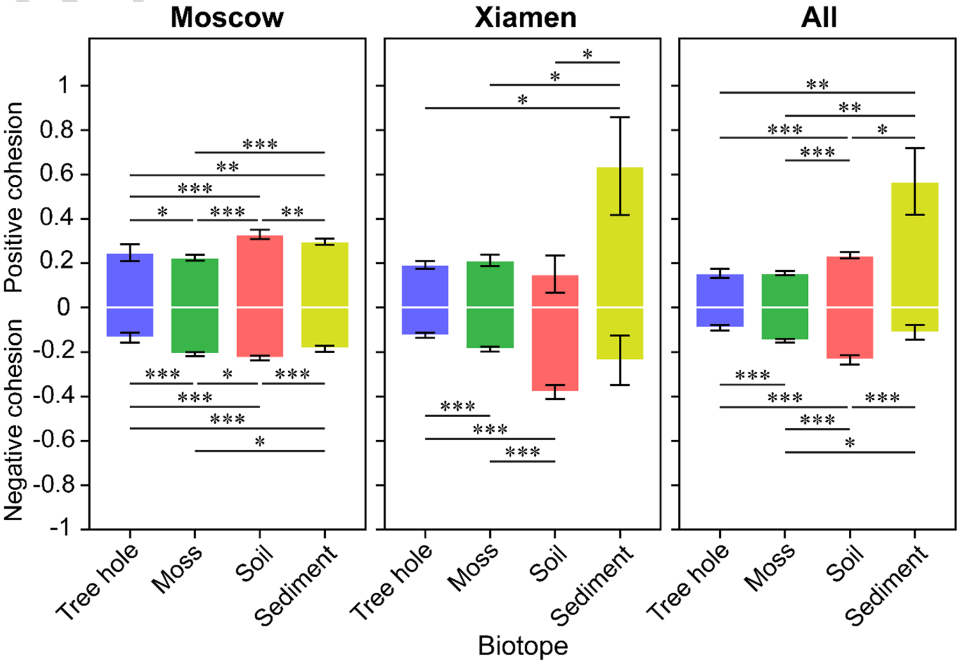

Fig. 5 Community dynamics (top panel) and community complexity (bottom panel) of testate amoebae in four studied biotopes. Significant levels: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. The number of total samples in Moscow and Xiamen parks is 63 and 69, respectively

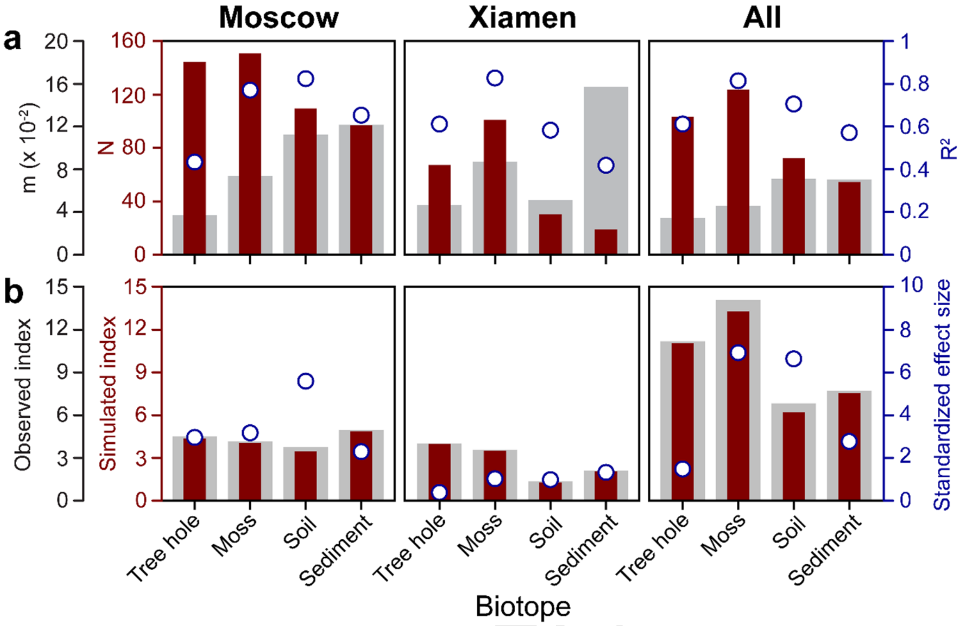

Fig. 6 Ecological processes shaping the testate amoeba assemblages. **a** Variation of stochastic processes across biotopes is shown by fit to Sloan's neutral community model. *m* indicates the immigration rate (grey columns), and *N* shows the metacommunity size (dark red columns). R^2 indicate the fit to the neutral model (blue circles). **b** Variation of deterministic processes across biotopes is shown by null model

Arcella, *Centropyxis*, *Cyphoderia*, *Difflugia*, *Euglypha*, and *Galeripora* (Ogden and Hedley 1980; Gilbert et al. 2000). At the same time, the wide diversity of microhabitats in urban ponds for different ecosystem services such as hydrological, geochemical, ecological, aesthetic, and cultural functions can provide numerous niches for testate amoebae (Ogden and Hedley 1980), hence the percent of biotope-specific morphospecies is highest in pond sediments (Fig. 3b). The species richness per biotope, which is quite high in moss (Fig. 4), clearly indicates that epiphytic mosses in urban parks form an important biotope for testate amoebae with cosmopolitan distribution, just like in natural forests (Davidova 2008).

Soil harboured fewer biotope-specific and more ubiquitous testate amoebae (Fig. 3b). This is because soil environment is multiple association biotope; thus, movements of some species through soil and from the soil in other ecotopes are an integral part of the ecology of testate amoebae in their search for better environment and more abundant food (Yang et al. 2010; Nielsen et al. 2014). As an example, *Euglypha laevis* (Supplementary Tables S2) has been largely reported from soil, but it can also be found in other biotopes, including moss and aquatic sediment (Vincke et al. 2004; Siemensma 2021).

Stochastic and deterministic processes shaping the testate amoeba community

The cohesion metrics allow an insight into how the testate amoeba communities are influenced by their environment (Ndayishimiye et al. 2021). The testate amoeba communities, which are complex across four studied biotopes in Moscow and Xiamen parks (Fig. 5), obviously indicate a variation in testate amoeba diversity, which is congruent with literary data (Hamm and Drossel 2017). Different ecological processes have been suggested to explain changes in testate amoeba diversity along environmental gradients, one of convincing explanations is that species diversity is generally regulated by inter-species relationships (e.g., competition, predation, mutualism, and parasitism) (Wang et al. 2020). Both deterministic and stochastic processes simultaneously shaped the community composition of testate amoebae, but their relative importance was neither equal nor constant (Fig. 6). It suggests that the ecological selection caused by both abiotic and biotic factors may control organismal fitness through changes in the community composition and the relative abundance of species (Zhou and Ning 2017). The stochastic processes, species interactions or priority effects may affect the testate amoeba diversity by changing the number of individuals or species in the community (Ren et al. 2018; Wang et al. 2020). In this work, the neutral community model successfully estimated the relationship between the occurrence frequency of morphospecies and their relative abundance variations, but with a substantial difference in explained community variance among four studied biotopes in both Moscow and Xiamen parks (42–83%, Fig. 6a). There are several possible explanations for this difference based on the number of biotope-specific and ubiquitous morphospecies found in each biotope and in comparison with the findings from other researches. For example, morphospecies belonging to the same group generally respond to stochastic processes similarly, which is contrary to what can be seen in an environment with more biotope-specific morphospecies. These conclusions are in line with the findings of Ren et al. (2018), who demonstrated that the importance of stochastic processes on the amoeba communities varies depending on the habitat type, and Wang et al. (2020), who showed that the stochastic processes are particularly marked for the rare testate amoebae. The high SES for C-score shown by four studied biotopes in Moscow parks (Fig. 6b) might be an indication of enhanced effect of deterministic processes on the testate amoeba communities. The low SES for C-score (Fig. 6b) and a low frequency of occurrences of proliferous *Difflugia* (e.g., *D. elegans*, *D. globulosa*, and *D. limnetica*) and *Netzelia* (e.g., *N. gramen*) in Xiamen parks (Supplementary Table S2) might be an indication of the minor impact of deterministic processes on the testate amoeba communities. The relative importance of stochastic or deterministic processes in four studied biotopes may further suggest that variables influencing the community composition of testate amoebae in heterogeneous environments are different (Ren et al. 2018; Wang et al. 2020; Ndayishimiye et al. 2021).

Ecological impact and implications for future research

To keep track of potential environmental issues, it is essential to consider the microorganisms and factors influencing their communities in an urban environment (Elmqvist et al. 2013). Parks normally are characterized by diverse microhabitats and represent important biodiversity hotspots in the cityscape (Nielsen et al. 2014; Hill et al. 2016). For all examined testate amoeba communities in Moscow and Xiamen parks, our findings consistently show that urban parks are rich in testate amoeba. This shows that urban parks should be the main concern in ecology and protection programs (Sushinsky et al. 2013; McDonald et al. 2020). An integrated assessment of the habitat suitability, microbial community stability, and socio-economic benefits of parks might possibly offer more opportunities for holistic solutions in urban environmental management and protection that can bring multiple benefits for public health (Malone et al. 2018).

The samples were only taken once in each park in Moscow and Xiamen, emphasizing that this investigation only considers spatial variation in testate amoeba communities and that a further assessment of the spatio-temporal dynamics of testate amoebae in the studied biotopes would be necessary for a better understanding of diversity and biogeography. Additionally, it is not determined whether changes in the local environment progressively increase the importance of deterministic processes or whether disturbance promotes a time-dependent shift in the relative influence of stochastic and deterministic processes (Mo et al. 2021). The priority scopes for any further research must take into account the above-shown limitations, more locations, and anthropogenic impacts to get a broader view of our conclusions across a wide range of climate zones.

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Authors' contributions Yu.M., J.Y., and A.S. conceived the study and secured funding. Yu.M., N.M., A.N.T., A.B., A.I., and D.S. conducted fieldwork in Moscow; J.C.N., P.N., K.X.R., M.A.A., H.H.C., W.P.W., and J.Y. conducted fieldwork in Xiamen; K.B. conducted microscopic analysis. J.C.N. performed statistical analysis and wrote the first draft of the manuscript. All authors made substantial contributions to the intellectual content, analysis and interpretation of the literature review, and editing of the manuscript.

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Declarations

Consent for publication All authors have approved the manuscript and agree with its publication in Urban Ecosystems.

Conflict of interest The authors declare that they have no conflict of interest.

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