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Effect of ecosystem type on soil heterotrophic flagellate communities under forest-steppe conditions

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Summary

The species diversity and patterns of soil heterotrophic flagellate community structure in the forest-steppe of the Middle Volga Region were investigated in accordance with the spatial heterogeneity of forest-steppe ecosystems. Fifty-three species and forms were identified. Eurybiontic *Bodo designis* (revealed in 100% of biotopes), *Paraphysomonas* sp. (100%), *Spumella* sp. (100%), *Heteromita minima* (94%), *Phyllomitus apiculatus* (88%), *Ploeotia obliqua* (88%), *Heteromita globosa* (82%), *Goniomonas truncata* (82%) were the most common species. Species richness of the genus *Cercomonas* was the highest. Communities could be divided into three groups by the species composition: i) from xerophytic grass and shrubby phytocenosis; ii) from meso-xerophytic pine forests and parvifoliate forests; iii) from mesophytic broad-leaved forests. The specificity of composition of heterotrophic flagellates increased with the decrease in the soil moisture and was reflected in the high diversity of amoeboid flagellates, which are well adapted to habitation on the surface of soil particles and in thin capillary spaces of dry soils. The study of general patterns of changes in community structure of soil protists in the "steppe-forest" gradient as well as in accordance with the secondary succession of forests after felling revealed rather homogeneous communities of heterotrophic flagellates in comparison with a very structured community of testate amoebae.

Key words: heterotrophic flagellates, soil protozoa, protozoan community, grassland-woodland transect, forest-steppe, Middle Volga Region

Introduction

Protists are the most numerous and cosmopolite group of terricolous organisms (Geltser, 1993). Many investigations are dedicated to the analysis of their role in terrestrial ecosystems (Darbyshire, 1994). It is experimentally shown that protists are active in normally wet soil: they feed, move, multiply and excrete waste products into the environment (Lozina-Lozinsky, 1930; Nikoluk,

1956, Geltser, 1960). However, a lot of issues concerning proto-zoan community organization in soils are still poorly known, one of the reasons being that such investigations are very laborious. Initially, the importance of protists in soil biodynamics was underestimated (Alexeev, 1925). At present, however, soil protistofauna is known to be an obligatory, plentiful and trophically active component of soil biota (Sandon, 1927; Brodsky, 1935a, 1935b; Singh, 1955; Nikoluk, 1956; Bonnet, 1964; Bamforth, 1971; Lepinis et al., 1973; Alabouvette et al., 1981; Page, 1988; Foissner, 1991; Geltser, 1993; Korganova, 1997; Korganova and Rakhleeva, 1997; Ekelund et al., 2001; Acosta-Mercado and Lynn, 2004; Smirnov and Brown, 2004; Chao et al., 2006; Esteban et al., 2006).

The overwhelming majority of papers dealing with soil protists are dedicated to investigations of biodiversity and community structure of testate amoebae (Gilbert and Mitchell, 2006; Mazei and Tsyganov, 2007). At the same time, heterotrophic flagellates are another important component of soil protozoan communities (Sandon, 1927; Lepinis et al., 1973; Coleman, 1994; Adl and Gupta, 2006). Recently, studies of terricolous heterotrophic flagellate communities have been confined to agricultural soils (Ekelund and Rшnn, 1994; Ekelund and Patterson, 1997; Ekelund, 1998; Ekelund et al., 2001). The data on heterotrophic flagellates from intact soils are extremely rare (Geltser, 1984).

Thus, the aim of our study was to investigate species diversity and patterns of community structure of soil heterotrophic flagellates in the forest-steppe of the Middle Volga Region in accordance with spatial heterogeneity of forest-steppe ecosystems. Two variants of ecosystem heterogeneity were analyzed in order to clarify, if soil protozoan community structure corresponded to changes of the terrestrial ecosystems. The first case represented investigation of various transitions from grasslands to woodlands. The second case interpreted research of changes in heterotrophic flagellate communities in accordance with stages of progressive forest succession after felling. Transition between grasslands to woodlands was studied in two variants: through shrubby edges (Chistyakova, 1993) and steppe forests (Novikova and Sokolova, 2008).

Material and methods

This investigation of soil heterotrophic flagellate communities was carried out in July 2006 in the forest-steppe of the Middle Volga Region (Fig. 1).

The first study area was "Ostrovtsovskaya" forest-steppe (Fig. 1). It contained all the stages of transition from xerophytic steppes (through xero-mesophytic meadows and edge shrubby complexes) to mesophytic bushy and tall forests. Leached and typical chernozems dominated. Forest-steppe gradient started from meadow steppe in feather grass (O1) and clover (O2) associations through steppe meadow in bush grass forb meadow (O3), lime grass forb wet meadow (O4), dense broom grove (O5), cherry bush (O6), blackthorn bush (O7), bird-cherry-tree wood (O8) to aspen forest (O9).

The second study area was "Kuncherovskaya" forest-steppe (Fig. 1). It contained all the stages of transition from xerophytic steppes through xero-mesophytic meadow steppes and steppe oak forests, mesophytic oak forests and aspen forests to hygrophytic osier-beds. Leached chernozems and taupe forest soils dominated. Forest-steppe gradient started from psammophilous steppe in forb feather grass fescue association with developed mossy (K1) and lichen (K2) cover through steppe meadow in feather grass forb association (K3), bush grass forb birch oak wood in the center of meadow steppe (K4), meadow brome forb oak forest edge (K5), forb maple oak forest (K6), euonymus oak lime-tree forest (K7), ash weed aspen forest (K8) to great nettle osier-bed (K9).

The third study area was "Zasurskyi forest" (Fig. 1). It was dominated by light-gray forest sandy and podzolized soils under the deciduous, mixed and pine forests. Much of the forested area had been cut down at different times in the 20th century. Therefore, the present-day pattern of forest heterogeneity is represented by wooded areas of different age (Antonov and Savoley, 1990). The biotopes for sampling were selected in accordance with the main directions of the secondary forest succession. The initial stages of succession are represented by young birch groves (Z-1a and Z-1b – two different groves). Further succession developed in several directions, depending on soil type and moisture level of the biotope. **Hydroseries** is formed in the most humid hollows on swamp peat-gley soils. The second stage (after the birch grove) is represented by birch aspen forests (Z-2-hydro). The third stage is represented by birch aspen pine boggy forests (Z-3-hydro). **Mesoseries** is developed in the medium moist conditions on the typical gray forest soils. The second stage (after the birch grove) is represented by a birch maple oak tillet wood (Z-2-meso), and the third stage, by the oak forests (Z-3-mezo). **Xeroseries** is formed at the most arid conditions on sandy shallow light-gray forest soils. The second stage (after the birch grove) is represented by birch forests with the underwood of pine (Z-2-xero) and the third stage, by the pine forests (Z-3-xero).

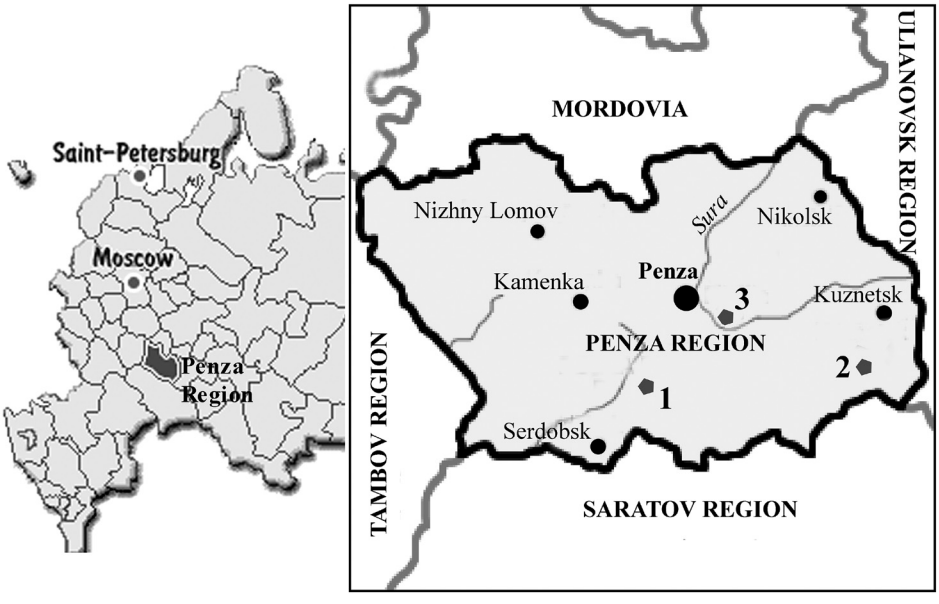

Fig. 1. Map of the study area. 1 – Ostrovtsovskaya forest-steppe (between the Alfer'evka and Berezovka villages, Kolyshleiskyi district); 2 – Kuncherovskaya forest-steppe (between the Staryi Chirchim and Krasnoe Pole villages, border of Kuznetskyi, Kameshkirskyi and Neverkinskyi districts); 3 – Zasurskyi forest (right shore of Penza Reservoir).

Three soil samples (replicates) were collected at a distance of 2–3 m from each other in each of the selected biotopes. Each sample was divided into two subsamples investigated separately: soil horizon A0 and the top 5 cm of horizon A1. The soil from each layer was placed into plastic sachets and transported to the laboratory. Simultaneously, a neighbouring piece of soil was placed into a separate package to determine the field water capacity.

To investigate the species richness of heterotrophic flagellates, artificial initiation of heterotrophic succession in microcosms (Tikhonenkov, 2007/8; Tikhonenkov et al., 2008) was carried out. One gram of dry soil was transferred into a Petri dish with 5 ml of Pratt medium. After that, the samples were enriched with suspension of *Pseudomonas fluorescens* bacteria (0.15 ml) and maintained in darkness at 25°C in the thermostat. Analysis of these samples (each a microcosm per se) at various time intervals allowed us to estimate the cryptic (potential) species diversity, which, being considerably higher than the active one (at the moment of sampling), reflects the latter in many respects (Tikhonenkov, 2007/8). As a result, investigating all successional stages in the microcosm, which are different in the sets of trophic, taxonomic and morphophysiological groups of flagellates, we can identify more comprehensively the species composition of the heterotrophic flagellate communities. Species diversity was studied on the 3rd day of exposition, the 6th day (which corresponded to the first, the most complete and heterogeneous stage of succession in the microcosms) and the 9th day (which corresponded to the second and the third stages with a regular species structure), in accordance with the results of our previous investigation (Tikhonenkov et al., 2008). Four microcosms (replicates) were studied from each biotope and each soil horizon.

Light microscopic observations were made with the aid of Biolam-I microscope (Russia) equipped with phase contrast and water immersion objectives giving a total magnification of 770×, as well as a Reichert microscope (Austria) with Nomarski interference contrast and glycerin immersion objectives (1000×). The microscopes were equipped with an analog video camera AVT HORN MC-1009/S, which was connected to a Panasonic NV-HS 850 video recorder. Image acquisition in VHS and S-VHS modes, followed by digitization of images and preservation of fragments of video film as AVI files, was carried out in order to facilitate more precise identification of heterotrophic flagellates.

The system of eukaryotes and protists by Adl et al. (2005) was used.

The classification of communities by their species structure (qualitative data) was conducted with the aid of hierarchical cluster analysis (complete linkage) based on the Jaccard indices similarity matrix. Principal component analysis (PCA) was carried out in order to reveal general patterns of differences between local communities by species distribution. All computations were performed with the aid of the program package PAST 1.89.

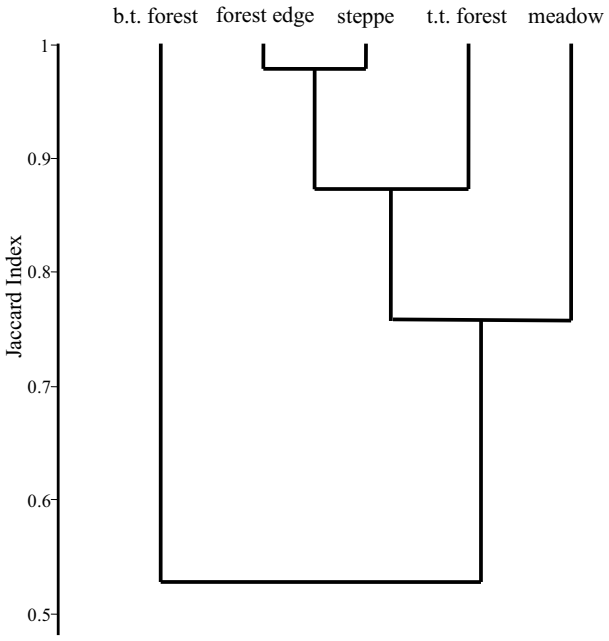

Fig. 2. Classification of communities of heterotrophic flagellates from the different-type biotopes of Ostrovtsovskaya forest-steppe by species composition: "steppe" – meadow steppes; "meadow" – steppe and boggy meadows; "forest edge" – bushy phytocenoses: broom grove and cherry bush; «b.t. forest» – bushy tree forests: blackthorn bush and bird-cherry-tree wood; "t.t. forest" – tall tree forest: aspen forest.

Results

Changes of protozoan communities along the grassland-woodland transect.

Forty species and forms of heterotrophic flagellates were identified in the soils of Ostrovtsovskaya forest-steppe (Table 1). Six species (*Bodo designis, Goniomonas truncata, Paraphysomonas* sp., *Phyllomitus apiculatus, Ploeotia obliqua, Spumella* sp.) were found in all biotopes. All of them are very widespread in the plankton and benthos of fresh-water bodies, as well as in sphagnum bogs (Zhukov, 1993; Tikhonenkov and Mazei, 2009). *Paraphysomonas* sp. and *Spumella* sp. belong to the Chrysophyceae Pascher, 1914 and are sedimentators (creating with their flagella a current of fluid and food particles absorbed directly on the cell surface). Other species actively search and capture food.

Communities of heterotrophic flagellates from bushy tree forests (blackthorn and bird cherry tree) were characterized by the maximum species richness. Thirty species of flagellates (22-23 species in certain biotopes) were found there. Up to 13-17 species inhabit other ecosystems.

Communities from bushy tree forests were distinguished by species composition (Fig. 2). Five characteristic species for these biotopes (*Apusomonas proboscidea, Bodo caudatus, Bodo* sp., *Monosiga ovata, Goniomonas* sp.) were identified only there. No specific flagellates were found in the aspen forest, while in the remaining habitats up to 1-3 species were unique for the biotope. The pattern of community heterogeneity at the level of both biotopes and soil horizons were not so distinct (Fig. 3). Protozoan communities from different biotopes and horizons (point level) were often very similar. All this suggests a random nature of the differences in flagellate communities on the point level.

Local communities are divided into 3 variants by species composition (Fig. 4). More than 50% of the differences in the set of dominating species were caused by distinctions between communities of steppe habitats (with predominance of *Ploeotia obliqua* and *Spumella* sp.) and other local communities, dominated by *Paraphysomonas* sp., *Phyllomitus apiculatus, Bodo designis.* About 30% of total variance was concerned with specificity of protozoan communities from bushy tree forests, with predominance of *Goniomonas truncata* and *Dimastigella trypaniformis.* It is important to underline that within the whole set of the dominant species, only the last one is not known to have a wide distribution but has preference to bogs and soils (Zhukov, 1993; Tikhonenkov and Mazei, 2009). All other species are eurybionts and have a wide geographical distribution.

Table 1. Species list of soil heterotrophic flagellates observed in the different habitats.

Species and forms	Concrete biotopes (see Material and Methods for details)																									Biotope types						
	O1	O2	O3	O4	O5	O6	O7	O8	O9	K1	K2	K3	K4	K5	K6	K7	K8	K9	Z–1	Z–2–hydro	R Z –2–meso	Z–2–xero	Z–3–hydro	Z–3–meso	Z–3–xero	1	2	3	4	5	6	%
Choanomonada Kent, 1880																																
Codonosiga botrytis Kent, 1880	+	+	+	+	+	+	+	+	–	–	–	–	–	+	+	–	+	–	–	–	–	–	+	–	–	+	+	+	+	+	+	47
Monosiga ovata Kent, 1880	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	6
Incertae sedis AMOEBOZOA: Spongomonadida (Hibberd, 1983) emend. Karpov, 1990																																
Phalansterium solitarium Sandon, 1924	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	6
Cercozoa Cavalier–Smith, 1998, emend. Adl et al., 2005																																
Allantion tachyploon Sandon, 1924	–	+	+	–	–	+	+	–	–	+	–	–	+	–	–	+	–	+	–	–	+	+	–	+	+	+	+	+	+	+	+	71
Allantion sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	6
Cercomonas aff. *agilis* (Moroff, 1904) Mylnikov and Karpov, 2004	–	+	–	–	+	–	–	+	–	–	–	–	–	–	–	+	+	–	+	–	–	+	–	+	–	+	+	+	+	+	+	47
C. breviantennatus Skuja, 1956	+	–	–	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	24
C. cfr. *cometa* Hollande, 1942	–	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	–	+	–	+	+	+	+	+	+	+	–	+	+	+	53
C. crassicauda (Dujardin, 1841) Mylnikov and Karpov, 2004	+	+	+	–	–	+	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	+	+	–	+	–	29
C. granulifera (Hollande, 1942) Mylnikov and Karpov, 2004	+	–	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	+	+	–	–	24
C. longicauda Dujardin, 1841	–	–	–	–	+	–	+	+	–	–	–	–	+	–	–	–	–	–	–	+	+	+	–	–	+	–	+	+	+	+	+	41
C. norviscensis Skuja, 1956	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	+	–	–	–	+	–	–	–	–	–	+	+	–	–	+	–	24
C. ovatus (Klebs, 1892) Tikhonenkov, 2007	–	–	–	–	–	–	–	+	+	+	+	+	–	+	+	+	+	+	–	–	+	–	+	+	–	+	+	+	+	+	+	59
C. radiatus (Klebs, 1892) Mylnikov and Karpov, 2004	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	6
Cercomonas sp.1	+	+	+	+	+	+	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	–	+	+	+	+	+	+	+	+	76
Cercomonas sp.2	–	–	–	–	–	–	–	–	–	+	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	+	+	–	+	+	–	29
Heteromita globosa Stein, 1878	+	+	–	–	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	+	+	+	+	+	+	+	+	82
H. minima (Hollande, 1942) Mylnikov and Karpov, 2004	–	+	+	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	94
H. reniformis (Zhukov, 1978) Mylnikov and Karpov, 2004	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	–	+	–	+	–	+	+	+	+	+	+	71
Heteromita sp.	+	+	–	+	–	–	–	+	–	+	+	–	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	71
Protaspis simplex Vørs, 1992	–	+	+	–	–	+	+	+	+	–	–	–	–	+	–	+	–	–	–	+	–	+	–	+	+	+	+	+	+	+	+	65
Protaspis sp.	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	–	+	–	18
Thaumathomonas lauterborni De Saedeleer, 1931	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	12
Thaumathomonas sp.	–	–	–	–	–	–	+	–	+	–	–	–	–	+	–	–	+	–	–	+	–	–	–	–	–	–	+	+	+	+	–	29

Table 1. (Continuation 1).

Species and forms	Concrete biotopes (see Material and Methods for details)																									Biotope types						
	O1	O2	O3	O4	O5	O6	O7	O8	O9	K1	K2	K3	K4	K5	K6	K7	K8	K9	Z–1	Z–2–hydro	R Z –2–meso	Z–2–xero	Z–3–hydro	Z–3–meso	Z–3–xero	1	2	3	4	5	6	%
Cryptophyceae Pascher, 1913, emend. Schoenichen, 1925																																
Goniomonas truncata (Fresenius, 1858) Stein, 1887	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	–	–	+	+	+	+	+	+	+	+	82
Goniomonas sp.	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	6
Stramenopiles Patterson, 1989, emend. Adl et. al., 2005																																
Cyathobodo sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	+	–	–	–	–	+	+	–	24
Paraphysomonas sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Spumella elongata (Stokes, 1886) Belcher and Swale, 1976	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	6
Spumella sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Incertae sedis Alveolata																																
Colponema loxodes Stein, 1878	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	6
Euglenozoa Cavalier–Smith 1981 emend. Simpson 1997																																
Bodo angustatus (Dujardin, 1841) Bütschli, 1883	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	6
B. caudatus (Dujardin, 1841) Stein, 1878	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	+	–	+	–	–	+	–	+	–	+	+	+	–	29
B. designis Skuja, 1948	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Bodo cfr. *globosus* Stein, 1878	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	6
B. minimus Klebs, 1893	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	+	+	+	–	–	–	–	+	–	–	+	+	–	29
B. rostratus (Kent, 1880) Klebs, 1893	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	6
B. saltans Ehrenberg, 1832	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	+	–	–	–	+	–	+	24
Bodo sp.	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	6
Dimastigella mimosa Frolov, Mylnikov and Malysheva, 1997	–	–	–	–	–	+	+	–	–	–	–	–	+	–	+	+	–	–	+	–	–	+	–	–	–	–	+	+	+	+	+	35
D. trypaniformis Sandon, 1928	–	–	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	+	–	–	+	18
Dimastigella sp.	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	6
Parabodo nitrophilus Skuja, 1948	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	–	12
Petalomonas minor Larsen and Patterson, 1990	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	12
P. minuta Hollande, 1942	+	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	+	–	–	–	–	–	+	+	–	–	+	–	24
P. pusilla Skuja, 1948	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–	12
Petalomonas sp.	+	–	–	+	+	+	+	+	–	–	+	+	–	–	–	+	+	–	–	–	+	+	–	+	–	+	+	+	+	+	+	65

Table 1. (Continuation 2).

Species and forms	Concrete biotopes (see Material and Methods for details)																																				Biotope types						
	01	02	03	04	05	06	07	08	09	K1	K2	K3	K4	K5	K6	K7	K8	K9	Z-1	Z-2-hydro	R Z -2-meso	Z-2-xero	Z-3-hydro	Z-3-meso	Z-3-xero	1	2	3	4	5	6	%
Ploeotia obliqua Schroeckh, Lee and Patterson, 2003	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	88
Ploeotia sp.	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	18
Rhynchobodo sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	12
Rhynchomonas nasuta (Stokes, 1888) Klebs, 1892	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	12
Incertae sedis Alveolata																																
Apusomonas proboscidea Alexeieff, 1924	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	6
Phyllomitus apiculatus Skuja, 1948	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	88
Number of species																																53

«+» – presence of the species in the samples, «–» – absence of the species in the samples.
Biotope types: 1 – grasslands (steppes, meadows); 2 – forest edges (broom groves, cherry bushes and steppe oak forests); 3 – bushy tree woods (blackthorn bushes and bird-cherry-tree woods);

Thirty five species and forms of heterotrophic flagellates were identified in the soils of Kuncherovskaya forest-steppe (Table 1). Four species *Bodo designis, Paraphysomonas sp., Phyllomitus apiculatus, Spumella* sp. (the same organisms as in Ostrovtsovskaya forest-steppe) as well as *Heteromita minima* were found in all the biotopes.

Communities from the driest (psammophilous steppe) and the moistest (osier-bed) biotopes were characterized by the minimal species richness (11 species). Up to 16–19 species inhabit other ecosystems. Amoeboid flagellates from the genus *Cercomonas* were characterized by the highest diversity (10 species out of 35). These flagellates glide on the surface of the substrate and are well adapted to habitation in thin capillary spaces of soil.

Local communities were divided into 2 variants by species composition (Fig. 5). The first one is from grasslands and is characterized by 4 species (*Bodo caudatus, Cercomonas breviantennatus, Colponema* sp., *Protaspis* sp.). The second one is from woodlands and has 6 characteristic species (*Cercomonas crassicauda, C. granulifera, C. longicauda, Petalomonas minor, P. pusilla, Rhynchobodo* sp.).

Point communities were rather clearly divided into 2 groups at the level of biotopes and soil horizons (Fig. 6): from the humus layer A1 and grass or forest litter (A0).

The community was very homogeneous by species composition (Fig. 7). More than 65% of the differences were caused by the predominance of *Paraphysomonas* sp. and *Bodo designis* in maple oak wood and oak tillet wood. These flagellates are a part of the dominating species complex in other communities of protists. Other 20% of the species composition variance is associated with the distinctions between communities of meadow steppe (with the predominance of *Ploeotia obliqua* and *Heteromita minima*) and aspen forest and osier-bed, dominated by *Spumella* sp., *Phyllomitus apiculatus, Goniomonas truncata.*

Changes of protozoan communities along the forest succession series.

Thirty seven species and forms of heterotrophic flagellates were identified in the soils of Zasurskyi forest (Table 1). Three species (*Bodo designis, Paraphysomonas* sp., *Spumella* sp.) were found in all biotopes (the same as in Ostrovtsovskaya and Kuncherovskaya forest-steppes). The maximum number of species was found in the birch maple oak tillet wood (21 species, Z-2-meso) and in oak forest

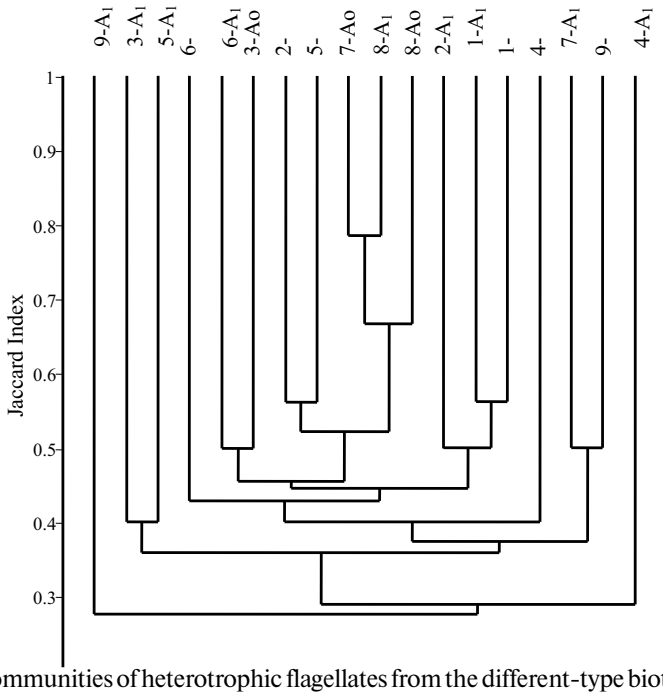

Fig. 3. Classification of communities of heterotrophic flagellates from the different-type biotopes and soil horizons of Ostrovtsovskaya forest-steppe by species composition: O1-O9 – different biotopes (see Material and methods for details).

(20 species, Z-2-meso). Boggy birch pine forest (Z-3-hydro) was characterized by the lowest species richness (10 species).

The communities within the hydroseries were the most heterogeneous in terms of species composition (Fig. 8). Point communities from boggy forests also differed from the others at the level of biotopes and soil horizons (Fig. 9). Local communities from boggy birch pine forest with the predominance of bodonids *Bodo saltans* and *B. designis* and those from pine forest dominated by *Goniomonas truncata* were distinguished by the species structure (Fig. 10). Communities from the other biotopes belong to another type with characteristic species *Phyllomitus apiculatus, Paraphysomonas* sp., *Heteromita minima, Ploeotia obliqua, Spumella* sp. Thus, the local communities of heterotrophic flagellates are the most heterogeneous within the climaxes of xero-and hydroseries, ending with the pine forests.

General patterns of heterotrophic flagellate communities in soils of forest-steppe ecosystems

The total of 53 species and forms of heterotrophic flagellates were identified in the soils of the Middle Volga region (Table 1). Eurybionts *Bodo designis* (revealed in 100% of biotopes), *Paraphysomonas* sp. (100%), *Spumella* sp. (100%), *Heteromita minima* (94%), *Phyllomitus apiculatus* (88%), *Ploeotia obliqua* (88%), *Heteromita globosa* (82%), *Goniomonas truncata* (82%) were the most common species. Species richness of amoeboid flagellates from the genus *Cercomonas* was the highest. At the same time, the greatest diversity of cercomonads was observed in dry habitats (steppes, meadows, shrubby edges, steppe forests). Communities could be divided into three groups by the species composition (Fig. 11): i) from xerophytic grasslands and bushes; ii) from meso-xerophytic pine forests and parvifoliate forests; iii) from mesophytic broad-leaved forests. The results of classification of point communities on the level of biotopes and the soil horizons show the specificity of species composition in the most humid boggy forests (Fig. 12). Most likely, local communities from other habitats represent a single variant of the community.

Discussion

Species composition and distribution of heterotrophic flagellates in virgin and cultivated soils of the Moscow Region (Russia) were investigated earlier (Geltser, 1984). Thirty-eight species of heterotrophic flagellates from 12 genera were identified. Genera *Bodo* and *Cercomonas* (*Cercobodo*) were represented by the greatest number of species, which corresponds fully with the results of our work. About a half

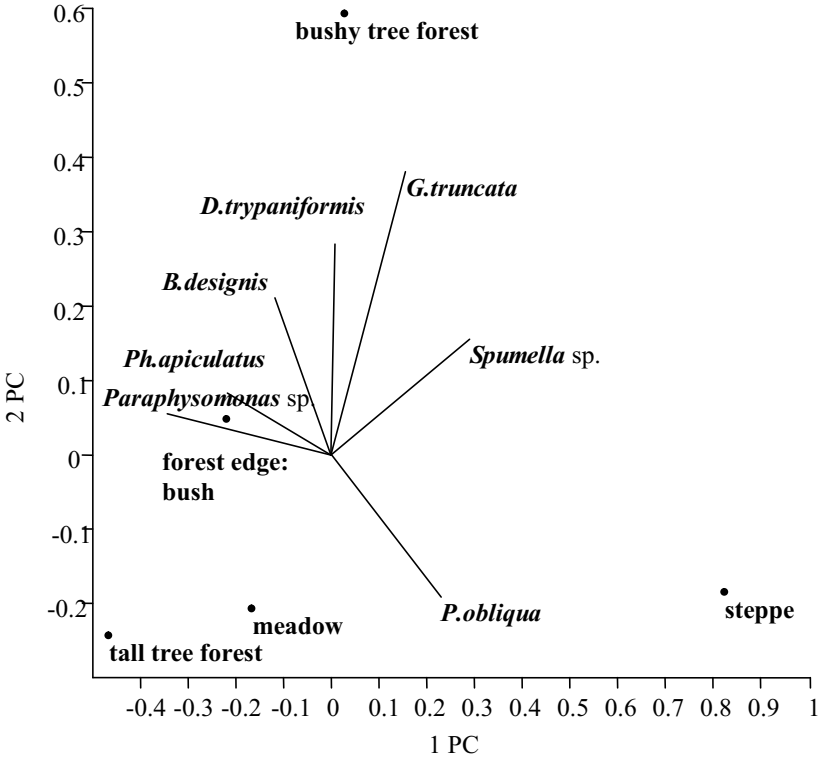

Fig. 4. Results of ordination (PCA) of communities of heterotrophic flagellates from the different-type biotopes of Ostrovtsovskaya forest-steppe by species composition: 1PC – first principal component (explains 54.0% of the total variance of species structure); 2PC – second principal component (27.2%).

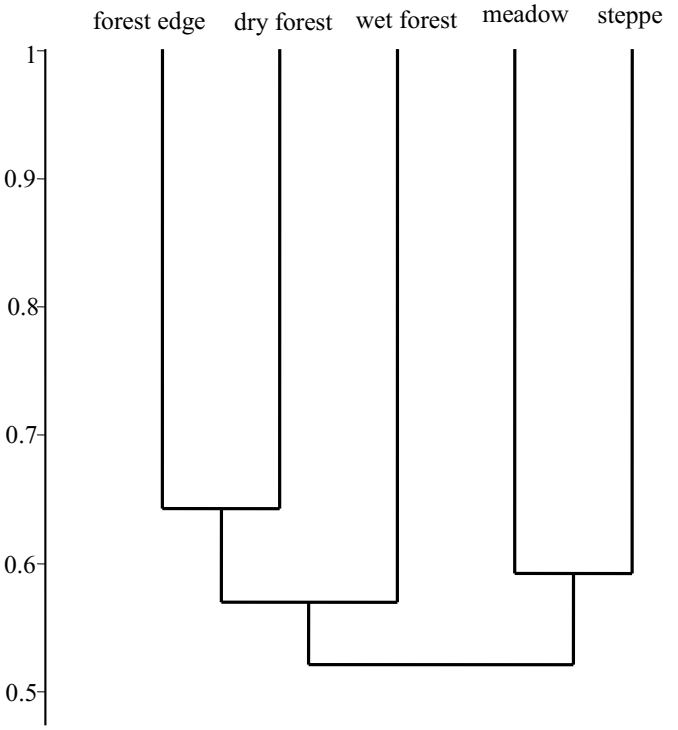

Fig. 5. Classification of communities of heterotrophic flagellates from the different-type biotopes of Kuncherovskaya forest-steppe by species composition: "steppe" – psammophilous steppe; "meadow" – meadow steppe; "forest edge" – forb and steppe oak forest; "dry forest" – maple oak forest and oak tillet forest; "wet forest" – aspen forest and osier-bed.

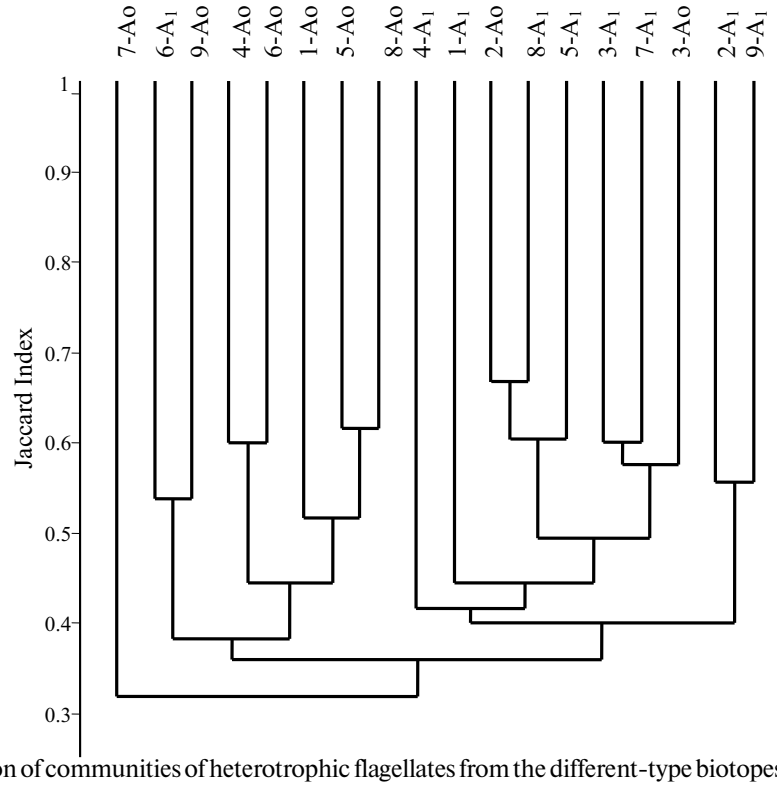

Fig. 6. Classification of communities of heterotrophic flagellates from the different-type biotopes and soil horizons of Kuncherovskaya forest-steppe by species composition: K1-K9 – different biotopes (see material and methods for details).

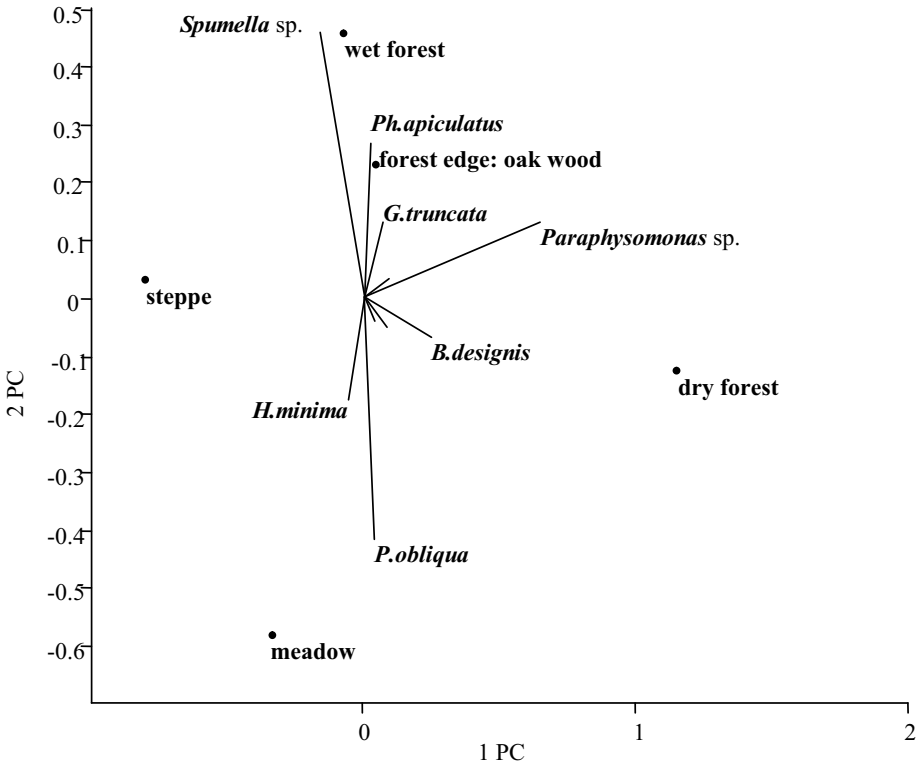

Fig. 7. Results of ordination (PCA) of communities of heterotrophic flagellates from the different-type biotopes of Kuncherovskaya forest-steppe by species composition: 1PC – first principal component (explains 68.7% of the total variance of species structure); 2PC – second principal component (19.8%).

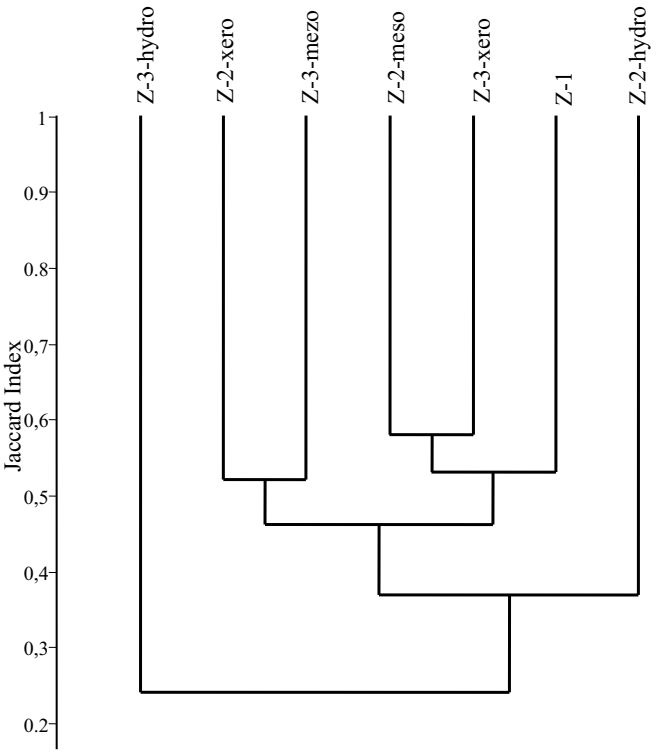

Fig. 8. Classification of communities of heterotrophic flagellates from Zasurskyi forest (see Material and methods for details).

of the species list of heterotrophic flagellates from the virgin soils of Moscow region were observed in the soils of forest-steppe of Penza region.

Soil protists are immigrants from the aquatic environment and are physiologically aquatic organisms. So, their presence in terrestrial ecosystems depends on the ability to withstand the lack of water as well as abrupt fluctuations in its content. Active life in the soil is confined to water films around soil particles and to capillary and drip gravitational moisture, and is limited by the conditions in these peculiar microreservoirs. The features of soil water (pH, chemical composition, the presence of soil colloids, low freezing point and the gas composition), distinguishing it from the surface (ground) water, have an essential significance. The necessity of movement in narrow inter-soil spaces (pores, capillaries), "discontinuity" of the distribution of the water and the diversity of its physical forms, as well as the volatility of moisture content determine the peculiarity of the soil as a habitat for protists (Fedorov, 1972). In fact, 49% of the identified species are characterized by metabolic (flexible) cell body and/or amoeboid stages in the life cycle. Perhaps, amoeboid cell is the most adaptive to habitation in micropits and thin water film surrounding soil particles (Ekelund and Patterson, 1997). Ability to form cysts, which aid to survive under the conditions of the lack of water as well as soil freezing in winter, is authentically known for 13 of the species found (the life cycle of many flagellates, especially cercomonads, are not studied).

Seventy-four percent of the total species diversity of flagellates from forest-steppe soils of the Middle Volga Region was noted earlier from different freshwater basins of the Sura River within the Middle Volga Region (Tikhonenkov and Mazei, 2005, 2007, 2008). Only 14 soil species have not been identified earlier in the water bodies of Middle Volga Region, but these organisms are not rare and are frequently observed in other freshwater habitats of the European part of Russia (Zhukov et al., 1998). It should be noted that choanoflagellates, which always accompany the dominant cercomonads and eugledinds in freshwater bodies, rarely occur in soil samples.

The community composition of soil heterotrophic flagellates is the most similar to that of heterotrophic flagellates in sphagnum. In both cases, the major component of the communities is amoeboid organisms (Tikhonenkov et al., 2006; Tikhonenkov, 2007/8). This fact is perhaps explained by the similarity of microniches in soils and in sphagnum (habitation in thin water films, micropores, micropits under the conditions of periodically changing water regime).

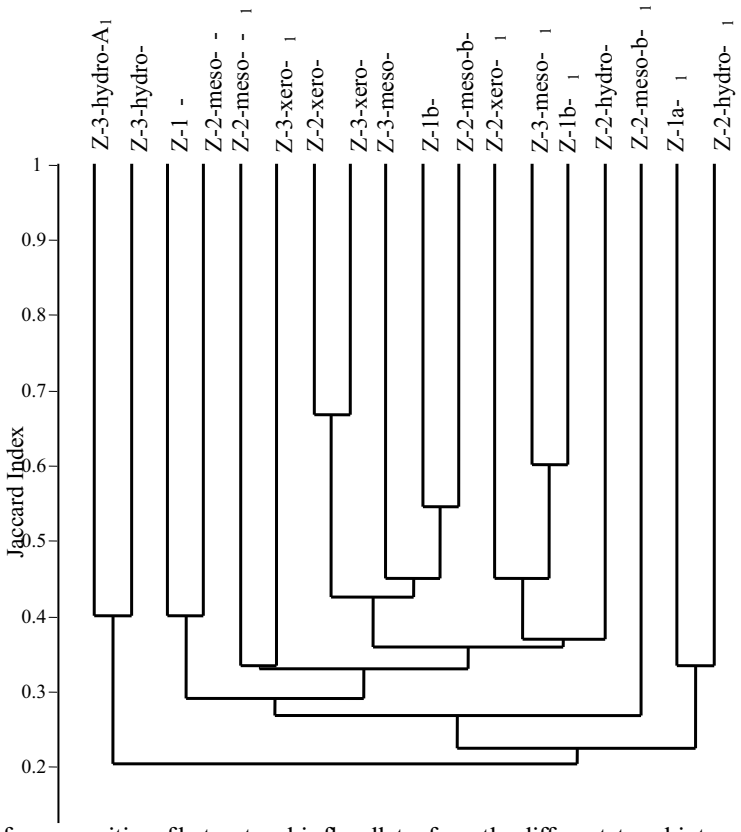

Fig. 9. Classification of communities of heterotrophic flagellates from the different-type biotopes and soil horizons of Zasurskyi forest by species composition (see material and methods for details).

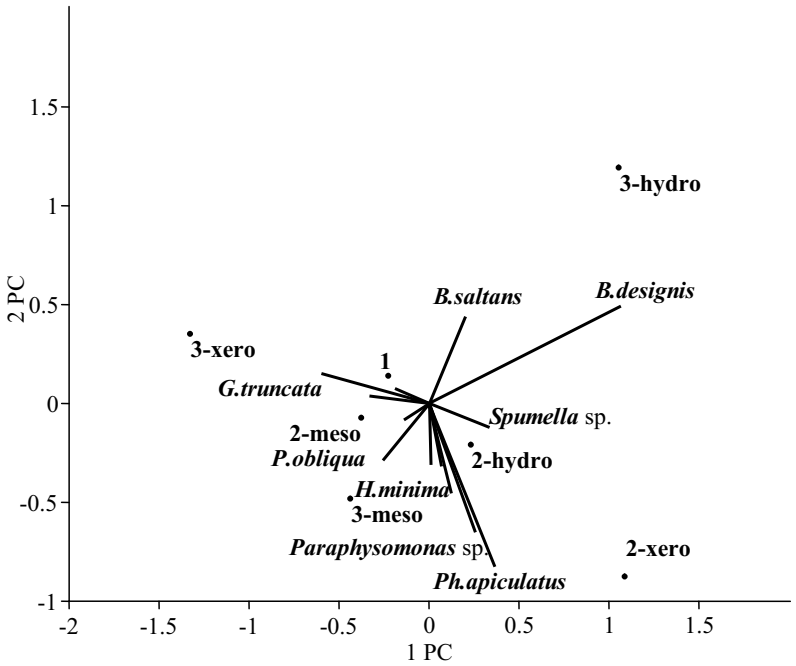

Fig. 10. Results of ordination (PCA) of communities of heterotrophic flagellates from the different-type biotopes of Zasurskyi forest by species composition: 1PC – first principal component (explains 47.1% of the total variance of species structure); 2PC – second principal component (27.2%).

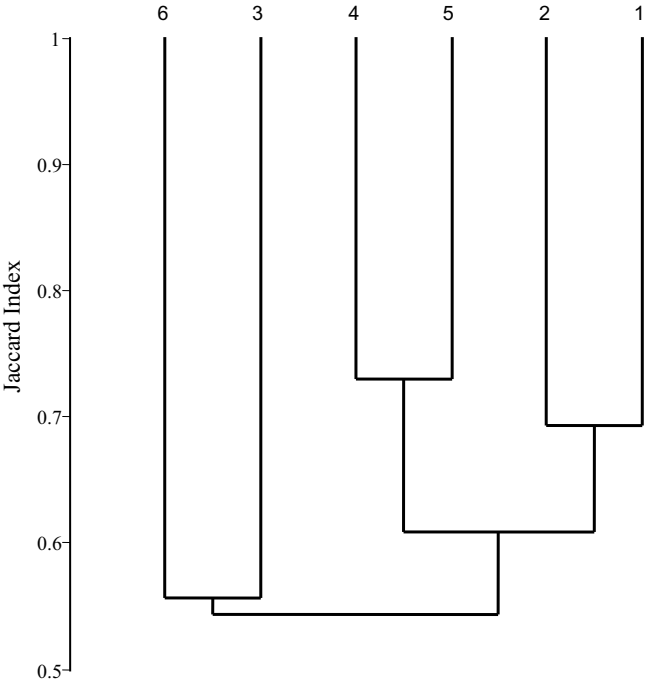

Fig. 11. Classification of communities of heterotrophic flagellates from the different-type biotopes by species composition: 1 – grasslands (steppes, meadows); 2 – forest edges (broom groves, cherry bushes and steppe oak forests); 3 – bushy tree woods (blackthorn bushes and bird-cherry-tree woods); 4 – small-leaved forests (birch forests and aspen forests); 5 – broad-leaved forests (maple tillet forests and oak forests); 6 – mixed and coniferous forests (pine forests).

Testate amoebae communities have been investigated simultaneously with those of soil heterotrophic flagellates, in the same biotopes and in accordance with the same aims (Mazei and Embulaeva, 2008, 2009). These different ecological and size groups of protists were shown to have different reactions to the spatial heterogeneity of forest steppe ecosystems.

In particular, we found that the specificity of community composition of heterotrophic flagellates increases with the decrease in the moisture of the biotope and is reflected in the high diversity of amoeboid flagellates. In contrast, the dominant complex of testate amoebae is more diverse in boggy, broad-leaved and coniferous forests (includes lobose and filose testaceans of plagiostomic, centrostomic and acrostomic life forms) in comparison with xerophytic meadow steppes, shrubby edges, steppe forests and birch groves (Mazei and Embulaeva, 2008, 2009).

In contrast to the heterotrophic flagellates, the patterns of testate amoebae communities in accordance with grassland-woodland transect depend on the characteristics of soils and vegetation in specific areas. In particular, along the transect on typical and leached chernozems at well-developed ecotones represented by bushes, the testate amoebae community differentiated into the variants corresponding to meadow steppes, tall forests and bushes. On transitional soils (between the dark-gray forest soils and podzolized chernozems), in the ecotones represented by steppe oak forests, specific rhizopods communities are absent, too (Mazei and Embulaeva, 2008, 2009). Heterotrophic flagellate communities do not show such a pronounced tendency to change along this gradient. The dominant complex is formed by eurybiont species with a wide geographic distribution. The differences of local communities are determined by recombination of the dominants as well as by the random distribution of rare species.

Testaceans and heterotrophic flagellates respond to secondary forest succession in different ways. In this context, directional change from the predominance of testate amoebae of plagiostomic life forms in the young birch groves to the dominance of rhizopods with centrostomic shells in maple tillet woods and oak forests was found. However, a single variant of the testate amoebae communities, slightly distinguishable from the original in birch groves, is formed within the trajectories of ecosystem development under wet (aspen forests, boggy birch

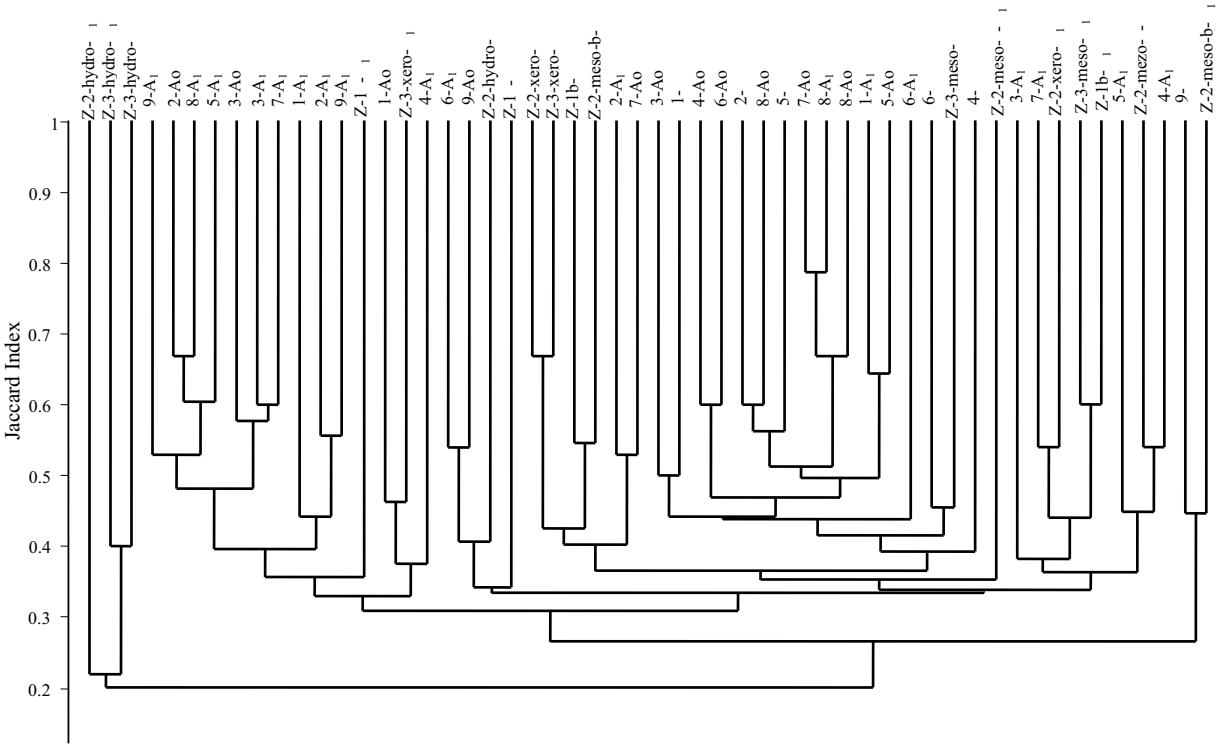

Fig. 12. Classification of communities of heterotrophic flagellates from the different-type biotopes and soil horizons by species composition (see Material and methods for details).

pine forests) or dry (mixed forests, pine forests) conditions. Heterotrophic flagellate communities show other tendencies. Species complexes of heterotrophic flagellates from oak forests (at a middle level of moistening) and from boggy forests (at an increased moistening) are the most different from the initial variant. Thus, heterotrophic flagellates and testate amoebae have different patterns of changes in accordance with the successional stage of forest phytocenoses. Testate amoebae communities are the most heterogeneous within the mesoseries, whereas the communities of flagellates, within the climaxes of xero- and mesoseries.

In conclusion, the first special investigation of general patterns of changes in community structure of soil protists in the "steppe-forest" gradient as well as in accordance with the secondary succession of forests after felling revealed a very structured community of testate amoebae and rather homogeneous communities of heterotrophic flagellates. Fifty-three species and forms of heterotrophic flagellates were identified in the soils of the Middle Volga Region forest-steppe. Eurybiontic *Bodo designis* (revealed in 100% of biotopes), *Paraphysomonas* sp. (100%), *Spumella* sp. (100%), *Heteromita minima* (94%), *Phyllomitus apiculatus* (88%), *Ploeotia obliqua* (88%), *Heteromita globosa* (82%), *Goniomonas truncata* (82%) were the most common species. Species richness of the genus *Cercomonas* was the highest. The communities can be divided into three groups by the species composition: i) from xerophytic grasslands and bushes; ii) from meso-xerophytic pine forests and parvifoliate forests; iii) from mesophytic broad-leaved forests. The specificity of composition of heterotrophic flagellates increases with the decreasing moisture in the biotope and and is reflected in the high diversity of amoeboid flagellates, which are well adapted to habitation on the surface of soil particles and in thin capillary spaces of soil. The dominant complex is formed by eurybiont species with a wide geographic distribution. The differences between the local communities are determined by recombination of the dominants as well as the random distribution of rare species.

Acknowledgements

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