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Diversity of Naked Amoebae in Soils of Forest Areas of Zhytomyr Region (Ukraine)

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Abstract

Here we consider the morphotypes and changing species composition of naked amoebae in soils of forest areas in Zhytomyr region (Ukraine). The species composition and morphotypes of amoebae are influenced by the microclimate of soil and litter. Complexes of naked amoebae in the study area constitute two clusters: one of broadleaf (oak) and mixed forests, another of coniferous forests. The most similar species compositions were found in soils of oak and mixed forests (64 % similarity). The least similar were amoebae complexes of coniferous forests and oak forests, and coniferous forests and mixed forests. Non-parametric multidimensional scaling (MDS) showed that species complex of amoebae of mixed forests and oak forests had moister and more acidic soils compared to coniferous forests.

Key words: naked amoebae, morphotypes, soils, Zhytomyr region

Introduction

Soil invertebrates play an important role in natural biocoenoses: they participate in biochemical soil processes, decompose organic products, change soil dispersivity, activate biological fixation of nitrogen, ammonification, cellulose decomposition (Helzer et al., 1980). To evaluate the state of soil, researchers commonly assess the indicator organisms including protists, which directly reflect their environment (Bulatova, 2010). Naked amoebae, very sensitive to soil changes are the least studied soil protists. We present the first study of naked amoebae distribution and their morphotypes in soils of Zhytomyr region and Ukraine as a whole. In our previous work presents data on features of distribution of naked amoebae in different types of water bodies of Ukraine (Patcyuk & Dovgal, 2012; Patsyuk, 2014; 2015; 2016; 2017; 2018; 2019). In addition, the ecology of naked amoebae remains poorly understood both in Ukraine and in the world as a whole. Most of the works of foreign scientists are devoted to the taxonomy and phylogeny of this group of protists (Glotova et al., 2018; Kudryavtsev et al., 2018; Bondarenko et al., 2019). As for other protozoa, there are works in which peculiarities of distribution testate amoebae and heterotrophic flagellate in the soils of different regions of the study (Trulova et al., 2011; Mazei et al., 2013; 2015; Shimano et al., 2017).

Materials and Methods

In 2016–2018, soil samples were collected at five cm depth in forests of various types (oak, mixed and coniferous) of Zhytomyr region. Sampling was accompanied by measuring acidity, humidity, temperature and soil type, tree litter and vegetation specifics.

Mixed forests are characterized by very diverse tree species: *Quercus robur* L., *Tilia cordata*, *Fraxinus excelsior* L., *Ulmus laevis*, *Acer platanoides*, *Sorbus sp.*, *Populus tremula* L.; the understory mostly consisted of *Corylus avellana*, *Eubatus* sp., *Rosa acicularis*, *Rubus idaeus* and other shrubs. The herbaceous layer is dense and includes grasses (Gramineae), *Asarum europaeum*, *Stellaria* sp., *Galium odoratum* L., *Polygonatum officinale*, *Pulmonaria* sp., *Achillea millefolium*, *Matricaria* sp. L., etc. Early spring flowers are represented by *Galanthus nivalis*, *Scilla*

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sp., *Corydalis* sp., *Anemone nemorosa*, etc. In oak forests the prevailing tree species is *Quercus robur* L. with the occasional *Tilia cordata*, *Corylus avellana*, etc. The herbaceous layer consists of *Vaccinium myrtillus*, *Peucedanum oreoselinum* (L.) *Moench*, *Pteridium aquilinum*, *Convallaria majalis*, *Polygonatum odoratum*, etc. Coniferous species are dominated by *Pinus sylvestris* L., accompanied by *Larix decidua*, *Picea* and *Abies*. The understory includes *Betula pendula*, *Prunus padus*, *Sorbus aucuparia*, and others. The herbaceous story is poor, tree litter consisting mostly of pine needles. There is a sparse growth of *Thymus serpyllum*, *Cladonia rangiferina*, *Pyrola* sp., *Vaccinium vitis-idaea* etc.

Soil acidity (Table 1) was measured using a laboratory pH-meter 150 M. Soil temperature was measured at 5 cm using soil thermometer, temperature under the litter was evaluated with mercury meteorological thermometer. The amoebae were cultured in 100 mm Petri dishes on non-nutrient agar (Page, 1988, 1991). Cultures were kept at room temperature. The protists were observed and photographed using light microscope Axio Imager M1 (Centre for collective usage of scientific equipment «Animalia» of I. I. Schmalhausen Institute of Zoology) with differential interferential contrast.

TABLE 1. Acidity of the studied soils in forest areas of Zhytomyr region.

№	Study area	Depth of collecting, cm	Soil pH
1	Berdychiv district	Up to 5	5.6–5.8
2	Popilnya district	Up to 5	5.5–5.6
3	Novograd-Volhynsky district	Up to 5	5.8–6.3
4	Baraniv district	Up to 5	6.4–6.6
5	Lyubar district	Up to 5	5.9–6.0
6	Olevsk district	Up to 5	4.6–4.8
7	Ovruch district	Up to 5	4.3–4.7

The amoebae were identified in two stages. First, their morphotypes were determined using special literature (Smirnov & Goodkov, 1999; Smirnov, 2008). If there were enough data for further determination, I used Page's key (Page, 1988; Page & Siemensma, 1991) and later publications on naked amoebae systematics (Smirnov, 2008; Smirnov et al., 2011; Adl et al., 2012). Fauna lists were compared with Chekanovsky–Sørensen index. The clusters were calculated with bootstrap support and multidimensional analysis was carried out using PAST 1.18 (Hammer et al., 2001).

Results and discussion

In forest areas of Zhytomyr region, naked amoebae are represented by 14 species from 10 families and 11 genera.

Class Tubulinea Smirnow et al., 2005
Order Euamoebida Lepsi, 1960
Family Amoebidae Ehrenberg, 1838
Genus *Deuteramoeba* Page, 1987
Deuteramoeba mycophaga Pussard, Alabouvette et Pons, 1980
Family Hartmannellidae Volkonsky, 1931
Genus *Saccamoeba* Frenzel, 1892
Saccamoeba stagnicola Page, 1974
Saccamoeba sp.

Class Discosea Cavalier - Smith et al., 2004
Subclass Flabellinia Smirnov et al., 2005
Order Dactylopodida Smirnov etr al., 2005
Family Paramoebidae Poche, 1913
Genus *Korotnevella* Page, 1981
Korotnevella stella Schaeffer,1926

Family Vexilliferidae Page, 1987
Genus *Vexillifera* Schaeeffer, 1926
Vexillifera sp.
Order Vannellida Smirnov et al., 2005
Family Vannellidae Bovee, 1970
Genus *Vannella* Bovee, 1965
Vannella lata Page, 1987
Order Himatismenida Page, 1987
Suborder Tectiferina Smirnov, Nassonova, Chao et Cavalier-Smith, 2011
Family Cochliopodiidae De Saedeleer, 1934
Genus *Cochliopodium* Hertwig et Lesser, 1874
Cochliopodium sp.
Subclass Longamoebia Smirnov, Nassonova, Chao et Cavalier-Smith, 2011
Order Dermamoebida Cavalier-Smith et al., 2004
Family Mayorellidae Schaeffer, 1926
Genus *Mayorella* Schaeffer, 1926
Mayorella cantabrigiensis Page, 1983
Mayorella sp.
Order Thecamoebida Smirnov, Nassonova, Chao et Cavalier-Smith, 2011
Family Thecamoebidae Schaeffer, 1926
Genus *Thecamoeba* Fromentel, 1874
Thecamoeba striata Penard, 1890

Class Variosea Cavalier-Smith et al., 2004
Family *Filamoebidae* Cavalier-Smith et al., 2004
Genus *Filamoeba* Page, 1967
Filamoeba nolandi Page, 1967

Class Heterolobosea Page et Blanton, 1985
Order Schizopyrenida Singh, 1952
Family Vahlkampfiidae Jollos, 1917
Genus *Vahlkampfia* Chatton et Lalung-Bonnaire, 1912
Vahlkampfia sp.(1)
Vahlkampfia sp.(2)
Genus *Naegleria* Alexeieff, 1912 emend. Calkins, 1913
Naegleria gruberi Schardinger, 1899

Almost 50 % of the species belong to the class Discosea. Classes Tubulinea and Heterolobosea are represented by three species each, comprising 21.4 % of the found species. Class Variosea is represented by a single species, *F. nolandi* (7 %) (Fig. 1). The least diversity is found in soils of coniferous forests (four species), the highest diversity is observed in oak forests (10) and mixed forests (12).

Two amoeba species, *Vahlkampfia* sp. (1) and *Vahlkampfia* sp. (2), are common for all study areas. These species are present in all samples and so can be considered eurybionts. Species composition of other amoebae is uneven (Table 2). Such amoeba species as *D. mycophaga, T. striata, V. lata, M. cantabrigiensis, K. stella, Vexillifera* sp., *Cochliopodium* sp. are typical for soil fauna of oak forests and mixed forests of Zhytomyr region. The common species in soils of mixed and coniferous forests is *N. gruberi*. Only *F. nolandi* is recorded in a coniferous forest, *Saccamoeba* sp. is observed in oak forests, *Mayorella* sp. and *S. stagnicola*—in mixed forests of Zhytomyr region. *Saccamoeba* sp. is typical only for moss carpets. The poor species composition of soils under coniferous forests is explained by higher acidity and by the tree litter specifics. Litter of coniferous trees is slowly mineralized.

Current methods provide no data on amoeba numbers. Hence, we tried to analyze the frequency of findings of naked amoebae in the study area. Out of fourteen identified species, *Vahlkampfia* sp. (1), *Vahlkampfia* sp. (2), *T. striata, V. lata, M. cantabrigiensis, Cochliopodium* sp. are collected in most samples, amounting to 43 % of total

species number (Fig. 2; Table 2). Frequency of their findings in the studied samples is 65.5 %, 53.58 %, 47.62 %, 53.58 %, 61.9 % and 52.38 %, respectively. The species with average frequency of findings was *K. stella* (23.81 %). *N. gruberi* (13.10 %)*, D. mycophaga* (15.48 %), *S. stagnicola* (16.67 %), *Mayorella* sp. (4.77 %); *Vexillifera* sp. (15.48 %), *F. nolandi* (2.38 %), *Saccamoeba* sp. (2.38 %) can be considered not numerous in soils. The latter group includes 50 % of the total number of species. In our study, they are found several times in 84 studied samples.

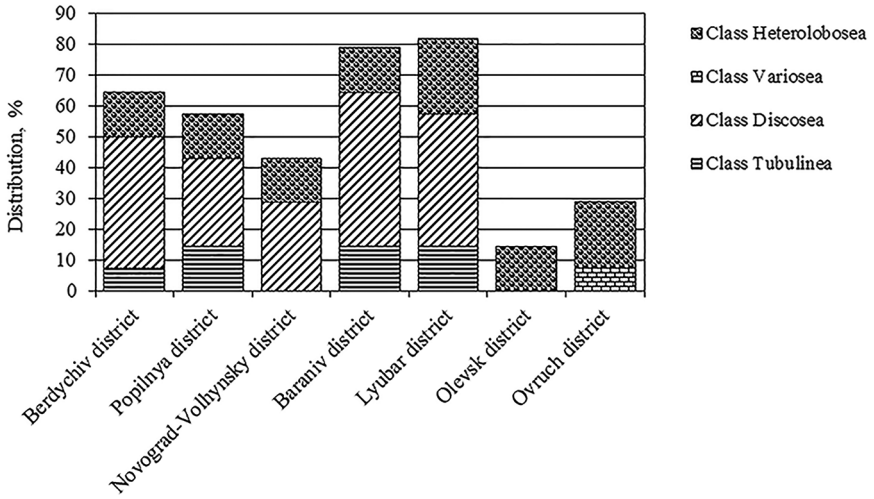

FIG. 1. The distribution of amoeba classes in study areas.

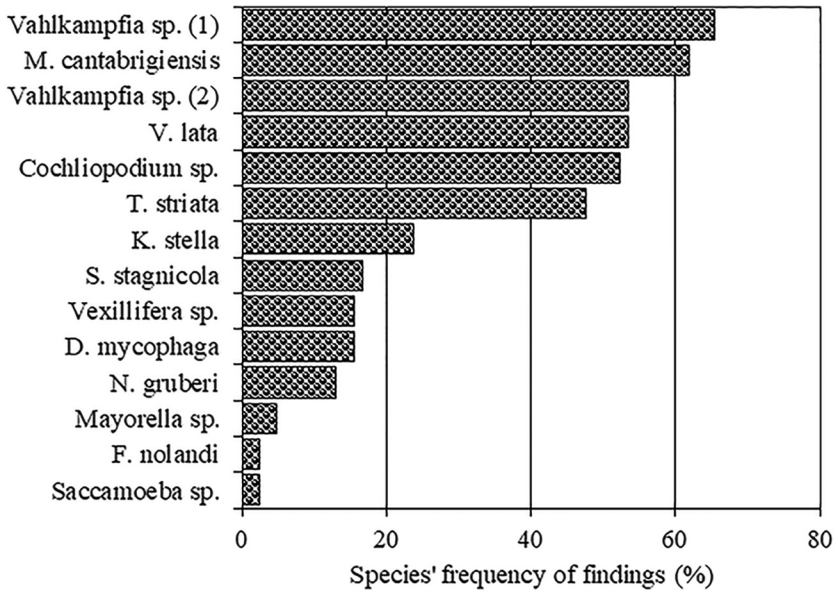

FIG. 2. Frequency of naked amoebae findings in forest areas of Zhytomyr region.

The found amoebae belong to nine morphotypes. The most morphotypes are found in soils of oak and mixed forests. These soils contain amoebae of all identified morphotypes (Table 2). Three species of naked amoebae, *Vahlkampfia* sp. (1) and *Vahlkampfia sp.* (2), *N. gruberi*, belong to the eruptive morphotype, typical to all soils of forest areas in Zhytomyr region. Amoebae of the monotactic (*Saccamoeba stagnicola, Saccamoeba* sp.), mayorellian (*M. cantabrigiensis, Mayorella* sp.) and dactylopodial (*K. stella, Vexillifera* sp.) morphotypes are typical for soils of oak and mixed forests. Similarly, amoebae of the orthotactic (*D. mycophaga)*, striate (*T. striata)*, fan-shaped (*V. lata)* and lens-like (*Cochliopodium* sp.) morphotypes (Table 3) are typical for those forest areas of Zhytomyr region.

For certain morphotypes, we analyzed the frequency of findings in soils of forests of Zhytomyr region. The most wide-spread are amoebae of eruptive (79.77 %), striate (47.62 %), fan-shaped (53.58 %), mayorellian (61.91 %) and lens-like (52.38 %) morphotypes, comprising 55.5 % of all morphotypes (Fig. 3–4). The least common are

orthotactic (15.47 %), monopodial (19.05 %), dactylopodial (17.86 %) and acanthapodial (2.4 %) amoeba morphotypes, which are 44.4 % of all morphotypes. Therefore, soils of Zhytomyr region are characterized by a diverse species composition of naked amoebae and their morphotypes. The richest diversity of naked amoebae and their morphotypes is seen in mixed forests and oak forests, as a result of well-developed understory, herbaceous layer, mixed tree litter, and soil acidity nearing neutral. All of this creates conditions favorable to the amoebic development.

TABLE 2. Species composition of naked amoebae in forest areas of Zhytomyr region.

№	Amoebae species	Forest type		
		Coniferous	Oak	Mixed
1.	*Vahlkampfia* sp. (1)	+	+	+
2.	*Vahlkampfia* sp. (2)	+	+	+
3.	*N. gruberi*	+	—	+
4.	*D. mycophaga*	—	+	+
5.	*S. stagnicola*	—	—	+
6.	*Saccamoeba* sp.	—	+	—
7.	*T. striata*	—	+	+
8.	*V. lata*	—	+	+
9.	*M. cantabrigiensis*	—	+	+
10.	*Mayorella* sp.	—	—	+
11.	*K. stella*	—	+	+
12.	*Vexillifera* sp.	—	+	+
13.	*F. nolandi*	+	—	—
14.	*Cochliopodium* sp.	—	+	+
Total		4	10	12

Note: «+»—present, «—»—absent

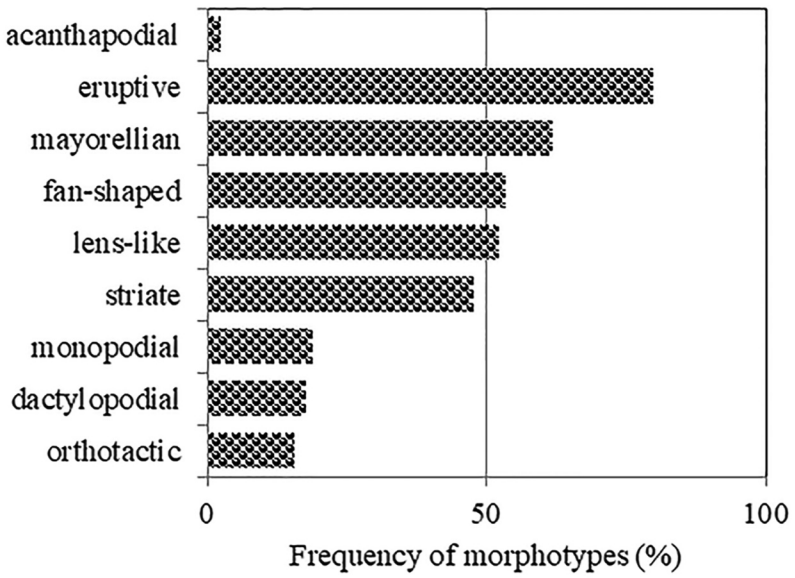

FIG. 3. Occurrence of naked amoeba morphotypes in soils of forest areas in Zhytomyr region.

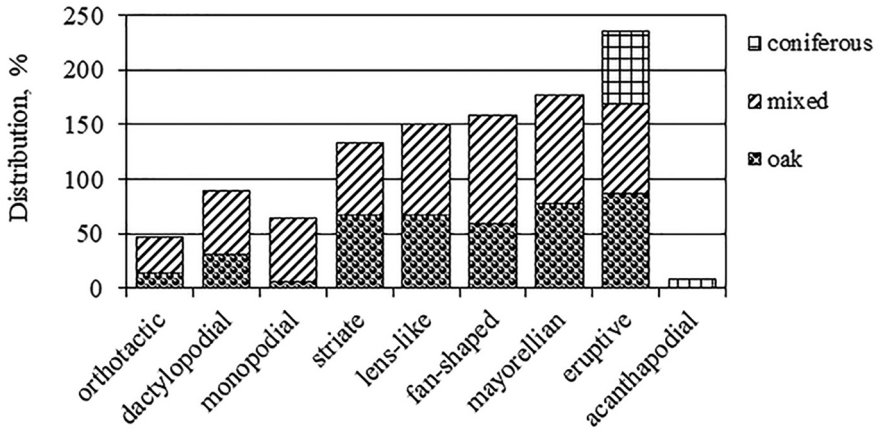

FIG. 4. Morphotypes distribution of naked amoebae in forest areas of Zhytomyr region.

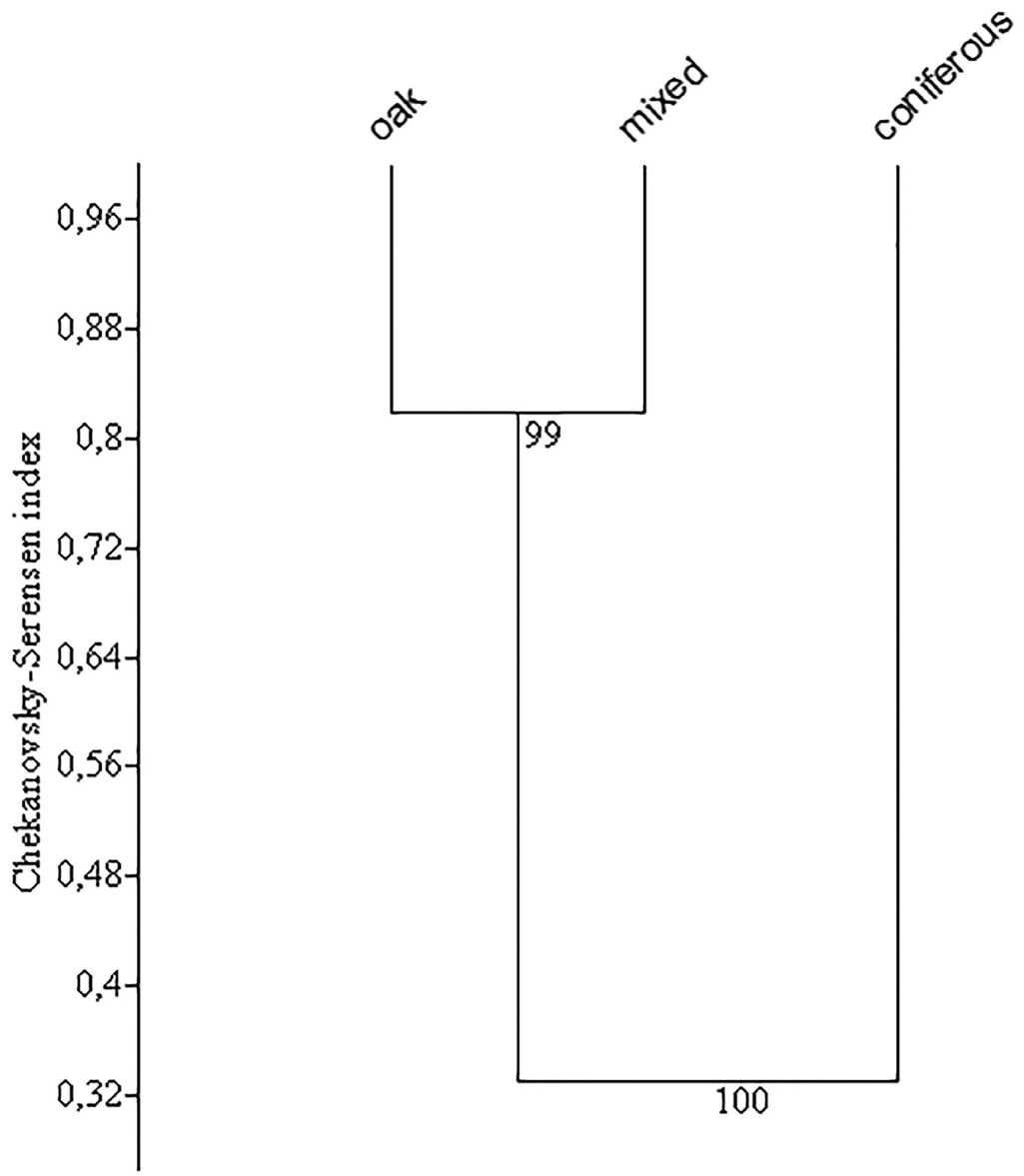

FIG. 5. Similarity of species composition of naked amoebae according to the Chekanovsky–Sørensen index (cluster significance at dengrogram nodes as % of 1000 permutations).

High specificity of naked amoebae complexes in soils of forest areas in the region is confirmed by cluster analysis presented on Figure 5. The dendrogram shows that the complexes of naked amoebae form two clusters, one containing species of oak and mixed forests, and the other those of coniferous forests. It is supported by 1000 boot-strap samples (cluster existence is 99 % and 100 %, respectively). Species composition of naked amoebae is formed in similar conditions in oak and mixed forests where the protistfauna is richest. It is confirmed by the Chekanovsky–Sørensen index: the most similar species composition was seen in soils of oak and mixed forests (0.81), where live 64 % of common species. The faunistic amoebic complexes of coniferous forests and oak and mixed forests (index values of 0.29 and 0.38, respectively) are least similar. We consider species composition to be influenced by such environmental factors as acidity, temperature and soil humidity.

According to the results of non-parametric multidimensional scaling (MDS), presented in Fig. 6, the mixed and oak forests have the highest soil moisture and acidity. Species composition in soils of coniferous forests is linked to lower soil acidity and moisture. As to the temperature factor, its effect on species complexes of oak, mixed and coniferous forests is weak in our study, perhaps due to the warm season of research.

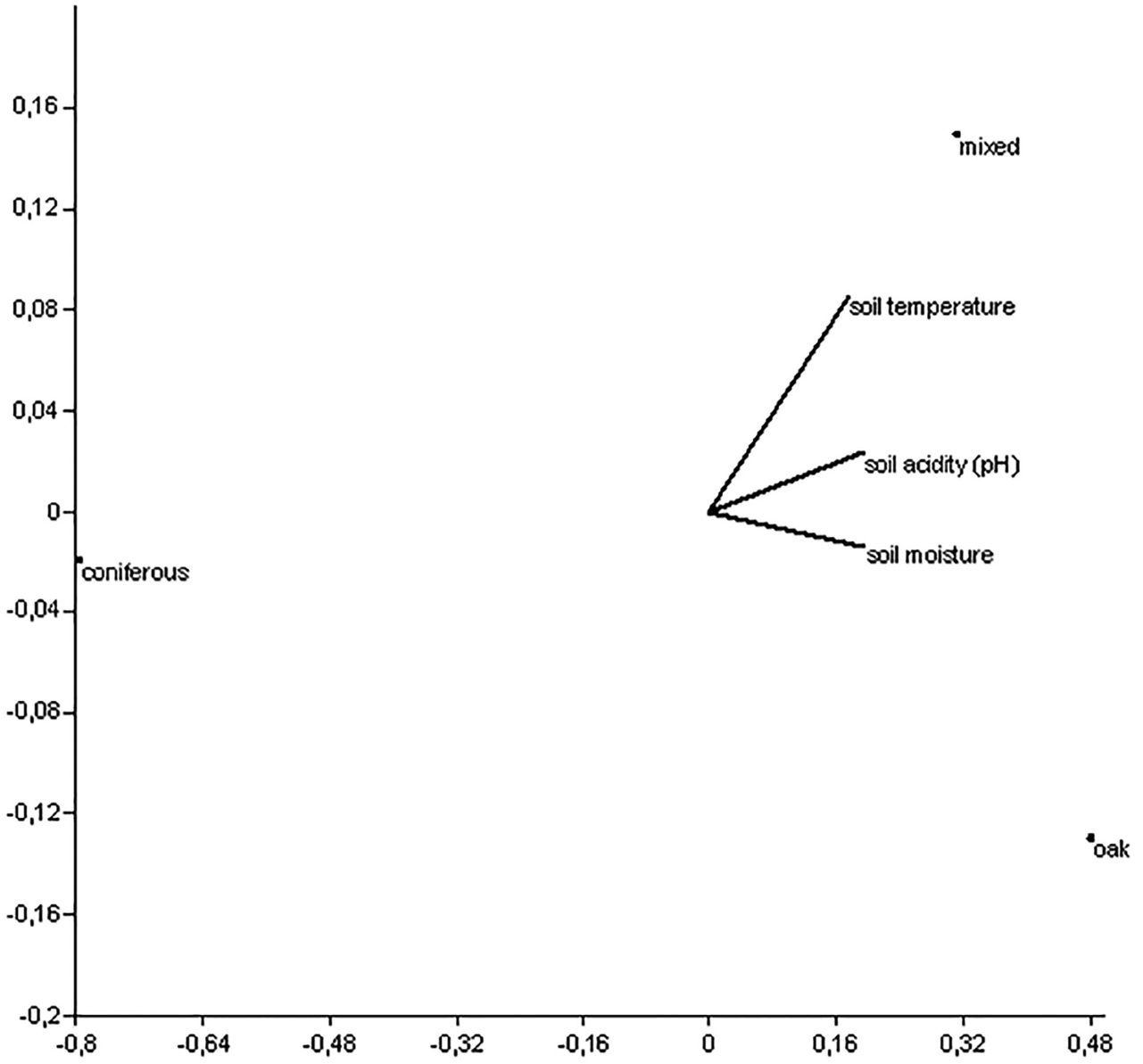

FIG. 6. Ordination of naked amoeba species that correspond to the forest areas and inhabit different microhabitats.

Another environmental factor is the soil type. In forest areas of Zhytomyr region, only *N. gruberi* and *F. nolandi* of 14 identified species prefer sod-podzolic soils, which is 14 % of all amoeba species. All other amoebae are found both in grey forest and sod-podzolic soils of forest areas in Zhytomyr region.

Fauna of the habitats is richer than the 14 species. This small species diversity can be a result of the prolonged time it took to deliver the samples to the laboratory. In our samples we identified representatives of most main taxa of naked amoebae.

In the soils of forest areas of Zhytomyr region we identified 14 species of naked amoebae of eight morphotypes. High variety of naked amoebae and their morphotypes is recorded in oak and mixed forests due to well-developed understory, presence of the herbaceous layer, neutral pH and high moisture of soil. The poorest amoeba diversity is in soils of coniferous forests of the region which are relatively acidic, drier and covered with coniferous litter. Two species, *N. gruberi* and *F. nolandi*, are added to the naked amoeba fauna of Ukraine.

TABLE 3. Morphotypes of naked amoebae in forest areas in Zhytomyr region.

Amoeba morphotype		Forest areas of Zhytomyr region						
		Oak forests			Mixed forests		Coniferous forests	
		Berdychiv district	Popilnya district	Novograd-Volhynsky district	Baraniv district	Lyubar district	Olevsk district	Ovruch district
1	Eruptive	+	+	+	+	+	+	+
2	Orthotactic	+	+	—	+	+	—	—
3	Monopodial	—	+	—	+	+	—	—
4	Striate	+	+	+	+	+	—	—
5	Fan-shaped	+	+	+	+	+	—	—
6	Mayorellian	+	+	+	+	+	—	—
7	Dactylopodial	+	—	—	+	+	—	—
8	Lens-like	+	+	+	+	+	—	—
9	Acanthapodial	—	—	—	—	—	—	+
Total		7	7	5	8	8	1	2
			8		8		2	

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