

The comparison of micromycetes and algae diversity in soils of various biocenoses of Eastern Forestial Steppe of Ukraine

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The species composition of soil algae and micromycetes was studied in forest plantations and cretaceous chalk outcrops in the Eastern Steppe of Ukraine within the Kharkiv region. The systematic structure of mycobiota and algaeflora of the studied plots was similar to the species contents in forest cenoses of Eastern Europe, with the prevalence of *Penicillium* fungi and Chlorophyta and Cyanophyta algae. The total species diversity of micobiota was equally rich in forest plantations and on chalk outcrops, whereas algae species diversity was higher in the forest soils. In forest plantations and on chalk outcrops with woody coverage the changes of the algae species number with the soil depths, as well as during comparison of different plots, were directed oppositely to that of changes of micromycetes species number. It was shown that the similarity in species composition of micromycetes and algae in all types of soils under woody covering depends mostly on the territorial distance between studied plots, and under grassy covering – mostly on the phytocenosis contents.

Key words: soil micromycetes, soil algae, species diversity, man-cultivated plantation forests, cretaceous chalk outcrops

ВІННИКОВА О.І. (2014). **Порівняння різноманітності ґрунтових мікроміцетів і водоростей різних біоценозів Східного Лісостепу України.** *Чорноморськ. бот. ж.*, **10** (1): 75-83. doi: 10.14255/2308-9628/14.101/8.

Досліджено видовий склад ґрунтових мікроміцетів і водоростей лісових насаджень та крейдяних оголень Східного Лісостепу України в межах Харківської області. Систематична структура міко- та альгофлори досліджених ґрунтів була подібною до складу мікроміцетів і водоростей лісових ценозів Східної Європи, з переважанням грибів роду *Penicillium* і водоростей відділів Chlorophyta та Cyanophyta. За загальною видовою різноманітністю мікобіота була однаково багата як в ґрунті лісонасаджень, так і на крейдяних оголеннях, а різноманітність водоростей була вищою в лісових ґрунтах. В лісових насадженнях та на ділянках крейдяних оголень з деревною рослинністю зміни числа видів водоростей за глибиною ґрунту чи при співставленні стаціонарів були протилежно спрямованими відносно змін числа видів мікроміцетів. Встановлено, що найсильніший вплив на подібність видового складу мікроміцетів і водоростей у будь-яких типах ґрунтів під деревною рослинністю має територіальна віддаленість стаціонарів, а під трав'яною рослинністю – склад фітоценозу.

Ключові слова: ґрунтові мікроміцети, ґрунтові водорості, видова різноманітність, лісові насадження, крейдяні оголення

Винникова О.И. (2014). **Сравнение разнообразия почвенных микромицетов и водорослей разных биоценозов Восточной Лесостепи Украины.** *Черноморск. бот. ж.*, **10** (1): 75-83. doi: 10.14255/2308-9628/14.101/8.

Исследован видовой состав почвенных микромицетов и водорослей лесных насаждений и меловых обнажений Восточной Лесостепи Украины в пределах Харьковской области. Систематическая структура мико- и альгофлоры изученных почв была сходна с составом микромицетов и водорослей лесных ценозов Восточной Европы, с преобладанием грибов рода *Penicillium* и водорослей отделов Chlorophyta и

Цианопхита. По общему видовому разнообразию микобиота было одинаково богата как в почве лесонасаждений, так и на меловых обнажениях, а разнообразие водорослей было выше в лесных почвах. В лесных насаждениях и на участках меловых обнажений с древесной растительностью изменения числа видов водорослей по глубине почвы и при сопоставлении стационаров были противоположно направленными относительно изменений числа видов микромицетов. Установлено, что наиболее сильное влияние на подобие видового состава микромицетов и водорослей в любых типах почв под древесной растительностью оказывает территориальная отдаленность стационаров, а под травяной растительностью – состав фитоценоза.

Ключевые слова: почвенные микромицеты, почвенные водоросли, видовое разнообразие, лесные насаждения, меловые обнажения

Introduction

Modern paradigm in ecologically focused biodiversity studies declares that edaphic algae and soil micromycetes must be considered as an essential part of terrestrial biocenoses. These systematic groups represent, respectively, the autotrophic and heterotrophic segments of soil microorganisms and thus predetermine the intensity of soil formation, its fertility and the overall rate of biogeochemical transformation of organic matter. During the last three decades the species contents and the systematic structure of micobiota and algaeflora have been actively investigated in various natural habitats, climate zones and ecosystems. Concurrently with the accumulation of the data on the biodiversity of soil algae and micromycetes, there were attempts to analyze the role of various factors in the formation of soil micobiota and algaeflora in particular locations and biocenoses. Such studies lead to rather controversial conclusions that occurred mainly due two reasons. First, many soil micromycetes and algae species are cosmopolites. Second, the methods of ecological floristic investigations have not been unified between these two microbial groups [METHODS..., 1991; SOIL ALGAE..., 2001; MARFENINA, 2005; KABIROV, 2007; AKSENOVA, 2010]. In the present work an attempt has been made to perform a combined study of species contents of mycobiota and algaeflora in different types of soil using the unified methodology of the floristic analysis in these two groups despite the differences in their basic trophic modes.

The aim of this research was to assess a possible influence of biotic and abiotic factors on the formation of soil mycobiota and algaeflora in various types of phytocenoses located in southern and northeastern parts of the Eastern Ukrainian Forest-Step.

Materials and methods

Thirteen plots were established in man-cultivated pine, birch and aspen plantation forests on the pine terrace of the river Seversky Donetz in the Zmiyiv district, the central part of Kharkiv region, Northeast Ukraine. Plots were located near Zadonetkoye village (7 plots) or around Borovoye lake (6 plots). In further analysis the plots were referred by a combination of site location (Z – near v. Zadonetkoye, B – around l. Borovoye), wetness level (1 – dry, 2 – fresh, 3 – wet) and the tree strain (p – pine, b – birch, a – asp). Additionally, one of the fresh pine stands at the Borovoye site (B–2p*) was heavily injured by the root pathogenic fungus *Heterobasidion annosum* (Fr.) Bref., and the nearest plot (B–2p) was established as a non-injured control. The soil type at all plots was low humus and weakly structured sandy loam. Plots Z–2p, Z–2b, Z–2a, B–2p, B–2p*, B–3p and B–3a had no plants in the understorey (grass vegetation covering less than 2 %). On remaining plots the grass covering varied from 50 to 70 % and consisted mainly of *Calamagrostis epigeios* (L.) Roth (Z–3p, Z–3b, B–1p), *Poa nemoralis* L. (Z–1p), *Poa trivialis* L. (Z–1b) or *Poa pratensis* and *Elitrigia repens* L. (B–3b). Other vascular plants were extremely rare on the studied sites. The

data comparison within selected pairs of plots allowed differential evaluation of the effect produced by a certain factor (e.g. wetness or tree strain) keeping other conditions identical.

Also eight plots were established on the chalk outcrops near villages Volchanskiye Hutora and Mala Volchja, situated on the right bank of river Volchiya, the tributary of river Seversky Donetz, in Vovchansk district, the north-eastern part of Kharkiv region. In further analysis the plots were referred by a combination of site location (V – near v. Volchanskiye Chutora, M – near v. Malaja Volchja) and type of covering: 1 – chalk hills with grass covering about 10 % (in case of M-group also totally covered by the lichen *Colemma tenax* (Swark) Ach. em.), 2 – chalk hills with grass covering about 20-30 %, 3 – chalk hills covered with thickets of *Prunus spinosa* L. or man-planted *Pyrus communis* L., 4 – hills with gleysol outcrops covered with thickets of *P. spinosa* or man-planted *P. communis*. The maximum distance between the plots within one location did not exceed 15 km. Geographically the studied chalk outcrops are situated approximately 100-110 km to North-north-east from the forest plantations.

Samples were collected in May and October, from semi-decomposed litter and soil horizons of 0-5 cm (fermentation and humus horizon) and 15-20 cm (maximum depth reached by plough at the time of planting the seedlings). Litter and soil samples were gathered using the techniques, typically employed in practice of mycology and algaecology [METHODS..., 1991; SOIL ALGAE..., 2001]. Micromycetes were isolated by the method of deep inoculation of the water suspension of litter or soil into melted Chapeck's mediums and mash-agar in Petri dishes, which were kept then in the incubator at 24±1°C [METHODS..., 1991]. Algae species contents was studied using the method of water-dissolved soil cultures, cultures on the fouling slides and on agarised Bold's medium [SOIL ALGAE..., 2001]. Algae cultures were grown at room temperature and under the lightening 2000 lk during 16 h per day. Fungi and algae strains, which showed growth in Petri dishes, were isolated into culture tubes with respective medium for further identification and storage.

The rate of mycobiota and algaeflora resemblance on different plots was estimates by Ward's cluster analysis method, employing Pearson – Bravet coefficient, which was calculated from a criss-cross comparison of species contents on each plot with each other. That was done using the software package Statistica v. 5.5 A (Stat Soft Inc., USA) [BOROVIKOV, BOROVIKOV, 1998].

Results and discussion

In total, 90 micromycetes species (93 intraspecie taxons) and 79 algae species were identified in litter and soil of cultivated forest plots. Fungi were represented by 21 Zygomycota species (24 taxons) and 69 Ascomycota species. Algae comprised Cyanophyta (4 species), Euglenophyta (2), Bacillariophyta (13), Xanthophyta (11), Chlorophyta (46), Cryptophyta (1), Chrysophyta (1) and Eustigmatophyta (1).

In plots established on chalk outcrops the litter and soil horizons contained in total 58 micromycetes species (62 intraspecie taxons), particularly belonging to 4 Zygomycota genera, 10 Ascomycota genera and 1 genus of Basidiomycota. Algaeflora of these plots consisted of 36 species (42 intraspecie taxons), among which 17 Cyanophyta (21 intraspecie taxons), 10 Chlorophyta (12 intraspecie taxons), 5 Bacillariophyta, 3 Xanthophyta and 1 Eustigmatophyta were identified.

Thus, the systematic structure of mycobiota and algaflora in either forest plantations or chalk outcrops was typical for soils of woodland biocenoses. In cultivated forests *Penicillium* Link genus and Chlorophyta division made the largest contribution to the species diversity. In calcificated soils Cyanophyta dominated in algaeflora, and mucoral fungi and *Penicillium* genus comprised the most essential proportions in mycobiota, however *Fusarium* Link and *Aspergillus* Link genera also played a role, providing, 13 and 15 % of total fungi species

number, respectively. The detailed lists of species identified in two localities will be published elsewhere.

The relative similarity of soil mycobiota and algaeflora in different forest plantations, assessed by cluster analysis, and the respective numbers of species on these plots are presented on Fig. 1. The same data for chalk outcrops are shown on Fig. 2. The numbers of fungi species in the soil of cultivated forests varied with no clear pattern in relation to the tree strain or wetness level. In the systematic structure of soil mycobiota the number of species from *Mucor* Fresen. genus increased and the contribution of *Aspergillus* and *Penicillium* proportionally decreased from dry to wet plots. The richness of algaeflora had a clear positive correlation with the wetness level; all Cyanophyta species were found on wet plots only, where a higher number of Bacillariophyta species also occurred. The list of frequent species, which were found on at least 7 out of 13 plots, consisted of 19 fungi and 10 algae.

In the soil of chalk outcrops *Mucor* and *Penicillium* fungi and Cyanophyta algae were the most frequent on each plot. However, the majority representatives of these affluent taxons and were found on 1–4 out of 8 sites. Nevertheless, there were 9 fungi species and 13 algae species, which were identified on at least 5 out of 8 plots in this location. There was an overall tendency for higher number of fungi species and lower number of algae species on plots under *P. spinosa* and *P. communis*, in comparison with treeless chalk hills. The former effect was produced by the uniform elevation of the numbers of species from all main groups of fungi – mucoral, *Penicillium*s and other Ascomycota – in the soil with thickets covering. The higher diversity of algae species on the completely open chalk outcrops occurred nearly exclusively due to increased representation of Cyanophyta.

An interesting and unexpected tendency was established during the combined analysis of algaeflora and mycobiota data collected in forest plantation plots. The difference in algae species number between plots with different tree strains were always opposite to that in fungi species number. In the chalk outcrop soils this negative correlation between local densities of fungi and algae species occurred only when plots with thickets of *P. spinosa* or *P. communis* (i.e. with wooden covering) were compared. Thus, probably, this effect seems to be prominent specifically in woodland phytocenoses.

The analysis of clusters on Fig. 1 showed that for both groups of microorganisms the physical distance between compared plots appeared to be the most influential factor, which set the rate of resemblance of species contents. Such effect, produced by the distance between investigated locations coupled to the peculiarities of zonal distribution, was noted by I.Yu. Kostikov during the comparison of partial algaeflora in soils of Ukrainian Right-Bank Forrest-Step and other territories [KOSTIKOV, 2002]. A similar conclusion was made by T. M. Darienko [DARIENKO, 2000] in her study of soil algae in Mountain Crimea. This author found that the influence of physical distance between the plots on the algae species contents sometimes can exceed the impact of the type of phytocenosis. The present research shows that this effect can be detected even at a relatively short distance between studied locations: about 15 km between Zadonetskoye village and Borovoye lake. Within primary clusters formed by the territorial factor further grouping of the plots by the mycobiota showed some dependence on the wetness level, whereas the tree strain appeared to be more important for the algaeflora species contents. Possible reasons for such effects, respectively, can be a necessity in high humidity for Mucorales and specific impact of root, leaf and fir-needle excreta on algae.

On the chalk outcrops the overall principal role of physical distance between locations in the species contents resemblance was more pronounced for micromycetes than for algae (Fig. 2). The latter again showed some association with the presence of tree covering. In case of fungi the strongest linkage of species contents occurred between plots V1 and V2, which had rather similar type of covering, but also were situated on the shortest distance (about 800 m) among studied sites. The algae species contents appeared to be the most similar in the pair

of plots also from one location (V) and similar type of covering (thickets of *P. communis* or *Pr. spinosa*).

The lists of species found in forest plantations and chalk outcrops were juxtaposed between two locations. There were 23 species (26 intraspecies taxons) of micromycetes and 16 algae species, which appeared to be common for two locations; that comprised, respectively, 15,5 % of total mycobiota and 13,9 % of total algae flora diversity on studied territories. Common micromycetes species included *Absidia* v. Tiegh. (4 species), *Aspergillus* (2), *Cylindrocarpon* Wollenw. (1), *Mucor* (5), *Penicillium* (7), *Rhizopus* Ehrenb. (1) and *Trichoderma* Pers. (3); common algae species were represented by *Cyanophyta* (2 species), *Bacillariophyta* (5), *Xanthophyta* (1) and *Chlorophyta* (8).

Among these common species fungi *Mucor hiemalis* f. *corticola* (Hagem) Schipper and *Trichoderma koningii* Oudem, as well as algae *Pinnularia borealis* Ehrenberg, *Myrmecia biatorellae* (Tschermak-Woess et Plessl) B. Petersen and *Bracteacoccus minor* (Chodat) Petrova were found on more than a half of plots in each of two locations and thus belonged to a category of frequent and cosmopolite species. Fungi species *Tr. viride* Pers., *Absidia coerulea* Bain., *A. glauca* Hagem, *Penicillium severskii* Schekh., *P. citrinum* Thom, *M. plumbeus* Bonord. and algae species *Chlorococcum scabellum* Deason et Bold, *Coccomyxa solorinae* Chodat, *Stichococcus bacillaris* Nägeli, *Hantzschia amphioxys* (Ehrenberg) Grunov in Cleve et Grunov were rather frequent in cultivated forests, but relatively rare on chalk outcrops. The opposite effect, i.e. high frequency in calcificated soil but rare occurrence in forest soils, was observed for fungi *M. hiemalis* f. *hiemalis* Wehmer and *Rhizopus microsporus* var. *oligosporus* (Saito) Schipper & St., and for alga *Navicula cryptocephala* Kutzing.

In general, there was a moderate positive correlation between the frequency rate for common species in cultivated forest and chalk outcrops biocenoses; the numbers of plots in two locations, on which a particular specie occurred, correlated with $r = 0,38$ for micromycetes and $r = 0,41$ for algae ($p < 0,05$ for both groups of microorganisms).

Each plot either in forest plantations or on chalk outcrops contained fungi and algae species, which were common for two localities. Also, there was no clear dependence for the occurrence of common species on the tree strain and wetness level. Plots Z-1p, Z-2a, B-3b and V-3, M-3, M-4 appeared to be the most rich in cosmopolitan micromycetes, each carrying from 12 to 17 of such species. The highest numbers of common algae species – 10 or 11 – were present on plots Z-3p, Z-3b, V-3 and M-1. Pooling two locations together, the overall distribution of common species among all studied plots seems to be close to random, with some tendency of their higher numbers on the plots with leaf tree covering.

Noteworthy, some species appeared to be frequent in one type of biocenoses whereas absent in another one. In the forest plantations these included: fungi – *Tr. hamatum* (Bonord.) Bainier, *M. moelleri* (Vuill.) Lendn., *Mortierella longicollis* Dixon-Stew., *P. cremeo-griseum* Chalabuda, *P. aurantiogriseum* Dierckx, *P. aculeatum* Raper & Fennell, *P. paxilli* Bainier, *P. glabrum* (Wehmer) Westling, *P. diversum* Raper & Fennell, *Aspergillus parvulus* G. Sm., *Mort. verticillata* Linnem., and algae – *Neocystis bohémica* Kostikov, Darienko, Lukešová, Hoffmann, *Cylindrocystis brebissonii* Meneghini, *Chlorella vulgaris* Beijerinck. Species with the high frequency rate exclusively in the calcificated soils were: fungi – *Actinomucor elegans* (Eidam) C. R. Beni et Hesselt, *Rh. japonicus* Vuill., *T. atroviride* Kasten, *Asp. ustus* (Bainier) Thom & Church, *P. glauco-cinerascens* Chalabuda, and algae – *Phormidium breve* (Kutz. Ex Gomont) Anag. et Komar, *Plectonema terebrans* Born. et Flah., *Eustigmatos magnus* (B. Petersen) Hibberd, *Oscillatoria limosa* Agardh.

The species density on one plot varied from 20 to 35 (mean $29,6 \pm 1,4$) micromycetes species and from 8 to 27 (mean $18,8 \pm 1,5$) algae species in forest plantations, and on chalk outcrops these ranged from 12 to 33 (mean $20,6 \pm 2,8$) of micromycetes and from 8 to 20 (mean $15,6 \pm 1,5$) of algae species. However, the relative inter-plot variability of fungi species

contents was higher on the chalk outcrops; in particular, the proportion of fungi species found on one plot only was 44 % *versus* 33 % in cultivated forests. The opposite effect was observed for algae: the proportion of species found on one plot only was higher in forest soils (43 %) than in calcificated soils (36 %).

Thus, the species heterogeneity counterbalanced the mean species density in case of fungi, but these two parameters mutually enhanced one another in case of algae. Due to this, a formal comparison of the overall species diversity between two studied types of biocenoses showed that mycobiota was equally rich in cultivated forests and on chalk hills: both contributed on average 7,75 intraspecific taxons per plot. By contrast to that, the total abundance of soil algaeflora was on average higher in forest plantations (6,6 species contributed by one plot) than on chalk outcrops (4,5 species and 5,25 intraspecific taxons contributed by one plot).

That was rather unexpected finding. The type of trophism implies a much stronger dependence of fungi than of algae on soil richness in organic matter. The latter was markedly higher in forest litter and humus horizons, compared to calcificated soils, and for micromycetes that provided a positive effect on species density but not, for some reason, on the species diversity. Moreover, autotrophic algae have obvious needs in the insolation rate, which was definitely higher on the chalk outcrops than on the forest understore soil surface. However, in general comparison between forest and chalk outcrops in our study the overall species diversity of algae and their species density both demonstrated a negative correlation with the insolation factor, and the reasons for this remained unclear.

Conclusions

Such surprising, inverse effects highlight the necessity of further ecologically focused floristic studies attempting to reveal possible mechanisms, which might govern the formation of the overall species diversity and mean species density of different groups of soil microorganisms in different terrestrial ecosystems. The floristic analysis of micromycetes and algae in soils of man-planted forests and chalk outcrops showed that the systematic structures of mycobiota and algaeflora of studied territories appeared to be typical for Eastern European woodland soils, with the largest contributions to the total species numbers made by *Penicillium* fungi and Chlorophyta and Cyanophyta algae, respectively. Within the total list of species identified in this study about 16 % of micromycetes species and 14 % of algae species were simultaneously present in cultivated forests and chalk outcrops. The overall distribution of these common species among all studied plots seems to be close to random, with some tendency of their higher numbers on the plots with leaf tree covering.

The formal comparison between two studied types of biocenoses for the overall species diversity and the mean species density per plot showed that mycobiota was equally rich in cultivated forests and on chalk hills, but the total abundance of soil algaeflora was on average slightly higher in forest plantations than on chalk outcrops. Within each location, the diversity of soil micromycetes and algae showed a complex pattern of dependence on the soil characteristics, microclimate and phytocenotic factors. The species contents resemblance on different plots were primarily determined by the physical distance between the studied sites. This effect was more pronounced for micromycetes than for algae, meanwhile the latter's comparative diversity were also associated with the presence of tree covering and its strain. In forest plantations and on chalk outcrops with wooden covering the inter-plot variations in algae species number had opposite directions to the concurrent changes of fungi species number. The inverse correlations of floristic parameters between two groups of microorganisms with different type of trophism, as well as somewhat paradox dependence of their diversity on the environmental factors can be potentially applicable for the monitoring of possible climatic or anthropogenic impacts on various terrestrial ecosystems.

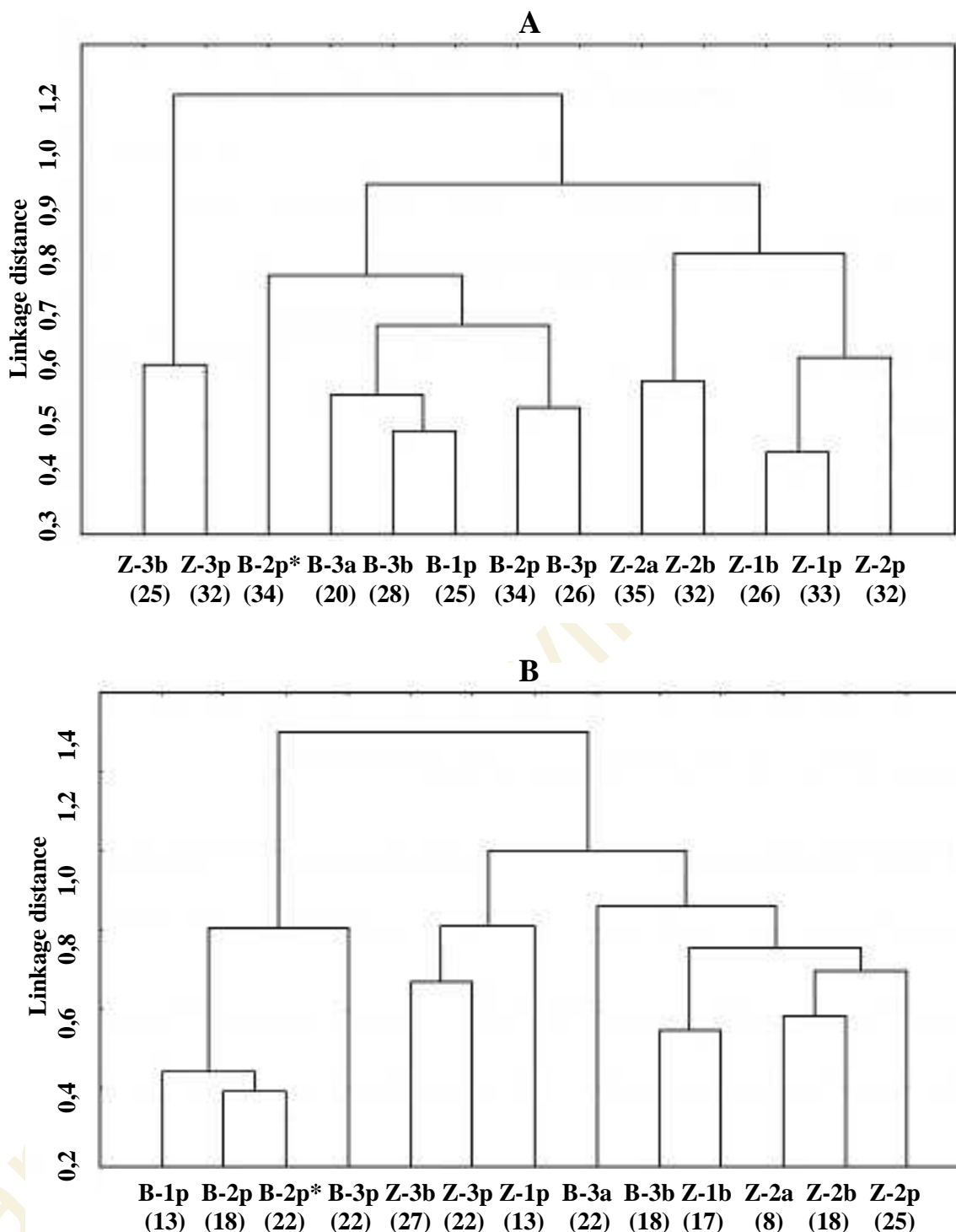


Fig. 1. A relative similarity of the species contents in cultivated forest plots: A – micromycetes, B – algae.

Plots are designated as location (Z – near v. Zadonetkoye, B – around l. Borovoye), wetness level (1 – dry, 2 – fresh, 3 – wet) and the tree strain (p – pine, b – birch, a – asp). Additionally, one of the fresh pine stands at the Borovoye site (B–2p*) was heavily injured by the root pathogenic fungus *Heterobasidion annosum*, and the nearest plot (B–2p) was established as a non-injured control. The linkage distance was estimated by Pearson – Bravet coefficient. The numbers of species found are given in parentheses under the plot code.

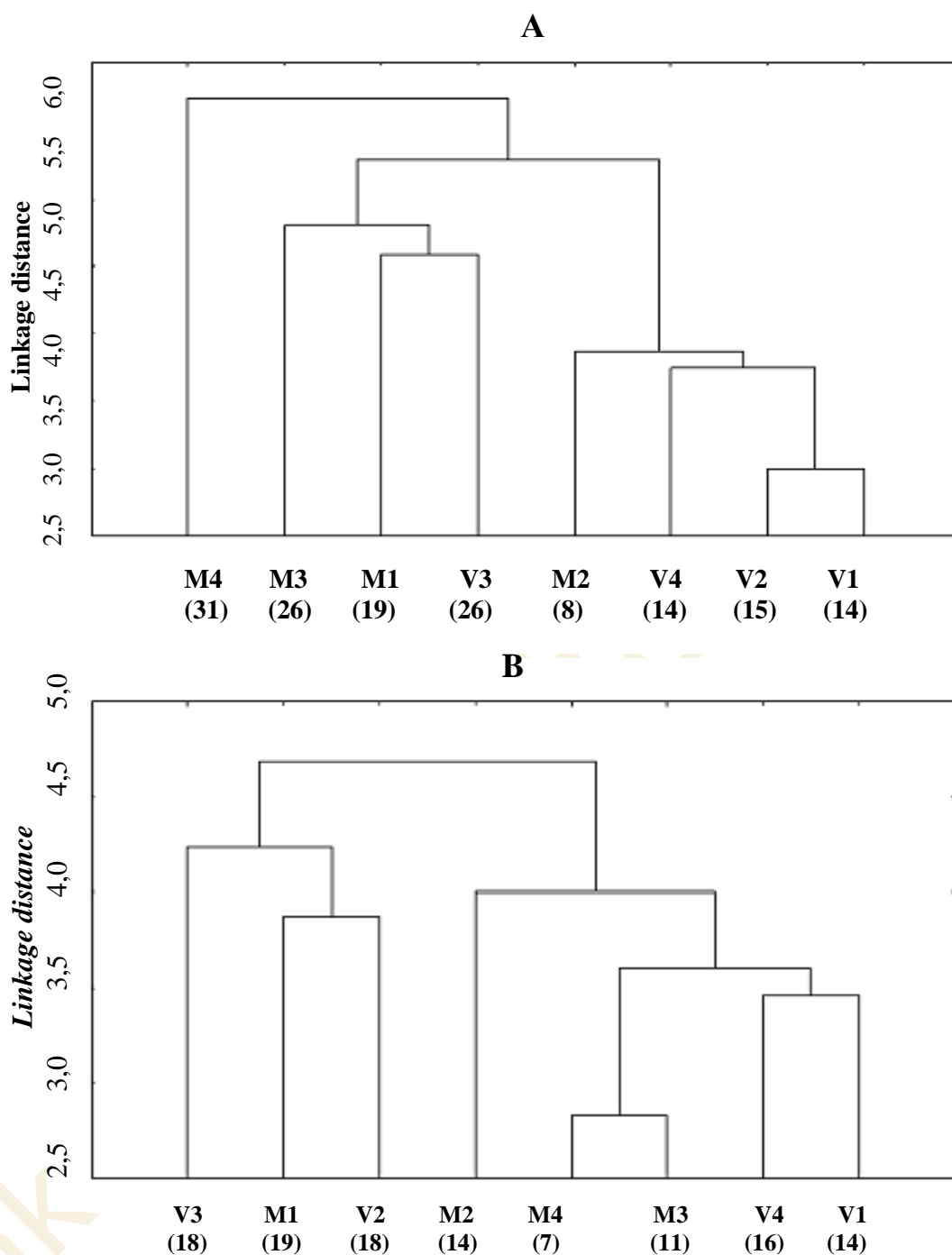


Fig. 2. A relative similarity of the species contents on the chalk outcrop plots, estimated by Pearson – Bravet coefficient: A – micromycetes, B – algae.

Plots are designated as location (V – near v. Volchanskiye Chutora, M – near v. Malaja Volchja) and type of covering: 1 – chalk hills with grass covering about 10 % (in M-group also totally covered by the lichen *Colemma tenax*), 2 – chalk hills with grass covering about 20-30 %, 3 – chalk hills covered with thickets of *Prunus spinosa* or man-planted *Pyrus communis*, 4 – hills with gleysol outcrops covered with thickets of *P. spinosa* or man-planted *P. communis*. The linkage distance was estimated by Pearson – Bravet coefficient. The numbers of species found are given in parentheses under the plot code.

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