Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
12(1): 136–146 (2022) ISSN 2229-2225
www.creamjournal.org
Article
Doi 10.5943/cream/12/1/11

Diversity of microfungi of coal mine spoil tips in the Magadan Region, Russia

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Iliushin VA, Kirtsideli IYu, Sazanova NA 2022 – Diversity of microfungi of coal mine spoil tips in the Magadan Region, Russia. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 12(1), 136–146, Doi 10.5943/cream/12/1/11

Abstract

The aim of this study was to explore the dynamics of microfungi complexes of coal mine spoil tips during the process of their overgrowth in the forest-tundra zone. The fungal complexes of the coal mine spoil tips around the town Susuman (Magadan Region, Russia) were investigated. Samples were taken from coal mine spoil tips with different ages (formed, 12-years, 25-years and 43-years). Forest-tundra soils were used as the control. The species composition, level of diversity, dominant groups of species and isolate density of fungal communities were studied. The rocky substrate of the spoil tips and harsh climatic conditions determined the relatively low diversity of microfungi. Only 12 species of microfungi were identified in the samples from the formed spoil tip, while in the samples from the 43-year-old tip the number of microfungi species increased to 24. The average density of microfungi (CFU/g) increased with the age of spoil tips. The composition of the dominant species of microfungi changed with the age of the spoil tips increased.

Keywords – biodiversity – coal mining – Far East – forest-tundra – microscopic fungi – succession

Introduction

The Magadan Region is located in the northern part of the Russian Far East. Most of the Magadan Region is located in the Kolyma River basin and is characterized by the greater climate continentality (Gerasimov 1970, Galanina et al. 2021). Despite the harsh climatic conditions, this region is of great economic importance in Russian.

Susuman is a town located in the forest-tundra zone. This territory is referred to the Verkhoyansk province of the East Siberian subregion of the Boreal floristic region (Yurtsev 1974). Despite the economic potential of the Magadan Region, few studies have been devoted to the mycobiota of this region. Most of the research is devoted to macrofungi of the Magadan Region (Sazanova 2015, Volobuev et al. 2019, Rebriev et al. 2020). Various studies have been devoted to fungal communities in the soils of tundra and forest-tundra (Egorova 1986, 2003, Grishkan 1994, 1997), larch forests (Egorova 2008, 2009) and phytopathogens (Azbukina & Karatygin 2010, Sazanova et al. 2017, Dokuchaeva & Dokuchaev 2020). However, the fungal communities of the territories of the Magadan Region, which were subjected to the greatest anthropogenic impact, have not been practically investigated.

Microfungi have a central role in the recovery of coal mine spoil tips and formation of young soils. They participate in biogenic weathering, organic matter decomposition, formation of humus

Submitted 18 March 2022, Accepted 16 August 2022, Published 6 September 2022
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(Detheridge et al. 2018). Some groups of fungi are also involved in the formation of mycorrhiza with plants-pioneers and are capable of destroying the structure of coal (Wise 1990, Detheridge et al. 2018).

The first studies of microfungi of coal mine spoil tips were carried out in Germany (Lieske & Hofmann 1928). In the Russian Far East, studies of the diversity of microfungi of spoil tips were conducted by Egorova et al. (2013). The Vorkuta district of the Komi Republic is the only northern area where the biodiversity of microfungi of coal mine spoil tips have been studied (Khabibullina et al. 2015, Iliushin & Kirtsideli 2021). The mycobiota of spoil tips of the Magadan Region has not yet been studied.

The purpose of this study was to explore the dynamics of microfungi complexes of coal mine spoil tips during the process of their overgrowth in the forest-tundra zone. Due to the presence of the spoil tips of different ages near Susuman, it is possible to compare the mycobiota of the spoil tips, taking into account the age that has passed since their formation.

Materials & Methods

Sampling

The research materials were taken from four spoil tips of the coal mine “Tal-Yuryakh” near the town of Susuman (62°47′ N, 148°09′ E) Magadan region in august 2020. The samples were collected from formed (exploited), 12-year-old, 25-year-old and 43-year-old spoil tips of the coal mine. Soils of the forest-tundra (78°05'50"N, 14°13'05"E) were used for comparison with the tips of the coal mine that we studied. The samples of spoil tip rock were collected from different depths. Geobotanical descriptions were performed on the selected sites, soil sections were made and average samples of soil and spoil tip rock were selected (Table 1). All samples were stored at 4°C.

Table 1 Sampling locations and descriptions.

Samples	Coordinates	Plant Projective cover	Sampling depths
Formed (exploited) spoil tip	63°20'10"N 146°37'10"E	0%	1 (0-10 cm)
12-year-old spoil tip	63°18'38"N 146°40'53"E	40%	1 (0-10 cm) 2 (10-20 cm) 3 (20-40 cm)
25-year-old spoil tip	63°18'23"N 146°43'22"E	50%	1 (0-10 cm) 2 (10-20 cm) 3 (20-40 cm)
43-year-old spoil tip	63°18'16"N 146°39'13"E	60%	1 (0-10 cm) 2 (10-20 cm) 3 (20-40 cm)
Control site (soils of Larch-dwarf cedar-shrub-lichen oppressed sparse forest, forest-tundra)	63°16'47"N 146°42'10"E	100%	1 (0-3 cm) 2 (3-20 cm) 3 (20-50 cm)

Isolation, incubation and identification

The soil dilution plate method was employed to isolate microfungi (Davet & Rouxel 2000). Fungi were cultivated on solid media Czapek agar (CZ) (Raper & Thom 1949) at 20 °C and 4 °C in the dark.

The isolates were initially identified by morphological identification using the most common determinant after their isolation in pure culture (Egorova 1986, Domsch et al. 2007). For micromorphological examination, microscopy by Carl Zeiss AxioImager A1 was employed.

The cultures with difficult morphological identification were also identified by molecular methods. DNA was extracted by using a DiamondDNA Plant kit (ABT, Russia, Barnaul) according to the manufacturer's instructions. Internal transcribed spacer rDNA region (ITS1-5.8S-ITS2) was applied as a phylogenetic marker and amplified using the PCR-primers ITS1 (5′-TCC-GTA-GGT-GAA-CCT-TGC-GG-3′) and ITS4 (5′-TCC-TCC-GCT-TAT-TGA-TAT-GC-3') (White et al. 1990).

Sequences were inspected using BioEdit version 7.1.9. The newly obtained sequences were compared with the available sequences by using a BLAST in the GenBank. The following criteria proposed by Godinho et al. (2013) were applied for the interpretation of sequences from the GenBank database: the genus and species were accepted for sequence identities ≥ 98%; only the genus was accepted for sequence identities between 95% and 97%.

The names of fungal taxa were unified using the Index Fungorum database (http://www.indexfungorum.org/).

Data analyses

The density of fungal isolates was expressed in colony-forming units per 1 g of absolutely dry soil (CFU/g). Colonies were counted after 10 days (20 °C) and 30 days (4 °C) of cultivation. The relative abundance of species and genera was used to assess the contribution of each species and genus to the structure of the mycobiota (Grishkan et al. 2008).

The tree clustering method was used to study the similarity of fungi complexes. The compositional distance was assessed using Percent disagreement. The distances between clusters were estimated using the Ward method (Ward 1963). The construction of dendrograms was carried out using the program Statistica 10.0.

We used the approach based on the algorithm for generating samples to estimate the expected number of species in the areas studied (Colwell et al. 2012). Statistical processing was performed using the program EstimateS 9.

Results

Structure of microfungal complexes

From the research, 58 species of microfungi from 33 genera were identified (Table 2). The specific abundance of the main genera of fungi in coal mine spoil tips is shown in Fig. 1.

Only 12 species of microfungi from nine genera were identified in the samples of formed (exploited) spoil tip. The percentage of *Pseudogymnoascus* isolates was 46%, the share of the genus *Penicillium* was 16%, and the percentage of *Microsphaeropsis* was 14%. The specific abundance of each genus *Cladosporium*, *Leucosporidium*, *Mortierella*, *Phialocephala* and *Tricladium* did not exceed 10%.

The species diversity of microfungi increased to 17 species from 12 genera in the rock of the 12-year-old spoil tip. The percentages of the genera *Penicillium*, *Pseudogymnoascus* and *Cladosporium* were 36%, 25% and 19%, respectively. The genus *Pseudogymnoascus* was represented by only one species *Pseudogymnoascus pannorum*.

Representatives of 22 species from 15 genera were identified in samples taken from the 25-year-old spoil tip. The dominant genera were also *Penicillium* and *Pseudogymnoascus*. The percentages of these genera were 46% and 25%, respectively. The percent of other genera did not exceed 7%.

The species diversity in the 43-year-old spoil tip was 24 species from 16 genera. The percentage of *Pseudogymnoascus* was the same (25%) as in the previous spoil tip. The percentage of fungi of the genus *Penicillium* decreased to 37%. The shares of *Ochrocladosporium* (10%) and *Mortierella* (11%) were significant.

As a result of the research conducted, 41 species of microfungi belonging to 28 genera were identified in the soils of the forest-tundra (Table 2). The species and genera diversity of forest-tundra soils were significantly higher than in the spoil tips. The dominant genera were *Pseudogymnoascus* (21%), *Penicillium* (16%), *Glaciozyma* (10%), *Mortierella* (10%) and *Aspergillus* (8%).

Table 2 Density of culturable microfungi of coal mine spoil tips and soil of the forest-tundra (control), CFU/g. (sampling depths: 1 – upper layer, 2 – middle layer, 3 – bottom layer).

	Accession Number	**Formed spoil tip**	**12-year-old spoil tip** (sampling depths)			**25-year-old spoil tip** (sampling depths)			**43-year-old spoil tip** (sampling depths)			**Horizons of control** (soils of forest-tundra)		
			1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Alternaria consortialis (Thüm.) J.W. Groves & S. Hughes	-	0	0	0	0	252	0	0	0	0	0	2982	462	34
Alternaria phragmospora Emden	**OL989268**	0	11	0	0	0	0	0	0	0	0	0	0	0
Articulospora sp.	**OL989295**	0	0	0	0	0	0	0	0	0	0	1260	84	0
Aspergillus terreus Thom	-	0	0	0	0	1764	252	0	1904	336	0	7098	84	34
Botrytis cinerea Pers.	-	0	0	0	0	0	0	0	112	0	0	0	0	0
Cadophora melinii Nannf.	**OL989263** **OL989293**	0	4	4	7	101	662	189	448	0	14	84	126	336
Cladophialophora humicola Crous & U. Braun	**OL989265**	0	0	0	0	151	32	63	0	0	0	0	0	0
Cladosporium cladosporioides (Fresen.) G.A. de Vries	-	0	39	4	0	101	32	0	112	0	0	504	126	0
Cladosporium herbarum (Pers.) Link	-	17	116	25	4	101	32	0	560	112	0	1092	42	34
Cladosporium sinuosum K. Schub., C.F. Hill, Crous & U. Braun	**OL989276**	6	70	7	0	0	0	0	0	0	0	0	0	0
Cladosporium sphaerospermum Penz.	-	0	7	0	0	101	95	189	336	224	14	210	252	571
Cosmospora berkeleyana (P. Karst.) Gräfenhan, Seifert & Schroers	-	0	0	0	0	2974	284	32	112	0	14	42	42	34
Cyathicula sp.	**OL989273**	0	0	0	0	0	0	0	0	0	0	0	0	168
Fusarium fujikuroi Nirenberg	-	0	0	0	0	0	0	0	4368	448	14	84	126	0
Fusarium oxysporum Schltdl.	-	0	4	4	4	0	0	0	0	0	0	0	0	0
Fusarium tricinctum (Corda) Sacc.	-	0	0	0	0	0	0	0	0	0	0	0	42	0
Fusicolla aquaeductuum (Radlk. & Rabenh.) Gräfenhan, Seifert & Schroers	-	0	0	0	0	0	0	0	0	0	0	42	0	0
Fusicolla merismoides (Corda) Gräfenhan, Seifert & Schroers	-	0	0	0	0	0	0	0	112	0	0	42	0	0
Geomyces vinaceus Dal Vesco	**OL989294**	0	53	53	18	0	0	0	0	0	0	126	2940	34
Glaciozyma antarctica (Fell, Statzell, I.L. Hunter & Phaff) M. Groenew. & Q.M. Wang	-	0	0	0	0	0	0	0	0	0	0	6048	2226	67

Table 2 Continued.

	Accession Number	**Formed spoil tip**	**12-year-old spoil tip** (sampling depths)			**25-year-old spoil tip** (sampling depths)			**43-year-old spoil tip** (sampling depths)			**Horizons of control** (soils of forest-tundra)		
			1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Humicolopsis cephalosporioides Cabral & S. Marchand	**OL989261** **OL989262**	0	0	0	0	202	0	0	0	0	0	2100	2982	101
Hyalodendriella betulae Crous	**OL989275**	0	0	0	0	0	0	0	0	0	0	42	0	0
Hyaloscypha hepaticicola (Grelet & Croz.) Baral, Huhtinen & J.R. De Sloover	**OL989260**	0	0	0	0	0	0	32	112	0	0	42	42	370
Juxtiphoma eupyrena (Sacc.) Valenz. - Lopez, Crous, Stchigel, Guarro & Cano	-	0	7	0	11	504	725	1575	560	112	210	126	588	874
Leptodontidium sp.	**OL989266**	0	0	0	0	0	0	0	0	0	0	0	42	0
Leucosporidium creatinivorum (Golubev) M. Groenew. & Q.M. Wang	**OL989287** **OL989288**	11	0	0	0	554	0	32	0	0	0	0	0	67
Leucosporidium drummii Yurkov, A.M. Schäfer & Begerow	**OL989286**	11	0	0	0	454	158	284	0	0	0	168	630	1579
Microsphaeropsis olivacea (Bonord.) Höhn.	**OL989274**	45	0	0	0	0	0	0	0	0	0	0	0	0
Mortierella alpina Peyronel	**OL989271**	6	11	49	28	403	158	32	3248	3360	280	2268	3528	1882
Mucor hiemalis Wehmer	-	0	4	4	0	504	1985	32	112	224	140	2184	252	168
Ochrocladosporium frigidarii Crous & U. Braun	**OL989269**	0	0	0	0	0	0	0	3360	3920	70	0	0	0
Penicillium camemberti Thom	**OL989291**	28	0	0	7	50	284	441	0	0	0	0	0	0
Penicillium canescens Sopp	-	0	0	0	0	0	0	0	112	2464	112	0	0	0
Penicillium citrinum Thom	-	0	4	0	0	0	0	0	0	0	0	0	0	0
Penicillium dipodomyicola (Frisvad, Filt. & Wicklow) Frisvad	**OL989283**	0	0	0	0	0	0	0	8848	1344	1078	0	0	0
Penicillium glabrum (Wehmer) Westling	-	0	0	0	0	1260	1071	95	336	4032	28	5082	420	68
Penicillium italicum Wehmer	-	0	0	0	0	0	0	0	112	112	14	42	0	0
Penicillium janczewskii K.W. Zaleski	-	0	350	105	18	0	0	0	1344	0	0	6384	420	0
Penicillium jensenii K.W. Zaleski	-	22	0	0	0	0	0	0	0	0	0	0	0	0

Table 2 Continued.

	Accession Number	Formed spoil tip	**12-year-old spoil tip** (sampling depths)			**25-year-old spoil tip** (sampling depths)			**43-year-old spoil tip** (sampling depths)			**Horizons of control** (soils of forest-tundra)		
			1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Penicillium lanosum Westling	**-**	0	0	0	0	0	0	0	0	0	0	0	42	336
Penicillium maclennaniae H.Y. Yip	**OL989280**	0	0	0	0	0	0	0	0	0	0	42	0	0
Penicillium oxalicum Currie & Thom	**-**	0	0	0	0	3276	5922	347	0	0	0	0	0	0
Penicillium solitum Westling	**-**	0	0	0	14	0	0	0	0	0	0	0	0	0
Penicillium spinulosum Thom	**OL989277**	0	0	0	0	5090	3465	1103	1232	0	0	0	42	101
Penicillium velutinum J.F.H. Beyma	**-**	0	0	0	0	101	32	0	0	0	0	336	84	0
Periconia sp.	**OL989264**	0	0	0	0	0	0	0	0	0	0	0	42	0
Phialocephala dimorphospora W.B. Kendr.	**OL989267**	6	0	0	0	0	0	0	0	0	0	0	0	0
Phialocephala fortinii C.J.K. Wang & H.E. Wilcox	**OL989259**	0	0	0	0	0	0	0	0	0	0	630	84	0
Phialocephala lagerbergii (Melin & Nannf.) Grünig & T.N. Sieber	**OL989292**	0	0	0	0	0	0	0	0	0	0	0	0	67
Phoma herbarum Westend.	**-**	0	0	0	0	353	252	284	0	0	14	42	84	302
Pseudogymnoascus pannorum (Link) Minnis & D.L. Lindner	**OL989278** **OL989279** **OL989284** **OL989289** **OL989290**	123	14	284	56	4334	5859	1670	8512	7952	518	7938	4872	3931
Pseudogymnoascus roseus Raillo	**OL989270**	22	0	0	0	0	0	0	0	0	0	0	0	0
Solicoccozyma aeria (Saito) Yurkov	**OL989285**	0	0	0	0	0	0	0	0	0	0	42	1302	34
Tolypocladium inflatum W. Gams	**OL989282**	0	0	0	0	0	0	0	0	0	0	0	126	0
Tolypocladium sinense C. Lan Li	**OL989281**	0	0	0	0	0	0	0	0	0	0	0	0	134
Trichoderma aureoviride Rifai	**-**	0	7	0	0	0	0	0	112	112	14	0	42	168
Trichoderma viride Pers.	**-**	0	0	0	0	0	0	0	336	112	0	0	462	0
Tricladium obesum Marvanová	**OL989272**	22	0	0	0	0	0	0	0	0	0	0	0	0

Density of microfungi

The density of microfungi (CFU/g) in the formed and 12-year-old spoil tips was very low (Fig. 2). The density increased with the age of spoil tips. The density of microfungal isolates in the 12-year-old spoil tip was 45 times lower than in the 43-year-old spoil tip (cultivation at 20 °C). In turn, the density of microfungi in the 43-year-old spoil tip almost reached the density in the control site and was only 1.3 times lower. The density of microfungal isolates decreases with depth in coal mine spoil tips.

The density also increased with age of spoil tips at 4 °C. However, the density of microfungal isolates in this cultivation regime was lower since fungi did not develop, for which the minimum growth temperature was higher than 4 °C.

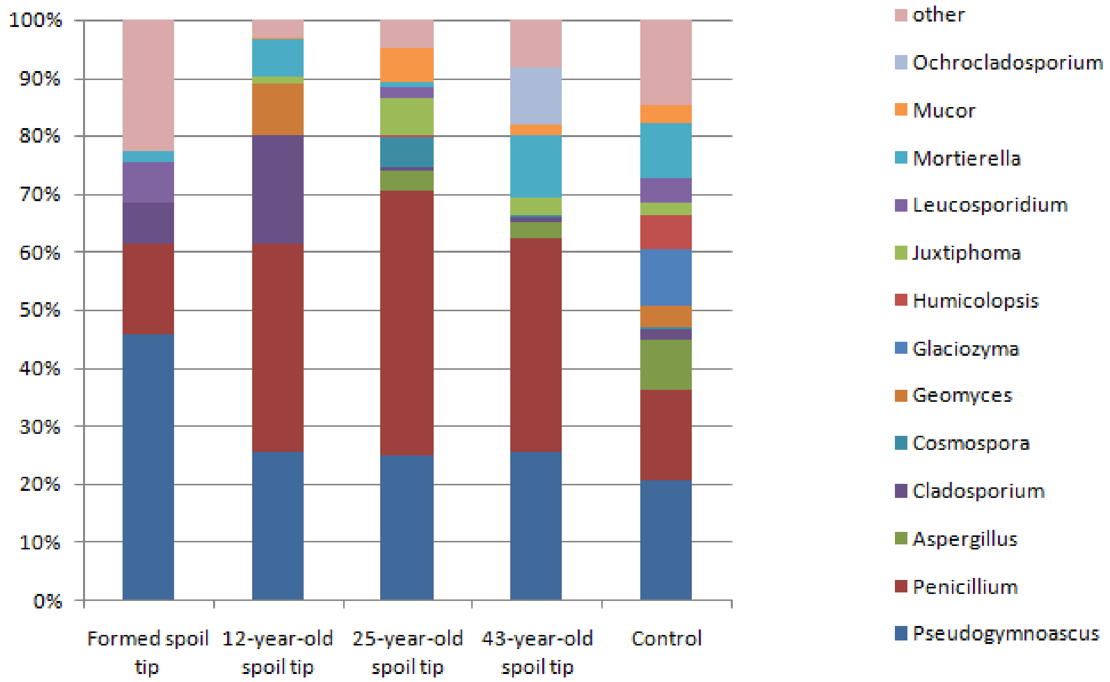

Fig. 1 – Specific abundance of the genera of microfungi in coal mine spoil tips.

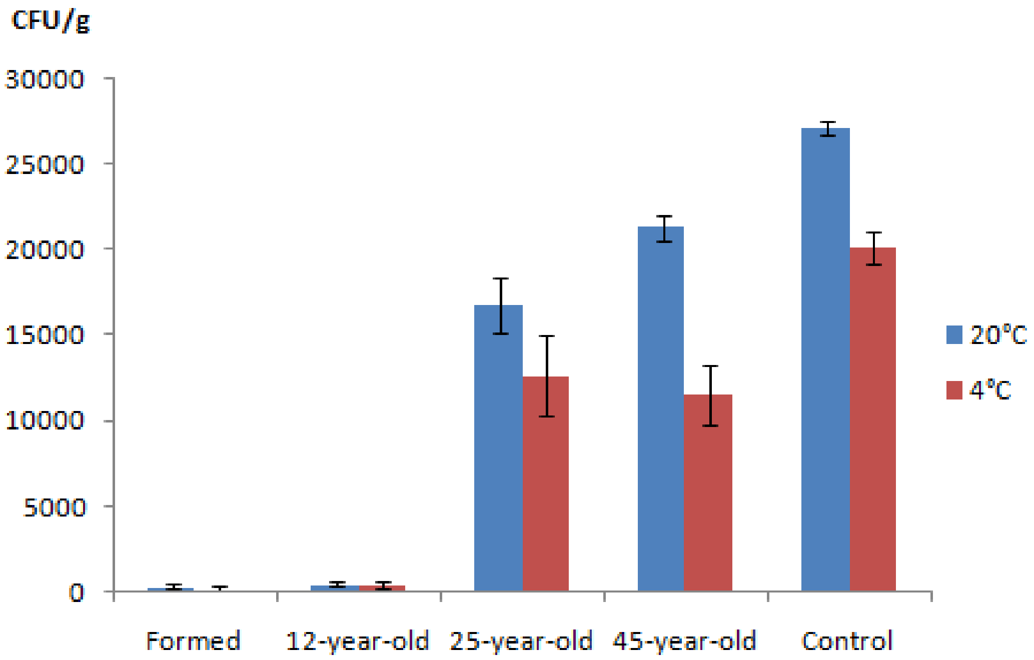

Fig. 2 – The density of microfungi (CFU/g) in the coal mine spoil tips at 20 °C and 4 °C (medium for sampling depths).

Comparison of the fungal complexes

From the data presented in the dendrogram (Fig. 3), complexes of microfungi are divided into three big clusters. The first cluster combines microfungi complexes from the formed spoil tip and different sampling depths of the 12-year-old spoil tip. The second cluster consists of microfungi complexes from the 25-year-old spoil tip and 43-year-old spoil tip. It should be noted that the second cluster also includes the third soil horizon of the control site. The similarity to the poorest soil horizon indicates that the process of the formation of mycobiota, which is characteristic of the natural soils of this region, is underway. The third cluster includes complexes of microfungi from two upper horizons of forest-tundra soil.

Thanks to the calculation of the maximum average Chao1 index, we have identified most of the expected species in the formed, 12-year-old and 25-year-old spoil tips (92%, 100% and 100%, respectively). 83% of expected species were identified in the 43-year-old spoil tip. The lowest level of species identification was expected in the richest in species control soil site (75%).

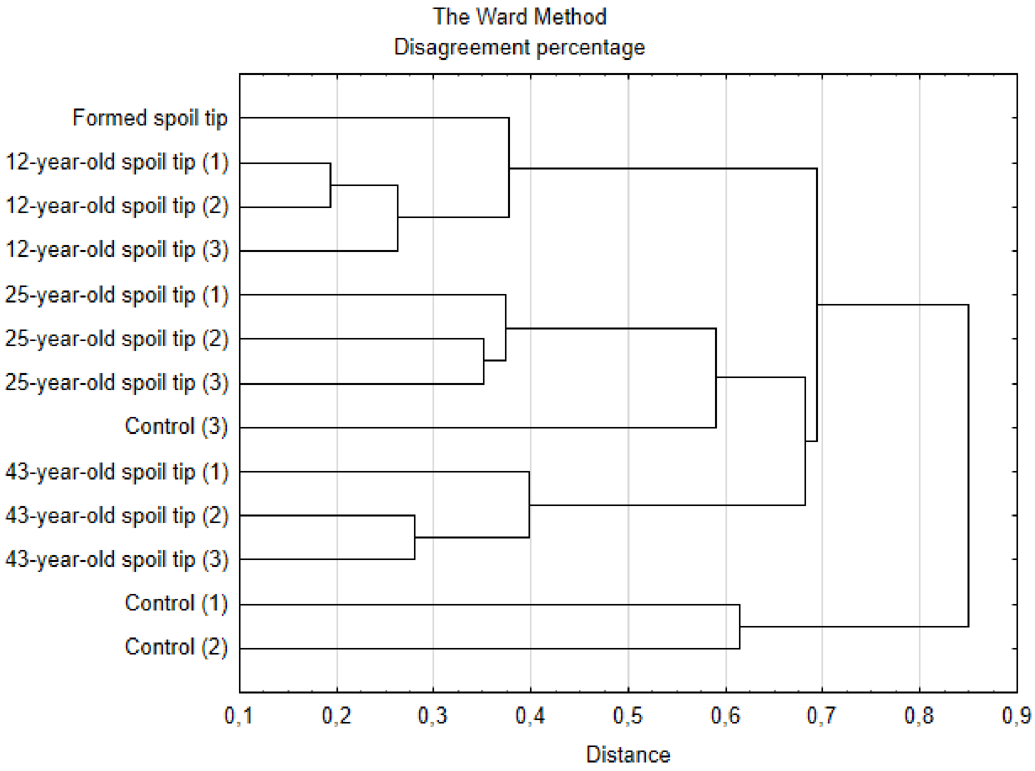

Fig. 3 – Dendrogram of the similarity of the microfungal complexes of coal mine spoil tips, taking into account separation by layers (sampling depths).

Discussion

The presence of *Pseudogymnoascus* was observed in all studied spoil tips of coil mine with a percentage of 25–46%. This genus was represented by two species *P. pannorum* and *P. roseus*. *P. pannorum* is noted as dominant in Arctic and Antarctic soils and permafrost samples. The ability to colonize substrates unsuitable for other species explains the prevalence of *P. pannorum* in spoil tips (Hayes 2012). *P. pannorum* is also found in northern disturbed ecosystems. For example, it was discovered in the coal mine spoil tips of the Vorkuta district of the Komi Republic (Khabibullina et al. 2015, Iliushin & Kirtsideli 2021).

The high percentage of *Penicillium* species in spoil tips may be attributed to its omnipresent nature. This genus is widespread and has been observed in a wide variety of habitats because it can colonize different substrates and can displace other less competitive species (Visagie et al. 2014). *Penicillium* species are present in many rock tips of different natures, including coal spoil tips (Elhottova et al. 2006, Marescotti et al. 2013, Khabibullina et al. 2015).

The percentage of fungi of genus *Cladosporium* was at the level of 7–19% in the formed and 12-year-old spoil tips. Microfungi of this genus are distributed throughout our planet and occupy many ecological niches (Bensch et al. 2012). On the contrary, fungi of the genus *Aspergillus* presented only in late tips and control soils.

The density of microfungi (CFU/g) increased with the age of spoil tips. This indicator was due to the gradual improvement of soil habitat. So, it was noticed that the density of fungi increased as the coal tips were recovered and what is it related to organic matter accumulation (Mummey et al. 2002).

The soils of the north are characterised by a predominance of microfungi in the upper horizon. The density of propagules decreases with depth, from the litter to the mineral horizons (Kirtsideli et al. 2011). We observed this trend in all spoil tips.

On the whole, these data indicate a significant change in the isolate density and species composition of microscopic fungi in coal mine spoil tips in the process of succession. The studied spoil tips represent a series of habitats in which the species composition and dominant groups of these species change, and the isolate density and diversity level gradually increases. The convergence of the structure of microfungal complexes of spoil tips and control site soils indicates a gradual increase in the stability of mycobiota in disturbed areas.

Acknowledgements

This study was carried out as part of the state assignment according to the thematic plan of the Botanical Institute of the Russian Academy of Sciences (theme No AAAA-A19-119020890079-6). The research was done using equipment of The Core Facilities Center “Cell and Molecular Technologies in Plant Science” at the Komarov Botanical Institute RAS (St.-Petersburg, Russia). The authors are grateful to the employees of Kolymskaya Coal Company LLC S.V. Smirnov, A.N. Filipov, A.K. Zubitsky for help with sampling.

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