Microbiology, Vol. 74, No. 1, 2005, pp. 91–96. Translated from Mikrobiologiya, Vol. 74, No. 1, 2005, pp. 104–110.

EXPERIMENTAL ARTICLES

The Structure of Micromycete Communities and Their Synecologic Interactions with Basidiomycetes during Plant Debris Decomposition

V. A. Terekhova*, and T. A. Semenova*,****

**Institute of Soil Science, Moscow State University and Russian Academy of Sciences, Moscow, Russia*

***Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii pr. 33, Moscow, 117071 Russia*

Received March 24, 2003; in final form, June 10, 2004

Abstract—We investigated the interactions between micromycetes and basidiomycete mycelium on plant substrates in the course of their 3-year incubation in the litter of ecologically intact spruce forests of the Central Forest State Biosphere Reservoir (Nelidovo district, Tver oblast). Only 40–60% of the micromycetes were involved in direct antagonistic interactions with basidiomycetous fungi. In terms of the ratio between physiologically active strains and those that did not interact with basidiomycete mycelium, we revealed differences in the structure of micromycete communities developing on various types of substrates (wood, bark, sphagnum moss, leaves, needles, litter, and cotton grass). The micromycetes tested belonged to 49 species. At the end of the observation period, the fraction of microscopic fungi that actively influenced basidiomycete mycelium was four times lower in the inactive litter fraction (lignin-containing wood debris) than in the active fraction (grass substrates). The mechanisms of indirect regulation of the structure and functions of micromycete communities are discussed, which may be based on the accumulation of phenolic compounds in the medium and changes in the enzyme activities of basidiomycete mycelium.

Key words: micromycetes and basidiomycetes, structure of fungal complexes, decomposition of plant debris.

Mutual regulation of the structure of the main components of the mycobiota is one of the key mechanisms that determine the rate of mineralization of plant debris in natural ecosystems. The mycelium of basidiomycetes, which produces active complexes of oxidative exoenzymes, is the main decomposer of cellulose- and lignin-containing substrates. Agaricoid fungi exert a significant influence on the microbiota formation under natural conditions, as shown for soil ecosystems [1]. In addition, microscopic soil fungi regulate the development of basidiomycetes.

The results of experimental studies reported in the literature reveal a wide spectrum of micromycete-produced biologically active compounds that control plant debris bioconversion [5]. The mechanisms of action of these substances and the structural and functional properties of the mycobiota performing various stages of plant mineralization in nature are difficult to study because the dynamics of plant decomposition should be investigated in conjunction with concomitant changes in micro- and macromycete communities.

This work used model systems in an attempt to single out binary interactions between macro- and micromycetes from the whole variety of intrasystem relationships, namely, to elucidate specific reactions involving colonies of soil micromycetes and those of a basidial macromycete. The goal of the work was to reveal changes in the structure of micromycete communities and in their interactions with basidiomycetes that occur in the process of decomposition of various kinds of plant debris.

MATERIALS AND METHODS

Micromycetes were isolated from plant substrates on permanent experimental grounds within the biotopes of typical southern taiga ecosystems of the Central Forest State Biosphere Reservoir (CFSBR, Tver oblast). Plant substrates of various kinds (bark, wood, sphagnum moss, cotton grass, spruce needles, and forest litter) were placed by sanctuary personnel in the litter of nemoral and boreal spruce forests located on experimental sites. Substrate samples were placed without disintegration in plastic net bags (10×10 cm) with a mesh diameter of 1 mm. The outdoor experiment was conducted for three years. The bags (ten for each of the experimental variants) were collected in spring and in autumn (at 6-month intervals) during the whole experiment. Micromycete colonies were obtained by a routine method: a water extract of the plant material was plated onto Czapek agar acidified to suppress the growth of bacteria [6]. The agaricoid Oyster fungus

0026-2617/05/7401-0091

Average values of certain parameters characterizing the structure of micromycete communities

Parameter	Nonwoody plant substrates	Woody substrates	All plant substrates
Micromycete density, N ($\times 10^3$ CFU/g substrate)	93.2	113.0	103.1
Species number, N_{sp}	6.06	5.48	5.77
Diversity of complexes, the Shannon criterion (H)	1.78	1.70	1.74

Pleurotus ostreatus Kuhn was used as a test culture. This fungus is an active decomposer of plant debris in the forest, concomitantly degrading lignin and cellulose-based biopolymers. This work used the collection strain H-5-3. An agar disk (0.6 cm in diameter) with 7- to 10-day-old basidiomycete mycelium was placed in the center of a Petri dish with malt agar. Micromycete inoculum isolated from plant substrates was placed on the periphery of the Petri dish, which was then incubated in a thermostat at 24°C for 4–7 days. Micromycetes were classified into three groups, depending on the pattern of their interactions with the macromycete. Group 1 included micromycetes that suppressed the development of the basidiomycete mycelium. Group 2 was composed of micromycetes that had no effect on the basidiomycete mycelium. Group 3 included micromycetes that stimulated the development of the basidiomycete mycelium.

RESULTS

A total of 142 species of microscopic fungi representing 40 genera were isolated from the plant substrates incubated in the litter of various ecosystems during the three-year experiment. Each sampling procedure yielded a relatively low number of species: the average species diversity index was 5.77. A comparative study of fungi from woody substrates and other habitats (leaves, needles, litter, and cotton grass) revealed no considerable differences (see table). However, the differences in the degree of substrate degradation were significant. Litter and leaves were virtually completely decomposed by the end of the experiment; cotton grass and needles were mineralized to a significant extent; and only sphagnum revealed no appreciable visible changes. In contrast, bark and wood fragments did not undergo such drastic changes during the incubation period, and only maceration of cellulose fibers occurred in some samples.

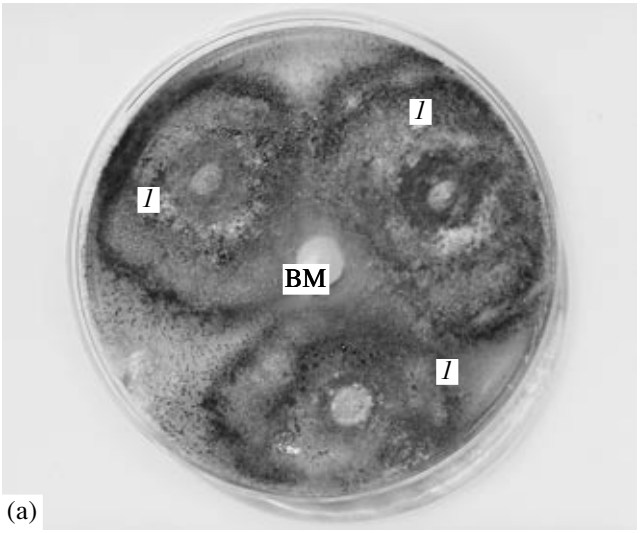
Typical inhabitants of the tested substrates included species of the genera *Penicillium, Thysanospora, Talaromyces, Paecilomyces, Oospora, Phialospora, Phoma, Septonema, Scopulariopsis*, and *Rhizopus*. A test study with basidiomycete mycelium used an array of colonies of micromycetes belonging to 49 species and displaying one of the three above interaction patterns. There were fungi with pronounced amylolytic (*Aureobasidium* sp. and *Mucor* spp.) and pectinolytic (*Phoma* sp.) activities, typical xylotrophs (*Chloridium* sp.), saccharolytic (*Verticillium* spp.) and cellulolytic (*Trichoderma* spp. and *Penicillium* spp.) species, and species that combine amylolytic and xylotrophic activities (*Acremonium* spp.). The tested fungi included a large number of producers of antibiotics and other biologically active substances [7].

The results obtained revealed that a majority (62.4%) of the micromycetes were fairly active with regard to the basidiomycete mycelium (Fig. 2a). Inhibitory activity was characteristic, e.g., of *Acremonium butyry, A. charticola, A. fusidioides, Aureobasidium pullulans, Monocillium dimorphosporum, Mortierella vinacea, Penicillium canescens, P. chrysogenum, P. crustosum, P. dierckxii, P. expansum, P. glabrum, P. griseofulvum, P. hirsutum, P. humuli, P. janczewskii, P. janthinellum, P. lividum, P. pinophilum, P. viridicatum, Phoma* sp., *Trichoderma aureoviride, T. harzianum, T. koningii, T. polysporum, T. virens*, and *T. viride.*

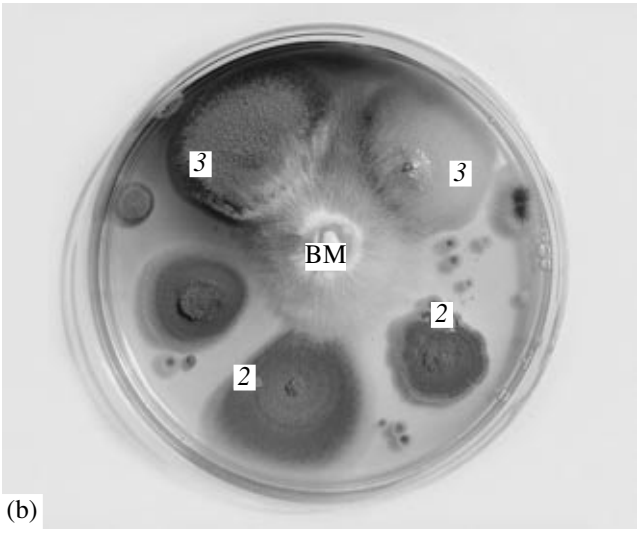
A significant part of the micromycete colonies (24.25%) had no effect on developing basidiomycete mycelium, and the micromycete hyphae only penetrated the basidiomycete colony or overgrew it without encountering visible obstacles. This pattern was displayed by *Chloridium viride, Cladosporium cladosporoides, C. sphaerospermum, Mortierella ramanniana, Paecilomyces lilacinus, Penicillium brevicompactum, P. aurantiogriseum, P. spinosulum, P. thomii*, and *P. verrucosum.*

Some micromycetes (14.2%) exerted a specific stimulatory influence on the development of oyster fungus mycelium, resulting in the development of more abundant aerial mycelium than under standard conditions or in the formation of peculiar tongue-shaped mycelium protrusions and mycelium strands in the part of the basidiomycete colony facing the micromycete colony (Fig. 1). This pattern was chiefly characteristic of some fungi of the genera *Acremonium, Mucor plumbeus, Mycelia sterilia*, (*Moniliaceae*), *Penicillium velutinum, P. rugulosum, P. purpurogenum, Gilmaniella humicola, Gliomastix murorum, Paecilomyces carneus*, and *P. farinosus.*

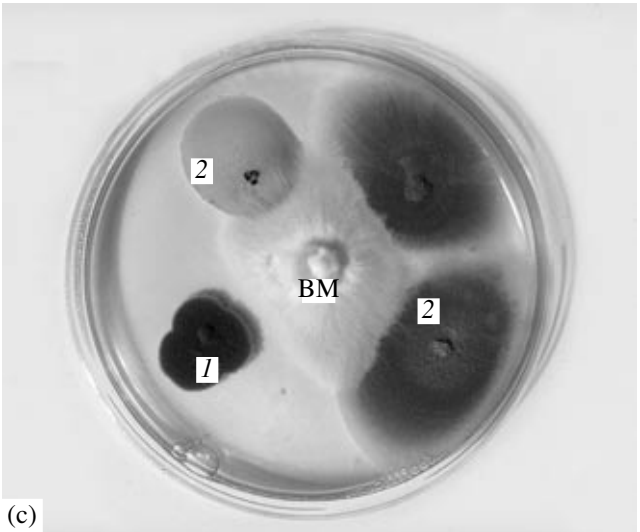
We performed a comparative analysis of the structure of the micromycetes developing on woody substrates (bark and wood) and on substrates arbitrarily termed herbal (litter, leaves, and cotton grass), which do not contain large bark or wood fragments and decompose in a relatively short time. This substrate type does not include sphagnum, which harbored



(a)



(b)



(c)

Fig. 1. Main types of effects of micromycete colonies on basidiomycete mycelium (BM): (*1*) inhibition; (*2*) no effect; (*3*) stimulation.

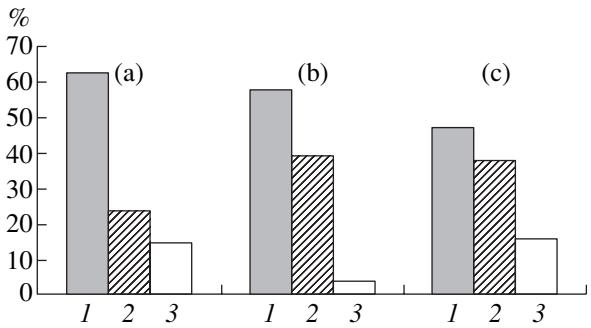


Fig. 2. Structure of micromycete communities (a) on all substrates, (b) on woody substrates, and (c) on herbal substrates in terms of the ratio between the colonies characterized by different effects on the mycelium of the basidiomycete test culture: (*1*) inhibitory; (*2*) neutral; (*3*) stimulatory.

numerous propagative structures of micromycetes but was not mineralized.

Based on the average values of the data obtained during the three-year research period, significant differences were revealed in the structure of micromycete complexes from different tested substrates (Figs. 2b, 2c). The percentage of micromycetes that inhibited the development of basidiomycete mycelium was lower on herbal than on woody substrates. However, the percentage of stimulatory micromycetes was almost four times lower in the complexes isolated from herbal debris. As for woody micromycete complexes, they were characterized by a high percentage of inhibitory micromycetes (45–75%), while the percentage of micromycetes that stimulated the development of Oyster fungus did not exceed 16%.

We carried out an analysis of the structure of micromycete communities developing on plant debris under various ecological conditions. We also investigated the dynamics of their interactions with basidiomycetes (Fig. 3). In contrast to woody micromycete complexes, fermentation of easily degradable substrates, such as foliage, cotton grass, and litter, resulted in dramatic changes in the structure of herbal micromycete complexes. This trend manifested itself in considerably different contents of physiologically active and inactive (with respect to the test basidiomycete culture) micromycetes at different degradation stages. Micromycete complexes on original herbal substrates (before their incubation in spruce forests) contained 66.7% basidiomycete-inhibiting fungi, 25.2% "neutral" fungi not affecting basidiomycete growth, and 8% basidiomycete-stimulating fungi. Amylolytic and pectinolytic fungi prevailed at the early stages of fungal succession. A large number of these fungi were capable of outcompeting the basidiomycete test culture and suppressing its development. Subsequently, fungi of the genus *Penicillium* also made a major contribution to the decomposition process. Initially, it was the oyster fungus–inhibiting species that predominantly developed. The later succession stages were characterized by the pres-

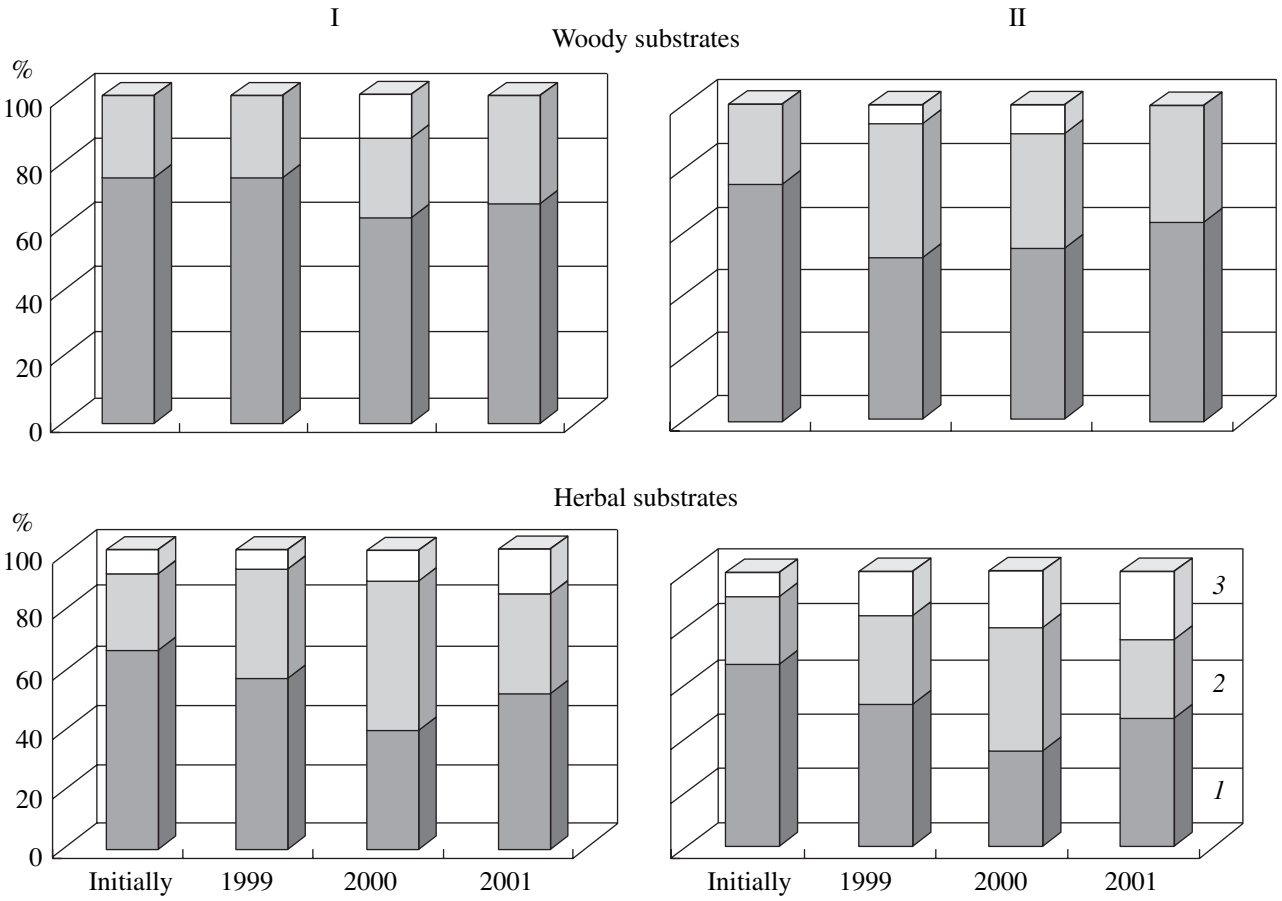


Fig. 3. Yearly dynamics of the structure of micromycete communities growing on woody debris in (I) boreal and (II) nemoral spruce forests, analyzed in terms of the ratio of micromycetes producing (*1*) inhibitory, (*2*) neutral, and (*3*) stimulatory effects on basidiomycete mycelium.

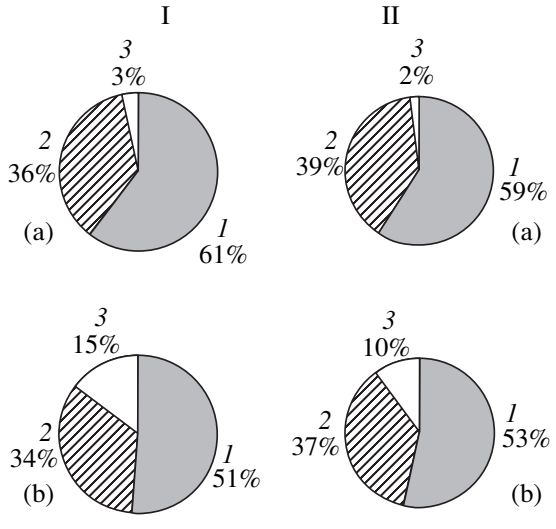


Fig. 4. Structure of the micromycete communities isolated in autumn from (a) woody and (b) herbal substrates in (I) boreal and (II) nemoral spruce forests. Micromycete effect on basidiomycete mycelium: (*1*) inhibitory; (*2*) neutral; (*3*) stimulatory.

ence of *Penicillium* species that had no appreciable effect on the basidiomycete development. The sterile mycelium of *Mycelia sterilia* (*Moniliaceae*) predominantly developed at the final stages of the decomposition process. It slightly stimulated the development of the oyster fungus in a number of studies. The percentage of basidiomycete-stimulating fungi increased two- to threefold (Fig. 3b) by the end of the observation time, when the bulk of the herbal substrates was already degraded.

The incubation conditions characteristic of plant substrates in the litters of the different tested ecosystems (the boreal and the nemoral spruce forests) did not produce any significant effect on the ratio between the three investigated groups of fungi in micromycete complexes. This follows from the data (Fig. 4) characterizing the micromycete structure on woody and herbal substrates.

DISCUSSION

The boreal and nemoral types of spruce forests of the CFSBR are characterized by different litter quanti-

ties. A number of hypotheses have been put forward to account for this difference in the tempo of plant debris decomposition [8]. Depending on the hypothesis adopted, the properties of the plant material, the hydrothermal conditions, or the structure of the biotic components of the soil are envisaged as the key factors. Obviously, all these factors are interrelated and the quantitative parameters of this relationship vary depending on the decomposition stage, as was shown in several studies on soil profiles [9–12].

Different species dominate the horizons of the forest litter at different decomposition stages. *Autobasidium pullulans, Mucor, Cladosporium*, and the macromycetes *Marasmius* and *Collybia* form the basis of the L layer. The F layer, which is characterized by the most active processes of cellulose and lignin degradation, is dominated by the cellulolytic species *Trichoderma, Chaetomium*, and *Mycogone* and by basidiomycetous fungi. The humus-rich H layer is predominantly inhabited by basidiomycetes. Of special interest are the functional mechanisms that account for the maintenance of these fungus–substrate relationships at various stages of plant debris mineralization and the factors that influence them.

In this work, we investigated the decomposition of two substrate types in terms of the structure and the dynamics of the synecologic relationships among the fungi involved. These substrate types are characterized by different amounts and properties of the plant debris accumulating on the soil surface in the tested plant ecosystems. Leaves, needles, and grass debris, along with tree bud scales, seeds, and flosculi, make up the active (rapidly decomposed) and the most abundant part of leaf debris. The inactive part of tree leaf debris (branches and bark) typically accounts for about 30% of the total quantity.

We revealed major differences between the biopolymer complexes of the tested types of plant substrates in terms of the relative contents of polysaccharides (cellulose and hemicellulose) and lignin and the element composition and structure of lignin macromolecules in various plants. In particular, grass lignin contains coniferyl and coumaryl alcohols, the wood of angiosperm trees contains coniferyl and sinapyl alcohols, and the wood of conifers contains coniferyl alcohol only.

The studies conducted by us confirm the fact that various mycobiota components are involved in complex synecologic interactions in the process of plant substrate decomposition. Importantly, we succeeded in obtaining experimental data that the micromycete groups discerned by us are significantly different in respect to their influence on basidiomycete mycelium. It seems likely that the suppression of the development of the basidiomycete is due to the direct effect of biologically active compounds with antibiotic properties that are produced by the micromycetes classified into group 1 (the inhibitory group) by us [7].

Other species, such as *Penicillium purpurogenum*, did not suppress basidiomycete growth but even stimulated it. This is in good agreement with published data that no antagonistic interactions occur between colonies of the agaricoid basidiomycete *Lepista nuda*, a litter saprotroph, and those of the micromycete *P. purpurogenum*. The micromycete was isolated from the active growth and fruition zone of *L. nuda*. The metabolites of the basidiomycete exerted no inhibitory influence on *P. purpurogenum* under experimental conditions.

The interactions between the basidiomycete mycelium and the micromycete group arbitrarily termed stimulatory (group 3) are apparently mediated by the complex effects of micromycetes on basidiomycete metabolism. Such effects were extensively investigated in studies on the involvement of soil surface micromycetes in fruiting body formation by the cultured champignon *Agaricus bisporus* (J. Lge) Imbach [2, 3, 14]. It was established that the presence of certain micromycete species in a soil substrate hampers the accumulation of phenolic substances and their oxidized forms, which inhibit fruiting body formation. Micromycetes decrease the level of the oxidative activity of *A. bisporus*. Only a part of micromycete species is directly antagonistic to the macromycete, while the other micromycetes are capable of indirect regulation of the functional activity of the basidiomycete mycelium by controlling the accumulation of aromatic compounds in the environment. Their quantity varies depending on whether the available plant substrates are easy or difficult to hydrolyze. The activity of some micromycete species may be conditional on the quantity of aromatic compounds in the environment. This is one of the factors accounting for the differences revealed by us in the mycobiota structure on herbal and woody substrates.

In our work, we used a unique method based on investigation of the dynamics of plant debris decomposition in combination with monitoring of the changes in communities of mycelial and basidial fungi. This method enabled us to reveal differences between the active and the inactive parts of plant debris in terms of the mycobiota structure and function and the dynamics of debris mineralization in nature. Differences in the substrate composition account for the different makeup of respective micromycete communities.

The data obtained confirm the idea that differences in the amounts of organic substances that occur in the soils of various ecosystems of the CFSBR are primarily due to the different characteristics of the available plant debris. If the characteristics of plant debris samples are unified, their decomposition rate is the same, at least at the initial stages of the decomposition process [15]. In line with these data, our research revealed virtually no differences in the ratio of basidiomycete-inhibiting, -stimulating, and neutral micromycete species between different ecosystems provided the same substrates were used.

REFERENCES

1. Sidorova, I.I. and Velikanov, L.L., Biologically Active Compounds of Agaricoid Basidiomycetes and Their Possible Role in the Regulation of the Structure of Myco- and Microbiota of the Soils of Forest Ecosystems. I. Antibiotic Activity of Water Extracts of Basidiomes of Some Dominant Species of Agaricoid Basidiomycetes, *Mikol. Fitopatol.*, 2000, vol. 43, no. 3, pp. 11–17.
2. Shumskaya, G.G. and Garibova, L.V., The Role of Micromycetes of the Surface Soil Layer in the Mechanism of Fruiting Body Formation by the Cultivated Champignon *Agaricus bisporus* (J. Lge) Imbach. I. Dynamics of the Population Density of Soil Micromycetes, *Mikol. Fitopatol.*, 1986, vol. 20, no. 1, pp. 26–29.
3. Shumskaya, G.G. and Garibova, L.V., The Role of Micromycetes of the Surface Soil Layer in the Mechanism of Fruiting Body Formation by the Cultivated Champignon *Agaricus bisporus* (J. Lge) Imbach. II. Species Composition of Soil Micromycetes, *Mikol. Fitopatol.*, 1986, vol. 20, no. 2, pp. 109–113.
4. Straatsma, G., Olijnsma, T.W., Gerrits, J., Amsing, J., Op Den Camp, H., and Van Griensven, L., Inoculation of *Scytalidium thermophilum* in Button Mushroom Compost and Its Effect on Yield, *Appl. Environ. Microbiol.*, 1999, no. 9, pp. 3049–3054.
5. Raudoniene, V., Micromycetes—Producers of Phenol Oxidases and Their Significance in Bioconversion of Plant Waste, *Summary of Doctoral Thesis (Biomed. Sci.)*, 2003, Inst. Bot., Vilnius, 2003.
6. *Metody eksperimental'noi mikologii* (Methods of Experimental Mycology), Bilai, V.I., Ed., Kiev: Naukova Dumka, 1982.
7. Domsh, K.H., Gams, W., and Anderson, T.-H., *Compendium of Soil Fungi*, Eching: IHW, 1993, vol. 1.
8. Dobrovol'skii, G.V., Trofimov, S.Ya., Dorofeeva, E.I., Luzikov, A.V., and Gei, K.A., The Rate of Decomposition of Forest Litter in Spruce Forests of Southern Taiga, *Lesovedenie*, 1999, no. 1, pp. 1–9.
9. Chastukhin, V.Ya. and Nikolaevskaya, M.A., *Biologicheskii raspad i resintez organicheskikh veshchestv v prirode* (Biological Decomposition and Resynthesis of Organic Compounds in Nature), Moscow: Nauka, 1969.
10. Ozerskaya, S.M. and Mirchink, T.G., Succession of Micromycetes during the Decomposition of Fallen Birch Leaves, *Mikol. Fitopatol.*, 1981, vol. 15, no. 2, pp. 97–101.
11. Frankland, J.C., in *The Fungal Community, Its Organization and Role in the Ecosystem*, Wicklow, D.T. and Carroll, G.C., Eds., New York: Marcel Dekker, 1981, pp. 403–426.
12. Terekhova, V.A. and Trofimov, S.Ya., Effect of Cross Introduction of Micromycetes on the Mineralization of the Litter in Two Types of Preserved Spruce Forests, *Mikol. Fitopatol.*, 2001, vol. 35, no. 4, pp. 53–58.
13. Sidorova, I.I. and Velikanov, L.L., Biologically Active Compounds of Agaricoid Basidiomycetes and Their Possible Role in the Regulation of the Structure of Myco- and Microbiota of the Soils of Forest Ecosystems. I. Antibiotic Activity of Cultures of the Litter Saprotroph *Lepista nuda, Mikol. Fitopatol.*, 2000, vol. 43, no. 4, pp. 10–16.
14. Garibova, L.V., Reshetnikova, I.A., and Shumskaya, G.G., Effect of Phenolic Compounds on the Growth and Development of Micromycetes of the Surface Soil Layer and the Study of Some Oxidative Enzymes of the Cultivated Champignon *Agaricus bisporus* (J. Lge) Imbach, *Mikol. Fitopatol.*, 1987, vol. 21, no. 1, pp. 28–36.
15. Dorofeeva, E.I., Distribution, Reserves, and Forms of Organic Matter in Soils of Southern Taiga (by the Example of the Central Forest Reservoir), *Cand. Sci. (Biol.) Dissertation*, Moscow: Moscow State Univ., 1997.