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CULTIVABLE FUNGAL DIVERSITY IN THE FOREST SOIL DURING LITTER DEGRADATION: MICROCOSM STUDY AT DIFFERENT TEMPERATURE REGIMES

Research article

Abstract

In the soils of the forest ecosystems litter decomposition is an important part of the carbon cycle. Soil fungi play fundamental role in this process, however, our knowledge about their successive changes is very limited, and their potential abilities under global warming are unknown. The objective of this study was to evaluate the diversity of the cultivable soil fungi during the annual cycle of aspen leaves and branches decomposition in laboratory experiments at different temperatures. Monthly soil fungi were quantified by plating technique followed by estimation of the morphological data and identification to genus level by the authentic manuals. Successional changes in fungal communities were revealed, and 102 cultures of the frequent fungal morphotaxa were isolated. It was found that the fungal communities were dominated by Ascomycota and Mortierellomycota. Eleven representative isolates were chosen for further molecular phylogenetic analysis of the nuclear ribosomal internal transcribed spacer (ITS1 and ITS2) DNA sequencing. It was found that *Penicillium*, *Mucor* and *Mortirella* were dominated at the early stage of succession and occurred most frequently throughout the decomposition process. During litter decomposition the predominance and diversity of Ascomycota increased, especially at 12°C. The frequency of *Pseudogymnoascus*, *Cephalotrichum* and *Trichocladium* was higher at 2°C, whereas temperature rise to 22°C led to an increase in *Oidiodendron* abundance. We propose that succession was driven by a decrease in the easily degradable carbohydrates and a rise in stable compounds content. Our study demonstrated that temperature was a strong determinant of the soil fungi species composition during litter decomposition.

Keywords: forest litter, cultivable fungi, global warming.

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РАЗНООБРАЗИЕ КУЛЬТИВИРУЕМЫХ ГРИБОВ В ЛЕСНОЙ ПОЧВЕ ПРИ РАЗЛОЖЕНИИ ПОДСТИЛКИ: ИССЛЕДОВАНИЕ МИКРОКОСМОВ ПРИ РАЗЛИЧНЫХ ТЕМПЕРАТУРНЫХ РЕЖИМАХ

Научная статья

Аннотация

В почвах лесных экосистем разложение подстилки является важной частью углеродного цикла. Почвенные грибы играют фундаментальную роль в этом процессе, однако наши знания об их последовательных изменениях очень ограничены, и их потенциальные возможности в условиях глобального потепления неизвестны. Целью данного исследования была оценка разнообразия культивируемых почвенных грибов во время годичного цикла разложения листьев и ветвей осины в лабораторных экспериментах при различных температурах. Ежемесячные почвенные грибы были количественно определены методом посева с последующей оценкой морфологических данных и идентификацией на уровне родов с помощью аутентичных руководств. Были выявлены сукцессионные изменения в грибных сообществах, и было выделено 102 культуры часто встречающихся грибных морфотаксов. Было обнаружено, что в грибных сообществах преобладали Ascomycota и Mortierellomycota. Одиннадцать репрезентативных изолятов были отобраны для дальнейшего молекулярно-филогенетического анализа секвенирования рибосомного внутреннего транскрибируемого спейсера (ITS1 и ITS2). Было обнаружено, что *Penicillium*, *Mucor* и *Mortirella* доминировали на

ранней стадии сукцессии и встречались наиболее часто на протяжении всего процесса разложения. Во время разложения подстилки преобладание и разнообразие аскомикоты увеличивались, особенно при 12°C. Частота встречаемости *Pseudogymnoascus*, *Cephalotrichum* и *Trichocladium* была выше при 2°C, тогда как повышение температуры до 22°C приводило к увеличению численности *Oidiodendron*. Мы предполагаем, что последовательность была обусловлена уменьшением количества легко разлагаемых углеводов и увеличением содержания стабильных соединений. Наше исследование показало, что температура является сильным фактором, определяющим видовой состав почвенных грибов во время разложения подстилки.

Ключевые слова: лесной опад, культивируемые грибы, глобальное потепление.

1. Introduction

Soil micromycetes are active participants in various biogeochemical processes in the soil, including both the transformation of mineral compounds and organic matter [1]. Fungi make up about 80% of the soil microbial biomass, are active under various conditions and react quickly to stress [2]. Fungi demonstrated high genetic and metabolic plasticity, which gave them the opportunity to colonize a variety of ecological niches [3].

Plant litter entering the soil makes up a significant part of the primary production of plants [4]. In the mixed forests of the Moscow region, the value of litter is about 2.2-7.0 t ha^{-1} per year. [5]. Currently, there are numerous works in the literature that are devoted to the study of changes in the chemical composition of plant litter during decomposition and related changes in the composition of soil [6]. However, the authors are not aware of the works devoted to assessing the impact of global warming on changes in the structure of communities of soil fungi involved in the decomposition of plant residues.

Climate warming leads to significant changes in the main constituent parts of the global carbon cycle, which, in turn, leads to a change in the magnitude and direction of greenhouse C-gases fluxes [7]. Modern studies based on the approaches of metagenomics and metatranscriptomics have demonstrated the important role of soil fungi in these processes [8] and gave the opportunity to assess the genomic potential and contribution of individual taxa [9].

The leading role of soil fungi in the decomposition of plant residues is determined by their ability to form a variety of extracellular enzymes that can decompose various plant polymers, including lignin [10]. The process of microbial decomposition of plant substrates is usually divided into successive stages, at which various intermediates are formed and, as a result, the composition of fungal community. When easily degradable carbon compounds enter the soil, this causes the restructuring of the fungal community in favor of primary colonizers (r-strategists) and the suppression of cellulolytic and chitinolytic fungi (K-strategists) [11]. It was shown that up to 95% of the carbon originated from plant residues pass through the soil microbial biomass [12]. Thus, changes in the hydrolytic fungi can be an important in the determining the rate of litter decomposition.

It was shown that microscopic soil fungi are very responsible to changes in the environmental conditions. So, their characteristics may be useful to assess the quality of natural environments. Despite the great taxonomic variety, the characteristics of fungal communities are quite informative to evaluate certain types of impacts. Most often the taxonomic composition of the fungal community, indexes of species diversity, mycelium and spore mass is used as indicators.

Earlier, we described the results of microcosm studies of temperature influence on the succession changes of saprotrophic soil bacteria after wood litter addition [13]. This article is a more in-depth analysis fungi behavior in the same experiment. The aim was to evaluate changes in the number and composition of soil fungi during the decomposition of Alnus leaves and branches at different temperatures.

2. Methods

Soil samples were harvested in September 2020 in a secondary forest (*Acer platanoides, Betula sp., Populus tremola, Alnus sp.*) in Serpukhov District, Moscow Region, Russian Federation (54.8 °N; 37.6 °E). Fallen leaves and thin *Alnus* branches were collected in October 2020 immediately after the natural fall, dried at 65 °C and ground to a particle size of 3-10 mm.

The soil was determined as a *gray* forest loamy soil (Gleic Phaeozems). The organic soil horizon has 2.5–4.0 cm thick with a pH 5.28. It contained 2.53% soil organic carbon (SOC) and 1.48 mg /100 g mineral N. Total soil carbon (C) and nitrogen (N) content were estimated by dry combustion on an elemental analyzer (LECO CHNS-932, USA). In soil samples C content was 1.880± 0.048%, N content was 0.179±0.011% and C: N ratio was 10.50. In leaves C content was 42.853±0.580%, N content was 0.923±0.023% and C: N ratio was 46.43; for branches these values were 46.540±0.447%, 0.738±0.029% and 63.06, respectively.

The microcosms prepared in March 2021 consisted of 100 ml vials with 10 g of fresh soil and 0.05 g of litter. The moistened to 60% water content soil microcosms were incubated at 2 °C, 12°C and 22°C in the dark for 360 days. Every month, three microcosms for each treatment were subjected to destructive disposal for microbiological analysis.

The number of colony-forming units (CFU) was quantified on Chapek nutrient medium with streptomycin (100 mg l^{-1}). The plates were incubated at 25°C and colonies number estimation and taxonomic identification were carried out on day 7-10. The composition of fungal communities was characterized by coefficients of frequency and relative abundance. The fungal taxon was considered as frequent when the frequency was greater than 30%.

We have applied fluorescent microscopy to determine the length of the mycelium and the number of spores of soil fungi (Calcofluor White dye, Sigma-Aldrich, USA; Axioplan 2, microscope, Zeiss, Germany). The biomass of the fungi was calculated from the lengths and diameters of the hyphae, as well as numbers and diameters of the spores, assuming a density of 0.628 g cm^3 and 0.837 g cm^3 for the hyphae and spores, respectively.

Fungal isolates were identified using a combination of morphological assessments using identification keys [14] and DNA sequencing. Colonies with different morphotypes were selected and axenic cultures were obtained. Samples of each isolate are available in UNIQEM's main culture collection at the biotechnology research center in Moscow, Russia.

The total genomic DNA was extracted from the fresh fungi. The isolated DNA was amplified by PCR of the ribosomal

operon ITS1 - 5.8 s - ITS2 - rRNA with the primer system ITS4-ITS5. The amplified PCR products were sequenced by Sanger sequencing at the basic facility "Bioengineering" of the Biotechnology Research Center of the Russian Academy of Sciences. All nucleotide sequences were used to generate consensus sequences by searching for BLASTn in the NCBI. The sequences were deposited in GenBank (MT348600 - MT348610). The current names and positions of microscopic mushroom taxa have been adjusted using the index Fungorum database.

The data are presented as averages over five samples. In all studies, the observed effects of treatment were considered statistically significant at $p<0.05$. The statistical analysis was carried out using Excel (Microsoft Office Excel 2011)

3. Results and discussion

Global warming can change the processes of soil organic matter transformation, mainly humus mineralization, and soil may to loss their biospheric functions [15]. Earlier studies have shown that the main driving factors of these processes may be the structure of the plant materials, hydrothermal features, and microbial community's patterns [16]. It is different to evaluate the relationships between these factors in situ because they are interdependent and different during plant residue decomposition period.

Soil fungi participate in the circulation of carbon soil compounds, and that's why their abundance, biomass and diversity are used in soil diagnostics. It is important that fungi, mainly Basidiomycetes, are active in the destruction of the complex polymers such as cellulose, hemicellulose, pectin, and lignin [17].

Figure 1 shows the data on the dynamics of the CFU number during incubation experiments. At the beginning fungal CFU was 8.9×10^3 g^{-1} of soil and during the first 5-6 months of incubation no significant changes were found in all variants (Fig. 1). This may be due to the predominant development of bacteria in the early stages, which we have shown in our earlier study [13]. After 6-7 and 10 months of incubation CFU number reached 1.3×10^7 g^{-1} soil in variants with leaves addition. The maximum CFU numbers were found in variants with branches incubated at 2ºC.

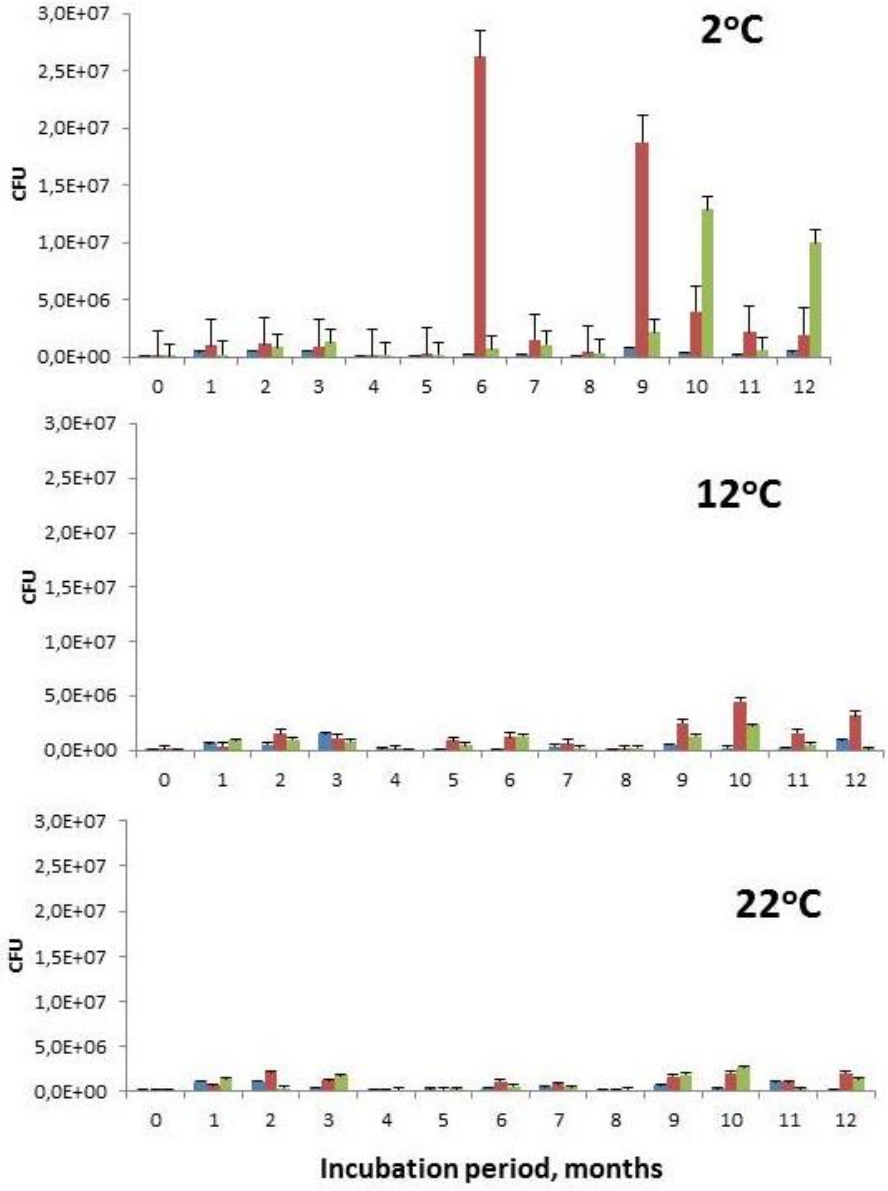

Fig. 1 − Dynamics of the cultivable fungi CFU:
blue – control, without substrate addition; *red* – leaves addition; *green* – branch addition

It is interesting that the greatest response of the fungal community to the substrate addition was found at the 2ºC. It can be assumed that soil fungi are psychrotolerant. Temperature rise may inhibit the growth of slow-growing species and germination of spores.

Direct microscopy analysis gives possibility to evaluate fungi that do not form colonies in nutrient media, but not distinguish viable and non-viable cells. Initially mycelium biomass was 0.4-0.6 mg g^{-1}, addition of leaves and branches have increased it after 6 months up to 0.7-1.7 and 0.4-1.6 mg g^{-1}, respectively (Fig. 2, 3). The spore`s biomass was greatly lower than the biomass of mycelium and ranged from 0.06 to 0.25 mg g^{-1} of soil.

Typical fungi were identified, isolated into a pure culture and their taxonomic affiliation was determined. The results of the analysis are given in the Table 1. Initially *Penicillium* and *Mucor* predominated and their total CFU number was 2.7×10^4 CFU g^{-1} of the soil, and *Penicillium* was absolute dominant with relative abundance 80-95%. For *Mucor* relative abundance was 6-16%, while for other micromycetes was less than 1%. *Penicillium* and *Mucor* use a wide range of substrates and predominate in the soils of different northern regions [18].

Taxonomic structure of fungal communities in the initial stage was slightly different from the composition of communities in the original soil and three dominants were found, namely *Penicillium* (Ascomycota), *Mucor* and *Mortirella* (Zygomycota) (Fig.4). At the early stage (one month) high abundance of *Mortirella* (24-31%) was found. Mortierellales and Mucorales are fast-growing fungi using simple substrates [19]. Usually at the first decomposition stages predominated saccharolytic fungi (Phycomycetes, *Mucor*), and epiphytic fungi *Phoma*. We did not find epiphytic fungi, probably because we used dried at 60ºC plant material.

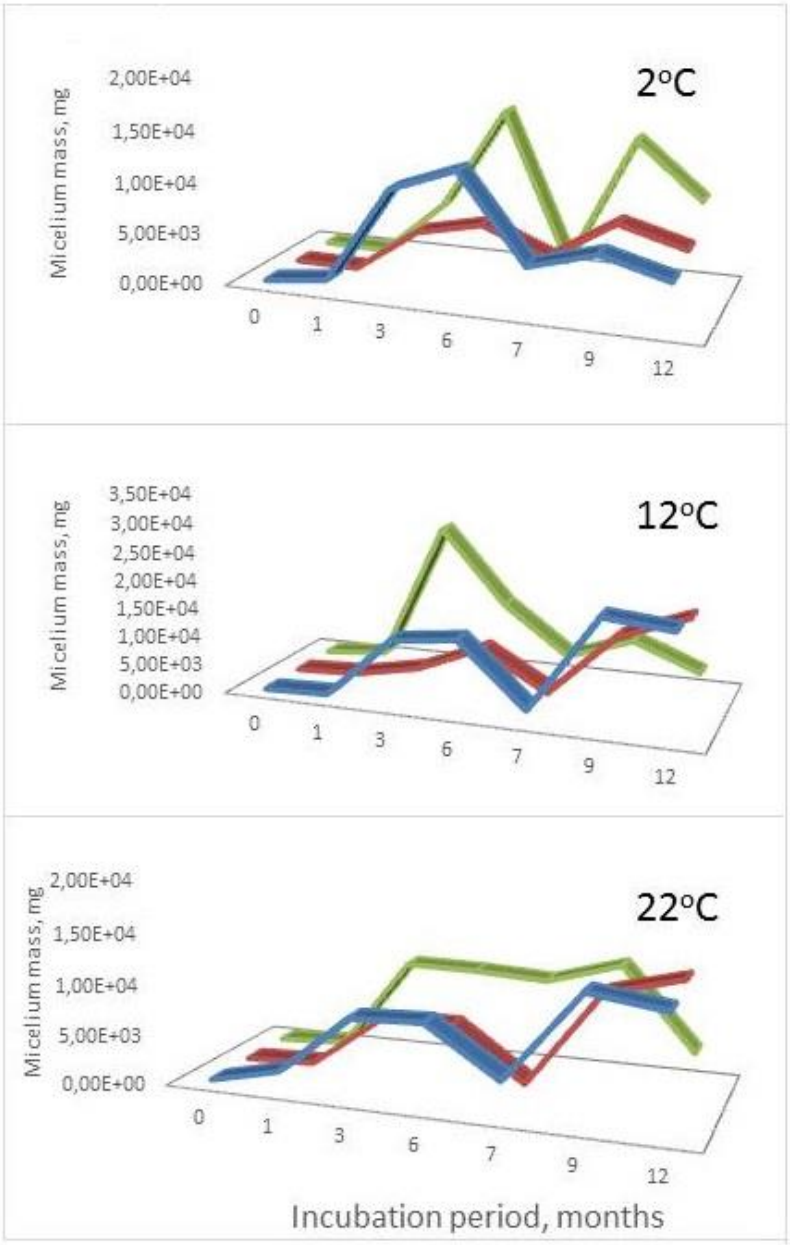

Fig. 2 − Changes of fungal mycelium biomass in soil at different succession stages

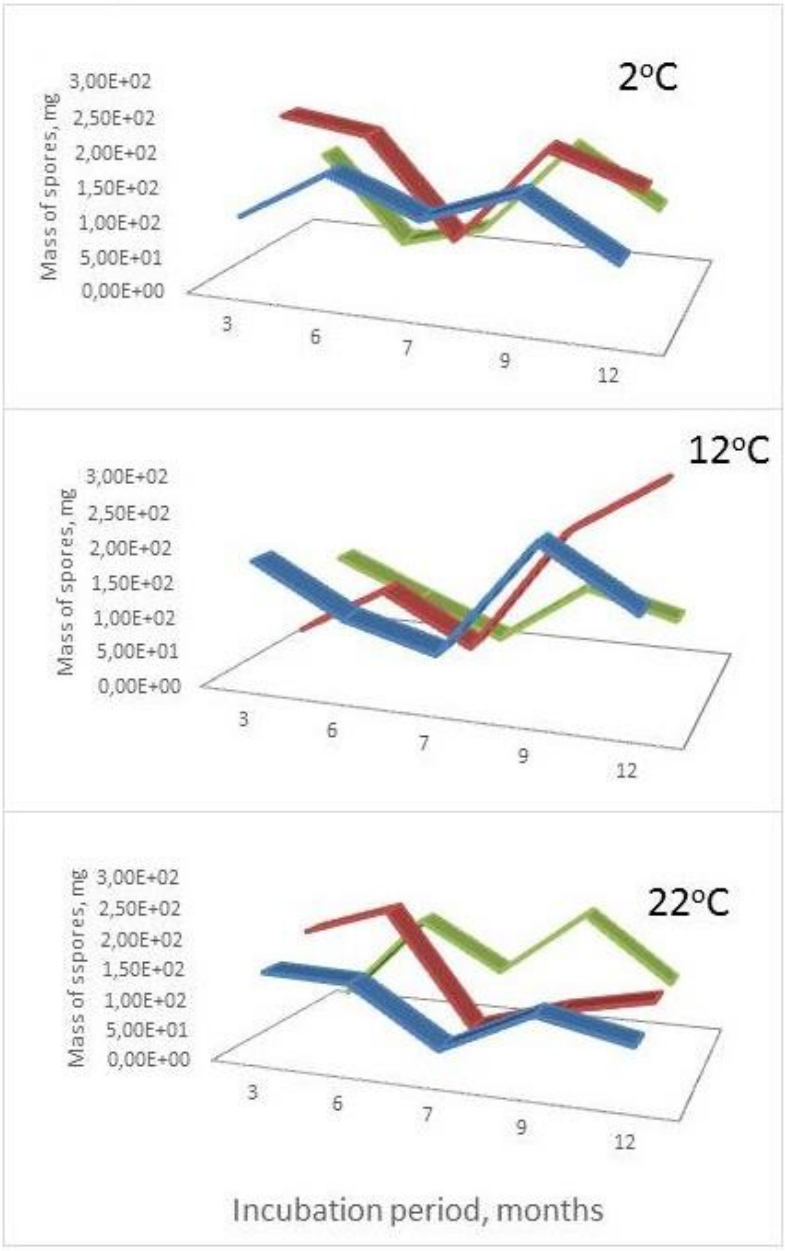

Fig. 3 − Changes of spores biomass in soil at different succession stages

Table 1 − Molecular identification of the frequent soil fungi by sequence analysis of ITS region pPHK

#	Fungal strain identifier (GenBank accession number)	Closest relative organism (GenBank accession number)	Query cover, %	Percent identity
1	BB22 (MT348600)	*Penicillium restrictum* CBS 130073 (MH865736.1)	100	99,27
2	AB22 (MT348601)	*Penicillium philippinense*CBS 623.72 (MH860600.1)	100	99,08
3	CB22 (MT348602)	*Oidiodendron* sp. UAMH8513 (AF062806.1)	97	99,42
4	DB12 (MT348603)	*Trichoderma crassum* 164916 (NR_134370.1)	100	100
5	IL12 (MT348607)	*Umbelopsis* CBS 236.82 (KC489499.1)	99	97,36
6	HL12 (MT348606)	*Trichocladium asperum*AM292050.1)	100	99,45
7	GL12 (MT348605)	*Mucor moelleri* CBS 406.58 (MH857827.1)	100	99,83
8	FL12 (MT348604)	*Mortierella* AD073 (MG052958.1)	95	99,66
9	ML2 (MT348610)	*Pseudogymnoascus roseus* CBS 127335 (MH864527.1)	100	99,62
10	KL2 (MT348609)	*Cephalotrichum nanum* NBRC 31239 (LC177640.1)	99	99,82
11	JL2 (MT348608)	*Trichocladium asperum* (LT993634.1)	96	98,11

At the second stage of the succession (three months) we found an increase in diversity and the appearance of new taxa. *Penicillium* and *Mortierella* were dominant. In samples incubated at 2 and 12ºC, the rate of *Mucor* decreased, but the rate of *Trichocladium* increased. No significant changes were observed in the samples without litter (Fig.4).

At the late succession stage (10-12 months) the fraction of initially dominating fungi decreased, but initially minor genera became dominant. *Oidiodendron, Trichoderma, Umbelopsis, Pseudogymnoascus, Cephalotrichum* which were negligible at the beginning of experiment, were detected in the fungal communities at the late stages (Fig.4). All of them relate to Ascomycetes and are capable to destroy complex substrates

Basidiomycota were not found in this study. It may be due to their possibility to hydrolyze lignin and get preferential development at the late stages of litter decomposition Another explanation may be that basidiomycetes do not form conidia on the cultural media.

In variants amended by leaves *Umbrellaopsis* (12%), *Trichocladium* (20%), *Mortierella* (15%) were dominated, but they were not detected in all other variants (Fig.4). The relative abundance of *Trichoderma* (17%) increased greatly in variants with branch addition. A significant increase in *Pseudohymnoascus* (19%), *Cephalotrichum* (16%) and *Trichocladium* (31%) was noted at 2°C in variants with leaves addition. At 22° the number of *Oidiodendron* was doubled in variants with leaves and branches (Fig. 4).

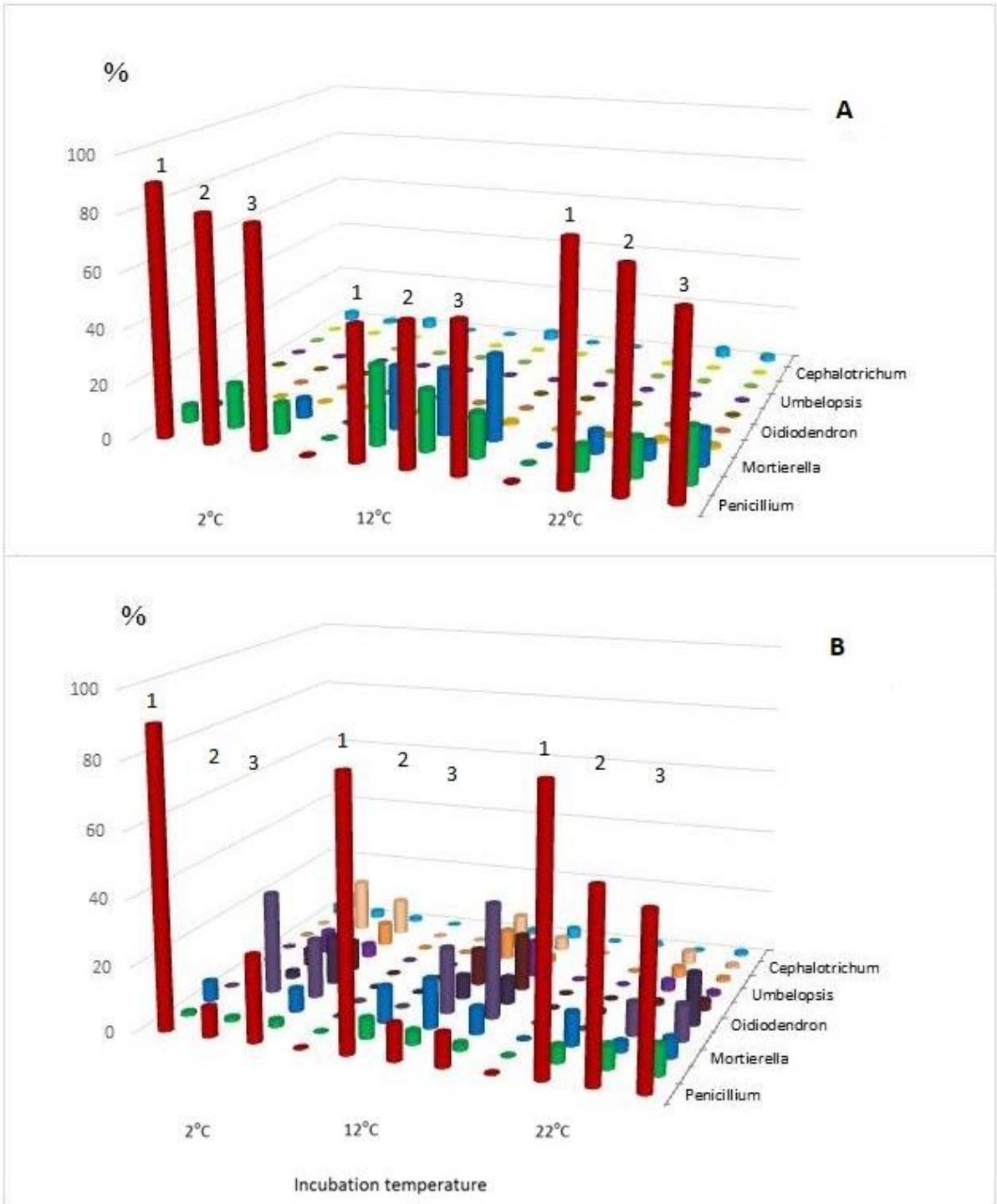

Fig. 4 − The relative abundances of the fungal genera in the initial *(A)* and late *(B)* successional stages in microcosm studies at different temperature regimes:
1 – control, without substrate addition; *2* – lives addition; *3* – branch addition

It is interest to note the changes in the proportion of *Penicillium* in the fungal communities during the succession. In all control samples (without substrates), the relative abundance of *Penicillium* was 80-95%, and it was the absolute dominant. After 12 months of incubation with substrates its abundance was 10-11% at 12ºC and 51-56% at 22ºC.

4. Conclusion

Soil fungi take an active part in the processes of transformation of carbon compounds, ensuring the growth of plants and the development of microbial communities. Our studies have confirmed the previously obtained results of other researchers that the decomposition of plant substrates is carried out by a diverse community of soil fungi.

Studies have shown that the introduction of plant materials causes changes in both quantitative (CFU quantity, mycelium biomass) and taxonomic (community composition) characteristics. The most significant changes in the composition of communities were detected after 10-12 months. A significant increase in the number of colonies at 2oC suggests that soil fungi are psychrotolerant.

The substrates led to the changes in the structure of the community: the relative abundance of dominant autochthonous genera has decreased, and the miners became dominant. During the leaf's decomposition *Umbrella, Trichocladium, Mortierella*, which were not detected in other variants, were dominated. When the temperature dropped to 2°C, the leaves addition stimulated the active development of *Pseudogymnoascu*s and *Cephalotrichum*. At high temperatures, a greater variety of *Penicillium* species and a high abundance of *Oidiodendron* were found.

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Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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