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Effect of elevated temperatures on composition and diversity of microfungal communities in natural and urban boreal soils, with emphasis on potentially pathogenic species

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ABSTRACT

Elevated temperatures differentially affect the development of cultivated microfungal assemblages, including species potentially pathogenic for humans, in various natural and anthropogenic soils. For that reason it is important to know whether the relative abundance and diversity of fungal pathogenic species increase at elevated soil temperatures. We compared successional changes in microfungal species diversity in natural (Histic Leptosol, Umbric Albeluvisol) and anthropogenic (Urbic Technosol) soils of the boreal zone of European Russia at temperatures of 10, 20, 30, and 35 °C, and at a water holding capacity of 60% using the soil dilution plate method. The greatest fungal diversity was detected at the lowest but common temperature (10 °C) in the investigated regions. The most significant changes in the diversity of fungal assemblages in the process of succession occurred at 20 °C. Elevated soil temperatures (30 and 35 °C) induced a drastic decrease in species diversity compared with what was observed at 10 °C.

During the successions, microfungal assemblages in both types of natural soils had the most pronounced differences in species composition at minimal (10 °C) and maximal (35 °C) soil temperatures. In the anthropogenic soil, microfungal communities at varying stages of succession were more similar at different temperatures, with the exception of the final stages of succession at the highest temperature of 35 °C. Independently of soil type, elevated temperature increased (up to 40–90%) the relative abundance of potentially pathogenic microfungal species dangerous for humans. Potentially pathogenic *Aspergillus fumigatus* was dominant in the natural soils, while *Scedosporium aurantiacum* dominated in the urban soil. Elevated soil temperatures in combination with high humidity due to climate warming may drastically accelerate the development of potentially pathogenic microfungal species.

1. Introduction

Temperature is one of the crucial ecological factors determining the development of soil biota in a changing global climate. Global warming may, inevitably and significantly affect the structure and functioning of soil communities (Zogg et al., 1997; Waldrop and Firestone, 2004; Zhang et al., 2005; Castro et al., 2010).

Fungi are an important group of soil organisms and one of the main decomposers of organic matter (Carlie et al., 2001; Gadd, 2007). Temperature changes due to global warming may have wide-reaching impacts on different taxonomic and ecological groups of soil fungi. Such impacts have already been observed on the fungal fruiting and fruit body yield of saprotrophic and ectomycorrhizal basidiomycetes (Boddy et al., 2014), the relationships between mycorrhizal fungi and plants (Fitter et al., 2004; Hawkes et al., 2008; Simard and Austin, 2009; Compant et al., 2010), the community composition and taxonomic richness of soil ascomycetes in dry and moist tundra (Semenova et al., 2015), the soil fungal communities in alpine meadows (Xiong et al., 2014), and the structure of active fungal communities in an Alaskan boreal forest soil (Allison and Treseder, 2008). In contrast, no effects of warming on fungal communities have been found in soils of more southern ecosystems, e.g., in the Kansas tallgrass prairie (Jumpponen and Jones, 2014), California's annual grassland (Gutknecht et al., 2012), and in the disturbed soil of burnt upland boreal ecosystem (Allison et al., 2010). Thus, more information is

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required to clarify the different responses of particular fungal groups in various types of soils to elevated temperatures.

Many of the above mentioned studies have been conducted using molecular techniques, which have greatly expanded our knowledge on the composition of the soil mycobiota. However, molecular approaches are limited as they do not allow isolation of fungal strains for further investigations. Fungal strain properties, specifically, cannot be determined yet these are particularly important in the research for fungal metabolites. Fungal cultivation, another useful tool, is the sole approach for many investigation such as the initial characterization of fungal communities' ecology (Bills et al., 2004; Schmit and Lodge, 2005).

One of the most important aspects of research into soil microfungal assemblages under climate warming is the assessment of the occurrence of fungi known to be potentially pathogenic and recognized to be dangerous for human health (Hoog et al., 2000). One of the reasons for their pathogenicity is their ability to grow at human body temperature. Consequently, it is essential to understand whether the relative abundance and diversity of potentially pathogenic species increase at elevated soil temperatures. This is especially important for urban soils where human population density is highest. However, few studies have addressed this question.

Microscopic fungi, the subject of our study, are widespread in different types of soils and belong to the anamorphic (asexual) state of Ascomycota. Indeed, most filamentous fungi recognized as potentially pathogenic are anamorphs of the Ascomycetes (Hoog et al., 2000).

The goal of our investigation was to compare the influence of elevated soil temperatures on the composition and diversity of cultivated microfungal assemblages from different types of natural and urban boreal soils in European Russia, with special attention to the isolation and identification of potentially pathogenic fungal species. The objectives of the study were to determine the density of microfungal isolates, as well as the species composition and diversity level of cultivated soil microfungal assemblages at different soil temperatures (10, 20, 30, 35 °C) during fungal succession. We hypothesized that (1) the community composition and diversity of cultivated soil microscopic fungi would change under warmer conditions, (2) the responses of soil microfungal assemblages would be stronger in the northern natural soil than in soils of temperate latitudes, and (3) the warming would favour the growth of potentially pathogenic microfungi in soils.

2. Materials and method

2.1. Location of the study area

Our studies were conducted in two types of natural soils – Histic Leptosol, typical in Northern areas, and Umbric Albeluvisol typical in temperate latitudes of the boreal zone of European Russia (Dobrovol'skii and Urusevskaya, 2004), and in one type of anthropogenic soil, Urbic Technosol (Moscow). The soils were classified according to the World Reference Base (IUSS Working Group WRB, 2015).

The Histic Leptosol site is located on the territory of the White Sea Research Station of the Moscow State University (66° 33′ N, 33° 06′ E). The vegetation of this area is dominated by *Pinus sylvestris* with birch undergrowth (*Betula alba*) and a cover of *Vaccinium* species, mosses (*Sphagnum* spp.) and lichens (*Cladonia* spp.). The average annual soil temperature at a depth of 0.2 m in this region is +3.1 °C while the average soil temperature during the warmest and coldest months reaches +13.6 °C and −5.3 °C, respectively (Dimo, 1972). According to our measurements with thermochrones (iButton), daytime soil temperature on open sites at a depth of 5 to 10 cm on some days during July, 2014 reached +33.0 °C.

The Umbric Albeluvisol site is located in the Moscow Region within the conservation area of Aleshkino Forest Park (55° 52′ N, 37° 25′ E) in a pine (*Pinus sylvestris*) nemoral forest with abundant herbaceous plants and a well-developed litter layer (Stroganova et al., 2008).

The Urbic Technosol soil is located 3.5 km to the southeast of the Umbric Albeluvisol site in a residential area constructed nearly 40 years ago (55° 51′ N, 37° 25′ E). The vegetation of the site is represented by birches (*Betula alba*), linden (*Tilia cordata*), acer (*Acer platanoides*) and ash (*Fraxinus excelsior*) planted there 35–40 years ago. This area is characterized by a very poorly developed grass cover. There is no litter.

The average annual soil temperature at a depth of 0.2 m in the region of both study sites is +6.1 °C while the average soil temperature during the hottest and coldest months has been recorded as +17.7 °C and −1.1 °C, respectively (Dimo, 1972). Data from the Moscow State University Meteorological Observatory for the hot summer of 2013 showed a soil temperature in this region at a depth of 5 cm of +35.0 °C during the day in July.

2.2. Soil sampling and analyses

In all examined soils, fungal assemblages were studied in the upper humus horizons. Soil samples from five replicates of the Umbric Albeluvisol and Urbic Technosol sites were collected from the A horizon (at a depth of 5–10 cm) in June 2013. In the northern Histic Leptosol the upper soil horizon is considered as OF + H. Samples of this soil were collected from this horizon (at a depth of 1–3 cm) in five replicates in July 2014 from 10 by 10 m plots. The samples were placed into sterile paper bags and air dried. The dry samples were stored (for 2–3 months) at +4 °C before processing. The morphological, physical and chemical properties of the soils have been described in detail in previous articles: the pH of the water extract (1:5) of the examined soil horizons of the Histic Leptosol, Umbric Albeluvisol and Urbic Technosol is 3.9, 4.19 and 6.4, respectively. The total organic carbon content of the soils is 36.09, 2.82 and 1.60% of dry soil, respectively (Stroganova et al., 2008; Perverzev and Litvinova, 2008).

2.3. Experimental design

Air-dried soil samples were sieved (3-mm-pore size) and five subsamples were combined into a composite sample – mixed samples are widely used in such experiments (Allison and Treseder, 2008; Pietikäinen et al., 2005; Bárcenas-Moreno et al., 2009). The pooled sample was distributed between eight plastic containers to a depth of 4–5 cm. The soil was moistened to 60% of water holding capacity (WHC) after which the containers were covered with Parafilm M (to protect samples from contamination and evaporation) and placed for a month in thermostats with constant temperatures of 10, 20, 30 and 35 °C (two containers for each temperature). The composition and diversity level of soil fungal assemblages were determined starting from the 3rd day after the addition of moisture and every seventh day thereafter. The containers were periodically ventilated. For technical reasons, the community of Histic Leptosol was not analyzed on the seventeenth day of succession.

2.4. Cultivation, isolation and identification of microfungi

Microfungi were isolated by serial dilutions of soil samples in sterile water (Davet and Rouxel, 2000) with subsequent plating on solid Czapek's agar (CzA) media with 2% sucrose. Streptomycin at a concentration of 100 mg l^{-1} was added to the medium to suppress bacterial growth. For Umbric Albeluvisol and Urbic Technosol soils, the dilution was 1:100; for the Histic Leptosol, 1:1000 and 1:10