Structure and dynamics of microfungal communities in soils with different humus content under polymetallic pollution

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1. Introduction

Heavy metals (HM) pollution is a priority negative impact on soils [1]. The sorption of HM cations by soils, and consequently the toxicity of these pollutants, depends on granulometric and mineralogical soil properties, organic matter content (humus status) and carbonates content, pH, and absorption capacity [2]. The humus status of soils can be modeled by the introduction of carbon containing compounds (humus preparations, biochar, and nanomaterials) [3,4]. The functions and structure of soil microbial communities can be transformed under HM. Among microscopic fungi, which are an integral component of terrestrial ecosystems, it is possible to increase the proportion of melanin-containing or opportunistic mycromycetes [5]. Despite the high degree of variability, mycological indicators can be highly informative for certain kinds of anthropogenic impacts [6]. Structural indicators of communities, proportion of resistant melanin containing species, indices of species diversity of fungi can be considered as the most informative.

The aim of the study was to characterize the structure and dynamics of fungal communities under polymetallic pollution in soils with different humus content without and with carbonaceous amendments.

2. Materials and methods

In pot experiments two different soils were used for the model HM pollution: chernozem and agrozem (organic carbon content $5.5\pm0.02\%$ and $1.5\pm0.02\%$, respectively). Pre-dried, crushed soil samples were leveled at humidity (60%) and were kept at room temperature for 5 days to achieve equilibrium. Then soil samples had been polluted by a dry mixture of HM (Zn, Cu and Pb salts). After 14 days the amendments were applied: 5 % biochar in dry form, 0.25 % lignohumate in the form of an aqueous solution, separately and as a mixture. Thus, 8 experimental variants were formed for each type of soil: 4 samples without HM and 4 HM polluted samples. The duration of the experiment after treatment with amendments was 90 days at a temperature of $22-25^{\circ}C$. The sampling for mycological analyses was carried out on the 7th, 30th, 60th and 90th days of the experiment.

The inoculation of microfungi from each experimental variant was carried out on three nutrient medium (agar Chapek medium, potato-dextrose agar, and Hutchinson medium (3-5 Petri dishes) to more precise identify and describe the structure of microfungal communities. Accounting of colony forming units (CFU) and taxonomic identification of spore – forming forms was carried out on 14-16 days. Also the assessment of microbial community structure differences in polluted and amendment soils samples were conducted by means of lipid analysis.

3. Results and discussion

At the beginning of the experiment only minor fluctuations in fungal species diversity, which were little dependent on the type of soil, were revealed for all nutrient media between the experimental variants. In all samples of chernozem there were 4-7 species; representatives of the genera *Trichoderma, Penicillium, Fusarium, Humicola* dominated. In samples of agrozem there were 6-8 species; representatives of the genera *Trichoderma, Penicillium, Fusarium, Humicola* dominated. In samples of agrozem there were 6-8 species; representatives of the genera *Trichoderma, Penicillium, Clonostachys, Chaetomium* dominated.

Microfungal communities have undergone a transformation during the exposition. The dynamics of the taxonomic composition of fungi and the number of CFU in soils of different humus status differed. We can assume that at the end of the exposure (figure):

1) species diversity of micromycetes increased in the both types of soils, but it was higher in agrozem samples in comparison with chernozem samples;

2) polymetallic pollution had a stressful effect, resulting in an increase in the number of species in the most experimental variants, especially in chernozem samples.

In the control samples of chernozem, representatives of the genera *Penicillium* and *Acremonium* dominated, in samples with addition of biochar, representatives of *Trichoderma* appeared. In chernozem samples with addition of HM, representatives of the genera *Trichoderma* and *Penicillium* actively developed; representatives of the genera *Acremonium* less actively developed; dark-colored forms appeared and *Aspergillus*. In control samples of agrozem, a great diversity of species *Penicillium* and the constancy of species *Trichoderma* were observed; representatives of the genera *Fusarium* and *Mucor* appeared in samples with addition of remediates. Representatives of the genera *Penicillium*, *Trichoderma, Clonostachys, Paecilomyces*, and *Acremonium* are abundant in the agrozem samples with addition of HM.



Figure 1. Total number of fungal species in the soil samples of different humus status without and with the introduction of HM and carbon containing compounds (at the end of the experiment)

According to results of lipid analysis of soils the fungal biomass of *Aspergillus* sp. (indicator of soil contamination) increased sharply under TM in agrozem (from 668.6 in control to 1184.5 mkg/g, i.e. more than 77% under HM), and it decreased when the samples were enriched with carbon after treatment with biochar and lignohumate. On the contrary, in humus-rich soil - chernozem, the effect of both TM and amendments was not so noticeable (362.3 and 299.5 mkg/g).

4. Conclusions

Our results confirm that humic substances perform a protective function in polluted soils. Differences in the transformation of the structure and dynamics of the quantitative parameters of the fungal communities suggest that the effect of polymetallic pollution differs in humus-rich and humus-poor soils. In a humus-poor agrozem *Aspergillus*-biomass sharply increases with pollution. We believe that it can be attributed to the compensatory mechanisms of the negative effect TM on this soil. The study was supported by RFBR, grant 18-04-01218_a.

5. References

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