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SYSTEMATIC STUDY OF ARID TERRITORIES

Dynamics of Microbiological Diversity of Soils in the Chu Valley during Land Use Change in Pastures

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Abstract—Soil microflora is one of the first to feel a negative impact and can serve as a biological indicator of changes in the soil structure and the degree of impact on the soil ecosystem. In 2020, studies were carried out at two sites located in the Shamshy Gorge in the Chui region of the Kyrgyz Republic. One of the plots was withdrawn from pasture use for one year in 2020 and two years in 2019 as compared to the actively used control option. The microbiological diversity was studied with conventional methods of microbiology. The micromycetes of the studied soils were represented in the dominance of species resistant to adverse environmental factors, *Trichoderma*, *Cladosporium,* and *Alternaria,* i.e., the species diversity was insignificant, with a predominance of representatives of dominant species. A decrease in the anthropogenic load in the studied areas has led to the activation of microbiological processes and a significant increase in soil microbiodiversity, which is indicative of improvement in the enzymatic properties and an acceleration of the soil self-healing process. Studies have also shown that soils in the regime of unregulated overgrazing are significantly susceptible to drying and trampling, which leads to a decrease in microbiological diversity in the soil ecosystem and the dominance of microorganism forms that are resistant to adverse environmental factors. The obtained data suggest that overgrazing affects the structure of soil microbiocenosis, replacing it with more drought-resistant species. Thus, the microbiological diversity of soils subjected to varying degrees of anthropogenic pressure can serve as a biological indicator of the state of the soil ecosystem. It is also necessary to resolve the issue of the regulation of the rotational grazing of livestock, which can also ensure the preservation of pasture productivity and contribute to the conservation of the biological diversity of flora and fauna of pastures, respectively.

Keywords: microbiological diversity, anthropogenic pressure, soil monitoring, ecosystem, type of pasture use, pasture degradation

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INTRODUCTION

According to the International Fund for Agricultural Development, the Chui region of the Kyrgyz Republic belongs to the category of regions vulnerable to climate change (Fig. 1).

According to the national action plan to combat desertification in the Kyrgyz Republic, the main environmental problem in the agricultural sector is degradation caused by uncontrolled grazing and the large numbers of animals. Overgrazing has caused severe pasture deterioration, which has resulted in a loss of productivity, severe soil erosion, wind erosion, deforestation, and more frequent mudflows (National action plan ..., 2000). Although the Chui region accounts for only 9% of the pastures of the republic, their utilization is more than 200%, and the potential for growth in the number of livestock has been exhausted (National Statistical Committee of the Kyrgyz Republic, 2018). The degree of degradation of pastures and their conditions are well studied, but the microbiological diversity of pasture soils with varying degrees of load is of particular interest. The species diversity in communities of soil microorganisms allows the maintenance of important ecosystem functions despite the changes in communities that occur under the influence of anthropogenic pollution (Bagyaraj and Ashwin, 2017; Mathew et al., 2012; Morrien et al., 2017). Microorganisms are sensitive to any changes, even minor ones, which makes them an indispensable object for monitoring of the soil environment. The study of soil microflora is also important in terms of its ability to self-repair due to negative impacts (Bastida et al., 2017). Thus, the composition of the microorganism community in soils is a sensitive indicator of the state of the soil as compared to the general soil characteristics (Blagodatskaya and Kuzyakov, 2013; Cardoso et al., 2013; Dengler et al., 2014) and makes it possible to assess the state of the studied

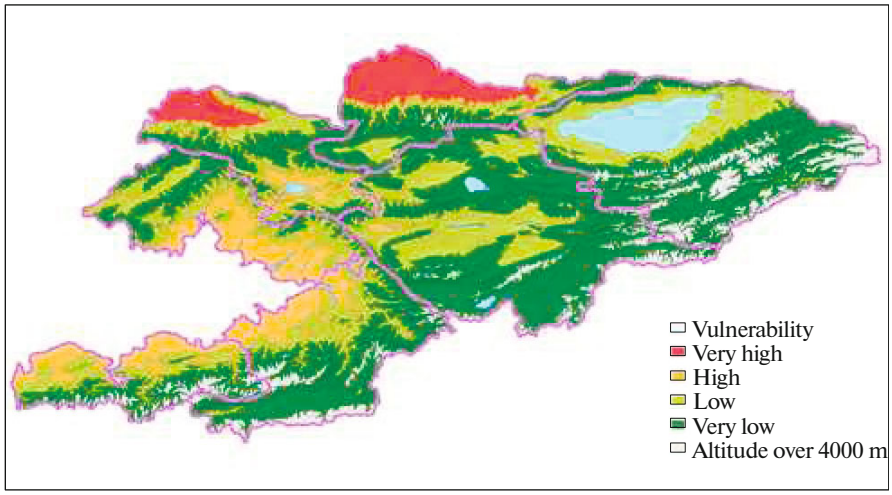

Fig. 1. Sketch map of levels of vulnerability to climate change in the Kyrgyz Republic (*Programma*, 2017).

ecosystem and to determine the degree anthropogenic load (Leff et al., 2015).

Even slight anthropogenic soil pollution causes changes in the quantitative characteristics of soil microbiota (Kirtsideli, 2019; Kutovaya et al., 2018). Among the dominant species with significant soil pollution appear those such as *Aspergillus fumigatus, Humicola grisea*, *Monodictys levis*, which are not observed even with the minimum frequency of occurrence in control soils (Huhe et al., 2017; Ferris and Tuomisto, 2015). Typical microorganism groups are present in different soil types (Lavelle et al., 2016). The relevance of their study has especially increased due to the increased dynamics of ecosystems under the influence of various environmental and anthropogenic factors (Phillips, 2017; Maron et al., 2018). The goal of the work was to study the dynamics of the microbiological diversity of pasture soils under the usual congestion of pastures and when they were withdrawn from general use for 1 and 2 years in the conditions of the Chui valley of the Kyrgyz Republic.

MATERIALS AND METHODS

The objects of our research were the pastures of the Chui region of the Kyrgyz Republic. The Chui region is located in the northern part of the country. It borders the Republic of Kazakhstan in the north and west, the Talas and Jalal-Abad regions in the southwest, the Naryn region, in the south, and the Issyk-Kul region in the southeast.

In order to study the microbiological state of pasture soils, we took soil samples from experimental plots fenced with a metal mesh with a total area of 1.0 ha (100 × 100). A detailed description of the experimental plot is given in Table 1. These areas are pastures with mountain chestnut soils with meadow vegetation.

The soil sampling and preparation for microbiological analysis were carried out in accordance with GOST 14.4.4.02-84 (*GOST*..., 1986). The soil sampling was carried out with the envelope method. Twenty-five samples were taken from each study site. Next, the samples were homogenized via dismounting to obtain representative samples. The soil samples were taken in sterile parchment bags. To take a soil sample with a sterile knife (*GOST 23707-79*, 1979), the top layer of soil (1.5–2.0 cm) was taken to remove foreign microflora on the site. Next, 100–200 g of soil was taken with a shovel and placed in a sterile parchment bag. The cut was laid immediately before sampling. Soil samples for microbiological studies were dried at a temperature not exceeding 30°C. Dry samples were stored in sterile parchment bags placed in linen bags. Before drying, the samples were evenly distributed on sheets of paper in low cuvettes with a layer of 1–1.5 cm. Stones, glass, large chips, and other inclusions were removed, and large lumps were crushed with a smooth wooden stick. During the drying period, the samples were mixed several times and protected from dusting. The soil was crushed in a porcelain mortar and sieved through a sieve 1 mm in diameter (Zenova et al., 2001).

Preparation of a soil sample for analysis. The preparation of a soil sample for microbiological analysis consists in the removal of large roots, the destruction of soil aggregates, the desorption of microorganisms from the surface of soil particles, and the disaggregation of microcolonies of microorganisms. For the microorganism desorption and microcolony disaggregation, the soil samples were treated with ultrasound at the UZDN-1 installation in the following mode: sample processing time of 4 min, current strength of 0.44 A, and a frequency of 15 kHz. To account for mycelial organisms in the soil, the soil samples were moistened to a pasty state for 3–5 minutes and rubbed

Table 1. Characteristics of soil sampling sites

Identifier, no.	Sampling date	Sampling coordinates	Height, m a.s.l. BS	Air temperature (on the day of sampling)	Site description
1	Sep. 13, 2020	42°35′29.1″ N, 75°24′08.53″ E, 42.591411, 75.402.371	1654	14°C	Plot with a total area of 1.0 ha fenced in 2020 (Fig. 3)
2	Sep. 13, 2020	42°35′29.1″ N, 75°24′08.53″ E, 42.591411, 75.402.371	1654	14°C	Plot with a total area of 0.1 ha in 2019 (Fig. 3)
3	Sep. 13, 2020	42°35′17.64″ N, 75°27′06.49″ E, 42.588233, 75.451805	1948	14°C	Plot with a total area of 1.0 ha fenced in 2020
4	Sep. 13, 2020	42°35′17.64″ N, 75°27′06.49″ E, 42.588233, 75.451805	1948	14°C	Unfenced site in close proximity to site no. 3

in a porcelain mortar with a rubber pestle or a finger in a rubber fingertip.

Isolation of microorganisms in the laboratory. Meat-peptone agar (meat broth, 1 L; agar, 20 g) and Czapek's medium for bacteria (in g/L, KCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; K_2HPO_4,1; $FeSO_4 \cdot 7H_2O$, 0.01; $NaNO_3$, 2.0; $CaCO_3$, 3.0; sucrose, 20; agar, 20) are used to isolate bacteria from the soil. Casein-glycerol agar is used to isolate actinomycetes from the soil (g/L): casein hydrolyzate with yeast extract, 0.3; glycerin, 10 mL; KNO_3, 2, K_2HPO_4, 2; $MgSO_4 \cdot 7H_2O$, 0.05; $FeSO_4 \cdot H_2O$, 0.01; $CaCO_3$, 0.02; NaCl, 2; agar, 20. For the identification and accounting for soil micromycetes from the soil via seeding from dilutions of soil suspensions on solid nutrient media, the most commonly used media are acidified with lactic acid (4 mL/L), wort-agar, and Czapek's medium of the following composition (g/L): sucrose, 20.0; $NaNO_3$, 2.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5; $FeSO_4 \cdot H_2O$, 0.01; agar, 20.0.

Preparation of soil suspensions. Prior to sowing, wet or dry soils were well mixed, poured onto a watch glass that had been wiped with alcohol, and then freed from foreign inclusions. A sample of 1 g was used after appropriate tillage via rubbing or otherwise transferred to a flask with 100 mL of sterile tap water. Dilutions of soil suspension were prepared, for which 1 mL of soil suspension from a flask (dilution 1 : 100) was sequentially transferred to a series of test tubes with 10 mL of sterile tap water. The soil suspension was sown on dense media from dilutions of 1 : 10, 1 : 100, 1 : 1000, etc., depending on the taxonomic affiliation of the microorganisms taken into account, the soil type and moisture content, and other factors. At least three to five repeated weights were taken from each soil sample, and each weight was sown on three to five cups with each medium.

Calculation of the number of colony-forming units of microorganisms in 1 g of soil. The number of colonies on the dish is counted from the bottom of the dish in transmitted light with a colony counter. Having counted the number of colonies on all parallel plates, we calculate their average number on the plate and then recalculate the content of colony-forming units (CFUs) in 1 g (CFU/g) of soil according to the formula

$$A = BCD,$$

where A is CFU/g of soil, B is the average number of colonies per dish, C is the dilution of soil suspension from which the inoculation was made, D is the number of drops in 1 mL of suspension (number of drops per 1-mL pipette used for inoculation).

Assessment of species and generic diversity of actinomycetes. The assessment was carried out with the Shannon diversity index, H (Odum, 1963). Statistical processing of experimental data was carried out with the STATISTICA 5.0 package for Windows.

RESULTS AND DISCUSSION

The number of actinomycetes in the studied soils dominated over other types of bacteria and micromycetes (Fig. 2). Actinomycetes are a link in the trophic chain that acts as decomposers. Their main role is to decompose complex polymers such as lignin, chitin, cellulose, etc. According to Marfenina and Ivanova (2017), an increase in the number of soil actinomy-

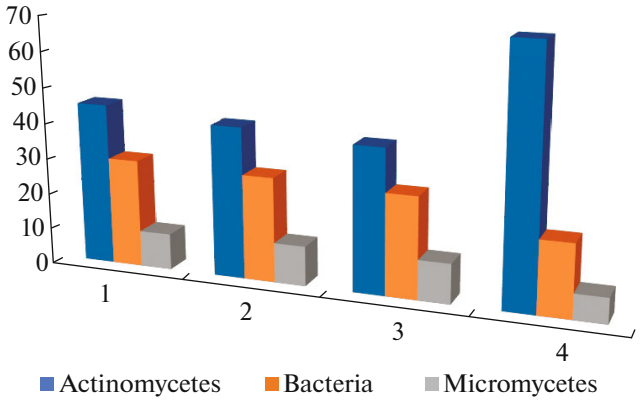

Fig. 2. Number of soil microbiota in the experimental variants, thousand CFU/g of air-dried soil.

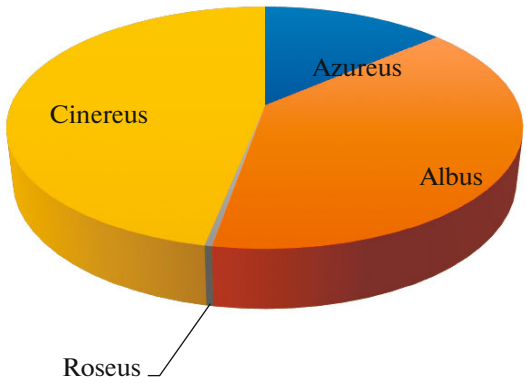

Fig. 3. Taxonomic composition and frequency of occurrence of streptomycetes, %.

cetes occurs at the late stages of microbial succession, when the fungal biomass begins to decrease. However, it was noted (Ovchinnikova et al., 2010; Svistova et al., 2003; Nazarenko, 2013) that actinomycetes are more resistant to anthropogenic impact than other groups of soil microorganisms; they predominate in technozem soils and may indicate a disturbance of the soil microbial system as a whole (Solov'eva, 2014). This may also indicate an increase in the aridization of the soil system (Dorokhova et al., 2014; Kurapova et al., 2012). This may be due to overgrazing, which leads to pasture degradation. This is rather alarming in the light of the above problems of possible aridization of the country.

The number of actinomycetes in all of the studied areas is high, and the micromycete number is minimal (Fig. 2). At the same time, actinomycetes predominated in the unfenced area; in the territory with the maximum load (no. 4), and in plots no. 3, 2, and 1, the actinomycetes CFUs were not so numerous. Thus, our studies also confirm that actinomycetes are resistant species under conditions of increased pasture pressure and can serve as indicators of the state of the pastures.

However, analysis of the taxonomic composition and frequency of occurrence of species showed that the species diversity of soil streptomycetes was higher and represented by representatives of four sections in plot no. 2, which indicates a decrease in anthropogenic impact, while representatives of the section Ginereus and Albus dominated, which are more resistant to anthropogenic impact, in plot no. 4. This also indicates an increased anthropogenic impact on the studied objects and the need to use rotational grazing in pastures (Fig. 3).

The bacterial diversity of soil samples is another factor indicative of the aridity of the studied soils (*Programma*..., 2017).

Thus, they were dominated by bacteria of the actinomycete line, which are characterized by the formation of carotenoid and melanoid pigments. The predominance of actinomycete bacteria in desert soils, most of which have hydrolytic activity, correlates with the rapid destruction of plant litter in desert ecosystems (Kurapova et al., 2012). The obtained data suggest that overgrazing may affect the structure of soil microbiocenosis, replacing it with more drought-resistant species.

The micromycetes of the studied soils were represented in the dominance of the species *Trichoderma*, *Cladosporium* and *Alternaria*, those. species diversity was insignificant and dominated by representatives of dominant species, which characterizes soils as being subjected to anthropogenic pressure. Further research is required to determine whether this means that the studied objects should be characterized as a zone of "anthropogenic stress," which is "a single, interdependent system of adaptations of the biocenosis to changing environmental conditions" according to the theory of ecological modifications (Abakumov, 1991).

The conducted studies have shown that a decrease in the anthropogenic load, which changed in the process of unregulated grazing upon its withdrawal from use, leads to the activation of microbiological processes and an increase in the species diversity of microorganisms in the soil. This indicates an acceleration of the process of self-recovery of degraded pastures. Soils that are in the mode of unregulated overgrazing are significantly susceptible to a decrease in microbiological diversity in the soil ecosystem and the dominance of forms of microorganisms resistant to adverse environmental factors. Thus, the dynamics of the microbiological diversity of pasture soils subjected to varying degrees of anthropogenic pressure can serve as an indicator of the state and aridization of pastures.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving humans or animals performed by any of the authors.

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