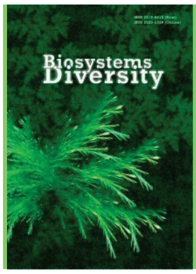

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Microbiota of the rhizosphere zone of *Calamagrostis epigeios* from a coal mine waste dump

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The microbiota plays an important role in the processes of plant overgrowth of coal mine waste dumps, enabling the transformation of numerous compounds into forms available to plants. The overgrowth of coal mine dumps is influenced by many factors. Pioneers are plant species that have a wide ecological and phytocenotic amplitude. *Calamagrostis epigeios* (L.) Roth. occupies a special place among them. The composition of the microbiota of the rhizosphere zone of *C. epigeios* was studied in relation to the age of the plants and the stage of the succession of the "Vizeyska" mine dump (Ukraine). It was established and confirmed as a result of two-factor variance analysis that the growth phase of *C. epigeios* and the stage of the succession of coal mine waste dumps have different effects on the number of microorganisms from the rhizosphere zone of plants. The number of pedotrophic microorganisms, microorganisms that metabolize nitrogen of organic compounds, cellulose-degrading microorganisms, and microscopic fungi depended more on the age of *C. epigeios*, and not on the stage of the succession of the studied area. The number of chemolithotrophic bacteria, particularly thiobacteria, decreased with the change of the growth phase of *C. epigeios*. The number of acidophobic thiobacteria depended more on the stage of succession, and the number of acidophilic thiobacteria depended more on the age of the *C. epigeios*. The number of microorganisms that metabolize nitrogen of organic compounds, oligonitrophilic microorganisms and microorganisms that metabolize nitrogen of inorganic compounds in the samples of tailings from the area with grasses and perennials and from the area with grasses, shrubs, and sun-loving trees was higher, compared to the number of these groups of microorganisms in the control and changed with the change in the growth phase of *C. epigeios*. The number of microorganisms that metabolize nitrogen of organic compounds, oligonitrophilic microrganisms and microorganisms that metabolize nitrogen of inorganic compounds was the highest in the samples from the area with grasses, shrubs, and sun-loving trees during the adult growth phase of *C. epigeios*. In the area where *C. epigeios* dominated within the vegetation, the highest number of microorganisms that metabolize nitrogen of organic compounds was also during the adult phase of *C. epigeios*, and the number of bacteria that assimilate mineral forms of nitrogen and oligonitrophilic microorganisms was the highest during the sub-adult stage. The index of pedotrophicity is higher in the samples taken in the area where *C. epigeios* prevails among other herbaceous plants, and where in the tree layer there are *Betula pendula*, *Populus tremula* with an admixture of *Pinus sylvestris*. Pedotrophicity indices which were calculated for these samples do not depend on the growth stage of *C. epigeios* and are higher than for the control area. Immobilization-mobilization of nitrogen indices in samples of tailings from the area with grasses and perennials and from the area with grasses, shrubs, and sun-loving trees ranged from 1.94 to 3.52 and were higher compared to the control site.

Keywords: recultivation; coal mine dumps; stages of succession; bacteria of nitrogen cycle; bacteria of sulfur cycle; cellulose-degrading microorganisms; pedotrophic microorganisms.

Introduction

Mining activities significantly impact the environment, especially in regions where land is set aside for mining and waste storage. The negative impact on the environment is observed throughout the life of a coal mine, starting with exploration, the production period and after its closure (Boldy et al., 2021). Coal mining is one of the main global geogenic and anthropogenic sources of environmental pollution with heavy metal compounds due to the formation of highly polluted drainage waters from mine tailings and rock dumps (Ovetibo et al., 2021), the cause of land degradation, loss of biodiversity, significant changes in natural areas and disappearance ecosystems, etc. (Harfoot et al., 2018; Poberezhsky et al., 2019; Boldy et al., 2021).

On the territory of the Chervonograd mining-industrial district (Ukraine) unvegetated and partially rehabilitated coal mining waste dumps of the Central Enrichment Factory "Chervonohradska", rehabilitated as a result of applying a layer of soil mixture coal mining waste dumps of the active mine "Nadia" , and naturally overgrown coal mining waste dumps of the inactive coal mine "Vizeyska" are located. The dumping of the tailings of the "Vizeyska" coal mine began in 1960. Its height reaches 30 m and the base area is more than 10 hectares. The modern plant cover of the upper part, slopes, and foot of the coal mining waste dump appeared as a result of self-growth and is represented mostly by pine, alder, and birch. Plant cover began to develop on dumps after 10–15 years according to the zonal type (Bashutska, 2006). The results of landscape and ecological studies of the "Vizeyska" coal mine waste dump are covered in the publication (Rudko et al., 2019). The authors demonstrated that the process of soil formation on the coal mining waste dump develops under the simultaneous interaction of many factors, as a result of which various techno-soils are formed. Features of techno-soils are determined by the composition of bulk sandy-lithogenic or weathered biotic-lithogenic and metamorphosed fine soils. Such techno-soils are developed on 30–40% of the coal mining waste dump area. The zone of active formation of soil and plant cover is located within the oldest coal mining waste dump, which is more than 50 years old.

The natural overgrowth of coal mine dumps is influenced by toxic substances in the dump substrates, water and temperature conditions, and meso-relief. The allelopathic effect is revealed by the exudates of plants that populate the substrate of dumps (Bashutska, 2006; Baranov, 2008; Popovych et al., 2011; Kompała-Bąba et al., 2019). As a result, a certain set of ecological niches with different regimes of humidification, heat supply, and soil nutrition is created, causing the heterogeneity of vegetation successions in such landscapes. Pioneers are species that have a wide ecological and phytocenotic amplitude. They are characterized by a powerful root system, a pillow-like aerial part or a basal rosette of leaves, pubescence, or a wax coating on leaves and stems (Safonova & Reva, 2009). Among them, a special place is occupied by *Calamagrostis epigeios* (L.) Roth., which grows in pine and mixed forests, and also settles on substrates with a low humus content, in particular, on fellings, burnings, and mine waste dumps (Kompała-Bąba et al., 2020; Kompała-Bąba et al., 2021a). *Calamagrostis epigeios* is a perennial herbaceous plant of the Poaceae family with a long creeping rhizome. The plant tolerates salinity, quickly captures substrates, and is resistant to the influence of heavy metals (Beshley et al., 2010; Beshley, 2011; Kompała-Bąba et al., 2021b).

The microbiota, which is the first soil-forming factor and is responsible for the transformation of many compounds into forms available to plants, plays an important role in the processes of plant settlement of mine tailings (Kumar & Verma, 2018; Gazitúa et al., 2021). Microorganisms of mine tailings provide many important biogeochemical processes, in particular, carbon (Sun et al., 2018) and nitrogen fixation (Sun et al., 2019, 2020a; Li et al., 2022b), cycles of metals and metalloids (Sun et al., 2020b; Li et al., 2021). As a result of their metabolism, microorganisms affect the ecosystem and create conditions for the overgrowth of mine tailings (Li et al., 2022a). The insufficient content of nutrients is one of the factors that limit the phytoremediation of mine tailings. Bacteria, microscopic fungi, and algae play an important role in the solubilization of phosphorus-containing minerals, potassium-containing feldspar, etc., improving the availability of these elements for plants. Microorganisms synthesize biologically active substances, for example, organic acids, amino acids, vitamins, indole acetic acid, phytohormones, and siderophores, which improve plant growth. Plant growth is positively affected by enzymes of microbial origin, in particular, 1-aminocyclopropane-1-carboxylate deaminase, which provides a decrease in ethylene content (Singh et al., 2022). Microorganisms of the rhizosphere can also be one of the factors that increase the tolerance of plants to low pH values, metal compounds, drought, salinity, high temperatures, etc. (Wang et al., 2017; Etesami & Beattie, 2018; Gupta & Pandey, 2019; Mghazli et al., 2021).

Microorganisms of mine tailings are characterized by various adaptations that ensure survival in such environments, so they are promising for the creation of phytoremediation technologies. Effective *in situ* bioremediation can be based on a deep understanding of the composition and functioning of the rhizosphere microbiota of plants inhabiting mine tailings (Sun et al., 2018). Data on the microbiota of the rhizosphere of plants inhabiting coal mine tailings are scarce.

The work aimed to analyze the composition of the microbiota of the *C. epigeios* rhizosphere zone, depending on the age of the plants and the stage of succession, of the naturally overgrown coal waste dumps of the "Vizeyska" mine in the Chervonograd mining-industrial district.

Materials and methods

Areas of the coal waste dumps of the "Vizeyska" mine of the Chervonograd mining-industrial district were selected on a terrace of at least 5 m^2, which was overgrown with *C. epigeios*. Plants were selected from areas of two types, which corresponded to two stages of succession for *C. epigeios*: the first area – with grasses and perennials, where this species dominates within the vegetation, and the second – with grasses, shrubs, and sun-loving trees, where *C. epigeios* prevails among other herbaceous plants, and in the tree layer, where there were *Betula pendula* Roth, *Populus tremula* L. with an admixture of *Pinus sylvestris* L. Because *C. epigeios* can be in different age states (Beshley, 2011), individuals of seedlings, juveniles, sub-adult, and adults were selected. The control was samples of the coal tailings without plants from the same mining waste dump. For analysis, the root system was taken from which the tailings were shaken and placed into sterile plastic containers. In laboratory conditions, the tailings adjacent to the roots were carefully scraped into a petri dish with a sterile scalpel. A weight of 1 g of tailings was added to 10 mL of sterile isotonic solution (0.9% NaCl). Serial dilutions were sown on appropriate nutrient media (Hudz et al., 2014). The number of microorganisms of different groups was determined by the number of colony forming units (CFU) on appropriate media. Soil agar (SA) was used to count the number of pedotrophic microorganisms (50.0 g of coal tailings poured with 1.5 L of water and autoclaved – 30 min at 121 °C). After that obtained extract was filtrated through a paper filter. After filtration 0.5 g $CaCO_3$ was added to the hot filtrate, mixed, and filtered again. To the resulting mixture 0.2 g of K_2HPO_4 was added, the volume brought to 1 L, pH to 7.0, and autoclaved – 30 min at 121 °C. The number of microorganisms that metabolize nitrogen of organic compounds was determined on meat peptone agar (MPA – meat peptone broth – 1 L, agar – 20.0 g, pH 7.0), oligonitrophilic bacteria, including nitrogen-fixing microorganisms – on Ashby's mannitol agar ($C_6H_{14}O_6$ – 20.0 g, K_2HPO_4 – 0.2 g, NaCl – 0.2 g, $MgSO_4$•$7H_2O$ – 0.2 g, K_2SO_4 – 0.1 g, $CaCO_3$ – 5.0 g, agar – 20.0 g, distilled water – up to 1 L, pH 7.0), microorganisms that metabolize nitrogen of inorganic compounds – on starch-ammonia agar (SAA) (starch – 10.0 g, $(NH_4)_2SO_4$ – 2.0 g, K_2HPO_4•$3H_2O$ – 1.0 g, $MgSO_4$•$7H_2O$ – 1.0 g, $CaCO_3$ – 3.0 g, agar – 20.0 g, distilled water – up to 1 L, pH 7.0). The number of microorganisms involved in the transformation of sulfur compounds was determined: on the Beyerink medium for acidophobic thiobacteria (solution A: $Na_2S_2O_3$•$5H_2O$ – 5.0 g, $NaHCO_3$ – 1.0 g, NH_4Cl – 0.1 g, Na_2HPO_4•$12H_2O$ – 0.2 g, $MgCl_2$•$6H_2O$ – 0.1 g, agar – 20.0 g, water – up to 900 mL; solution B: $FeSO_4$•$7H_2O$ – 1.0 g, $Na_3C_6H_5O_7$ – 3.0 g were dissolved in 100 mL of distilled water. After sterilization, 1 mL of solution B was added to solution A, pH 9.2–9.4; on Silverman–Lundgren medium 9K for acidophilic thiobacteria (solution A: $(NH_4)_2SO_4$ – 3.0 g, KCl – 0.1 g, K_2HPO_4•$3H_2O$ – 0.5 g, $MgSO_4$•$7H_2O$ – 0.5 g, $Ca(NO_3)_2$ – 0.01 g, agar – 20.0 g, distilled water – up to 700 mL. Solution B: $FeSO_4$•$7H_2O$ or $Na_2S_2O_3$•$5H_2O$ – 44.2 g, distilled water – up to 300 mL. Solution C: 10 n H_2SO_4 solution. After sterilization, 1 mL of solution C was added to solution B, and the resulting mixture of solutions B and C was mixed with solution A; pH 3.5–4.0); on the Kravtsov–Sorokin medium for sulfate-reducing bacteria (Na_2SO_4•$10H_2O$ – 0.5 g, NaH_2PO_4 – 0.3 g, K_2HPO_4•$3H_2O$ – 0.5 g, $(NH_4)_2SO_4$ – 0.2 g, $MgSO_4$•$7H_2O$ – 0.1 g, $NaC_3H_5O_3$ – 2.0 g, agar – 8.0 g, water – 50 mL, distilled water – 950 mL, pH 7.0–7.5); on Kravtsov–Sorokin medium without SO_4^{2-} for sulfur-reducing bacteria (S^0 – 2.5 g, NaH_2PO_4 – 0.3 g, K_2HPO_4•$3H_2O$ – 0.5 g, NH_4Cl – 0.16 g, $MgCl_2$•$6H_2O$ – 0.1 g, $NaC_3H_5O_3$ – 2.0 g, agar – 8.0 g, water – 50 mL, distilled water – 950 mL, pH 7.0–7.5). Hutchinson's medium (filter paper; K_2HPO_4•$3H_2O$ – 1.0 g, $CaCl_2$ – 0.1 g, $MgSO_4$•$7H_2O$ – 0.3 g, NaCl – 0.1 g, $FeCl_3$•$6H_2O$ – 0.02 g, $CaCO_3$ – 2.5 g, $NaNO_3$ – 2.5 g, agar – 20.0 g, distilled water – up to 1 L, pH 7.0) was used to determine cellulose-degrading microorganisms. Microscopic fungi were isolated on beer mash agar (BMA) (mash – 500 mL, agar – 20.0 g, distilled water – 500 mL, pH 7.0).

The directedness of microbiological processes in the samples of tailings was determined according to (Andreyuk et al., 2001; Volkogon et al., 2010). The coefficient of mineralization and immobilization of nitrogen ($K_{m.-im.}$) was calculated according to the formula: $K_{m.-im.} = C_{SAA} / (C_{MPA} + C_{BMA})$, where C_{SAA}, C_{MPA}, and C_{BMA} are the amount of CFU/g abs. dry tailings on SAA, MPA, and BMA. The index of pedotrophicity ($K_{ped.}$) was calculated according to the formula: $K_{ped.} = C_{SA} / (C_{MPA} + C_{BMA})$, where C_{SA} is the amount of CFU/g abs. dry tailings grown on SA; C_{MPA}, C_{BMA} – number of CFU/g abs. dry tailings on MPA and BMA.

Statistical processing of results and graphs were conducted using OriginPro 8.5 (OriginLab Corporation, USA, 2010). Results are presented as mean value with correction for standard deviation (x ± SD). The reliability of the data and the difference between them were estimated by the Student coefficient. A difference with a significance level of $P < 0.05$ was considered reliable. The two-way analysis of variables (ANOVA) followed by Bonferroni test was used to compare the effects of plant growth phases and succession stages on the number of different groups of microorganisms.

Results

The number of microorganisms of different groups during different phases of growth of *C. epigeios* in coal tailings samples from the areas of the naturally overgrown coal waste dumps of the "Vizeyska" mine, which correspond to different stages of succession, was studied: grasses and perennials, where this species dominates within the vegetation, and grasses, shrubs, and sun-loving trees, where *C. epigeios* prevails among other herbaceous plants, and in the tree layer there were *B. pendula*, *P. tremula* with an admixture of *P. sylvestris*. The control was tailings samples from the site without plants of the "Vizeyska" mine of the Chervonograd mining-industrial district.

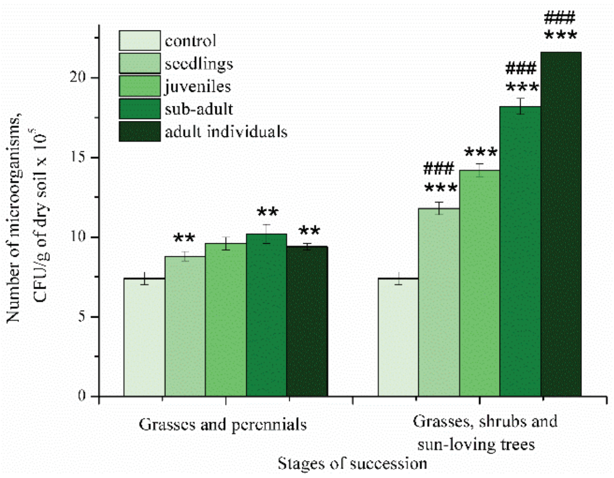

Fig. 1. The number of pedotrophic microorganisms in the rhizosphere zone of *Calamagrostis epigeios*, depending on the phase of plant growth and the stage of succession, of the naturally overgrown coal waste dumps of the "Vizeyska" mine of the Chervonograd mining-industrial district ($x \pm SD$, ** – $P < 0.01$, *** – $P < 0.001$, $n = 5$ – significant changes compared to control, ### – $P < 0.001$, $n = 5$ – significant changes in the number of microorganisms in samples from rhizosphere zone of *C. epigeios* from the area with grasses, shrubs, and sun-loving trees, compared to the number of microorganisms in samples from rhizosphere zone of *C. epigeios* from the area with grasses and perennials)

The number of pedotrophic microorganisms in samples of tailings from the area with grasses and perennials increased when *C. epigeios* was at the stages of juvenile and sub-adult phases of growth. During the adult phase of growth, their number slightly decreased and differed little from the number at the seedling stage (Fig. 1). The number of pedotrophs in the sample from the area with grasses, shrubs, and sun-loving trees also increased with the change in the growth phase of *C. epigeios*. During the adult growth phase of *C. epigeios* the number of pedotrophic microorganisms was higher by 1.8 times, compared to the number of these microorganisms in the sample during the seedlings stage of *C. epigeios*. The number of pedotrophic microorganisms in samples with vegetation at various stages of succession was higher, compared to the number of these microorganisms in samples without plants, which were the control (Fig. 1).

The number of microorganisms that metabolize nitrogen of organic compounds, oligonitrophilic microorganisms and microorganisms that metabolize nitrogen of inorganic compounds in samples from both areas with vegetation was higher, compared to the number of microorganisms of these groups in the control sample (Fig. 2a, 2b, 2c). The number of microorganisms of these groups changed with the change in the growth phase of *C. epigeios*. The highest number of microorganisms that metabolize nitrogen of organic compounds, oligonitrophilic microorganisms and microorganisms that metabolize nitrogen of inorganic compounds in the samples of tailings from the area with grasses, shrubs, and sun-loving trees was during the adult growth phase of *C. epigeios*. In the area, where *C. epigeios* dominated within vegetation, the highest number of microorganisms that metabolize nitrogen of organic compounds was also during the adult phase of plant growth. At the same time, the number of bacteria that assimilate mineral forms of nitrogen and oligonitrophilic bacteria, including nitrogen-fixing microorganisms, was the highest in the sub-adult growth phase.

The number of sulfur-reducing microorganisms in samples of tailings from the area with grasses and perennials varied slightly during different growth phases of *C. epigeios* (Fig. 3a), and differed slightly from the number of these microorganisms in the sample without plants.

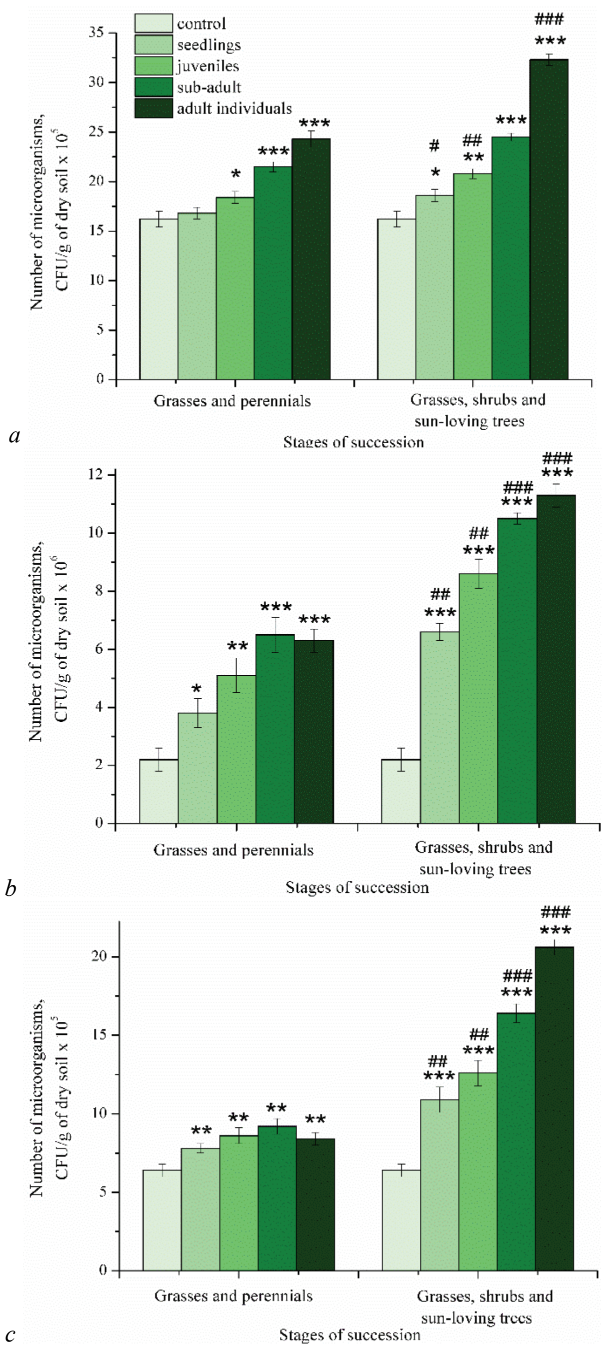

Fig. 2. The number of microorganisms involved in the transformation of nitrogen compounds in the rhizosphere zone of *Calamagrostis epigeios*, depending on the phase of plant growth and the stage of succession, of the naturally overgrown coal waste dumps of the "Vizeyska" mine of the Chervonograd mining-industrial district: *a* – the number of microorganisms, that metabolize the nitrogen of organic compounds, *b* – the number of bacteria that assimilate mineral forms of nitrogen, *c* – the number of oligonitrophilic, including nitrogen-fixing, microorganisms; $x \pm SD$, * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$, $n = 5$ – significant changes compared to control, # – $P < 0.05$, ## – $P < 0.01$, ### – $P < 0.001$, $n = 5$ – significant changes of the number of microorganisms in samples from rhizosphere zone of *C. epigeios* from the area with grasses, shrubs, and sun-loving trees, compared to the number of microorganisms in samples from rhizosphere zone of *C. epigeios* from the area with grasses and perennials)

The number of sulfur-reducing bacteria in the sample from the area with grasses, shrubs, and sun-loving trees changed with the change in the growth phase of *C. epigeios*. During the sub-adult and adult phases of growth, the number of sulfur-reducing bacteria in the sample of tailings from the area with grasses, shrubs, and sun-loving trees was 2.50–2.75 times higher, compared to the number of these microorganisms in the sample without plants (Fig. 3a). The number of sulfate-reducing bacteria in the sample of tailings from the area with grasses and perennials and from the area with grasses, shrubs, and sun-loving trees decreased with the change in the growth phase of *C. epigeios*. The number of sulfate-reducing microorganisms was also significantly lower, compared to the number of these microorganisms in the sample of tailings without plants (Fig. 3b).

Thiobacteria provide oxidation of reduced sulfur compounds, using them as electron donors. The number of acidophilic and acidophobic thiobacteria in the investigated samples of tailings slightly decreased during different phases of plant growth, and was also lower at the adult stage, compared to the sample of tailings from the site without plants. In the sample of tailings from the site with grasses, shrubs, and sun-loving trees, the number of thiobacteria was the highest during the stage of seedlings of *C. epigeios*, and since their number decreased (Fig. 3c, 3d).

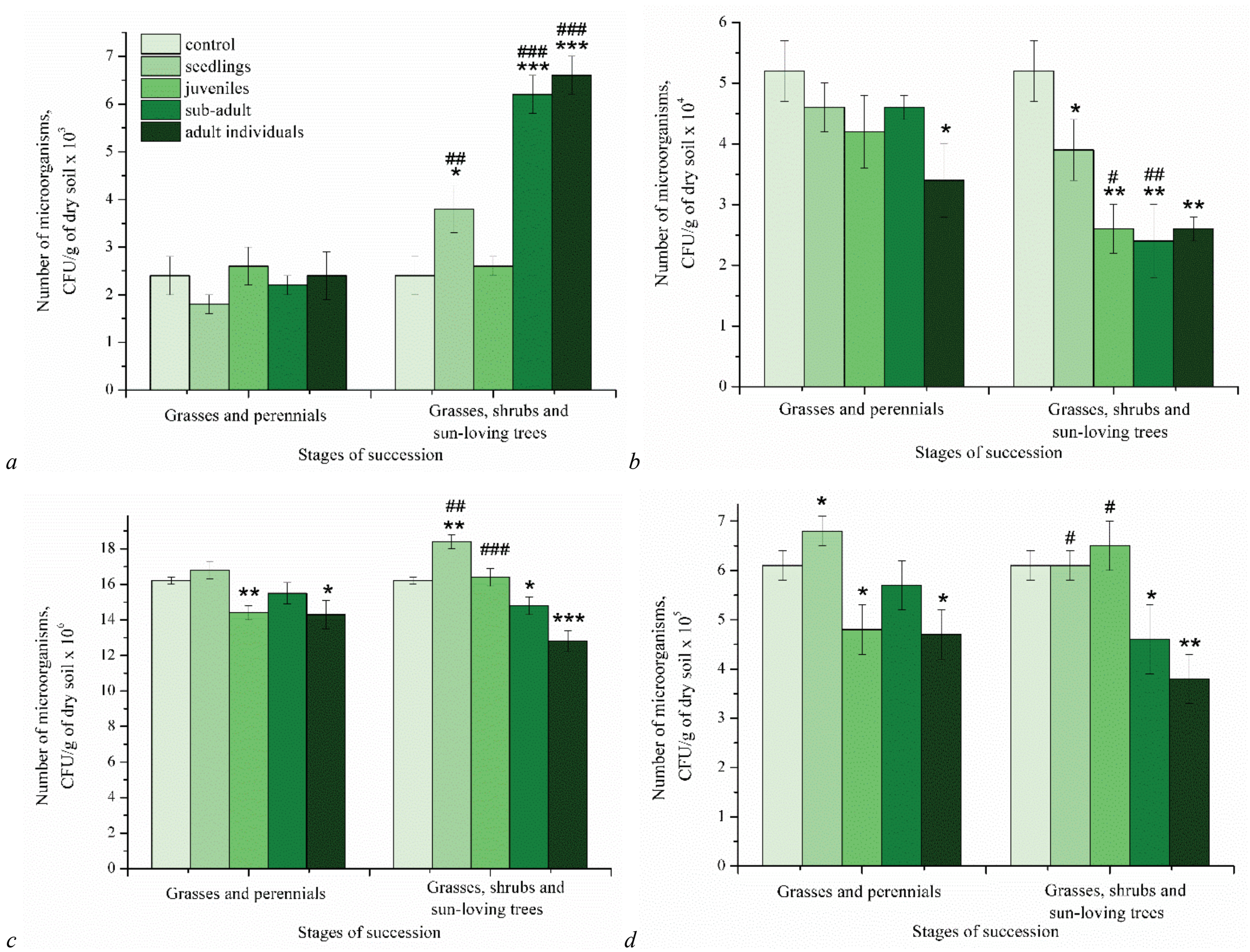

Fig. 3. The number of microorganisms involved in the transformation of sulfur compounds in the rhizosphere zone of *Calamagrostis epigeios*, depending on the age of the plants and the stage of succession of the naturally overgrown coal waste dumps of the "Vizeyska" mine in the Chervonograd mining-industrial district: *a* – the number of sulfur-reducing bacteria, *b* – number of sulfate-reducing bacteria, *c* – number of acidophilic thiobacteria, *d* – number of acidophobic thiobacteria; x ± SD, * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$; n = 5 – significant changes compared to control, $^{\#}$ – $P < 0.05$, $^{\#\#}$ – $P < 0.01$, $^{\#\#\#}$ – $P < 0.001$; n = 5 – significant changes of the number of microorganisms in samples from rhizosphere zone of *C. epigeios* from the area with grasses, shrubs, and sun-loving trees, compared to the number of microorganisms in samples from rhizosphere zone of *C. epigeios* from the area with grasses and perennials

The number of cellulose-degrading microorganisms was also the highest during the adult phase of plant growth in samples of tailings from the area with grasses and perennials and from the area with grasses, shrubs, and sun-loving trees. During the adult phase of growth, the number of cellulose-degrading microorganisms in the samples of tailings from the area with grasses and perennials was higher by 72 times, compared to the number of these microorganisms in the samples of tailings without plants. The number of cellulose-degrading microorganisms in the samples of tailings from the area with grasses, shrubs, and sun-loving trees during the adult growth phase exceeded the number of these microorganisms in the samples without plants by 81 times (Fig. 4a). The number of microscopic fungi in the samples of tailings with plants was lower by 4–6 times, compared to the control. The number of microscopic fungi in the samples of tailings from the area with grasses and perennials did not significantly increase with the change in the plant growth phase, and in the samples from the area with grasses, shrubs, and sun-loving trees, an increase in the number of microscopic fungi was found during the sub-adult and adult phases of plant growth, compared to the number of these microorganisms during the seedlings and the juvenile growth phase (Fig. 4b).

A two-factor analysis of variance (ANOVA) of the influence of the growth phase of *C. epigeios* and stages of the succession of the studied areas, as well as their cumulative impact (Table 1), was carried out for a comprehensive assessment of the variability of the number of microorganisms of different groups. It was established that the factors "stage of the succession of the area" and "the growth phase of *C. epigeios*" reliably affect the change in the number of microorganisms of different groups in the studied samples (Fig. 5). The eta square (%) of influence of unaccounted factors doesn't exceed 20.0%. A significant degree of influence of the factor "the growth phase of *C. epigeios*" was revealed on the number of microorganisms that metabolize nitrogen of organic compounds, acidophilic and acidophobic thiobacteria, cellulose-degrading microorganisms, and microscopic fungi. The highest eta square (%) of the influence of the factor "the growth phase of *C. epigeios*" was for cellulose-degrading microorganisms. For these five groups of microorganisms, the influence of the factor "stage of the succession of the area" and the cumulative impact of the two studied factors were much smaller. A significant influence of the factor "stage of the succession of the area" was found on the number of pedotrophic, oligonitrophilic microorganisms, microorganisms that metabolize nitrogen of inorganic compounds, sulfur-reducing microorganisms, and sulfate-reducing microorganisms.

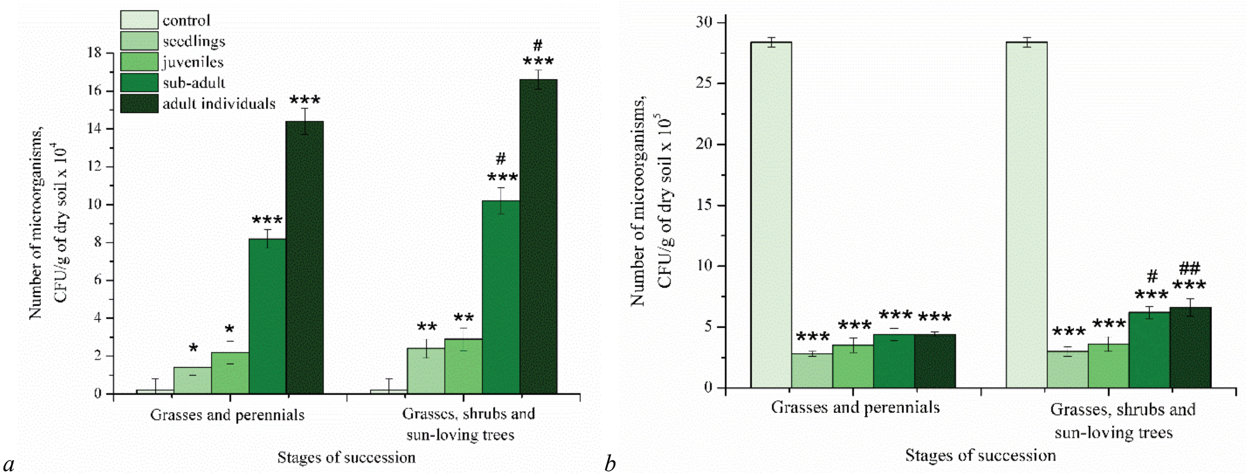

Fig. 4. The number of cellulose-degrading microorganisms and microscopic fungi in the rhizosphere zone of *Calamagrostis epigeios*, depending on the age of the plants and the stage of succession, of the naturally overgrown coal waste dumps of the "Vizeyska" mine of the Chervonograd mining-industrial district: *a* – the number of cellulose-degrading microorganisms, *b* – the number of microscopic fungi; x ± SD, * – P < 0.05, ** – P < 0.01, *** – P < 0.001, n = 5 – significant changes compared to control; # – P < 0.05, ## – P < 0.01, n = 5 – significant changes of the number of microorganisms in samples from rhizosphere zone of *C. epigeios* from the area with grasses, shrubs, and sun-loving trees, compared to the number of microorganisms in samples from rhizosphere zone of *C. epigeios* from the area with grasses and perennials

Table 1
Two-way ANOVA of the dependence of the number of microorganisms of different groups in the rhizosphere zone of *Calamagrostis epigeios* on the phase of plant growth and the stage of succession in the samples of the naturally overgrown coal waste dumps of the "Vizeyska" mine of the Chervonograd mining-industrial district

Group of microorganisms	Factor	F	P
Pedotrophic microorganisms	Stage of the succession of the area	222.36	$5.55 \cdot 10^{-16}$
	The growth phase of *C. epigeios*	27.05	$6.63 \cdot 10^{-9}$
	Cumulative impact	12.81	$1.15 \cdot 10^{-5}$
Microorganisms that metabolize nitrogen of organic compounds	Stage of the succession of the area	421.61	0
	The growth phase of *C. epigeios*	631.14	0
	Cumulative impact	58.98	$3.92 \cdot 10^{-13}$
Oligonitrophilic microorganisms	Stage of the succession of the area	1330.02	0
	The growth phase of *C. epigeios*	158.91	0
	Cumulative impact	128.10	0
Microorganisms that metabolize nitrogen of inorganic compounds	Stage of the succession of the area	700.87	0
	The growth phase of *C. epigeios*	132.48	0
	Cumulative impact	10.25	$6.97 \cdot 10^{-5}$
Sulfur-reducing microorganisms	Stage of the succession of the area	472.91	0
	The growth phase of *C. epigeios*	67.58	$6.14 \cdot 10^{-14}$
	Cumulative impact	70.48	$3.43 \cdot 10^{-14}$
Sulfate-reducing microorganisms	Stage of the succession of the area	81.18	$2.72 \cdot 10^{-10}$
	The growth phase of *C. epigeios*	12.59	$1.33 \cdot 10^{-5}$
	Cumulative impact	5.81	0.003
Acidophobic thiobacteria	Stage of the succession of the area	2.60	0.116
	The growth phase of *C. epigeios*	35.38	$2.81 \cdot 10^{-10}$
	Cumulative impact	17.88	$5.36 \cdot 10^{-7}$
Acidophilic thiobacteria	Stage of the succession of the area	4.03	0.053
	The growth phase of *C. epigeios*	91.33	$8.88 \cdot 10^{-16}$
	Cumulative impact	24.17	$2.32 \cdot 10^{-8}$
Cellulose-degrading microorganisms	Stage of the succession of the area	66.68	$2.51 \cdot 10^{-9}$
	The growth phase of *C. epigeios*	1252.33	0
	Cumulative impact	4.16	0.013
Microscopic fungi	Stage of the succession of the area	47.41	$8.59 \cdot 10^{-8}$
	The growth phase of *C. epigeios*	67.85	$5.79 \cdot 10^{-14}$
	Cumulative impact	11.99	$2.00 \cdot 10^{-5}$

To reflect the directedness of the processes occurring in the studied areas, some relative values were calculated, in particular the pedotrophicity index and the coefficient of mineralization and immobilization of nitrogen (Table 2). The pedotrophicity index reflects the functional structure of the soil microbial communities and the degree of consumption of organic matter. It is expressed as the ratio of the number of microorganisms growing on soil agar to the number of microorganisms growing on rich nutrient media. An increase in the pedotrophicity index in mine tailings is a consequence of the intensity of decomposition of humic substances. Its values are higher in samples taken in the area where *C. epigeios* dominates within the vegetation, and samples from the area where *C. epigeios* prevails among other herbaceous plants, and in the tree layer there were

B. pendula, *P. tremula* with an admixture of *P. sylvestris*. Pedotrophicity indices, which were calculated for these areas, do not depend on the growth phase of *C. epigeios*. In areas where *C. epigeios* dominates within the vegetation, this index is lower. For both groups of succession, the index of pedotrophicity is higher than for the control area.

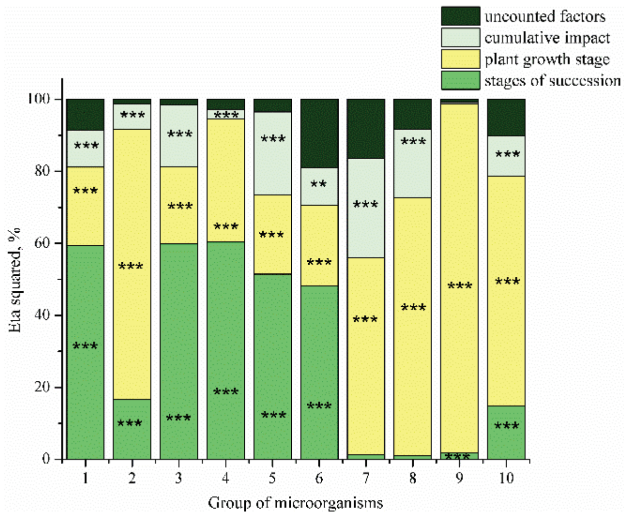

Fig. 5. Dependence of the number of microorganisms of different groups in the rhizosphere zone of *Calamagrostis epigeios* on the phase of plant growth and the stage of succession in the samples of the naturally overgrown coal waste dumps of the "Vizeyska" mine of the Chervonograd mining-industrial district: *1* – pedotrophic microorganisms, *2* – microorganisms that metabolize nitrogen of organic compounds, *3* – oligonitrophilic microorganisms, *4* – microorganisms that metabolize nitrogen of inorganic compounds, *5* – sulfur-reducing microorganisms, *6* – sulfate-reducing microorganisms, *7* – acidophobic thiobacteria, *8* – acidophilic thiobacteria, *9* – cellulose-degrading microorganisms, *10* – microscopic fungi,* – significant differences within variables P < 0.05, ** – significant differences within variables P < 0.01, *** – significant differences within variables P < 0.001

Table 2
The directedness of microbiological processes in samples of naturally overgrown coal waste dumps of the "Vizeyska" mine, Chervonograd mining-industrial district with different stages of succession

Stages of succession	Growth phase	Index of pedotrophicity	Index of mineralization and immobilization of nitrogen
Control	Without plants	0.17	0.49
Grasses and perennials	Seedlings	0.45	1.94
	Juveniles	0.44	2.33
	Sub-adult	0.39	2.51
	Adult individuals	0.33	2.20
Grasses, shrubs, and sun-loving trees	Seedlings	0.55	3.06
	Juveniles	0.58	3.53
	Sub-adult	0.59	3.42
	Adult individuals	0.56	2.91

The coefficient of mineralization-immobilization of nitrogen reflects the intensity of the processes of mineralization and assimilation of nitrogen-containing compounds in the soil. The immobilization-mobilization of the nitrogen index in samples of tailings from the area with grasses and perennials and from the area with grasses, shrubs, and sun-loving trees was higher, compared to this indicator in the sample of coal tailings without plants, and ranged from 1.94 to 3.52.

Discussion

The overgrowth of tailings in coal mine waste dumps is accompanied by the accumulation of organic compounds as a result of the vital activity of plants or the death of plant biomass. This assumption is supported by the calculation of the pedotrophicity index (Bulyhin & Tonkha, 2018; Yamborko et al., 2019), which is 3.3 to 3.5 times higher for sites where *C. epigeios* dominates among other herbaceous plants, and in the tree layer there are *B. pendula*, *P. tremula* with an admixture of *P. sylvestris*, compared to the samples of tailings from the area without plants. The accumulation of organic compounds promotes the development of various groups of chemoorganotrophic microorganisms, and most of all – cellulose-degrading ones in the rhizosphere zone of *C. epigeios*. The number of cellulose-degrading microorganisms in the samples of tailings from the area with grasses and perennials and from the area with grasses, shrubs, and sun-loving trees increased with the change in the phase of plant growth, which is probably due to the increase in the phytomass of *C. epigeios* (Beshley et al., 2010; Beshley, 2011), which is a substrate for microorganisms of this group. Cellulose-degrading microorganisms, releasing cellulases, contribute to the formation of humic substances from the products of cellulose decomposition. The intensity of cellulose decomposition can be used to assess the role of cellulose-degrading microorganisms in the formation of soils, especially in technogenically transformed areas (Dyakiv et al., 2016). We assume that the existing compounds are intensively metabolized by microorganisms in the samples of coal tailings from the investigated sites with plants, as evidenced by the increase of the immobilization-mobilization of nitrogen index. The directedness of microbiological processes in the coal tailings of the investigated dumps depends on physical and chemical factors and confirms the role of the natural overgrowth processes of the dumps in their remediation and soil formation processes (Kuzmishyna-Diakiv & Hnatush, 2015). The values of the studied indices were higher in the samples of tailings from areas with grasses, shrubs, and sun-loving trees, which is probably due to a higher phytomass and, accordingly, a higher content of nutrient compounds. In addition to the numerous advantages of phytoremediation of mine tailings, the immobilization of toxic compounds, in particular, heavy metals, is one of the most important (Gazitúa et al., 2021). It was established that the microbiota of the rhizosphere is involved in the process of plant tolerance to heavy metal compounds and other toxic compounds (Kumar & Verma, 2018). We assume that the increase in the number of microorganisms that metabolize the nitrogen of organic compounds, microorganisms that metabolize the nitrogen of inorganic compounds, oligonitrophilic microorganisms, cellulose-degrading microorganisms – that are chemoorganotrophs, in samples from areas with grasses, shrubs, and sun-loving trees, compared to samples from the area with grasses and perennials, or the area without plants, in addition to the increase in the content of nutrient compounds , is due to a decrease in the content or availability of heavy metals or other compounds. The number of pedotrophic microorganisms, microorganisms that metabolize the nitrogen of organic compounds, cellulose-degrading microorganisms, and microscopic fungi depended more on the age of *C. epigeios*, and not on the stage of the succession of the studied area, which is probably due to their trophic features.

The number of chemolithotrophic bacteria, particularly thiobacteria, decreased with the change in the growth phase of *C. epigeios* both in the samples from the area with grasses and perennials and in the samples from the area with grasses, shrubs, and sun-loving trees. Probably, the decrease in the number of thiobacteria is due to the increase in the content of organic compounds in the coal tailings. As a result of the conducted two-factor variance analysis (ANOVA) of the influence of the growth phase of *C. epigeios* and the stages of the succession of the studied areas, as well as their cumulative influence, it was established that the number of thiobacteria depended more on the age of *C. epigeios*. Probably, among the isolated thiobacteria, obligate chemolithotrophs, which are sensitive to the content of organic compounds in the substrate, were dominated.

Bacteria involved in the transformation of sulfur compounds play an important role in the functioning of mine tailing cenoses since sulfur is one of the common elements in coal tailings (Blayda, 2016). Sulfur-reducing bacteria are part of the group of factors that create prerequisites for the functioning of other groups of microorganisms in coal mine waste dumps under conditions of high concentration of heavy metal and sulfur compounds. These microorganisms, despite their low abundance in coal mine waste, reduce the mobility of heavy metal compounds as a result of dissimilatory sulfur and metal reduction (Kuzmishyna-Diakiv & Hnatush, 2015). Sulfur- and sulfate-reducing bacteria of mine tailings are involved in the reducing link of the sulfur cycle, ensuring the reduction of oxidized sulfur compounds to hydrogen sulfide, and later to metal sulfides due to the precipitation of ferrum, copper, and chromium (Dyakiv et al., 2017).

Conclusion

The number of pedotrophic microorganisms, microorganisms that metabolize nitrogen of organic compounds, cellulose-degrading microorganisms, and microscopic fungi depended more on the age of *C. epigeios*, and not on the stage of the succession of the studied area. The number of chemolithotrophic bacteria, particularly thiobacteria, decreased with the change of the growth phase of *C. epigeios*. The number of acidophobic thiobacteria depended more on the stage of succession, and the number of acidophilic thiobacteria depended more on the age of the *C. epigeios*. The number of microorganisms that metabolize nitrogen of organic compounds, oligonitrophilic microorganisms and microorganisms that metabolize nitrogen of inorganic compounds in the samples of tailings from the area with grasses and perennials and from the area with grasses, shrubs, and sun-loving trees was higher, compared to the number of these groups of microorganisms in the control and changed with the change in the growth phase of *C. epigeios*. The number of microorganisms that metabolize nitrogen of organic compounds, oligonitrophilic microorganisms and microorganisms that metabolize nitrogen of inorganic compounds was the highest in the samples from the area with grasses, shrubs, and sun-loving trees during the adult growth phase of the *C. epigeios*. In the area where *C. epigeios* dominated within the vegetation, the highest number of microorganisms that metabolize nitrogen of organic compounds was also during the adult phase of *C. epigeios*, and the number of bacteria that assimilate mineral forms of nitrogen and oligonitrophilic microorganisms was the highest during the sub-adult stage. The index of pedotrophicity is higher in the samples taken in the area where *C. epigeios* prevails among other herbaceous plants, and in the tree layer there are *B. pendula*, *P. tremula* with an admixture of *P. sylvestris*. Pedotrophicity indices, which were calculated for these samples, do not depend on the growth stage of *C. epigeios* and are higher than for the control area. Immobilization-mobilization of nitrogen indices in samples of tailings from the area with grasses and perennials and from the area with grasses, shrubs, and sun-loving trees ranged from 1.94 to 3.52 and were higher compared to the control site.

Thus, it was established and confirmed as a result of two-factor variance analysis that the growth phase of *C. epigeios* and the stage of the succession of coal mine waste dumps have different effects on the number of microorganisms from the rhizosphere zone of plants.

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