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DEGRADATION, REHABILITATION, AND CONSERVATION OF SOILS

Microbiological Indicators of Heavy Metals and Carbon-Containing Preparations Applied to Agrosoddy-Podzolic Soils Differing in Humus Content

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Abstract—The response of the microbial community (microbial biomass carbon (C_{mic}), basal respiration (BR), and functional diversity (FD)) of agrosoddy-podzolic soil (Albic Glossic Retisols (Loamic, Aric Cutanic, Ochric)) to pollution by heavy metals (HMs: Cu 660, Zn 1100, Pb 650 mg/kg) and carbon-containing preparations (5% of biochar and 0.25% of lignohumate) was studied in model experiment (30 days). Soils with different organic carbon contents (C_{org} 3.86 and 1.30%) were sampled at two sites (Chashnikovo, Moscow oblast). We determined C_{mic} by the substrate-induced respiration method and FD by multisubstrate testing (47 substrates). It was found that HMs application reduced C_{mic} on average by 49–57%, BR by 23–52%, and FD by 45%, but, on the contrary, increased the microbial metabolic quotient ($qCO_2 = BR/C_{mic}$) by 9–46%. The changes of these properties were most significant in the soil with low C_{org} content (1.30%). Carbon-containing preparations did not contribute to variations in C_{mic}, BR, and qCO_2 in both soils with HMs, but increased their FD. It is concluded that the studied microbiological parameters may be used as indicators for optimal assessment of soil quality: FD and C_{mic} are the more sensitive to HMs than BR and qCO_2.

Keywords: bioindication, soil quality assessment, organic carbon, microbial respiration, microbial biomass, functional diversity of microorganisms, chemical pollution, lignohumate, biochar

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INTRODUCTION

An efficient system for assessing soil quality should be based on a combination of sensitive indicators reflecting soil capacity to function [23] and to perform ecosystem services aimed at maintenance of nutrient cycles, degradation of pollutants, and climate regulation [46]. Soil microorganisms provide the decomposition of organic matter and the release of mineral nutrients and thus contribute to the diversity and productivity of plants [50]. Hence, soil microorganisms can be used as indicators of the status of soil cenoses. Microbial biomass and respiratory activity of soil may reflect to some extent its changes under different anthropogenic impacts [2, 44] and, thus, characterize soil health [23]. These parameters are included in the environmental monitoring programs of soils and terrestrial ecosystems in some European countries [30, 33]. The analysis of soil microbial communities by range of consumable organic substrates ("metabolic profiling") is a promising approach to assess soil quality. In the world practice, it is performed on the basis of the BIOLOG system [27]. In Russia, this technology is implemented by the method of multisubstrate testing (MST) termed Eco-Log [6].

Numerous studies devoted to soil quality assessment in agroecosystems [9, 23] are based on a wide range of indicators [23, 49]. The content of heavy metals (**HMs**) in soil is one of regularly evaluated parameters [4, 5]. It has been shown that HMs enter the soil of agrocenoses as a result of the application of mineral [38] and organic [29, 55] fertilizers, irrigation [32, 39], and the use of various soil amendments [8, 11]. In turn, HMs from soils may accumulate in agricultural products [31].

The contents of HMs, in particular, lead (Pb), zinc (Zn), and copper (Cu) in agricultural and urban soils are subjected to legislative monitoring in Russia and some other countries [3, 23]. These HMs are assigned to hazard classes I and II: highly (Pb and Zn) and moderately (Cu) toxic elements, respectively (*GOST 17.4.1.0283*).

The application of carbon-containing materials—biochar [28] and humic preparations—is one of the methods to reduce HMs content in soils [43].

Biochar is a product of pyrolysis of various materials, including plant debris, organic wastes, and sewage sludge; it is characterized by the high carbon content (70–80%) and high sorption capacity. These properties of wood biochar make it possible to actively immobilize various pollutants in environment and to increase the soil water holding capacity [25]. The role of the biochar in carbon sequestration and restoration of humus-depleted soils, as well as in remediation of polluted soils (including oil-polluted soils), is well studied [19, 22, 35, 40]. Biochar application results in a significant decrease in the soil content of exchangeable HMs [42]. This effect is most significant for humus-depleted and acid soils [45]. A decrease in the Cd and Pb contents in rice seeds grown on a slightly acid clay soil has been observed after the biochar application [21]. The adsorption capacity of the preparation in soil depends on its origin (material) and the HMs type. There are data that soil mulching by biochar increases the As and Cu mobility, but almost does not affect the Cd and Pb mobility [18].

Products of so-called "green chemistry" are also widely used to improve agroecosystem soils; they are mainly represented by humic preparations made of coal, peat, sapropels, and organic waste [43]. Their biological activity depends on the initial material [52]. Lignohumate produced of lignosulfonate (waste of the wood processing industry) is an efficient humic preparation, which contains high- and low-molecular-weight humus compounds (70–80%) and microelements.

There are also data on a positive remediation effect after the combined application of biochar and humic preparations to contaminated soils [15, 56]. The mobility of Zn, Cd, and Pb cations in alluvial soils decreases more significantly after the application of a mixture of lignohumate and biochar as compared to their separate use [15].

Changes in the microbial community functioning of variously humified agrosoddy-podzolic soils as a result of their contamination by HMs and application of carbon-containing preparations (biochar and lignohumate) were studied in a model pot experiment. The aim was to assess the bioindication role of microbial communities and to ensure plant productivity. We analyzed the responses of microbial communities by their functional and structural parameters (microbial biomass, basal respiration, metabolic quotient, and functional diversity) to evaluate soil degradation under HMs contamination and subsequent remediation with organic preparations.

MATERIALS AND METHODS

We studied plow horizons of clay loamy agrosoddy-podzolic soils (Albic Glossic Retisols (Loamic, Aric Cutanic, Ochric)) in the area of the Chashnikovo Training and Experimental Soil Ecological Center of Lomonosov Moscow State University (Solnechnogorsk district, Moscow oblast). The two analyzed plots were located at a distance of 1.1 km from one another (56°02′01.9° N, 37°10′04.9° E and 56°01′41.7° N, 37°11′04.3° E). The organic carbon content was high in soil of plot 1 (C_{org} = 3.86%, strongly humified soil) and low in soil of plot 2 (C_{org} = 1.30%, slightly humified soil). In early May 2019, soil samples were taken from the upper 20-cm layer from the corners and center of the test plots (40 m^2) with subsequent combine into one composite soil sample (25 kg), and transported to the laboratory for the pot experiment (the initial soil water content was 35–40%).

Water solutions (10 mL/kg) of copper ($CuSO_4$), zinc ($ZnSO_4$), and lead ($PbCl_2$) salts was added to soil samples to obtain Cu, Zn, and Pb concentrations equal to 660, 1100, and 650 mg/kg soil, respectively. These concentrations corresponded to five tentatively permissible concentrations (TPC) for each of these elements [5]. Parallel to the metals, carbon-containing preparations (biochar and lignohumate) were used separately and in combination in particular variants of the experiment.

Biochar (a pyrolysis product of birch wood, fractions 2–8 mm, produced by the Metakom Company, Russia) was added at the rate of 5% of the soil sample weight. Biochar contained C (88.2%) and N, H, and S (0.44, 0.82, and 0.19%, respectively), as well as ash (2.8%) and water (3%); pH_{CaCl_2} was 8.9, and the C/N ratio was 21.4. The content of Cu, Zn, and Pb cations in the biochar was no more than 0.02%. Potassium lignohumate was obtained by artificial humification of lignosulfonate (produced by NPO RET, Russia). Its ash content was 40%, the C, N, H, S, and K contents were 37.3, 0.5, 3.72, 4.84, and 9.0%, respectively; the C-to-N ratio was 134.7; pH_{CaCl_2} was equal to 9 (1% solution), and the content of humic acids was 58% of the organic matter. Water solution of lignohumate was applied to the soil (0.25% of its mass).

Soil samples from each plot were divided into two equal parts (soils 1 and 2). A mixture of water solutions of HM salts was applied to soil 1 and thoroughly mixed. Soil 2 was moistened with water (10 mL/kg), the volume of which was equal to that of water with HM salts. The water content of the soils was about 60% of the their water holding capacity. After these treatments, soils were left for seven days at room temperature for uniform distribution of water and solution of HM salts. Then soils 1 and 2 (with added metal salts and water) were divided into four parts (variants), one of which was used as a control for carbon-containing additives (biochar and lignohumate applied individually and in combination) and HMs. The prepared soils (eight variants for each soil) were incubated for seven days at room temperature. Then, the soil of each vari-

ant (2.5 kg) was placed in three vessels of 3 L in volume (replications). The experimental variants for soils of plots 1 and 2 were the following: control, biochar (B), lignohumate (L), biochar + lignohumate (BL), HM-control, HM + biochar (HMB), HM + lignohumate (HML), and HM + biochar + lignohumate (HMBL).

Samples of each variant were taken to determine chemical parameters of soil, and then seeds of mustard *Sinapis alba* L. (10 seeds per vessel) were sown. The vessels with soil were placed in an open greenhouse for 30 days (the mean daily air temperature was 16.8°C). The soil moisture was controlled during the experiment by weighing vessels for subsequent water addition. After the incubation was ended, mustard plants were removed from the vessels to determine their biomass, and microbiological parameters of soils were studied.

The characteristics determined in soil samples included: the content of organic carbon (C_{org}) (ISO 14235:1998); total (Elementar EL III CNHS analyzer), ammonium (*GOST 26489-85*, Hach DR 2800 photometer), and nitrate (Federal Environmental Regulations 16.1.8-98, Dionex ICS 2000 chromatograph) nitrogen (N_{total}, NH_4^+, NO_3^-); mobile compounds of phosphorus (P) and potassium (K) (approach by Kirsanov, Aglient 5110 ICP-OES spectrometer); and total Cu, Zn, and Pb (FR.1.29.2006.02149, Aglient 5110 ICP-OES spectrometer). We measured pH of water extract (soil : water = 1 : 4) by the potentiometric method (Hanna HI2211-02 pH meter).

The carbon content of microbial biomass (C_{mic}) was determined by the substrate-induced respiration (SIR) method [1]. The method is based on the respiratory response of soil microorganisms to available organic substrate addition (glucose) into the soil, which is proportional to the content of microbial biomass [16]. We placed soil sample (3 g) in a glass vial (15 mL), added drop by drop a glucose solution (0.2 mL/g) to obtain the resulting concentration of 10 mg/g, sealed it, and incubated for at least three hours at 22°C. Then, we took an air sample (0.5 mL) from the air phase of the vial by a syringe and injected it into a KristalLux 4000M gas chromatograph to measure the CO_2 concentration. The incubation time of the soil with glucose was fixed. The SIR rate (μL CO_2/(g h)) was calculated taking into account the CO_2 concentration, the volume of the gas phase in the vial, and the weight and incubation time of the soil sample. The C_{mic} content (μg C/g soil) was determined by the equation: $C_{mic} = SIR \times 40.04 + 0.37$ [16].

Basal respiration of soil (BR) was measured similarly to SIR with the application of distilled water to the soil instead of glucose solution. The incubation lasted 24 hours at 22°C, and the result was expressed as μg C-CO_2/(g h).

We calculated the BR/C_{mic} ratio, which reflected the specific respiration of microbial biomass (qCO_2) and characterized the ecological status of soil microbial community [2].

White mustard plants were extracted with their roots from the pots in each variant of the experiment and dried to a constant weight (105°C, 2 h). The resulting dry plant biomass was given in g/vessel.

The functional diversity of the microbial community was evaluated by the method of multisubstrate testing technology (branch of Biolog assay) (FR. 1. 37. 2010. 08619; patent of the Russian Federation no. 23355432335543). We placed a soil sample (0.7 g) in a centrifuge cup (50 mL), added 35 mL of distilled water, and placed it in a Vortex shaker (for 2 min) to separate microbial cells from soil particles. Then we centrifuged the suspension (10 min, 3000 rpm), separated supernatant, and added triphenyltetrazolium bromide as an indicator (2 mL of saturated solution per 20 mL of the supernatant). The supernatant aliquot with the indicator (200 μL) was placed in 96 wells (cells) of an Eco-Log plate that also contains previously dry added 47 test substrates (sugars, amino acids, polymers, nucleosides, salts of organic acids, and alcohols) and the mineral base (the control). The plates were incubated for 72 hours at 28°C until a visually detectable red color appeared in the cells due to reduction of triphenyltetrazolium to formazan during substrate utilization. After that, the optical density of each cell was measured in the range of 510 nm by the Eco-Log hardware-software complex. The formazan concentration and related to it optical density in the cells were determined from the intensity of the development of a group of microorganisms capable to consume a particular substrate [6]. The optical density was used for the calculation of the following parameters of the functional diversity of microbial community [6, 7]: the diversity (*N*) reflecting the amount of consumed substrates (from 0 to 47); the specific metabolic activity (*W*) determining the intensity of substrate utilization (the sum of the optical density of all consumed substrates divided by their number, from 0 to 4000 units); and the coefficient of the rank distribution of substrate utilization spectra (*d*) in the range from 0.01 to ≥2.00. Lower values of this coefficient characterize a wellbeing and stability of the soil microbial community, i.e., optimum conditions for its functioning, while higher values reflect unfavorable and stress conditions [6, 7].

The data on plant biomass and microbiological (C_{mic} and BR) and chemical parameters of soil for statistical processing were obtained in three replications. The C_{mic}, BR, and chemical parameters were calculated per the dry weight soil (105°C, 2 h). The substrate testing was performed for a mixed sample (three vessels) of each variant of the studied soils. The values of C_{mic}, BR, qCO_2, and mustard biomass for the experimental variants (biochar, lignohumate, and HMs) were compared by one-way analysis of variance (one-way ANOVA) followed by pairwise multiple comparison of means Tukey's test). The principal

Table 1. Chemical parameters of agrosoddy-podzolic soil sampled from different sites (0–20 cm; Moscow oblast)

Site no.	C_{org}	N_{total}	C : N	pH	NH_4^+	NO_3^-	P_2O_5	K_2O	Cu	Pb	Zn
	%				mg/kg						
1	3.86	0.33	12	6.74	21.9	60.7	1685.3	701.5	22.0	23.7	89.1
2	1.30	0.14	9.6	6.28	8.6	65.8	220.4	193.6	9.3	10.1	32.0

component analysis (PCA) was based on a correlation matrix between C_{mic}, BR, qCO_2, and mustard biomass for the experiment variants. The preliminary data preparation for the PCA included scaling according to the equation (x_i – mean)/standard deviation. The relationship between C_{mic} and mustard biomass was evaluated by the correlation analysis (the Pearson correlation coefficient). The statistical analysis and visualization of data were performed in the *R* software using the "FactoMineR", "factoextra" (PCA), "car" (one-way ANOVA), and "agricolae" (the Tukey's test) packages.

The cluster analysis of samples according to the spectrum of substrates utilization was performed using the Euclidean distance and the Ward method. The parameters of functional diversity were calculated using the Eco-log software [6] and the Statistica 7.0 program.

RESULTS

Chemical parameters of soil. The C_{org} content in the plow horizon of the high-humus soil on plot 1 was almost three times higher as compared to plot 2 (Table 1). The content of other nutrients in soil 1 was also higher than in soil 2 (two–three times for N_{total}, N-NH_4^+, and K and 7.6 times for P). The content of N-NH_4^+, the C : N ratio, and pH in the studied soils differed slightly. The amount of Cu, Pb, and Zn in soil 1 was 2.3–2.8 times higher than in soil 2, but significantly lower than their TPCs. Hence, the studied soils may be considered uncontaminated by HMs.

The C_{org} content increased by about 4% in comparison with the control variants as a result of biochar application but almost did not alter after the lignohumate addition. Soil pH did not change after biochar and lignohumate treatments.

Microbiological parameters of soil. *The* C_{mic} *content* in soil 1 with high C_{org} was, on average, almost two times higher than that in the C_{org}-depleted soil (326 and 173 μg C/g) (Fig. 1a). The application of HMs to the studied soils significantly decreased (by almost two times) the C_{mic} content. Addition of carbon-containing preparations into soils 1 and 2 (without HMs) did not cause a C_{mic} change, except for the stimulating effect of lignohumate in soil 2. The application of carbon-containing preparations into HMs contaminated soils also did not cause a significant change in the C_{mic} content.

The BR rate of soils 1 and 2 averaged 0.53 and 0.46 μg C-CO_2/(g h), respectively (Fig. 1b). This parameter did not significantly change under HMs contamination of soil 1 and decreased by 2.1 times for soil 2. The biochar and lignohumate addition (separately and in combination) into soils with HMs did not cause significant differences in the BR rate. In soils uncontaminated with HMs, biochar and lignohumate did not significantly affect the BR rate, except for the variant with their combined addition into soil 2, which increased the BR by almost 40%.

In the control variants of soil 1, qCO_2 was almost 1.7 times lower as compared to soil 2, which indicates stressed functioning of the microbial community of soil 2 (Fig. 2). An increase in the microbial metabolic quotient under HMs contamination, including the variant with carbon-containing preparations, was only significant for soil 1. Biochar and lignohumate did not cause considerable changes in qCO_2, but their combined addition significantly increased this parameter for soil 2 (by 43% on average) as compared to the control. However, no such effect was found for soil 1.

The relationship of microbial parameters (C_{mic}, BR, and qCO_2) *and plant biomass in soil samples with heavy metals and carbon-containing* preparations. The addition of HMs (Zn + Pb + Cu) into soils suppressed the plant growth: from 1.38 ± 0.05 (control) to 0.40 ± 0.13 g of dry biomass/vessel in high humified soil 1, and from 0.68 ± 0.09 g/vessel (control) to complete plant death in low humified soil 2. The plant biomass for the uncontaminated (without HMs) soil 1 was reduced to 0.77 ± 0.05 g/pot as a result of biochar application and to 0.70 ± 0.11 g/vessel as a result of the lignohumate application. In soil 2, biochar reduced the phytomass to 0.46 ± 0.11 g/pot, and lignohumate, on the contrary, increased to 0.83 ± 0.14 g/vessel (by 22%), but this change was not significant. Carbon-containing preparation treatments did not reduce the HMs toxicity in low-humus soil and slightly changed – in high-humus soil: the plant biomass increased to 0.47 ± 0.05 g/vessel after biochar addition, decreased to 0.28 ± 0.06 g/vessel after lignohumate addition, and was close to the control (0.41 ± 0.13 g/pot) after their combined use.

The PCA allows us to summarize the results and to identify patterns in the studied properties of soil microorganisms and its ability to sustain plant growth

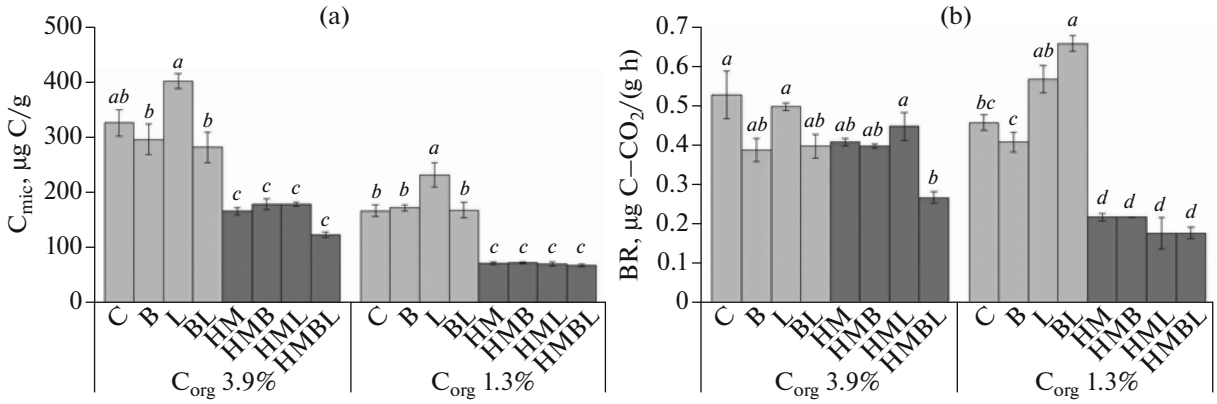

Fig. 1. (a) Microbial biomass carbon (C_{mic}) and (b) basal respiration (BR) in agrosoddy-podzolic soils (0–20 cm) with different organic carbon (C_{org}) contents and treatments. Here and in other figures: (C) control, (B) biochar, (L) lignohumate, (BL) (B + L), (HM) heavy metals, (HMB) HM + B, (HML) HM + L, and (HMBL) HM + B + L. Mean ± standard error ($n = 3$) values are given; values with different letters differ significantly ($p \le 0.05$) for each parameter and the C_{org} content ($p \le 0.05$, the Tukey criterion).

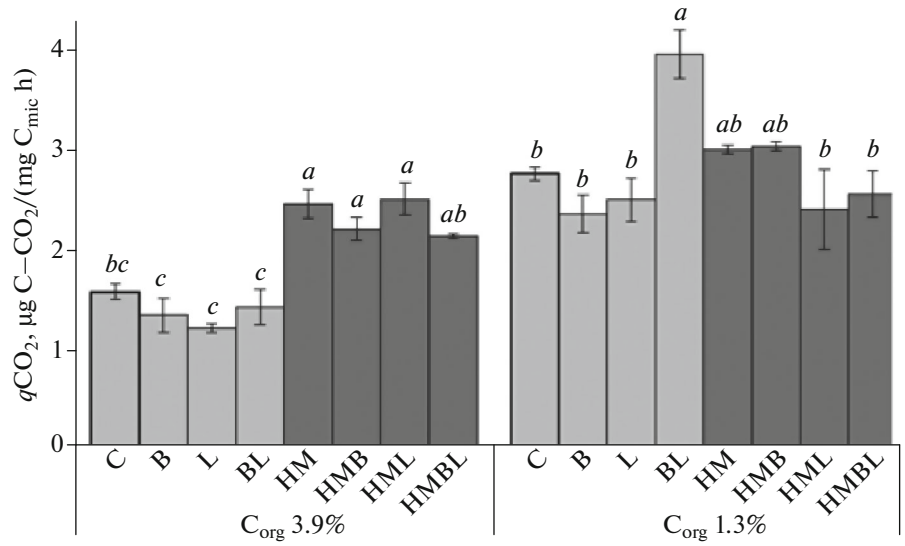

Fig. 2. The microbial metabolic quotient (qCO_2) of agrosoddy-podzolic soils (0–20 cm) with different organic carbon (C_{org}) contents and treatment methods.

after the application of HMs and carbon-containing preparations. The first two principal components are most significant (eigenvalues >1) and explain 95% of the total variance (Fig. 3).

The first principal component mainly shows the gradient of soil C_{mic} content and plant biomass for different experiment variants ($R^2 = 0.69$ and 0.67), and the second principal component characterizes qCO_2 changes ($R^2 = 0.55$). There is a clear differentiation of soils with (left) and without (right) HMs application along the first principal component. The distribution of soils along the second principal component is mainly related to the biochar and lignohumate application. The mustard biomass most closely correlates with the soil C_{mic} (the Pearson correlation coefficient $r = 0.81$, $p < 0.001$).

Functional diversity of the microbial community. Quantitative parameters of the microbial functional diversity of soils with different C_{org} contents are shown in Fig. 4. The number of substrates consumed by microbial community (N) of soil 1 was higher compared to soil 2 (37 and 11, respectively). The functional diversity of soil 2 significantly decreased after contamination with HMs (6 consumed substrates), but almost did not change for soil 1. Subsequent remediation of contaminated soil 2 with carbon-containing preparations significantly increased its microbial functional diversity: lignohumate almost restored it to the initial value (from 6 to 11), and in combination with biochar even increased it (from 6 to 16). For soil 1 contaminated by HMs, biochar slightly increased microbial functional diversity (from 37 to 40), lignohumate did

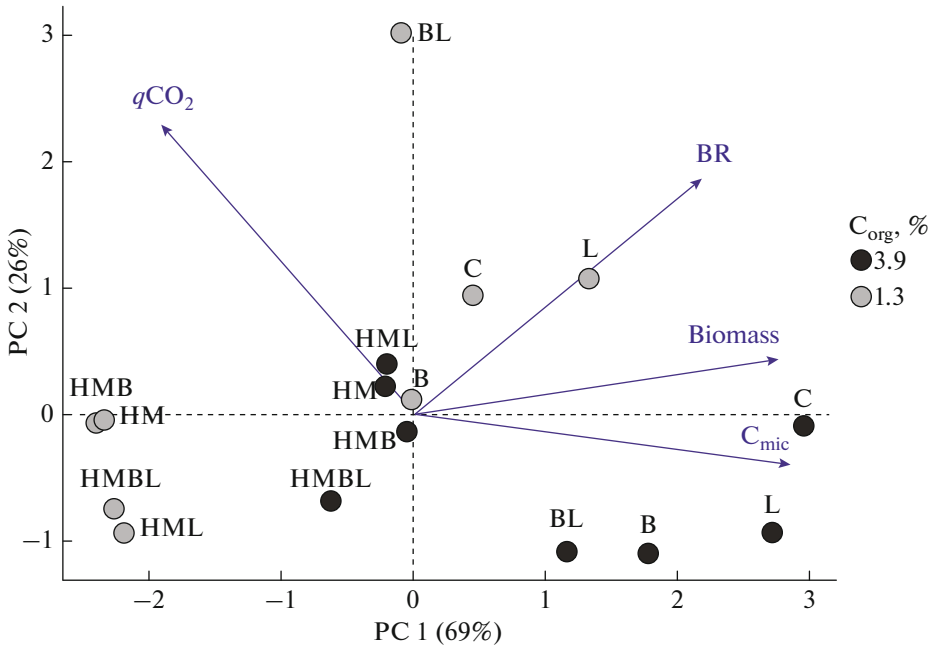

Fig. 3. Projection of parameters (microbial biomass carbon (C_{mic}), basal respiration (BR), microbial metabolic quotient (qCO_2), and biomass of mustard plants) of agrosoddy-podzolic soils with different humus contents on the first and second principal components (PC).

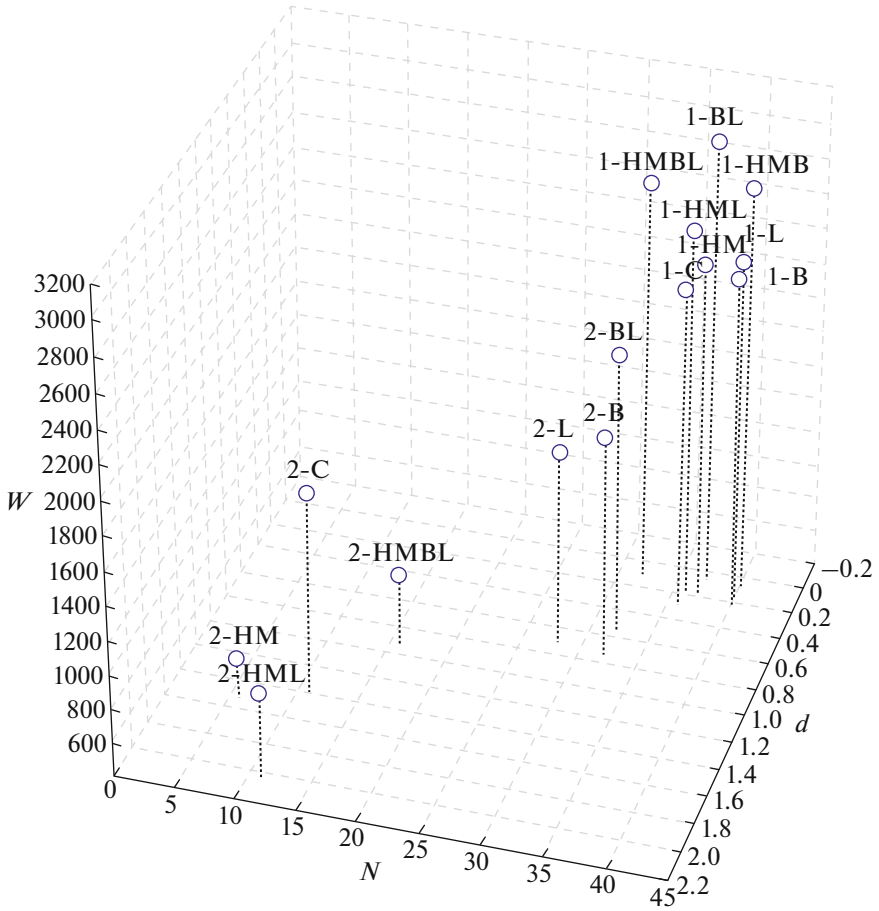

Fig. 4. Parameters of functional diversity of microbial communities of agrosoddy-podzolic soils (0–20 cm) with (1) high and (2) low C_{org} contents and different treatment variants. Here and in Fig. 5: (*N*) number of consumed substrates, units; (*W*) specific metabolic activity, rel. units; and (*d*) coefficient of rank distribution of the spectrum of consumption of substrates.

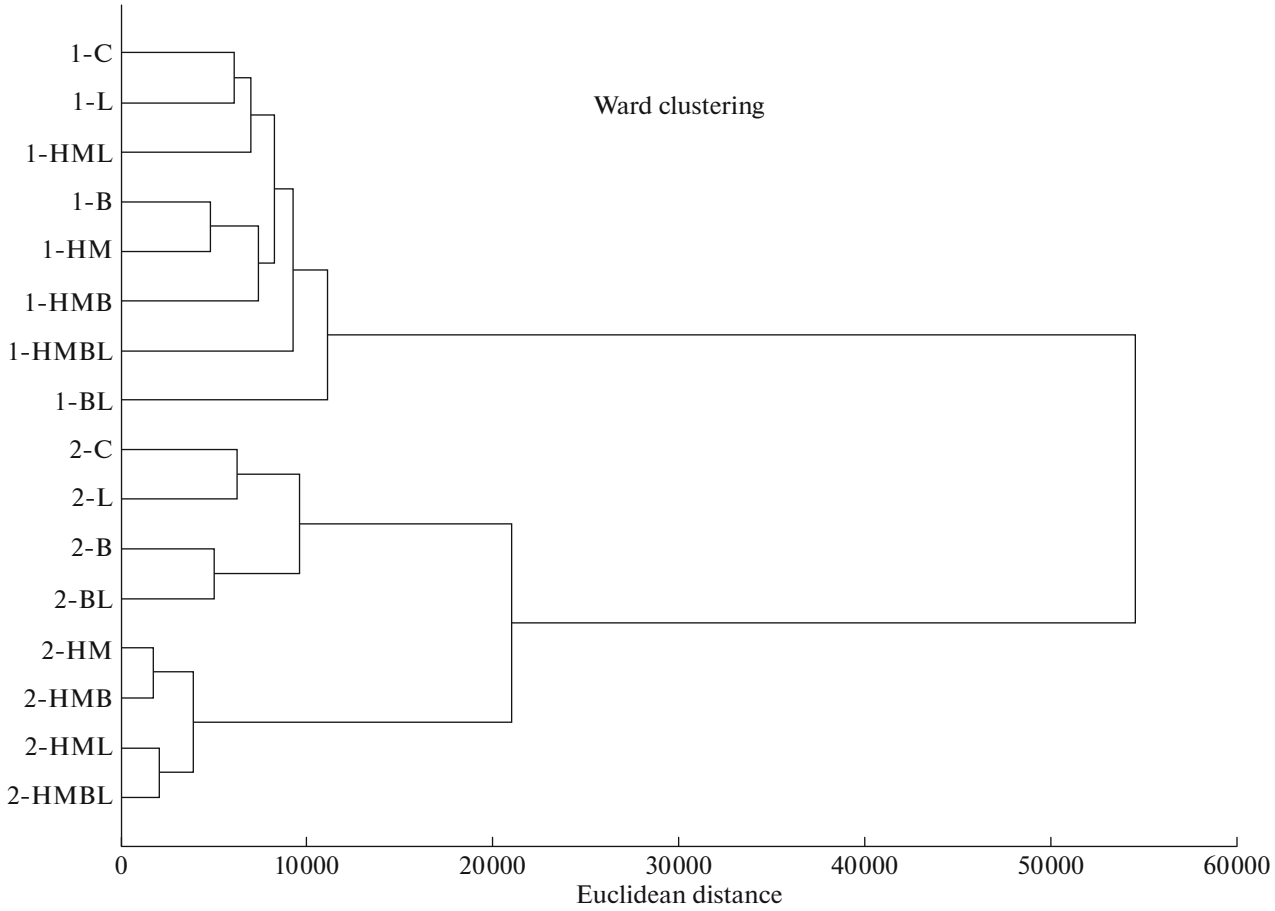

Fig. 5. Clustering of soil samples with (1) high and (2) low C_{org} contents relative to the spectra of substrate consumption (dendrogram of the hierarchical clustering analyzed with the use of the squared Euclidean distance and Ward clustering procedures).

not have a noticeable effect, and the combination of these preparations even reduced the parameter to 32. Organic preparation additions into uncontaminated soil 2 increased its microbial diversity ($N = 28$ and 32 for biochar and lignohumate, respectively). For uncontaminated soil 1, lignohumate and biochar additions was slightly increased microbial diversity ($N = 40$) compared to the control, while their combined use did not change it.

The microbial metabolic activity (W) of soil 2 was lower than soil 1 (1500 vs. 2200 units). HMs contamination of soil 2 (low C_{org} content) caused almost a twofold decrease its W, while for soil 1 (high C_{org} content), on the contrary, slightly increased this parameter (by ~100). Organic preparation treatments of contaminated soil 2 had no stimulating effect on W, but slightly increased it for soil 1 (by 200–300 units). In both uncontaminated soils, biochar and lignohumate additions increased W. Adding these preparations separately into soil 1 increased W by only 100 units, but their combined addition increased this parameter by 700 units (to the highest value of ~3000). For soil 2, application of biochar separately and in combination with lignohumate increased W by 200 and 500 units, respectively, however use of individually lignohumate had no effect.

The microbial community functioning of high humified soil 1 was more stable ($d = 0.29$) in comparison to low humified soil 2 ($d = 1.38$). The HMs contamination of soil 2 increased significantly this coefficient (to 2.03), which identify to deterioration in microbial community functioning. However, in high humified soil 1, this coefficient changed slightly ($d = 0.20$). Biochar treatment of contaminated soil 2 stronger decrease d (from 2.03 to 0.96) than lignohumate application (from 2.03 to 1.54). Carbon-containing preparation additions into contaminated soil 1also decreased this coefficient: from 0.20 to 0.05 (biochar), to 0.19 (lignohumate), and to 0.13 (combined application).

In general, the biochar and lignohumate decreased d coefficient in all the experiment variants, which contributed to increased tolerance of microbial communities to different pollutants due to upscale stability of microbial system.

The cluster analysis of MST results allow us to differentiate the microbial communities of the studied soils according to consumed substrates (Fig. 5). Two

large clusters corresponding to C_{org} content were identified. The effect of HMs contamination was more significant for low humified soil 2, the experiment variants of which clearly grouped into subclusters.

DISCUSSION

Heavy metals contamination of agrosoddy-podzolic soils (Cu, Zn and Pb concentrations: 660, 1100 and 650 mg/kg, respectively) with different C_{org} contents resulted in deterioration of microbial community functioning expressed in decreasing microbial biomass and basal respiration rate and increasing microbial metabolic quotient (qCO_2). Our results correspond to those obtained in the model experiment with solonetzic soil contamination by low and high concentrations of Cd and Pb [51].

Soil respiration, including CO_2 emission, are used as indicators of the soil ecological status in a number of countries [30, 33, 37]. It has been shown that soil contamination with HMs and metalloids inhibits decomposition processes and decreases the intensity of CO_2 release and the enzyme activity (polyphenol oxides, dehydrogenase, and lipase) [54]. However, low concentrations of some HMs may stimulate growth of microorganisms, acting, in particular, as coenzymes.

The indication capacity of functional microbial parameters obviously depends on soil conditions, in particular, the humus content. It is known that changes in soil microbial parameters depends on the initial humus content. Polymetal contamination of low humified soil resulted in decreasing microbial biomass by twofold, and for high humified soil—by only 30%. However, a significant decrease in BR was observed for only low humified soil, and an increase in qCO_2, was, on the contrary, in high humified soil.

It is shown that HMs contamination significant changed soil microbial biomass content regardless of the using carbon-containing preparations (Fig. 3). In a model experiment was shown that biochar addition (0.5, 1, and 3%) into the upper 20-cm soil layer did not significantly change BR, microbial biomass, and qCO_2 [47], which in turn causes excessive CO_2 emission from soil [35, 41]. In our experiment, the microbial biomass content significantly correlates with the plant biomass (Fig. 3), which indicates the important biodiagnostic role of this microbial parameter; in particular, for the characterization of soil fertility [50].

The effect of biochar and lignohumate on the soil microbial community functioning was different. Lignohumate had a stronger stimulating effect on microbial parameters than biochar, which may be explained by different mechanisms of their impact on soil. Lignohumate can stimulate the growth of soil microbial communities due to supply of nutrients—nitrogen and potassium [20]—and thus contributes to increasing microbial diversity, enzyme activity [14], CO_2 emissions, nitrogen fixation, and denitrification [12]. Biochar improves fertility of agroecosystem soil through increasing the cation exchange rate and decreasing HMs uptake by plants. In addition, biochar treatments of soils result in a significant decrease in the content of exchangeable forms of HMs [42] during short period. For example, on the 12th day after the biochar addition into soils, soluble Cd content decreased by almost 80% and Zn and Cd—by almost 90% [26].

The positive effect of biochar on the BR rate was only recorded in combination with lignohumate (Fig. 1). Their synergistic effect was shown for soddy-podzolic soils: stimulating BR, decreasing qCO_2 and mobility and toxicity of Cu cations for *Sinapis alba* and *Daphnia magna* [15].

The microbial functional diversity evaluated by utilization of different organic substrates demonstrates high indicative significance of FD for the assessing soil contamination with HMs [7, 53]. According to similarity of substrates utilization spectra by microorganisms, studied soils were clustered into two groups corresponding to their initial humus content (Fig. 5). The polymetal contamination impact was more significant in low-humus soil. Importantly, only for the low-humus soils, HMs contamination resulted in full death of mustard plants. Thus, low-humus soil depleted of nutrients is more sensitive to toxicants, which is diagnosed through decreasing the functional diversity and stability of the soil microbial community).

In the high-humus soil, the HMs toxic effect on microbial community functioning was essentially less compared in low-humus soil. The application of biochar, in combination with lignohumate, in particular, optimizes the parameters of microbial community.

After publication of the first schemes and methods for assessing and monitoring soil quality in the 1990s, more than 60 national and regional approaches have been developed mainly in North America, Europe, and China [23]. These approaches mostly focused on to soil fertility, which is considered as soil capacity to provide nutrients and water to plants (www.fao.org). In this regard, some authors [36] consider it necessary to supplement the characteristics of soil quality optimal for crop growth by parameters of biodiversity and functional activity of soil microbiota.

Soil organisms play the key role in soil functioning, so biological and biochemical parameters are important for modern approaches to soil assessment [9, 17]. Some authors emphasize that biological parameters of soil quality are necessary to understand the relationships between abiotic soil properties, ecological functions, and productivity of aboveground vegetation [34]. However, biological parameters are still insufficiently used in systems of soil quality assessment. These are mainly parameters of input–output models: microbial biomass and soil respiration [23].

Our data confirm the possibility and significance to use microbiological parameters (for example, microbial biomass) as indicators of the ecological status of soils [24]. It is obvious that various parameters of microbiota functioning should be differentiated with respect to their reliability and informative value for soil monitoring and assessment. Generalization of the obtained data allows us to conclude that the indicative value of soil microbial characteristics, such as the basal respiration and metabolic quotient, is lower than that of the microbial biomass and functional diversity of microbial community. This may be explained by the fact that the disturbance or stress may suppress microbial respiration of some species of soil microorganisms, but even stimulate it for others.

CONCLUSIONS

The results of our study indicate that structural and functional microbial markers determined by the MST method and data on the microbial biomass carbon provide more information on changes in soil microbial communities, including those soil contamination with heavy metals, in comparison with data on the intensity of microbial respiration and the ecophysiological state of soil microbial communities.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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