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SOIL BIOLOGY

Biomass and Functional Diversity of Microbial Communities in Catenas of Reserved and Arable Gray Soils and Chernozems

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Abstract—The biomass and functional diversity of microbial communities were studied in the watershed, transit, and accumulative positions in catenas composed of reserved gray soils (Luvic Retic Phaeozems) and chernozems (Haplic Chernozems) of the Belogorye Nature Reserve and arable variants outside the reserve. Microbial biomass was determined by the substrate-induced respiration (SIR) and the content of phospholipids. Multisubstrate testing of respiratory responses was carried out in the MicroResp system after the addition of amino acids, carboxylic acids, and carbohydrates. It was found that microbial biomass decreased in the reserved chernozem from the watershed towards the accumulative part of the slope; minimal values in the gray soil were recorded in the transit part of the catena. It was close in the plowed horizon of agrochernozems in all parts of the catena and 2–3.5 times less than in reserved chernozems. An increase in microbial biomass was recorded in the agrogray soils of the transit and accumulative parts of the catena. Cluster analysis of respiratory responses in the 0–10 and 10–20 cm layers identified two groups of the most demanded substrates. The first group in both layers included citric and ketoglutaric acids, the second group included fructose and succinic acid. Ascorbic acid, sucrose, and glutamine were included in the first group in the 0–10 cm layer and in the second group (along with asparagine and glycine) in the 10–20 cm layer. An increase in metabolic diversity was observed from the watershed to the accumulative position of the catena in all reserved and arable catenas. At the same time, plowing led to its decrease in the 0–10 cm layer: up to 1.5 times in chernozems and up to 4 times in gray soils. In the 10–20 cm layer, similar trend was observed, except for the agrogray soil in the transit part of the catena, where the number of significant responses increased 3.6 times in comparison with the reserved variant.

Keywords: reserved and arable catenas, MicroResp™, Luvic Retic Phaeozems, Haplic Chernozems

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INTRODUCTION

Years-long plowing and related changes in water and air regime of soil, removal of great amounts of phytomass, and application of chemical fertilizers and plant-protecting agents unavoidably have an effect on soil microbial communities. In such situation, the changes in the state of microbial communities related to anthropogenic effects can be assessed by comparison with reference soils, which may be soils of nature reserve areas. Such researches were carried out, for example, in the territory of Streletskaya Steppe of the Central Chernozemic Nature Reserve [3] and Belogorye Nature Reserve (Belgorod oblast). For the latter reserve, conditions of soil formation [9] and the soil cover [18] were studied. The sites Vorskla Forest [18] and Yamskaya Steppe [14, 15, 19] were studied the most extensively, and microbiological activity of reserved gray soils, fallow and arable soil was compared [11]. As a rule, soils of local watersheds, where erosion-accumulative processes did not manifest themselves, were of interest to researchers. However, the dissection of landscapes increases, beginning from the northern boundary of the steppe zone as climate humidity increased, and the areas of slope surfaces sharply increased. Physiological activity of soil microbial communities in different geomorphological positions within one slope is studied insufficiently [17, 30]. In this relation, the study of the state of soil microbial communities in catenas is actual.

Pronounced trend should be also noted for the study of quantitative characteristics of soil microbial community in comparison with the study of its functional state. Functional diversity of soil microbial communities is attributed to the capability of microorganisms to assimilate different groups of low-molecular compounds [28, 30, 31]. Garland and Mills [29] suggested the notion of community-level physiological profile (CLPP) to describe functional diversity of

soil microbial communities. This approach allows obtaining the information about the volume of microbial biomass and about the way, in which soils can respond to different disturbing factors [25]. Using of only one carbon substrate to obtain information about metabolic capacity of soil microflora led to the development of different systems, which can give quick and sensitive results. The systems Biolog [29] and Ekolog [5], in which growing characters of microbial communities are estimated, may serve as examples. Recording of their respiratory responses to the addition of different low-molecular substrates was one more variant in estimating the functional diversity of microbial communities [26]. Developed system MicroResp [24] with using colorimetric detection of microbial respiration and automated plate reader allows quick assessing of functional state of soil microbial biomass.

The aim of our work was to compare functional diversity of soil microbial communities in catenas of reserved gray soils and chernozems in the territory of Belogorye Nature Reserve and in catenas of arable soils under the same landscape-geomorphological and lithologic conditions. Working hypothesis presumed that plowing would cause the decrease of microbial biomass and, hence the decrease of functional diversity of agricultural soils. We proceeded from the assumption that metabolic profiles of agrogray soils and agrochernozems would be close in similar agrocenoses.

OBJECTS AND METHODS

The study was carried out in Belogorye Nature Reserve (Belgorod oblast) situated in the south of the Central Russian Upland. Relief of the terrain was typically erosional dominated by the slopes of different gradients, separated by gullies and ravines. The territory of reserve belongs to the basins of Don and Dnieper rivers. Groundwater depth varied in different sites from 3 to 30 m. Calcareous loess-like deposits predominate among parent rocks in the Reserve. Forest ecosystems predominate there: the occupy 65%, while steppe and meadow ecosystems occupy 24% of total area.

Two sites in the territory of the Reserve—Vorskla Forest and Yamskaya Steppe—and nearby areas of arable land were selected for the study.

Dark gray soils (Luvic Retic Phaeozems (Loamic, Pachic)) [13]) on loess-like loams, which thickness accounted for 5–10 m, occur in all parts of catena of reserved soils in the key site Vorskla Forest. The thickness of AU horizon reached 55–60 cm. Arable soils in the near-to-top part of local watershed and in transit zone were presented by agrogray eroded sandy loamy soils on loess-like loam (Luvic Retic Phaeozems (Aric, Loamic) [13]). Agrogray prograded loamy soils were identified in the accumulative position of the catena.

Migrational-mycellary deep heavy loamy deeply carbonate chernozems on calcareous loess-like loams (Haplic Chernozems (Loamic, Pachic)) [13]) were recorded in all positions of catena of reserved soils in the Yamskaya Steppe key site. The AU horizon depth regularly changed, increasing from 50–60 cm in the watershed and transitional position to 70–80 cm in the accumulative part of the catena. The upper boundary of BCAmc horizon was ranged within the depth of 77–85 cm. Segregational deep heavy loamy deeply carbonate on calcareous (carbonate-free in the accumulative zone) loess-like loams (Haplic Chernozems (Aric, Loamic, Pachic) [13]) were studied in the catena of arable soils. Some trend was observed to heavier texture and greater thickness of the remained part of AU horizon in the accumulative position of catena.

Plowing in the territories adjacent to the Vorskla Forest key site began in last third of XIX–early XX century. Plowing in the territory near the Yamskaya Steppe key site started in the 1930s. Both reference and arable plots were situated on the slopes of southern aspect with the same slope gradient (3°–4°).

Soils were studied in three positions of catenas: local watershed—transit zone—accumulative zone. Points of sampling of reserved and arable soils in catenas are presented in Fig. 1.

Two test pits 0.5 m deep were made in every point of catena. Morphological-genetic description of the profiles was carried out, and soil samples for chemical analyses were taken.

Soil samples to study the state of microbial communities were taken from the upper layers 0–10 and 10–20 cm, and placed into polyethylene bags representatively and preserving aseptic conditions. The samples were taken in May. After transportation to laboratory, average samples were taken; roots and plant residues were removed, the samples were sieved through the 2-mm sieve, and dried to air-dry state. The samples were kept up to beginning of the experiment in polyethylene bags at room temperature.

Physical and chemical soil properties were determined in Research Equipment Sharing Center, Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences. Content of organic carbon (C_{org}) in the layer 0.5 м were determined by titrimetric variant of I.V. Tyurin method in modification of St. Petersburg State University with oxidation in thermostat at the temperature of 140°C [4]. Carbonates were determined by acidimetry method, pH of water extract by potentiometry [2], and particle-size composition was determined with the pipette method of Kachinskii [12].

Microbial biomass was determined by the content of phospholipids (C-PL) as it was described earlier [20]. Phospholipids were extracted from soil sample by monophase mixture methanol: chloroform: phos-

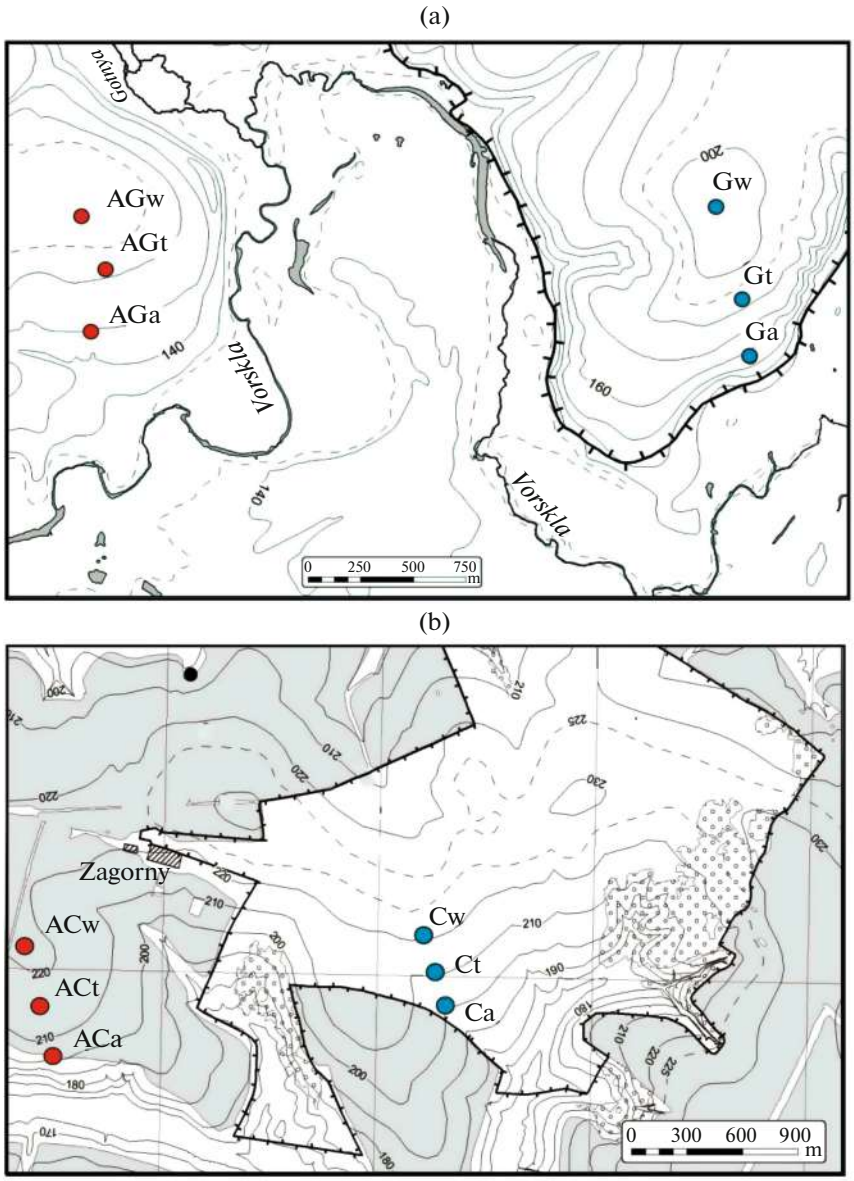

Fig. 1. Sampling points: (a) key plot Vorskla Forest; (b) key plot Yamskaya Steppe; G, reserved gray soils; AG, agrogray soils; C, reserved chernozems; AC, agrochernozems. Small indices: w, watershed; t, transitional zone; a, accumulative zone.

phate buffer (1 mM, pH 7.4) in the ratio 1 : 2 : 0.8; then the extract was separated into water and organic phases. Aliquots were taken from organic phase containing lipids; phosphate groups of phospholipids were split out in the reaction with potassium persulfate; concentrations of these groups were determined by spectrophotometric analysis (λ = 610 nm) after the reaction with ammonium molybdate and staining with malachite green. The content of phospholipids reduced to the units of carbon of microbial biomass, taking that 190 nM of phospholipids correspond to 1 mg of C_{org} was measured [27].

Microbial biomass was also determined with the method of substrate-induced respiration (C-SIR) in gas chromatograph Kristallyuks 4000M (Research Equipment Sharing Center, Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences), using in calculations the conversion factor 40.04 [1, 22].

Functional diversity of microbial communities was estimated with the method of multisubstrate testing of respiratory activity in the plates [23]. The following respiratory substrates were used in testing of microbial communities: L-isomers of amino acids [glycine (Gly), alanine (Ala), arginine (Arg), histidine (His), serine (Ser), asparagine (Asp), phenylalanine (Phe), leucine (Leu), glutamine (Gln), lysine (Lys), and cysteine (Cys)], carboxylic acids [ascorbic (Asc), citric (Cit), lactic (Lac), acetic (Ace), oxalic (Oxa), succinic (Suc), maleic (Mal), and ketoglutaric (Ket)], and sim-

ple carbohydrates [mannose (Mn), saccharose (Sc), arabinose (Ar), fructose (Fr), maltose (Ml), and glucose (Gl)]. All substrate solutions were brought to pH 5 by adding of 1 M NaOH or 1 M HCl. Final concentrations of substrate compounds were the following: amino acids—15 mM, salts of carboxylic acids—190 mM, simple carbohydrates—30 mM, as Degens and Harris recommended [26].

Weighed portions of soils (500 mg) were taken into the 96-well plates, moistened to 60% of field capacity, covered with parafilm, then preincubation was carried out, as it was specified by method developers [24]. The 25-μL solution portions of one of 25 substrates were added in triplicate to the wells with soil, and water was added to control wells. The plates were covered by covers with detection gel on the basis of agar and indication solution (KCl [150 mM], $NaHCO_3$ [2.5 mM], and cresol red [12.5 mM]) in the ratio of 1 : 2. The measurements were carried out in the reader xMark™ Microplate Spectrophotometer (Bio-Rad) at wavelength 570 nm according to recommendations of developers [24]. Emission of carbon dioxide resultong of respiration of microbial communities caused the change of detection gel color from crimson to yellow. The intensity of color of detection gel was calibrated via simultaneous measurement of CO_2 concentration in gas chromatograph Kristallyuks 4000M. Respiratory activity of microbial communities was calculated in μg C-CO_2/(g of soil h).

All analyses were made in triplicate. Statistical treatment of data was carried out with commonly adopted methods using data clustering in Heatmapper program and principal components method in PC-ORD 5 program.

RESULTS AND DISCUSSION

Physicochemical parameters of soils in the plot Vorskla Forest. Detailed description of chemical properties and particle-size composition of reserved gray soils in the Reserved Gray and agrogray soils in Agrogray catenas was presented earlier [6]. The content of silt in soil profile increased with depth from 20 to 43% (Table 1).

The fraction of coarse silt predominated in soils (up to 47%). Particle-size composition of arable soils was close to that of forest variants, but some shift of this parameter towards the increase of coarser fractions should be noted.

The values of C_{org} content in virgin soils in the watershed position and accumulative part of the slope were relatively close and were at the level of 2.7%. Some decrease of C_{org} value was observed in the upper 0–10 cm layer of the transitional part of the slope. The pH values were corresponded to slightly acid ones, close to neutral values, except for the transitional plot, where pH values were shifted to the acid area. Cation exchange capacity was much lower in these soils. The exchangeable bases was dominated by Ca^{2+}.

The pH values increased with depth to the values close to neutral in arable soils in the accumulative part of the slope. The content of C_{org} in the upper soil layers was low and reached 1% only in the accumulative part of the slope. Cation exchange capacity was minimal in soils of watershed area and gradually increased downslope.

Physicochemical parameters of soils at the Yamskaya Steppe key site. Particle-size composition of soils in catenas of chernozems and agrochernozems were relatively close with some trends towards more heavy texture in arable soils. The similarity should be noted of particle-size composition in all positions of catena.

The C_{org} content in soils of all positions of reserved catena accounted for 5–6%. These values were two times lower in arable soils in all positions of catena. The pH values were in neutral range, except for soils of the accumulative part of the slope, where the reaction was displaced to alkaline area. Soils were characterized by pronounced decrease of cation exchange capacity.

Particle-size composition and chemical properties of soil profiles of chernozems (0–50 cm) at the Yamskaya Steppe key site are presented in Tables S1 and S2.

Microbial biomass. The data on microbial biomass in the layers 0–10 and 10–20 cm of reserved and arable gray soils and chernozems estimated with two methods (C-PL and C-SIR) are presented in Fig. 2.

Microbial biomass in reserved soil in most cases was 1.5–2.5 times greater in the layer 0–10 cm than in the layer 10–20 cm. Plowing resulted in its decrease. The values of this parameter in the catena of agrochernozems decreased 2–3.5 times in the 0–10 cm layer and by 30–50% in the layer 10–20 cm. In the catena of agrogray soils, microbial biomass decreased 3.5 times in watershed and by 50% in transitional and accumulative positions. The difference in the values of this parameter between the upper and lower parts of plowed horizon was surprising. For example, microbial biomass in the layer 10–20 cm of plowed horizon was in some cases higher than that in upper layer. This was apparently related with the differentiation of initially homotypic soil material during the time passed between soil treatment and soil sampling, or with soil overturning so that surface layer rich in plant residues appeared to be covered by soil material from deeper layers.

Both methods demonstrated in most cases similar dynamics of microbial biomass. It gradually decreased downslope in the catena of reserved chernozems and was minimal in the transitional part in the catena of reserved gray soils.

The difference in microbial biomass determined with two methods was insignificant in the catena of agrochernozems. The situation was more complicated, when comparing the resulting data obtained by

Table 1. Some chemical properties of reserved and arable soils

Catena	Geomorphological position	Depth, cm	C_{org}	$CaCO_3$	pH	Clay, <1 μm	Silt, <10 μm
			%			%	
Key plot Vorskla Forest, gray soil							
Reserved Gray	Watershed	0–10	2.7	0.74	5.8	3	20
		10–20	1.7	0.67	5.5	5	25
	Transitional part of the slope	0–10	1.3	0.52	4.7	3	22
		10–20	0.9	0.45	4.9	3	22
	Accumulative part of the slope	0–10	2.7	0.89	5.7	6	24
		10–20	2.1	0.82	5.8	8	27
Agrogray	Watershed	0–10	0.7	0.45	4.4	4	14
		10–20	0.9	0.30	4.9	4	14
	Transitional part of the slope	0–10	0.8	0.59	4.9	8	19
		10–20	0.8	0.22	5.3	7	19
	Accumulative part of the slope	0–10	1.1	0.82	5.2	9	27
		10–20	1.1	0.30	5.3	9	26
Key plot Yamskaya Steppe, chernozems							
Reserved Chernozem	Watershed	0–10	6.2	1.11	6.2	9	28
		10–20	4.8	1.19	6.3	11	34
	Transitional part of the slope	0–10	6.2	1.04	5.9	8	29
		10–20	4.1	1.04	6.2	11	32
	Accumulative part of the slope	0–10	5.4	1.49	7.3	10	31
		10–20	4.0	1.41	7.4	13	35
Agrochernozem	Watershed	0–10	3.3	1.19	6.6	21	44
		10–20	3.1	1.04	6.5	21	43
	Transitional part of the slope	0–10	2.7	1.11	6.3	24	46
		10–20	4.8	1.04	6.3	23	43
	Accumulative part of the slope	0–10	3.0	1.19	6.9	23	46
		10–20	2.4	1.19	7.1	24	48

two methods in the catena of agrogray soil. The C-SIR method demonstrated similar dynamics of microbial biomass here, but the values of biomass were lower.

Hence, it can be said that plowing resulted in an expected decrease of microbial biomass in chernozems as well as in gray soils. However, despite general similarity of biomass dynamics in the catenas, some difference was observed. We consider that this difference was connected with the difference in life processes, on estimation of which these methods are based [respiration of microorganisms (C-SIR) and cell morphology, and wholeness of cell membranes (C-PL)]. The conversion factors, which also can introduce some distortion of real situation, were used in both methods.

Metabolic diversity of microbial communities, estimated by respiratory responses in the system of multisubstrate testing. The substrates of respiratory activity were presented by low-molecular compounds from the groups of amino acids, carboxylic acids, and carbohydrates. Sum total 11 amino acids, 8 carboxylic acids, and 6 carbohydrates were tested.

The range of responses to introduction of substrates of amino acids group increased in reserved catenas of both soils from watershed to accumulative part of the slope. Absolute values were 2–3 times lower in catenas of gray and agrogray soils (Fig. S1) than in catenas of chernozems (Fig. S2). The range of responses to carboxylic acids was more specific: it increased in the layer 0–10 cm down the catena in the chernozemic

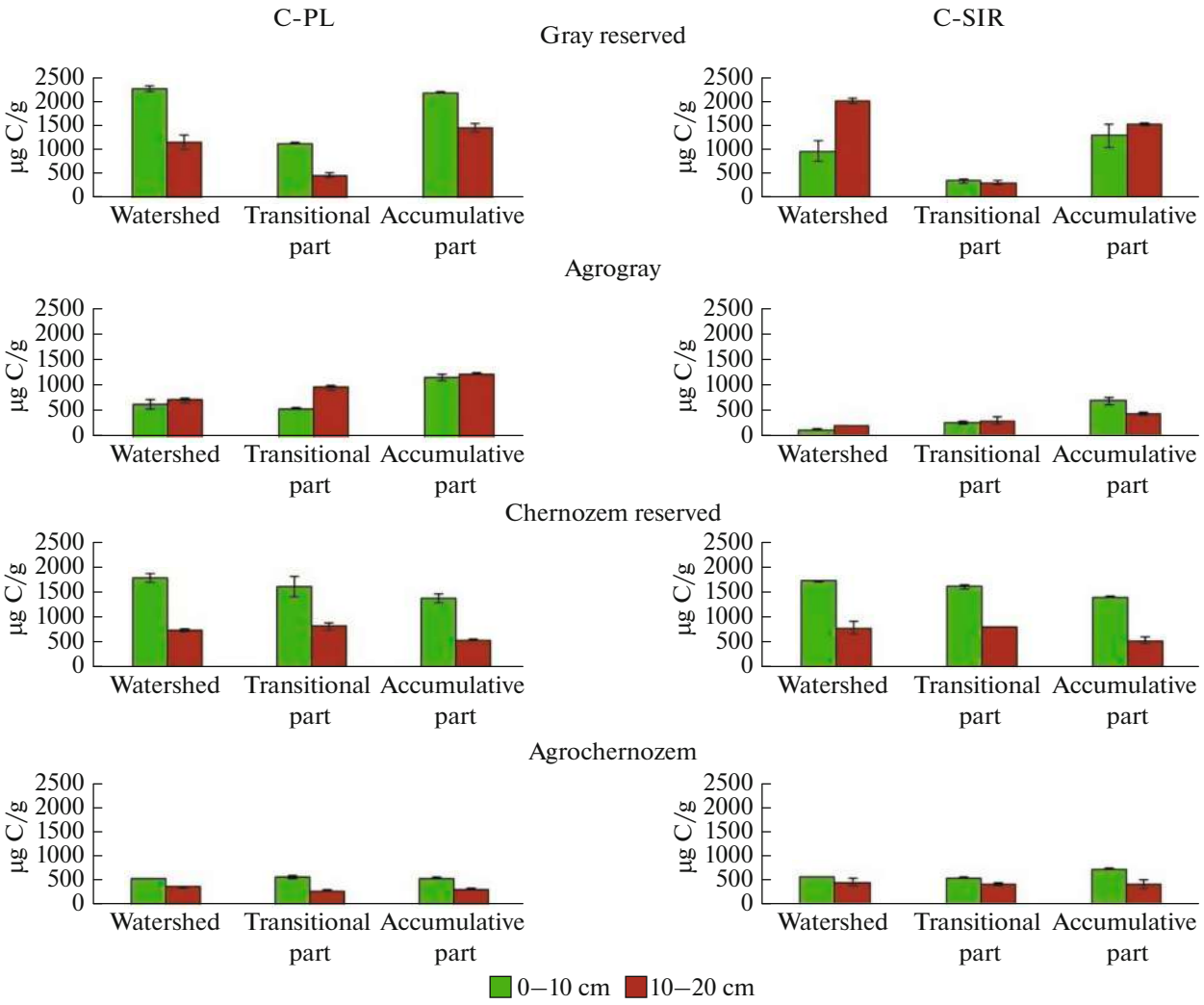

Fig. 2. Microbial biomass in catenas of reserved and arable soils, estimated with C-PL and C-SIR methods.

catena as well as in the catena of gray soils, but the range of responses decreased in the layer 10–20 cm of the former. The range of responses to carbohydrates in catenas of reserved chernozems and gray soils increased in the layer 0–10 cm from watershed to slope and decreased in the layer 10–20 cm. Respiratory responses in both studied soils were greater in the layer 0–10 cm than in the layer 10–20 cm: in gray soils in watershed in 1.3–2.9 times, in transitional part in 1.3–9 times, and in the accumulative part in 2–12 times; and in chernozems in 1.2–9 times, 1.7, and 2.5–10 times, respectively.

Respiratory activity of microbial communities in agrogray soils was 2–10 times lower than in reserved variants. The range of responses and the number of responses with high amplitude increased in the layer 0–10 cm down the catena of agrogray soils. In comparison with the upper soil layer, the range of responses to all groups of substrates in the layer of 10–20 cm of agrogray soils was greater in transitional part of the catena, but the responses in accumulative part were greater only to the carbohydrates.

Respiratory activity of microbial communities in plowed chernozems was 1.5–7 times lower in comparison with reserved variants. In both layers of agrochernozems, the number of substrates providing high responses and amplitude of responses increased down the catena. Based on the number of these respiratory substrates and their amplitude, we can state that microbial communities of soils in catenas of reserved soils were metabolically more diverse, because population densities of corresponding groups were greater than in plowed variants. Metabolic diversity in the catena of reserved chernozems was in turn also greater than in catena of reserved gray soils.

Since the values of microbial biomass in reserved and arable soils vary to a considerable extent, it is apparent that respiratory responses of microbial communities will be greater there, where microbial biomass is greater. In other words, absolute values of

respiratory responses, calculated per unit weight of soil, represent ecological parameter reflecting the state of soil in ecosystem, but not the state of soil microbial community. For comparative analysis of respiratory responses at the level of microbial community, normalizing per unit microbial biomass was carried out, i.e., specific metabolic diversity of microbial communities was estimated.

The difference in the values of specific metabolic diversity of reserved and arable variants (normalized values) characterizes the internal structure, state, and activity of microbial communities. It can be said that normalized per unit microbial biomass values of metabolic diversity reflect mostly the consequences of microbial communities transformations in response to the changes of soil formation conditions caused by economic activity.

General picture of metabolic diversity changed essentially after normalizing of the values of respiratory responses (Figs. S1 and S2). Specific respiratory responses of plowed soils appeared to be higher than those of reserved analogs practically in all cases. That's grounds for saying that a microbial biomass unit in plowed soils can assimilate more efficiently the available sources of nutrition than similar biomass unit in reserved soils. It should be noted that specific respiratory responses in both reserved and arable variants became greater in the layer 10–20 cm than in the layer 0–10 cm. This is explained by more stable hydrothermal conditions in the layer 10–20 cm in comparison with the uppermost soil layer.

Statistical treatment. The maps are presented in Fig. 3 of data clustering with correlation matrix of respiratory response values of microbial communities in the layers 0–10 and 10–20 cm of reserved and arable soils. For map drawing, the calculation was carried out of response values relative the maximal value in every group of low-molecular compounds.

The group of substrates (ascorbic, citric, and ketoglutaric acids, saccharose and glutamine) can be observed in the layer 0–10 cm of all analyzed soils, responses to which were maximal. Responses to ascorbic acid, glutamine, and saccharose were maximal within corresponding group. These responses fell on microbial communities in reserved chernozems: maximal response to saccharose was recorded in watershed, to glutamine in transitional part, and to ascorbic acid in the accumulative part of catena. Maximal responses of microbial communities in reserved gray soils were to ketoglutaric acid in transitional part and to citric acid in the accumulative part of catena.

Clustering of soils by the character of substrate consumption revealed two groups at the first stage. The small group included accumulative zones of reserved chernozems and gray soils and was characterized by high responses to most substrates. In the large group, separated into 2 subgroups, special attention attracted the subgroup of reserved chernozems of watershed and transitional part of catena, which were characterized by maximal respiratory responses of microbial communities to glutamine and saccharose. In the transitional part of catena, unlike to watershed, pronounced responses were observed to the group of the following substrates: cysteine, histidine, lysine, leucine, and oxalic acid. The subgroup included all variants of arable soils and reserved gray soils in watershed and in the transitional part of catena, it was characterized by low responses to some these substrates.

The next stage of clustering revealed separated subgroup of agrochernozems in transitional and accumulative parts of catena. Significant similarity of metabolic diversity of microbial communities was observed between these soils. Unlike chernozems, in which reserved and arable variants were separated to particular groups, such regularity was not observed in gray soils. Transitional part of catena of gray soils and accumulative part of agrogray catena were included to the same subgroup, in which the number of low responses increased. Another subgroup included transitional part of agrogray catena and virgin gray, agrogray soils and agrochernozems in watershed positions. Low responses here accounted for more than a half of all metabolic responses.

Hence, data clustering for soils of the layer 0–10 cm demonstrated essential similarity in metabolic diversity of microbial communities of virgin chernozems and gray soils in accumulative parts of catenas and similarity of virgin gray and agrogray soils with agrochernozem variant.

Calculation of total values of metabolic responses demonstrated that total responses of microbial communities in the upper layer decreased in the row watershed—transitional part—accumulative part in all catenas, both arable and reserved. The influence of plowing caused the decrease of total responses in 2–3 times, excluding transitional part of catena of virgin chernozems.

Data clustering is shown in Fig. 3b of responses of microbial communities in the layer 10–20 cm of all analyzed soils. The responses to ketoglutaric acid and citric acid and to saccharose and glutamine were the most significant. Strong responses were recorded to ascorbic acid, glycine, asparagine, succinic acid, and fructose. Maximal responses fell on catena of chernozems, as it was reported for the upper layer: to ketoglutaric acid and saccharose in watershed and to glutamine in the transitional part.

The layers 10–20 cm at the first stage of clustering were subdivided to groups, one of which included the watershed and transitional part of catena of reserved chernozems and watershed part of agrochernozems; the other group included all other variants separated to two subgroups. First subgroup included watershed parts of gray and agrogray catenas, transitional part of agrogray catena, and accumulative part of gray catena; second subgroup included transitional part of gray

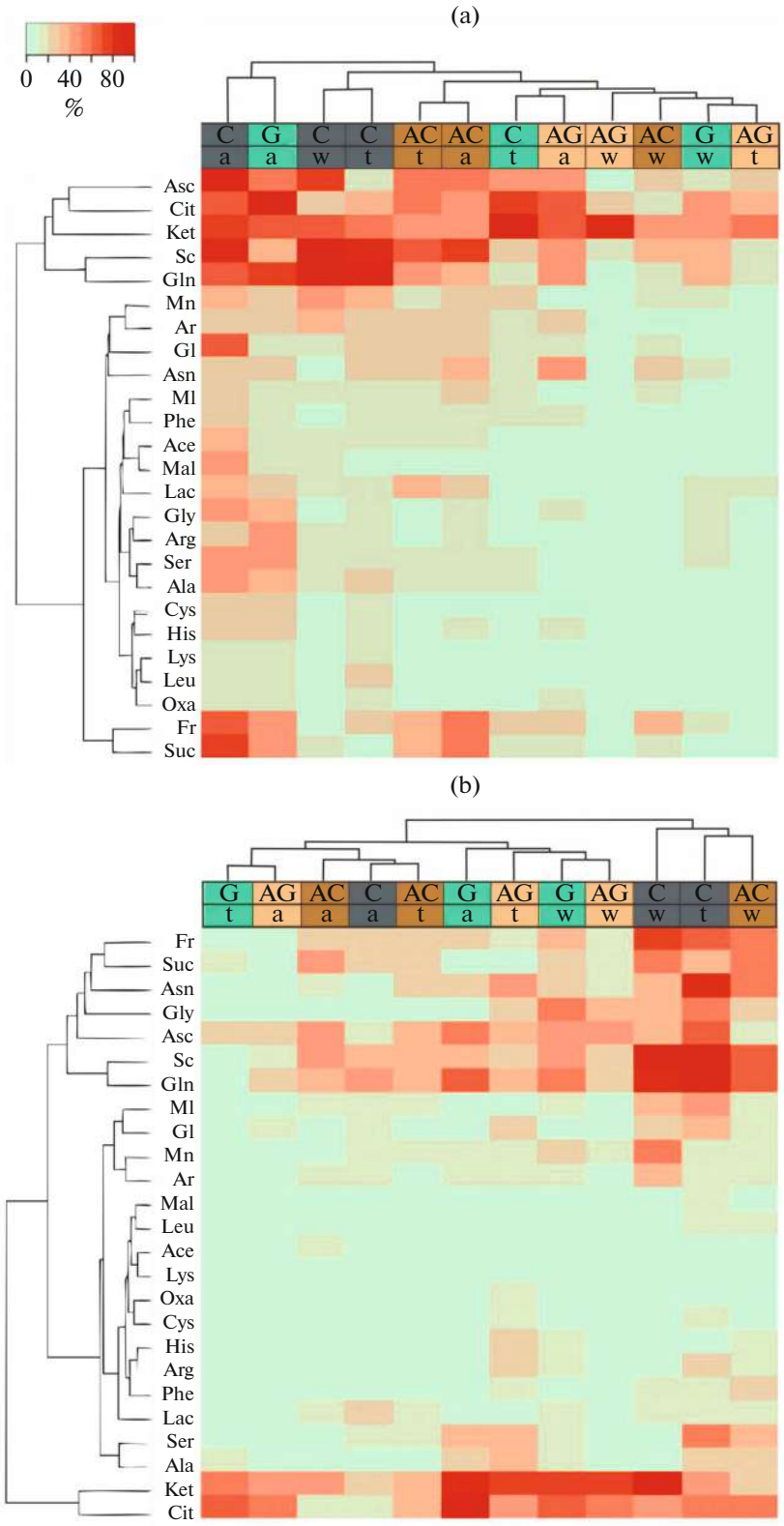

Fig. 3. Clustered heat map of metabolic diversity of microbial communities in reserved and arable soils: (a) layer 0–10 cm; (b) layer 10–20 cm. In the vertical line L-isomers of amino acids [glycine (Gly), alanine (Ala), arginine (Arg), histidine (His), serine (Ser), asparagine (Asp), phenylalanine (Phe), leucine (Leu), glutamine (Gln), lysine (Lys), and cysteine (Cys)], carboxylic acids [ascorbic (Asc), citric (Cit), lactic (Lac), acetic (Ace), oxalic (Oxa), succinic (Suc), maleic (Mal), and ketoglutaric (Ket)], and simple carbohydrates [mannose (Mn), saccharose (Sc), arabinose (Ar), fructose (Fr), maltose (Ml), and glucose (Gl)]. In the horizontal line the clustering is given of reserved and arable soils. Indices: G, reserved gray soil; AG, agrogray soil; C, reserved chernozem; AC, agrochernozem. Small indices: w, watershed; t, transitional zone; a, accumulative zone.

catena and accumulative part of agrogray catena together with reserved chernozems (accumulative part) and agrochernozems (transitional and accumulative parts of catena).

Hence, microbial diversity of reserved chernozems was close in both layers, whereas layer-by-layer comparison of responses to presented substrates demonstrated the difference in reserved gray soil and agrogray soil and agrochernozem. The trend can be observed in the layer 10–20 cm to merging agrogray soils and reserved chernozems.

All variants of soils in Fig. 4 were considered relative to the influence on metabolic diversity of microbial communities of geomorphological position and plowing. To construct this heat map, a convention was accepted that all responses below 10% of maximal value in every group, corresponded to green fields in Fig. 3, and are considered as insignificant. The number of insignificant responses accounted for 38 and 47% in the layers 0–10 and 10–20 cm, respectively.

The changes in metabolic diversity of microbial communities were estimated by the changes in the number of significant responses. Subdividing the substrates into three primary groups, corresponding to the first and second stages of clustering, did not change relative to results of cluster analysis obtained in the case of differentiated approach to the values of low responses.

The upper 0–10 cm layer in all variants of soils were characterized by high responses to the first group of substrates of energy metabolism included citric and ketoglutaric acids, included to tricarboxylic acid cycle.

This group included also ascorbic acid, saccharose, and glutamine, which can be connected with Krebs cycle because glutamine can be transformed via glutamate to α-ketoglutarate. Among other substrates, the succinic acid, which also is included to tricarboxylic acid cycle, and fructose should be noted. These two substrates included in the second group were demanded by microbial communities less than substrates of the first group: pronounced responses in the layer 0–10 cm were observed only in 60% of all soil variants. All other substrates, included those in the third group, demonstrated maximal variability in the sequence of reserved and arable soils.

The number of significant responses accounted for 48% of their total number in gray reserved soil of the watershed. Plowing resulted in the 4 times decrease of the number of significant responses. Microbial community of the watershed agrogray soil gave a significant responses only to saccharose, citric acid, and especially to ketoglutaric acid, and it is not surprising, because the response to glutamine, which can be the additional source of ketoglutarate, decreased.

Metabolic diversity of virgin soil was higher in transitional zone than in the watershed soil, and the number of significant responses here accounted for 60%. Significant responses were found to six substrates: arabinose, glucose, maltose, phenylalanine, alanine, and succinic acid, which were absent in the upper layer in watershed soil. The responses to only three substrates, glycine, arginine, and lactic acid, decreased to the values less than 10% of maximal values. The effect of plowing on metabolic diversity of microbial community in the upper layer of gray soil was less pronounced in the transitional part of catena in comparison with that on the watershed. The number of significant responses here was 2.5 lower in comparison with the reserved variant. All responses to the first group of substrates, including glutamine, remained significant; and additionally significant response appeared to lactic acid, which was absent in the reserved soil.

Maximal metabolic diversity was found in the upper layer of reserved gray soil in the accumulative part of catena. The responses to all 25 substrates were significant here, and high values of responses were observed not only to introduction of substrates of the first and second groups, but also to some substrates of the third group, and especially to serine and arginine. Plowing resulted in 1.8 times decrease of the number of significant responses, and this suggested a lower influence of plowing on gray soil in the accumulative part of catena in comparison with watershed and transitional parts, where similar decrease accounted for 4 and 2.5 times respectively.

Hence, the increase was observed of metabolic diversity of microbial communities in the upper layer of gray reserved soils and agrogray soils in the sequence: watershed—transitional zone—accumulative zone, and the influence of plowing decreased in the same order. The difference in metabolic diversity of the upper layer of agrogray soils in different geomorphological positions increased significantly relative to reserved variants owing to plowing. For example, the number of significant responses in the catena of virgin soils increased 2 times from watershed to accumulative zone and 4.6 times in plowed variants.

Similar trend was observed in the upper layers of chernozems. The number of significant responses in reserved variants accounted for 68, 92, and 100% of total number of responses in watershed, transitional, and accumulative zones, respectively. It decreased after plowing 1.5, 1.4, and 1.3 times in comparison with reserved soils. High responses to the first group of substrates remained in all plowed variants. Significant responses to fructose and asparagine, which were absent in reserved chernozem, were found in agrochernozem on zone watershed. Despite a general decrease of metabolic diversity, significant response appeared in the transitional part of catena in chernozem to succinic acid, markedly increased the responses to ascorbic and lactic acids and to saccharase and fructose. All responses in the accumulative part of catena were lower in plowed variant than corresponding responses in reserved variant. In general, the number of significant responses in catena of virgin soils increased 1.5 times from the watershed to the accumulative zone

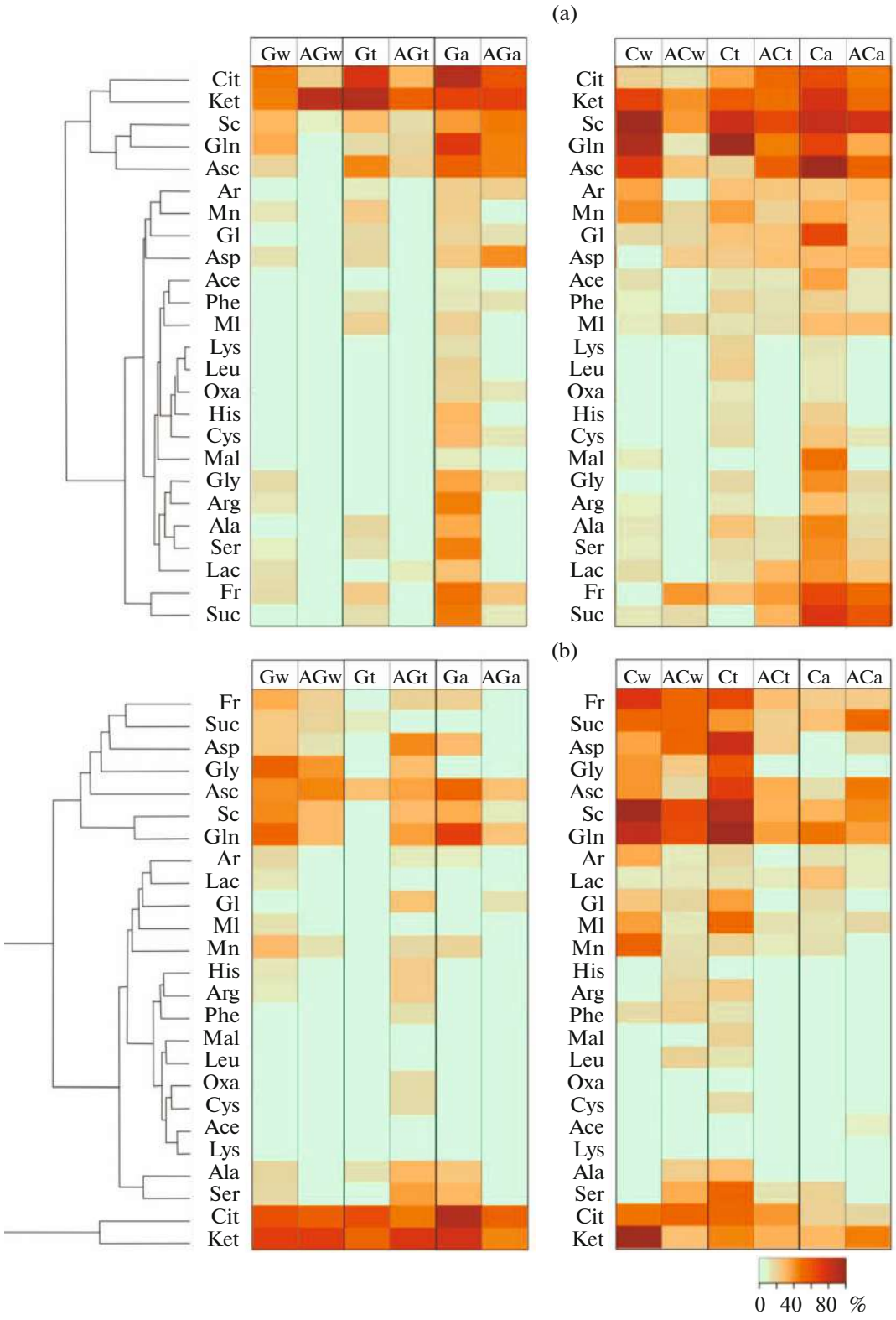

Fig. 4. Clustered heat map of changes in metabolic diversity of microbial communities in reserved and arable soils under the influence of plowing in different parts of catena: (a) layer 0–10 cm; (b) layer 10–20 cm. In the vertical line L-isomers of amino acids [glycine (Gly), alanine (Ala), arginine (Arg), histidine (His), serine (Ser), asparagine (Asp), phenylalanine (Phe), leucine (Leu), glutamine (Gln), lysine (Lys), and cysteine (Cys)], carboxylic acids [ascorbic (Asc), citric (Cit), lactic (Lac), acetic (Ace), oxalic (Oxa), succinic (Suc), maleic (Mal), and ketoglutaric (Ket)], and simple carbohydrates [mannose (Mn), saccharose (Sc), arabinose (Ar), fructose (Fr), maltose (Ml), and glucose (Gl)]. Indices: G, reserved gray soil; AG, agrogray soil; C, reserved chernozem; AC, agrochernozem. Small indices: w, watershed; t, transitional zone; a, accumulative zone.

and 1.8 times in plowed variants. Hence, the influence of position in catena on chernozems was much lower in comparison with gray soils catena, where similar decrease reached 2 and 4.6 times, respectively.

So, the trend of changes in metabolic diversity of microbial communities of the upper soil layer depending on soil plowing and on its position in catena was common for gray soils and chernozems. Metabolic diversity increased from the watershed to the transitional part of catena, and plowing resulted in its maximal decrease in watershed and minimal decrease in the accumulative position. The increase in the number of significant responses from watershed to accumulative part correlated in gray soils, as well as in chernozems, with the increase of silt and clay content down the catena with correlation coefficient 0.7 for reserved variants and 0.8 for plowed variants. It is evident that the downslope moving of soil material had essential influence on the upper soil layer, and this influence greater in plowed variants was than in reserved ones because of the disturbance of the upper layer structure.

Maximal values of responses in every group of substrates, taken to calculate metabolic diversity of microbial communities in the layer 10–20 cm (Fig. 4b), were lower than maximal values in the layer 0–10 cm: by 86% for the group of amino acids and by 13 and 23% for the groups of carboxylic acids and carbohydrates, respectively. Clustering of substrates in this layer, as well as in the layer 0–10 cm, allowed isolating three primary groups. First group with high responses in all soil variants included only citric and ketoglutaric acids. Saccharose, glutamine, and ascorbic acid observed in the first group in the layer (Fig. 4a), fell here to the second group, in which high variability was recorded. This group included also fructose and succinic acid, the only substrates of the second group in the layer 0–10 cm, and asparagine and glycine. The remained substrates formed the third group with maximal variability of responses in the set of reserved and arable soils. Coefficients of variation in the first, second, and third groups accounted for 54, 92, and 116% for the layer 0–10 cm, and 40, 75, and 158%, respectively, for the layer 10–20 cm.

Unlike the upper layer of gray and agrogray soils, where plowing in watershed resulted in significant decrease of amplitude of responses in all clusters of substrates, the layer 10–20 cm demonstrated such decrease only in the third cluster (Fig. 4b). Practically, all significant responses were lost here, excluding the response to mannose adding.

The layer 10–20 cm of reserved gray soil was characterized in the transitional part of catena by extremely low metabolic diversity. Only 20% of responses were significant here, and the responses were found in the second group to ascorbic and succinic acids and in the third group to alanine. Plowing in the transitional zone led the microbial community of lower layer of agrogray soil to significant increase of metabolic diversity. The number of significant responses increased here 3.6 times in comparison with reserved variant and was accompanied by an increase of microbial biomass. The comparison of data obtained for the layers 0–10 and 10–20 cm of agrogray soil in transitional part of catena attested to better conditions for microbial community functioning in the lower layer. It is likely that tillage had a maximal effect here, since the surface soil layer enriched with plant residues appeared to be overlain by soil material from deeper layers.

Significant responses to succinic acid and glycine, lactic acid, maltose, histidine, and arginine were lost in the accumulative part of catena in comparison with the variant on the watershed. The number of significant responses decreased here 1.8 times, and high responses were lost to most substrates of the second cluster: fructose, succinic acid, asparagine, and glycine. If we compare the upper and lower layers of reserved gray and agrogray soils in accumulative zone, we can see that the number of significant responses decreased 2.3 times with depth in both cases. The increase of metabolic diversity with depth was observed on the contrary in the watershed; the number of significant responses increased 1.4 times in reserved gray soil and 3.3 times in agrogray soil.

Similar trend was observed in the layer 10–20 cm of reserved chernozems. In virgin variants, the number of significant responses accounted for 60, 84, and 52% of the total number of responses in watershed, transitional zone, and accumulative zones, respectively. This number decreased in arable variants, excluding agrochernozem on the watershed, where the number of significant respiratory responses was 1.3 times higher than in reserved chernozem. High responses to the first group of substrates remained in the layer 10–20 cm of agrochernozems as well as in all other cases. Significant responses were found in agrochernozems in watershed and in transitional parts of catena to the same substrates as in reserved chernozems (excluding serine in agrochernozem in watershed which was absent in reserved soils in the same position of catena), though they were not so high. The appearance of response to acetic acid in the accumulative part of catena should be noted, which was not found in all points of reserved catena, and higher response to succinic acid in comparison with the response in the accumulative part of reserved catena of chernozems. In the aggregate, the numbers of significant responses in the layer 10–20 cm in the catena of virgin chernozems were close in watershed and accumulative part of catena and increased 1.5 times in transitional part of catena. The number of significant responses decreased 1.7 times in agrochernozems from watershed to transitional zone and did not change in accumulative zone relative the transitional zone. Hence, the influence of position in catena on chernozems differed from gray soils, where the decrease was found in 1.5 and 1.7 times respectively in the direction watershed – transit part – accumulative part of catena.

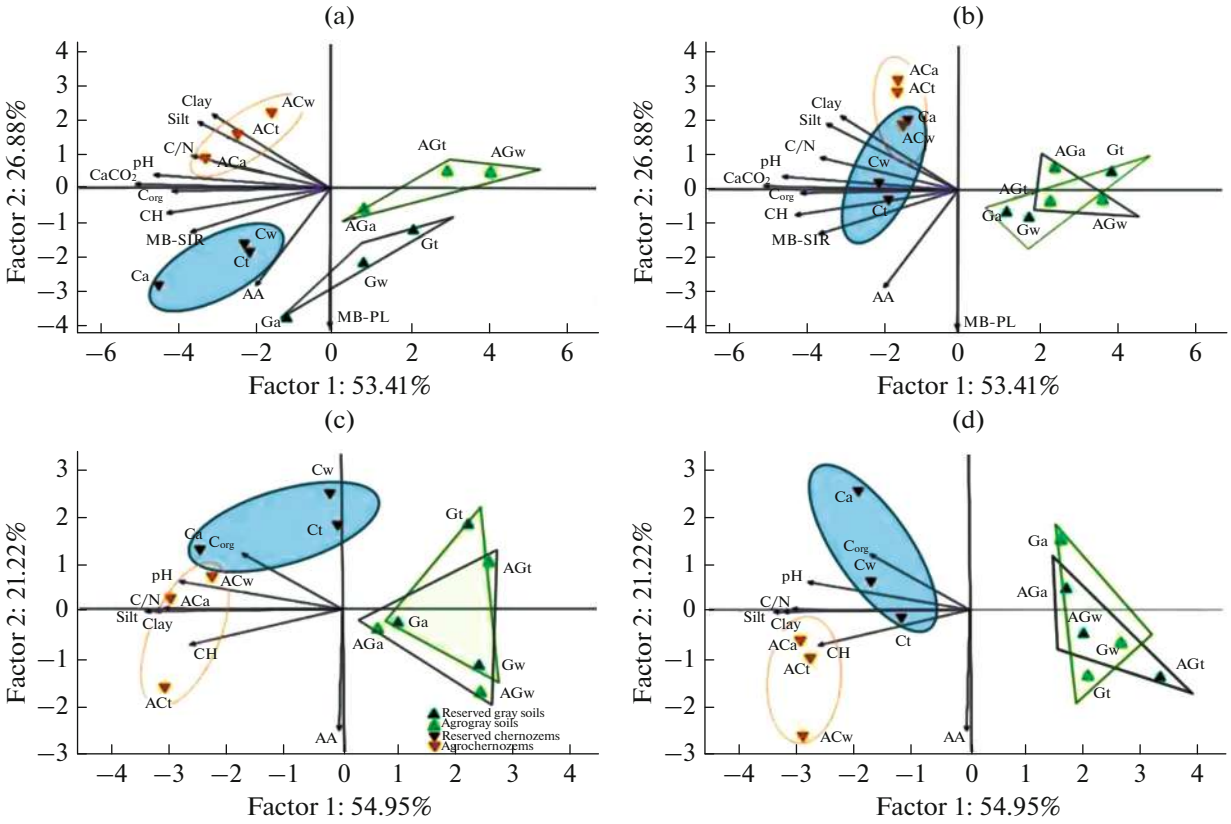

Fig. 5. Positions of microbial communities in different parts of reserved and arable catenas on the plane of principal components: absolute values of respiratory responses ((a) layer 0–10 cm; (b) layer 10–20 cm), normalized values ((c) layer 0–10 cm; (d) layer 10–20 cm). Indices: G, reserved gray soil; AG, agrogray soil; C, reserved chernozem; AC, agrochernozem. Small indices: w, watershed; t, transitional zone; a, accumulative zone; AA, aminoacid group; CH, carbohydrate group.

The layer 10–20 cm in gray soils fell out the trend: the influence was probable of southern slope aspect in virgin variant (sharp decrease of the number of significant responses), and mixing of the layers during plowing was in plowed variant.

To reveal the interrelations between metabolic diversity of microbial communities and physicochemical soil properties in the catenas of reserved and arable gray soils and chernozems, we used the principal components method, in which obtained parameters (microbial biomass determined by concentrations of phospholipids and by substrate-induced respiration of microorganisms, MB-PL and MB-SIR) and total responses of microbial communities to efficient substrates of the group of carbohydrates CH, carboxylic acids CA, amino acids AA) (Fig. 5). Analysis of absolute values of respiratory responses on factor plane demonstrated that factor 1 responsible for 53% of variation mostly depended on pH, concentrations of C_{org} and $CaCO_3$, the C/N ratio, C-SIR biomass, and response to the group of carbohydrates. Respiratory response to the group of amino acids demonstrated weak negative correlation with both factors, and mostly with factor 2, which is responsible for 27% of variation.

Negative correlation with factor 2 was demonstrated for microbial biomass C-PL and responses to amino acids, indicating the effect of soil type and plowing as the main causes of difference in metabolic diversity of microbial community.

The assessment of specific (normalized) characteristics of functional diversity of microbial communities demonstrated that silt and clay content, and the C/N ratio were related to factor 1 (55% of variation) on factor plane, and this resulted in grouping gray soils in the right and chernozems in the left half-plane. The closest positive correlation with factor 2 (21% of variation) was found for respiratory response to introduction of amino acids: reserved chernozems were grouped in the upper and agrochernozems in the lower half-plane. It should be noted that respiratory responses of microbial communities were higher in the catena of agrochernozems than in the virgin variant, whereas such difference was nor found in the catenas of gray soils (both virgin and arable). Grouping of objects by soil type was determined by climatic difference and availability of nutrients for microbial communities under forest litter in gray soils and under herbaceous vegetation in chernozems. Chernozems were characterized in general by expectedly higher metabolic diversity of

microbial communities in comparison with gray soils. It should be also noted that the upper and lower soil layers (0–10 and 10–20 cm, respectively) differed in reserved catenas, and especially in that of gray soils, where essential difference in the value of microbial biomass affected the separation of transitional part in the statistical analysis of data.

It is obvious that disappearance (weakening) of some metabolic responses of microbial community and appearance (strengthening) of other responses was caused by the change in the environmental conditions. The typologic biodiversity changes at the level of microbial community [7, 20]. The replacement of one trophic groups of microorganisms by other groups was caused not only by the change of taxonomic diversity in the course of microbial succession, but also by metabolic rearrangement of microorganisms, trophic preferences of which could vary significantly under different conditions. The changes in the content and composition of soil organic matter, water and aerial regimes, disturbance of soil structure in the result of plowing and application of fertilizers form numerous variants of nutritional medium in microzones [8]. It is known that variability of microorganisms depending on conditions of their growth was often connected with the capability of cell to pass into dormant state [10]. Biochemical plasticity, which manifests itself in the diversity of metabolism ways, is caused by the capability of bacteria and fungi to synthesize alternative enzymes. Unlike isoenzymes, which catalyze the key biochemical reactions of central metabolism, alternative enzymes responsible for performing similar reactions, are encoded by the other genes and are characterized by another structure of molecule from primary amino acid sequence to configuration of active center [15]. Hence, maximal total respiratory response would depend on the particular set of low-molecular substrates, which are especially demanded by microbial community at particular moment, due to plasticity of metabolism of soil microorganisms in response to environmental changes.

CONCLUSIONS

An assumption that plowing would cause a decrease of microbial biomass, hence, the decrease of functional diversity of arable soils was only partly confirmed. Plowing caused the decrease of microbial biomass in agrogray soils and agrochernozems, but the trends of changes of this parameter differed in soils of different types. For example, mitigating of this parameter was observed in the plow horizon of agrochernozems in all positions of catena, whereas the decrease of microbial biomass in agrogray soils was true mostly for the watershed and transitional parts of catena.

Cluster analysis of respiratory responses revealed three groups of substrates, two of which were highly-demanded by microbial communities and accounted for 28 and 36% for the layers 0–10 and 10–20 cm, respectively. The third group included all other substrates. The decrease of the number of significant responses from the first to the third group accounted for 97–71–48 and 100–81–35% in the layers 0–10 and 10–20, respectively. Coefficient of variation increased from 54–40 in the first group to 116–158% in the third group. First group in both layers included citric and ketoglutaric acids; second group included fructose and succinic acid. Ascorbic acid, saccharose, and glutamine were included in the thirst group in the 0–10 cm layer and in the second group (together with asparagine and glycine) in the 10–20 cm layer.

Metabolic diversity of microbial communities in reserved soils was unsurprisingly greater in the catena of chernozems. Metabolic diversity of chernozems and gray soils became closer in the upper layer from the watershed to the accumulative part of catena. The number of significant responses in reserved variants in watershed and transitional parts was 1.5 times higher in chernozems than in gray soils, but this number did not differ in the accumulative part and reached 100% of the total number of significant responses. The difference in the number of significant responses in the upper layer of plowed variants between agrochernozems and agrogray soil also decreased down the catena from 3.7 to 1.5 times.

Pronounced increase of the number of significant responses from the watershed to theaccumulative position was found in the upper soil layers in all catenas and correlated with the increase in silt and clay contents down the catena with correlation coefficient 0.7 for reserved variants and 0.8 for plowed variants.

As a rule, the decrease was observed in the layer 10–20 cm of the number of significant responses in comparison with the upper layer. An exception in reserved variants was the gray soil in watershed, where the increase was recorded of the number of significant responses by 1.4 times. In plowed variants, the number of significant responses in the lower layer of agrogray soil increased in watershed and in the transitional part of catena and only in the watershed one in chernozem.

Cluster analysis of soils of the 0–10 cm layer demonstrated essential similarity of metabolic diversity of microbial communities in reserved chernozems and gray soils in accumulative parts of catenas and similarity of reserved gray and agrogray soils with agrochernozem variant.

SUPPLEMENTARY INFORMATION

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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