= SOIL BIOLOGY =

Soil Microbial Biomass and Functional Diversity of Microbial Communities in Native and Arable Soils of the Belogor'e Reserve

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Abstract- Microbial biomass and functional diversity of soil microbial communities in forest soils of the Belogor'e Nature Reserve and in arable soils formed under similar geomorphologic and lithological conditions were determined. We have analyzed the content of carbon of total microbial biomass (C-TMB), of the biomass of living microorganisms by the content of soil phospholipids (C-PL.), and of biomass of microbial cells capable of glucose assimilation (C-SIR). The respiratory response of the soil microbial community to the introduction of different groups of organic compounds-inductors (amino acids and carboxylic acids) was assessed by the method of multisubstrate testing (MST). It is shown that anthropogenic transformation of natural ecosystems results in a decrease in the total microbial biomass, biomass of living cells, and cells that give a respiratory response to glucose. The functional diversity of microbial communities in soils in natural and transformed ecosystems significantly differs. Soil plowing has resulted in a significant decrease in the ability of the soil microbial community to assimilate low-molecular organic compounds. This is most typical for amino acids: arginine, alanine, and glycine (a 2.7-, 5.4-, and 7.1-time decrease, respectively, as compared to native soils). Among carboxylic acids, the decrease in the respiratory response to the introduction of succinic acid is the most pronounced (8.7 times). It has been revealed that the geomorphologic position in natural ecosystems does not affect the biomass of the microbial community and its functional diversity, while the arable soils are characterized by a clear tendency to an increase in these parameters on the lower part of the slope.

Keywords: total microbial biomass, Belogor'e Nature Reserve, respiratory response of microbial community, plowland, multisubstrate testing, geomorphologic position, catena

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INTRODUCTION

Anthropogenic activity causes significant changes in soil microbial communities, which are the most essential component of ecosystems. The total microbial biomass, its living part, and the biomass of cells that give a respiratory response to glucose application are the most widely used ecological parameters, characterizing the status and diversity of microbial communities in soils [14, 18–20].

A significant part of the microbial community in soils is resting, therefore, the determination of carbon of the total microbial biomass (**C**–**TMB**) is informative for studying soil microbial communities. This biomass is represented by cells that do not grow on nutrient substrates, do not respond to glucose application, and are not destructed by fumigants due to organomineral shells [8, 10, 11, 30, 41].

The content of carbon of living microbial biomass determined by the amount of phospholipids in soils (C-PL) is an important parameter of assessing the

status of soil microbial community. Phospholipids are components of cell membranes of bacteria, actinomycetes, fungi, and lower plants and are quickly destructed after cell death. The content of phospholipids in cells of microorganisms is constant [34], which enables the estimation of living microbial biomass in soils, in habitats with very low organic carbon (C_{org}) content in particular. The content of phospholipids may be given in units of C_{org} or in the number of cells [34–36]. This parameter is widely used for the study of soil microbial communities in various natural ecosystems [16, 17, 22, 25–27, 33, 48].

The biomass of microorganisms, which respond to glucose amendment and are determined by the substrate-induced respiration method (C-SIR), is the most conventional parameter of the soil microbial community status [1, 2, 28, 44, 46, 47].

Comparison of C–TMB, C–PL, and C–SIR with the C_{org} content in soil enables more comprehensive assessment of the status of its microbial community.

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The C–PL to C_{org}, C–PL to C–TMB, C-SIR to C_{org}, and C-SIR to C-TMB ratios may indicate changes in the functional diversity of microbial communities [7, 25, 39, 40]. This approach has been successfully tested on buried soils of different ages [25, 40].

Anthropogenic load can cause changes not only in quantitative parameters of soil microbial biomass, but also in the functional diversity of the soil microbial community. This aspect of soil functioning in agrocenosis is insufficiently studied. The method of multisubstrate testing is used to assess the ability of soil microorganisms to assimilate various types of organic compounds. The basis of the methodological approach was laid by S.N. Vinogradskii, who analyzed the spectrum of low-molecular organic compounds consumption by soil microbial community [3]. These works resulted in the elaboration of the BIOLOG system [31] transformed into the ECOLOG system by Gorlenko and coauthors [5, 6].

The aims of the study were to identify specific features of changes in microbial biomass and its components, as well as of the microbial functional diversity of soils along the catena, which included the watershed and the middle and lower parts of the slope, i.e. different zones of the soil profile disturbance (erosion, erosion + transit, and accumulation, respectively).

OBJECTS AND METHODS

Objects of research. We have analyzed dark gray forest soils of the Belogor'e Nature Reserve located in Belgorod oblast (a key site of the Les na Vorskle oak forest on the Vorskla River) and agro-gray soils on a plowed plot. The virgin and arable key sites are characterized by similar lithological and geomorphologic conditions (Fig. 1), which enables us to consider the anthropogenic activity (arable land) as the only factor of possible changes in chemical properties and in the status of microbial communities of these soils. The research area is located on the southwestern slope of the Middle Russian Upland at 200-250 m above sea level. The studied plot of Les na Vorskle is bordered from three sides by the Vorskla, Gotnya, and Loknya rivers. The climate in the oblast is moderately continental (the mean annual air temperature is $+6.0^{\circ}$ C, and the maximal one is $+40^{\circ}$ C (above $+50^{\circ}$ C on the open surface of the plowed land). The minimal air temperature is -37° C. The snow cover persists during 3-4 months, and its mean thickness is 20-25 cm [9]. Soil-forming rocks in the reserve and on adjacent plowland are dominated by loess-like sediments. The soil cover of the Les na Vorskle test plot is mainly formed by dark gray forest soils (Luvic Retic Greyzemic Phaeozem (Siltic)). The reserve area and adjacent plowland have been repeatedly investigated [9, 15, 21].

Soil sampling. Soil samples were taken along the catenas on forested and arable plots. We laid soil pits

on southern slopes under similar lithological conditions and small pits (0.5-m-deep in two replications) on the watershed and in the middle and low parts of the slope. The morphologic-genetic description of soil profiles was performed. Soil samples were taken from 10-cm-thick layers to a depth of 50 cm, large roots and other plant residues were removed, and soil was airdried and sifted through sieves with mesh diameters of 1 mm and 2 mm. We used the former for physicochemical analyses of all the sampled layers and the latter for microbiological studies of only the 0- to 10-cm layer. We determined microbiological parameters (C-TMB and C–PL) in air-dry soil and performed MST analysis and C-SIR determination for soil preliminary moistened to 60% of FC and incubated at a temperature of 22°C during a day.

Methods. We determined the organic carbon content in the samples by the method of wet combustion elaborated by Tyurin and modified by the St. Petersburg State University with oxidation by concentrated sulfuric acid in a thermostat at T = 140°C, the content of carbonates by the acidimetric method, pH_{H₂O} by the potentiometric method, CEC by the Bobko-Askinazi method modified by the Central Institute of Agrochemical Service (TsINAO), and the particlesize composition by the pipette method [4].

The assessment of the abundance and total biomass (C-TMB) of soils included the following stages. Soil sample (3 g) was stirred in 90 mL of 0.5% sodium pyrophosphate solution treated by two pulses of ultrasound of 30 s long with a pause of 30 s between them [27], using an UZG 13-0.1/22 ultrasonic generator at a power of 50 W and a frequency of 22 kHz. The extract with microbial cells was separated from the soil sediment by centrifugation at 1000 rpm during 30 min under cooling (4°C). Sodium pyrophosphate solution (90 mL) was added to the soil precipitate, and all the procedures were repeated. The extraction was performed in three replications. Soil precipitates obtained from 3 g of soil after three treatments were brought to 30 mL and sequentially diluted by distilled water 100 times to the rate of 1000 per 1 g of soil. After three treatments, extracts of the microbial fraction were combined, brought to 300 mL, and aliquots of 1 mL were taken for direct quantification. Aliquots were diluted ten times to the rate of 1000 per 1 g of soil. To obtain micro-preparations, 10 µL of finally diluted soil sediments and extracts of the microbial fraction were put on slides, and the drop was evenly distributed over an area of 600 mm². The preparations were slightly dried, flamed, and stained by the DAPI solution $(5 \,\mu\text{g/mL})$ during 15–20 minutes, and microbial cells were quantified under a Leica 2000 fluorescent microscope in 60 fields of view with an area of 0.02 mm^2 (1000-time magnification). The number of cells per one gram of soil was determined by the formula:

$$N = a \times 29857 \times 100 \times 1000,$$

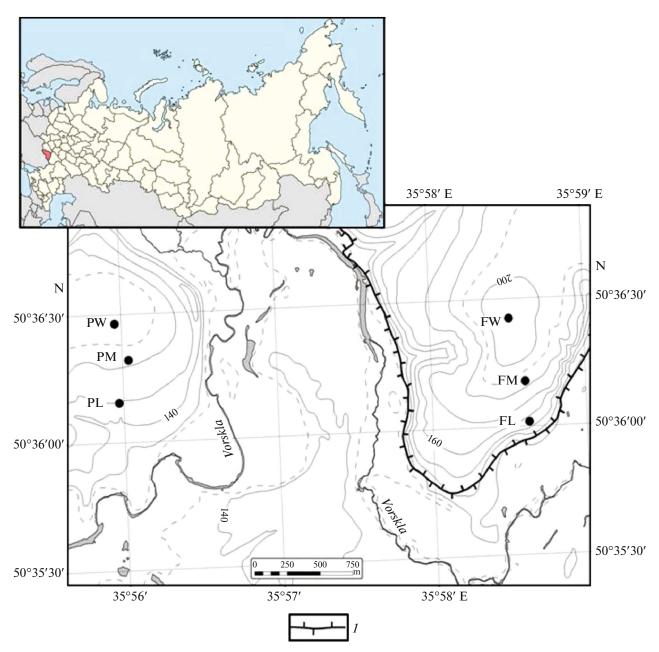


Fig. 1. Map of the location of catenas on plowed gray forest soils (PW—watershed, PM—middle part, and PL—lower part of the slope) and gray forest soils (FW—watershed, FM—middle part, and FL—lower part of the slope). (*I*) The boundary of the protected area.

where N is the number of cells in the extracted microbial fraction or in sediment from 1 g of soil, a is the mean number of cells in one field of view, 29857 is the number of fields of view in 600 mm², 100 is the number of aliquots of 10 µL in 1 mL, and 1000 is the final dilution per 1 g of soil.

The completeness of extraction (CE) of microbial fraction (percentage) was calculated by the formula:

$$CE = N_{\rm MF} / (N_{\rm MF} + N_{\rm SS}) \times 100$$

where N_{MF} and N_{SS} are the number of cells in the microbial fraction and in soil sediment.

Extracts of the microbial fraction were precipitated at 12000 rpm. After drying at 105°C, the mass of the separated microbial fraction and the content of organic carbon in it were determined by wet combustion with spectrophotometric determination of residual bichromate. Carbon of the total microbial biomass was calculated by the formula

$$C-TMB = C(MF)/CE \times 100,$$

where C(MF) is the content of organic carbon in the extracted microbial fraction, and CE is the completeness of its extraction [10, 24, 38, 42].

Carbon of living microbial biomass was calculated by the content of phospholipids in cell membranes of soil microorganisms (C-PL) [25, 35]. The method is based on extraction of phospholipids from a soil sample in a single-phase mixture (methanol: chloroform: phosphate buffer in the ratio between volume fractions of 1:2:0.8). The single-phase mixture (18.3 mL) was added to the weighted soil sample (2 g) extracted by shaking (two hours at room temperature), and the soil sediment was precipitated from the supernatants by centrifugation (15 min). The obtained supernatants were separated, 5 mL of a single-phase mixture was added to the sediment, and it was centrifuged again. Chloroform and phosphate buffer solution (6.2 mL) were added to the combined supernatants of both centrifugation stages for stratification. The upper water layer with the buffer solution was removed, and the lower organic layer with lipids, phospholipids in particular, was analyzed. The aliquot (1 mL) of the organic layer was placed to analytical cups of 2 mL in volume and evaporated in nitrogen current at 34.5°C. Then, a saturated potassium persulfate solution (900 µL) was added to the cups, and they were placed in a thermostat (98°C) for four days to separate phosphate groups from phospholipids. After that, ammonium molybdate (200 μ L) was added to each cup for the reaction with phosphate groups, a solution of malachite green $(900 \ \mu L)$ was applied, and the mixture was analyzed on a spectrophotometer.

Carbon of microbial biomass, which gives a respiratory response to glucose amendment (C-SIR) was determined by substrate-induced respiration (V_{SIR}) on a Kristallyuks 4000M gas chromatograph at the Common Use Center of the Institute of Physicochemical and Biological Problems of Soil Science of the Russian Academy of Sciences. Weighted portions (1 g) of each soil sample were placed in tubes (15 mL) with plastic plugs and incubated for 12 hours at 22°C. After ventilation, 200 µL of 1% glucose solution was added, and the tubes were plugged. Within four hours of incubation $(22^{\circ}C)$, the amount of released CO₂ was measured on a Kristallyuks 4000M gas chromatograph at the Common Use Center of the Institute of Physicochemical and Biological Problems of Soil Science of the Russian Academy of Sciences. We calculated the respiratory activity of microbial communities in $\mu g C - CO_2/(g h)$ and recalculated it in $\mu g C/(g h)$, using the coefficient 40.04 [1, 29].

The functional diversity of microbial communities was assessed, using the multisubstrate testing (MST) [5, 6, 12, 13, 37, 43]. The microbial community was tested for amino acids: glycine, alanine, arginine, histidine, tyrosine, and cysteine (L-isomers) and carboxylic acids: ascorbic, citric, lactic, acetic, oxalic, and succinic. The concentrations of these substrates corresponded to those recommended [32] for amino acids (15 mM) except for tyrosine (5 mM), and the concentrations of salts of carboxylic acid varied from 20 to 190 mM according to the anion: 20 mM for citric, 30 mM for oxalic, 50 mM for succinic, 100 mM for ascorbic, 160 mM for lactic, and 190 mM for acetic acid. The reaction of solutions of carboxylic acids and amino acids was brought to pH = 6-7 by adding 1 n NaOH or 1 n HCl. The MST analysis was performed similarly to C-SIR determination [23]. Weighted portions of each soil sample (1 g) were placed in tubes (15 mL) with plastic plugs and incubated during 12 hours at 22°C. One of the substrates (200 uL) was added to the tubes after ventilation, and the tubes were plugged. After four hours of incubation at 22°C, the amount of released CO2 was measured on a gas chromatograph (Kristallyuks 4000M at the Common Use Center of the Institute of Physicochemical and Biological Problems of Soil Science of the Russian Academy of Sciences). The respiratory activity of microbial communities was calculated in μg of C–CO₂/(g h).

Statistical processing of data. All analyses were performed in three replications. The graphs and tables show the means \pm standard error (SE). The data were statistically processed, using the principal component method, in the Statistica 10 program.

RESULTS

The analysis of the particle-size composition of soils on catena "Forest" shows a significant increase in the content of physical clay with depth from 20 to 43% (Table S1). Soils of the watershed plot are enriched with the coarse silt fraction: its content in the upper layer reaches 47%. There is a pronounced rise in the amount of fine sand in soils of the middle slope part in comparison with the watershed and the lower slope part, where it is noticeably smaller. In the middle part of the slope, the content of the clay fraction is minimal (from 4 to 13%).

There is a rise in the content of fine sand in arable soils on the watershed and in the middle slope part as compared to the pits on the middle and lower slope, which may be related to the lithological heterogeneity of soil-forming rocks. The content of coarse silt is 9– 22% and slightly increases only on the lower slope. The clay content varies from 4 to 8% in the upper layer and rises with depth to 18%. The particle-size composition of arable soils is similar to forest analogues.

The C_{org} content in soil profiles of the natural ecosystem on the watershed and on the lower part of the slope is similar (Table S2). In the middle part of the slope, it is 1.3% in the upper 10-cm layer, sharply decreases down the profile, and does not exceed 0.2% at a depth of 20–30 cm. The reaction is weakly acid (close to neutral) except for soils of the middle slope, where pH is lower. The cation exchange capacity here is significantly smaller. Exchange cations are dominated by Ca²⁺.

There is a pronounced increase in pH with depth in arable soils in the lower part of the slope. The C_{org} con-

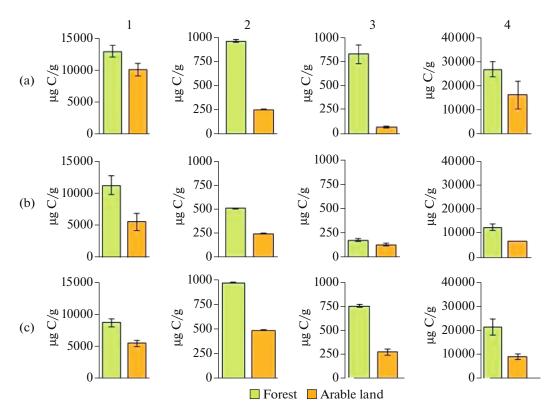


Fig 2. Biomass of microbial community (1, C–TMB; 2, C–PL; 3, C–SIR; and 4, C_{org} , $\mu g/g$ abs. dry soil) of forest and plowed soils in different geomorphic positions: (a) watershed, (b) middle part of the slope, and (c) lower part of the slope.

tent in the upper soil layers is low and reaches 1% only in the deep layer of the lower slope. The cation exchange capacity of soils on the watershed is very low and gradually increases down the slope.

Carbon of total microbial biomass (C-TMB). Forest soils of the catena are characterized by a considerable variation in C_{org} , and differences in the C-TMB content are insignificant and are not reliable in soils of various parts of catenas (Fig. 2).

Differences between arable soils on various slope parts are more significant. The C_{org} content decreases two times due to plowing, and C–TMB drops more slightly (20–35%). The greatest decrease (two-time) in C–TMB is recorded in soils of the middle part of the slope.

Carbon content of living microbial biomass (C-PL). The content of carbon of living microbial biomass in plowed soils changes more significantly as compared to C-TMB. It decreases four times in arable soils of the middle part of the slope and two times in the lower one as compared to reserved soils.

The content of carbon of microbial biomass (C–SIR). The decrease in active biomass is the most pronounced. For example, C–SIR is 832 μ g/g of soil in native soils of the watershed plot and does not exceed 100 μ g/g of soil on the plowland. In the lower slope part, this parameter is three times smaller in the plowed soil (277 μ g/g of

soil) than in the corresponding native soil. This trend is not revealed on the middle slope.

Thus, the C–TMB content in soils of the natural ecosystem is higher than in soils of the arable land at all geomorphologic positions. This is especially the case for C–PL, which is clearly seen for soils of the watershed, where this parameter is four times lower than in soils of the natural ecosystems. The portion of microorganisms with the respiratory response to glucose amendment (C–SIR) is about 30%. The same regularity persists on the lower part of the slope, but the portion of active cells (C–SIR) in the living microbial biomass (C–PL) is slightly higher (57%) in arable soils. The revealed differences are less pronounced in the middle part of the slope.

Functional diversity of microbial communities. The response of microbial communities to the introduction of various substrates differs significantly on forested and arable lands (Table 1). In soils of "Forest" catena, differences in respiratory responses to the introduction of carboxylic (ascorbic, lactic, citric, and succinic) acids and amino (glycine, arginine and alanine) acids are the strongest (Fig. 3). The respiratory response of the microbial community of the forest soil to the ascorbic acid application is the strongest and reaches 68 μ g C–CO₂/(g h) in the lower part of the slope. It is also significantly weaker (28 μ g C–CO₂/(g h))

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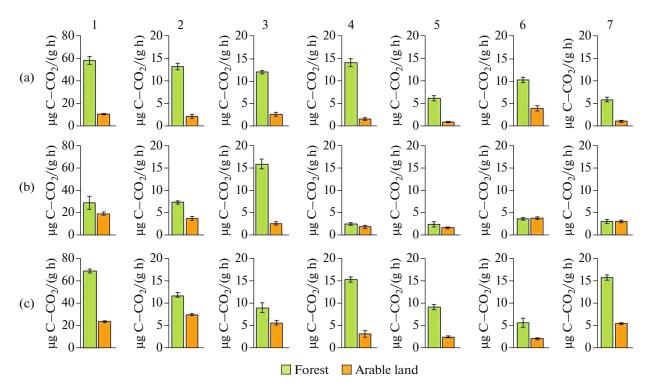


Fig. 3. Comparison of multisubstrate testing (MST) of respiratory activity of microbial communities (μ g C-CO2/(g h)) of catena "Arable land" and catena "Forest" on (a) watershed, (b) middle part of the slope, and (c) lower part of the slope in variants with application of: 1—ascorbic acid, 2—lactic acid, 3—citric acid, and 4—succinic acids and amino acids: 5—glycine, 6—arginine, and 7—alanine.

on the central slope. The similar pattern is revealed for respiratory responses to lactic and succinic acids.

The response to the introduction of acetic and oxalic acids in soils of all the analyzed geomorphologic positions of catena "Forest" is minimal and does not significantly differ.

Contrary to carboxylic acids, the respiratory responses of the microbial community of soils of catena "Forest" to amino acids are in general low. The responses to glycine, histidine, arginine, and alanine at different parts of the catena are similar to the response to ascorbic acid.

The respiratory response of the microbial community in arable soils to the application of ascorbic acid is maximal in the lower slope part $(23 \ \mu g \ C-CO_2/(g \ h))$. It is slighter on the middle one and decreases to $10 \ \mu g \ C-CO_2/(g \ h)$ on the watershed. The respiratory response to other carboxylic acids is in general similar:

Soil	AsA	LA	CA	AcA	OA	SA	Gly	Arg	His	Ala	Cys	Tyr
	$\mu g C - CO_2/(g h)$											
Catena "Forest"												
Watershed plot	57.7	13.2	11.9	2.5	4.8	14.0	6.2	10.2	6.3	5.9	4.0	3.4
Middle part of the slope	28.4	7.4	16.0	2.1	4.4	2.5	2.6	3.6	3.2	3.0	3.8	2.9
Lower part of the slope	68.6	11.7	9.0	1.6	4.1	15.4	9.3	5.7	5.7	15.8	3.6	3.2
Catena "Arable land"												
Watershed plot	10.3	2.2	2.6	1.2	1.6	1.6	0.9	4.0	1.3	1.1	2.8	1.1
Middle part of the slope	18.7	3.8	2.7	0.8	2.5	1.9	1.7	3.8	1.9	3.0	2.2	2.3
Lower part of the slope	23.5	7.5	5.6	1.8	3.2	3.2	2.6	2.1	3.5	5.5	3.7	2.7

Table 1. Results of multisubstrate testing of respiratory activity $(\mu g/(g h))$ of microbial communities of soils (from 0- to 10- cm layer)

Carboxylic acids: AsA—ascorbic, LA—lactic, CA—citric, AcA—acetic, OA—oxalic, and SA—succinic. Amino acids: Gly—Glycine, Arg—Arginine, His—Histidine, Ala—alanine, Cys—Cysteine, and Tyr—Tyrosine.

it is the lowest on the watershed, slightly higher in the middle, and the highest in the lower part of the slope.

The respiratory response to arginine is the most pronounced in soil of the watershed (4 μ g C–CO₂/(g h)) and decreases to 2 μ g C–CO₂/(g h) down the slope. Alanine application results in the opposite regularity: 5.5 μ g C–CO₂/(g h) on the lower slope and a drop to 1.1 μ g C–CO₂/(g h) on the watershed.

The comparison of the respiratory response of the microbial community to carboxylic (ascorbic, lactic, citric, succinic) acids and amino (glycine, arginine and alanine) acids shows that it is more pronounced on all parts of catena "Forest" as compared to catena "Arable land". The respiratory response to ascorbic acid is the most significant: $69 \ \mu g \ C-CO_2/(g h)$ on the lower part of the slope of catena "Forest", which exceeds the values for catena "Arable land " by $45 \ \mu g \ C-CO_2/(g h)$. The regularity on the watershed is similar.

The respiratory responses of microbial community to the application of the most significant substrates on the watershed and lower slope of catena "Forest" significantly exceeds those on similar elements of catena "Arable land". The respiratory response of microbial community to succinic acid, glycine, arginine, and alanine on the middle slope of both catenae does not significantly differ.

Statistical analysis of biomass and functional diversity of microbial communities of forest and plowed soils is given in Fig. 4. The decrease in respiratory responses to the application of low-molecular compounds as a result of arable land is not the same on different elements of the catena (Fig. 4 I). The respiratory response to glucose in the arable soil of the watershed is eight times weaker as compared to forest soil. Respiratory responses to alanine, glycine, and histidine, as well as to succinic, ascorbic, and lactic acids here varies 5–9 times, and the difference is maximal for succinic acid. There are 1.5–4.5-time variations in responses to arginine, tyrosine, and cysteine and to oxalic, citric, and acetic acids. The difference is the lowest in the variant with cysteine introduction.

Respiratory responses to glucose in forest and arable soils on the same slope parts practically do not differ. In soils of the middle part of the slope, respiratory responses vary 1.5–2.5 times in case of application of cysteine and acetic, oxalic, lactic, and ascorbic acids, and six times in the variant with citric acid. The responses to the application of glycine, arginine, alanine, and succinic acid in soils of the middle slope do not significantly differ. The greatest variations (three–five times) on the lower part of the slope are revealed, when glycine, arginine, and alanine, as well as ascorbic and succinic acids are added.

Figure 4 II shows the most significant changes in respiratory responses to the of introduction lowmolecular weight compounds in arable soils as compared to forest ones. The response to succinic acid is the most strongly related to the living and active microbial biomass (K = 0.96 - 0.98). The response to alanine is in close correlation with the portion of living microbial (K = 0.89) and active microbial biomass (K = 0.90) in the total microbial biomass. There is a relationship between the response to applied glycine and the cation exchange capacity of soil (K = 0.98)and between arginine and the organic carbon content (K = 0.88) and the total microbial biomass (K = 0.70). Organic carbon and the total microbial biomass in plowed soils regularly decrease 1.3-2.3 times on all elements of the catena. The drop in living and active microbial biomass is to four and ten times, respectively, on the watershed and is 1.5-3 times on the slope. The differences are the smallest on the middle part of the slope, where C-PL and C-TMB in the upper layer of the forest soil are minimal.

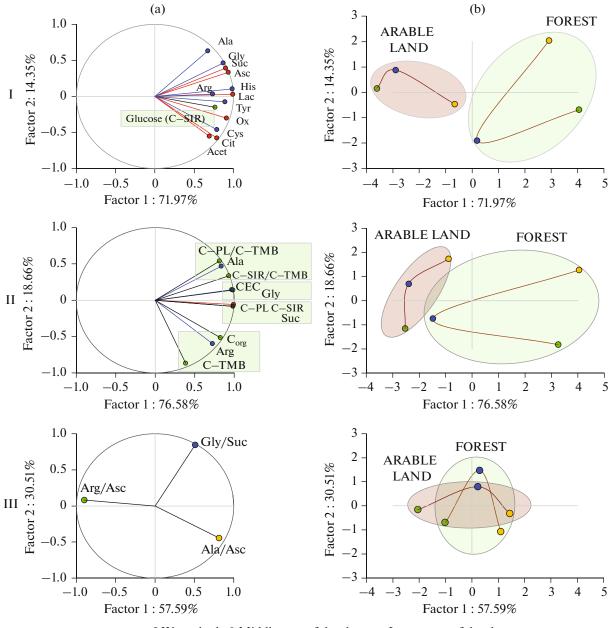
It is proposed to use the ratio between respiratory responses to added amino acids and carboxylic acids to detect the presence of organic substances in gray forest and chestnut soils [23]. Soils on different elements of catenas significantly differ in the ratios between respiratory responses to glycine and succinic acid, alanine and ascorbic acid, and arginine and ascorbic acid (Fig. 4 III). The similarity between arable and forest soils on the same catena elements is maximal here.

Thus, the respiratory response of soil microbial community to the introduction of low-molecular weight compounds is significantly stronger in soils of the natural ecosystem than in arable soils in all parts of the catena (watershed and middle and lower slopes). Therefore, anthropogenic activity causes a sharp drop in the ability of microbial community to assimilate carboxylic acids and amino acids. The decrease in the functional diversity is especially pronounced in soils of the watershed, where erosion is the strongest. For example, the decrease in respiratory response to ascorbic acid in plowed soil on the watershed is five—six times stronger than in soils of natural ecosystems in the same geomorphologic positions. The regularities for other carboxylic acids are similar.

The dependences in the middle part of the slope of both catenas are different. The regularities of respiratory responses on watershed sites are found only in case of citric acid application. The differences between arable and forest soils are not so pronounced for lactic and ascorbic acids, and are unreliable in the variant with succinic acid.

The ability of microbial community to assimilate low-molecular compounds in soils of the lower part of the slope is greater, which is related to the accumulation of humus material in the lower part of the slope.

The analysis of responses of microbial community to the application of amino acids shows the same regularity: they are significantly stronger in soils of natural ecosystems. The respiratory response of soil microbial community to these substrates in natural undis-



• Watershed • Middle part of the slope • Lower part of the slope

Fig. 4. Statistical analysis of biomass and functional diversity of microbial communities of forest and plowed soils on various elements of topography, using the method of principal components: coordinates of quantitative characteristics of the status of microbial communities on the factor plane determined by the value and direction of mutual correlation (a) and the corresponding location of the study objects on the factor plane (b). I—respiratory responses to the introduction of amino acids and carboxylic acids as indicators of the functional diversity of the microbial community; II—respiratory responses, correlating with microbial biomass, organic carbon content, and cation exchange capacity of forest and plowed soils; and III—ratios between respiratory responses, reflecting the location of soils on various topographic elements.

turbed soils at different geomorphologic positions is different. For example, the response to arginine of watershed soils is stronger than in soils of the lower part of the slope, while the regularities for glycine and alanine are opposite. In the middle part of the slope, respiratory responses to amino acids and succinic acid in soils of natural and human-transformed ecosystems are insignificant and do not reliably differ. We cannot explain the nature of this phenomenon.

CONCLUSIONS

The total microbial biomass (C–TMB) and the biomass of living cells (C–PL) and of cells with respi-

ratory response to glucose amended (C–SIR) are stronger in forest soils than in their plowed analogues. Anthropogenically transformed soils are characterized by a noticeable decrease in C–SIR, less noticeable drop in C–PL, and minimal differences in C–TMB. There is a tendency to an increase in C–SIR and C–PL in arable soils on the lower part of the slope.

The functional diversity of microbial communities of soils in natural and human-transformed ecosystems significantly differs. Arable land results in a pronounced decrease in the ability of soil microbial community to assimilate low-molecular organic compounds. The ability of microbial community of plowed soils to assimilate arginine and alanine is 2.7 and 5.4 times lower as compared to forest soils, respectively. The decrease is the greatest in the variants with glycine and succinic acid: 7.1 and 8.7 times, respectively.

Geomorphologic position in natural ecosystems does not affect the biomass of soil microbial community and its functional diversity. In plowed soils, there is an increase in C–TMB, C_{org} , and C–PL on the lower part of the slope. A tendency to a drop in biomass and functional diversity is revealed in the transit geomorphologic position of the middle part of the slope in both catenas.

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SUPPLEMENTARY INFORMATION

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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