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Soil microbial biomass and gas-production activity (CO_2) in Chernozems of different land use

Kristina Ivashchenko[1],*, Nadezhda Ananyeva[1,2], Vyacheslav Vasenev [2, 3], O. Ryzhkov [4], V. Kudeyarov [1] , R. Valentini [2,5]

[1]Institute of Physicochemical and Biological Problems in Soil Science of the Russian Academy of Sciences, Pushchino, Russia
[2]Laboratory of Agroecological Monitoring, Prediction and Modeling of Ecosystems, RSAU, Moscow, Russia
[3]Peoples' Friendship University of Russia, Miklukho-Maklaya str. 6, Moscow, Russia
[4]The Central Chernozems State Biosphere Reserve named by V. Alekhin, Zapovednoe, Kursk region, Russia
[5]University of Tuscia, Via S. Camillo de Lellis, Viterbo, Italy

*Corresponding author e-mail : ivashchenko-kv@rambler.ru
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Abstract

The typical chernozems (Kursk region, Russia) in undisturbed (virgin and mowing steppe) and disturbed (pasture, black fallow, arable and including urban) ecosystems were studied. In each ecosystem and urban functional zone (recreational, residential and industrial) soil samples were collected in 3-5 randomly chosen locations from the depths 0-10, 10-50, 50-100 and 100-150 cm (total 124). In the collected samples soil microbial biomass (C_{mic}) was analyzed by substrate-induced respiration method and basal (microbial) respiration (BR) was measured. The ratio of BR / C_{mic} = qCO_2 and portion of C_{mic} in soil organic carbon (C_{org}) were estimated. In the soils (0-10 cm) under black fallow, arable and urban ecosystems the C_{mic} content and C_{mic} / C_{org} ratio were almost two times less compared to undisturbed ecosystems. Reduction of Cmic, BR and C_{mic} / C_{org} and increase of qCO_2 value down the chernozem's profile was reported. The C_{mic} stocks over the chernozem's profile were 2-4 times higher in undisturbed ecosystems than in disturbed ones. Topsoil 10 cm contributed to the major part of the total C_{mic} and BR over the profile (41-79%). The excess of CO_2 production (BR) in urban soils was shown.

Keywords: Typical chernozems, soil microbial biomass carbon, soil respiration, land use

INTRODUCTION

Chernozems, widely recognized as the etalon soils, occupy about 6% of Russian territory, but represent an important natural resource of the country (Kovda, 1983; Avetov et al., 2011). The unique features of chernozems include high fertility, large stocks of carbon and nutrients over the whole profile. Incorporation of chernozems into intensive agriculture reaches 50-80%, which provides 2/3 of all agricultural products of the country. In result, the 20-30% loss of soil carbon over the last 150 years due to chernozem's degradation is reported (Mikhailova, Post, 2006). Recently Chernozemic region is getting more and more urbanized with the proportion of urban population increased by 10% over 2010-2014 years (Assessment of residential…, 2014). These factors lead to severe anthropogenic impact on terrestrial ecosystems, including soils.

Chemical, physical and morphological features of chernozems are well studied and presented in literature (Rodionov et al., 2001; Shein et al, 2011; Kogut et al, 2012). Quite a few publications also focused on biological properties of chernozems under different land use. (Stakhurlova et al., 2007; Blagodatskii et al., 2008; Polyanskaya et al., 2010). However, comparative analysis of soil gradient from natural to anthropogenic-transformed ecosystems (arable and urban) are still lacking (Chen et al., 2001; Vasenev et al 2012; Zhao et al., 2013). Moreover, changes in the biological properties of the single soil type (typical chernozems) under different ecosystems, taking into account their spatial variability and spatially discrete distribution, are still poorly understood.

Soil microorganisms represent a living and active soil part, very sensitive to various natural and anthropogenic influences. The microbial component provides the main CO_2 flux from soil to atmosphere (Cornard, 1996; Zavarzin, Kudeyarov, 2006) and is responsible for the optimal functioning of terrestrial ecosystems (Martens, 1995). It's well assumed that long-term agricultural use (chernozems plowing) leads to a dramatic reduction of microbiological parameters. For instance, soil microbial biomass content in arable chernozems was four times (Senicovscaia, 2012) and 50% (Balashov, Buchkina, 2011) less than in fallow ones. The C_{mic} / C_{org} ratio in arable (76 yrs.) chernozems was almost two times less compared to mowing meadow (Blagodatskii et al., 2008).

This research was focused on analyzing soil microbial component in typical chernozems, its respiration and ecophysiological activities in natural and anthropogenic-transformed ecosystems, including urban ones. The aim of this study was to identify features of the microbial community's functioning in chernozems, considering their profile distribution, following anthropogenic transformation.

MATERIAL AND METHODS

Sites and Soil sampling

Typical chernozems (Luvic Chernozems; WRB, 2014) in Kursk region (Russia), under natural (virgin and mowing steppe) and anthropogenic disturbed (pasture, black fallow, arable and urban) ecosystems were studied. Kursk region locates in the south-western part of the middle-Russian plateau. Annual average air temperature for the region is +4.6-6.1°C and annual precipitation amounts to 475-640 mm. Sampling campaign was carried out in summer period of 2012-2013. Sampling points were selected randomly in each observed ecosystem and functional zone of Kursk city (recreational, residential, and industrial) (Fig. 1). In each observation point mixed sample was taken from a 2x2 m plot (corners and centre), from 0-10, 10-50, 50-100 and 100-150 cm layers (total 124 samples). Bulk sample for each site and layers was prepared. Soil samples were delivered in laboratory and stored in natural moisture condition with air exchange (8-10°C) no more than 4 weeks before the use in experiments.

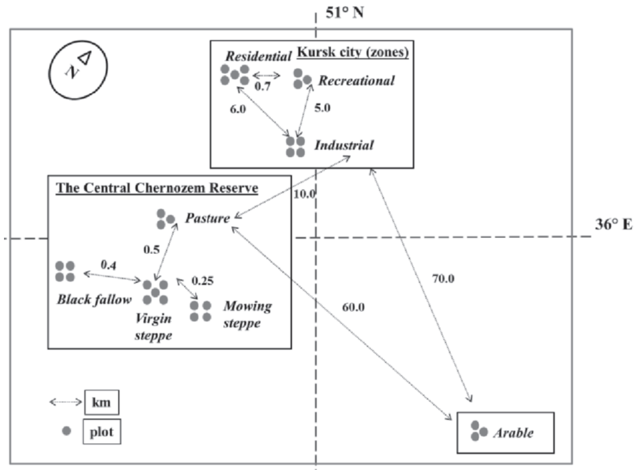


Figure 1. Localization of soil sampling points in Kursk region

Microbiological Analyses

Soil substrate-induced respiration (SIR) based on additional respiration response of soil microorganisms (initial maximum CO_2 production) enriched by available substrate (glucose) was measured (Anderson, Domsch, 1978; Ananyeva et al., 2011). Soil samples (2 g) were placed into a 15 ml vial, a solution of glucose was added (0.1 ml; 10 mg g^{-1} soil), vial was closed hermetically and time was recorded. The vial was incubated (3-5 h, 22°C) and an air sample was taken and injected into a gas chromatograph (KristaLLyuks 4000M, thermal conductivity) for measuring CO_2 production. Carbon of the microbial biomass was calculated according to: C_{mic} (µg C g^{-1}) = SIR (µl CO_2 g^{-1} h^{-1}) × 40.04 + 0.37 (Anderson, Domsch, 1978). Basal (microbial) respiration (BR) was measured as described for SIR, although glucose solution was substituted by distilled water (0.1 ml g^{-1} soil) and incubated (24 h, 22°C). The BR rate was expressed in µg CO_2-C g^{-1} soil h^{-1}. Specific respiration of the microbial biomass (the microbial metabolic quotient, qCO_2) was estimated as the ratio of BR / C_{mic} = qCO_2 (µg CO_2-C mg^{-1} C_{mic} h^{-1}). The ratio between Cmic and soil organic carbon content (C_{org}) was calculated and expressed in %.

Prior to the estimation of SIR and BR all soil samples (0.3-0.5 kg) were sieved (mesh, 2 mm), moistened up to 50-60% water holding capacity and pre-incubated in aerated bags at 22°C for 7 days to avoid excess CO_2 production after mixing, sieving and moistening of soil sample (Ananyeva et al., 2008; Creamer et al., 2014).

Chemical Analyses

In the collected soil samples C_{org} content was measured by the dichromate oxidation, the ammonium nitrogen – by calorimetry with Nessler reagent, nitrate nitrogen – by Grandval-Lyazhu technique, soluble potassium – by flame photometry and phosphorus – by Kirsanov. The acidity of soil solution (soil : KCl solution = 1 : 2.5) was determined using potentiometer.

Statistical Analyses

The C_{mic} and BR measurements were performed in three replicas and results were expressed per dry weight of soil (105°C, 8 h) as mean ± standard deviation. Distribution of the experimental data was analyzed using Statistica 10.0 package (box-and-whisker diagram or box plot), which illustrated the mean, standard deviation, minimum and maximum values. Analysis of variance (ANOVA, post-hoc analysis by Tukey test) was carried out using R software (2.14.1, http://www.r-project.org/). The experimental data was processed by correlation analysis (Pearson coefficient, the relationship between soil chemical and microbiological parameters). A principal component analysis and experimental data ordination were performed in PCord 4.27 program.

RESULTS AND DISCUSSION

Average C_{org} content in soils of undisturbed or lightly disturbed ecosystems (steppe, pasture) was almost 50% higher, than in disturbed ones and the lowest C_{org} (1.4%) was obtained in the residential zone (Table 1). Soil pH values increased in a row from the undisturbed ecosystems to disturbed ones. The ammonium nitrogen content was in average higher in soil of the virgin and mowing steppes, pasture and recreational zone compared to the black fallow, arable and residential areas. The highest content of nitrate nitrogen was obtained in the virgin steppe soil and the lowest was in the black fallow (80 and 4.6 mg kg^{-1}, respectively). The other nutrients (P, K) contents were respectively three-four times higher in the soils of disturbed (urban) ecosystems compared to undisturbed ones.

The C_{mic} content in typical chernozems (0-10 cm) ranged from 84 μg C g^{-1} (industrial zone) to 1954 μg C g^{-1} (virgin steppe) (Fig. 2 A). The highest average values of C_{mic} were found in chernozems of virgin and mowing steppes, pasture (1254 ± 413 and 1337 ± 413, 1088 ± 392 μg C g^{-1}, respectively), and the lowest were in the black fallow and arable plots (377 ± 136 and 340 ± 53 μg C g^{-1}, respectively). Moreover, C_{mic} values in the soil of industrial zone were two times less compared to recreational one (in average 308 and 630 μg C g^{-1}, respectively). In other words, the tillage (plowing) led to essential decrease in soil microbial biomass (almost up to 3-4 times). Microbial carbon content in chernozems of lightly disturbed ecosystems (steppe, pasture) was significantly higher than in anthropogenic disturbed ones, including urban ($p \leq 0.05$, "a" group, Tukey test). It should be also noted that the C_{mic} in urban soil was very variable (coefficient of variance, CV = 41-72%) compared to other ecosystems (CV = 31-37%).

The rate of basal (microbial) respiration in studied chernozems ranged from 0.20 to 1.57 μg CO_2-C g^{-1} h^{-1} (industrial zone) (Fig. 2 B). The highest average BR value was observed in the virgin steppe chernozems (1.24 μg CO_2-C g^{-1} h^{-1}),

Table 1. Chemical properties of typical chernozems (Kursk region) in different ecosystems and functional areas of Kursk city (upper 10 cm mineral layer, the average for mentioned sites ± standard deviation)

Ecosystem / Zone (n) [a]	Management history (yrs)	C_{org}, %	pH_{KCl}	NH_4^+	NO_3^-	P_2O_5	K
				mg kg^{-1}			
Virgin steppe (2)	74	3.0±0.2	6.8±1.0	13.0±5.4	79.9±4.7	101±2	142±22
Mowing steppe (2)		2.4±0.5	6.3±0.3	18.0±3.3	19.6±4.1	98±6	126±11
Pasture (4)	79	3.1±0.3	6.4±0.5	10.7±4.6	10.3±2.9	107±14	97±22
Black fallow (3)	60	2.0±0.2	6.1±0.5	8.9±3.3	4.6±0.9	130±46	97±29
Arable (2)	60	2.1±0.0	6.2±0.6	9.3±0.8	11.8±9.3	168±12	130±13
R, park (1)	40	2.1±0.0	7.2±0.0	17.7±1.8	10.5±2.1	791±95	582±58
Residential (3)	50	1.4±0.6	7.6±0.6	8.7±3.3	16.5±14	203±52	401±323
I, factory (1)	68	2.0±0.0	7.2±0.0	12.9±0.0	10.8±0.0	229±0	212±0

[a] R, recreational; I, industrial; n, number of soil sampling sites

that was significantly ($p \leq 0.05$) higher compared with other ecosystems. The lowest average BR was reported for the black fallow, arable land and residential zone of the city (0.47, 0.54 and 0.56 µg CO_2-C g^{-1} h^{-1}, respectively). The CV of this parameter in urban soils was significantly higher (49-75%) compared to other ecosystems (16-28%). Interestingly, no significant difference was found between the highest BR in urban soils of recreational and industrial zones (1.19 and 1.57 µg CO_2-C g^{-1} h^{-1}, respectively) and chernozems in the virgin steppe (1.11 µg CO_2-C g^{-1} h^{-1}). This evidences on the high potential of disturbed soils including urban ones to emit carbon dioxide flux into the atmosphere.

Average qCO_2 values in chernozems varied from 0.50 µg CO_2-C mg^{-1} C_{mic} h^{-1} (residential zone) to 6.83 µg CO_2-C mg^{-1} C_{mic} h^{-1} (industrial zone) (Fig. 3). The increased of qCO_2 was reported in a raw from virgin steppe to industrial zone of the city, following an increase in anthropogenic pressure, however, statistically significant difference was not found.

The highest C_{mic} / C_{org} ratio in chernozems of studied ecosystems was obtained for the virgin steppe (3.7%), whereas the index for black fallow, arable land and industrial zones were in average two times less (1.5, 1.6 and 1.2%, respectively) (Fig. 4). It should be mentioned, that the soil C_{mic} / C_{org} ratio in residential zone was considerable (average 2.7), but the variability of this parameter was also quite high (CV = 66%).

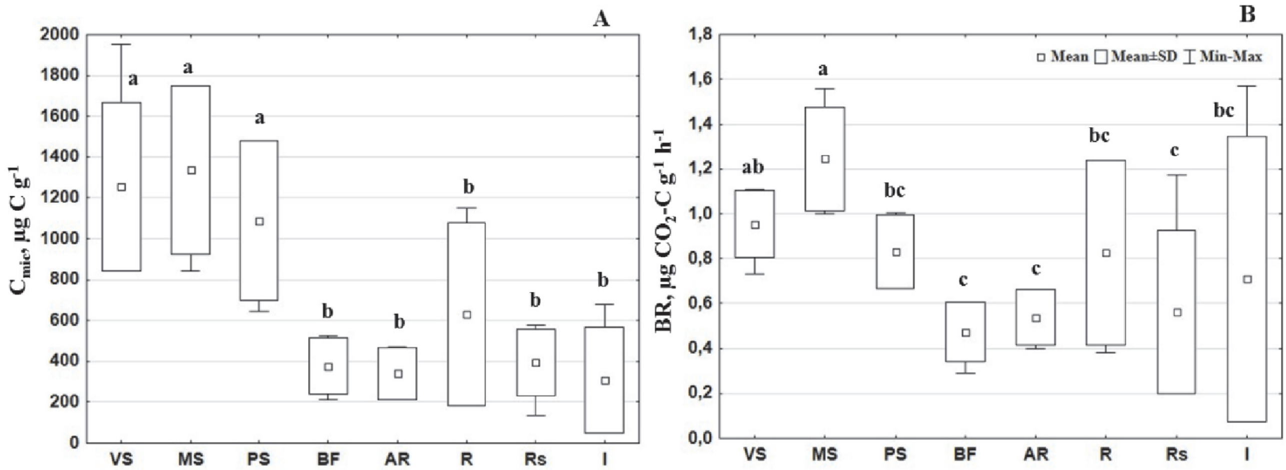


Figure 2. Distribution of soil microbial biomass carbon (A) and basal respiration (B) in typical chernozems (0-10 cm) of different ecosystems and functional zones (VS, virgin steppe; MS, mowing steppe; PS, pasture; BF, black fallow; AR, arable; R, recreational; Rs, residential; I, Industrial, n=5, 4, 3, 4, 3, 3, 5, 4, respectively; the different letters indicate the significant difference, p≤0.05)

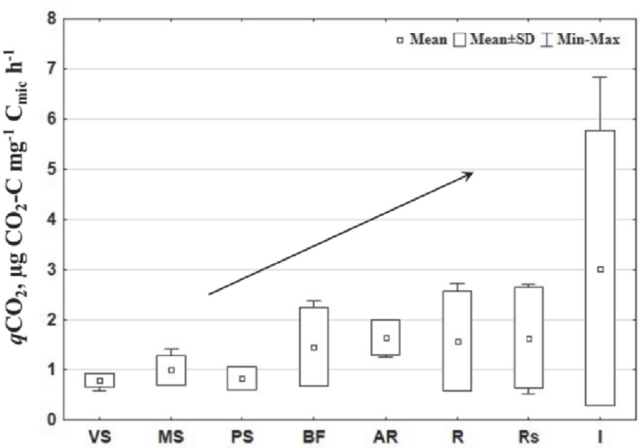


Figure 3. Distribution of microbial metabolic quotient (qCO_2) in typical chernozems (0-10 cm) of different ecosystems and functional zones (indication as in Figure 2; no significant difference, p≤0.05)

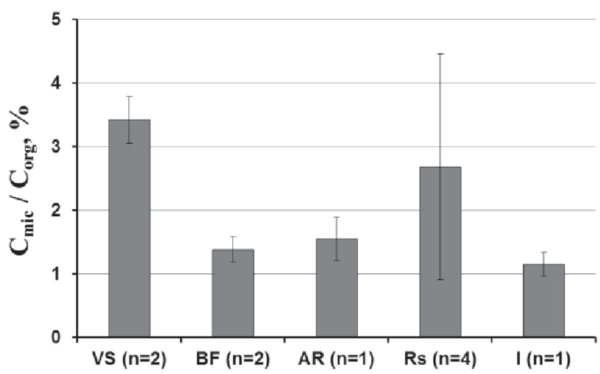


Figure 4. The ratio of soil microbial biomass carbon (Cmic) to soil organic carbon (Corg) in typical chernozems (0-10 cm) of different ecosystems and functional zones (n, number of soil sampling, indication as in Figure 2)

We found a significant (almost two times) decrease of microbial biomass and its portion in the soil organic carbon in typical chernozems of disturbed ecosystems (black fallow, arable and urban areas) compared to undisturbed ones. Increase of specific microbial respiration (qCO_2) in soils of disturbed ecosystems was also shown, that might indicate high energy losses of soil microorganisms and shows stress of their physiological status. Alteration in functioning of microbial community and soil in general in disturbed ecosystems likely occur due to continuous anthropogenic influence on soil (vegetation change, removal of crop residuals, pollution and mixing of soil horizons). One of the results is shift of gas exchange (CO_2 emissions) under anthropogenic pressure.

We found decrease in C_{mic} and BR over the profile of virgin steppe chernozems – subsoil values (for the layers 10-50, 50-100 and 100-150 cm) were in average of 3, 9 and 33 times, and 2, 4, 23 times lower respectively. In the urban industrial zone C_{mic} and BR values in subsoil layers were lower by an average of 5, 5, 8 and 5, 6, 6 times for 10-50, 50-100, 100-150 cm, respectively, compared with topsoil 0-10 cm layer. This profile distribution of measured microbiological features showed a significant role of the topsoil mineral layer with the contribution of 56-79 and 41-77% to the total C_{mic} and BR, respectively (Fig. 5). The contribution of the bottom layer (100-150 cm) to the total profile pool C_{mic} and BR in natural ecosystems was not significant (1-3 and 3-7% respectively), although in urban soils it was considerably higher (10-12 and 8-13%, respectively).

We described the increase of qCO_2 values down the chernozems' profile. In the subsoil layers of virgin steppe the qCO_2 values were higher compared with the topsoil ones (in average by 2, 3, 3 times for 10-50, 50-100 and 100-150 cm, respectively). Increase in qCO_2 along the profile of arable soils was less noticeable (in average 2 times). No significant changes in qCO_2 over the urban soil profile were found. The C_{mic} / C_{org} ratio mainly decreased down the chernozems profile (from 1.3 to 41 times).

The total C_{mic} stock in 1.5 m layer of chernozems under studied ecosystems ranged from 134 μg C g^{-1} (industrial zone) to 2618 μg C g^{-1} (mowing steppe) (Fig. 6). The highest

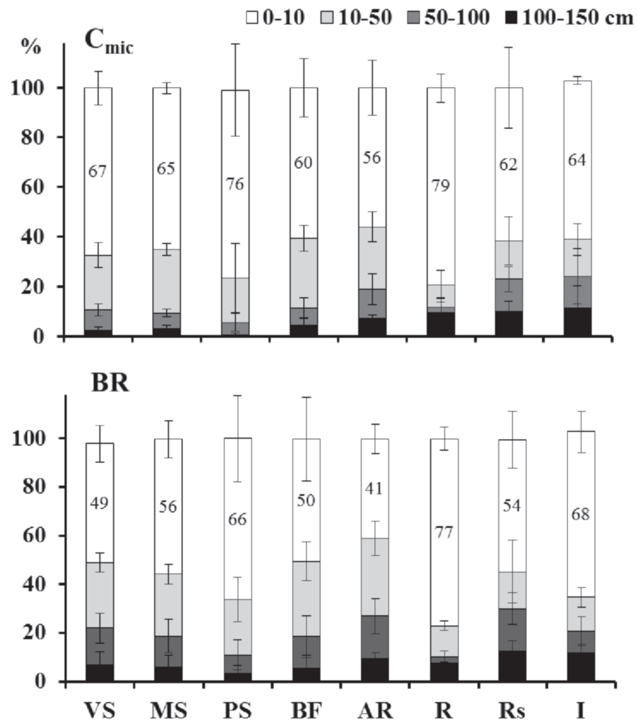


Figure 5. Contribution of variuos layers of typical chernozems to total profile stocks of soil microbial biomass carbon (C_{mic}) and basal respiration (BR) for different ecosystems and functional zones (average for mentioned soil sampling sites indication as in Figure 2)

average C_{mic} was obtained in undisturbed ecosystems (virgin and mowing steppes) - 1852 ± 511 and 2049 ± 620 μg C g^{-1}, respectively. In the urban the Cmic stocks in urban soils of recreational zone reached 783 ± 526 μg C g^{-1}, which was almost two times higher than in the industrial area. As for the black fallow and arable land Cmic stocks were significantly ($p \leq 0.05$) lower (607 ± 115 and 598 ± 139 μg C g^{-1}, respectively) than for the virgin steppe and similar to the urban soils. Total microbial production of CO_2 from 1.5 m chernozems' profile ranged from 0.35 μg CO_2-C g^{-1} h^{-1} (industrial area) to 2.74 μg CO_2-C g^{-1} h^{-1} (mowing steppe), although the difference between different ecosystems was not always significant. However, it was noted that the BR values in urban residential and industrial zones were similar with soil microbial respiration in natural ecosystems (virgin and mowing steppes).

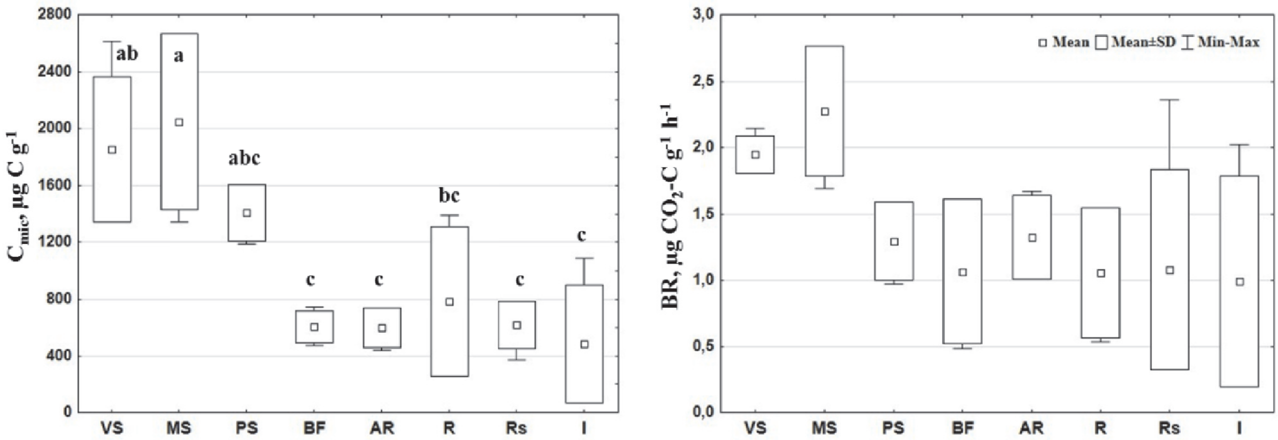
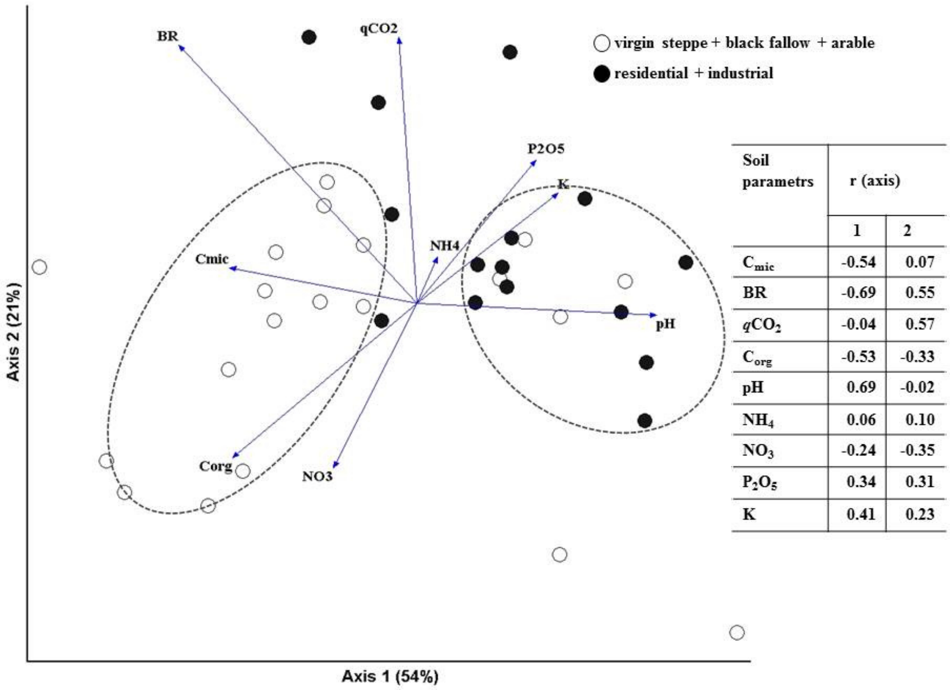


Figure 6. Distribution of soil microbial biomass carbon pool (Cmic) and basal respiration (BR) in typical chernozems profile (1.5 m) for different ecosystems and functional zones (indication as in Figure 2)



Soil parametrs	r (axis)	
	1	2
C_{mic}	-0.54	0.07
BR	-0.69	0.55
qCO_2	-0.04	0.57
C_{org}	-0.53	-0.33
pH	0.69	-0.02
NH_4	0.06	0.10
NO_3	-0.24	-0.35
P_2O_5	0.34	0.31
K	0.41	0.23

Figure 7. Principal component analysis for chemical (C_{org}, pH, NH_4, NO_3, P_2O_5) and microbiological (C_{mic}, BR, qCO_2) parameters of various chernozems layers (different ecosystems and functional zones; n=35))

Thus, major microbiological indicators (C_{mic}, BR and C_{mic} / C_{org}) decreased down the chernozem's profile, whereas the specific microbial respiration (qCO_2) mainly increased. It was identified that the microbial biomass and respiration activity (41-79%) was concentrated in the top 10 cm mineral layer of chernozems. The highest C_{mic} stocks in chernozems' profile was found in undisturbed ecosystems, which were 2-4 times larger than in the arable and urban soils. It was shown that total microbial respiration of the soil profile in urban ecosystems (residential and industrial zones) was comparable to one of undisturbed ecosystems, which highlights considerable contribution of disturbed ecosystems to the emission of CO_2 this area.

Significant positive correlation between C_{mic} and BR, C_{org}, NH_4^+, NO_3^- was found (r = 0.60, 0.56, 0.59, 0.88, respectively) (Table 2). The microbial respiration significantly and positively correlated with C_{org}, NH_4^+, NO_3^-, P_2O_5, K (r=0.39, 0.55, 0.36, 0.60, 0.36, respectively), however the correlation coefficient with pH was negative (r=-0.41). The

Table 2. Correlation coefficient (Pearson) between microbiological (C_{mic}, BR) and chemical (C_{org}, pH_{KCl}, NH_4+, NO_3-, P_2O_5, K) parameters, and studied soil layers (0-10, 10-50, 50-100 and 100-150 cm; virgin steppe, black fallow, arable and, residential, industrial zones, n=35; bold italic is significant, p <0.05)

Parameter	C_{mic}	BR	C_{org}	pH_{KCl}	NH_4^+	NO_3^-	P_2O_5	K	Layer
C_{mic}	1.00								
BR	0.60	1.00							
C_{org}	0.56	0.39	1.00						
pH_{KCl}	-0.26	-0.41	-0.29	1.00					
NH_4^+	0.59	0.55	0.50	-0.24	1.00				
NO_3^-	0.88	0.36	0.61	-0.03	0.57	1.00			
P_2O_5	0.16	0.60	0.00	-0.10	0.32	0.09	1.00		
K	0.17	0.36	0.04	0.16	0.09	0.21	0.63	1.00	
Layer	-0.61	-0.69	-0.42	0.25	-0.63	-0.44	-0.58	-0.38	1.00

negative correlation between soil depth (layers) and microbiological and chemical parameters was shown, indicating decrease of those parameters with depth.

The principal component analysis (PCA) (ordination of experimental data) showed that the contribution of parameters in variance of Axis 1 and 2 amounted to 54 and 21%, respectively. The Axis 1 is most closely describes the change C_{mic}, BR, C_{org}, pH, P_2O_5 and K (r= -0.54, -0.69, -0.53, 0.69, 0.34 and 0.41, respectively) and Axis 2 is gradient of BR, qCO_2 and NO_3^- (r=0.55, 0.57 and -0.35, respectively). As the result of PCA, soils of natural and slightly disturbed ecosystems located mainly in the left part of the resulting plot, whereas urban soils were rather in the right part. This evidences of the lower C_{org} and C_{mic} stocks and higher nutrient (P and K) contents and pH in the latter ones.

CONCLUSION

We found considerable changes of soil microbial community in typical chernozems (Kursk region, Russia) along a gradient from undisturbed areas (virgin steppe) to disturbed agricultural (pasture, arable land, black fallow) and urban ecosystems. The changes included decrease of microbial biomass, its respiration activity and portion in soil organic carbon and the increase of specific respiration of the microorganism, indicating a stressful condition of soil microbial component. The significant reduction of microbial biomass stocks and less significant decrease in respiration activity over the soil profile along the gradient of ecosystems were found, that might indicate the excess CO_2 fluxes by soils of disturbed (urban) ecosystems.

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