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Carbon dioxide emission and soil microbial respiration activity of Chernozems under anthropogenic transformation of terrestrial ecosystems

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Abstract

The total soil CO_2 emission (EM) and portion of microbial respiration were measured (*in situ*; May, June, July 2015) in Chernozems typical of virgin steppe, oak forest, bare fallow and urban ecosystems (Kursk region, Russia). In soil samples (upper 10 cm layer), the soil microbial biomass carbon (C_{mic}), basal respiration (BR) and fungi-to-bacteria ratio were determined and the specific microbial respiration (BR / C_{mic} = qCO_2) was calculated. The EM was varied from 2.0 (fallow) to 23.2 (steppe) g CO_2 m^{-2} d^{-1}. The portion of microbial respiration in EM was reached in average 83, 51 and 60% for forest, steppe and urban, respectively. The soil C_{mic} and BR were decreased along a gradient of ecosystems transformation (by 4 and 2 times less, respectively), while the qCO_2 of urban soil was higher (in average by 42%) compared to steppe, forest and fallow. In urban soil the C_{mic} portion in soil C_{org} and C_{fungi}-to-C_{org} ratio were by 2.6 and 2.4 times less than those for steppe. The relationship between microbial respiration and BR values in Chernozems of various ecosystems was significant (R^2 = 0.57).

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Introduction

The circulation of carbon dioxide between soil and atmosphere provided by two main processes: plants photosynthesis and respiration of soil. Soil respiration (soil CO_2 emission), in turn, is provided by respiration of soil microorganisms and plant roots. It is believed that about 70% of total soil CO_2 emission was derived by soil microbial respiration (Zavarzin and Kudeyarov, 2006). According to many researchers the portion of respiration of soil microorganisms and plant roots in total soil CO_2 emission depends on hydrothermal conditions (Ryan and Law, 2005; Martin and Bolstad, 2005), photosynthesis activity (Kuzyakov and Gavrichkova, 2010) and soil organic matter composition (Metting, 1993), but it mainly remains still unclear (Hanson et. al, 2000; Kuzyakov and Larionova, 2005). In addition, the separation of these soil CO_2 fluxes in

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natural condition is difficult to defined (Kuzyakov and Larionova, 2005) and time- and labor-consuming (Yevdokimov et al., 2010). Moreover, the determination of these two portions in total soil CO_2 emission might be very important for studying carbon cycle and modeling carbon change in terrestrial ecosystems (Wie et al., 2010).

Chernozems is an important natural resource of Russia, the distribution of Chernozems area is about 6% of the country (National Soil Atlas..., 2011). Plowing Chernozems is currently reached up 50-60%, which made almost 2 / 3 of all agricultural production in Russia. For the last 150 years the carbon content of Chernozems was decreased by 20-30% (Mikhailova and Post, 2006). Besides, the urban area in Chernozems zone increases by 10% for 2010-2015 yrs (Statistical Pocketbook, 2015).

Anthropogenic transformation of terrestrial ecosystems (agricultural use, urbanization) leads to the changes of soil microbial community functioning. It was shown that in arable Chernozems the soil microbial biomass content was dramatically decreased by almost 3-4 times (Senicovscaia, 2012; Ivashchenko et al., 2015). In Chernozems of Voronezh region (Russia) the soil microbial biomass carbon and its portion in total soil organic carbon were decreased by 2 times and by 40%, respectively, compared to natural analogue (Blagodatskii et al., 2008).

Our study was focused on: i) the measurements of total CO_2 emission from Chernozems and portion of soil microbial respiration *in situ* in natural and anthropogenically transformed ecosystems; ii) the parameters estimation of soil microbial community functioning (soil microbial biomass carbon content, basal respiration, specific respiration of microbial biomass and fungi-to-bacteria ratio); iii) the assessment of relationship between soil microbial respiration measured *in situ* and laboratory conditions.

Material and Methods

Location

The Chernozems typical (Kursk region, Russia: 51°33′50″-51°39′40″ N / 36°04′58″-36°07′41″ E) of natural (virgin steppe, oak forest) and anthropogenically transformed (bare fallow, urban) ecosystems was studied. The steppe, forest and fallow are located in the Central-Chernozemic State Biosphere Nature Reserve area (12 km from Kursk city), urban ecosystem is an industrial zone of Kursk city (near the factory "Kurskrezinotechnika").

Field measurement

The total soil CO_2 emission was measured (closed-chamber, LI-820) in five spatially distant points on the plot (20 × 20 m) of each ecosystem (ground vegetation cut) and expressed as g CO_2 m^{-2} d^{-1} (20 totally). In each point of the ecosystem the soil temperature and soil moisture were recorded at 10 cm depth. The measurements were carried out in early May, June and July, 2015 yr. Soil samples were taken from 10-cm layer for chemical and microbiological (60 totally) analyzes.

Soil microbial respiration *in situ* was determined by substrate induced respiration method (Larionova et al., 2006; Yevdokimov et al., 2010). Into soil steppe, forest and urban the four "collar-base" were cut in 10-cm depth on the distance 1-2 m from points for total CO_2 emission measurement (Figure 1). In two "collar-base" it was unsieved soil (with roots), in the other two it was sieved soil (mesh 3 mm, roots excluded). The measurement of soil CO_2 from the surface of four "collar-base" started not earlier than in half an hour. The water or glucose solution was added (slow penetration) into unsieved and sieved soils of "collar-base" then. In preliminary experiments it was found that the volume (water or glucose solution) provided slow penetration of liquid through 10 cm soil layer was equaled 0.6, 0.9 and 1.0 L for forest, steppe and urban, respectively. The glucose concentration provided the highest initial soil substrate-induced respiration (Anderson and Domsch, 1978) was amounted 5 mg g^{-1} soil in our experiments. The time between addition of liquid to soil and soil CO_2 measuring was 4 h (preliminary experiment).

Soil microbial respiration (MR) of unsieved (with roots) and water-moistened soil was calculated according by following:

$$MR = (GL - W)_{UNS} \Big/ \left(\frac{GL}{W}\right)_S \times \frac{(GL - W)_{UNS}}{(GL - W)_S}$$

where MR is soil microbial respiration, g CO_2 m^{-2} d^{-1}; GL is CO_2 emission from enriched glucose soil, g CO_2 m^{-2} d^{-1}; W is CO_2 emission from soil with water, g CO_2 m^{-2} d^{-1}; UNS is unsieved soil (with roots); S is sieved soil (without roots).

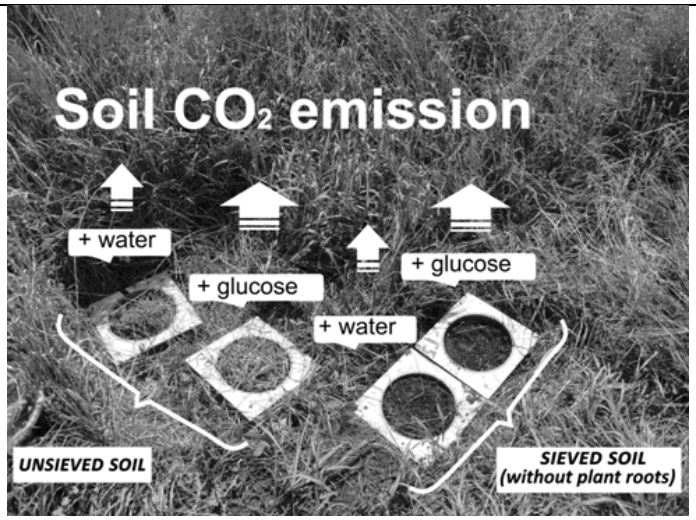


Figure 1. Design of soil microbial respiration measurement in field conditions

The $(GL/W)_S$ ratio was characterized the excess of CO_2 emission from sieved soil added glucose compared to soil added water. The $(GL\text{-}W)_{UNS}$ / $(GL\text{-}W)_S$ ratio was characterized the soil disturbance (sieving). The MR portion in total CO_2 emission from unsieved (with roots) and water-moistened soil was expressed as MR / W_{UNS} ratio (%). Then we calculated the MR portion in total soil CO_2 emission from nearest point without any liquid addition. Soil MR of steppe, forest and urban was measured in two replicates.

Lab measurement

Soil microbial biomass carbon (C_{mic}) was measured by substrate-induced respiration (SIR) method (Anderson and Domsch, 1978; Ananyeva et al., 2011). Briefly, soil subsamples (1 g) were placed into a 15 ml vial, a glucose solution was added dropwise (0.1 ml, 5 mg glucose 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 by syringe and injected into a gas chromatograph (KristaLLyuks 4000M, thermal conductivity detector) for measuring CO_2 production. The soil C_{mic} (μg C g^{-1} soil) was calculated by SIR (μl CO_2 g^{-1} soil h^{-1}) × 40.04+0.37 (Anderson, Domsch, 1978).

Soil basal (microbial) respiration (BR) was measured as described for SIR, instead glucose the water added (0.1 ml g^{-1} soil) and incubated (24 h, 22ºC). The soil BR rate was expressed in μg CO_2-C g^{-1} soil h^{-1}.

Specific respiration of soil microbial biomass (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}).

Fungi and bacteria contribution to total SIR in steppe and urban soils was determined by selective inhibition technique (Lin and Brookes, 1999; Bailey et al., 2002). Streptomycin sulfate (water solution) and cycloheximide (powder) were added separately and both into soil subsamples (1 g) for the highest SIR inhibition. More details see in the papers (Susyan et al., 2005; Ananyeva et al., 2014). The fungi-to-bacteria ratio was calculated.

Prior to the lab measurements 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 plastic bags at 22°C for 7 days to avoid excess soil CO_2 production after these manipulations (Ananyeva et al., 2008; Creamer et al., 2014).

Statistical analysis

The measurements were performed in three replicates for SIR, BR and fungi-to-bacteria ratio. The results were calculated for dry soil (105°C, 8 h) and expressed as mean ± standard deviation (Excel). Significance of the difference in total soil CO_2 emission, hydrothermal and microbiological soil parameters between ecosystems was tested by one-factor analysis of variance (ANOVA) and Tukey's multiple comparison test. Statistic tests were chosen based on the preliminary analysis: normality distribution of experimental data was checked by Shapiro-Wilk test, variance homogeneity was checked by Levene's test. Relationship between MR and total soil CO_2 emission, microbiological and hydrothermal soil parameters was analyzed by Pearson's correlation coefficient. Relationship between MR and BR was analyzed nonlinear regression. All experimental data was statistically analyzed and visualized (box-plot) using Statistica 10.0 software. A principal component analysis and ordination of experimental data was carried out by PCord 4.27.

Results and Discussion

The soil organic carbon content (C_{org}) of natural ecosystems was by approximately 2 times higher than that of anthropogenically transformed, and the pH value of steppe and forest was by about one unit less than bare fallow and urban (Table 1). The CO_2 emission from Chernozems in various months was ranged from 2.0 (fallow) to 23.2 (steppe) g CO_2 m^{-2} d^{-1}, these values differ by almost an order of magnitude (Table 2). In May the highest average soil CO_2 emission was found in steppe, forest and urban ecosystems, and the lowest value was in fallow, wherein the soil temperature in forest was significantly low and the soil moisture was high compared to other studied ecosystems. In June a significantly high soil CO_2 emission was found in steppe, and the low one was in fallow, the soil of which was significantly high temperature and low moisture. In the warmest month (July) the soil CO_2 emission from fallow was also the lowest (high soil temperature and low soil moisture). During the observed period (May-July) the high soil CO_2 emission was found in steppe, and the low was in fallow (in average 20.3 and 3.6 g CO_2 m^{-2} d^{-1}, respectively), the difference between these values was almost 6 times (Figure 2).

Table 1. Ecosystem, history treatment (HT), soil organic carbon content and soil acidity (C_{org}, pH, respectively, mean, n = 15) of Chernozems typical (Kursk region, Russia)

Ecosystem		HT, yrs	C_{org}, %	pH_W
Natural	Virgin steppe	75	4.9	5.8
	Oak forest	80	4.8	6.2
Anthropogenically transformed	Bare fallow	60	2.0	7.0
	Urban	70	2.3	7.4

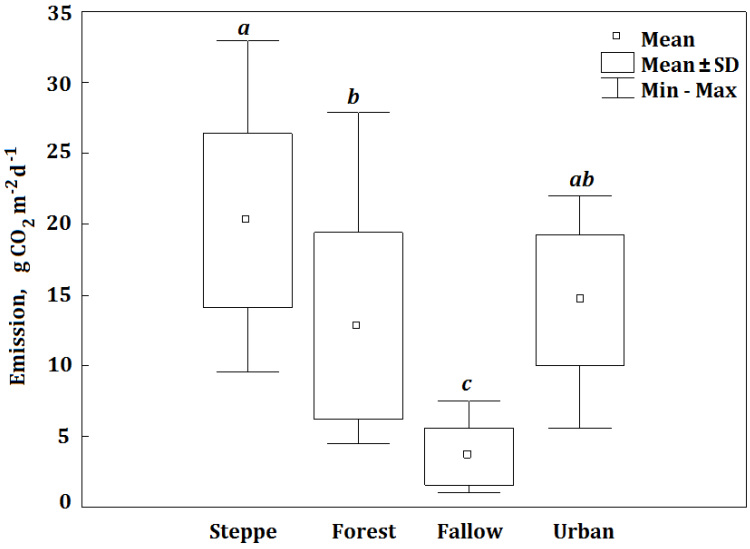


Figure 2. Distribution (box-plot) of CO_2 emission in Chernozems of different ecosystems (n = 15, May-June-July, 2015). The values with different letters were significantly (p ≤0.05) different each other

The soil C_{mic} and BR of various ecosystems for May-June-July were ranged from 284 (urban) to 1710 (steppe) μg C g^{-1} soil and from 0.28 (fallow) to 1.64 (steppe) μg CO_2-C g^{-1} soil h^{-1}, respectively (Table 3). The soil C_{mic} and BR values of steppe and forest were mainly significantly higher than fallow and urban for each studied month. The qCO_2 value in urban soil was on the contrary significantly high in May and June, however in July, the difference of this index for studied ecosystems was not found. For the observed period, the soil C_{mic} content and BR rate of natural ecosystems (steppe, forest) were significantly higher than the anthropogenically transformed (fallow, urban), however the qCO_2 of urban soil was significantly higher compared to other ecosystems (Figure 3). Therefore, there is a base to consider the “deterioration” of soil microbial community functioning in anthropogenically transformed ecosystems compared to natural analogues. In our experiments the highest SIR inhibition by antibiotics both was achieved 41-51% (Table 4). The fungi portion in urban and steppe soils was almost the same (82-85%), wherein the fungi / bacteria ratios were also approximately equal (3.4 and 3.8, respectively). However, the C_{mic} / C_{org} (as an indicator of soil organic matter "quality") and C_{fungi} / C_{org} ratios for urban soil were 2.6 and 2.4 times less than those for steppe. It might be indicated the essential "deterioration" of soil microbial community functioning under anthropogenic impact.

Table 2. Soil CO_2 emission (g CO_2 m^{-2} d^{-1}), soil temperature (T) and soil water content (W) of Chernozems typical in different ecosystems. The values with different letters were significantly ($p \leq 0.05$) differ for each parameter separately

Ecosystem	May			June			July		
(n = 5)	Emission	T,°C	W, %	Emission	T,°C	W, %	Emission	T,°C	W, %
Steppe	23.2 ± 3.4 *a*	11 ± 1 *b*	27 ± 1 *b*	17.6 ± 6.7 *a*	17 ± 1 *b*	13 ± 4 *ab*	20.0 ± 7.5 *a*	18 ± 0 *c*	19 ± 5 *ba*
Forest	12.1 ± 9.3 *bc*	9 ± 0 *c*	36 ± 2 *a*	9.6 ± 5.2 *ab*	19 ± 2 *b*	21 ± 3 *a*	16.7 ± 2.4 *a*	19 ± 0 *c*	30 ± 1 *a*
Fallow	3.3 ± 1.1 *c*	15 ± 2 *a*	20 ± 4 *b*	2.0 ± 0.8 *b*	24 ± 2 *a*	12 ± 4 *b*	5.6 ± 2.0 *b*	33 ± 1 *a*	18 ± 2 *b*
Urban	15.7 ± 2.6 *ab*	13 ± 2 *ab*	27 ± 7 *b*	9.6 ± 3.0 *ab*	20 ± 4 *b*	13 ± 7 *ab*	18.7 ± 2.1 *a*	22 ± 3 *b*	24 ± 7 *ab*

Table 3. Temporal changes of soil microbial biomass carbon (C_{mic}, µg C g^{-1} soil), basal respiration (BR, µg CO_2-C g^{-1} soil h^{-1}) and specific respiration of microbial biomass (qCO_2, µg CO_2-C mg^{-1} C_{mic} h^{-1}) of Chernozems (0-10 cm) in different ecosystems (Kursk region, 2015). The values with different letters were significantly ($p \leq 0.05$) differ for each parameter separately

Ecosystem	May			June			July		
(n = 5)	C_{mic}	BR	qCO_2	C_{mic}	BR	qCO_2	C_{mic}	BR	qCO_2
Steppe	1710 ± 370 *a*	1.01 ± 0.17 *a*	0.60 ± 0.10 *b*	1132 ± 167 *a*	1.22 ± 0.39 *a*	1.07 ± 0.23 *ab*	1414 ± 233 *a*	1.64 ± 0.6 *a*	1.14 ± 0.32 *a*
Forest	1660 ± 488 *a*	0.92 ± 0.11 *a*	0.59 ± 0.18 *b*	1369 ± 342 *a*	0.69 ± 0.04 *b*	0.52 ± 0.10 *c*	1356 ± 378 *a*	1.16 ± 0.12 *a*	0.91 ± 0.26 *a*
Fallow	372 ± 130 *b*	0.28 ± 0.10 *b*	0.76 ± 0.21 *b*	310 ± 45 *b*	0.29 ± 0.03 *b*	0.92 ± 0.11 *b*	397 ± 33 *b*	0.41 ± 0.09 *b*	1.05 ± 0.26 *a*
Urban	284 ± 101 *b*	0.48 ± 0.15 *b*	1.72 ± 0.30 *a*	439 ± 158 *b*	0.55 ± 0.19 *b*	1.28 ± 0.26 *a*	351 ± 140 *b*	0.43 ± 0.07 *b*	1.36 ± 0.50 *a*

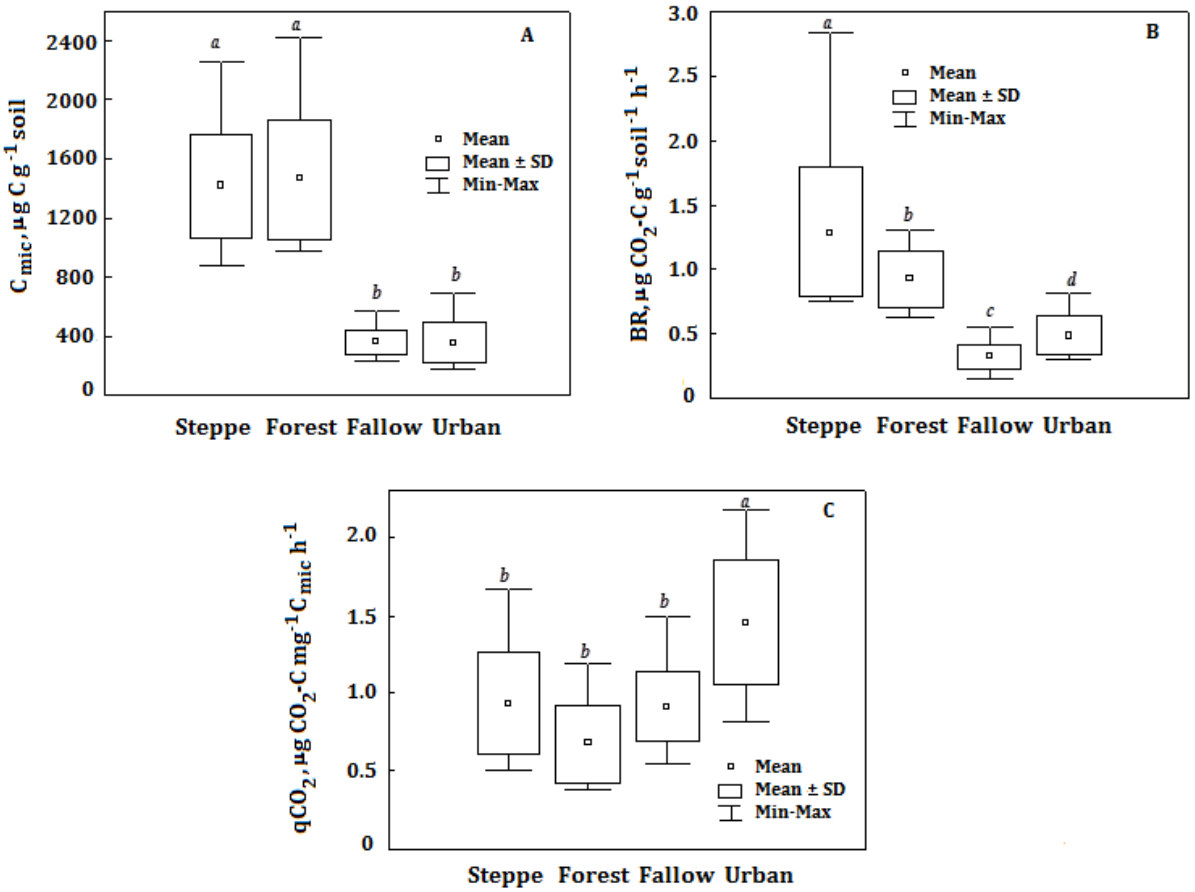


Figure 3. Distribution (box-plot) of soil microbial biomass carbon content (C_{mic}, µg C g^{-1} soil), basal respiration (BR, µg CO_2-C g^{-1} soil h^{-1}) and specific respiration of microbial biomass (qCO_2, µg CO_2-C mg^{-1} C_{mic} h^{-1}) in Chernozems (0-10 cm) of different ecosystems (n = 15, May-June-July, 2015). The values with different letters were significantly ($p \leq 0.05$) different each other

Table 4. Soil organic carbon content (C_{org}), soil microbial biomass carbon (C_{mic}), C_{mic} portion in C_{org}, the highest inhibition of substrate-induced respiration (SIR) by streptomycin and cycloheximide both, fungi / bacteria (F / B) and C_{fungi} / C_{org} ratios in different ecosystems of typical Chernozems

Ecosystem	C_{org}, %	pH_w	C_{mic}, µg C g^{-1} soil	C_{mic} / C_{org}, %	F / B	SIR inhibition, %	C_{fungi} / C_{org}, %
Steppe	5.57	6.24	1606 ± 130	2.9	3.8 ± 1.2	51	2.4 ± 0.43
Urban	1.71	7.97	191 ± 32	1.1	3.4 ± 0.1	41	1.0 ± 0.0

The soil MR of steppe, forest and urban ecosystems for three months of observation was varied from 4.8 (urban) to 17.5 (forest) g CO_2 m^{-2} d^{-1} and amounted in average 6.9, 9.1 and 10.8 g CO_2 m^{-2} d^{-1} for urban, steppe and forest, respectively, however these values were not significantly differ (data not shown). The MR portion in total soil CO_2 emission in May was varied from 27% in urban to 91% in forest (Figure 4). The highest portion of MR was found in forest, it was 76 и 91% for two replicates (points). The highest difference of MR between replicates was in forest and urban. In the first replicate of forest it was a rich undergrowth of shrubs (more roots), and in the second replicate it was rare (less roots). The first replicate of urban industrial zone was covered by rich grasses and had sod cover (less MR), the second replicate was almost without grass cover (more MR). The MR portion in total soil CO_2 emission for the three studied months was the highest in forest and amounted in average 83% (Figure 5). The MR portion in total soil CO_2 emission of steppe and urban was less and amounted in average 51 and 60%, respectively.

Between MR and soil total CO_2 emission (or BR, C_{mic} and soil water content) the positive correlation was found (r = 0.48, 0.51, 0.34, respectively) for studied months. Between MR and soil temperature the correlation was weak (r = 0.06). Between MR (*in situ*) and BR (*lab test*) was revealed the regression relationship with high satisfactory R^2 (Figure 6). So, the relationship might be allowed to predict soil MR (time- and labor-consuming procedure) by soil BR measurement.

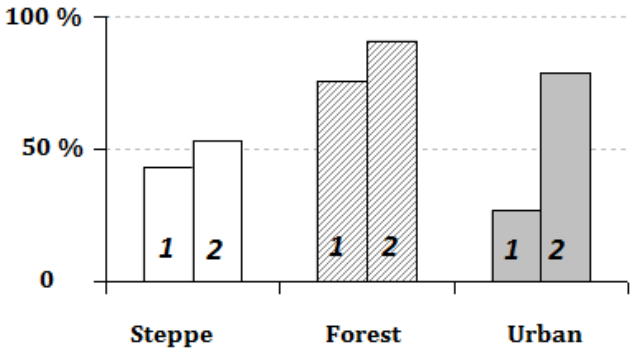


Figure 4. The portion of soil microbial respiration in total soil CO_2 emission of Chernozems in different ecosystems (1 and 2 are numbers of measurement point, May, 2015)

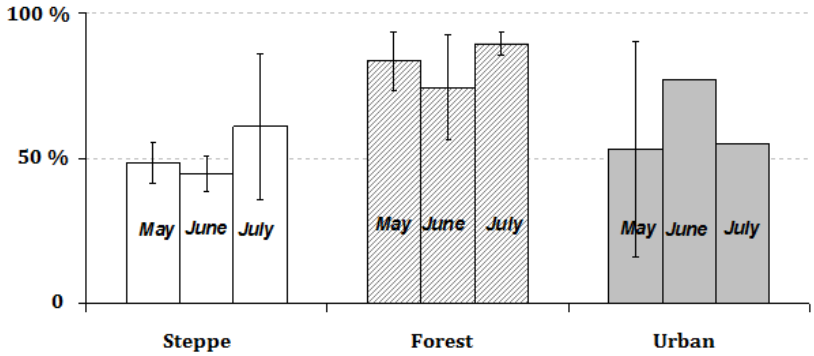


Figure 5. The portion of soil microbial respiration in total soil CO_2 emission of Chernozems in different ecosystems (n = 2, May-June-July, 2015)

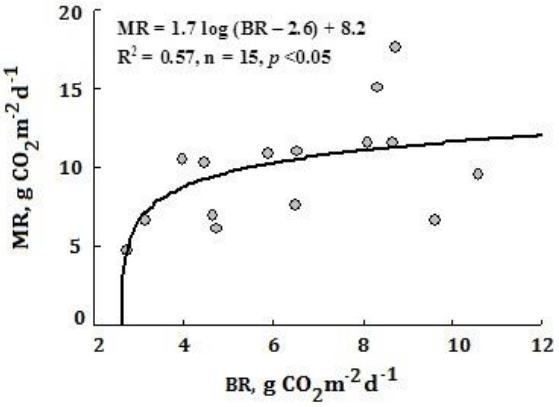
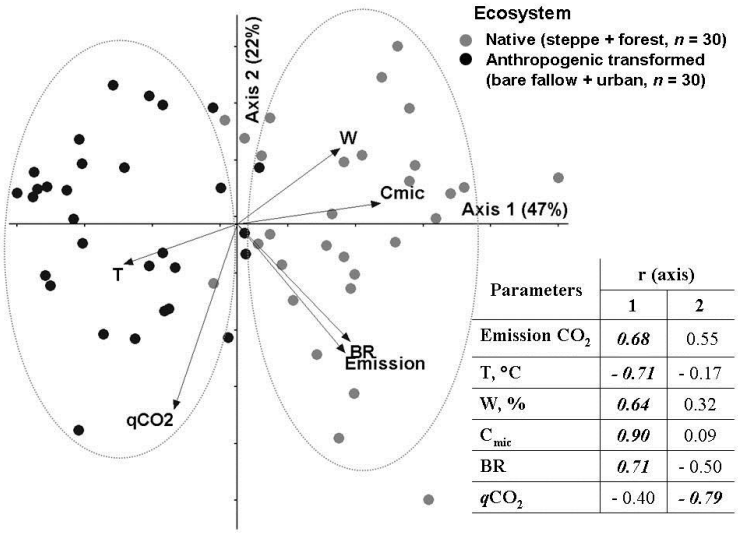


Figure 6. Relationship between microbial (MR) and basal (BR) respirations in Chernozems of steppe, forest and urban ecosystems

A principal component analysis showed that the first and second axes (components) produce 47 and 22% of the experimental data variation, respectively (Figure 7). The highest correlation of the first axis was found with the content of C_{mic}, BR, CO_2 emission, soil temperature and moisture. The highest correlation of the second axis was found with qCO_2 value. The first axis can be considered as the gradient of ecosystem changes. The soils of undisturbed (natural) ecosystems are mainly collected in the right part of figure, and the soils of disturbed (anthropogenically transformed) ecosystems are located in the left part. This might be indicated a more "optimum" functioning of the soil microbial community in Chernozems of natural ecosystems.



Parameters	r (axis)	
	1	2
Emission CO_2	*0.68*	0.55
T, °C	*- 0.71*	- 0.17
W, %	*0.64*	0.32
C_{mic}	*0.90*	0.09
BR	*0.71*	- 0.50
qCO_2	- 0.40	*- 0.79*

Figure 7. PCA ordination of Chernozems CO_2 emission (g CO_2 m^{-2} d^{-1}), hydrothermal (T, W) and microbiological (C_{mic}, µg C g^{-1} soil; BR, µg CO_2-C g^{-1} soil h^{-1}; qCO_2, µg CO_2-C mg^{-1} C_{mic} h^{-1}) parameters of natural and anthropogenically transformed ecosystems (Kursk region)

Conclusion

Along a gradient of Chernozems ecosystems (steppe, forest, fallow, urban) the significant decrease of soil C_{mic}, BR and C_{fungi} / C_{org} ratio was found (by 2-4 times less), while the qCO_2 value increased. It might be illustrated an "deterioration" of soil microbial community functioning under anthropogenic transformation of terrestrial ecosystems. Soil basal respiration (*lab test*) might be characterized soil microbial respiration *in situ*.

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