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Biomass of microbial communities in catenas of virgin and arable chernozems and gray forest soils

K S Dushchanova^{1*}, T E Khomutova¹, P A Ukrainski², F N Lisetski² and A V Borisov¹

¹ Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences, 2, Institutskaya st., Pushchino 142290, Moscow Region, Russian Federation

² Belgorod State National Research University, 85, Pobedy st., Belgorod 308015, Belgorod region, Russian Federation

*Email: kamilla.dushchanova@gmail.com

Abstract. The biomass of microbial communities was studied in catenas of virgin chernozems and dark-gray forest soils within the “Belgor’e” natural reserve and vicinal arable soils developed in the same geomorphological and lithological conditions. Living and active microbial biomass was estimated from the point of the phospholipid content and respiratory responses of cells to glucose amendment respectively. In catena of virgin chernozem of the northern exposure, the biomass of microbial community changed slightly, while in catena of the southern exposure, a decrease of biomass was observed in soil on the lower part of the slope. The opposite trend of accumulation of microbial biomass towards the lower part of the slope was observed in catena of arable chernozems. In virgin gray forest soils, the biomass of microbial community did not differ on the watershed and the lower part, but distinctly decreased in the middle part of the catena. In agro-gray forest soils, the biomass tended to increase towards the lower part of the slope. In both soils plowing has led to a decrease in living and active microbial biomass.

1. Introduction

Microbial community is a key participant in a variety of soil processes, and its biomass is a most widely used environmental indicator of the state of natural and anthropogenically disturbed ecosystems. Living microbial biomass, determined by the content of phospholipids in soils (C-PL), is promising because the phospholipids are an essential component of all living cells being a part of the cell membranes of bacteria, actinomycetes, fungi, and lower plants. After cell death, they are quickly destroyed and are no longer found in cellular storage products. It is shown that the content of phospholipids in microbial cells is constant, which makes it possible to estimate the amount of living microbial biomass in soils, as well as to prove the presence of living microorganisms (active and dormant) in various natural biotopes with extremely low organic carbon content. The phospholipid content can be converted to organic carbon units or cell numbers, which allows us to compare the values for biomass obtained by different methods and estimate the number of cells in different natural ecosystems [1–8]. Another generally accepted indicator of the state of the soil microbial community is the biomass measured by the substrate-induced respiration (C-SIR) method [9, 10].



Currently, many studies are devoted to the analysis of microbial biomass in virgin and arable soils [11–15]. As a rule, these works are carried out on reference sections located mainly on watersheds or flat plots. However, we more often deal with landscapes with expressed relief and slopes. The spatial variation of microbial biomass in these cases is not studied. Another unstudied aspect of spatial variation of soil microbial biomass is related to the influence of slope exposure, illumination, and heat supply.

The aim of the work was to assess spatial variability of microbial biomass in soils of catenas, which include watershed, middle and lower parts of the slope of natural ecosystems developed on soils of different types and anthropogenically disturbed vicinal lands.

2. Objects of studies

The “Les na Vorskle” (forest on the Vorskla River) and the “Yamskaya steppe” sections of the “Belogor’e” nature reserve and currently arable land located in close vicinity to the reserved area were selected for the study, which allows us to consider anthropogenic impact as the only factor explaining possible changes of chemical properties and state of microbial communities in soil (figures 1 and 2).

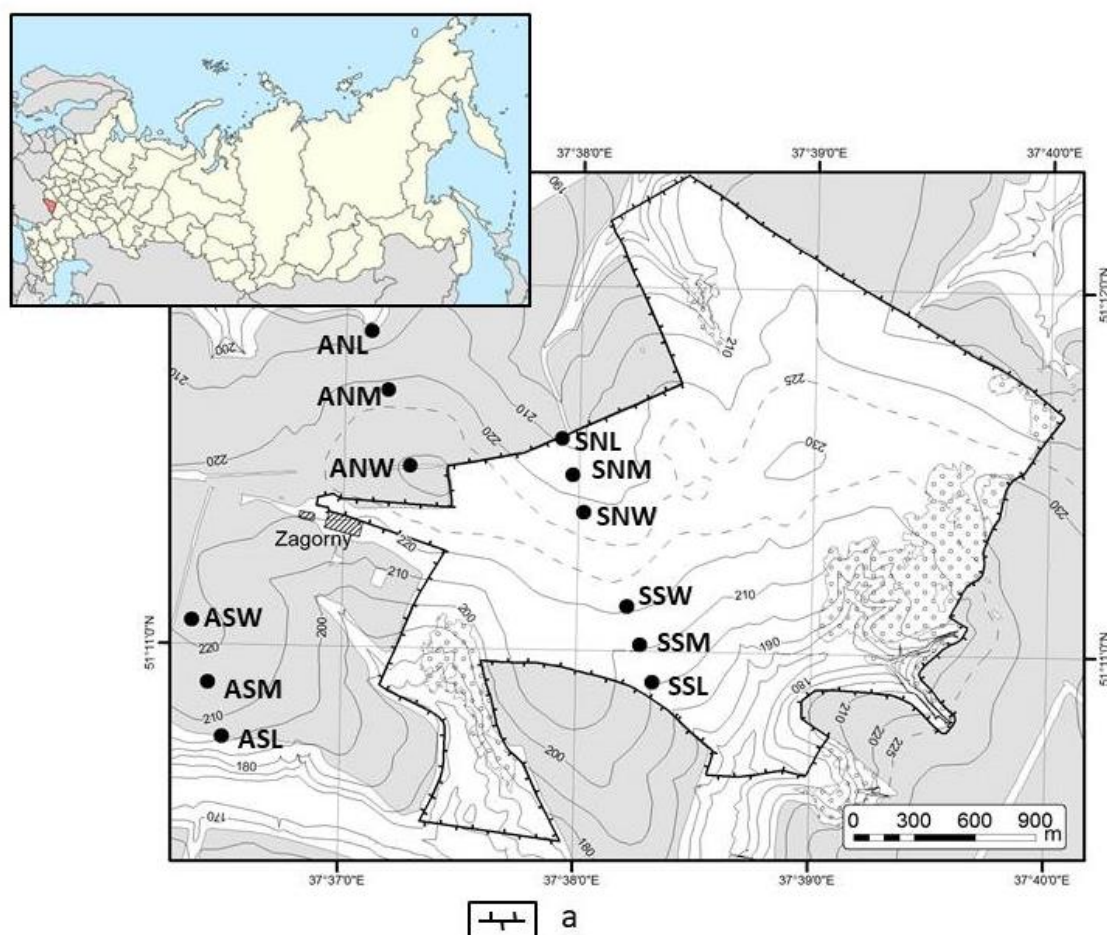


Figure 1. Location of catenas of the northern and southern exposure of the steppe chernozems in the reserved area and vicinal arable lands outside of the reserve: “Steppe North” (SNW – watershed, SNM – middle part, SNL – lower part of slope) and “Steppe South” (SSW – watershed, SSM – middle part, SSL – lower part of slope); “Arable North” (ANW – watershed, ANM – middle part, ANL – lower part of slope) and “Arable South” (ASW – watershed, ASM – the middle, ASL – lower part of slope) and, a – border of the protected area.

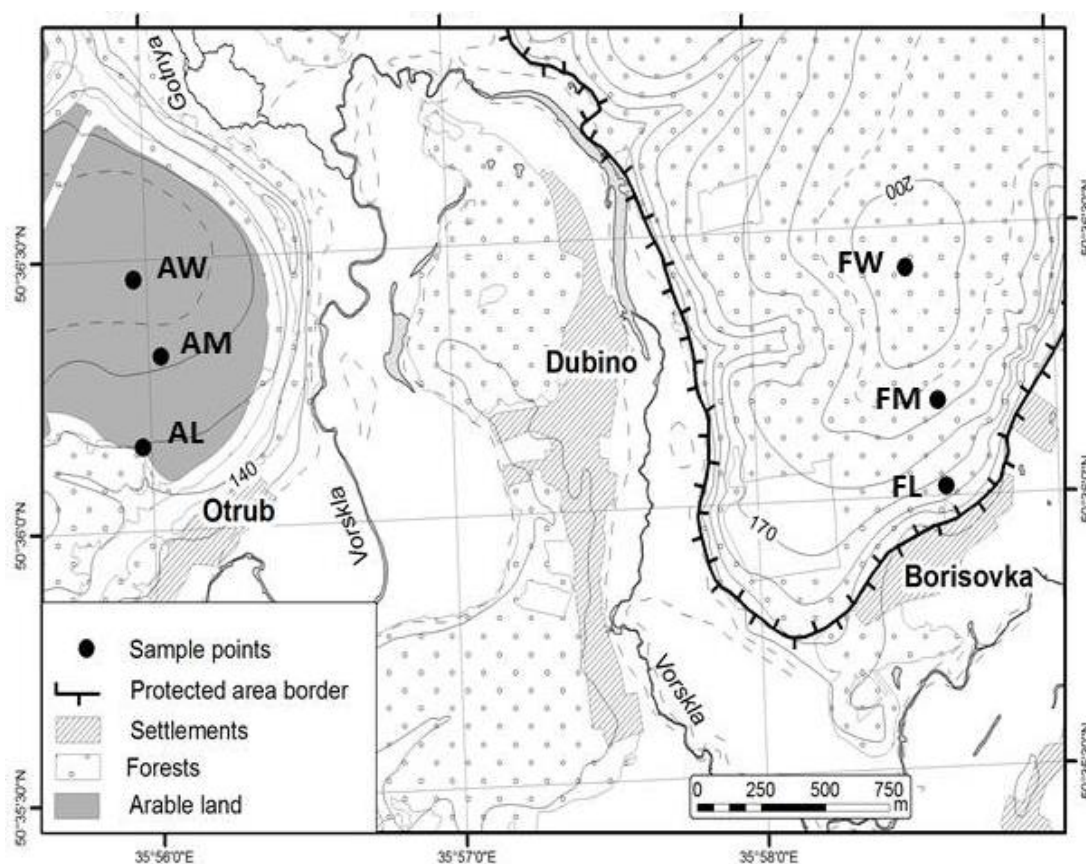


Figure 2. Location of the catenas of southern exposure of gray forest soil in the reserved area and vicinal agro-gray soils outside of the reserve: “Forest” (FW – watershed, FM – middle part, FL – lower part of the slope); “Arable Land” (AW – watershed, AM – middle, AL – lower part of the slope).

The research area is located in the southern part of the Central Russian Upland. The average annual temperature is +6.5 °C, the maximal air temperature is +40 °C. The soil temperature below 0 °C lasts on average 3–4 months a year (December–March). Snow cover lies for 3–4 months; its average thickness is 20–25 cm. The average annual precipitation is 530–589 mm, about half of the precipitation falls in summer. The terrain is typically erosional; slopes are divided by gullies. The territories of the reserve sections belong to the Don and Dnieper basins. The depth of water table varies from 3 to 30 meters. The predominant parent rocks on the territory of natural reserve are carbonated loess-like deposits. Forest ecosystems occupy 65% of natural reserve; steppe and meadows occupy 24%. Plowing of the territories adjacent to the “Les na Vorskle” site was started in the last third of the XIX-early XX, and in the area of the “Yamskaya steppe” site – in the 30s of the XX century. Protected and adjacent arable areas were repeatedly studied previously [16–18].

The “Yamskaya steppe” section has a less pronounced erosion division. The terrain is dominated by convex and concave forms of slopes. From west to east, the “Yamskaya steppe” crosses the watershed of two tributaries of the Oskol River – the Dubenka and Chufichka rivers. The vegetation of the steppe is represented by feather-grass, motley-grass, and meadow-grass associations. In this key area, we studied virgin migration-micellar chernozem on the slopes of the Northern and Southern exposures (“Steppe North” and “Steppe South” catenas). Both catenas were laid on similar slopes (3–4°). Height differences in catenas from the watershed to the middle part and from the middle part to the lower part of the slope were as follows: “Steppe North” – 6 and 8 m; “Steppe South” – 10 and 11 m. Arable analogues were also studied in catenas on similar slopes of the Northern and Southern exposure (“Arable North” and “Arable South” catenas). Height differences in catenas were as follows: “Arable North” – 8

and 20 m; “Arable South” – 5 and 15 m. The lithological conditions and particle size distribution in soils were similar.

The “**Les na Vorskle**” section is inclosed by the Vorskla, Gotnya, and Loknya rivers on three sides. The territory is characterized by an erosive type of terrain: slopes are divided by ravines and gullies. Vegetation is represented by perennial mountain oak forest (*Quercus robur*). The forest understory is composed of the European spindle tree, *Euonymus verrucosa*, English field maple. Within the framework of this work, virgin dark-gray forest soils in the “Forest” catena and agro-gray soils in the “Arable land” catena on the adjacent arable land were studied in the key area “Les na Vorskle”. Both catenas were laid on the slopes of similar steepness of the southern exposure. Height differences in catenas from the watershed to the middle part and from the middle part to the lower part of the slope were as follows: “Forest” – 9 and 28 m; “Arable Land” – 8 and 13 m.

All arable sites were involved in crop rotation and in the year of sampling were under bare fallow. In all cases, two digs 0.5 m deep were laid on the watershed, the middle and the lower part of the slope. Morphology and genetic horizons of soil profiles were described and soil samples were taken representatively for chemical analysis. For microbiological studies soil samples were taken representatively and aseptically from the upper layer 0–10 cm into plastic bags and stored at a temperature corresponding to the time of sampling until the tests were done. Roots and plant residues were removed; samples were averaged and sieved through a 2 mm screen.

3. Methods

Physicochemical characterization of samples of dark-gray forest soils was performed using the facilities of the Institute of Physicochemical and Biological Problems in Soil Science. Physicochemical characterization of migration-micellar chernozem samples was performed earlier [17] in the sections most closely located to the plots studied. Determination of the pH and size-particle distribution was performed by standard methods, organic carbon content (C_{org}) was determined by wet combustion with titrimetric measurement [19].

The “living” microbial biomass (C-PL) was estimated by the content of phospholipids of soil microorganisms. The method is based on the extraction of lipids from a soil sample with a single-phase mixture (chloroform: methanol: phosphate buffer solution 1:2:0.8), determining the content of phospholipids in the color reaction with malachite green after oxidation with potassium persulfate by spectrophotometric method. The results were expressed as $\mu\text{g C/g}$ of soil using the proportion that 190 nmol PL corresponds to 1 mg C [2, 4, 6].

The “active” microbial biomass or SIR-microbial biomass (C-SIR), was determined in soil samples after glucose amendment using gas chromatograph “Crystallux 4000M”, expressed as $\mu\text{g C/g}$ of soil using a conversion coefficient of 40.04 that indicates living non-resting cells [9, 10].

Analyses were performed in three replicates and the data processing was performed using standard methods.

4. Results and discussion

4.1. Physicochemical characterization of 0–10 cm layer of the soils studied

The virgin soils of the key area “Yamskaya steppe” in the “Steppe North” catena had a medium-loamy composition. The silt content was the same on the watershed and the middle part of the slope (15%), and increased 1.2 times on the lower part of the slope. The content of clay on the watershed was similar to that in the middle part (46%), and slightly decreased by 2% in the lower part of the slope. The organic carbon content in soils in all geomorphological positions was 4.7%. The pH values were close to neutral. In the “Steppe South” catena, the soils had a heavy loamy granulometric composition. The content of silt on the watershed amounted to 28% and decreased down the slope. The clay content on the watershed was 57% and did not differ from the lower part of the slope. On the middle part of the slope it was 1.3 times lower. The organic carbon content was 4.3% on the watershed and lower part of the slope, and was 1.2 times higher on the middle part of the slope. The pH values were neutral.

The virgin soils of the key section “Les na Vorskle” had a light loamy granulometric composition. The silt content was the same on the watershed and the middle part of the slope (3%), and on the lower part of the slope it increased 2 times. The clay content on the watershed was 20% and increased downward by 10% on the middle part and 20% on the lower part. The organic carbon content was the same on the watershed and the lower part of the slope (2.7%), on the middle part of the slope it decreased almost 2 times. The pH values were slightly acidic.

The soils of the “Arable Land” catena, similar to the reserved area, had a light loamy granulometric composition. The silt content on the watershed was 4% and increased 2 and 2.3 times in the middle and lower parts, respectively. The clay content on the watershed was 14% and increased downward by 40% in the middle part and by 90% on the lower part. The organic carbon content was the same on the watershed and in the middle part of the slope (0.7%); it increased almost twice on the lower part of the slope. The pH values tended to be acidic.

4.2. Biomass of microbial communities in the soils studied

4.2.1. The key area “Yamskaya steppe”. The data on the living microbial biomass (C-PL) and active biomass of microorganisms that respond to glucose (C-SIR) in the soils of the “Steppe North” and “Steppe South” catenas of migration-micellar chernozems are shown in figure 3. In soils of the “Steppe North” catena the living microbial biomass was about 2000 $\mu\text{g C/g}$ and did not differ significantly throughout the catena, although there was a tendency to decrease in C-PL downward the slope. At the same time, the active microbial biomass decreased downward from 2,000 to 1,500 $\mu\text{g C/g}$. All microbial communities on the watershed were alive and able to respond to glucose amendment, and on middle and lower parts of the catena, about 17% of the microbial community were resting/dormant.

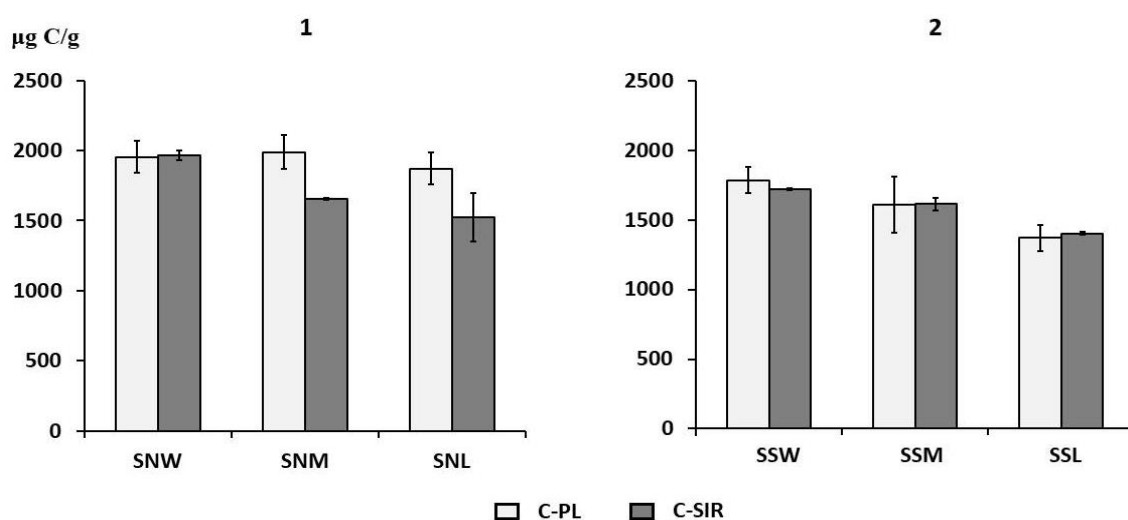


Figure 3. “Living” (C-PL) and “active” (C-SIR) microbial biomass in catenas of Northern and Southern exposure of the steppe chernozems in the reserved area: 1 – “Steppe North” (SNW – watershed, SNM – middle and SNL – lower parts of the slope) and 2 – “Steppe South” (SSW – watershed, SSM – middle and SSL – lower parts of the slope).

On soils of the catena “Steppe South”, the maximal value of C-PL was 1790 $\mu\text{g C/g}$, which is 20% lower than on the slope of the Northern exposure. Down the slope, the trend of decreasing of microbial community was more expressed (by 15–20% in lower part of the slope). At the same time, C-SIR values did not differ significantly from C-PL, which points to completely alive and active state of microbial communities.

In catena of arable chernozems of the Northern exposure “Arable North”, the value of C-PL increased down the slope (figure 4). On the watershed, the C-PL value was minimal and amounted to over 500 $\mu\text{g C/g}$ of soil. It increased downward the catena and it was 22% higher on the middle part, and 33% on the

lower part of the slope. At the same time, on the watershed and the middle part of the slope, the C-SIR value did not differ significantly from C-PL, that points out that almost all microbial cells gave a respiratory response to the glucose amendment. On the lower part of the slope, 84% of microbial community remained active.

In catena of arable chernozems of the Southern exposure “Arable South” on the watershed, the value of C-PL was 530 $\mu\text{g C/g}$, which was 5% higher compared to the watershed of the Northern slope. The trend of increasing of living microbial biomass downward the slope remained. The maximal value of C-PL in “Arable South” catena was 900 $\mu\text{g C/g}$ on the lower part of the slope that did not differ significantly from the value for the middle part of the slope and exceeded respective values in “Arable North” catena by 17%. The C-SIR and C-PL values also did not differ significantly that meant that all cells were alive and responded to the glucose amendment.

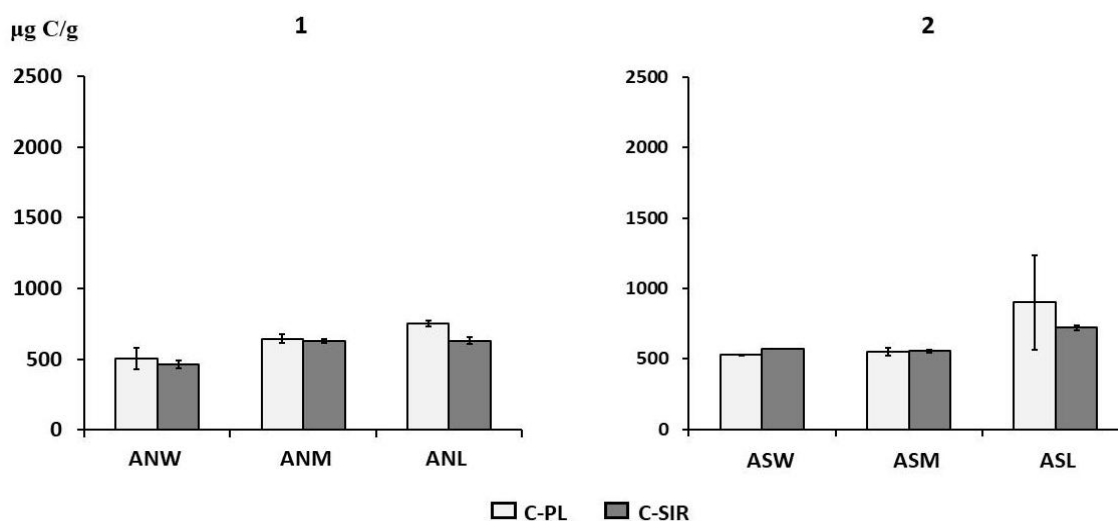


Figure 4. “Living” (C-PL) and “active” (C-SIR) microbial biomass in catenas of Northern and Southern exposure of vicinal arable lands outside of the reserve: 1 – “Arable North” (ANW – watershed, ANM – middle and ANL – lower parts of the slope) and 2 – “Arable South” (ASW – watershed, ASM – middle and ASL – lower parts of the slope).

Therefore, the values of living microbial biomass in the soils of steppe catenas were 2–4 times higher than the ones in the soils of arable catenas. Most of microbial communities were living and active. The trend of a decrease of microbial biomass downward steppe catenas and its increase downward arable catenas of both exposures was revealed.

4.2.2. *The key area “Les na Vorskle” (forest on the Vorskla River).* On the dark gray forest soils of the southern “Forest” catena maximal values of C-PL were observed on the watershed and the lower part of the slope (about 970 $\mu\text{g C/g}$), and on the middle part of the slope they were 2 times lower (figure 4). In this case, 86 and 78% of microbial community on the watershed and lower part of the slope respectively remained active, while on the middle part of the slope only 34% of the community were active. This shows that more than half of the community were resting/dormant cells.

On the dark gray forest soils of the southern “Arable land” catena (figure 5), the living microbial biomass increased downward the catena. On the watershed and middle part, the C-PL values were about 250 $\mu\text{g C/g}$, and in the lower part they were 2 times higher. Active microbial biomass increased downward the catena, being about 30% of microbial community on the watershed and 50–57% on the middle and lower parts of the slope, respectively.

In the key section “Les na Vorskle”, the living microbial biomass in the “Arable land” catena was 2–3 times less than in the “Forest” catena, while the maximal values of C-PL in the arable land were comparable to the minimal values of the forest ecosystem.

4.2.3. *Comparison of the steppe and forest catenas within natural reserves.* The value of microbial biomass in the forest and steppe catenas of the southern exposure (“Forest” and “Steppe South”) differed significantly (figure 6). The values of living microbial biomass in the “Steppe South” catena were almost 2 times higher than in the “Forest” catena. Downward the “Step South” catena, the microbial biomass tended to decrease, and in the catena "Forest" the biomass was 2 times lower on its middle part, compared to the watershed and the lower part of the slope. At the same time, the microbial community of the steppe ecosystem was completely active, while in the forest the proportion of dormant microorganisms reached 66% on the middle part of the slope.

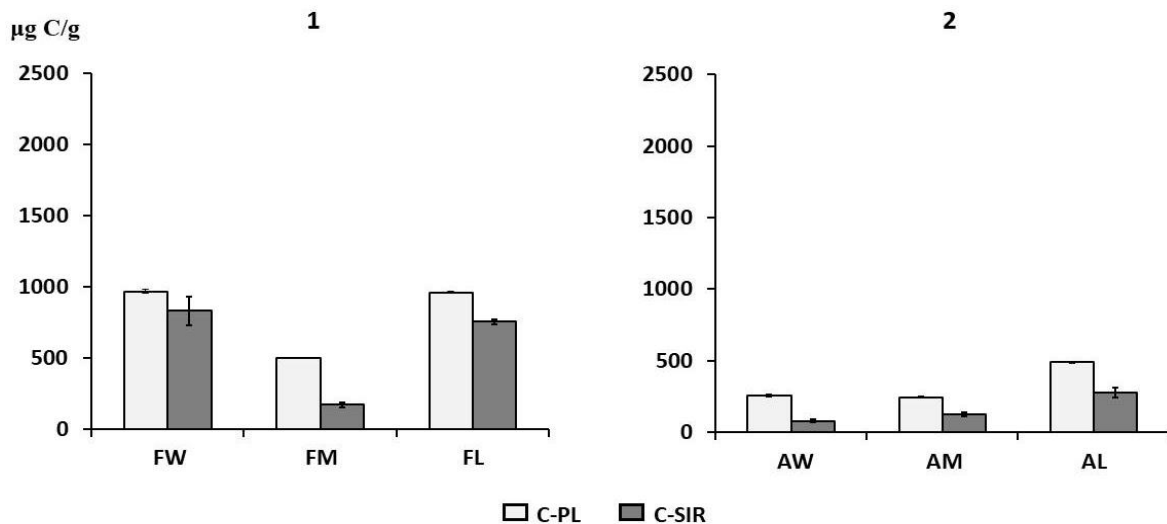


Figure 5. “Living” (C-PL) and “active” (C-SIR) microbial biomass in catenas of Southern exposure of natural forest ecosystems and vicinal arable lands outside of the reserve: 1 – “Forest” catena (FW – watershed, FM – middle and FL – lower parts of the slope) and 2 – “Arable Land” catena (AW – watershed, AM – middle and AL – lower parts of the slope).

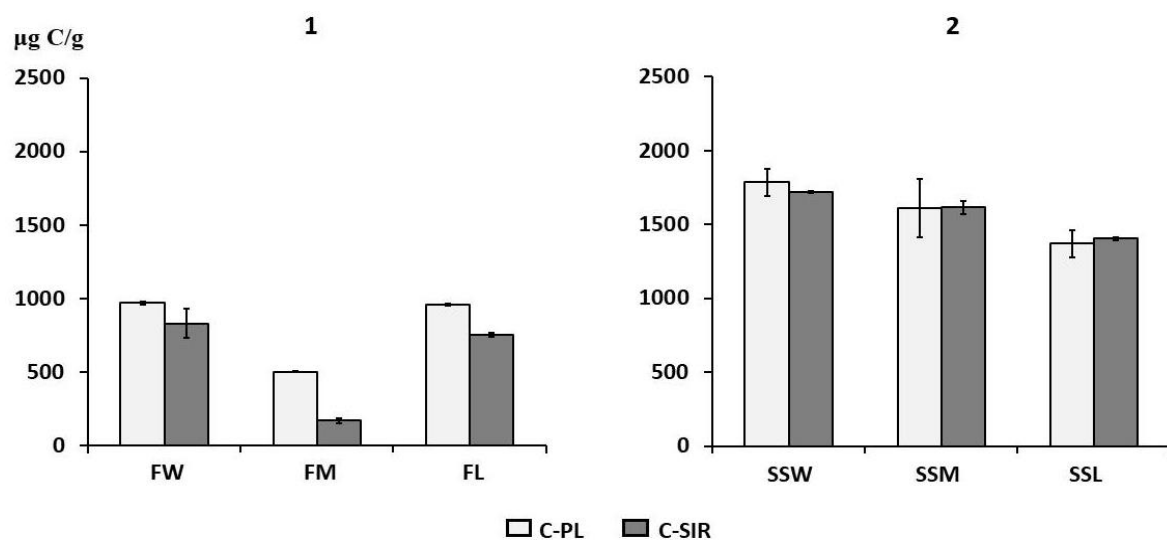


Figure 6. “Living” (C-PL) and “active” (C-SIR) microbial biomass in catenas of Southern exposure of the natural forest and steppe ecosystems: 1 – “Forest” catena (FW – watershed, FM – middle and FL – lower parts of the slope) and 2 – “Steppe South” catena (SSW – watershed, SSM – middle and SSL – lower parts of the slope).

5. Conclusions

In the steppe chernozem catena of Southern exposure, the maximal living microbial biomass was 1790 µg/g and was 20% less than in the catena of the Northern exposure. There was a downward trend for the biomass decrease, more pronounced in the catena of the Southern exposure. In the “Arable South” catena on the watershed, the value of C-PL was 530 µg C/g of soil, which was 5% higher than on the watershed of the “Arable North” catena. On both arable catenas, there was an increasing trend for the living microbial biomass, but on the “Arable South” catena it was less expressed. In the soils of steppe catenas, the living microbial biomass was 2–4 times higher compared to the soils of arable catenas. Most of microbial communities were living and active microorganisms. A decreasing trend for the microbial biomass downward steppe catenas and an increasing one downward arable catenas were revealed.

In the dark gray forest soils of the “Forest” catena, the living microbial biomass was 2–3 times higher than in the “Arable land” catena, and the maximal values of C-PL on the arable land were comparable to the minimal values of the forest ecosystem.

The values of microbial biomass in the forest and steppe catenas of the southern exposure (“Steppe South” and “Forest”) differed significantly. They were almost 2 times higher in the “Steppe South” catena compared to the “Forest” catena. While downward the “Step South” catena the microbial biomass tended to decrease, in the “Forest” catena on its middle part the biomass was 2 times lower than on the watershed and the lower part of the slope. At the same time, the microbial community of the steppe ecosystem was completely active, while in the forest the proportion of resting/dormant microorganisms reached 66% on the middle part of the slope.

Living microbial biomass in arable chernozem catena was twice higher than in arable agro-gray soil. The microbial community in chernozem catena was completely active, while in agro-gray soil up to a half of the community was resting/dormant on all parts of the catena studied.

We can conclude that lower values of microbial biomass in arable catenas of chernozems and dark gray forest soils compared to steppe and forest catenas of natural reserves are associated with the disposal of plant mass during land use. The increasing trend for the microbial biomass downward the arable catenas is connected with a change of granulometric composition of soils and flushing of fine-grained particles downward the catena. In the forest catena, in contrast to the steppe one, the decrease in microbial biomass in its middle transit part is associated with the peculiarities of soil formation processes on the slope.

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