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MICROBIOLOGICAL AND ECOPHYSIOLOGICAL PARAMETERS OF SOD-PODZOLIC SOIL UPON LONG-TERM APPLICATION OF STRAW AND MINERAL FERTILIZERS, THE CORRELATION WITH THE YIELD

I.V. RUSAKOVA

All-Russian Research Institute for Organic Fertilizers and Peat — Branch of Upper Volga Federal Agrarian Research Center, 2, ul. Pryanishnikova, Vyatkino, Sudogodskii Region, Vladimir Province, 601390 Russia, e-mail rusakova.iv@yandex.ru (✉ corresponding author)
ORCID:
Rusakova I.V. orcid.org/0000-0002-5085-0578
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Abstract

In modern agriculture, top priority is given to requirements for environmentally friendly application of fertilizers, providing for the intensification of use of biological sources of soil fertility recovery, primarily bioresources of farming ecosystems. One of significant, easily renewable biological resources is field residues of agricultural crops, which, according to many researchers, are the key to sustainable crop production and biosphere preservation. In this regard, one of main requirements for biologically based resource-saving methods and agrotechnologies is the returning of afterharvesting residues back into the soil without alienating the ones from the field, which ensures the enhancement of organic carbon input, improvement of biological status of soils, and their fertility and productivity, in general. A number of domestic and foreign papers prove the plant residues (PR), the structure of which consists of over 80 % of straw of cereals and leguminous crops, to be important for the preservation of favorable microbiological state of the soil. However, there is uncertainty in the qualitative and quantitative evaluations of the effect of the straw on the soil microbial community since the PR burial that may have both positive and negative consequences, which is often noted when introducing straw with a wide C to N ratio. Experimental data available in the scientific literature were obtained mainly when conducting research in laboratory and short-term field experiments with single use of straw as a fertilizer. The data of long field experiments with the repeated introduction of straw in crop rotation are useful for a more complete understanding of the straw effect on the microbial community and the use of this knowledge for the development of effective methods for managing the plant residues. The purpose of this study was to assess the effect of long-term use of straw of cereals and leguminous crops and mineral fertilizers (MF), separately and in combination, on the biological status of sod-podzolic sandy-loam soil. The indicators characterizing the composition, structure and metabolic activity of the microbial community of sod-podzolic soil were determined at the end of the 4th rotation of the 5-course row-crop rotation in a long field experiment: microbial biomass (C_{mic}.), microbial number and ratio of ecotrophic groups of microorganisms (ETGM), basal respiration (BR), and ecophysiological coefficients. It has been established that the return of afterharvesting residues in combination with medium doses of MF provides a balanced supply of nutrients and organic carbon to the microbial community and contributes to the reduction of mineralization processes and to the accumulation of C_{mic}. The microbial biomass closely correlated with the content of total ($r = 0.94$, $p < 0.05$) and easily decomposable carbon ($r = 0.89$, $p < 0.05$) and nitrogen ($r = 0.95$, $p < 0.05$) in the soil, and the yield of annual grasses closely correlated with the most part of indicators being determined in the experiment.

Keywords: microbial community, microbial biomass, soil, straw, mineral fertilizers

Soil fertility and its rational use are largely determined by the intensity and orientation of the biochemical activity of microorganisms involved in the destruction and mineralization of soil organic matter (SOM). Soil microbial biomass (MB) is a very sensitive pool of SOM, and any change in the management of agroecosystems affects the structure and content of MB much faster than the content of total organic matter [1-3].

Agricultural land use, including regular mechanical tillage, the use of various agro-chemicals, the annual alienation of most of the phytomass, often leads to a decrease in MB reserves, significant violations in its structure and functions compared to natural ecosystems [4, 5]. The unfavorable effect of agricultural production on the microbiological quality of the soil is becoming a global challenge [6]. A number of studies [7, 8] show the negative effect of mineral fertilizers (MF), primarily nitrogen fertilizers, on MB, which is explained by acidification, as well as a decrease in bioavailable carbon reserves.

Organic matter is the main limiting factor for microbial activity in arable soils [7, 9, 10]. Methods which ensure high exogenous carbon entry into the soil, including by returning post-harvest plant residues (PR) as a trophic and energy source for microorganisms [11], contribute to MB conservation and growth. Thus, the return of straw provided more favorable microbiological and biochemical soil conditions compared to its removal [12, 13]. In the scientific literature there also data on the absence or negative effect of grain straw on biological activity and effective soil fertility [14]. However, little is known about the consequences of prolonged use of straw for the microbial community.

This paper is our first report on long-term (20 years) application of straw in combination with mineral fertilizers. As a result, the biological state of sod-podzolic soil have become more favorable, approaching natural fallow lands in a number of microbiological parameters with prevailing microbial and soil carbon accumulation and yield increase.

The aim of the study was to assess the effect of repeated application of straw of cereals and leguminous crops in grain crop rotation (separately and in combination with mineral fertilizers) on biological parameters f sod-podzolic soil and the yield of annual grasses.

Materials and methods. The studies were carried out in a long-term, since 1996, field experiment of the All-Russian Research Institute of Organic Fertilizers and Peat (sod-podzolic soil, crop rotation — winter wheat, grain lupine, potatoes, barley, annual grasses lupine + oats). The experimental design included the following treatment: no fertilizers (control), MF (annual average dose $N_{54}P_{51}K_{57}$ applied before sowing), straw of winter wheat, lupine, and barley (3 t/ha each applied in the fall after harvesting grain and leguminous crops), straw of winter wheat, lupine and barley (3 t/ha each). In total, for four crop rotation, 36 t/ha straw was incorporated into the arable layer of the soil. The experiment was arranged in 2-fold temporal repetition and 3-fold spatial repetition (42-47 m^2 plots).

Soil samples were collected at the end of the 4th crop rotation (2016-2017) with a reed drill (0-20 cm layer) 2 weeks after harvesting the green mass of annual grasses (lupine of Crystal variety, oats of Amigo variety). Sets of 20-30 individual samples from each plot were combined into single sample. At the same time, samples of natural bare fallow soils and grass fallow soils (0-20 cm layer) located close to arable soils and being their genetic analogues were collected.

The microbiological and ecophysiological parameters most common in domestic and foreign studies were determined [7-9, 15]. The number of proteolytic microorganisms was counted on meat peptone agar (MPA), amylolytic microorganisms on starch-ammonia agar (SAA), oligotrophs on minimal agar (MA), oligonitrophic microorganisms on Ashby nitrogen-free medium [16].

The carbon of microbial biomass ($C_{mic.}$) was quantitated by the rehydration method. Basal respiration (BR, mg $C\text{-}CO_2 \cdot kg^{-1}$ dry soil $\cdot$ day^{-1}) was determined by the rate of soil CO_2 release for 24 hours of lab incubation at 22 °C and 60% humidity. For CO_2 absorption, 0.5 N NaOH was used followed by titration using 0.2 N HCl in the presence of phenolphthalein. The coefficients of oligotrophicity ($C_{oligt.}$) and oligonitrophilicity ($C_{olign.}$) were calculated as the ratio of the number of microorganisms on MA to MPA (MA/MPA), and on Ashby to MPA (Ashby/MPA), respectively [16]. Microbial factor was calculated as $C_{mic.}/C_{org.}$ (%). Microbial metabolic coefficient (specific respiration of MB) qCO_2 was calculated as the ratio $BR/C_{mic.}$ (mg $C\text{-}CO_2 \cdot kg^{-1} \cdot C_{mic.}{}^{-1} \cdot h^{-1}$). We determined total organic carbon ($C_{org.}$) by wet “burning” method ($K_2Cr_2O_7 + H_2SO_4$, 20 min in a drying cabinet at 160 °C) with a photometry (λ = 590 nm); cold water extractable organic carbon (C_w) in extracts after shaking for 3 minutes (soil:water = 1:20); hot water extractable organic carbon (C_{hw}) after 1-hour boiling soil suspension (soil:water = 1:5) in a water bath (80 °C) followed by C determination in filtered extracts after evaporation of aliquots (similar to the $C_{org.}$ analysis). Total nitrogen ($N_{tot.}$) was measured by the indophenol greens photometric method; hydrolyzable alkaline (N_{ha}) as per Kornfield’s method using 1.0 N NaOH for hydrolysis of the soil in Conway plates (48 h at 28 °C), 2% boric acid solution to absorb the released NH_3 and titration using 0.02 N H_2SO_4. Assays were performed in 3-6-fold repetition; microbiological tests were carried out with fresh samples on the day of soil sampling, chemical analyses were performed with air-dry samples and re-calculated for dry soil.

The green mass yield of annual herbs (lupine-oat mixture) was recorded for each plot (16.8 m^2 area) and expressed as dry matter.

Statistical processing was performed with STAT.EXE software (Pryanishnikov All-Russian Research Institute of Fertilizers and Soil Science, Moscow) by the method of univariate analysis of variance (p = 0.05) with calculation of mean (*M*) and standard deviations (±SD) using the Fisher *F*-test and LSD to assess the significance of the difference between the means. Correlation coefficients were calculated with Statistica 6.0 software (StatSoft, Inc., USA) ($p \leq 0.05$).

Results. Plant residues are a complex nutrient and energy substrate, the main source of bioavailable carbon for heterotrophic microorganisms [9, 11]. Nutrients entering the soil from MF, especially nitrogen, are also important for their active life

During the experiment, 14.4 t/ha of organic carbon (about 90% of the initial reserves) were delivered to the arable layer in treatment with straw. At the end of the 4th rotation, $C_{org.}$ in treatment MF + straw supplement was 0.567%, which was 1.22 and 1.13 times higher ($p < 0.05$) compared to the control and MF application, respectively (Table 1).

Easily degradable, rapidly transformed components of organic matter are the most essential to maintain microbial activity [17]. Water-soluble organic compounds, i.e. simple amino acids, monosugars, partially fulvic and humic acids, are the most available carbon source for soil microorganisms [18]. The concentration of C_w in the control and upon MF application was very low, 47.1 and 49.7 mg/kg soil, respectively (comparable to fallow). The highest C_w level was characteristic of arable layer with straw incorporation, 59.4 mg/kg, which was 1.26 and 1.20 times higher ($p < 0.05$) than without fertilizers and with MF, respectively. Highest C_{hw} value (188 mg/kg) was noted under MF + straw (1.20 and 1.08 times higher than without fertilizing and with MF), but the differences between the variants were not significant ($p > 0.05$). C_w and C_{hw} of the natural fallow amounted to 122 and 363 mg/kg, which was 2.05 and 1.93 times higher ($p < 0.05$) even compared to the maximum values for these fractions in the crop rotation soil (see Table 1).

1. Biological and agrochemical estimates of soz-podzolic soil upon multiple straw incorporation and application of $N_{54}P_{51}K_{57}$ mineral fertilizers (MF) **in grain-tillage crop rotation** (*M*±SD; Vladimir Province, Sudogodskii District, 2016-2017)

Option	$C_{org.}$	$C_{mic.}$	C_w	C_{hw}	BR	$C_{mic}/C_{org.}$	qCO_2
No fertilazers	0.463 ± 0.030^a	317 ± 42^a	47.1 ± 0.9^a	157 ± 9^a	3.7 ± 0.8^a	6.85	0.48
MF	0.501 ± 0.029^b	346 ± 38^a	49.7 ± 0.9^a	174 ± 17^a	7.5 ± 2.1^{bc}	6.91	0.90
MF + straw	0.567 ± 0.009^c	465 ± 42^b	52.3 ± 4.1^{ab}	188 ± 9^a	8.1 ± 1.4^{bc}	8.20	0.73
Straw	0.524 ± 0.037^{bc}	383 ± 29^a	59.4 ± 5.6^b	182 ± 29^a	6.7 ± 1.2^b	7.31	0.73
Bare fallow	0.400 ± 0.008^d	187 ± 12^c	48.1 ± 3.1^a	116 ± 11^b	4.2 ± 0.1^{ab}	4.68	0.94
Grass fallow	0.778 ± 0.052^e	609 ± 68^d	122 ± 8.8^c	363 ± 26^c	9.2 ± 1.5^c	7.83	0.63

Note. $C_{org.}$ — total carbon, %; $C_{mic.}$ — microbial carbon, mg/kg soil; C_w — cold water extractable organic carbon, mg/kg soil; C_{hw} — hot water extractable organic carbon, mg/kg soil; BR — basal respiration, mg $C\text{-}CO_2$/kg soil; $C_{mic.}/C_{org.}$ — microbial factor, %; qCO_2 — microbial metabolic coefficient, mg $C\text{-}CO_2 \cdot kg^{-1} \cdot C_{mic.}^{-1} \cdot h^{-1}$. For the experiment design description, see Materials and methods section. Identical letter indices indicate the absence of a statistically significant difference ($p > 0.05$).

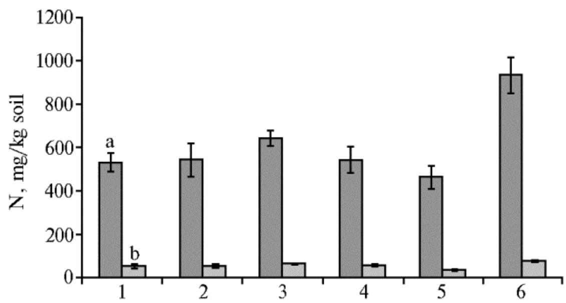

Fig. 1. Total (a) **and hydrolyzable alkaline** (b) **nitrogen in sod-podzolic soil upon multiple straw incorporation and application of $N_{54}P_{51}K_{57}$ mineral fertilizers in grain-row crop rotation:** 1 — no fertilizers, 2 — $N_{54}P_{51}K_{57}$, 3 — $N_{54}P_{51}K_{57}$ + straw, 4 — straw, 5 — bare fallow, 6 — grass fallow (*M*±SD; Vladimir Province, Sudogodskii District, 2016-2017). For the experiment design description, see Materials and methods section.

Nitrogen is a factors significantly limiting soil microbial activity. The $N_{tot.}$ was the highest upon long-term incorporation of straw in combination with MF (640 mg/kg), though the differences with other options were not significant ($p > 0.05$). In this treatment, the concentration of easily hydrolyzable nitrogen N_{ha} was significantly higher and reached 62.5 mg/kg which was 1.2 times as much as the same indicator without fertilizers and with MF. However, in the soil of grass fallow these indicators were significantly ($p < 0.05$) higher, 930 mg/kg soil for $N_{tot.}$ and 75.6 mg/kg soil for N_{ha} (Fig. 1).

Soil microbial biomass is a living component of SOM, mainly due to archaea, bacteria and eukaryotes, with the exception of roots and animals [19]. MB measurement is widely used as a relatively simple means of assessing the impact of environmental and anthropogenic changes on soil microorganisms [20]. As per meta-analysis of reported data (414 observations), the content of $C_{mic.}$ in arable soils of various types ranged from 43 to 2155 mg/kg with an average value of 365 mg/kg soil [7]. As per our observation, the microbial carbon content was minimal in long-fallowing soil (187 mg/kg) and maximal in grass fallow (609 mg/kg). In our field experiment, $C_{mic.}$ value was the maximum (465 mg/kg) upon application of MF + straw, being higher than in the other variants ($p < 0.05$). This is consistent with the results of studies [3, 10, 21, 22] reporting an increase in $C_{mic.}$ when incorporating straw in the soil. The annual use of MF did not contribute to the growth of $C_{mic.}$, providing only 9% growth of the value compared to control (see Table 1). Generalization of data array by Kallenbach et al. [7] showed that most studies also revealed a negative effect of MF on microbial communities, which is explained by their acidifying effect and the deficit of available carbon sources after an initial increase in mineralization activity. Different reports note that in post-agrogenic soils of natural fallow status the conditions (absence of mechanical treatments, constant soil cover with vegetation, and accumulation of easily decomposable organic matter) contribute to an increase in MB to a greater extent than in arable soils [23]. Overall increase in $C_{mic.}$ in soils is regarded as an absolutely positive fact, increasing their biological status [20].

$C_{mic.}/C_{org.}$ ratio is an indicator of the availability of soil organic carbon [24]. Moreover, a high proportion of $C_{mic.}$ indicates the fixing of organic carbon in MB and favorable conditions for the microbial community functioning. Low microbial factor indicates a decrease in the supply of microflora with available organic matter. $C_{mic.}/C_{org.}$ ratio in soils most often varies between 1-5% [7]; some authors give values up to 10% and higher [17]. In our research, the value of $C_{mic.}/C_{org.}$ ranged from 6.85 (control) to 8.20% (MF + straw), which indicates an improvement in SOM quality, accessibility to microflora and a large accumulation of carbon in MB during long-term use of straw in combination with MF. Kallenbach et al. [25] and Miltner et al. [26] also emphasize that the carbon accumulated in MB makes an important contribution to the humus soil pool formation.

Microbial respiration is an integral parameter that quantitates the overall metabolic activity of heterotrophic soil microflora. Microbial CO_2 production, determined in laboratory conditions, should be evaluated as the potential activity of MB under optimal temperature and humidity [15]. The lowest BR value (3.7 mg C-CO_2/kg soil) we recorded where crops in the rotation were grown without fertilizers, using only basic soil resources. As per the BR values, straw used separately had a significant effect on microbial metabolism, increasing the respiratory rate 1.8-fold ($p < 0.05$). Mineralization processes proceeded most actively and at approximately the same rate in the MF and MF + straw treatment, where the BR rate was 2.03 and 2.20 times higher ($p < 0.05$) than in the control (see Table 1).

Metabolic coefficient qCO_2 which characterizes the efficiency of substrate use by microorganisms is an informative indicator of the ecophysiological state of the soil microbial community [27]. For arable soils, qCO_2 most often varies from 0.5 to 2.0 mg C-$CO_2 \cdot kg^{-1} \cdot C_{miv.}{}^{-1} \cdot h^{-1}$ [28]. High values indicate a very significant need for energy sources or low efficiency of the use of organic substrate. The qCO_2 value in our experiments depended on the land use conditions and applied fertilizers and varied from 0.48 mg C-$CO_2 \cdot kg^{-1} \cdot C_{miv.}{}^{-1} \cdot h^{-1}$ in the control to 0.90 C-$CO_2 \cdot kg^{-1} \cdot C_{mic.}{}^{-1} \cdot h^{-1}$ upon MF and 0.94 C-$CO_2 \cdot kg^{-1} \cdot C_{mic.}{}^{-1} \cdot h^{-1}$ in the bare fallow soil (see Table 1). According to Zhang et al. [22], ecologically, the high microbial metabolic coefficient qCO_2 reflects the need for heterotrophs in carbon, and if the carbon that is lost by respiration is not replenished in the soil, MB decreases. When straw was used in combination with mineral fertilizers, qCO_2 value was 1.5 times higher than in the control, but 1.2 times lower ($p < 0.05$) compared to MF alone. That is, the high supply of microorganisms with nutrients during the annual introduction of MF without a sufficient amount of easily decomposable organic matter did not contribute to efficient consumption of carbon, which was lost to a greater extent during respiration than on biomass synthesis, leading, in turn, to a reduced pool of the soil carbon. The low qCO_2 value without fertilizers may indicate better consumption of carbon by soil biota when root residues of crop in the rotation are only sources of nutrition and energy. The high qCO_2 value (0.94 mg C-$CO_2 \cdot kg^{-1} \cdot C_{mic.}{}^{-1} \cdot h^{-1}$) in the soil of fallow, indicating a loss of carbon, can be explained by stimulation of the respiratory activity of the soil due to regular mechanical treatments.

The abundance and structure of the community of soil microorganisms are of paramount importance for understanding microbiological processes in the soil [4] and can be characterized by the number and ratio of microorganisms from various physiological or ecological trophic groups. It must be borne in mind that, given the size of a particular group, it is possible to conclude only about the physiological potential of soil microorganisms, but not about its performance in natural conditions [29]. The highest abundance of ecotrophic groups of microorganisms was characteristic of MF and MF + straw variants. However, lower C_{oligt}. (0.95 and 1.09) and $C_{olign.}$ (1.33 and 1.21), when straw was repeatedly used together with MF, testified to the relative dominance of copyotrophic microflora under these conditions as compared to oligotrophic microflora (Table 2).

2. Abundance and structure of microbial community in sod-podzolic soil upon multiple straw incorporation and application of $N_{54}P_{51}K_{57}$ mineral fertilizers (MF) **in grain-row crop rotation** (*M*±SD; Vladimir Province, Sudogodskii District, 2016-2017)

Option	Number of microorganisms, mln CFU/g soil				C_{oligt}.	$C_{olign.}$
	proteolytic	amylolytic	oligotrophic	oligonitrophilic		
No fertilizers	6.0±0.9^{ab}	7.9±2.8^{a}	8.6±2.2^{a}	9.0±2.1^{a}	1.43	1.50
MF	14.1±4.1^{c}	19.6±3.6^{b}	18.4±2.9^{b}	21.7±4.1^{b}	1.31	1.54
MF + straw	12.3±2.2^{bc}	14.1±1.5^{c}	11.7±2.8^{a}	16.4±3.9^{ab}	0.95	1.33
Straw	11.3±3.2^{bc}	13.3±2.2^{c}	12.3±2.5^{a}	13.7±3.2^{a}	1.09	1.21
Bare fallow	2.8±0.2^{a}	3.1±0.7^{a}	3.8±0.9^{c}	2.2±1.1^{c}	1.36	0.79
Grass fallow	7.9±2.5^{b}	12.8±4.2^{c}	10.8±3.4^{a}	12.5±4.8^{a}	1.37	1.58

N o t e. C_{oligt}. and $C_{olign.}$ — coefficients of oligotrophicity and oligonitrophilicity. For the experiment design description, see Materials and methods section. Identical letter indices indicate the absence of a statistically significant difference (p > 0.05).

According to Kolodyazhny et al. [30], the incorporation of straw into sod-podzolic soil contributed to a 2.5-fold increase in the number of copyotrophs (ammonifying and amylolytic microorganisms) and a 1.5-fold decrease of oligotrophs and pedotrophs. Higher indices of the absolute and relative abundance of oligotrophs under annual introduction of MF indirectly confirm the prevalence of humus destruction. In general, in most cases, the presence of crop residues in the soil had a beneficial effect on microbial communities [6, 7, 13].

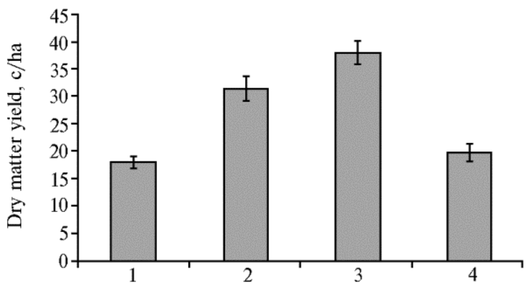

Fig. 2. Yield of annual grasses (lupine + oats) on sod-podzolic soil upon multiple straw incorporation and application of $N_{54}P_{51}K_{57}$ mineral fertilizers in grain-row crop rotation: 1 — no fertilizers, 2 — $N_{54}P_{51}K_{57}$, 3 — $N_{54}P_{51}K_{57}$ + sraw, 4 — straw (*M*±SD; Vladimir Province, Sudogodskii District, 2016-2017). For the experiment design description, see Materials and methods section.

Long-term incorporation of straw in combination with MF provided the maximum yield of leguminous grass herbs, 38.2 c/ha dry matter, which was significantly higher ($p < 0.05$) compared to not only the non-fertilized variant, but also to the MF application (Fig. 2). This is consistent with research data [21, 31] which also established a positive effect of straw on soil fertility and crop productivity.

In this work, we evaluated a quantitative relationship of biological indicators with the total C and N content, as well as with easily metabolized fractions. The yield of annual grasses significantly ($p < 0.05$) and positively correlated with $C_{org.}$, $N_{tot.}$, C_{hw}, $C_{mic.}$, $C_{mic.}/C_{org}$. and also with the counts of microorganisms on MPA (r = 0.88-0.93). A rather close, but insignificant negative correlation was revealed between productivity and $C_{oligt.}$ (r = –0.60). Microbial carbon significantly ($p < 0.05$) and closely correlated with $C_{org.}$ (r = 0.94), $N_{tot.}$ (r = 0.95), and C_{hw} (r = 0.89), as it is often observed in long-term field experiments. In a long field experiment on loamy loess soils of Germany in grain-cultivated crop rotation with mineral and organic fertilizers a significant positive correlation was found between $C_{mic.}$ and $C_{org.}$, and also between $C_{mic.}$ and C_{hw}, $r = 0.71$ and $r = 0.65$, respectively [32].

Thus, the long-term (12-fold over a 20-year period of field test) application of straw and mineral fertilizers in five-course crop rotation significantly affected microbial activity in sod-podzolic soil. With the annual application of mineral fertilizers (MF) and the removal of all post-harvest residues, the high functional activity of microorganisms was maintained, as evidenced by the high values of basal respiration and the microbial metabolic coefficient qCO_2. However, with regard to low of $C_{mic.}$, $C_{mic.}/C_{org.}$, $C_{org.}$ values, destructive mineralization prevails that is not favorable for carbon accumulation in the microbial biomass and in the soil. Under such conditions, microorganisms use the organic substrate less efficiently, spending C on respiration and increasing the loss of carbon from the soil. The introduction of straw without MF in the crop rotation does not have a negative effect on the biological properties and yield. This is possibly due to alternation of cereal straw and lupine straw, which differ in biochemical composition, during crop rotation. Regular incorporation of straw in combination with medium doses of mineral fertilizers maintains a balanced supply of microbial community with nutrients and carbon, increasing carbon accumulation in microbial biomass. The $C_{mic.}/C_{org.}$. value in this variant was comparable with that for natural fallow soil. Our findings confirm the importance of regular application of grain straw and leguminous straw for favorable C regime in soil. This contributes to providing soil microbial community with biologically available organic matter and an increase in soil microbial biomass.

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