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SOIL CHEMISTRY

Identification of Labile and Stable Pools of Organic Matter in an Agrogray Soil

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Abstract—The intensity of decomposition of the organic matter in the particle-size fractions from a agrogray soil sampled in a 5-year-long field experiment on the decomposition of corn residues was determined in the course of incubation for a year. The corn residues were placed into the soil in amounts equivalent to the amounts of plant litter in the agrocenosis and in the meadow ecosystem. A combination of three methods—the particle-size fractionation, the method of ^{13}C natural abundance by C3–C4 transition, and the method of incubation—made it possible to subdivide the soil organic matter into the labile and stable pools. The labile pool reached 32% in the soil of the agrocenosis and 42% in the meadow soil. Owing to the negative priming effect, the addition of C4 (young) carbon favored the stabilization of the C3 (old) carbon in the soil. When the young carbon was absent, destabilization or intense decomposition of the old organic matter was observed. This process was found even in the most stable fine silt and clay fractions.

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INTRODUCTION

Soil organic matter (**SOM**) is a complex system of organic and organo-mineral compounds. Owing to its heterogeneity, the SOM represents a continuous series (continuum) of organic compounds differing in their resistance to decomposition by soil microorganisms: from labile pools that are renewed within hours and days to very stable pools that are preserved in the soil for millennia [4, 7, 21]. The partitioning into the labile and stable pools is performed using physical, isotopic, and chemical methods.

The most widespread approaches to the physical fractionation are the aggregate-size, particle-size, and density analyses [2, 4, 21, 26]. Using the first two methods, the fractionation is performed according to the size of the waterproof aggregates and primary soil particles; in the latter case, the bulk density of the particles is taken into account. To distinguish between the free and tightly bound SOM pools, the method of particle-size–density fractionation is often used [2, 4, 21]. The fractions isolated by this method significantly differ in the contents of carbon and mineral components. However, they are not homogenous with respect to the time of the SOM renewal. In addition, the disaggregation of the soil (during its mechanical grinding or ultrasonic treatment) and the interaction of the soil particles with water and chemicals composing heavy liquids can significantly change the initial composition of the fractions obtained in the course of the fractionation procedure [23].

At first glance, isotopic methods (radiocarbon dating, the study of the natural ^{13}C abundance upon changes in the composition of the vegetation (C3 and C4 plants), and the decomposition of labeled plant residues) allow one to separate the SOM into more homogeneous pools according to the time of their renewal: the young pool, which accumulates after sharp changes in the isotopic composition of the plant residues, and the old stable pool. The method of the natural ^{13}C abundance upon the C3–C4 vegetation changes is often used for estimating the rate of the SOM renewal in the field [4, 8, 25]. In the course of growing C4 plants (for instance, a corn monoculture with $\delta^{13}C = -12...-14‰$) on a soil that was previously used for growing C3 plants ($\delta^{13}C$ $-26...-27‰$), the old C3 carbon is gradually replaced by the young C4 carbon of the corn residues. The young carbon pool is usually considered as labile; however, it may not always be called homogeneous. Under the long-term input of labeled plant material into the soil, some part of the young carbon is transformed into a stable organic matter pool. In order to assess the carbon sta-

bilization in the soil, the direct determination of its stability is necessary by the carbon losses as CO_2 in the course of the SOM decomposition in long-term incubation experiments.

The direct assessment of the stability of the particle-size-density fractions by the intensity of the CO_2 emission is rarely used, though this method allows gaining important information about the SOM stability in different pools. Christensen [13] found that the maximum stability was characteristic of the organic matter of the silt particles; on the contrary, the carbon of the coarse fractions is the most labile. The stability of the humus in the clay particles is intermediate between that of the silt and sand particles. The difference between the intensities of the SOM decomposition in the silt and clay fractions may be insignificant [24]. However, the high intensity of the SOM decomposition and the high rate of its renewal in the coarse (>50 μm) fractions as compared with the fine (<20 μm) fractions was found in many investigations [10, 18–21].

The differentiated estimate of the $^{12}CO_2$ and $^{13}CO_2$ emissions in long-term experiments (using the combination of the isotope and incubation approaches) allows comparing the renewal of the young and old SOM. The determination of the dynamics of the CO_2 emissions and their isotopic composition in the course of a 3-yr-long incubation experiment performed by Collins et al [12] showed that both pools—the young C4 and the old C3—consisted of labile and stable components. The labile C3 and C4 components were renewed with the same rate, and the time of the renewal of the stable C4 carbon fraction was approximately three times lower than that of the old C3 carbon. In the experiments performed, samples of different soil types were used. They were taken under corn that had been cultivated for 8–35 years. During this period, the young carbon was stabilized in rather stable pools with a renewal time of 12–28 years. The incubation of the density- and particle-size fractions isolated from the same soils [20] allowed establishing that the most stable pool of young carbon was mainly stabilized in the fine (silt and clay) fractions.

Thus, the isotope method enables determining the SOM stability in the particle-size fractions and the degree of stabilization of the young organic matter. In addition, the measurements of the CO_2 losses in the course of long-term incubation permit one to determine the qualitative changes in the SOM that take place upon the fractionation. The comparison of the respiration activity of the undisturbed soil and the weighted average of the CO_2 emissions from all the particle-size fractions with due account for their masses leads to contradictory conclusions. In Christensen's opinion [13], fractionation does not change the SOM resistance to decomposition by microorganisms. Marschner et al. [23] state that the disaggregation of the soil particles and the sedimentation analysis in water significantly increase the respiration activity of the organic matter in the soil fractions. The most significant changes occur in the combination of the particle-size- and density analyses, since the heavy liquids (for instance, sodium polytungstate) that are used in the separation of the particles by their density inhibit the activity of soil microorganisms [15].

We suggest that the combination of the particle-size fractionation of the carbon pools with the subsequent incubation and investigation of the isotopic composition of the CO_2 emissions will make it possible to subdivide the SOM into the most homogeneous pools and to estimate their sizes in the main soil types. In this work, the integrated approach combined both field and laboratory experiments. For the incubation, samples were taken from the soil developed under the C3 vegetation in the field experiment on the decomposition of corn (C4 plants) residues.

The aim of this work was to compare the rates of the young (C4) and old (C3) carbon renewal in the particle-size fractions from a agrogray soil for separating the SOM into the homogeneous labile and stable pools.

OBJECTS AND METHODS

The agrogray soil (C_{org} = 1.1%, pH_{KCl} 4.65) was studied in 2000–2005 at the experimental field station of the Institute of Physicochemical and Biological Problems of Soil Science of the Russian Academy of Sciences (Pushchino, Moscow oblast).

The rate of the carbon renewal in the agrogray soil was determined in a field experiment performed on the long-term fallow plot that served as the control. Every year for 5 years, milled green corn residues were applied to the upper 25-cm-thick soil layer of the test plots at the rates of 1.0 and 3.0 kg/m^2 (dry mass) corresponding to 1.9 and 5.8 kg C/m^2 for the whole period. The first rate corresponded to the amount of organic carbon entering the soil with the plant residues and organic fertilizers in the intensive farming practices; the second rate corresponded to the input of carbon into the soil in highly productive meadow communities. The C : N ratio in the corn residues was 27. The area of the plots was 1 m^2; two replicates were used. The test plots were isolated along their perimeter with PVC (Viniplast) frames to the depth of the plow layer and covered with wooden grates protecting the soil against drying. Every year, the soil was tilled in the fall, when corn residues were applied into it, and, in the spring, for its loosening and maintaining the fallow state. In the growing seasons, the water content in the upper 25 cm varied within 17–25% (50–75% of the water-holding capacity) during the entire experiment.

At the end of the experiment, soil samples were taken from the upper 25-cm-thick layer at 5 points per plot; mixed samples were then prepared and analyzed.

The soil samples were separated into particle-size fractions. The air-dry soil (50 g) was mixed with water (25% of the soil mass) and ground for 30 min. The obtained paste was sifted through 1.0- to 0.1-mm sieves. The coarse fraction (0.1–1.0 mm) proved to be heterogeneous. It was then separated into the organic and mineral components using flotation in water. The further analysis showed that this particle-size fraction includes the carbon of the light fraction (LF) representing fragments of humified plant residues and the sandy fraction consisting of mainly mineral components. The fraction <0.1 mm was treated with ultrasound (100 W) for 15 min and separated into particle-size fractions by sedimentation in water [1]. After the full extraction, the fractions were isolated by centrifuging (a K-70 centrifuge) for 30 min at 4000 rpm (1600 g).

In the soil samples and isolated fractions, the contents of organic carbon and nitrogen were measured, as well as the content of the ^{13}C isotope using a Euro EA (Eurovector, Italy) element analyzer coupled with a MAT 253 mass spectrometer (Thermo Electron, Germany). The analytical signals for the ^{13}C isotope were expressed in $\delta^{13}C$ of the international VPDB standard:

$$\delta^{13}C = [(R_{sample}/R_{stand}) - 1)] \times 1000, \quad (1)$$

where R_{sample} and R_{stand} are the $^{13}C/^{12}C$ ratios in the sample and standard. The $\delta^{13}C$ for the VPDB is 0‰, and $R_{stand} = 0.0111802$.

Simultaneously, the isotopic composition of the corn residues was determined: $\delta^{13}C$ in the corn green mass was −12.0‰.

The portion of C4 (corn) carbon in the soil was calculated according to the following equation:

$$\delta^{13}C_n = f\,\delta^{13}C4 + (1 - f)\,\delta^{13}C3, \quad (2)$$

where $\delta^{13}C_n$ is the $\delta^{13}C$ in the soil sample, $\delta^{13}C4$ is the $\delta^{13}C$ in the young organic matter formed during the decomposition of the corn residues, $\delta^{13}C3$ is the content of ^{13}C in the initial soil samples collected before the beginning of the experiment, and f is the portion of C4 organic matter (i.e., newly formed humus accumulated due to the decomposition of the corn residues over the 5 years of the experiment).

The constants of the organic matter decomposition in the soil fractions were determined from the cumulative curves of the CO_2 emission upon the incubation. As the mass of the light organic fraction in the composition of the sand fraction (0.1–1.0 mm or 100–1000 μm) did not exceed 2%, the latter was used as a whole (LF + sand) in the incubation experiments. Along with the coarse (sand) fraction, the coarse silt (10–100 μm), medium silt (5–10 μm), fine silt (1–5 μm), and clay (<1 μm) fractions were incubated. These particle-size fractions were incubated at constant temperature and moisture for a year. For this purpose, 1 g of the fractions was placed into a flask (15 ml) and moistened up to 70% of the water-holding capacity. The fine fractions (the medium and fine silt and clay) were mixed with 1 g of sand (after its calcination and treatment with HCl). The hermetically closed flasks were incubated at 22°C. The constant moisture was controlled by the changes in the soil's mass. Gas samples were taken on the 1st, 3rd, 5th, 7th, 10th, and 14th days and then every week. The respiration intensity of the soil and fractions was determined from the data on the CO_2 enrichment in the time intervals between the gas sampling. The flasks were periodically ventilated after the CO_2 concentration in the gas samples exceeded 2%. The CO_2 concentration was determined using a chromatograph (Kristallyuks-4000) with a detector of the thermal conductivity. The gas mixture was separated in 3-m-long columns filled with Porapack-Q at 30°C.

The constants of the decomposition of the labile and stable carbon were determined using two methods. In the first method, the cumulative curve of the CO_2 emission was approximated for the time of the experiment using the equation

$$C_t = A_1 e^{-k_1 t} + (1 - A_1) e^{-k_2 t}, \quad (3)$$

where C_t is the organic carbon (C_{org}) content in the soil after the cumulative losses of $C–CO_2$ during the time t (the portion of the initial C_{org} content in the soil); A_1 is the portion of the labile pool; and k_1 and k_2 are the constants of the decomposition in the labile and stabile pools, respectively.

In the second method, the decomposition constants (k) of the young (C4) and old (C3) carbon were calculated by the equation based on the exponential decomposition of both the labile organic matter of the corn residues and the stabile SOM [8]:

$$C_t = e^{-kt}, \quad (4)$$

$$k = -\ln(C_t)/t. \quad (5)$$

The MRT of the carbon used in both methods was calculated as follows:

$$MRT = 1/k. \quad (6)$$

The equations were approximated using the Markwardt algorithm. The differentiated accounting for the young and old organic matter losses was performed on the basis of the data on the total losses of ^{13}C per year obtained from the results of the isotopic analysis of the carbon at the beginning and at the end of the incubation. The total annual losses of the old C3 carbon were calculated as the difference between the cumulative CO_2 emission in the course of the annual incubation and the losses of the young C4 carbon. The experi-

ments were performed in 3–4 replicates; the results of the analyses were calculated per oven-dried soil mass.

RESULTS AND DISCUSSION

Cumulative losses and constants of the SOM decomposition. The intensity of the respiration during the soil incubation points to the different resistance of the organic matter in the different particle-size fractions to decomposition by the soil microorganisms (Fig. 1, Table 1). The annual losses of carbon amounted to 7–26% of the initial C_{org} content in the particle-size fractions. The minimum carbon losses were found in the clay fraction, and the maximum losses were in the coarse and medium silt fractions. In the particle-size fractions, as well as in the whole soil, the $C{-}CO_2/C_{org}$ ratio in the control variant significantly exceeded the portion of the annual carbon losses as CO_2 in the variants with the application of plant residues into the soils.

A decrease in the potential carbon losses from the soil in the course of the respiration, erosion, or leaching of the SOM beyond the soil profile is considered as the SOM stabilization [27]. An opposite phenomenon is the SOM destabilization, i.e., an increase in the carbon losses due to the enhanced mineralization of the SOM and its loss under the impact of erosion and intrasoil runoff. Thus, after the application of the plant residues to the agrogray soil for 5 years, the carbon stabilization predominated. On the contrary, in the control variant, after the 5-year-old bare fallow, destabilization and mineralization of the SOM prevailed. These differences were clearly observed in the light fraction. In the control variant, the annual carbon losses in the course of the incubation amounted to about 20% of the initial C_{org} content. At the same time, in the variants with corn residues, the losses decreased approximately by two times.

The calculation of the constants of the SOM decomposition according to Eq. (3) shows that the differences in the SOM stability are mainly manifested upon the decomposition of the stable pool of organic matter with the decomposition constant being k_2. Unlike the k_1 constants for the labile pool, which insignificantly differ for the different particle-size fractions, the k_2 values, as well as the values of the annual carbon losses, point to the high decomposabity of the organic matter in the coarse and medium silt fractions. The organic matter in the fine silt and clay fractions is the most stable. Ambiguous data were obtained for the LF + sand fraction in different variants of the experiment. In the control variant, this fraction represented the most labile component of the SOM with the maximum k_2 value. In the variants with the application of plant residues, it was more stable: the k_2 values were comparable with those for the fine silt and clay fractions. The presence of a small (4.5% of C_{org}) labile pool even in the stable fine fractions confirms the conclusion about their heterogeneity we came to in the earlier study [21]. The values obtained by us are in agreement with the literature data attesting to an increase in the constant of the SOM decomposition in the fine fractions [10, 18–20].

The annual cumulative carbon losses from the soil in our experiment were almost equal to the $C{-}CO_2$ emission calculated as the sum of the carbon losses from the particle-size fractions (with due account for their weight in the soil). In the control soil and the variants with the addition of plant residues (1.9 and 5.8 kg C/m^2), the annual losses upon the incubation of the nonfractionated soil reached 183, 181, and 220 mg $C{-}CO_2$/100 g, respectively; for the sums of the separate fractions subjected to incubation, they comprised 196, 178, and 233 mg $C{-}CO_2$/100 g, respectively. As such, the closeness of these values does not prove the absence of changes in the SOM quality upon the particle-size fractionation. If fact, the constants of the decomposition of the organic matter in the separate fractions significantly differed from the constants calculated for the whole soil. The process of the fractionation is related to the destruction of the soil aggregates and the sedimentation of the particles in water, which led to oppositely directed changes in the k_1 and k_2 values. The fractionation lowered the k_1 and the size of the labile pool (A_1), because these values for the whole soil were higher than those for any of the separated particle-size fractions in all the experimental variants. Hence, during the fractionation, some part of the labile pool of the SOM was lost. Probably, the losses were associated with the dissolution of the most decomposable part of the labile pool in water. The constant of the decomposition of the stable pool (k_2) in any fraction was much higher than k_2 in the whole soil. The increase in the rate of the decomposition of the stable pool after the fractionation was probably associated with the destruction of the aggregates (mechanical grinding and ultrasonic treatment) during the preparation of the soil for the particle-size analysis. In this connection, we consider the conclusion of Christensen [13] about the insignificant influence of the physical (aggregate) stabilization on the organic matter decomposition reached on the basis of the cumulative CO_2 fluxes to be insufficiently substantiated. Thus, the data of the incubation experiment can only be used as relative values for comparing the stability of the soil fractions, since the disturbance of the physical stabilization (which is an important mechanism of the SOM stability) is accompanied by significant changes in the organic matter in the isolated particle-size fractions.

The constants of the decomposition of the old and young carbon of the SOM. The isotopic composition of

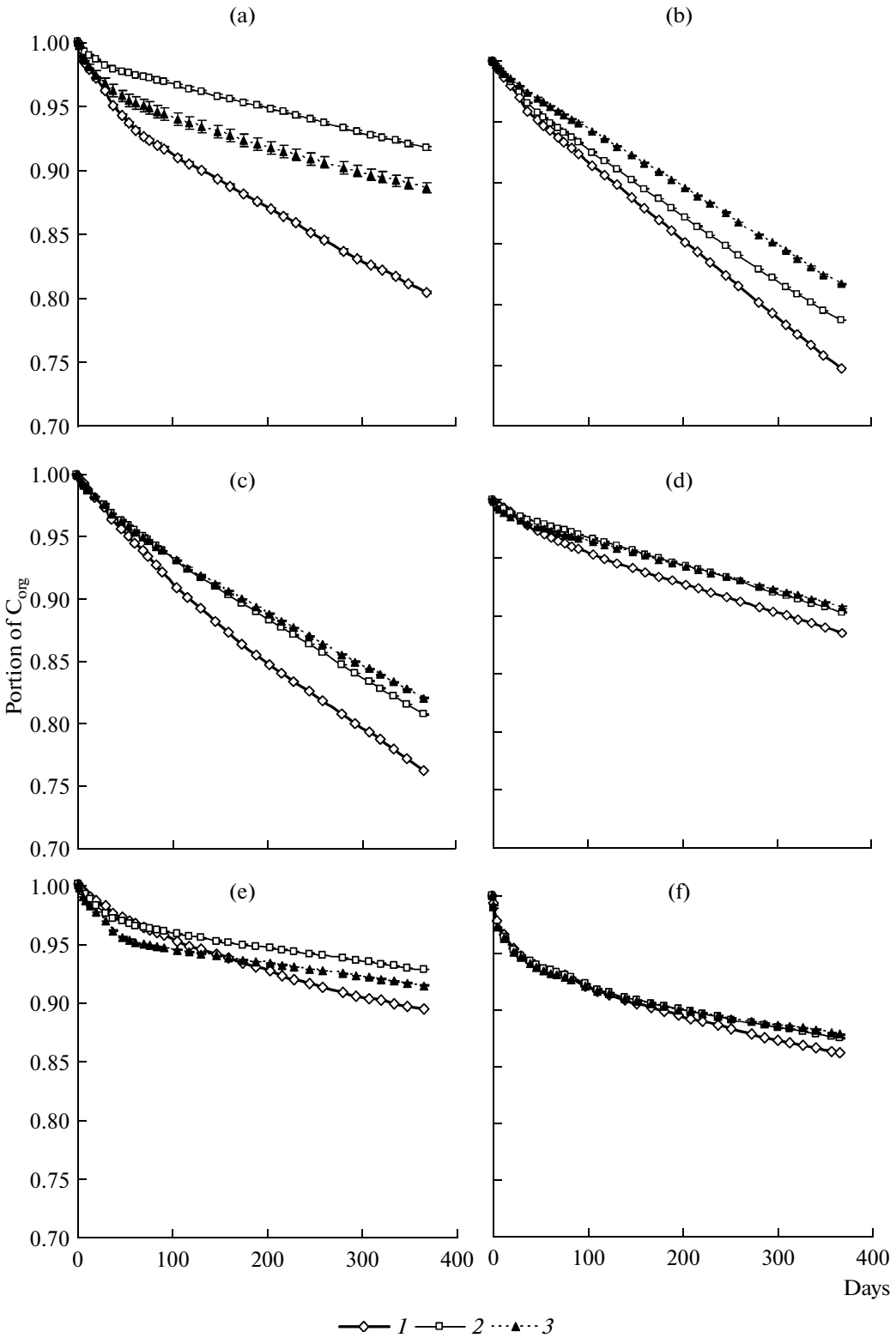

Fig. 1. Contents of C_{org} in the particle-size fractions of the agrogray soil without carbon losses as CO_2 in the (*1*) control variant and after the application of (*2*) 1.9 kg of C/m^2 and (*3*) 5.8 kg of C/m^2 of corn residues. Fractions: (a) LF + sand (100–1000 μm), (b) coarse silt (10–100 μm), (c) medium silt (5–10 μm); (d) fine silt (1–5 μm), and (e) clay (<1 μm); (f) whole soil.

Table 1. Constants of the organic matter decomposition in the particle-size fractions of the agrogray soil (0–25 cm) calculated according to Eq. (3) (mean ± STD)

Variant	Fraction	Annual carbon loss		A_1	k_1, yr^{-1}	MRT_1, yr	k_2, yr^{-1}	MRT_2, yr
		mg $C-CO_2$/100 g	$C-CO_2/C_{org}$					
Control	Nonfractionated soil	189 ± 6	0.139 ± 0.003	0.055 ± 0.003	23.5 ± 2.5	0.043 ± 0.004	0.097 ± 0.002	10.3 ± 0.2
	Sand + LF, 100–1000 μm	317 ± 2	0.195 ± 0.005	0.045 ± 0.002	13.6 ± 0.9	0.074 ± 0.005	0.171 ± 0.002	5.8 ± 0.1
	Coarse silt, 10–100 μm	92 ± 1	0.252 ± 0.004	0.011 ± 0.002	23.7 ± 7.4	0.042 ± 0.019	0.276 ± 0.001	3.6 ± 0.1
	Medium silt, 5–10 μm	379 ± 4	0.237 ± 0.004	0.014 ± 0.003	12.1 ± 2.2	0.082 ± 0.018	0.263 ± 0.001	3.8 ± 0.1
	Fine silt, 1–5 μm	372 ± 3	0.114 ± 0.003	0.022 ± 0.001	8.1 ± 0.6	0.123 ± 0.010	0.099 ± 0.001	10.1 ± 0.1
	Clay, <1 μm	435 ± 19	0.106 ± 0.003	0.030 ± 0.001	6.9 ± 0.7	0.145 ± 0.016	0.084 ± 0.002	11.9 ± 0.3
1.9 kg C/m^2	Nonfractionated soil	185 ± 9	0.125 ± 0.006	0.057 ± 0.004	25.5 ± 3.5	0.039 ± 0.002	0.079 ± 0.003	12.6 ± 0.4
	Sand + LF, 100–1000 μm	280 ± 5	0.082 ± 0.004	0.037 ± 0.001	3.5 ± 0.2	0.287 ± 0.018	0.046 ± 0.001	21.9 ± 0.6
	Coarse silt, 10–100 μm	102 ± 1	0.213 ± 0.003	0.014 ± 0.001	15.4 ± 2.2	0.065 ± 0.011	0.226 ± 0.001	4.4 ± 0.1
	Medium silt, 5–10 μm	340 ± 4	0.192 ± 0.004	0.014 ± 0.001	17.1 ± 2.7	0.058 ± 0.011	0.199 ± 0.001	5.0 ± 0.1
	Fine silt, 1–5 μm	335 ± 6	0.097 ± 0.002	0.022 ± 0.002	3.0 ± 0.2	0.330 ± 0.027	0.078 ± 0.001	12.8 ± 0.2
	Clay, <1 μm	325 ± 2	0.072 ± 0.003	0.031 ± 0.002	14.8 ± 0.6	0.068 ± 0.003	0.044 ± 0.001	22.5 ± 0.3
5.8 kg C/m^2	Nonfractionated soil	225 ± 4	0.122 ± 0.005	0.062 ± 0.003	23.1 ± 2.1	0.043 ± 0.004	0.070 ± 0.002	14.2 ± 0.4
	Sand + LF, 100–1000 μm	582 ± 21	0.113 ± 0.003	0.037 ± 0.001	16.9 ± 2.5	0.059 ± 0.010	0.085 ± 0.002	11.7 ± 0.3
	Coarse silt, 10–100 μm	112 ± 1	0.183 ± 0.004	0.014 ± 0.001	2.6 ± 0.4	0.383 ± 0.063	0.186 ± 0.001	5.4 ± 0.1
	Medium silt, 5–10 μm	456 ± 7	0.186 ± 0.005	0.022 ± 0.001	10.0 ± 1.3	0.100 ± 0.015	0.175 ± 0.002	5.7 ± 0.1
	Fine silt, 1–5 μm	357 ± 1	0.092 ± 0.005	0.022 ± 0.001	8.2 ± 0.6	0.122 ± 0.009	0.071 ± 0.001	14.0 ± 0.1
	Clay, <1 μm	394 ± 13	0.086 ± 0.004	0.045 ± 0.002	14.8 ± 1.2	0.068 ± 0.006	0.043 ± 0.002	23.3 ± 1.0

Table 2. Constants of the C4 and C3 carbon decomposition in the course of the annual incubation of particle-size fractions of the agrogray soil (0–25 cm) (mean ± STD)

Variant	Fraction	Initial data			After the annual incubation			Loss of $C–C_4/C–C_{4org}$	$k\,C_4$, yr^{-1}	Loss of $C_3–CO_2/C–C_{3org}$	kC_3, yr^{-1}
		C_{org}, %	$\delta^{13}C$ ‰	$C_4–C/C_{org}$	C_{org}, %	$\delta^{13}C$ ‰	$C_4–C/C_{org}$				
Control	Nonfractionated soil	1.36 ± 0.02	−25.74 ± 0.21	Not det.	1.26 ± 0.02	−26.10 ± 0.04	Not det.			0.139 ± 0.006	0.149 ± 0.021
	Sand + LF, 100–1000 μm	1.63 ± 0.02	−26.19 ± 0.05	″	1.31 ± 0.02	−26.65 ± 0.03	″			0.195 ± 0.002	0.217 ± 0.025
	Coarse silt, 10–100 μm	0.37 ± 0.02	−26.39 ± 0.02	″	0.27 ± 0.02	−26.67 ± 0.02	″			0.249 ± 0.001	0.290 ± 0.022
	Medium silt, 5–10 μm	1.77 ± 0.01	−26.45 ± 0.03	″	1.26 ± 0.03	−26.74 ± 0.02	″			0.214 ± 0.004	0.270 ± 0.031
	Fine silt, 1–5 μm	3.25 ± 0.10	−25.77 ± 0.05	″	2.88 ± 0.05	−26.02 ± 0.05	″			0.114 ± 0.003	0.121 ± 0.020
	Clay, <1 μm	4.11 ± 0.01	−25.16 ± 0.04	″	3.66 ± 0.02	−25.59 ± 0.03	″			0.106 ± 0.009	0.115 ± 0.052
1.9 kg C/m^2	Nonfractionated soil	1.48 ± 0.01	−24.35 ± 0.25	0.101 ± 0.004	1.40 ± 0.02	−24.90 ± 0.02	0.078 ± 0.005	0.352 ± 0.009	0.433 ± 0.039	0.139 ± 0.005	0.098 ± 0.024
	Sand + LF, 100–1000 μm	3.40 ± 0.02	−21.97 ± 0.02	0.330 ± 0.004	3.12 ± 0.02	−23.28 ± 0.03	0.187 ± 0.005	0.250 ± 0.008	0.288 ± 0.032	0.107 ± 0.005	0.033 ± 0.024
	Coarse silt, 10–100 μm	0.48 ± 0.01	−23.64 ± 0.05	0.191 ± 0.005	0.38 ± 0.01	−24.46 ± 0.04	0.131 ± 0.004	0.356 ± 0.012	0.440 ± 0.051	0.255 ± 0.003	0.206 ± 0.015
	Medium silt, 5–10 μm	1.81 ± 0.01	−24.54 ± 0.01	0.132 ± 0.003	1.39 ± 0.01	−25.26 ± 0.02	0.09 ± 0.002	0.441 ± 0.015	0.581 ± 0.059	0.218 ± 0.005	0.174 ± 0.014
	Fine silt, 1–5 μm	3.46 ± 0.02	−24.8 ± 0.02	0.070 ± 0.005	3.15 ± 0.02	−25.39 ± 0.01	0.043 ± 0.003	0.405 ± 0.011	0.519 ± 0.074	0.104 ± 0.006	0.078 ± 0.012
	Clay, <1 μm	4.51 ± 0.12	−24.29 ± 0.06	0.066 ± 0.004	4.18 ± 0.03	−24.98 ± 0.03	0.043 ± 0.006	0.360 ± 0.009	0.445 ± 0.065	0.077 ± 0.004	0.055 ± 0.013
5.8 kg C/m^2	Nonfractionated soil	1.85 ± 0.05	−21.9 ± 0.18	0.279 ± 0.008	1.67 ± 0.02	−22.99 ± 0.04	0.180 ± 0.005	0.289 ± 0.006	0.340 ± 0.035	0.168 ± 0.005	0.057 ± 0.009
	Sand + LF, 100–1000 μm	5.13 ± 0.03	−17.62 ± 0.01	0.587 ± 0.002	4.55 ± 0.03	−18.75 ± 0.02	0.350 ± 0.002	0.176 ± 0.008	0.193 ± 0.031	0.182 ± 0.015	0.074 ± 0.013
	Coarse silt, 10–100 μm	0.61 ± 0.01	−20.70 ± 0.02	0.395 ± 0.003	0.50 ± 0.01	−21.57 ± 0.02	0.258 ± 0.003	0.264 ± 0.009	0.306 ± 0.053	0.260 ± 0.003	0.163 ± 0.017
	Medium silt, 5–10 μm	2.35 ± 0.02	−22.58 ± 0.01	0.268 ± 0.002	2.00 ± 0.02	−23.44 ± 0.01	0.183 ± 0.002	0.293 ± 0.010	0.347 ± 0.038	0.264 ± 0.007	0.171 ± 0.020
	Fine silt, 1–5 μm	3.88 ± 0.01	−23.12 ± 0.02	0.192 ± 0.005	3.52 ± 0.02	−23.81 ± 0.02	0.136 ± 0.002	0.233 ± 0.009	0.266 ± 0.021	0.110 ± 0.002	0.067 ± 0.012
	Clay, <1 μm	4.61 ± 0.02	−23.08 ± 0.10	0.158 ± 0.009	4.22 ± 0.02	−23.93 ± 0.04	0.108 ± 0.003	0.273 ± 0.011	0.318 ± 0.035	0.100 ± 0.004	0.058 ± 0.012

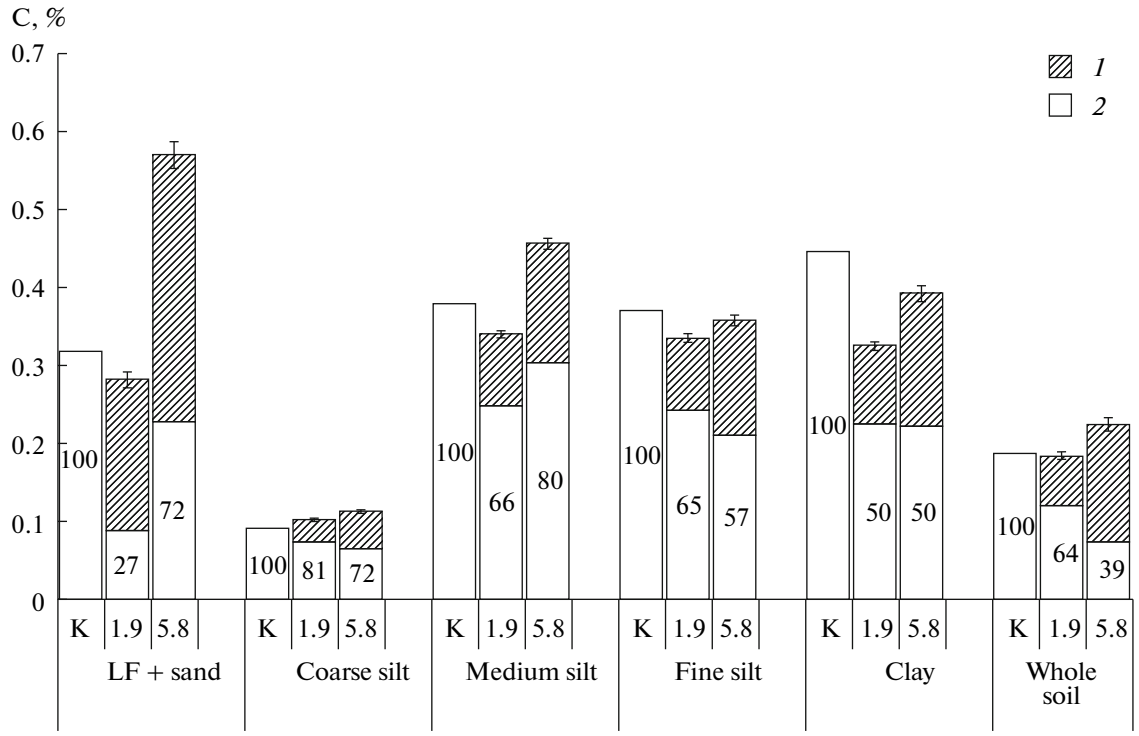

Fig. 2. Cumulative losses of (*1*) C4 and (*2*) C3 carbon as CO_2 upon a year-long incubation of the particle-size fractions from the agrogray soils of the control (K, without corn residues) plot and the plots with added corn residues (1.9 and 5.8 kg C/m^2). The figures indicate the loss of C3 carbon, % of its loss in the control.

the particle-size fractions before and after the incubation attested to a decrease in the $^{13}C/^{12}C$ ratio not only in the soils with corn residues but also in the control soil (Table 2). The isotopic composition of the control soil (0.2–0.4‰) changed to a lesser degree than that of the soil with the corn residues (0.5–1.3‰). The alteration of the isotopic composition in the course of the incubation of the C3 soil without C4 carbon is usually related to changes in the substrate quality in the course of the SOM decomposition by microorganisms. At the late stages of this process, lipids and lignin (stable compounds depleted of the ^{13}C isotope [9, 14]) are destroyed, whereas the carbohydrate components enriched in the heavy carbon isotopes [17] are mainly decomposed during the initial stages. It is difficult to determine whether the isotopic composition of the young C4 organic matter changes in the course of the incubation. It is known that both the depletion and the enrichment of the $^{13}CO_2$ released from the soil in the course of the decomposition of the heavy carbon may take place (as compared with the initial content of heavy carbon in the decomposing substrate) [14]. In the long-term experiments on the decomposition of plant residues without their mixing with the soil, the $\delta^{13}C$ in the released CO_2 was approximately 12‰; i.e., it did not significantly differ from the isotopic ratio in the corn [12, 28]. Therefore, to calculate the *f* value in Eq. (1), we used the same $\delta^{13}C_4$ value and different $\delta^{13}C_3$ values at the beginning and at the end of the incubation.

The differentiated estimate of the losses in the C4 and C3 organic matter using eqs. (5) and (6) (Table 2) showed a more intense emission of C4 carbon, which is in agreement with the published data [12, 20]. In our experiment, the losses of young (C4) carbon during a year of incubation varied within 30–47%, whereas the losses of old (C3) carbon varied within 6–25%.

The constants of the decomposition of the young organic matter (k_{C4}) and its mean residence time (MRT_{C4}) were approximately equal in all the fractions and in the whole soil (Fig. 2). Even in the fine fractions that are traditionally considered as resistant to decomposition [2, 21, 22, 25], the young C4 pool was as labile as that in the coarse fractions. The high rate of decomposition of the C4 carbon in the fine fractions showed that the young organic matter was unstable in the course of the 5-year experiment. Even during 10 years, no stabilization of the young carbon was observed in the experiments on the decomposition of forest litter in a field [16]. For the formation of tight bonds between the mineral and organic components of a soil, a longer period is needed. This complicates the study

of the mechanisms of the carbon stabilization in soils. Only in the experiments on growing a corn monoculture for 30 years and longer does the MRT_{C4} in the fine fractions significantly increase [20].

In contrast to the young carbon, the constants of the decomposition of the old (C3) organic matter depended on the size of the fractions: a low rate of decomposition characterized the fine fractions, and a high rate was characteristic of the coarse soil particles. In the control variant, the constants of the organic matter decomposition in all the particle-size fractions and in the whole soil were higher than the k_{C3} values in the variants with the application of plant residues. Consequently, the old organic matter (C3) in the soil with the corn residues was decomposed more slowly than the organic matter in the soil of the fallow (without plant residues). In the fallow soil without the input of labile carbon with plant residues, the carbon of the stable pool should be decomposed. Our data on the total CO_2 emission without the analysis of its isotopic composition, the differentiated estimates of the C4 and C3 losses, and the calculation of the decomposition constants for the SOM point to the more intensive decomposition of the humus in the control fallow soil as compared with the decomposition of the C3 organic matter in the soil with corn residues applied for five years. This effect was observed for all the particle-size fractions and for the whole soil. The emission of old C3 carbon from the soil with the corn residues comprised 27–81% of the emission in the control variant.

In the nonfractionated soil, the value of the old C3 carbon emission was inversely proportional to the amount of C4 carbon introduced into the soil with the plant residues. In the particle-size fractions, this dependence was less distinct, probably, due to the small losses and disturbances of the physical stability of the C4 organic matter in the course of the particle-size fractionation.

Mechanisms of the organic matter stabilization. The decrease in the loss of the old (stable) SOM with the addition of the young (labile) carbon into the soil manifested itself as a negative priming effect; i.e., the destruction of the stable C3 pool was slowed down due to the predominant decomposition of the readily decomposable C4 substrate. We did not find any signs of stabilization of the young carbon in the soil. However, its presence in the soil favored the stabilization of the old carbon due to the negative priming effect, when the soil microorganisms began to utilize the more labile C4 substrate.

In soil studies, great attention is usually paid to the influence of the positive priming effect on the SOM decomposition. This effect consists of the increasing rate of the C3 carbon decomposition as labile energy substrates (sugars, amino acids, and other readily decomposable compounds comprising root exudates) enter the soils [23, 24]. Unlike the positive priming effect leading to the soil humus decomposition, the negative priming effect can be considered as a mechanism of the SOM stabilization. The importance of this mechanism has to be assessed for other soil types and ecosystems. However, even nowadays, there are literature data that confirm the possibility of such a mechanism. The account for the ^{14}C isotope that entered the atmosphere during the nuclear weapon tests in the 1950s–1960s and was included into the carbon cycle shows that up to 95% of the soil respiration is related to the young CO_2, the age of which does not exceed ten years [29]. Consequently, the predominant decomposition of young labile substrates is the main mechanism determining the carbon budget in the soil.

The manifestation of the negative priming effect in our experiments points to the significance of the chemical stability of the SOM as the main mechanism of the carbon stabilization in soils. However, other mechanisms of the old C3 carbon stabilization are also possible. The negative priming effect can also be due to the fact that the old carbon, unlike the young C4 carbon, occurs in the soil for a longer time and interacts with its mineral stabilizing components (clay minerals, bi- and trivalent cations). The predominant utilization of the young carbon added to the soil by microorganisms favors the slow decomposition of the old carbon and its interaction with the mineral soil components. Future studies should clarify the major stabilization factor: the longer time of the C3 presence in the soil with the formation of additional organo-mineral bonds or the slowing down of the decomposition of the C3 SOM due to the predominant destruction of C4 organic matter.

When plant residues do not enter the soil, microorganisms begin to decompose the old organic matter more actively. In our experiment, when plant residues (in the control variant) were absent, k_2 and k_{C3} increased even in the stable fine silt and clay fractions. Thus, the absence of fresh plant residues appears to destabilize the organic compounds bound with clay minerals. This suggestion is confirmed by the results obtained by Chenu et al. [11]. In this work, the destabilization of the clay–humus compounds was observed even upon a sharp decrease in the amount of plant residues entering the soil. The carbon content significantly decreased both in the soil and in its clay fraction upon the land use change of forest for arable lands and their cultivation for 35 years. The carbon content in the clay fraction of the agrocenosis was 41% of the initial C_{org} content in the particles <2 μm in the soil under the forest [11].

Destabilization of the organo-mineral compounds seems to take place in the deep soil horizons, where the input of young organic matter is limited (as in the control variant). This fact can explain the high rates of

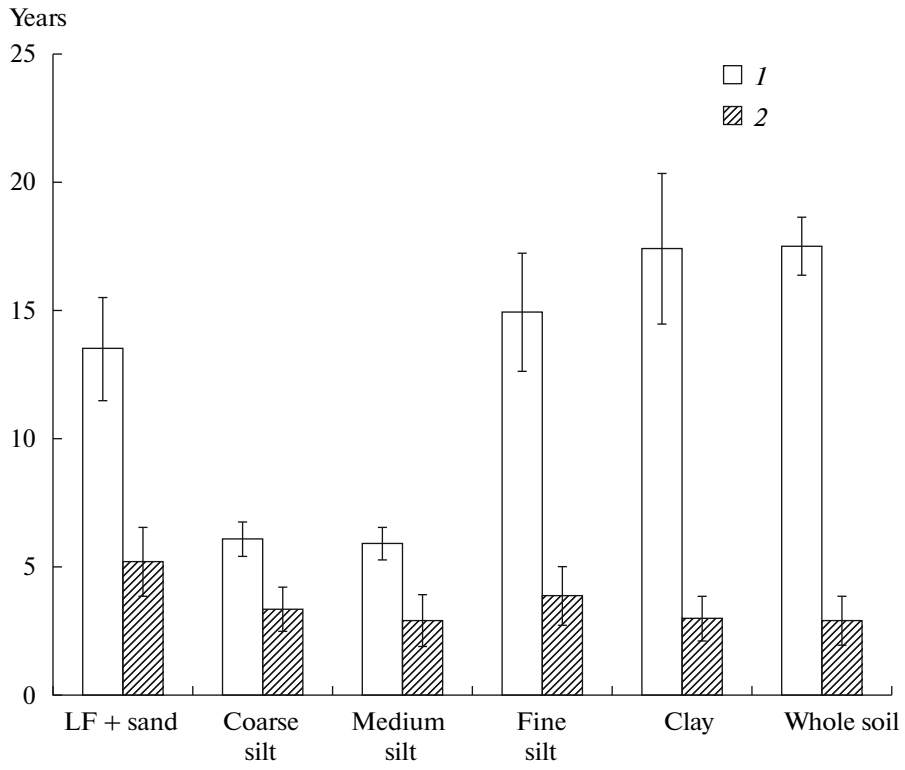

Fig. 3. The mean residence time (MRT) of the (*1*) C4 and (*2*) C3 carbon in the particle-size fractions of the agrogray soil.

the carbon renewal in the deep soil horizons upon the substitution of C4 plants for C3 plants [12]. The high priming effect due to the application of glucose and other energy substrates to the soil taken from the Bs horizon (as compared to the topsoil) [24] can be also related to the destabilization of the soil organic matter in the deep horizons.

Some authors consider the SOM of long-term fallows or deep soil horizons as a stable carbon pool, since the input of labile carbon is limited or absent. In our opinion, this carbon pool should be considered more correctly as the pool of organo-mineral compounds at the stage of destabilization. A search for an inert pool (stabile under any conditions) can hardly be successful, since the stability of the soil organic and organo-mineral compounds is related to regular inputs of fresh organic substances. In the absence of fresh plant litter for several years, it may significantly decrease.

The role of the chemical resistance of the SOM as a mechanism for its stabilization has been actively discussed [22, 23]. The significance of this process is disputable due to the intense decomposition of the stable individual organic compounds in soils [23]. This mechanism is suggested to be important only at the initial stages of the decomposition of plant residues (at the early stages of humification) upon the formation of the light fraction of the SOM [22]. The mechanism of the chemical stability is alternative to the stabilization of the organic matter in the course of its interaction with the mineral soil components and the formation of stable organo-mineral complexes with clay minerals.

As soil is an open system freely exchanging matter and energy with the environment, the budget of organic matter and the time of its renewal in different soil fractions follow the laws of dynamic equilibrium [6]. Along with the accumulation of organic matter in the soil, its decomposition takes place. During carbon accumulation in the soil or when its budget is close to zero (the variants with the application of 5.8 and 1.9 kg of C/m^2 of corn residues), the chemical stability of the organic molecules is the main mechanism of the carbon stabilization. Under a positive or zero organic matter budget in the soil, the k_2 and k_{C3} in the LF + sand and in the fine organo-mineral (fine silt and clay) fractions were approximately equal. Therefore, upon the humus degradation, the stability of the organo-mineral compounds is higher than that of the organic matter in the LF + sand fraction. In this case, the interaction of the SOM with clay minerals is the main mechanism slowing down the decomposition of the humus and ensuring the presence of organic matter for a long time.

Thus, our data point to the importance of the chemical stability as a mechanism of the carbon stabilization in ecosystems with SOM accumulation. In

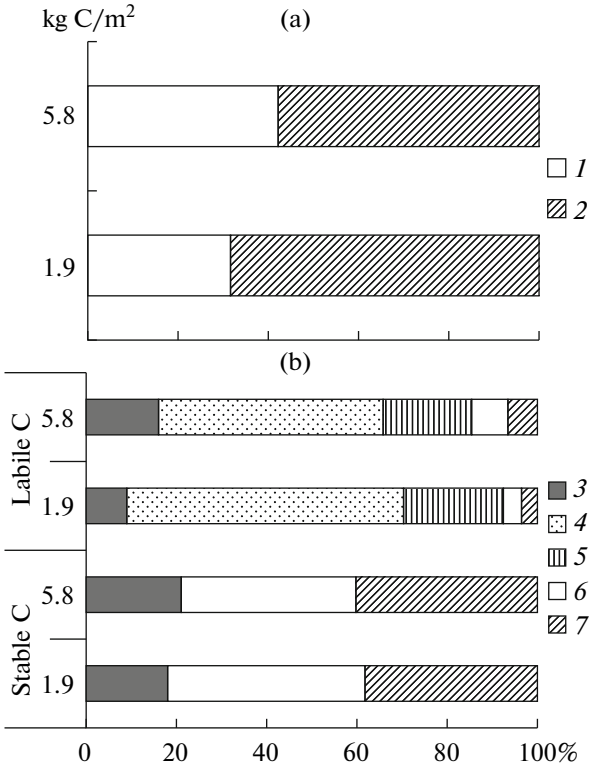

Fig. 4. (a) Labile (*1*) and stable (*2*) carbon pools in the bulk mass of the agrogray soil and (b) the contributions of the isolated particle-size fractions to the labile and stable soil organic matter pools: (*3*) LF + sand, (*4*) coarse silt, (*5*) medium silt, (*6*) fine silt, and (*7*) clay.

other words, the predominant decomposition of the newly entering plant residues by the soil microorganisms (in comparison with the decomposition of the old SOM), or the negative priming effect, promotes the carbon stabilization in the soil. In the situations when a carbon cycle with losses is formed (a negative carbon budget in the soil), the carbon stabilization in the form of organic compounds strongly bound with clay minerals acquires special importance.

The proportion between the stable and labile organic matter pools in the soil.. The labile A_1 pool with k_1 did not exceed 6.2% of the total C_{org} content; in this case, the approximation of the cumulative carbon losses as CO_2 according to the double exponent law (Eq. (1)) indicates that more than 90% of the organic matter can be considered as the stable pool.

The labile pool's size estimated as the portion of the young carbon in the SOM turned out to be higher and amounted to 7.8 and 18.0% of the total C_{org} content in the variants with the application of 1.9 and 5.8 kg of C/m^2, respectively. The constants of the labile pools calculated by the two methods were different: the k_{C4} in all the variants was lower than the k_1. Thus, the constants k_1 and k_{C4}characterize two different labile pools. The first pool is the most labile (1–6% of the total C_{org} content) with the k_1 decomposition constant and with the MRT of 10 to 60 days. By its size and renewal time, it approximately corresponds to the microbial biomass, which decomposes polymer substrates in the soil [5]. The second pool is larger and more stable; it is characterized by the k_{C4} decomposition constant and the MRT of 3–5 years, it includes derivates of plant residues at the early stages of their decomposion. The values of k_{C3} were close to those of k_2. Both constants characterize the stable SOM pool irrelative of the calculation method.

The values of k_{C4} were higher than those of k_{C3}. Therefore, the young carbon can be considered as a labile pool. However, the k_{C4} and k_{C3} in the particle-size fractions were different. The young and old SOM pools in the coarse and medium silt fractions were almost the same in their stability. Therefore, we suppose that the whole carbon of these fractions can be considered as labile. Especially sharp differences (by 4–10 times) between k_{C4} and k_{C3} were observed in the light, fine silt, and clay fractions. Consequently, the young C4 carbon in these fractions is considered as part of the labile pool, whereas the old (C3) carbon represents the stable SOM pool in the same fractions. Thus, the labile carbon pool is actually the sum of several pools with high constants of carbon renewal irrelative of its origin (C4 or C3). The following components contribute to the labile C pool: (a) the total carbon (C_{org}) in the coarse and medium silt fractions and (b) the young (C4) carbon in the light, fine silt, and clay fractions. Taking into account the masses of the fractions, the labile pool amounts to 32 and 42% in the variants with the application of 1.9 and 5.8 kg C/m^2, respectively (Fig. 3a). Thus, the size of the labile pool increased upon the application of the high rate of plant residues. Taking into account that the low and high doses of plant residues were equivalent to the carbon amounts that entered the soil with the plant litter in the fertilized agrocenoses and in the meadow ecosystem, respectively, the labile pool in the natural ecosystem is significantly higher than that in the agroecosystem. This fact well agrees with the literature data [11, 25].

The distribution of the labile and stable pools of organic matter by the particle-size fractions with due account for their masses (Fig. 3b) shows that the most significant contribution to the labile carbon pool (61%) is associated with the coarse silt fraction due to its high mass and the high renewal rate in its C3 and C4 labile components. Unlike the coarse silt fraction, the light fraction, which is traditionally considered labile [2, 14, 21, 25], consists of both labile and stable components and contributes to about 10–15% of the labile soil carbon pool and 17–21% of the stable soil carbon pool. The stable pool is mainly composed of the old

C3 carbon of the fine silt and clay fractions in equal portions.

CONCLUSIONS

The combination of three methodological approaches—the particle-size fractionation, the study of the ^{13}C abundance upon the C3–C4 vegetation changes, and the incubation method—allowed separating the soil organic matter into the labile and stable pools after the decomposition of corn residues in the agrogray soil formed under C3 plants. The heterogeneity of the particle-size fractions was determined using a differentiated estimate of the young carbon (C4) of the plant residues and the old carbon (C3) of the soil organic matter and the $^{13}C : {}^{12}C$ ratio in these fractions. The size of the labile pool depended on the amounts of C4 litter and comprised 32 and 42% of the C_{org} content in the experiments with the addition of corn residues in amounts equivalent to the input of plant residues in a fertilized agrocenosis and in a natural meadow, respectively. The stable organic matter pool was predominantly formed from the old carbon of the fine silt and clay fractions in equal proportions. A small contribution (17–21%) to the stable pool was made by the C3 carbon of the light fraction separated the sand fraction. The labile SOM pool consisted of the C_{org} (C3 + C4) of the coarse and medium silt fractions and of the young C4 carbon in the light, fine silt, and clay fractions. The contribution of the carbon of the coarse silt fraction was the highest (61%) due to the high content of this fraction in the soil mass.

The application of C4 carbon provoked a negative priming effect in the soil, which inhibited the organic matter decomposition and favored the stabilization of the old C3 carbon. On the contrary, in the absence of fresh plant residues in the fallow soil, the destabilization of the organic matter was observed even in the stable fine silt and clay fractions.

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