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SOIL CHEMISTRY

# Assessing the Stability of Soil Organic Matter by Fractionation and $^{13}C$ Isotope Techniques

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**Abstract**—Carbon pools of different stabilities have been separated from the soil organic matter of agrochernozem and agrogray soil samples. The work has been based on the studies of the natural abundance of the carbon isotope composition by C3–C4 transition using the biokinetic, size-density, and chemical fractionation (6 M HCl hydrolysis) methods. The most stable pools with the minimum content of new carbon have been identified by particle-size and chemical fractionation. The content of carbon in the fine fractions has been found to be close to that in the nonhydrolyzable residue. This pool makes up 65 and 48% of $C_{org}$ in the agrochernozems and agrogray soils, respectively. The combination of the biokinetic approach with particle-size fractionation or 6 M HCl hydrolysis has allowed assessing the size of the medium-stable organic carbon pool with a turnover time of several years to several decades. The organic matter pool with this turnover rate is usually identified from the variation in the $^{13}C$ abundance by C3–C4 transition. In the agrochernozems and agrogray soils, the medium-stable carbon pool makes up 35 and 46% of $C_{org}$, respectively. The isotope indication may be replaced by a nonisotope method to significantly expand the study of the inert and medium-stable organic matter pools in the geographical aspect, but this requires a comparative analysis of particle-size and chemical fractionation data for all Russian soils.

*Keywords*: labile and stable pools labile and stable pools of soil organic matter, C3–C4 transition, particle-size and density fractionation, nonhydrolyzable organic matter, $CO_2$ emission from the soil

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## INTRODUCTION

Soils are the main sink of organic carbon in the terrestrial ecosystems retaining it in their humic substances for a long time and regulating the gas composition of the atmosphere [4]. Therefore, information about the rate and time of carbon turnover in the soils of Russia is necessary for predicting the implications of global climate changes.

Data on the sizes and mean residence times of different carbon pools and the stabilization mechanisms of the soil organic matter (OM) are necessary for understanding the regulation mechanisms of the carbon cycle under variable environmental conditions and modeling the carbon cycle in terrestrial ecosystems.

The simulation of the carbon cycle in ecosystems and the study of the physical and chemical mechanisms of OM stabilization involve the subdivision of the OM into the labile and stable pools [3–5, 13, 20–22, 24]. Soil scientists use a wide range of methodological approaches for this purpose. The OM is most frequently subdivided into the labile and stable pools by the biokinetic method based on the mathematical approximation of cumulative carbon loss during the long-term incubation of soil by a double or triple exponential decay model [5, 12, 13, 21, 22]. The two-pool model of OM most reliably describes the OM loss in the form of $CO_2$ with a double exponential cumulative curve [22]. This method conceptually subdivides the OM into the labile and recalcitrant pools. The mean residence time of the labile OM pool varies from several days to several weeks; therefore, its size (1–5% of $C_{org}$) can be relatively exactly determined in an incubation experiment. The stable OM pool, which makes up 95–99% $C_{org}$, is heterogeneous. From the approximation data, its mean residence time is 10–30 years [16, 21, 22], although the OM age determined by radiocarbon dating exceeds 1000 years [14, 21]. Hence, the incubation experiments cannot reliably determine the size of the most stable OM pool with a mean residence time of hundreds and thousands of years.

Along with the biokinetic approach, physical and chemical fractionation techniques are used for the partitioning OM into components of different stabilities [3, 20]. The microbial biomass and free organic substances (semidecomposed plant residues of low bulk density) are usually classified as labile components [1, 3, 5, 17, 24]. The specific humic substances strongly bound to the mineral soil component are considered stable [3, 10, 20, 21]. The free and strongly bound organic components are frequently separated by particle-size and density fractionation (combination of methods for particle separation by size and density) and chemical extraction [1, 3, 5, 17, 20, 21]. The labile components enter in so-called light fractions (LFs), which are separated by the density fractionation of soil in heavy liquids [3, 15]. The heavy fractions, on the contrary, belong to the stable OM pools [20]. The stable OM of fine-silt and clay particles is separated by particle-size fractionation [1, 3], and the stable OM that cannot be extracted, oxidized, or hydrolyzed by chemical reagents is separated by chemical fractionation [10, 20, 21].

The combination of mathematical simulation and fractionation revealed relationships between the conceptual and physical OM pools. The stable, slowly decomposable OM pools include either the clay fraction [1, 24] or the OM pool resistant to acid and alkaline hydrolysis [16, 21].

Another approach to the partitioning OM into the labile and stable pools is based on the method of $^{13}C$ natural abundance by C3–C4 transition, e.g., at the growing of C4 corn ($\delta^{13}C$ is –12 to –14 permille) in a monoculture after C3 plants ($\delta^{13}C$ is –26 to –27 permille) [5, 20]. This approach allows assessing the relatively stable carbon pool with a mean residence time of decades to several hundreds of years [5, 8, 20]. The resistance of this pool to decomposition under variable temperature and soil moisture is of great importance in relation to the greenhouse effect, because climate changes are usually predicted for 50–100 years, i.e., within the pool time period [4, 24].

Each of the above methods has advantages and shortcomings. In distinction from the mathematical approximation, the partitioning OM into pools of different stabilities using the $^{13}C$ isotope method and physical and chemical fractionation allows determining the sizes of the functional pools rather than the conceptual ones [8, 16, 17, 21, 24]. At the same time, the labor intensity and the change in the stability of the OM pools during the separation procedure are disadvantages of physical and chemical fractionation. An important shortcoming of the method of $^{13}C$ abundance is related to the predominance of C3 plants in the temperate zones; therefore, the applicability of this method is limited to the continuous corn agroecosystems.

We think that the combination of all the approaches in field experiments with C3–C4 transition will not only reveal the stable OM fractions using independent methods but also select the simplest and most accessible procedure for the determination of the stable OM pool in the main soil types of the Russian Federation.

The aims of this study were (1) to identify the stable OM pool using the physical and chemical fractionation in combination with the method of $^{13}C$ natural abundance by C3–C4 transition and (2) to compare the size of this pool with the conceptual stable OM pool calculated by the biokinetic method.

## OBJECTS AND METHODS

The studies were performed on agrogray soils ($C_{org}$ 1.8%, $pH_{KCl}$ 4.6) of the Experimental Field Station of the Institute of Physicochemical and Biological Problems of Soil Science (Pushchino, Moscow oblast) in 2000–2010 and on an agrochernozem ($C_{org}$ 3.4%, $pH_{KCl}$ 5.3) of the experimental plots (established in 1966) of the Voronezh Branch of the All-Russian Research Institute of Corn in 2008–2010.

The carbon turnover rate in the agrogray soil was determined in a stationary microplot experiment established on a continuous fallow field. Cut green corn residues were annually incorporated into the 0- to 25-cm soil layer at a rate of 3 kg dry matter/$m^2$ during 5 years, which gave 5.8 kg C/$m^2$ for the entire period. The C : N ratio in the original material was 27. The plot area was 1 $m^2$; the experiments were performed in triplicate. The soil was annually trenched during the embedding of organic materials in the fall and ripped in the spring to retain the fallow. After the end of each experiment, soil samples were taken from a depth of 0 to 20 cm at 5 points on each plot to prepare a mixed sample.

Agrochernozem samples were taken from continuous corn at a depth of 0 to 20 cm in the treatment with the complete mineral fertilizer N120P60K60. The soil was sampled from 15 points on each plot. A mixed sample was prepared from each 5 individual samples. All the characteristics were determined in the mixed samples in triplicate. Samples of the original soil taken before the establishment of the experiment in 1966 were simultaneously analyzed.

**Soil fractionation.** The soil material was separated into particle-size fractions according to the standard procedure [2]. For this purpose, 50 g of air-dry soil was mixed with water (25% of the soil weight) and dispersed by trituration for 30 min. The coarse fraction (>100 μm) was separated into the organic (LF) and mineral components by water flotation. The fraction <0.1 mm was sonificated at 100 W for 15 min and separated into particle-size fractions by sedimentation in water. After an exhausting extraction, the fractions were precipitated by centrifugation at 4000 rpm (1600 g) for 30 min.

The primary particles distribution in the studied soil was characterized by the pipette method after the dispersion of aggregates with sodium pyrophosphate.

The density fractionation was performed by the separation of a 10-g dry soil sample with water solu-

tions of sodium polytungstate (1.4, 1.8, and 2.2 g/cm$^3$) [19]. The soil material was separated into the density fractions <1.4, 1.4–1.8, 1.8–2.2, and >2.2 g/cm$^3$. After an exhausting extraction, the fractions were washed with water from sodium polytungstate and precipitated by centrifugation as the particle-size fractions.

The acid hydrolysis was performed with 6 M HCl in soil samples of 2 g at 120°C for 20 h according to the procedure described for the hydrolysis of the humic and fulvic acids [9].

**Carbon isotope composition.** The contents of organic carbon and nitrogen and the $^{13}C$ : $^{12}C$ ratio in the soil samples and the separated fractions were determined on a Thermo MAT 253 mass spectrometer (Finnigan, Germany) equipped with a Eurovector Euro EA elemental analyzer (Italy). The content of $^{13}C$ was expressed in $\delta^{13}C$ units against the international Vienna-Pee Dee Belemnite (VPDB) standard:

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

where $R_{sample}$ and $R_{stand}$ denote the $^{13}C$ : $^{12}C$ ratios in the sample and the standard, respectively. The $\delta^{13}C$ value for VPDB is 0 promille; $R_{stand} = 0.0111802$.

The isotope composition of the corn residues were simultaneously determined: the value of $\delta^{13}C$ in the corn belowground material and stubble was –12.0 permille in the agrogray soil and –11.6 permille in the agrochernozem.

The portion of the C4 corn carbon in the soil was calculated from the equation

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

where $\delta^{13}C_s$ is the $\delta^{13}C$ value in the soil sample; $\delta^{13}C_4$ is the $\delta^{13}C$ value in the young organic matter resulting from the decomposition of the corn residues; $\delta^{13}C_3$ is the $\delta^{13}C$ value in the original soil samples taken before the establishment of the experiment; and $f$ is the portion of $C_4$-type organic matter, i.e., newly formed humus accumulated during 5 years in the agrogray soil and during 44 years in the agrochernozem.

The subdivision of the OM into the labile and recalcitrant pools and the calculation of their decomposition constants were based on the approximation of the cumulative $CO_2$ emission during the experiment from the equation

$$Y = A_1 e^{-k_1 t} + A_2 e^{-k_2 t}, \quad (3)$$

where $Y$ is the content of organic carbon ($C_{org}$) in the soil minus the cumulative $C-CO_2$ loss during the time $t$ expressed as the portion of the initial $C_{org}$ in the soil; $k_1$ and $k_2$ are the decomposition constants of the labile and stable pools, respectively; $A_1$ and $A_2$ are the sizes of the labile and recalcitrant pools, respectively; and $A_2 = 1 - A_1$.

To separate the inert pool, the cumulative $C-CO_2$ loss was approximated by a double exponential function with a constant. Then, Eq. (3) takes the form

$$Y = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_3, \quad (4)$$

i.e., the third OM pool appears in the model, which is not decomposed during the incubation and is calculated as the difference between the $C_{org}$ and two OM pools ($A_1$ and $A_2$): $A_3 = 1 - A_1 - A_2$.

The separation of the inert OM pool by physical and chemical fractionation techniques allows assessing the size recalcitrant of OM pool by an independent method. Then, Eq. (3) takes the form

$$Y = A_1 e^{-k_1 t} + (1 - A_1 - A_3) e^{-k_2 t} + A_3. \quad (5)$$

According to Eq. (5), the $A_3$ value is set in the model as a parameter, and the second OM pool is calculated as the difference $A_2 = 1 - A_1 - A_3$ with the decomposition constant $k_2$. Thus, Eqs. (4) and (5) are equivalent. They subdivide the OM into three pools: labile pool $A_1$, recalcitrant pool $A_2$, and inert pool $A_3$. In Eq. (4), the sizes of all the pools are determined during the approximation, while, in Eq. (5), the stable OM pool is studied by fractionation, and the two other pools are calculated from the exponential equation.

The mean residence time (MRT) of a pool was calculated as the inverse value of the decomposition constant

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

Thus, the values inverse to the constants $k_1$ and $k_2$ correspond to $MRT_1$ and $MRT_2$ for the labile and recalcitrant pools, respectively.

The cumulative $CO_2$ loss by emission during the OM decomposition was determined in an incubation experiment at constant temperature and water content for 430 days. For this purpose, a soil sample of 10 g was placed in a 120-mL vial and wetted to 70% of the water holding capacity (WHC). The sealed vials were incubated at a constant temperature of 22°C. The constant water content of the samples was controlled by weighing. Gas samples were taken on the 1st, 3rd, 5th, 7th, 10th, and 14th days and then weekly. The soil respiration rate was determined from the enrichment of $CO_2$ in the intervals between the gas samplings. The vials were periodically ventilated when the concentration of $CO_2$ in the gas samples exceeded 2%. The concentration of $CO_2$ was determined on a Kristallux-4000 chromatograph with a thermal conductivity detector. The gas mixture was separated in 3-m columns packed with Porapak-Q at 30°C.

## RESULTS AND DISCUSSION

From the data on the $^{13}C$ natural abundance, the portion of the new OM carbon resulting from the decomposition of corn residues in the upper 0- to 20-cm layer was 7% in the agrochernozem and 28% in

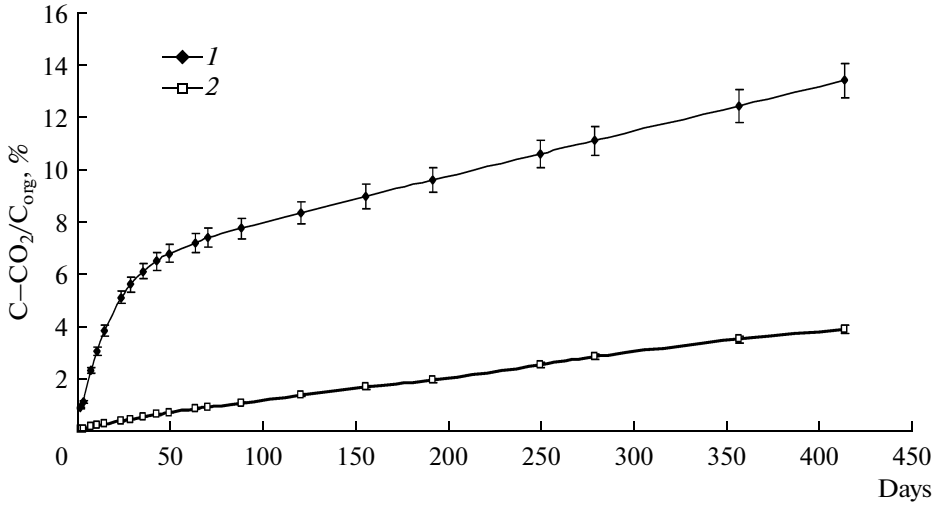


**Fig. 1.** Cumulative emission of $CO_2$ during the incubation of (*1*) an agrogray soil and (*2*) agrochernozem at 22°C and 70% of the WHC.

the agrogray soil. Hence, the OM pool of old carbon formed from the residues of C3 plants grown before continuous corn was 93% in the agrochernozem and 72% in the agrogray soil. As was shown earlier [8], the low enrichment of the OM with new carbon in the agrochernozem is related to not only the high stability of the OM but also its low input with plant residues at the growing of corn for silage. In the agrogray soil, on the contrary, the input of new carbon with plant residues was significantly higher than in the agrochernozem: 1160 and 110–155 g C/m$^2$ per year, respectively. The agrogray soil and the agrochernozem also differed in OM storage: the carbon reserve in the upper 100-cm layer was 24–27 kg C/m$^2$ for the agrochernozem and did not exceed 6–7 kg C/m$^2$ for the agrogray soil. Thus, the slow substitution of old carbon by new carbon from corn was also due to the significant excess of the carbon storage in the agrochernozem over that in the agrogray soil.

The method of $^{13}C$ natural abundance by C3–C4 transition allows separating the OM pool with a turnover time of decades to 200 years [20]. The size and turnover time of the pool depend on the amount of plant residues put into the soil and the duration of cropping plants with another type of photosynthesis [8]. In field experiments, the duration of continuous corn varied significantly: 44 years on the agrochernozem and only 5 years on the agrogray soil. Thus, the comparison of the carbon turnover rates in the field experiments under C3–C4 transition characterizes the apparent stability of the OM and does not allow unambiguous conclusions to be drawn about the actual stability of the OM and the stable carbon pool in the soil to be separated. The independent assessment of the actual stability of the OM was performed using the biokinetic method based on the determination of the $CO_2$ emission during the decomposition of the OM in controlled incubation experiments.

The cumulative carbon loss as $CO_2$ during the entire incubation period indicates a higher stability of OM in the agrochernozem than in the agrogray soil: the loss of OM in the agrochernozem at the optimum temperature and water content during 430 days of the experiment was lower than that in the agrogray soil by 3.4 times (3.9 and 13.2% of $C_{org}$, respectively) (Fig. 1). The calculation of the two-pool model parameters from Eq. (3) confirms this conclusion (Table 1): the size of labile pool $A_1$ and both decomposition constants $k_1$ and $k_2$ in the agrogray soils were higher than in the agrochernozem. In both soil types, the OM was divided into two uneven parts: a very small labile pool and a large recalcitrant pool. The size of the labile pool was 6% of $C_{org}$ in the agrogray soil and only 0.3% of $C_{org}$ in the agrochernozem; the mean residence times were 23 and 16 days, respectively. The recalcitrant pool reached more than 90% of the OM in the agrogray soil and 99% of the OM in the agrochernozem with the mean residence times being 14 and 30 years, respectively.

The stability of the OM in the agrochernozem is due to the increased content of stable organic and mineral soil components. The OM of the agrochernozem was characterized by an increased content of aromatic functional groups. As was noted earlier, the portion of aryls in the OM was 35 and 25% of $C_{org}$ in the agrochernozem and the agrogray soil, respectively [6]. The determination of the primary particles distribution (Table 2) indicates a high content of physical clay in the agrochernozem compared to the agrogray soil, which determines the classification of the soils into different groups. The agrochernozem has a heavy loamy texture, and the agrogray soil has a medium loamy texture [2]. Hence, along with the chemical

**Table 1.** Approximation parameters of the cumulative carbon loss in the form of $CO_2$ during the incubation of an agrogray soil and an agrochernozem at 22°C and 70% of the WHC

| Assessment method | $A_1$ | $k_1$, days$^{-1}$ | $MRT_1$, days | $A_2$ | $k_2$, years$^{-1}$ | $MRT_2$, years | $A_3$ | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| | Agrogray soil | | | | | | | |
| Biokinetic method, Eq. (3) | 0.062 ± 0.002 | 0.062 ± 0.007 | 16.2 ± 0.4 | 0.938 ± 0.002 | 0.070 ± 0.002 | 14.2 ± 0.4 | 0 | 0.982 |
| Biokinetic method, Eq. (4) | 0.040 ± 0.001 | 0.177 ± 0.014 | 5.7 ± 0.5 | 0.090 ± 0.011 | 2.26 ± 0.11 | 0.44 ± 0.01 | 0.87 ± 0.02 | 0.999 |
| Combination of fractionation with the biokinetic method | 0.061 ± 0.002 | 0.065 ± 0.008 | 15.5 ± 0.8 | 0.46 ± 0.06 | 0.151 ± 0.008 | 6.6 ± 0.1 | 0.48 ± 0.01 | 0.983 |
| | Agrochernozem | | | | | | | |
| Biokinetic method, Eq. (3) | 0.003 ± 0.001 | 0.044 ± 0.009 | 22.8 ± 0.9 | 0.997 ± 0.001 | 0.033 ± 0.002 | 30.1 ± 0.2 | 0 | 0.999 |
| Biokinetic method, Eq. (4) | 0.002 ± 0.000 | 0.055 ± 0.01 | 18.1 ± 0.8 | 0.29 ± 0.01 | 0.123 ± 0.029 | 8.1 ± 0.4 | 0.71 ± 0.06 | 0.999 |
| Combination of fractionation with the biokinetic method | 0.002 ± 0.000 | 0.052 ± 0.01 | 16.2 ± 0.4 | 0.35 ± 0.03 | 0.099 ± 0.001 | 10.0 ± 0.1 | 0.64 ± 0.08 | 0.999 |

**Table 2.** Primary particles distribution in an agrogray soil and agrochernozem, %

| Soil type | Fractions, mm | | | | | | Total particles, mm | |
|---|---|---|---|---|---|---|---|---|
| | 1–0.25 | 0.25–0.05 | 0.05–0.01 | 0.01–0.005 | 0.005–0.001 | <0.001 | physical clay <0.01 | physical sand >0.01 |
| Agrogray soil | 2 | 13 | 47 | 14 | 12 | 12 | 37 | 63 |
| Agrochernozem | 8 | 12 | 24 | 10 | 21 | 26 | 57 | 43 |

**Table 3.** Distributions of soil mass, carbon, and the C : N ratio among the fractions of an agrogray soil and agrochernozem

| Method | Fraction, μm, g/cm$^3$ | Mass | $C_{total}$ | $\frac{C}{N}$ | Mass | $C_{total}$ | $\frac{C}{N}$ |
|---|---|---|---|---|---|---|---|
| | | % | | | % | | |
| | | Agrogray soil | | | Agrochernozem | | |
| Unfractionated OM | | 100.0 | 1.86 ± 0.05 | 10.2 ± 0.2 | 100.0 | 3.4 ± 0.31 | 12.4 ± 0.3 |
| Particle-size fractionation | Sand + LFs, 100–1000 | 6.2 ± 0.9 | 5.13 ± 0.21 | 15.0 ± 0.2 | 25.0 ± 2.3 | 0.89 ± 0.11 | 17.5 ± 0.4 |
| | Coarse silt, 10–100 | 56.6 ± 1.3 | 0.61 ± 0.02 | 12.3 ± 0.1 | 32.2 ± 2.1 | 1.12 ± 0.10 | 17.0 ± 0.3 |
| | Medium silt, 10–5 | 5.6 ± 0.9 | 2.55 ± 0.03 | 12.0 ± 0.2 | 5.6 ± 0.8 | 5.52 ± 0.12 | 15.4 ± 0.2 |
| | Fine silt, 1–5 | 11.0 ± 1.1 | 3.87 ± 0.10 | 10.2 ± 0.2 | 15.8 ± 1.5 | 6.64 ± 0.15 | 13.6 ± 0.2 |
| | Clay, <1 | 9.6 ± 1.2 | 4.61 ± 0.02 | 8.6 ± 0.1 | 21.6 ± 2.1 | 5.41 ± 0.08 | 10.4 ± 0.2 |
| Density fractionation | <1.4 | 0.7 ± 0.2 | 28.91 ± 1.09 | 20.0 ± 0.8 | 0.6 ± 0.1 | 27.94 ± 1.81 | 21.6 ± 0.8 |
| | 1.4–1.8 | 0.6 ± 0.3 | 12.37 ± 0.78 | 14.8 ± 0.5 | 19.2 ± 1.3 | 9.61 ± 0.52 | 13.3 ± 0.3 |
| | 1.8–2.2 | 9.3 ± 1.1 | 6.11 ± 0.52 | 9.0 ± 0.3 | 24.3 ± 2.4 | 3.96 ± 0.21 | 7.7 ± 0.3 |
| | >2.2 | 86.0 ± 2.9 | 0.82 ± 0.12 | 6.8 ± 0.3 | 49.1 ± 2.7 | 0.71 ± 0.04 | 7.6 ± 0.2 |
| Hydrolysis with 6 M HCl | Nonhydrolyzable OM | 91.5 ± 1.3 | 1.06 ± 0.07 | 27.2 ± 0.9 | 80.0 ± 2.4 | 2.9 ± 0.09 | 22.8 ± 0.8 |

recalcitrance, another mechanism of OM stabilization—the formation of stable clay–humus complexes—plays an important role in the formation of the high actual stability of the OM in the agrochernozem.

The biokinetic method allowed comparing the stability of the OM in the studied soils regardless of the environmental conditions; however, the MRT values of the recalcitrant pools ($MRT_2$) were relatively low: 14 and 30 years in the agrogray soil and the agrochernozem, respectively. It is known that the age of the OM in gray forest soils and chernozems determined by radiocarbon dating is about 1000 years and 3500 years, respectively [14]; therefore, the recalcitrant pool with the decomposition constant $k_2$ is heterogeneous and can be subdivided into a recalcitrant pool and an inert pool. According to Eqs. (4) and (5), the inert OM pool $A_3$ is almost not decomposed in the 430-day-long incubation experiment; i.e., it does not contribute to the emission of $CO_2$. In the predictions of climatic changes for the next 100 years, a low contribution (about 5%) to the carbon dioxide fluxes into the atmosphere is usually attributed to the inert pool [11, 14, 23]. In distinction from the short-term prognoses for the variations of the carbon cycle related to the current climate changes, the simulation of the carbon cycle during the soil evolution with consideration for diagenesis should consider this pool as recalcitrant, i.e., decomposing at a very low rate, and not inert.

Though the method of variations in the natural abundance of $^{13}C$ upon C3–C4 change in vegetation did not allow us to determine the real stability of OM, its application in combination with the particle-size, densimetric, and chemical fractionation of OM made it possible to determine the stability of separated fractions from data on the proportion between "new" and "old" carbon in them and to estimate the size of inert pool $A_3$.

The distributions of the soil mass and OM among the particle-size fractions in both soils are similar (Table 3). The coarse silt fraction makes the largest contribution. The main differences between the two soil types are due to the double excess of the fine fractions (fine-silt and clay) in the agrochernozem over their content in the agrogray soil. An inverse relationship was observed between the fraction size and the content of OM, as well as a direct relationship between the fraction size and the C : N ratio, which well agrees with the literature data [1, 3]. The content of $C_{org}$ in all the fractions of the agrochernozem was higher than in the agrogray soil, except for the LFs. The concentration of OM in the LF of the agrogray soil is higher than in that of the agrochernozem because of the input of a large amount of plant residues into the agrogray soil.

The distribution of OM among the fractions with consideration for the content of $C_{org}$ indicates that the major part of the OM (60–70%) in both soils is concentrated in the fine fractions (Fig. 2a). The portion of the LFs in the OM of the agrogray soil was higher than in the OM of the agrochernozem by more than 2 times. The contribution of the fine fractions, on the contrary, was larger in the agrochernozem due to the high carbon concentration and the larger amount of fine-silt and clay particles in this soil.

The content of new carbon in the particle-size fractions of both soils was contrasting and monotonically decreased with the decreasing size of the particle-size fractions (Fig. 3a).

The determination of the isotope composition of the fractions confirmed their heterogeneity and allowed assessing the content of old carbon in the LFs, which are traditionally considered labile, as well as the content of new labile C4 carbon in the fine fractions, which predominantly contain the stable carbon forms. Even at the very high input of plant residues into the agrogray soil significantly exceeding the carbon input in the agroecosystem, the OM of the coarsest fraction (250–1000 μm) contained only 69% new carbon (Fig. 3a), and the old carbon composed the remaining 31%. The carbonaceous particles and metal–humic compounds present in the LFs could favor the partial stabilization of organic carbon in these fractions of the studied soils [3].

At the same time, the content of old carbon in the stable fine-silt and clay fractions significantly exceeded the portion of new carbon. The contribution of new carbon to the OM of the stable clay fraction did not exceed 15% in the agrogray soil (Fig. 3a). Thus, the portion of new carbon in the OM of the most depleted clay fraction was about 4 times smaller than that in the most enriched coarse fraction of 100–1000 μm. Hence, the method of $^{13}C$ natural abundance by C3–C4 transition confirmed the known thesis that the clay fraction, which contained the minimum amount of new carbon, is the most stable. The difference in its content between the clay and fine-silt fractions is insignificant; therefore, both fine fractions can be combined into the inert pool.

In distinction from the particle-size fractions, the distribution of soil material among the density fractions significantly varied between the soils (Table 3). The heaviest fraction >2.2 g/cm$^3$ made up the major part of the agrogray soil (more than 80%), while its portion in the agrochernozem composed only half of the mass. The content of the lighter fractions (1.4–1.8 and 1.8–2.2 g/cm$^3$) in the agrochernozem was several times higher than in the agrogray soil. The large amount of these fractions can be related to the presence of amorphous silica and fine clay particles in the agrochernozem.

The content of carbon and the C : N ratio in the density fractions is inversely proportional to their densities, which agrees with the data obtained by other authors [1, 15, 17, 20].

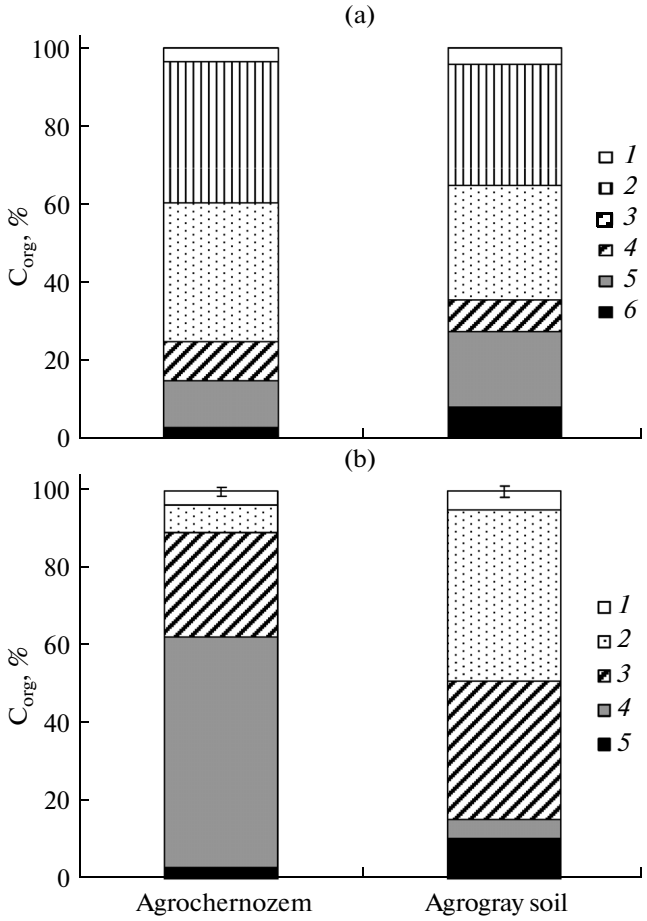


**Fig. 2.** Distribution of OM among the (a) particle-size fractions ((*1*) DOC; (*2*) clay; (*3*) fine silt; (*4*) medium silt; (*5*) coarse silt; (*6*) LF) and (b) density fractions (g/cm$^3$) ((*1*) DOC; (*2*) >2.2; (*3*) 1.8–2.2; (*4*) 1.4–1.8; (*5*) <1.4) of an agrogray soil and agrochernozem with consideration for the weight and content of carbon in the fractions.

The distribution of OM among the density fractions with consideration for the content of $C_{org}$ in the studied soil types was contrasting (Fig. 2b). The main differences between the agrochernozem and the agrogray soil are related to the contents of the fraction of 1.4–1.8 g/cm$^3$ (54 and 5% of $C_{org}$, respectively) and the heaviest fraction >2.2 g/cm$^3$ (10 and 44% of $C_{org}$, respectively). The distribution of the agrogray soil OM among the density fractions is typical for an arable mineral soil under intense biological turnover [1], while the major part of the OM in the agrochernozem is concentrated in the fraction of1.4–1.8 g/cm$^3$. The large portion of OM in the LFs is typical for organic peat soils and mineral soils with a retarded biological turnover [1]. In the agrochernozem, a soil of the steppe series, the predominant accumulation of OM in the LFs was due to the high content of fine particles in the organomineral complexes rather than to the low rate of biological turnover, which is confirmed by the

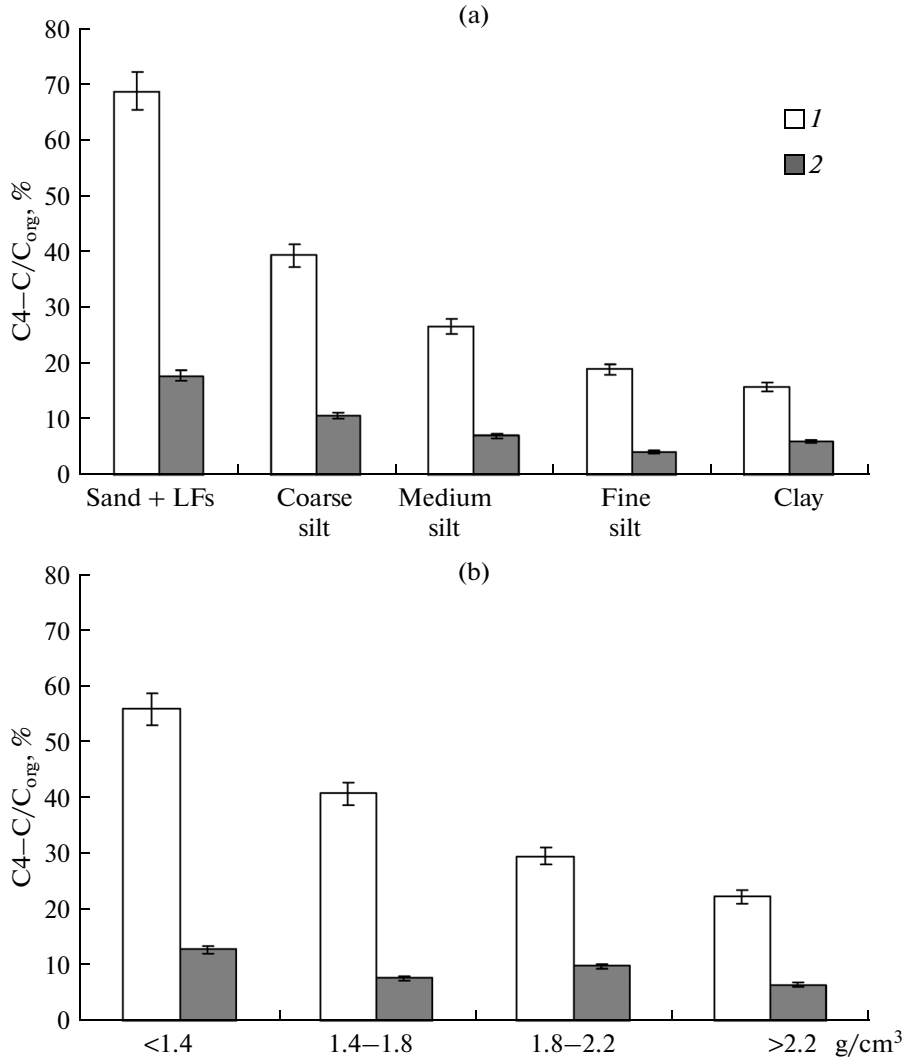


**Fig. 3.** Content of C4 carbon in the (a) particle-size and (b) density fractions of (*1*) an agrogray soil and (*2*) agrochernozem.

high content of the fine-silt and clay particle-size fractions in the agrochernozem (Tables 2, 3).

The total content of $C_{org}$ in the all the fractions was lower than 100%; i.e., the fractionation was accompanied by its loss in the form of dissolved organic carbon (DOC). Similar loss values were observed at the particle-size and density fractionation: 3–5% of $C_{org}$ (Fig. 2).

The separation into the density fractions also allowed the carbon pools with different new-to-old carbon ratios to be isolated; however, the distribution of new carbon among the light and heavy fractions was less differentiated than that in the particle-size fractions (Fig. 3b). In the agrogray soil, the portion of new carbon was 56 and 22% in the lightest (<1.4 g/cm$^3$) and heaviest (>2.2 g/cm$^3$) fractions, respectively. At the same time, the portions of new carbon in the coarsest and the finest clay fractions were more different: 69 and 15%, respectively. In the agrochernozem, the distribution of new carbon among the density fractions was nonuniform, but the density fractionation did not separate the carbon pools strongly differing in the content of new carbon. For example, the portion of new carbon in the fraction of 1.8–2.2 g/cm$^3$ was slightly larger (10%) than in the lighter fraction of 1.4–1.8 g/cm$^3$ (7.8%). Thus, the particle-size fractionation is more preferable than density fractionation for the separation of the OM into pools with contrasting contents of new carbon.

The lowest content of new carbon among the all the studied pools was found in the OM of the residue after the thermal hydrolysis of the soil with 6 M HCl (Fig. 4). This treatment allowed almost completely extracting the new carbon from the agrochernozem and decreasing its content in the agrogray soil by about 6 times compared to the OM of the unfractionated soil. Hydrolysis with 6 M HCl under heating is traditionally used for the separation of the aliphatic and aromatic

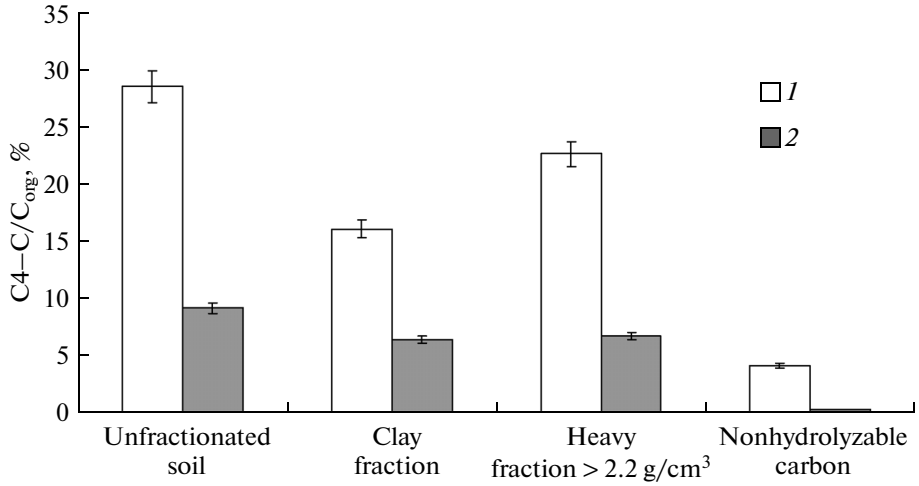


**Fig. 4.** Content of C4 carbon in the most stable fractions of OM separated by different fractionation methods from an (*1*) agrogray soil and (*2*) agrochernozem.

parts of humic and fulvic acid [10]. The results obtained for the entire OM pool confirm that only the nonhydrolyzable part of the humus acids is most stable and not the entire molecules [11]. The comparative analysis of the acid hydrolysis data with those of the particle-size and density fractionation indicates low enrichment of the nonhydrolyzable OM pool with new carbon compared to the least enriched clay fractions and the heavy density fractions (Fig. 4).

Thus, hydrolysis with 6 M HCl allows separating the most stable OM pool with the minimum content of new carbon. This conclusion is confirmed by the literature data: the radiocarbon dating and $^{13}C$ natural abundance under C3–C4 transition showed that the nonhydrolyzable OM has a very low turnover rate compared to the carbon of the unfractionated soil [16, 21]. Nonetheless, the content of new carbon in the nonhydrolyzable OM of the agrogray soil remains high: about 4%. It is known that the HCl hydrolysis can isolate the OM pool derived from the old carbon alone only at the low content of cellulose in the soil [20, 21]. The content of cellulose in the agrogray soil can be high because of the high input of plant residues: 5.8 kg C/m$^2$ for 5 years of the experiment. The treatment with hydrochloric acid does not completely hydrolyze the cellulose polysaccharides. Hydrolysis with 80% sulfuric acid under heating is used for this purpose [20, 21]. This can be a promising procedure for further experiments on the separation of the inert OM pool.

It should be noted that the partial oxidation of OM with chlorine, the extraction of mineral components (calcium, magnesium, aluminum, etc.), and the dissolution of aluminosilicates occur, along with the hydrolysis of proteins and polysaccharides, during the heating of soil [21]. Minimum iron compounds are extracted in the course of hydrolysis. The new OM is probably stabilized very slowly. It remains in the form of complexes with mobile extractable forms of mineral components even several tens of years after the input of plant residues into the soil (in the case of agrochernozems). The inert OM pool composed of the old carbon most probably includes stable organomineral complexes consisting of a condensed nonhydrolyzable aromatic part of humus acids with iron compounds. The validity of this supposition should be verified in further studies.

In the first approximation, the identified pool of OM nonhydrolyzable by hydrochloric acid can be used as the inert pool in the models of the carbon cycle in soils and terrestrial ecosystems. Nitrogen was hydrolyzed to a larger extent than carbon, and the nonhydrolyzable nitrogen pool made up only 23.2 and 29.5% of $N_{total}$ in the agrogray soil and the agrochernozem, respectively. Therefore, the C : N ratio in the nonhydrolyzable inert pool was 23–28, which exceeded the values typical for OM. In distinction from the nonhydrolyzable OM pool, the fine particle-size fractions had C : N ratios (7–9) closer to the values typical for OM. In spite of the similar sizes of the inert OM pool determined by the particle-size fractionation and 6 M HCl hydrolysis (Fig. 5), the compositions of the pools isolated by different methods were different. The obtained differences can be related to the changes in the OM composition during fractionation. The acid hydrolysis of OM involves a harder treatment than the other procedures of OM separating into the labile and stable fractions; therefore, the nonhydrolyzable OM pool is chemically disturbed and can be used only for determining the size of the inert OM. The method of density fractionation has an analogous disadvantage. The interaction of OM with sodium polytungstate during the separation results in significant changes of the OM mineralizability in the density fractions [15]. The fine fractions separated by the less destructive particle-size fractionation in water com-

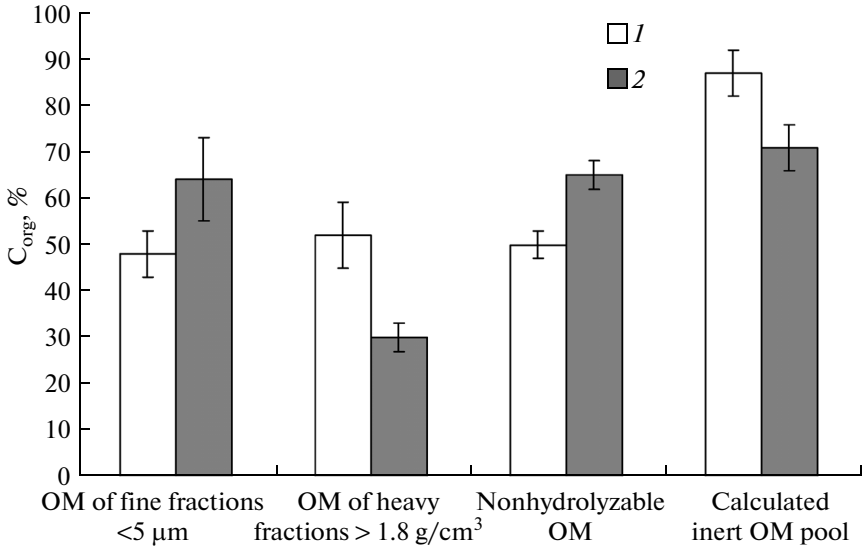


**Fig. 5.** Size of the inert OM pool in an (*1*) agrogray soil and (*2*) agrochernozem determined by different fractionation methods.

pose a pool close to the native inert OM. Thus, the content of OM nonhydrolyzable with 6 M HCl can be used as a preliminary assessment of the inert carbon pool. In distinction from the chemically disturbed nonhydrolyzable OM, the fine particle-size fractions represent a good physical model of the inert pool for simulating the effect of different environmental and anthropogenic factors on the mineralization of the stable OM in controlled laboratory conditions.

The results of long-term incubation experiments were used for assessing the decomposition constants of OM in the studied soils with consideration for a specific inert pool (Fig. 1). The introduction of the third inert pool according to Eq. (4) increased the decomposition constants of the two other OM pools $k_1$ and $k_2$ in both the agrogray soil and agrochernozem (Table 1). The size of the inert pool was 87 and 71% of $C_{org}$ in the agrogray soil and the agrochernozem, respectively; i.e., this pool is the predominant component of the OM. The inert pool $A_3$ calculated from Eq. (4) takes the highest values compared to the inert pools determined by the fractionation methods (Fig. 5).

An obvious advantage of the calculation method is the determination of the inert OM pool size using a mathematical model; i.e., its identification requires no additional soil analyses. Nonetheless, the size ratio between the inert OM pools in the two soil types leaves doubt in the reliability of this approximation. The contradiction is that the inert pool in the agrogray soil with the less stable OM is larger than that in the agrochernozem, where OM is decomposed more slowly. In addition, the size of the calculated inert pool largely depends on the duration of the incubation experiment: the shorter the incubation, the larger the inert pool undecomposed during the experiment. The incubation for a year is probably insufficient, and a longer experiment should be performed for the reliable determination of pool $A_3$ by calculation.

The determination of the inert OM pool by chemical fractionation and the calculation of the decomposition constants of the labile and recalcitrant OM pools from Eq. (5) gave different results. In this case, the size of inert pool $A_3$ was determined as the OM fraction nonhydrolyzable by 6 M HCl minus the content of new OM from C4 plants in this pool. The content of the inert pool in the agrochernozem was almost equal to that of the nonhydrolyzable OM, because the portion of new carbon was only 0.2% (Fig. 4). Because of the higher content of new carbon in the nonhydrolyzable OM of the agrogray soil, which was equal to 4%, the inert pool in the agrogray soil was smaller than the nonhydrolyzable pool by this value, in distinction from the agrochernozem. Thus, the size of the inert pool was 65 and 48% of $C_{org}$ in the agrochernozem and the agrogray soil, respectively. The higher content of the inert pool in the agrochernozem agrees with the known high stability of OM in these soils and the carbon loss as $CO_2$ observed in the incubation experiment.

At the use of fractionation in combination with the biokinetic approach, the size and decomposition rate of the labile pool (parameters $A_1$ and $k_1$) in Eq. (5) are comparable to the analogous parameters determined from Eq. (3) using the two-pool model. The recalcitrant OM pool with the decomposition constant $k_2$ was decomposed more rapidly than the analogous pool in Eq. (3) but more slowly than the inert OM pool in Eq. (4). The mean residence time of the recalcitrant pool $A_2$ in Eq. (5) was 6.6 and 10 years in the agrogray soil and the agrochernozem, respectively.

The actual turnover rate of pool $A_2$ under field conditions will be lower than the $k_2$ value, because the incubation proceeded at the optimum temperature

and water content: 22°C and 70% of the WHC. The calculation of the temperature coefficients $Q_{10}$, which we performed earlier for the agrochernozem [7], indicates a decrease of the OM decomposition constant by 3 times, when the temperature falls from 22 to 12°C, and its further decrease by 2 times, when the temperature falls from 12 to 2°C. For most soils of Russia occurring in the temperate climatic zone, the mean annual temperature is in the range of 2–12°C. Under field conditions, the mean residence time of the medium-stable OM with consideration for the obtained coefficients $Q_{10}$ can be 30–60 years. Consequently, the medium-stable OM pool with a mean residence time of several tens of years can be identified from incubation experiments performed at the temperature and water content similar to their field values. In the studied soils, the size of the medium-stable pool calculated from Eq. (5) was higher in the agrogray soil than in the agrochernozem: 46 and 35% of $C_{org}$, respectively.

Such a pool is usually identified from the OM substitution rate with new carbon in field experiments with C3–C4 transition [8, 17, 20]. However, similar field experiments are very rare in Russia; therefore, the determination of the medium-stable OM pool in laboratory experiments combining the biokinetic approach with soil fractionation can be promising in the zonal and regional aspects. The validity of this supposition should be verified by studying the zonal and intrazonal soils developed on different parent materials. The final choice of the fractionation method for determining the size of the inert OM pool also faces some difficulties. Taking into account the distributions of $C_{org}$ and $^{13}C$ in the density fractions of the agrochernozem and agrogray soil, we may state at this stage of investigation that density fractionation is the least suitable method for the determination of inert pool $A_3$. The distributions of $C_{org}$ and $^{13}C$ in the particle-size fractions and the fractions isolated by acid hydrolysis were similar in both soils in spite of significant differences in their particle-size compositions, the inputs of corn residues, and the durations of the continuous corn cropping. Particle-size and chemical fractionations give almost the same size of the inert OM pool in the same soil. For the final choice of fractionation method, the results of the particle-size and chemical fractionation of the OM in the zonal soil series should be compared.

It should be noted that the proposed approaches allow determining only the sizes of the labile, medium-stable, and inert OM pools. The assessment of the mineralization rates of the pools under varied climatic conditions requires further studies of the $CO_2$ emission in incubation experiments at different temperatures and soil water contents.

## CONCLUSIONS

The use of particle-size, density, and chemical fractionation, in combination with the method of the natural abundance of the carbon isotope composition, allowed identifying the most stable carbon pools in the soil. Particle-size fractionation or thermal hydrolysis with 6 M HCl is recommended for the determination of the most stable OM pool. The carbon of the fine (fine-silt and clay) fractions or the nonhydrolyzable residue can be considered as a stable or inert pool in the modeling of the current carbon cycle in terrestrial ecosystems. The studies showed that this pool makes up 65 and 48% of $C_{org}$ in agrochernozems and agrogray soils, respectively. The combination of the biokinetic approach with particle-size or chemical fractionation (OM hydrolysis with 6 M HCl) allowed assessing the size of the medium-stable OM pool, which is usually identified from the $^{13}C$ natural abundance by C3–C4 transition. The size of the medium-stable carbon pool with a renewal time of several years to several decades was 35 and 46% of $C_{org}$ in the agrochernozem and the agrogray soil, respectively. The possibility of substitution the isotope identification by a nonisotope method for the determination of the OM pools should be assessed in further studies in order to identify the medium-stable and inert OM pools in a wide range of soils.

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