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SOIL CHEMISTRY

Distribution of Stable Carbon Isotopes in an Agrochernozem during the Transition from C_3 Vegetation to a Corn Monoculture

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Abstract—The distribution of carbon in an agrochernozem's profile was studied by the natural ^{13}C abundance method during the C_3–C_4 vegetation transition and the analysis of the soil phytolith complex under a continuous corn monoculture. A young pool of soil organic matter (SOM) formed during 43 years of monoculture growing was detected by the isotope analysis in the 0- to 60-cm layer, while the analysis of the phytolith complex identified this pool deeper: corn phytoliths were detected in the 0- to 80-cm layer. The maximum size of the young pool was found in the upper soil horizon; it reached 6.4% of the SOM in the 0- to 20-cm layer. The apparent time of the SOM turnover was 635 and 2225 years in the 0- to 20- and 40- to 60-cm layers, respectively. The high values of the mean residence time were related to the low input of plant residues to the soil at the growing of corn for silage and the high initial content of organic carbon in the chernozem. The changes in the isotope composition after the decalcification of the soil to remove carbonates and the variation of the $\delta^{13}C$ in the corn biomass during the vegetation period significantly affected the calculated value of the mean residence time.

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INTRODUCTION

The most progress in studying the global carbon cycle of terrestrial ecosystems was achieved in the determination of the above-ground productivity [1]. The turnover of carbon in their belowground parts are much more poorly understood, although the major carbon pools are concentrated in the organic matter and the carbonate pedofeatures of the soil [4]. The use of methods for the indication of stable carbon isotopes in ecological studies can fill the existing gaps in the assessment of the role of soil as a biosphere component in the carbon cycle.

The fractionation of the ^{12}C and ^{13}C carbon isotopes allows determining the carbon sources in different pools of soil organic matter (SOM) and assessing the rates of the carbon fluxes between the separate pools. The most common method for in situ isotope indication is the study of the C_3 and C_4 plants' transition because of the significant difference in the discrimination of the heavy ^{13}C isotope depending on the type of photosynthesis. The analysis of the isotope composition of different C_3 and C_4 plant species performed for a large sample set ($n = 965$) showed that the average enrichment in $\delta^{13}C$ in the C_3 plants was $-26.7 \pm 2.3‰$. In the C_4 plants, the average enrichment was $-12.5 \pm 1.1‰$ [15]. During the C_3–C_4 transition, any carbon pool in the soil is a two-component mixture consisting of old (C_3) and young (C_4) carbon [5, 11]. It is supposed that the relative difference in the $\delta^{13}C$ values of the C_3 and C_4 plants remain during the humification of the plant material, its transformation to SOM, the emission of CO_2, and the formation of pedogenic carbonates observed in arid regions after the dissolution of the CO_2 derived from the soil respiration [28]. This characteristic difference in the enrichment with ^{13}C was used for the differentiated dynamics study of the carbon derived from C_3- and C_4-type photosynthesis in the SOM [13], the CO_2 released from the soil [14], and the pedogenic carbonates [28].

The fractionation of carbon isotopes during the decomposition of the SOM, the diffusion of CO_2 in the soil profile, and the formation of pedogenic carbonates also result in the significant spatial and temporal variation of the $\delta^{13}C$ value in the soil because of the effects of several factors. During the destruction of plant residues, the enrichment of the SOM with the heavy carbon isotope occurs most frequently [19]; however, a depletion of the SOM compared to the plant material is observed in some cases. The differences between the SOM and the phytomass vary from

−6.1 to +4.4‰ depending on the plant material, the weather conditions, the degree of decomposition of the plant material, and the growth rate of the microbial community [25, 29, 30].

In long-term experiments on growing corn in a monoculture, it was shown that the isotope composition of the carbon became heavier during the humification of the above ground plants' biomass to a greater degree than during the decomposition of their belowground organs [19]. The differences between the $\delta^{13}C$ values of the corn plants and the SOM varied among the years from 0.2 to 4.5‰ [19].

The qualitative composition of the organic compounds decomposed in the course of the humification also affected the isotope composition of the SOM carbon. The secondary metabolites (aromatic molecules, proteins, and isoprene compounds) are depleted of ^{13}C, while the primary metabolites (mono- and polysaccharides) are enriched with ^{13}C compared to the phytomass of the plant residues [25]. In addition, molecules depleted of ^{13}C are predominantly used in catabolic reactions with the formation of the final destruction product (CO_2), and molecules with the increased content of ^{13}C are used in the synthesis of the microbial biomass [25]. The average weighted values of the SOM enrichment with the ^{13}C isotope compared to the plant residues (equal to 2‰ [25, 29]) are usually used in calculations. The insufficient consideration of this value in the calculations increased the rate of the SOM turnover by 10–15%, and the neglect of the $\delta^{13}C$ dynamics during humification can increase the error of the determination to 28% [19].

The difference between the enrichments of the carbonates and SOM was 15‰ on the average [5], but it could increase to 20‰ under very arid conditions [28]. In addition, the global and local changes in the content of ^{13}C in the atmospheric air determine the values of $\delta^{13}C$ in almost all the carbon pools of the ecosystem, including the SOM, the soil-air CO_2, and the pedogenic carbonates [28]. Thus, in the soils containing both C_{org} and C_{inorg}, the interpretation of the data for the carbon isotope composition in the ecosystems where a C_3–C_4 transition occurred is a difficult problem. The inorganic carbonate carbon is most frequently removed from the soil before the determination of the SOM isotope composition during the decalcification of a sample with dilute HCl [13, 24].

It is known that, along with calcium carbonates, fulvic acids of the 1a fraction (the 0.1 N H_2SO_4-extractable FA1a fraction) are also extracted from the soil during decalcification. Their content in the upper horizons of soils in the arid regions (chernozems and chestnut soils) makes up 2–4% of C_{org} [6]. In the deep horizons, the content of the FA1a fraction reaches 9%. Taking into consideration that the multiple acid treatment to a negative reaction to Ca^{2+} ions is used during the removal of carbonates, the carbon loss can exceed 9%. It is still unclear to which depth the substitution of young (C_4-type) carbon for old (C_3-type) carbon occurs in a soil with vegetation changes; therefore, significant losses of C_{org} during the removal of carbonates are especially undesirable in both the upper and lower soil horizons.

For determining to which extent the removal of carbonates affects the carbon isotope composition in the SOM, this work included the comparative analysis of the $\delta^{13}C$ values in the SOM of decalcified soil samples and those untreated with HCl, which were taken from the upper horizons of an agrochernozem containing carbonates in trace amounts.

Microscopic residues of plant origin are permanently accumulated and preserved in soils and sediments. A peculiar group of plant microresidues includes siliceous structures of specific shapes: phytoliths, which are formed in plant tissues and get into the soil or sediments after the death of plants. These structures remain unchanged for a long time and form own phytolithic complexes. Phytoliths of cultivated plants, including corn, are well identified in soil samples [21–23]. Therefore, their profile distribution can be an additional indicator for the presence of corn residues in the soil.

The aims of this work were to study the distribution of the stable carbon isotopes in the SOM and pedogenic carbonates in the soil profile, to assess the effect of the soil decalcification on the isotope composition of the SOM carbon, and to determine the penetration depth of the carbon derived from the C_4-type photosynthesis using the methods of isotope indication and the identification of corn phytoliths in the profile of an agrochernozem under a continuous corn monoculture.

OBJECTS AND METHODS

Studies were performed in 2008 on leached agrochernozem (C_{org} 3.2%, pH_{KCl} 5.3) on experimental fields of the Voronezh branch of the All-Russian Research Institute of Corn (VNIIK), which was established in 1966.

The soil was sampled from under a continuous corn monoculture (treatment with a complete mineral fertilizer, N120P60K60) and a continuous fallow; samples were taken by layers with a 20-cm interval to a depth of 100 cm. The soil was sampled from 15 points per plot. A mixed sample was prepared from every five points. Thus, all the parameters were determined in mixed samples in triplicate. Samples of the initial soil taken before the establishment of the experiment in 1966 were also analyzed.

Two portions were taken from each soil sample. One portion was used for the determination of the carbon isotope composition without pretreatment. The other portion was freed from calcium and magnesium carbonates by the procedure usually used before the particle-size analysis of calcareous soils [2]. The soil was treated with 0.2 N HCl until the cessation of effervescence and with 0.05 N HCl to a negative reaction to

Ca^{2+} ions. The noneffervescent samples from the upper horizons were treated only with 0.05 N HCl to a negative reaction to Cl^- ions and dried at 60°C to a constant weight.

The isotope composition of the carbonate carbon was determined after the dry combustion of the organic matter from the soil sample in a muffle furnace at 500°C for 14 h.

The content of the heavy ^{13}C isotope in the soil and carbonates was determined using a Thermo Finnigan MAT 253 mass spectrometer combined with a Euro EA elemental analyzer (Eurovector, Italy). The analytical signal for the ^{13}C isotope was expressed in $\delta^{13}C$ units relative to the VPDB international standard:

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

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

The contents of ^{13}C in the belowground mass and stubble of the corn grown on the chernozemic soil were also determined; the average value of $\delta^{13}C$ was −11.6‰.

The portion of corn carbon (from the C_4-type photosynthesis) in the soil was calculated from the equation

$$\delta^{13}C_s = f\delta^{13}C_4 + (1 - f)\delta^{13}C_3, \quad (2)$$

where $\delta^{13}C_s$ is the $\delta^{13}C$ value in the soil sample; $\delta^{13}C_4 - \delta^{13}C$ value in the young organic matter resulting from the decomposition of the corn residues; $\delta^{13}C_3$ is the content of the heavy ^{13}C isotope in the initial soil samples taken before the establishment of the experiment in 1996; and f is the portion of C_4-type organic matter, i.e., the newly formed humus accumulated during the last 42 years under the monoculture.

The constant (k) and the mean residence time (MRT) of the SOM during the time t were calculated from the following equation (under the supposition that the decomposition of the corn organic matter follows an exponential law [11]):

$$k = -\ln(1 - f)/t, \quad (3)$$

$$MRT = 1/k. \quad (4)$$

The humification coefficient of the corn residues was calculated as the ratio between the C_4-type carbon pool in the 0- to 60-cm layer of the soil under the monoculture and the amount of carbon arriving to the soil with the root and harvest residues. The pool of C_4-type carbon was calculated from the C_{org} pool and the f value found using Eq. (2) for the 0- to 20-, 20- to 40-, and 40- to 60-cm layers. The average annual input of plant residues into the soil under the monoculture was determined in a previous study [8]. In this work, the mass of the root residues in the 0- to 40-cm layer was calculated. For the root mass in the 40- to 60-cm layer, the results of Ivannikova [3] were taken, according to which the root mass in the 40- to 60-cm layer makes up 7–8% of the total root mass in the 0- to 60-cm layer. The results of the analyses were calculated for oven-dry matter.

The phytolith complexes were isolated to a depth of 100 cm with an interval of 20 cm from the soil sampled under the continuous corn monoculture (treatment with the complete mineral fertilizer N120P60K60). For this purpose, a soil sample of 30 g was freed from carbonates by 10% HCl and from clay and fine-silt particles by elutriation, and the dried residue was treated with a heavy liquid (a mixture of cadmium iodide and potassium iodide water solutions) 2.3 g/cm^3 in density. The precipitate (particles with a density >2.3 g/cm^3) was rejected, and the fraction of ≤2.3 g/cm^3 was washed with water, dried, and microscoped.

The examination of the preparations and the identification and counting of the phytoliths were performed with a Carl Zeiss HBO 50 (AC) microscope at 100 and 400 magnifications. Ten top-down runs were made on an object plate 24 × 24 mm in size. Then, two more object plates were examined for new morphotypes. Next, the numbers of total phytoliths and each of their forms were found. The calculated phytoliths were separated into the groups of cereals, corn, weeds, and other.

RESULTS AND DISCUSSION

The results of determining the carbon pool in the agrochernozem (Table 1) indicated the high stability of its organic matter. In the 0- to 80-cm layer of the soil, the storages of carbon remained at a high level (from 27 to 28 kg C/m^2) and did not differ significantly among the following treatments: before the corn growing, the unfertilized control, NPK under a 42-year-long corn monoculture, and a 42-year-old black fallow. Small differences among the SOM pools in the experimental treatments were detected only as a tendency: the minimum carbon pool was found in the fallow soil, and its maximum pool was found in the fertilized soil under the corn monoculture. These differences were similar in the 0- to 20-cm plow layers, as well as in the deeper soil horizons.

Inorganic carbon in the form of carbonates was almost absent in the soil under the corn to a depth of 80 cm. A nonuniform distribution of carbonates was observed in the 80- to 100-cm layer: the standard deviation exceeded the average value of C_{inorg} in the soil. In distinction from the soil under the corn, an appreciable content of carbonates was observed in the fallow soil already at a depth of 60 cm.

In contrast to the carbon pools, the isotope composition of the carbon significantly varied among the experimental treatments. In the initial soil sampled before the establishment of the experiment and in the soil of the black fallow, the values of $\delta^{13}C$ were typical for the C_3-type vegetation and varied from −25 to −27‰. Small

Table 1. Organic and inorganic carbon pools (mean ± standard deviation) in the experimental treatments on a leached chernozem

Depth, cm	C_{org}, %	Storages, g of C_{org}/m^2	C_{inorg}, %	Initial reserves, g of C_{inorg}/m^2
Corn monoculture, NPK, 2008				
0–20	3.42 ± 0.22	8348 ± 538	0.011 ± 0.005	28 ± 11
20–40	3.21 ± 0.30	8904 ± 841	0.013 ± 0.003	35 ± 9
40–60	2.47 ± 0.53	6731 ± 1435	0.012 ± 0.003	33 ± 9
60–80	1.55 ± 0.48	4674 ± 1441	0.043 ± 0.114	130 ± 343
80–100	0.96 ± 0.38	3104 ± 1211	0.159 ± 0.307	513 ± 989
0–80		28658 ± 2266		226 ± 343
Corn monoculture, control, 2008				
0–20	3.37 ± 0.25	8223 ± 612	0.013 ± 0.004	31 ± 11
20–40	3.16 ± 0.30	8758 ± 830	0.012 ± 0.004	34 ± 12
40–60	2.21 ± 0.43	6026 ± 1175	0.012 ± 0.005	33 ± 13
60–80	1.50 ± 0.34	4522 ± 1037	0.017 ± 0.017	51 ± 52
80–100	1.02 ± 0.47	3277 ± 1500	0.109 ± 0.338	352 ± 1087
0–80		27528 ± 1876		149 ± 57
Fallow, 2008				
0–20	3.12 ± 0.12	7800 ± 295	0.018 ± 0.005	44 ± 11
20–40	3.04 ± 0.10	8421 ± 290	0.015 ± 0.004	43 ± 12
40–60	2.19 ± 0.51	5979 ± 1668	0.012 ± 0.002	33 ± 7
60–80	1.58 ± 0.28	4756 ± 849	0.636 ± 0.017	1913 ± 51
80–100	0.96 ± 0.51	3091 ± 1642	1.683 ± 0.060	5418 ± 193
0–80		26955 ± 1917		2033 ± 54
Initial soil, 1966				
0–20	3.19 ± 0.05	7980 ± 125	0.021 ± 0.002	51 ± 5
20–40	3.12 ± 0.10	8642 ± 277	0.012 ± 0.001	33 ± 3
40–60	2.22 ± 0.07	6061 ± 191	0.020 ± 0.003	54 ± 8
60–80	1.54 ± 0.10	4635 ± 301	0.030 ± 0.006	89 ± 18
0–80		27318 ± 468		138 ± 20

variations in the $\delta^{13}C$ value were observed down the profile of the initial soil. In the lower horizons, the content of ^{13}C was slightly higher than that in the upper horizons, although the differences between the horizons did not exceed 0.5‰. This distribution pattern is typical of soil profiles [5], although the reasons for this phenomenon have yet to be clarified. It is supposed that the formation of SOM, which presently occurs in the deep horizons, took place in the atmosphere more enriched with the heavy carbon isotope in comparison with the modern epoch.

An abrupt increase of the ^{13}C portion in the total carbon was observed in the fallow soil at 60–80 cm, which coincided with the appearance of an appreciable content of carbonates (Table 2) more enriched with ^{13}C than the SOM.

The value of $\delta^{13}C$ (the C_{total} in the untreated soil and the C_{org} in the decalcified soil) in the soil under the monoculture was higher than that in the analogous layer of the initial soil by 1–2‰ to a depth of 40 cm and by 0.3‰ in the 40- to 60-cm layer, which indicated a gradual substitution of old carbon by young SOM developed at the decomposition of corn residues. The carbon derived from C_4-type plants was accumulated in both the upper and lower horizons of the soil. However, the largest differences in the $\delta^{13}C$ values were observed in the upper horizon, and the smallest differences were found in the 60- to 80-cm layer. Thus, the rate of the young carbon accumulation in the soil decreased with the depth.

The most significant differences between the decalcified and HCl-untreated samples were noted in the lower horizons of the fallow soil. In the 60- to 80- and 80- to 100-cm layers, the value of $\delta^{13}C$ in the decalcified samples was significantly lower than that in the soil untreated with HCl (–26.6 and –9.5‰, respectively). The comparison of the $\delta^{13}C$ values after the decalcification of the samples indicated that the iso-

Table 2. ^{13}C isotope ($\delta^{13}C$, ‰; mean ± standard deviation) in the organic matter and carbonates from the leached chernozem of the experimental treatments

Depth, cm	C_{total}	C_{org}	C_{inorg}
	untreated soil	decalcified soil	incineration residue
Corn monoculture, NPK			
0–20	−24.47 ± 0.12	−25.79 ± 0.18	−19.67 ± 0.17
20–40	−24.90 ± 0.04	−26.15 ± 0.15	−19.13 ± 0.16
40–60	−25.26 ± 0.06	−26.40 ± 0.07	−18.44 ± 0.20
60–80	−25.93 ± 0.16	−26.41 ± 0.15	−19.15 ± 0.16
80–100	−24.93 ± 0.49	−26.23 ± 0.35	−19.82 ± 1.70
Fallow			
0–20	−25.34 ± 0.25	−26.62 ± 0.04	−20.43 ± 0.09
20–40	−25.27 ± 0.15	−26.77 ± 0.04	−21.25 ± 4.56
40–60	−25.54 ± 0.13	−25.72 ± 0.12	−20.20 ± 0.65
60–80	−17.51 ± 0.25	−26.65 ± 0.02	−9.50 ± 0.01
80–100	−14.91 ± 0.38	−26.46 ± 0.27	−9.55 ± 0.11
190–200 lime nodules	−9.82 ± 0.13	absent	−9.86 ± 0.16
Initial soil, 1966			
0–20	−25.35 ± 0.17	−26.57 ± 0.20	−20.36 ± 0.13
20–40	−25.37 ± 0.12	−26.55 ± 0.12	−20.88 ± 0.11
40–60	−25.45 ± 0.15	−26.71 ± 0.15	−20.72 ± 0.15
60–80	−24.98 ± 0.16	−26.32 ± 0.40	−20.20 ± 0.11

tope composition of the SOM carbon in the initial and fallow soils was similar. In long-term field experiments, the initial soil samples do not always remain, but the missing information on the carbon isotope composition in the old SOM can be derived from the study of soil samples from a continuous fallow. Because of the absence of an initial soil sample (before the establishment of the experiment) from a depth of 80–100 cm, we used a decalcified sample of fallow soil for the determination of the $\delta^{13}C$ in the SOM of C_3 origin.

The isotope composition of the SOM in the soil under the C_3 vegetation and corn monoculture significantly changed after the decalcification. Unidirectional changes were observed: a depletion of the heavy ^{13}C carbon isotope occurred in the SOM of both the C_3 and C_4 origin. The difference in the $\delta^{13}CC$ value between the untreated and decalcified soil samples from the upper horizon was 1.5‰. The decalcification of the soil not only removed carbonates but it extracted the most mobile FA1a fraction from the soil. This fraction could lose a significant part of the carbohydrate components highly enriched with ^{13}C; their removal resulted in the relative enrichment in lignin and lipid compounds with a low content of ^{13}C [18, 27]. The negative effect of the decalcification on the $\delta^{13}C$ value was mainly observed in the upper horizon of soil. The value of $\delta^{13}C$ in the untreated soil under the monoculture exceeded its value in the initial soil by 1.13‰, and the corresponding excess for the decalcified samples was only 0.78‰. Thus, the decalcification before the determination of the isotope composition of the young SOM is undesirable because of its possible loss during this procedure.

The isotope composition of the inorganic carbon significantly varied among the experimental treatments. In the upper and medium parts of the soil profile to a depth of 60 cm, inorganic carbon was present in trace amounts (from 0.01 to 0.04%), and its enrichment in all the experimental treatments was higher than the content of ^{13}C in the SOM by 5–6‰. It is known that the isotope composition of the carbonate carbon is determined by the isotope composition of the CO_2 released during the soil respiration and the differences in the precipitation rates of the $Ca^{12}CO_3$ and $Ca^{13}CO_3$ [5]. The carbon dioxide in the soil air is usually enriched by 5‰ compared to the SOM. A heavier isotopic composition of carbon in the carbon dioxide in comparison with the SOM is usually attributed to the more rapid diffusion of $^{12}CO_2$ compared to $^{13}CO_2$ [5]. Thus, the lighter CO_2 molecules diffuse into the atmosphere, and the heavier molecules are mainly retained in the soil profile or dissolved in the soil solution. During precipitation from the soil solutions, the isotope composition of the carbonate carbon became heavier by 10‰ more. Thus, the fractionation of ^{13}C during the diffusion of CO_2 and the precipitation of carbonates resulted in a heavier isotope composition

(by 15‰ on the average). The very low content of ^{13}C and the increase of the $\delta^{13}C$ in the carbonates from the upper horizons of the studied soils by only 5–6‰, rather than by 15‰, indicated that the precipitation of carbonates was insignificant under the natural conditions. The content of ^{13}C in the carbonates from the upper horizons corresponded to $\delta^{13}C$ in the CO_2 of the soil air. Hence, the inorganic carbon of the upper horizons entered into the carbonates of the soil solutions and the CO_2 dissolved in the soil water and precipitated as carbonates after its evaporation, i.e., during soil drying.

The isotope composition of the inorganic carbon in the upper horizons of the soil to a depth of 100 cm, where its content was 0.01–0.16%, differed between the initial soil with the C_3-type SOM and the soil under the C_4-type corn plants. In the upper 0- to 20-cm layer of the soil under the corn, both the SOM and C_{inorg} were enriched with ^{13}C compared to the soil under the C_3-type plants by 1.67 and 1.33‰, respectively. In the lower horizons, the C_{inorg} was enriched with ^{13}C to a greater extent than the SOM. For example, the SOM carbon in the 60- to 80-cm layer of the soil under the monoculture was enriched by 0.55‰, and the difference in the $\delta^{13}C$ of the carbonates in the soil under the monoculture and the initial soil was 1‰. This could be related to the more rapid transfer of CO_2 in the gas phase, its dissolution in the soil solution, and its precipitation in the form of carbonates compared to the transport of organic carbon down the profile. Thus, if the intense formation of pedogenic carbonates presently occurred in the soil studied, the long-term growing of the corn monoculture would determine the isotope composition of the C_{inorg} not only in the upper but also in the lower horizons of the soil.

The increase in the content of C_{inorg} in the soil was accompanied by a significant increase in the content of the heavy carbon isotope in the carbonates. In the fallow soil, a significant content of carbonates with high enrichment (–9.5‰) was found already at the depth of 60 cm. A similar content of ^{13}C was observed in calcareous formations (lime nodules) at the depth of 190–200 cm, i.e., at the level of the parent material. The difference between the values of $\delta^{13}C$ in the carbonates and the SOM of the fallow soil at a depth of 60–80 cm was 17.1‰, which was slightly higher than the theoretical heavying by 15‰ at the dissolution of CO_2 and the precipitation of carbonates [5]. Thus, it can be concluded that, in the absence of plants in the fallow soil, the content of lithogenic carbonates enriched with the heavy carbon isotope increased in the colloid solutions (i.e., without exchange with the soil CO_2) because of the intense evaporation of water and the increase in the storages of inorganic and total carbon in the soil profile. The possibility of such transfer under the enhanced aridization of the climate was shown earlier with chernozemic paleosols as an example [10].

Along with the soil carbonates, the fractionation of the ^{13}C isotope in corn plants also affects the calculation of the SOM turnover rate at the C_3–C_4 transition in vegetation [15]. The value of $\delta^{13}C$ in the aboveground organs of the corn varied from –13.8 to –11.3‰ at the end of the vegetation period. In the belowground organs, the ^{13}C enrichment at the end of the vegetation period was slightly lower: –11.8‰. The average weighted $\delta^{13}C$ value with consideration for the proportions of the above- and belowground phytomasses in the total mass of the plant residues was used in the calculation of the SOM turnover rates. After the harvesting of the corn, the average weighted value of $\delta^{13}C$ with consideration for the proportions of stubble and roots was –11.6‰.

The interannual variation of the isotope composition of the after harvesting residues was lower than the intra-annual variation of the ^{13}C in the corn phytomass. The enrichment of $\delta^{13}C$ in the stubble of the corn harvested in 2006, 2008, and 2010 was –11.6, –11.3, and –11.5‰, respectively. The after harvesting residues are characterized by the low interannual variation of the carbon isotope composition, and the major part of the young carbon arrives into the soil after the corn harvest; therefore, the content of ^{13}C in the phytomass at the end of the vegetation period should be used in Eq. (2) as the $\delta^{13}C_4$ value. At the same time, the SOM turnover rates were also calculated at the minimum enrichment of the plant residues equal to –13.8‰ for assessing the sensitivity of the SOM residence time to variations in the content of ^{13}C in the corn phytomass (figure).

The variation of the isotope composition of the C_4 plants affected the SOM residence time similarly to the significant loss of young SOM of C_4 origin after the soil decalcification. The contribution of the C_4-type SOM after the decalcification was the lowest and amounted to 5.4%. Decalcification was mainly used to remove the carbonates from the lower soil horizons. In order to unify the procedure of the samples' preparation for the analysis of the carbon isotope composition, the decalcification of the soil samples from the upper horizons containing insignificant amounts of $CaCO_3$ was also performed. Taking into consideration the loss of the young SOM carbon during the decalcification, the procedure of the soil preparation for the analysis of the ^{13}C should differ for samples taken from the upper and lower horizons. Only the soil from the lower horizons should be decalcified. At this stage of the study, we consider correct the values of the *f* and MRT obtained for the undecalcified soil at $\delta^{13}C$ = –11.6‰ in the corn residues (figure, treatment *1*). The contribution of young carbon to the SOM in the 0- to 20-, 20- to 40-, and 40- to 60-cm layers was 6.4, 3.0, and 2.0%, respectively, with the corresponding MRT values being 635, 1398, and 2225 years, respectively.

If the minimum and maximum enrichments of the corn residues equal to –13.8 and –11.6‰, respec-

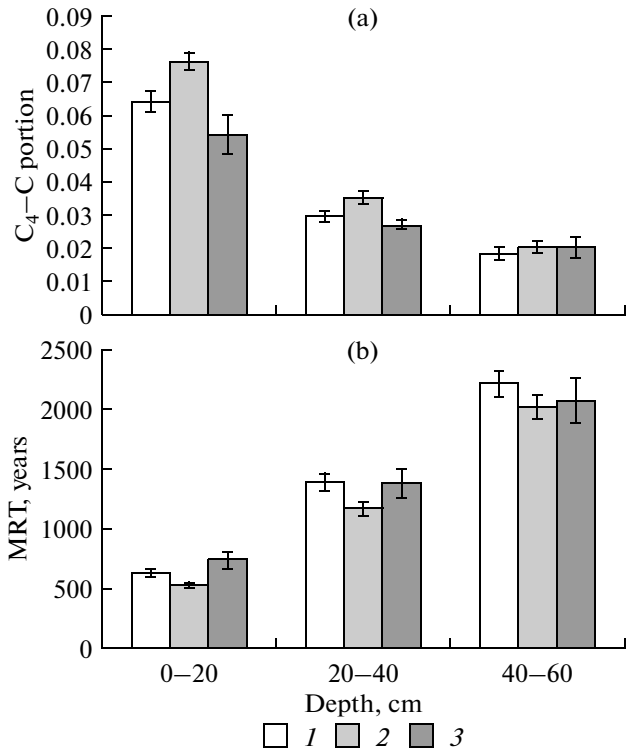

Fig. 1. The (a) content and (b) mean residence time of C_4 organic carbon in the agrochernozem of the NPK treatment; $\delta^{13}C$ in the corn residues, undecalcified soil: (*1*) –11.6‰, (*2*) –13.8‰; (*3*) decalcified soil: –11.6‰.

tively, are substituted in to Eq. (2), the portion of young carbon *f* in the 0- to 20-cm soil layer will be 7.5 and 6.4% of the C_{org}, and the residence time will be 635 and 742 years, respectively. The difference between the minimum and maximum enrichment of the plant residues was about 2‰, i.e. equal to the average increase in the SOM enrichment compared to the plant residues [25]. This difference in the studied soils disturbed the values of the *f* and MRT by only 15% in the upper 0- to 20-cm soil layer, which suggested that the obtained SOM turnover rate, even with this correction, was significantly lower than the values obtained by other authors. During the 30–40 years of the corn monoculture's growing, the SOM was usually renewed by 23–40% in the upper horizons and by 4–15% at a depth of 50–100 cm [13]. The maximum values reached 90% in the 0- to 5-cm layer of a soil without tillage during 40 years of corn growing in the Northern Appalachians [24]. In the cited works, all the soils were preliminarily decalcified; therefore, the minimum MRT estimates typical for the decalcified soil were used for the comparison. In other studies, MRT values of 18 to 96 years were the most typical for the upper soil horizon during the C_3–C_4 transition in the vegetation [13, 20, 24]; in some cases, the MRT values exceeded 200 years [17]. An anomalously long SOM residence time was observed in the chernozem under study. After the removal of the carbonates, the MRT was equal to 742 and 2342 years in the 0- to 20- and 40- to 60-cm soil layers, respectively.

It can be supposed that such a low carbon turnover rate in the SOM was related to several reasons: (1) the lower productivity of the corn grown in the monoculture compared to the crop rotation, (2) the high original reserves of organic carbon in the chernozem compared to the other soil types, and (3) the low input of plant residues under the intense withdrawal of the above-ground phytomass.

The productivity of the corn itself, as well as the input of the after-harvest residues into the soil, is higher in the 10-course crop rotation than in the monoculture by a factor of 1.5. At the same time, taking into consideration the high productivity of the corn compared to the other agricultural crops, the long-term annual average inputs of the plant residues into the soils under the crop rotation and the monoculture did not significantly differ from each other [7]. Therefore, we consider the high reserve of carbon in the chernozem and the low input of carbon into the soil during the growing of corn for green fodder to be the main reasons for the low rate of the carbon turnover in the studied soil. To confirm this supposition, we compared some parameters of the biological cycle of the carbon in agroecosystems of corn grown in permanent monocultures. The agrochernozem under study was characterized by extremely high reserves of SOM; therefore, its turnover will take a significantly longer time than in the other soil types of Europe and America (Table 3), even at the similar input of carbon with the plant residues.

In distinction from the turnover rate SOM, the humification coefficient (K_h) of the corn residues in the studied chernozem well agreed with the literature data. Its values did not differ statistically between the fertilized and control treatments (15 and 17% in the undecalcified soils and 13 and 16% in the decalcified soils). Similar K_h values were obtained in a series of 15 to 50-year-long experiments with corn monocultures in the United States and Western Europe. Thus, SOM is accumulated in the studied soil with a high rate, and, along with its high reserves, the small mass of plant residues arriving into the soil during the growing of corn for green fodder is the main reason for the slow turnover of carbon in the chernozem.

The order of the values characterizing the return of carbon into the soil in our experiment well agreed with the values obtained at the experimental stations in Halle and Askov, which are also located north of the 51st parallel, and where long-term experiments with growing corn for silage are being performed. At the growing of corn for grain in more southern regions (40°–48° N), the amount of organic matter returned into the soil with the after-harvest residues is larger by 4–5 times than at the growing of a monoculture for silage. Therefore, the substitution rate of the old carbon by the young corn carbon is also significantly

Table 3. Parameters of the biological carbon cycle in long-term experiments with a corn monoculture

Experiment	Coordinates	Treatment	Air temperature, °C*	Precipitation, mm**	SOM reserve, 0- to 60-cm layer, kg C/m^2	C–C$_4$ portion	Accumulation	Input	Kg	Source
						(f), % C_{org}	C–C$_4$, kg C/m^2 annually		%	
Voronezh branch, VNIIK	51°48′N 55°06′E	42-year-old monoculture	6.5	579	23.0	3.4	0.020	0.112	17.4	Our data
Voronezh branch, VNIIK	51°48′N 55°06′E	42-year-old monoculture N120P90K90	6.5	579	24.0	4.0	0.023	0.152	14.8	Our data
Lamberton, MN, USA	44°14′N 95°18′W	33-year-old monoculture NPK	6.2	630	8.6	35.4	0.095	0.570	16.6	Collins et al., 1999 [13]
Northern Appalachians, OH, USA	40°24′N 81°48′W	40-year-old monoculture	10.5	1000	6.2	54.0	0.084	0.640	13.2	Puget et al., 2005 [24]
Rotthalm@unster, Bavaria, Germany	48°21′N 13°12′E	24-year-old monoculture NPK	8.7	886	7.4	29.6	0.091	0.630	15.0	Flessa et al., 2008 [17]
Askov Experimental Station, Denmark	51°31′N 11°59′E	39-year-old monoculture NPK	9.2	465	9.1	9.9	0.023	0.080	30.0	Flessa et al., 2008 [17]
Askov Experimental Station, Denmark	55°28′N 09°07′E	14-year-old monoculture NPK	7.6	862	6.3	7.0	0.029	0.180	16.3	Kristiansen et al., 2005 [20]
Askov Experimental Station, Denmark	55°28′N 09°07′E	14-year-old monoculture NPK + 0.8 kg/m^2 annually for corn	7.6	862	6.4	18.0	0.080	0.500	16.0	Kristiansen et al., 2005 [20]

Notes: * Average annual value;
** Total annual.

higher. The portion of carbon in the C_4-type SOM is no higher than 10% at the growing of corn for silage, and more than half the SOM in the 0- to 60-cm layer can consist of young carbon at the growing of corn for grain.

It should be noted that the rate of the carbon turnover little depended on the soil type and the climatic conditions of its formation in the cases considered in Table 3. At the growing of corn for grain, a similar young carbon accumulation of 0.08–0.09 kg C/m^2 annually was observed in agroecosystems of Northern America and Europe regardless of the soil type and climatic parameters. The rate of the carbon accumulation during corn growing for silage was also similar: 0.02–0.03 kg C/m^2 annually. Thus, the amount of plant residues arriving into the soil is the main factor determining the rate of the SOM turnover under the current conditions. This conclusion is most clearly confirmed by the results of two parallel experiments at the Aksov experimental station. The annual application of 800 g/m^2 of corn green mass in addition to its amount arriving at the growing of corn for silage increased the rate of carbon accumulation in the soil from 0.029 to 0.08 kg C/m^2 annually and increased the content of young carbon from 7 to 18% C_{org}.

Taking into consideration the dependence of the apparent SOM turnover rate on the content of plant residues, the agreement between the MRT values obtained for the agroecosystems and the actual rates typical for natural phytocenoses should be assessed. We compared the productivity of perennial herbaceous plants (meadow ecosystems) with the input of carbon into the soils of agroecosystems. According to Titlyanova [9], the productivity of boreal forests in Europe is 15–17 t/ha. With consideration that the average content of C_{org} in the plant residues is 42%, the annual input of carbon in the meadow ecosystems of Europe and Northern America is 630–714 and 294–504 g of C/m^2, respectively. Hence, at the growing of corn for grain on both the European and American continents, the return of carbon with the plant residues corresponds to its amount arriving into the soil in highly productive meadows. At the growing of corn for silage, a correction for the withdrawal of the phytomass should be made. If the input of carbon into the soil in the studied agroecosystems on agrochernozems increases from 112–152 g of C/m^2, i.e., to the values typical for natural agroecosystems the SOM in the upper soil horizon will contain 30% young carbon, and the MRT will be about 100 years, i.e., typical for the values obtained by the natural ^{13}C abundance method during the C_3–C_4 transition.

This is an approximate calculation. For a more accurate comparison of the organic carbon MRT values in arable and natural ecosystems, not only the amount of phytomass arriving into the soil but also it structure should be considered. The above-ground phytomass prevails in the plant residues of agroecosystems of corn grown for grain, while the belowground organs of corn make the major contribution in meadow agrocenoses. The $C_{ab\text{-}gr}/C_{un\text{-}gr}$ ratio is 0.2–0.7 and 1.8–2.0 for meadows and agroecosystems, respectively [13]. It is known that the K_h at the decomposition of the underground corn organs exceeds the corresponding values at the destruction of the above-ground phytomass by 1.7 times [12]. In addition, the disturbance of the natural soil structure under tillage significantly decreases the humification: the K_h value was equal to 13 and 8% under zero and conventional tillage, respectively [24]. Thus, the humification in natural ecosystems is characterized by increased efficiency compared to agrocenoses. This fact should be taken into consideration in the calculation of the SOM MRT.

Nonetheless, the results of our studies and the literature data suggested that the same pool of young carbon with a residence time of 30–100 years was recorded in all the experiments with C_3–C_4 transition. The longer apparent residence time was related to the low productivity of the phytocenoses rather than to the increased resistance of this pool to decomposition. For the more accurate calculation of the SOM turnover rates in different ecosystems, manipulation experiments should be used to regulate the amount and structure of the phytomass with another isotope composition incorporated into the soil.

The value of $\delta^{13}C$ in the deep horizons of the soil under the monoculture was higher than that in the initial soil by less than 1‰; therefore, the thickness of the soil layer in which the accumulation of C_4-type carbon occurs and the time of its renewal cannot always be determined unambiguously. The amounts of corn phytoliths at different depths were studied for the independent assessment of the young carbon's penetration depth.

Corn phytoliths make up a very small portion (about 2–3%) of their total amount in the phytolith complex (Table 4). This is related to the fact that corn phytoliths were accumulated in the soil layer only under the current pedogenesis conditions during 42 years of monoculture growing. Phytoliths of other cereal plants compose the major part (about 85%) of the phytolith complex. The profile distributions of all the separated groups of the phytolith complex are similar: a gradual decrease to a depth of 60–80 cm and an abrupt (almost by an order of magnitude) decrease in the 80- to 100-cm layer. The phytoliths typical for corn plants are completely absent in the 80- to 100-cm layer. Thus, the phytolith analysis shows that the lower boundary of the young carbon accumulation occurs at a depth of 80 cm. From the isotope analysis of the soil, the accumulation of young carbon in the 60- to 80-cm layer was statistically unreliable; therefore, the phytolith analysis was found to be more sensitive than the isotope analysis.

The morphological parameters of the phytoliths detected in the soil under the corn monoculture in the upper and lower parts of the soil profile are typical for leaves rather for the belowground organs of corn. The

Table 4. Distribution of phytoliths in the profile of a leached chernozem under a corn monoculture

Depth, cm	Phytoliths	Corn			Weed plants	Other cereals	Other forms
		cruciform	bi- and trilbular forms	total			
0–20	707	7	9	16	29	598	64
20–40	415	1	8	9	26	397	68
40–60	542	5	10	15	32	401	104
60–80	375	2	4	6	19	261	89
80–100	66	0	0	0	0	36	30

penetration of phytoliths from the above-ground organs of the plants to a depth of 60–80 cm is due to the activity of burrowers and the fracturing of soil highly heated in the early summer and fall in the absence of plant cover, as well as due to the spilling of soil material from the upper to the lower horizons. Thus, young carbon arrives into the studied soil not only with the dead belowground organs and migrating soil water but also during the mechanical mixing of the soil. The importance of this process is also confirmed by the abundance of phytoliths from corn leaves in the middle part of the chernozem profile. The contents of phytoliths in the 0- to 20- and 40- to 60-cm layers are similar, which indicates the most significant mixing of the soil material to a depth of 60 cm.

CONCLUSIONS

The analysis of the distribution of the stable carbon isotopes (^{13}C and ^{12}C) in the soil on which a C_3–C_4 transition occurred allowed dividing the soil carbon into the young and old pools. The young carbon pool was formed after the transition, and the old pool with a longer carbon residence time was formed in the soil before the transition. In the studied agrochernozem under a continuous C_4-type monoculture (corn), the young pool was detected by the isotope analysis to a depth of 60 cm. The phytolith analysis showed that the young carbon also arrived to the deeper horizons to a depth of 80 cm not only with the root residues but also at the mechanical mixing of the soil.

In the upper part of the profile to a depth of 80–100 cm, changes in the isotope composition of the plant carbon affect the content of ^{13}C in the carbonates. In the upper horizons, carbonates are present in trace amounts and can be considered as precipitated during the soil drying from the soil solutions, where the soil-air carbon is dissolved. The latter forms the most mobile component of the soil carbon pool, which most rapidly responds to the occurring changes in the carbon isotope composition during the growing of C_4-type plants in a monoculture.

The size of the young carbon pool in the studied soils was found to be very small: the content of C_4-type SOM was 6.4% in the 0- to 20-cm soil layer and only 2% at a depth of 40–60 cm after the corn monoculture's growing for 42 years. The SOM residence time in the leached chernozem calculated by the natural ^{13}C abundance method was extremely high compared to other soils in the world: it was 697 and 2742 years at depths of 0–20 and 40–60 cm, respectively. The turnover rate of the SOM depends on the mass of the plant residues arriving into the soil; therefore, the long apparent SOM residence time in our experiment was related to the low input of carbon during the corn growing for silage rather than to the high stability of the SOM. Manipulation experiments with a regulated amount of corn residues incorporated into the soil should be used for the more accurate calculation of the actual SOM residence time.

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