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EXPERIMENTAL ARTICLES

Turnover of "New" and "Old" Carbon in Soil Microbial Biomass

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Abstract—The contributions of "new" carbon coming from plants with the C4-type of photosynthesis (maize) and "old" carbon from soil organic matter (SOM) formed under C3 vegetation as carbon sources for microorganisms was determined. Soil samples were taken from the plots of field experiments on Chernozem and Phaeozem. The values of $\delta^{13}C$ were determined in evolved CO_2, SOM, total microbial biomass (C_{mic}), and phospholipid fatty acids (PLFA), assuming that the PLFA markers for certain taxonomic groups of microorganisms enriched in C4 carbon indicated a more significant role of these microorganisms in the transformation of root exudates and plant residues. Carbon pools were arranged in the following order by the degree of their enrichment with "new" C: SOM < C_{mic} < CO_2. Consequently, the "new" carbon proved to be a more preferable substrate for microbial growth than the "old" one. The share of C4 in the markers varied from 18 to 60% (on average 38%) in Phaeozem and from 15 to 40% in Chernozem (on average 28%). The groups of microorganisms in Phaeozem were arranged in the following order by the degree of their enrichment with "new" carbon: protozoa < saprotrophic fungi < actinomycetes < gram-positive bacteria < gram-negative bacteria < mycorrhizal fungi. In Chernozem, the contribution of C4 to the carbon composition of PLFA did not differ significantly for various groups of microorganisms. The C4 content within the PLFA markers of fungi and gram-negative bacteria did not demonstrate any crucial contribution of these groups of organisms to the transformation of "new" C. The long-term C3–C4 transition probably results in formation of a broad range of carbon pools similar in their C4 content but different in resistance to mineralization; therefore, gram-positive bacteria could assimilate C4 from resistant C pools. The low content of "new" carbon in the PLFA markers of fungi may be explained by a considerable portion of dormant forms.

Keywords: microbial community structure, microbial biomass, ^{13}C, C3–C4 transition, "old" and "new" carbon, soil respiration

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Transformation of plant residues and root exudates is an important component of the carbon cycle in soil [1–3]. The data on these processes are taken into consideration developing of the management practices for enhancement of C sequestration in soil and, as a consequence, for climate change mitigation and conservation of the natural resources of our planet. The most important aspects of decomposition and transformation of organic matter in soil are (1) the structure and activity of the soil microbial community and (2) resistance of soil organic matter (SOM) to microbial decomposition. The structure and activity of the soil microbial community are of tremendous significance, because soil microorganisms are the driving force of the decomposition and humification of organic matter in soil. Although the mechanisms of SOM stabilization in soil are rather complicated and insufficiently studied, in general, they may be divided into three groups associated with (1) preferential preservation of stable organic compounds in soil, (2) SOM interaction with minerals and metal ions, and (3) spatial inaccessibility of SOM for microorganisms–decomposers [1, 4]. Therefore, new data on the ratio between plant- and humus-derived carbon within the biomass of microorganisms of different taxonomic groups are necessary. It allows identification of the microorganisms dominating in the decomposition of soil organic matter [5].

The most widespread method of studying the transformation of plant and SOM carbon in soil is the stable isotope method ^{13}C (stable isotope probation, ^{13}C-SIP). The input of ^{13}C into the belowground plant organs and the rhizosphere can be determined by labeling the plants with ^{13}C-enriched or depleted CO_2 or by using the phenomenon of natural carbon ^{13}C discrimination for plants with C3 ($\delta^{13}C \approx -27‰$) and C4 ($\delta^{13}C \approx -13‰$) photosynthesis. In other words, C4 plants are naturally enriched in the ^{13}C isotope than C3 plants. Cultivation of C4 plants on soils formed under C3 plants (the C3–C4 transition) or, vice versa, C3 plants on C4 soil (the C4–C3 transition) results in

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a shift between the $\delta^{13}C$ values in plant biomass, plant residues, and in SOM. Thus, there appears a possibility to differentiate the "new" carbon of plant residues and rhizodeposits and the "old" carbon of humus by the values of ^{13}C enrichment of the carbon pools [6]. The input of new and old C into microbial biomass, i.e., preferential utilization of C3 or C4 as carbon substrates, can be estimated by $\delta^{13}C$ for the microbial biomass in soil and in the rhizosphere. Moreover, the values of isotope enrichment in ^{13}C as a component of carbon dioxide evolved from the soil and rhizosphere make it possible to calculate the portions of mineralized C from C3 and C4 pools. Cultivation of C4 plants on C3 soil can be replaced by the addition of C4 plant material into C3 soil followed by long-term incubation under field or laboratory conditions.

In the past two decades, soil microbiologists have developed modifications of the method for detection of phospholipid fatty acids (PLFA), which may be used to determine the structure of soil microbial communities. PLFA are the most important membrane component in living cells; they are not incorporated into storage compounds and are quickly degraded in dead cells [7, 8]. Therefore, PLFA are good markers of the biomass of living organisms [9]. Since the differences in the chemical structure of PLFA (chain length, the position of substitution, branching or unsaturation, occurrence of cyclic structures) are indicators of affiliation with a certain taxonomic group, individual PLFA can be used as biomarkers for soil microorganisms [4]. Specific PLFA markers exist for methanotrophic archaea, gram-positive and gram-negative bacteria, mycorrhizal and saprotrophic fungi, and for other eukaryotes including plants. Specific PLFA markers with substitutions by methyl group in position 10 are used for actinomycetes belonging to gram-positive bacteria.

Gram-negative bacteria are known for their dominant role among the rhizosphere microorganisms, while gram-positive bacteria are distributed throughout the entire humus layer more uniformly [10]. Although individual PLFA markers can be used to determine the structure of soil microbial community only at the level of genera or physiological groups of microorganisms, their combination with the ^{13}C-SIP methods potentially makes it possible to identify the components of the soil microbial community involved in decomposition and humification of the recently incoming organic matter from root exudates and plant residues. However, a number of methodological complications such as natural variations in ^{13}C within the markers of the taxonomic groups, ambiguous subdivision of soil microbiota into rhizosphere and non-rhizosphere microorganisms, complicated recycling of "new" and "old" C in soil, etc., prevent univocal determination of the role of specific microorganisms in carbon transformation in soil by the value of ^{13}C enrichment [4, 5].

The goal of our work was to determine the contribution of new C4 and old C3 carbon as a source for microbial growth. Our working hypothesis was that, regardless of the method of C3–C4 transition (cultivation of C4 plants on C3 soil or ploughing the C4 plant material into C3 soil) and the type of soil, the changes in the structure of the soil microbial community and the input of new carbon into the biomass of specific taxonomic groups of microorganisms will proceed in approximately the same direction. Furthermore, in view of the inconsistency of the literature data, we decided to find out whether the content of C4 carbon could be used to assess the dominant role of gram-negative bacteria and saprotrophic fungi in the decomposition of plant residues and root exudates in soils under study.

MATERIALS AND METHODS

Soil. Soil was taken from the experimental plots for field experiments with the C3–C4 transition. Experiment 1 was performed on the field plots of the All-Russian Research Institute of Maize, Russian Academy of Agricultural Sciences (Voronezh oblast) on Chernozem (C 3.6%, N 0.3%, pH 6.8). In the field experiment, maize had been grown continuously since 1966. Individual replicates for laboratory experiments were selected from the mixed sample prepared for each of the three plots of 193 m^2. The control soil (C3 soil) was collected from four plots of the same size, where bare fallow had been maintained since 1966. The control plots were not fertilized. The rates of fertilizers at the plots with the C3–C4 transition were 120, 90 and 90 kg ha^{-1} for N, P, and K, respectively. The average annual carbon input with the crop residues of maize was 152 g C m^{-2}.

Experiment 2 was performed on Phaeozem on microplots (1 m^2, 3 replicates) founded on the fields of a multi-year experiment of the Institute of Physicochemical and Biological Problems of Soil Science, Russian Academy of Sciences (C 1.4%, N 0.1%, pH 6.0). Maize plant material was added to the soil of the plots each spring during 5 years at the rate of 1.16 kg C m^{-2}, which was equivalent to the mean annual C input into meadow ecosystems on this soil type. The plots of the microplot experiments were ploughed 5–6 times during a year for weed control. The control plots without addition of maize plant biomass were treated according to the same scheme. Both the controls and the plots with the C3–C4 transition received the annual rates of fertilizers: 60 kg ha^{-1} for N, P, and K, respectively. Before the beginning of the microplot experiment, the soil had been used in the grain–grass crop rotation.

In July 2008, soil samples were taken from the depth of 0–20 cm on the fields of both experiments. A mixed sample made of 5 individual sub-samples (200 g each) was prepared for each plot. Mixed samples were

used to make one representative soil sample of Chernozem (Experiment 1, "Ch-Control" and "Ch-C3–C4" treatments) and one representative sample of Phaeozem (Experiment 2, "P-Control" and "P-C3–C4" treatments). The content of total organic C and $^{13}C : {}^{12}C$ isotope ratio were determined in the soil samples taken from the plots of both field experiments. The isotope ratio was also determined in the samples of maize plants taken in Experiments 1 and 2. Fresh soil samples were passed through a 2-mm sieve; then small stones and visible root fragments were removed manually with forceps. The soil was dried at 20°C and stored at room temperature until the beginning of the incubation experiment in April 2009.

The scheme of the laboratory experiment. In the beginning of the experiment, the soil was wetted with deionized H_2O to 70% of water holding capacity (WHC) to activate the microorganisms and was divided into 2 series. In the first series, 20-g samples of dry soil were placed into 100-mL glass vials and wetted. Then the vials were tightly closed with rubber stoppers to isolate the space inside the vials from the atmosphere. Gas samples were periodically taken with a syringe to determine CO_2 concentration and isotope ratio. The vials were ventilated in order to maintain the carbon dioxide concentration at no more than 2% (aerobic conditions). In the second series, 100-g dry soil samples were placed into 500-mL glass vials, adjusted to 70% of WHC, and incubated according to the same scheme as the samples of series 1. After 10-day incubation, the soil from series 1 was analyzed for the content of dissolved organic matter (DOM) and microbial carbon (C_{mic}). The soil from series 2 was frozen at –22°C and stored till the analysis for PLFAs and the isotope ratio in PLFAs. The content of total C and the value of $\delta^{13}C$ were also determined in SOM and in the maize plant material.

Determination of the profiles of phospholipid fatty acids (PLFAs). PLFA analysis was performed in accordance with the procedures described by Zelles and Bai [11] and Gattinger et al. [8]. The lipids were extracted from fresh soil (25 g dry weight) with methanol, chloroform, and phosphate buffer. The lipids from this extract were separated into neutral lipids, glycolipids, and phospholipids (polar lipids) in a silica-bonded phase column (SPE-SI; Bond Elut, Varian, Palo Alto, United States) by elution with chloroform, acetone and methanol, respectively. The mixture was separated into methyl ethers of saturated (SATFA), monounsaturated (MUFA), and polyunsaturated (PUFA) lipids by extraction, separation, hydrolysis, and derivatization. The resultant fatty acid methyl esters (FAMEs) were separated and identified by gas chromatography/mass spectrometry (GC–MS) (5973MSD GC/MS Agilent Technologies, Palo Alto, United States) using polar columns (BPX-70, SGE GmbH, Griesheim, Germany), 60 m × 0.25 mm × 0.25 μm, coated with 70% cyanopropyl polysilphenylene-siloxane [12]. Fatty acid methyl esters were identified by comparing the retention time and mass spectra for the standard substances, the cells of pure microbial cultures, and environmental samples [7, 8]. The PLFA content was quantitatively assessed using the HP ChemStation software package for chromatography (SOVLVIT, Switzerland) by comparing the experimental chromatograms to the standard chromatograms for individual PLFA. Bacteria were identified by the following PLFAs: saturated (i15:0, a15:0, n15:0, i16:0, i17:0, a17:0, n17:0, cy17:0, and cy19:0) and monounsaturated (16:1ω7, 16:1ω9, and 18:1ω7). In the above PLFA set, fatty acids cy17:0 and cy19:0 were specific markers for gram-negative bacteria, while the acids i15:0, a15:0, i17:0 and a17:0 were specific markers for gram-positive bacteria [7, 13]. The specific markers for other groups were 18:2ω6,9 and 18:1ω9 for fungi [14, 15]; 20:2ω6,9c, 20:3ω6,9,12c, and 20:4ω6,9,12,15c for microeukaryotes [5]; and 10-methylated saturated PLFAs for actinomycetes [7].

The values of $\delta^{13}C$ for individual PLFAs were determined by online coupling of GC–MS system and isotopic mass spectrometry after combustion (GC Combustion III, Thermo Electron Cooperation, Bremen, Germany) and determining their isotope ratio in a mass spectrometer (IRMS, Delta Advantage, Thermo, Bremen, Germany). The actual $\delta^{13}C$ ratio of the individual FAME was corrected for the one C atom that was added during derivatization [16]. The isotope signal was expressed in the $\delta^{13}C$ versus the Vienna-Pee Dee Belemnite International Standard (V-PDB):

$$\delta^{13}C\ [‰\ VPDB] = [(R_{sample}/R_{V\text{-}PDB}) - 1] \times 1000, \quad (1)$$

where R_{sample} and R_{VPDB} are the $^{13}C/^{12}C$ ratios in the sample and in the V-PDB standard, respectively ($R_{V\text{-}PDB} = 0.0111802$ [17]).

Determination of carbon in soil microbial biomass. The carbon of soil microbial biomass was determined by the method of fumigation–extraction (FE) [18]. The FE method is based on extraction of the biomass of soil microorganisms killed by 24-h incubation in the atmosphere with chloroform vapors. For extraction, 0.5 M K_2SO_4 solution was used. Extraction was carried out at the soil to salt solution ratio of 1 : 4; the extracts were filtered through a dense paper filter, frozen at –20°C, and stored at this temperature up to the analysis for total soluble carbon on a TOC5050 analyzer (Shimadzu). Weighed wet soil samples corresponded to the dry weight of 10 g.

The microbial carbon pool size was calculated based on the value of "C flush", i.e. the difference between the C content in salt extracts from fumigated and non-fumigated (control) soils, with correction for the conversion factor $k_{EC} = 0.45$ (the extraction coefficient of killed microbial biomass):

$$C_{mic} - FE = C\ flush/k_{EC}. \quad (2)$$

Table 1. The values of $\delta^{13}C$ for plant material, SOM, microbial biomass, and CO_2 evolved from the control soil and from the soil under C3–C4 transition performed by the following methods: (1) cultivation of maize plants on C3 soil (Chernozem, Experiment 1) and (2) addition of plant C4 material (maize biomass) to C3 soil (Phaeozem, Experiment 2)

Soil type	Chernozem		Phaeozem		
$\delta^{13}C$ of maize, ‰	−11.6	(0.2)	−12.0	(0.1)	**
SOM in the control soil, %	3.2	(0.1)	1.4	(0.1)	***
SOM in soil under C3–C4 transition, %	3.4	(0.2)	1.8	(0.1)	***
$\delta^{13}C$ in SOM of control soil, ‰	−25.3	(0.2)	−25.8	(0.2)	**
$\delta^{13}C$ in SOM of soil under C3–C4 transition, ‰	−24.5	(0.1)	−21.8	(0.2)	***
New C4 in SOM under C3–C4 transition, %	6.4	(0.2)	27.2	(0.2)	***
$\delta^{13}C$ of C_{mic} in control soil, ‰	−22.9	(0.8)	−27.1	(0.3)	***
$\delta^{13}C$ of C_{mic} in soil under C3–C4 transition, ‰	−19.5	(1.1)	−21.6	(0.3)	***
New C4 of C_{mic} in soil under C3–C4 transition, %	24.1	(2.5)	39.7	(2.3)	***
$\delta^{13}C$ in CO_2 evolved from control soil, ‰	−23.5	(1.1)	−25.9	(1.1)	**
$\delta^{13}C$ in CO_2 evolved from soil under C3–C4 transition, ‰	−20.0	(2.2)	−19.7	(1.1)	NS
New C4 in CO_2 (soil under C3–C4 transition), %	25.6	(2.4)	45.2	(1.3)	***

Note: Symbols ** and *** denote the reliability of the differences between Chernozem and Phaeozem at $p < 0.05$ and $p < 0.01$, respectively (determined by the method of unpaired t test, $n = 5$). NS indicates statistically insignificant differences. The values of standard deviation are given in parenthesis.

The content of dissolved organic matter (DOM) in the K_2SO_4 extracts was determined on a total organic carbon analyzer (TOC5050, Shimadzu). The $\delta^{13}C$ value in salt extracts was determined by liquid chromatography/mass spectrometry of stable isotopes (LC-IRMS, Thermo Electron, Bremen, Germany) in accordance with the description given in the works [19] and [20]. The $\delta^{13}C$ value in microbial carbon ($\delta^{13}C_{mic}$) was calculated by the following formula [21]:

$$\delta^{13}C_{mic} = \frac{\delta^{13}C_f C_f - \delta^{13}C_e C_e}{C_f - C_e}, \quad (3)$$

where $\delta^{13}C_f$ and $\delta^{13}C_e$ are the values of $\delta^{13}C$ for extracts from the soil exposed to fumigation and from the fresh control soil; C_f and C_e are the contents of total C in extracts from the soil exposed to fumigation and from the fresh control soil.

Analysis of C and the isotope ratio in CO_2, soil, and plant material. The concentration of CO_2 and $\delta^{13}C$ values in the latter were determined by gas chromatography/mass spectrometry of stable isotopes (Delta plus, Finnigan MAT, Germany). Soil and maize plant samples were dried at 65°C and grounded on an automated tissue homogenizer (Retsch MM2, Retsch GmbH, Haan, Germany); weighed samples were collected into zinc capsules. The C content and $\delta^{13}C$ values were measured by Elemental Analyzer Isotope Ratio Mass Spectrometry (EA-IRMS; Eurovector, Milan, Italy) coupled to a Thermo Finnigan MAT 253 (Bremen, Germany). The size of the new (C4) carbon fraction within C pools and CO_2 (% of total C) was calculated by the following formula [5]:

$$F_C = \frac{\delta^{13}C_{C4} - \delta^{13}C_{C3}}{\delta^{13}C_{maize} - \delta^{13}C_{SOM}}, \quad (4)$$

where $\delta^{13}C_{C4}$ and $\delta^{13}C_{C3}$ are the $\delta^{13}C$ values for the carbon pools or for CO_2 evolved from the soil with C3–C4 transition and the control soil, respectively; $\delta^{13}C_{maize}$ and $\delta^{13}C_{SOM}$ are the $\delta^{13}C$ values for the biomass of C4 plants (maize) and SOM in the control soil. The total C content and the $^{13}C : {}^{12}C$ ratio in CO_2 were determined once in 1–3 days, depending on the intensity of carbon dioxide emission. C_{mic} and PLFAs were analyzed at the end of incubation.

Statistical analysis. The C content and the isotope ratio in SOM, maize biomass, DOM, C_{mic}, and evolved CO_2 were determined in 5 replicates, while PLFA was determined in 4 replicates. Unpaired t-tests were performed to reveal significant differences for $\delta^{13}C$ values in the C soil pools and in individual PLFAs, as well as for the share of contribution (%) of new (C4) carbon as a component of PLFA and soil C pools.

RESULTS

The $\delta^{13}C$ values and new (C4) carbon content in soil organic matter, C_{mic} and CO_2. The content of SOM in Chernozem was approximately twice as high as in Phaeozem, both in the controls and in the soil with C3–C4 vegetation succession (Table 1). At the same time, the control treatments for both soils showed approximately the same $\delta^{13}C$ values. Phaeozem with C3–C4 transition was more enriched in the ^{13}C iso-

tope that the respective treatment of Chernozem. Accordingly, Phaeozem contained more new carbon in SOM (27.2%) than Chernozem (6.4%). The $\delta^{13}C$ values for C_{mic} in the control Phaeozem (–22.9‰) and Chernozem (–27.1‰) differed significantly, whereas the difference for the soil exposed to C3–C4 transition was insignificant: –19.5 and –21.6‰, respectively. The microbial biomass of Chernozem showed only insignificantly higher values of $\delta^{13}C$ in the treatment with C3–C4 transition compared to the control soil (about 2‰). In Phaeozem, the effect of C3–C4 transition was much more pronounced: about 6‰ (Table 1). While $\delta^{13}C$ of CO_2 evolved from the control soil was higher for Chernozem, $\delta^{13}C$ for CO_2 from the soil after C3–C4 transition was approximately –20‰ of $\delta^{13}C$ regardless of the soil type. The percentage of new (C4) carbon in all carbon pools was significantly higher (at $p < 0.05$) for Phaeozem. In both field experiments, the share of C4 in the carbon pools followed the pattern $SOM < C_{mic} < CO_2$.

Phaeozem under C3–C4 transition was shown to have higher levels of microbial biomass at the end of incubation and of total CO_2 emission compared to the control treatment (Fig. 1). Chernozem showed another tendency: at a higher level of microbial biomass in the "Ch-C3–C4" treatment, total carbon dioxide emission was less than in the control (Fig. 1). It was most probably caused by higher resistance of SOM from Chernozem to microbial decomposition. Moreover, the relatively low soil respiration rate in the "Ch-C3–C4" treatment could be due to the fact that for ~40 years the plots under maize were treated with higher rates of mineral fertilizers than the bare fallow plots. This could result in a considerable increase in the yield factor (Y) of the soil microbial community after C3–C4 transition [22]. Both types of soil showed, however, the following fundamental pattern: the values of C3 in CO_2–C and C_{mic} in the control, which contained only C3 carbon by definition, were higher than the respective C3 values in the carbon pools of soil under conditions of C3–C4 transition. Thus, it was shown that C4 was a more preferred substrate for microbial growth and, therefore, the new carbon replaced a considerable part of the old one in microbial biomass and in the C pools utilized by soil microorganisms. As a result, gaseous losses of the old carbon from soil humus in the "C3–C4" treatments were lower than in the controls, i.e., we revealed a negative priming effect of organic compounds of plant origin on soil humus. This negative priming effect could probably be accounted for by C4 carbon being represented by easier decomposable organic substances compared to the old C3 in SOM: even after years and decades of C3–C4 transition, we observed no uniform labeling of all pools of soil organic matter by new carbon.

In spite of the fact that the soils in field experiments 1 and 2 differed in type, SOM content,

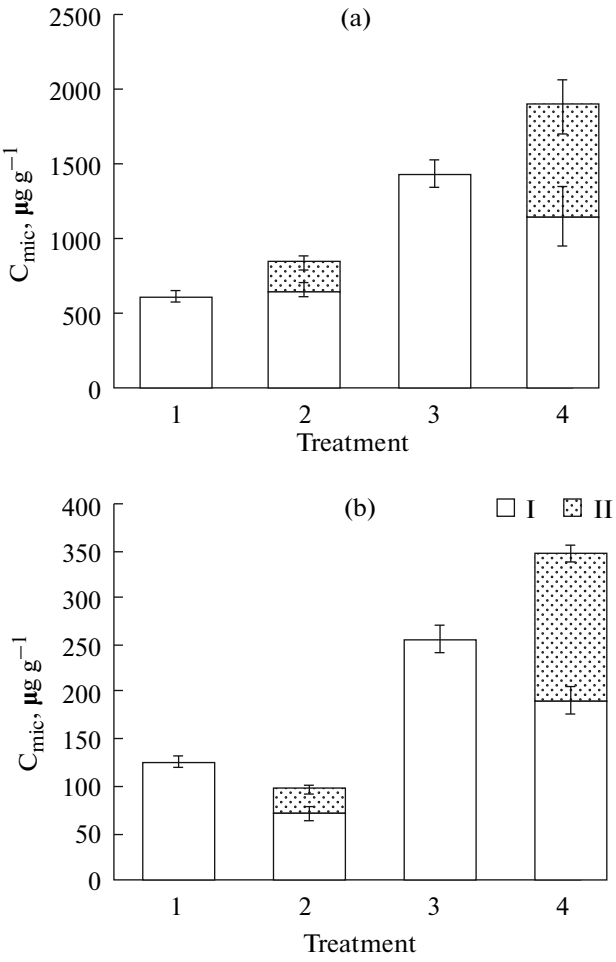

Fig. 1. Old C3 (I) and new C4 (II) carbon as components of the biomass of soil microorganisms (a) and CO_2 evolved from soil during 10 days of incubation (b) from Chernozem: control soil (1); soil under C3–C4 transition and Phaeozem (2): control soil (3); soil under C3–C4 transition, μg C g^{-1} of soil (4). Bars indicate the values of standard deviation (±SD).

and duration and annual input of plant-derived carbon to the soil, both soils showed similar tendencies: in the "C3–C4" treatments, the carbon of microbial biomass and the carbon of CO_2 were enriched in C4 compared to SOM.

The $\delta^{13}C$ values and the content of new (C4) carbon in phospholipid fatty acids. The $\delta^{13}C$ values in the individual PLFAs isolated from control soils varied from –33.4 to –20.1‰. For the soils under C3–C4 transition, the respective interval of values was –28.0...–14.9‰ (Table 3). This high variability in ^{13}C enrichment is in good agreement with the literature data. In the experiments of Kramer and Gleixner [5], $\delta^{13}C$ values for individual PLFAs varied from –19.9...–38.5‰ in the control and from –8.3... –25.7‰ in the soil under C3–C4 transition. Flessa et al. [4] also found the high variability of $\delta^{13}C$ for

Table 2. The content of individual PLFA isolated from the control soil and from the soils under C3–C4 transition performed by the following methods: (1) cultivation of maize plants on C3 soil (Chernozem, Experiment 1) and (2) addition of plant C4 material (maize biomass) to C3 soil (Phaeozem, Experiment 2)

Group of organisms	PLFA name	Chernozem					Phaeozem				
		control soil PLFA, nmol g^{-1}		soil under C3–C4 transition PLFA, nmol g^{-1}			control soil PLFA, nmol g^{-1}		soil under C3–C4 transition PLFA, nmol g^{-1}		
Gram-positive bacteria	br14:0	0.03	(0.02)	0.06	(0.02)	NS	0.04	(0.01)	0.05	(0.01)	NS
Bacteria	n14:0	0.19	(0.08)	0.25	(0.03)	ND	0.13	(0.04)	0.14	(0.02)	NS
Gram-positive bacteria	i15:0	0.70	(0.03)	1.28	(0.10)	***	0.48	(0.10)	0.52	(0.04)	NS
Gram-positive bacteria	a15:0	0.58	(0.07)	0.86	(0.08)	**	0.60	(0.14)	0.98	(0.04)	**
Bacteria	n15:0	0.12	(0.02)	0.23	(0.04)	**	0.07	(0.01)	0.11	(0.01)	**
Gram-positive bacteria	i16.0	0.27	(0.01)	0.49	(0.06)	***	0.19	(0.03)	0.23	(0.04)	NS
Gram-positive bacteria	a16:0	1.60	(0.17)	2.35	(0.10)	***	1.65	(0.14)	1.51	(0.14)	NS
Actinomycetes	10Me17:0	0.68	(0.11)	1.13	(0.18)	**	0.27	(0.06)	0.34	(0.07)	NS
Gram-positive bacteria	i17:0	0.29	(0.03)	0.50	(0.07)	**	0.14	(0.05)	0.15	(0.02)	NS
Gram-positive bacteria	a17:0	0.17	(0.01)	0.27	(0.03)	**	0.11	(0.03)	0.18	(0.04)	*
Gram-positive bacteria	11,17:0	0.29	(0.04)	0.51	(0.06)	**	0.14	(0.03)	0.14	(0.01)	NS
Gram-negative bacteria	cy17:0	0.34	(0.03)	0.55	(0.06)	**	0.47	(0.15)	0.44	(0.04)	NS
Actinomycetes	10Me18:0	0.10	(0.02)	0.18	(0.03)	**	0.03	(0.01)	0.05	(0.01)	NS
Gram-positive bacteria	br18.0	0.53	(0.04)	0.67	(0.02)	**	0.30	(0.04)	0.34	(0.02)	NS
Actinomycetes	10Me19:0	0.40	(0.03)	0.62	(0.08)	**	0.16	(0.06)	0.23	(0.02)	NS
Gram-negative bacteria	cy19.0	0.67	(0.05)	1.61	(0.16)	***	0.54	(0.17)	0.49	(0.09)	NS
Gram-positive bacteria	i20:0	0.25	(0.02)	0.42	(0.01)	***	0.14	(0.03)	0.20	(0.05)	NS
Protozoa	n22:0	0.09	(0.02)	0.13	(0.01)	**	0.12	(0.02)	0.12	(0.02)	NS
Protozoa	n24:0	0.05	(0.01)	0.09	(0.03)	*	0.09	(0.01)	0.06	(0.01)	**
Gram-negative bacteria	15:1ω6	0.01	(0.01)	0.03	(0.01)	***	0.01	(0.01)	ND	ND	ND
Arbuscular mycorrhiza	16:1ω5	0.08	(0.01)	0.13	(0.01)	***	0.07	(0.02)	0.08	(0.02)	NS
Gram-negative bacteria	16:1ω7	0.24	(0.03)	0.33	(0.02)	**	0.19	(0.07)	0.21	(0.06)	NS
Gram-negative bacteria	17:1ω8	0.10	(0.02)	0.18	(0.01)	***	0.03	(0.01)	0.03	(0.01)	NS
Methanotrophs	18:1ω7	0.27	(0.02)	0.36	(0.01)	***	0.19	(0.06)	0.20	(0.04)	NS
Saprotrophic fungi	18:1ω9	0.22	(0.02)	0.41	(0.03)	***	0.15	(0.03)	0.11	(0.02)	NS
Saprotrophic fungi	18:2ω6,9	0.11	(0.02)	0.26	(0.06)	**	ND	ND	0.15	(0.04)	ND

Note: Symbols *, **, and *** denote the reliability of differences at $p < 0.10$, $p < 0.05$, and $p < 0.01$, respectively (determined by the method of unpaired t-test, $n = 4$). NS indicates statistically insignificant differences; ND, PLFA was not determined. The values of standard deviation are given in parenthesis.

Table 3. The values of $\delta^{13}C$ and the $\delta^{13}C$ shift, ‰ for individual PLFAs under C3–C4 transition performed by the following methods: (1) cultivation of maize plants on C3 soil (Chernozem, Experiment 1) and (2) addition of plant C4 material (maize biomass) to C3 soil (Phaeozem, Experiment 2)

Group of organisms	PLFA name	Chernozem						Phaeozem					
		control soil		soil under C3–C4 transition			shift in $\delta^{13}C$	control soil		soil under C3–C4 transition			shift in $\delta^{13}C$
Gram-positive bacteria	br14:0	–24.4	(1.7)	–21.3	(0.9)	NS	3.1	–25.5	(2.2)	–19.9	(0.8)	**	5.6
Bacteria	n14:0	–27.0	(0.4)	–24.8	(0.7)	*	2.3	–26.3	(1.1)	–23.9	(1.0)	*	2.4
Gram-positive bacteria	i15:0	–22.8	(0.9)	–18.2	(0.3)	**	4.6	–24.4	(0.7)	–18.4	(0.6)	***	5.9
Gram-positive bacteria	a15:0	–20.6	(1.3)	–16.3	(0.9)	*	4.3	–22.0	(0.7)	–16.5	(0.6)	***	5.5
Bacteria	n15:0	–20.1	(2.4)	–17.3	(2.0)	NS	4.5	–24.3	(1.6)	–17.3	(2.0)	**	7.0
Gram-positive bacteria	i16.0	–21.5	(1.2)	–17.2	(0.8)	*	4.3	–24.6	(0.8)	–18.2	(1.4)	***	6.4
Gram-positive bacteria	a16:0	–22.5	(0.8)	–18.5	(0.4)	**	4.0	–24.6	(0.5)	–19.2	(1.3)	***	5.5
Actinomycetes	10Me17:0	–22.8	(0.9)	–18.4	(1.1)	*	4.5	–24.7	(0.8)	–19.9	(1.2)	***	4.8
Gram-positive bacteria	i17:0	–21.8	(2.1)	–16.1	(0.6)	*	5.7	–23.4	(1.0)	–16.9	(1.3)	***	6.6
Gram-positive bacteria	a17:0	–20.5	(1.2)	–14.5	(1.8)	*	6.0	–22.0	(1.3)	–16.7	(0.7)	***	5.2
Gram-positive bacteria	11,17:0	–22.5	(2.1)	–18.4	(1.2)	NS	4.1	–22.6	(2.6)	–14.9	(0.3)	**	7.7
Gram-negative bacteria	cy17:0	–23.3	(0.9)	–18.2	(0.9)	**	5.1	–25.7	(1.2)	–20.5	(1.5)	**	5.3
Actinomycetes	10Me18:0	–23.1	(1.5)	–17.9	(0.7)	*	5.2	–24.3	(1.3)	–20.8	(1.5)	*	3.5
Gram-positive bacteria	br18.0	–23.9	(0.3)	–20.0	(0.7)	**	4.0	–25.1	(0.6)	–22.5	(1.1)	**	2.6
Actinomycetes	10Me19:0	–23.0	(1.1)	–19.2	(1.3)	*	3.9	–25.2	(0.5)	–19.8	(0.8)	***	5.4
Gram-negative bacteria	cy19.0	–23.8	(0.8)	–19.9	(0.4)	**	3.9	–27.0	(0.8)	–21.0	(0.7)	***	6.0
Gram-positive bacteria	i20:0	–26.7	(0.8)	–23.6	(0.2)	**	3.1	–29.9	(0.7)	–25.7	(0.7)	***	4.2
Protozoa	n22:0	–27.0	(0.4)	–23.0	(0.2)	**	4.0	–29.6	(0.7)	–27.8	(0.5)	**	1.7
Protozoa	n24:0	–27.1	(3.2)	–22.8	(1.8)	NS	4.3	–29.7	(0.5)	–26.5	(1.3)	**	3.2
Gram-negative bacteria	15:1ω6	–25.8	(1.4)	–20.2	(0.7)	**	5.5	–29.1	(0.4)	ND	ND		ND
Arbuscular mycorrhiza	16:1ω5	–21.9	(1.4)	–17.1	(0.7)	*	4.7	–24.7	(0.8)	–17.1	(1.5)	***	7.6
Gram-negative bacteria	16:1ω7	–24.9	(1.5)	–21.6	(0.6)	*	3.3	–27.3	(0.4)	–21.6	(0.6)	***	5.7
Gram-negative bacteria	17:1ω8	–26.5	(1.3)	–23.1	(0.6)	*	3.5	–33.4	(1.6)	–23.9	(1.9)	***	8.3
Methanotrophs	18:1ω7	–26.0	(1.5)	–23.6	(0.7)	NS	2.4	–27.6	(1.3)	–22.4	(1.5)	**	5.3
Saprotrophic fungi	18:1ω9	–20.9	(1.4)	–18.1	(0.6)	*	2.7	–22.3	(1.2)	–19.0	(2.6)	NS	3.3
Saprotrophic fungi	18:2ω6,9	–22.5	(1.5)	–16.9	(0.7)	**	5.6	ND	ND	–18.7	(0.7)		ND

Note: Symbols *, **, and *** denote the reliability of differences at $p < 0.10$, $p < 0.05$, and $p < 0.01$, respectively (determined by the method of unpaired t-test, $n = 4$). NS indicates statistically insignificant differences; ND, PLFA is not determined. The values of standard deviation are given in parenthesis.

PLFA extracted from the soil under maize: from –11.0 to –27.7‰.

All PLFAs extracted in the "C3–C4" treatments showed higher ^{13}C concentrations than the controls, i.e., they were more enriched in the new (C4) carbon. The highest content of identified PLFAs was found in the "Ch-C3–C4" treatment (Table 2). As regards individual MUFAs, the maximum concentrations were shown for 18:1ω7 and 18:1ω9. Among monounsaturated PLFAs, 16:1ω5 and 18:1ω9 were the most enriched with ^{13}C. The maximum shifts in $\delta^{13}C$ values for PLFA under conditions of C3–C4 transition compared to the respective PLFAs in the control soil were observed for 16:1ω5 and 17:1ω8 in the "P-C3–C4" variant (7.6 and 8.3‰, respectively; Table 3).

Apart from monounsaturated PLFAs, the "Ch-C3–C4" treatment showed maximum concentrations of saturated PLFAs. Among the latter, the highest concentrations were determined for Cy19:0, i15:0, and a16:0. The highest concentrations in Phaeozem were shown for Cy19:0, a15:0, and a16:0 (0.49, 0.89, and 1.51 nmol g^{-1}, respectively). The maximum changes in $\delta^{13}C$ during C3–C4 transition were observed for 11,17:0 (7.7‰), i17:0 (6.6‰), and Cy19.0 (6.0‰) in Phaeozem; i17:0 (6.7‰) and for a17:0 (6.0%‰) in Chernozem (Table 3).

The only representative of the group of polyunsaturated PLFAs revealed in our soil samples, 18:2ω6,9 (the marker of saprotrophic fungi), was found only in Chernozem, in the soil with high SOM content, and in the "P-C3–C4" treatment enriched with C from maize biomass. This marker was not found in the control Phaeozem (Tables 2–4). The latter soil was shown to contain only the fungal marker 18:1ω9, a representative of the group of monounsaturated PLFAs, with a rather moderate isotopic shift (3.3‰) and C4 contribution to PLFAs (24%). The absence of the 18:2ω6,9 marker in soil with low SOM content is in good agreement with the literature data. For example, Kramer and Gleixner [5] revealed the 18:2ω6,9 marker only in a relatively fertile soil (Haplic Luvisol), while the extracts from a less fertile soil (Haplic Phaeozem) did not contain this PLFA.

The C4 contribution to PLFAs varied within 18 to 60% (an average of 38%) in Phaeozem and 15 to 40% in Chernozem (an average of 28%). The following PLFAs were most enriched with the new carbon: (a) 16:1ω5, the marker for arbuscular mycorrhiza (AM); (b) 11,17:0; i17:0 and 17:0, the markers for gram-positive bacteria; (c) Cy19.0 and 17:1ω8, the markers for gram-negative bacteria; and (d) 18:2ω6,9, the above mentioned marker for saprotrophic fungi (Table 4). The considerable content of C4 carbon in the PLFA marker for AM is easily explicable taking into consideration the close involvement of AM into the plant root system [23]. By the degree of enrichment with new carbon, the groups of microorganisms in Phaeozem were arranged as follows: protozoa < saprotrophic fungi < actinomycetes < gram-positive bacteria < gram-negative bacteria < mycorrhizal fungi (Fig. 2). The contribution of C4 carbon to PLFAs extracted from Chernozem did not differ significantly for different groups of organisms, with the statistically insignificant maximum for mycorrhizal fungi (Fig. 2).

It is notable that even after more than four decades of the maize monoculture (Experiment 1), the most of carbon in SOM, C_{mic}, and most of the detected individual PLFAs was represented by old carbon (Tables 1, 4). It is in good agreement with the data of other authors. For instance, the experiment of Kramer and Gleixner [5] showed that C4 carbon substituted only 20% of carbon in PLFAs and about 15% of carbon in SOM after 39 years of maize cultivation on C3 soil. Nearly half of the PLFA carbon was represented by old C even in the soil with a relatively high value of old carbon substitution in SOM (nearly one third of total SOM C).

DISCUSSION

Gram-negative bacteria are thought to prefer the new plant-derived carbon, while gram-positive bacteria are to be involved mainly in the transformation of the old SOM-derived carbon [4, 23, 24]. Therefore, it is usually supposed that the biomass of gram-negative bacteria is enriched with ^{13}C isotope compared to the biomass of gram-positive bacteria. However, in our experiment this tendency was rather "fuzzy" and statistically insignificant in Phaeozem with low SOM content, while it was absent in humus-rich Chernozem. Previously, Kramer and Gleixner [5] also mentioned that, although the PLFA markers of gram-negative bacteria were enriched with C4 carbon compared to the markers of gram-positive bacteria, the changes in isotopic enrichment of PLFAs in the more fertile soil were similar for gram-negative and gram-positive bacteria. In other words, the authors did not reveal any differences between gram-positive and gram-negative bacteria in the "strategy of nutrition", as it could be expected in terms of modern soil microbiology. Denef et al. [25] also showed gram-positive bacteria, along with gram-negative ones, to be able to immobilize considerable amounts of ^{13}C exudates after $^{13}CO_2$ pulse labeling of plants. Thus, the widespread notion about gram-negative bacteria as mostly associated with roots and gram-positive bacteria as evenly distributed in soil and therefore little involved in plant carbon transformation has not been experimentally confirmed by isotopic techniques.

The most probable reasons of the relatively low content of new carbon in PLFA in general and in the markers of fungi and gram-negative bacteria in particular are as follows:

(1) Carbon assimilation (recycling) by bacteria occurs from fungal necromass, including ^{13}C, rather than only directly from rhizodeposits or plant residues

Table 4. The portion of new (C4) carbon (%) for individual PLFAs under C3–C4 transition performed by the following methods: (1) cultivation of maize plants on C3 soil (Chernozem, Experiment 1) and (2) addition of plant C4 material (maize biomass) to C3 soil (Phaeozem, Experiment 2)

Group of organisms	PLFA namev	Chernozem		Phaeozem		Reliability
		% C4		% C4		
Gram-positive bacteria	br14:0	21	(6)	41	(5)	**
Bacteria	n14:0	15	(2)	18	(2)	NS
Gram-positive bacteria	i15:0	31	(2)	43	(4)	**
Gram-positive bacteria	a15:0	29	(3)	40	(2)	**
Bacteria	n15:0	30	(6)	51	(8)	**
Gram-positive bacteria	i16.0	29	(4)	46	(10)	*
Gram-positive bacteria	a16:0	27	(1)	40	(5)	**
Actinomycetes	10Me17:0	30	(5)	35	(8)	NS
Gram-positive bacteria	i17:0	38	(5)	48	(6)	NS
Gram-positive bacteria	a17:0	40	(7)	38	(8)	NS
Gram-positive bacteria	11,17:0	27	(3)	56	(2)	***
Gram-negative bacteria	cy17:0	34	(4)	38	(4)	NS
Actinomycetes	10Me18:0	35	(6)	25	(5)	NS
Gram-positive bacteria	br18.0	26	(1)	19	(2)	***
Actinomycetes	10Me19:0	26	(4)	39	(4)	**
Gram-negative bacteria	cy19.0	26	(3)	44	(8)	**
Gram-positive bacteria	i20:0	21	(1)	30	(7)	*
Protozoa	n22:0	27	(2)	23	(4)	NS
Protozoa	n24:0	29	(9)	23	(4)	NS
Gram-negative bacteria	15:1ω6	32	(4)	ND	ND	
Arbuscular mycorrhiza	16:1ω5	32	(2)	55	(14)	*
Gram-negative bacteria	16:1ω7	22	(1)	41	(11)	*
Gram-negative bacteria	17:1ω8	29	(2)	60	(23)	*
Methanotrophs	18:1ω7	16	(1)	38	(7)	**
Saprotrophic fungi	18:1ω9	18	(1)	24	(6)	NS
Saprotrophic fungi	18:2ω6,9	38	(8)	ND	ND	

Note: Symbols *, **, and *** denote the reliability of differences between Chernozem and Phaeozem at $p < 0.10$, $p < 0.05$, and $p < 0.01$, respectively (determined by the method of unpaired t-test, $n = 4$). NS, statistically insignificant differences; ND, PLFA is not determined. The values of standard deviation are given in parenthesis.

as such. Denef et al. [26] used this phenomenon to explain the fact that rapid ^{13}C input from the labeled exudates to the biomass of soil fungi was initially observed 10 h after $^{13}CO_2$ pulse labeling of the plants, but after 11 months the fungal PLFA markers were much less enriched with ^{13}C compared to the bacterial PLFA markers. The same phenomenon could partially account for the relatively low content of new carbon in 18:1ω9, one of the markers of saprotrophic fungi in our experiment.

(2) One of the reasons of the relatively low content of new carbon in fungal biomass may be the presence of a considerable portion of soil saprotrophic fungi as dormant forms (spores). According to Blagodatsky et al. [27], the biomass of microorganisms that could grow immediately after addition of an easily degradable substrate (glucose) to soil represents no more than 1% of the total soil microbial biomass. The portion of active microorganisms increased only to 4–10%, even in the course of long-term incubation experiments with periodical addition of large amounts of glucose. These data are in good agreement with those of Hobbie et al. [28], who combined the radiocarbon method and AMS spectroscopy and discovered that the "age" of carbon in the biomass of saprotrophic fungi (about 10 years) was much greater than that of mycorrhizal carbon (not more than 2 years). The same method in combination with fumigation showed also a considerable content of dormant forms in the biomass of soil

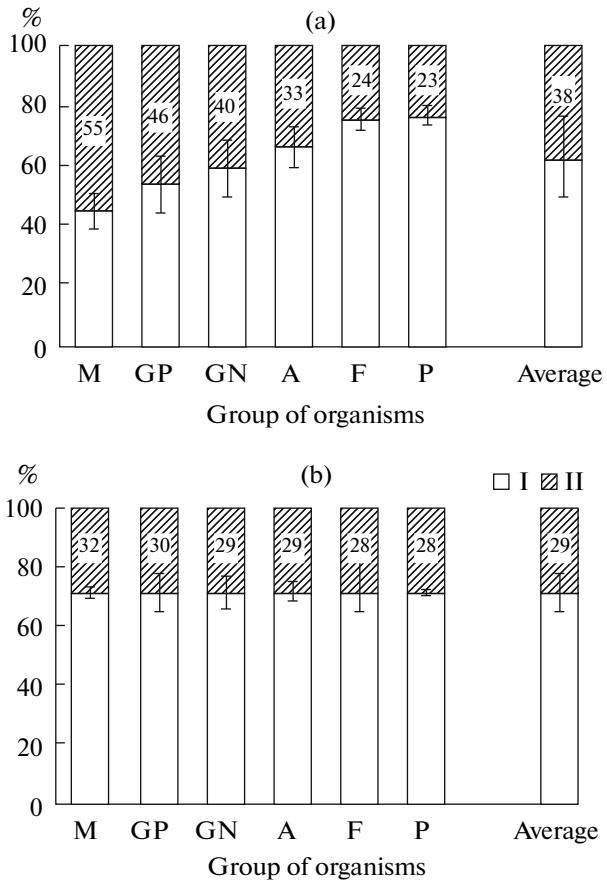

Fig. 2. The contributions of old C3 (I) and new C4 (II) to the biomass carbon of different groups of organisms determined by the method of PLFA profiling (%) in the soil under C3–C4 transition in Chernozem (a) and in Phaeozem (b). Designations of the groups of organisms: GP, gram-positive bacteria; A, actinomycetes; GN, gram-negative bacteria; M, arbuscular mycorhhizal fungi; F, for saprotrophic fungi; P, protozoa; av., the average for all groups of organisms. Bars indicate the values of standard deviation (±SD).

microorganisms [29]. Hence, it may be supposed that only a small part of soil fungal community in our experiments was actually involved in assimilation and mineralization of new carbon. Most of the fungal community biomass was represented by the biomass of organisms in the dormancy or maintenance status, or under conditions of inaccessibility of the plant-derived substrate in soil due to microhabitat diversity.

(3) Gram-positive bacteria can assimilate both the easily decomposable plant-derived organic substances and the stable SOM components, as has been shown by the method of combined ^{13}C and ^{14}C labeling [5]. Other authors also showed intensive reutilization of SOM carbon in soil [4, 29]. Thus, the ability of members of the soil microbial community to grow on certain carbon substrates depends much more on the substrate availability and other ecological conditions than on the structure of this community. In our experiments, the substances containing new carbon were largely transformed during the long-term C3–C4 transition in Chernozem and Phaeozem from easily decomposable rhizodeposits (the "Ch-C3–C4" treatment) or from newly added plant material (the "P-C3–C4" treatment) into a broad range of carbon pools with different recalcitrance to mineralization by soil microorganisms. In other words, the resultant carbon pools were available for utilization by both gram-negative and gram-positive bacteria.

The relative contribution of the above mechanisms to the regulation of turnover of the newly arrived C from plant residues, as well as humus mineralization in soil, can be estimated in further experiments where the comparative resistance of C4 and C3 compounds as components of the SOM fractions, as well as the patterns of new carbon input both into the biomass of different groups of soil microorganisms and into different fractions of soil organic matter will be studied.

Thus, our experiment to assess the carbon input from plants with C4 photosynthesis into the biomass of soil microorganisms showed that biomarker PLFA carbon for all taxonomic groups in Chernozem and Phaeozem was enriched in C4 carbon compared to SOM, with the maximum portion in the mycorrhizal biomass. We showed a tendency for preferential utilization by soil microorganisms of the newly incoming plant-derived C, rather than SOM-derived carbon, i.e., the negative priming effect. On the contrary, the dominance of gram-negative bacteria and saprotrophic fungi in plant carbon assimilation, which could be assumed on the basis of available literature data, was not revealed in our experiment. We believe that this phenomenon is accounted for by the fact that the long-term C3–C4 transition resulted in formation of a wide range of C4-containing carbon pools with different recalcitrance to mineralization and, as a result, soil microorganisms assimilated C from both easily decomposable and stable forms of C4 organic compounds. That was probably the reason why C4 was found not only in the biomass of the mainly rhizosphere microorganisms (e.g., gram-negative bacteria), but also by the microorganisms involved in mineralization of humus substances (e.g., gram-positive bacteria). Moreover, the portion of C3 and C4 carbon in the PLFA markers of specific groups of soil microorganisms was greatly influenced by the phenomenon that most of the biomass of the soil microbial community was represented by dormant, rather than by actively growing forms. Elucidation of the quantitative contribution of these mechanisms requires additional experiments with parallel determination of C4 carbon input into the biomass of different taxonomic groups of microorganisms and into different fractions of soil organic matter.

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