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SOIL CHEMISTRY

Transformation of Biochar from Plant Biomass in Gray Forest Soil: Evaluation by Isotopic Labeling Method

P. Yu. Galitskaya[a] (ORCID: 0000-0002-5070-786X), S. Yu. Selivanovskaya[a] (ORCID: 0000-0001-6379-7166), K. O. Karamova[a] (ORCID: 0000-0003-0846-251X), A. S. Gordeev[a, *] (ORCID: 0000-0002-1918-305X), P. A. Kuryntseva[a] (ORCID: 0000-0002-9274-7077), and P. Ghorbannezhad[b] (ORCID: 0000-0002-6146-8964)

[a] *Kazan Federal University, Kazan, 420008 Russia*

[b] *Shahid Beheshti University, Tehran, 198396 Iran*

**e-mail: drgor@mail.ru*

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Abstract—Pyrolysis is considered to be one of promising methods for processing agricultural waste and for producing fertilizers. The efficiency of the resulting biochar as a fertilizer has been proven, but the preferential way of decomposition of organic substances in it—biotic or abiotic—is still open to argument. The ways of transformation of biochar obtained from corn (a plant of the C_4 type of photosynthesis with an increased ^{13}C content) were assessed in this work, using the solid-phase CP/MAS ^{13}C NMR spectroscopy. Biochar was placed into the top layer of a monolith of gray forest soil, and the precipitation regime characteristic of Central Russia was simulated for 90 days. The peak at 129 ppm typical for aromatic compounds increased during the experiment in the obtained NMR spectra of soil samples with biochar in the upper soil layer, but not in other layers. This testifies that biochar particles do not migrate down the soil profile during one season. The intensity of cumulative microbial respiration in the presence of biochar increases from 85.0 g CO_2 kg^{-1} in the control sample to 201.4 g CO_2 kg^{-1} in the sample with biochar (the topsoil). According to the NMR spectra of the salt formed during mineralization of carbon dioxide released from the soil, it contains labeled carbon: there is a peak at 169 ppm characteristic of carbonates. The cumulative volume of CO_2 released from the soil with biochar is 1.9 times greater as compared to the control soil. The addition of microorganisms-decomposers caused an additional increase in the CO_2 volume: 2.4 times relative to the control, which indicates the role of microorganisms in the destruction of soil organic matter and of biochar. However, based on the stability of the total carbon content in the soil, it can be concluded that only a small proportion of biochar components is susceptible to biotic decomposition.

Keywords: carbon, biochar, isotope label, particle migration, respiratory activity

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INTRODUCTION

Biochar (biocoal, pyrocoal), which is a product of oxygen-free thermal decomposition of biomass (plants and organic agricultural, household, and industrial wastes), has attracted great attention in recent years of both scientists and representatives of the real sector of economy [25]. Biochar is mainly composed of carbon and hydrogen, as well as of oxygen, nitrogen, and sulfur, the proportion of which depends on the raw material and the conditions of biochar production [31, 35]. Biochar is characterized by high porosity and stability and thus may be used as a sorbent, for example, in wastewater treatment [6, 41, 50, 54], or as a soil ameliorant. It improves soil water and air exchange, creates ecological niches for beneficial microorganisms, and introduces additional sources of carbon and nitrogen nutrition of plants [51]. A reason for the use of biochar is the fact that its application as an ameliorant enables sequestering of a significant carbon amount in the soil, which is important for preventing global climate change [39, 40].

Despite the accumulation of a significant scientific pool of data on the use of biochar for improving soils, the ways of its transformation in soil are still insufficiently studied. It is known that it is slower than the transformation of other types of organic fertilizers, for example, compost. The long-term release of carbon and nitrogen compounds enables the application of biochar as a fertilizer once every few years and not annually [8, 32, 45]. The following problems demand solution: which processes—aerobic or anaerobic—in the biochar transformation predominate [27, 52], and into which compounds of humus and biomass, or gaseous substances biochar is transformed [52]. The study of biochar transformation causes some difficulties similarly to other carbon-containing fertilizers and soil ameliorants. The period of partial or complete transformation of biochar in soil is not clear: accord-

ing to some estimates, it may range from hundreds of hours to hundreds of years [4, 52]. It is traditionally supposed that the initial stages of biochar decomposition consist in splitting the easily available water-soluble fractions transported from biochar into the soil under the effect of moisture, decomposition of the ameliorant structure by soil biota, etc. It is assumed that the structure of biochar itself is changed with time: the so-called ageing of biochar [43]. However, there is more than one opinion of researchers about the features and nature of such changes, and the stability of biological coal depends on the conditions of its production and on raw materials used. The role, composition, diversity, and species composition of soil biota in degradation of biochar in the soil have not been comprehensively studied [12, 26].

The above discussed difficulties result in the use of the method of stable or radioactive isotopes for the study of transformation of carbon-containing objects [15]. Stable isotopes are an effective tool for modern environmental research to determine the main stages of the complete cycle of carbon transformation in the soil–plant–atmosphere system [26, 47]. The use of isotope-labeled biochar samples, containing ^{13}C and/or ^{14}C isotopes, enables us to trace the ways of fertilizer decomposition in soil by the difference in the content of isotopes in soil and in biochar samples [52]. Raw materials of plant origin with a high content of isotopes (for example, of ^{13}C) are used to obtain isotopically labeled carbon samples [47]. Such raw materials may be obtained by growing plants in a ^{13}C medium, where most of CO_2 in the atmosphere is replaced by $^{13}CO_2$. In this case, all processes of carbon absorption by plants from the air involve isotope-labeled CO_2, and the resulting plant raw materials contain to 99% of ^{13}C isotope [47]. This method is related to great technical difficulties and requires significant time and financial expenses; therefore, C_4-plants are used along with the plant raw materials obtained by this method. They accumulate ^{13}C in the green mass in natural habitats under normal conditions. There are examples in published works of the use of C_4-plants, most often of corn, to study the transformation of fertilizers and biochar in soil [26, 52]. In this case, the proportion of carbon replaced by its isotope is not constant and ranges from 1.1 to 6 wt % according to various estimates [47], depending on a number of conditions. The use of C_4-plants as raw materials to obtain biochar does not guarantee the required level of carbon substitution in biochar, which requires additional research. Nevertheless, the described approach has proven to be successful and effective in this kind of research.

Despite the fact that NMR is an effective method to study the structural and dynamic properties of a wide range of objects and systems, biochar studies with its application are single [22, 29, 47]. For example, the work by Cimo et al. (2014) presents CP/MAS ^{13}C NMR spectra of biochar samples made of different raw materials (chicken manure, coniferous trees, and poplar) and under various conditions (peak pyrolysis temperature) [7].These spectra consist of a single wide signal in the range from 100 to 160 ppm with the center at 126 ppm. According to [24, 34], the region between 110 and 160 ppm is usually assigned to aromatic systems or graphite-like microcrystallites.

This similarity of the CP/MAS ^{13}C NMR spectra is explained by the fact that the aromatic carbon signal increases with the rise in the pyrolysis temperature in the range of 250–450°C [3, 9, 13], i.e., an increase in the pyrolysis temperature causes transformation of the initial biomass into aromatic coal. Despite the structural similarity of the samples, their physicochemical properties differ [7, 21].

The shape and parameters of the NMR spectra of biochar strongly depend on the type of raw material, its moisture content, the presence of inclusions and impurities, the type of pyrolysis treatment, the parameters and mode of pyrolysis (for example, the peak temperature of pyrolysis and its duration), etc. [17, 29, 47]. Therefore, there are different points of view among researchers on the nature of the shape and parameters of NMR spectra. Nevertheless, most of published works [17, 22] contain information about the role of biochar and soil with biochar in the region of 120–130 ppm of CP/MAS ^{13}C NMR spectra typical for aromatic compounds. It is emphasized [37, 38] that there is a contribution of pyrolyzed organic material to the signal from alkyl groups and the weakening of aryl groups in isolated soil organic matter during the experiments with the introduction of biochar into the soil.

The method of labeled isotopes is extremely rarely used to study the biochar behavior in the environment, primarily in the soil, which is characterized by various simultaneous biotic and abiotic processes [2, 19].

The purpose of this work was to study the transformation of biochar in the soil based on plant residues. The tasks included the assessment of migration of biochar particles introduced into the upper soil layer down the soil profile, as well as the analysis of the decomposition rate of biochar in soil to CO_2 and of the role of soil microorganisms in this process. The experiment was performed in intact soil columns, and the biochar transformation was evaluated by the method of labeled isotopes.

OBJECTS AND METHODS

Production and verification of biochar with a high content of ^{13}C isotope. Phytomass of plants with the C_4-cycle of photosynthesis—corn (*Zea mays*)—was used to obtain biochar with a high content of ^{13}C isotope. Pyrolysis was performed on an experimental rotor-type installation at 400°C during 2 h. Biochar produced of substrate without an increased content of

^{13}C isotope—chicken manure—was used as a control. Preliminary burning of three portions of chicken manure was performed at 400°C to clean the pyrolysis chamber from trace amounts of decomposition manure products prior to the actual production of biochar with a high content of ^{13}C. According to the data of the elemental analysis performed according to ISO 13878:1998 and ISO 10694:1995, biochar used in the experiment contained 56.97 ± 0.08% of total carbon with an isotope fraction of ^{13}C of 7.0 ± 1.0% of the total mass of biochar and 1.09 ± 0.02% of the total nitrogen of the gross biochar mass. After pyrolysis, biochar became a powder, which was applied into the soil without fractionation. The particle size in the powder was characterized by laser diffraction on a Bluewave particle analyzer (Microtrac, United States) according to ISO 13320:2020. Particles from 3 to 700 μm predominated by fractions of 1–500 μm (96 vol %) were present in the powder. We took 1 g of biochar to analyze solid-phase spectra of CP/MAS ^{13}C NMR. No additional homogenization was performed.

Experiment with intact soil columns. Undisturbed soil monoliths were taken from a soil pit on the experimental plot of the Tatar Research Institute of Agriculture, Kazan Scientific Center, Russian Academy of Sciences located in Laishevskii district of the Republic of Tatarstan in the forest-steppe zone [1]. Soils in the columns were characterized prior to the experiment. The soil was assigned to the type of plowed gray forest soils (Phaeozem). The filtration coefficient was 43.2 mm/min, the total moisture capacity of the plow layer was 35.2%, and the field moisture capacity was 25.7%. The studied soil was characterized by a medium-loamy particle-size composition with the domination of the clay–coarse-silt fraction. Soil monoliths were taken by soil columns made of stainless steel: prisms with square cross-section, one of the faces of which was absent and was covered by a polymer film during the experiment. The distribution of soil particles by size was characterized, using a Bluewave laser diffractometer (Microtrac, United States), and was represented by a range from 0.5 to 500 μm with the domination of particles of 2–100 μm (88 vol %) [44]. The size of soil monoliths was 10 × 10 × 50 cm.

Five columns were assigned to the experiment (B), and the other five columns were used as control ones (K). Biochar was applied to the upper layer of the experimental columns at the rate of 8% (of the soil mass in the layer of 0–5 cm). We also laid columns D, in the upper layer of which, water extract from compost, containing microorganisms, was applied in addition to biochar at the volume concentration of 100 mL/kg. The extract contained 1×10^7 copies of 16S rRNA genes.

Then, we simulated precipitation in April–June, i.e. during the snowmelt period and the following 2 months [23, 53]. The following statistical meteorological data were used to assess irrigation regime and volume of experimental soil columns: the mean accumulation of solid precipitation during the cold period (from October to March) for the area of the Republic of Tatarstan is 165.2 mm, and the mean precipitation is 170.7 mm. The total precipitation was divided by 30 days of the experiment duration. The columns were incubated at a temperature of 20–22°C and the relative moisture of 70–75%.

Sampling was performed on the 3rd, 7th, 30th, 60th, and 90th days from the experiment start. Samples were taken from layers during the monolith division: the upper four layers of 5 cm thick (BT1a, BT1b, BT2a, and BT2b for experimental columns; KT1a, KT1b, KT2a, and KT2b for control columns; and DT1a, DT1b, DT2a, and DT2b for columns with microorganisms) and then three layers of 10 cm thick (BM1, BM2, and BL for experimental columns; KM1, KM2, and KL for control columns; and DM1, DM2, and DL for columns with microorganisms) (Fig. S1).

The content of total nitrogen and total carbon was determined in samples of 10-cm-thick layers of control soil samples KT1, KT2, KM1, KM2, and KL and of samples with biochar BT1, BT1, BM1, BM2, and BL on an automatic CN analyzer Vario Max Cube (Elementar, Germany) according to ISO 13878:1998 and ISO 10694:1995. Respiratory activity of soil microorganisms was measured is these samples according to ISO 16072:2002 by incubating soil samples in closed vessels and subsequent titrimetric determination of the released CO_2 after its capture by alkali. The physiological profile of the soil microbial community was evaluated by the method of multisubstrate testing by the Biolog Ecoplate system for those soil samples, which were taken by layers on the 90th day to determine total C and N. Suspensions (1 : 1000) prepared of these samples were dropped by 140 μL into 96-well Biolog Ecoplate tablets (Biolog, Int, United States). The tablets were incubated at 25°C for 5 days (until the growth of the degree of cell staining stopped), and the optical density was measured at a frequency of 24 h, using a Multiskan FC tablet reader (Thermo Fisher Scientific, United States). The intensity of substrate consumption and the total metabolic activity of microorganisms was determined by the mean staining intensity of medium in the cells (31 cells for each sample) summed for 5 days (AWCD). The Shannon index was calculated to assess the microbial functional diversity (31 substrates for each sample) [43].

Soil samples, as well as a mixture of alkali and sodium carbonate solutions formed after CO_2 capture were taken and prepared for the NMR spectroscopy.

NMR spectroscopy. The CP/MAS ^{13}C NMR spectroscopy was performed for the following solid samples: biochar, soil layer 0–5-cm-thick, and solid sediment after evaporation of the mixture of alkali solutions with sodium carbonate. The preparation of biochar consisted in taking a representative sample of 1.0 g immediately after the end of pyrolysis and cooling. The preparation of soil samples included drying

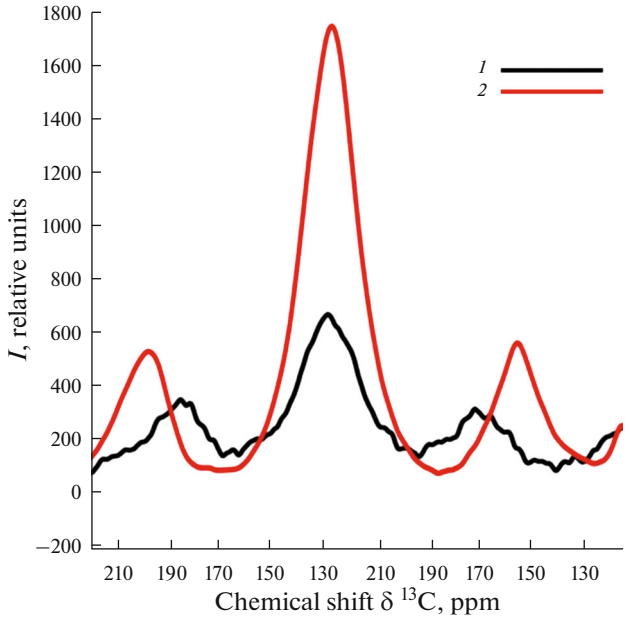

Fig. 1. CP/MAS ^{13}C NMR spectra of biochar samples obtained from (*1*) chicken manure and (*2*) corn at the pyrolysis temperature of 400°C and the retention duration of 2 h.

for 12 h at temperature of 105°C. Such preparation enabled neutralization of the effect of moisture on NMR data and met the requirements for the physical properties of samples for a solid-state NMR experiment with rotation of a special rotor in the sensor at a magic angle. The preparation of the solid precipitate was the following: an aliquot (5 mL) of a solution, containing NaOH alkali residues and sodium carbonate, was taken. The solution was evaporated at a temperature of 105°C for 12 h, and crystal samples were analyzed.

CP/MAS ^{13}C NMR spectroscopy was performed on a Bruker Avance III spectrometer with a magnetic field value of 9.4 T equipped with a 4 mm MAS probe. The constant temperature of 25.0 ± 0.1°C was maintained. The rotation frequency of the zirconium rotor at a magic angle was 7000 ± 1 Hz, the repetition time was 4 s, the contact time was 1 ms, and the number of scans was 10000. The comparison of the spectra of biochar samples obtained from corn and of biochar obtained from chicken droppings and prepared for the analysis in a similar way confirmed that pyrolysis of corn phytomass enabled to obtain biochar with an increased content of isotope ^{13}C (Fig. 1).

Statistical processing of the results. Each measurement was performed in three replications. Statistical data were analyzed in the Statistica software. One–factor ANOVA analysis and the Mann-Whitney multiple rank criterion were used to determine the statistical significance and the differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The effect of biochar on the content of total nitrogen and total carbon in soil. Figure 2 shows data on changes in the total carbon and total nitrogen in the soil. It is seen that the content of both elements was high and equal in layers KT1, KT2, and KM1 of the control column: 2.52–2.68% for C and 0.20–0.22% for N and did not change with time. Then, the content of the elements decreased down the profile and amounted to 1.18–1.58% for C and 0.08–0.11% for N in sample KL.

The biochar application at the rate of 8% caused an increase in nitrogen and carbon contents in the BT1 layer by 1.8 times for C and by 1.4 times for N. Fluctuations in the content of both elements with time in the BT1 layer may be explained by the initial uneven distribution of biochar in the soil, therefore, the impossibility to obtain homogeneous samples, as well as by more uniform distribution with time. Changes in the content of total nitrogen and total carbon in the experimental columns as compared with the control ones were recorded only for the upper BT1 layer, into which biochar was applied. Despite intensive watering and filtration of significant water amount down the soil profile, the presence of total nitrogen and total carbon in these layers was not recorded, which indicated the absence of migration of the main part of biochar and its components into the underlying soil horizons. From the practical point of view, this means that biochar should be applied immediately throughout the entire depth of the root layer in order to ensure the nutrition of plants and microorganisms of the rhizosphere.

The content of total carbon and nitrogen in columns D, into which biochar and microorganisms were introduced, did not significantly differ from those in columns B (Tables S1 and S2). The introduction of microorganisms can affect such parameters as the content of soluble organic carbon and easily hydrolyzable nitrogen, but not integral parameters, such as the content of total nitrogen and total carbon [14, 49].

The content of labeled carbon in soil columns. Let's consider changes in the NMR spectra of soil samples. Figure 3 shows the spectra for the BT1a layer on the 3rd and 7th days of the experiment with the columns. The spectrum for the control sample KT1a (without biochar) taken on the 3rd day of the experiment is given for comparison. This spectrum does not contain peaks useful for identification and subsequent analysis. The spectra of control samples for all days of sampling and for all soil layers are similar, which is the expected result, because the control samples do not contain biochar, and thus the ^{13}C content in them is not increased. The integral peak intensity for carboxylic groups and aliphatic compounds is close in value within the experimental error for samples taken on the 3rd and 7th days, while the peak typical for aromatic compounds increases with the time of the experiment.

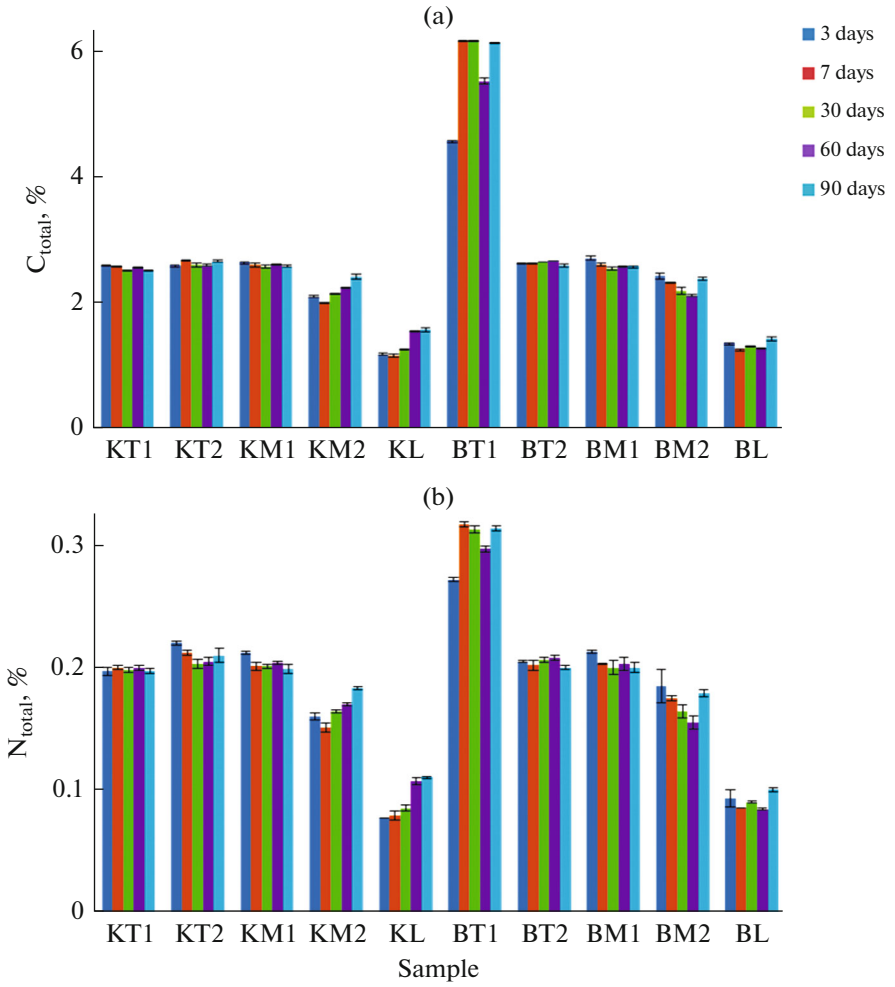

Fig. 2. Total carbon (a) and nitrogen (b) contents in control and experimental columns (with isotopically labeled biochar).

A similar trend may be seen with an increase in the experiment time (Fig. 3a): the NMR spectra of samples from the upper BT1a soil layer, into which biochar was directly applied, contain the main peaks represented in the spectrum of biochar with an increased content of ^{13}C. There is a tendency to an increase in the signal level for the main peaks, but a significant rise in the integral signal intensity is recorded only for a peak with the maximum in the region of 128 ppm assigned to graphite-like structures. Since 90% of the recorded NMR signal, in the dynamics of the experiment in particular, belong to aromatic groups, and there is no decrease in the level of this NMR peak with the experiment time, it may be assumed that the main part of biochar does not undergo transformation during its interaction with the soil for 90 days. In other words, there is rather redistribution between the components of biochar of different chemical nature, or the signal from carboxyl and aliphatic groups decreases and that from aromatic groups increases, which is seen, when comparing the shape of NMR spectra typical for the initial status of biochar, when it is mixed with soil. Changes in the structure of biochar with time are obviously related to its microbial transformation.

Microbial respiration of soil from soil columns. Microorganisms are the main agents of decomposition of organic matter in soil. Being an organic substance, biochar applied into the soil can differently affect soil microorganisms. On the one hand, it may become an additional source of their nutrition. In this case, their activity during biochar application will increase, which may result not only in quick decomposition of the applied fertilizer, but also in a change in the transformation rate of soil organic matter [10]. This will be reflected in an increase in the activity of enzymes, especially of those involved in the carbon cycle, and in soil respiration. If biochar components are used in catabolic processes, labeled carbon will be present in the composition of CO_2 released from soil. In case biochar is used for the synthesis of new organic compounds, labeled carbon will be recorded in soil by the NMR spectroscopy as part of peaks typical for soil improver and in new peaks. On the other hand, biochar may inhibit the activity of microorganisms due to

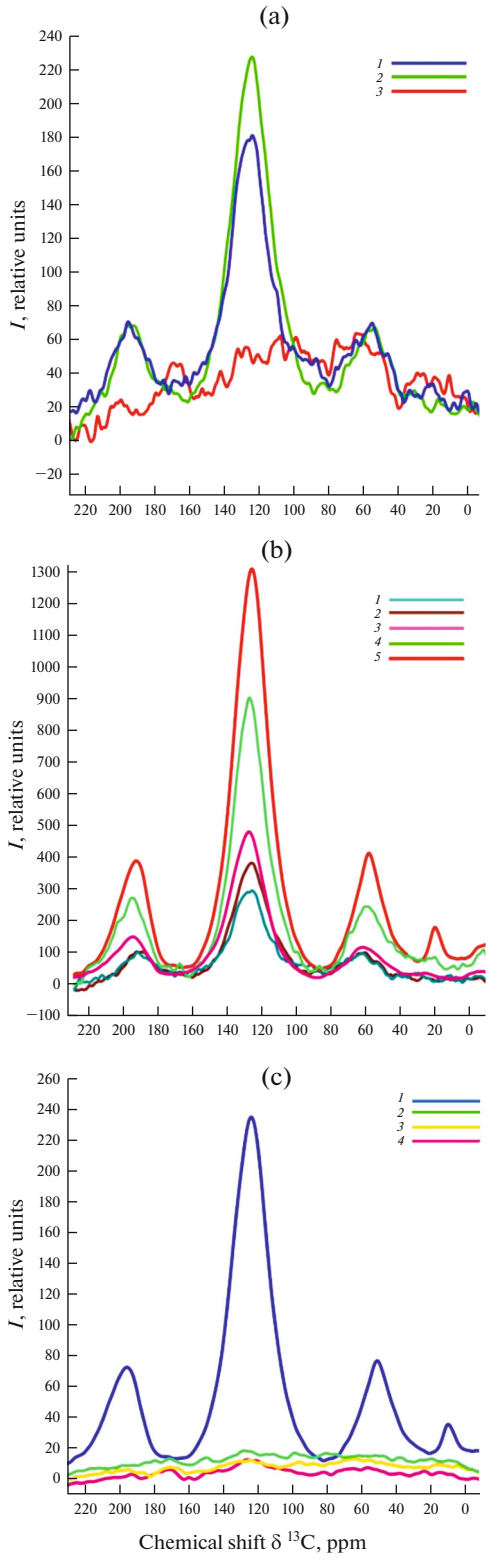

Fig. 3. CP/MAS ^{13}C NMR spectra of soil samples: (a) from the BT1a layer on the (*1*) 3rd day and (*2*) 7th day from columns with biochar and on the (*3*) 3rd day from the control column KT1a; (b) from the BT1a layer from columns with biochar on the (*1*) 3rd, (*2*) 7th, (*3*) 30th, (*4*) 60th, and (*5*) 90th day; (c) from the (*1*) BT1a, (*2*) BT1b, (*3*) BT2a (*3*), and (*4*) BT2b layers taken from columns with biochar on the 30th day of the experiment.

the presence of toxic components in it. In this case, there will be a decrease in the activity of microorganisms, resulting in lower soil respiration, enzymatic activity, etc.

Based on the above, we assessed the CO_2 release (respiration) and the presence of labeled carbon in layers BT1a and KT1a of experimental and control samples. Cumulative respiration curves from the experimental and control columns are shown in Fig. 4b. In addition, the experiment with soil column and biochar was duplicated to confirm the microbial component in the decomposition of organic compounds of biochar. Water extract from compost was added to biochar, assuming that such an extract is rich in destructive microorganisms (columns D, layer DT1a). The approach, when a community of destructive microorganisms is added to a substrate, and then an increase in the CO_2 emissions is measured to show the proportion of the biological component in the substrate mineralization, is widely used by researchers [16, 20, 35].

It is seen that the volume of $^{13}CO_2$ released from the soil with biochar was greater as compared to the control soil: the difference was 1.9 times on the 90th day of the experiment. The addition of destructive microorganisms caused an additional 2.4-fold increase in the volume of CO_2 released from the soil relative to the control, which testified to the role of microorganisms in the destruction of soil organic matter and biochar. In order to determine, whether the released CO_2 was a product of decomposition of fertilizer (and not only of soil organic matter), mixtures of alkali and carbonate salt obtained during CO_2 capture were analyzed after the experiment was completed. Figure 4b shows, for example, the results of the NMR spectroscopy of the crystalline phase obtained by evaporation of an alkali and carbonate solution on the 3rd day of the experiment to assess the respiratory activity of soils of the KT1a, BT1a, and DT1a layers. It is seen that there is a single narrow line in the area of 169 ppm in some parts of the spectrum. This peak is typical for carbonate-containing mineral phases [46]. The signal from the control sample is related to the natural content of ^{13}C, and the increased level from the sample with biochar confirms the fact that its components have been transformed into CO_2. The signal of the sample, containing both biochar and introduced destructive microorganisms, is even stronger, which confirms the role of microorganisms in the decomposition of organic matter of biochar. The amount of ^{13}C transferred to CO_2 from biochar is significantly smaller than the amount of ^{13}C remained in soil, but undergone transformation. This testifies that the microbial transformation of biochar is mainly aimed at the synthesis of new compounds. The amount of soil improver that was not transformed during incubation in the soil for 90 days is even greater, which confirms the stability of this substance and its ability to sequester carbon in soil [18, 31].

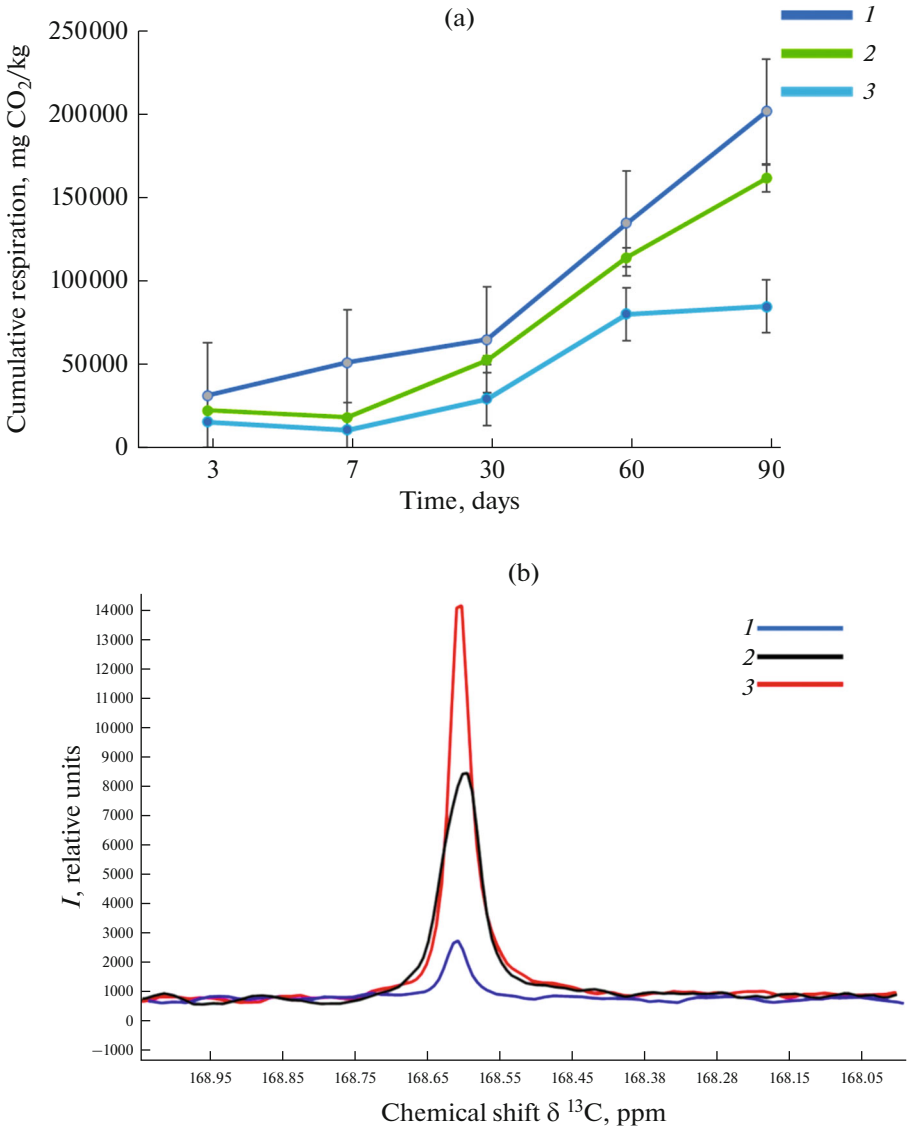

Fig. 4. (a) Cumulative respiration of the upper soil layer in (*1*) control soil columns (KT1a), (*2*) columns with biochar (BT1a), and (*3*) columns with biochar and applied destructor microorganisms (DT1a); (b) ^{13}C NMR spectra of alkali-trapped CO_2 released from the upper soil layer in (*1*) control soil columns (KT1a), (*2*) columns with biochar (BT1a), and (*3*) columns with biochar and applied destructor microorganisms (DT1a) during three days.

The physiological profile of microbial communities in soil columns. In addition to microbial respiration, changes in the functioning of microbial communities under the effect of biochar were evaluated in soil columns. For this, we analyzed the ability of communities to decompose various carbon-containing substrates, using the Biolog Ecoplate method in samples of control soil and of soil with biochar on the 90th day of the experiment. We used the average well color development index (AWCD) to characterize changes in the metabolic activity of soil community (Fig. 5) and the Shannon index to characterize variations in the biological diversity of the soil community (Fig. 6).

The metabolic activity (AWCD) decreased from the upper soil layer KT1 (2.84) to the lower KL (0.97) in the control samples. This trend corresponded to the published data and reflected the difference between the total number and activity of microorganisms in soil layers [5]. The biochar application did not cause significant changes in the metabolic activity of microorganisms in the samples taken in the corresponding layers. The absence of significant differences between control samples and those with ameliorant was also recorded by the Shannon index. Unlike other types of organic fertilizers such as compost, biochar does not produce an explosive change in the activity of soil microorganisms. On the contrary, it is a slow-acting ameliorant and causes a gradual, but not radical, shift in the composition of communities. Researchers consider such a mild effect of biochar on microbial com-

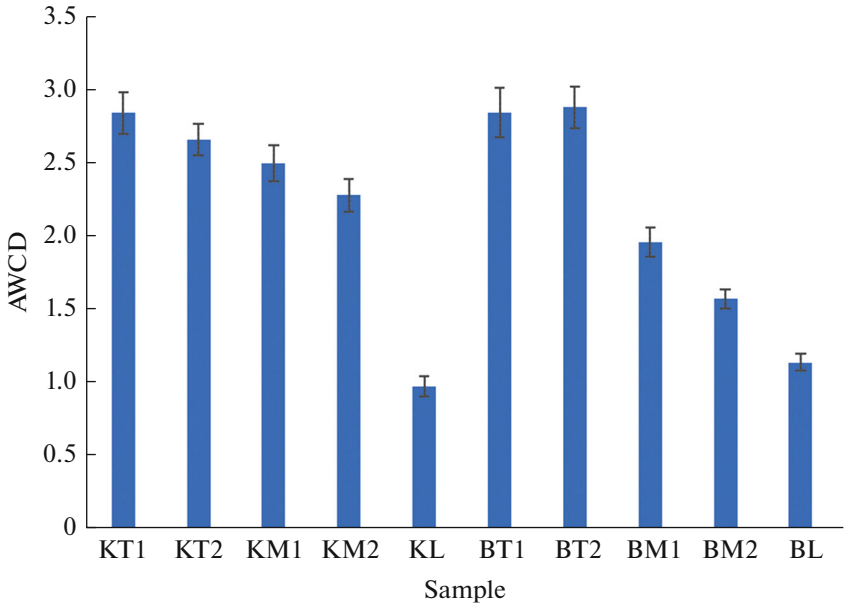

Fig. 5. Metabolic activity of soil microorganisms in control soil samples and samples from monoliths with biochar taken on the 3rd, 7th, 30th, 60th, and 90th days of the experiment.

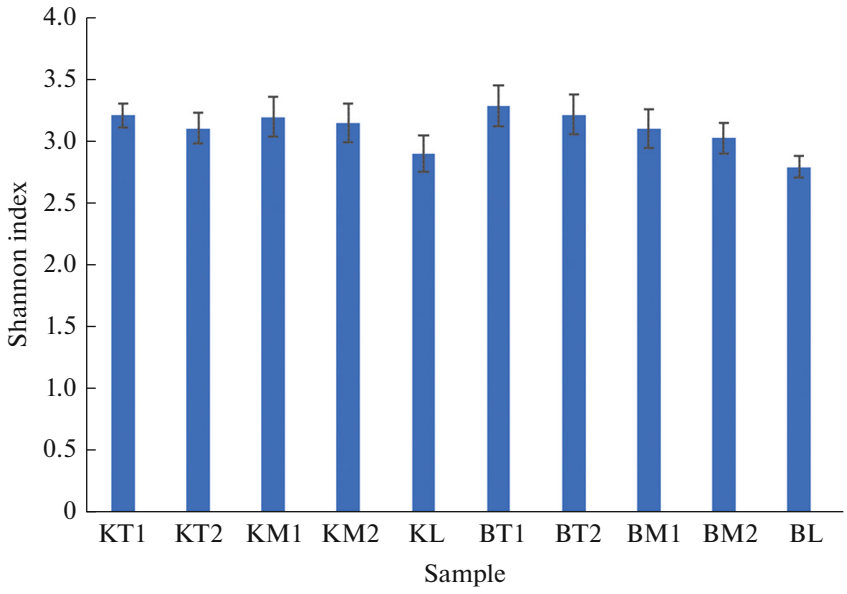

Fig. 6. Shannon index calculated for 120 h of incubation in control soil samples and samples with biochar taken on the 3rd, 7th, 30th, 60th, and 90th days of the experiment.

munities in combination with a stimulating effect on plant growth and development, as well as the possibility of carbon sequestration to be its advantage over other types of fertilizers [28, 30, 38, 55].

CONCLUSIONS

The transformation of biochar with an increased content of the ^{13}C isotope in soil has been studied in soil columns. It is shown that biochar applied into the upper layer completely remains there and does not migrate down the soil profile. The CP/MAS ^{13}C NMR spectra of soil samples with biochar with an increased content of ^{13}C isotopes selected during the experiment show the dominance of chemical groups of aromatic nature in biochar. They are most likely related to extended aromatic structures or graphite-like microcrystallites, which form the carbonaceous porous base

of fertilizer. There is also an increase in the proportion of aromatic and a decrease in the proportion of carboxylic and aliphatic groups during the experiment. The cumulative respiration from the upper 5-cm-thick soil layer increases almost twice during 90 days of the experiment, when ameliorant is applied. Labeled carbon isotopes ^{13}C are present in CO_2 released from soil, which indicates their origin from biochar. The role of microorganisms in the mineralization of biochar is proved. It is found that most of the fertilizer remains unchanged in the soil, and the main way of transformation of a smaller part consists in the release of terminal functional groups from the carbon base and in the synthesis of new organic compounds. The results obtained enable us to suggest that, from a practical point of view, biochar improves agricultural characteristics of soil, the content of carbon and nitrogen, in particular, does not cause inhibition of soil microbial communities, and its small proportion is involved in microbial transformation in the soil. The bulk of biochar is stored in soil for a long time, so it obviously may be used as a fertilizer once every few years. In addition, this testifies to its potential as a carbon sequester. Small-plot and long-term field experiments with biochar, using C_4-plants are required to develop practical recommendations on the use of this soil improver for these purposes.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://doi.org/10.1134/S1064229324601598.

Fig. S1. Scheme of layer-by-layer soil sampling from a monolith.

Table S1. Total carbon content in soil samples from columns with the addition of biochar and destructive microorganisms.

Table S2. Content of total nitrogen in soil samples from columns with the addition of biochar and destructive microorganisms.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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