AGRICULTURAL BIOLOGY,
2020, V. 55, № 1, pp. 163-173
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 631.58:631.461:577.2

doi: 10.15389/agrobiology.2020.1.163eng
doi: 10.15389/agrobiology.2020.1.163rus

INFLUENCE OF BIOCHAR ON THE TAXONOMIC COMPOSITION AND STRUCTURE OF PROKARYOTIC COMMUNITIES IN AGRO SODDY-PODZOLIC SOIL

V.Yu. SHAHNAZAROVA[1, 2], N.E. ORLOVA[1], E.E. ORLOVA[1], T.A. BANKINA[1], K.L. YAKKONEN[1], E.Ya. RIZHIYA[3], A.A. KICHKO[2]

[1]*Saint Petersburg State University,* 7/9, Universitetskaya nab., St. Petersburg, 199034 Russia, e-mail norlova48@mail.ru (✉ corresponding author), orlova55@mail.ru, bankinaagro@mail.ru, yakkonen@mail.ru;
[2]*All-Russian Research Institute for Agricultural Microbiology*, 3, sh. Podbel'skogo, St. Petersburg, 196608 Russia, e-mail shahnazarova-v@mail.ru, 2014arki@gmail.com;
[3]*Agrophysical Research Institute*, 14, Grazhdanskii prosp., St. Petersburg, 195220 Russia, e-mail alen_rizh@mail.ru
ORCID:
Shahnazarova V.Yu. orcid.org/0000-0001-9933-7591
Orlova N.E. orcid.org/0000-0002-6768-377X
Bankina T.A. orcid.org/0000-0002-6505-3467
Orlova E.E. orcid.org/0000-0001-9438-3812
Yakkonen K.L. orcid.org/0000-0002-9745-1654
Rizhiya E.Ya. orcid.org/0000-0001-7920-867X
Kichko A.A. orcid.org/0000-0002-8482-6226

The authors declare no conflict of interests
Acknowledgements:
The research was carried out using the equipment of the ARRIAM Center for Genomic Technologies, Proteomics, and Cell Biology.
Supported financially by Russian Foundation for Basic Research (project No. 18-016-00208a "The effect of biochar on biochemical and microbiological processes of the soil organic matter transformation")
Received September 30, 2019

Abstract

Currently the scientific literature is actively discussing the feasibility of biochar using in agriculture. Biochar is one of the new types of organic meliorants. It is obtained by pyrolysis of wood or other plant waste in an inert atmosphere converting carbon compounds to a stable state. Its use is recommended to increase the soils biological activity and the agricultural crops productivity and it is actively implement in agricultural technologies of foreign countries. However many aspects of the biochar influence on the agrocenoses properties and state have been poorly studied. There is information about both positive and negative processes occuring in soils under biochar. The main concern is the data on the biochar influence on humus mineralization, since dehumification can lead to loss of soil fertility and ecological stability. This is especially important for soddy-podzolic soils characterized by a low humus content and a weak degree of humification. Such soils initially have low ecological stability and are quite vulnerable to human impact. Therefore using soddy-podzolic soils in agriculture considerable attention should be paid to the microbiological and biochemical transformation of soil organic matter. Studies on the biochar influence on the soil microbiota composition and state in our country are isolated, and for soddy-podzolic soils of the North-Western region of Russia are conducted for the first time. The aim of this work was to assess the influence of biochar on the features of the agro soddy-podzolic soils prokaryotic community. The research was carried out in incubation experiments on well-cultivated agro soddy-podzolic sandy loam soil of the Leningrad region. The biochar was produced by fast pyrolysis of birch and aspen wood at 550 °C. Its concentration in the experiment was 1%. The incubation time was 7 and 90 days. The repeat of the variants of the experiment was 3-fold. The content of total organic carbon and nitrogen, mineral forms of nitrogen, and soil suspension pH were determined in soil samples using methods commonly used in agrochemical practice. The method of sequencing the variable region of the 16S rRNA gene was used to determine the taxonomic composition of soil prokaryotes. The sequence clustering and the taxonomic identification of the taxonomic units (OTU, Operational Taxonomic Unit) were performed using the QIIME program. The diversity and evenness of agro soddy-podzolic soil bacterial communities was estimated by the OTU number and Shannon index. Statistical data processing was performed using IBM SPSS Statistics, Version 25 (IBM, USA). The reliability of the differences between the variants was measured by one-factor variance analysis using the Duncan's or Student-Newman-Keuls test at $p < 0.05$. The intensification of the processes of mineralization of soil organic matter occurred under biochar. The humus content in the soil decreased from 4.41 to 3.83 % which is 11 % more than in the control during the observation period. Activation of organic matter transformation processes took place simultaneously with changes in the state of the prokaryotic community. This community was represented mainly by the bacteria phyla *Actinobacteria*, *Firmicutes*, *Proteobacteria*, *Chloroflexi*, *Acido-*

bacteria, *Planctomycetes*, *Verrucomicrobia*. The biochar application was accompanied by an increase in the total bacteria diversity and by the abundance of phyla *Planctomycetes*, *Verrucomicrobia*, *Proteobacteria* and FBP representatives but also by a decrease in the abundance of phyla *Actinobacteria*, *Nitrospirae, Firmicutes* and *Fibrobacteres* representatives. In general biochar application leads to increase in the oligotrophs abundance and to reduce the copiotrophic proportion in prokaryotic community. The inhibition of bacteria from phylum *Nitrospirae* can be explained by a decrease in the concentration of available ammonium. In addition biochar application leads to increase in the abundance of some taxa containing active hydrolytics of natural polymers (orders *Myxococcales* and *Xanthomonadales*, class *Sphingobacteriia* etc.). Most likely this is due to the intensification of the difficult mobilizing organic substances transformation in agro soddy-podzolic soils under biochar.

Keywords: sequencing, structure of microbocenosis, bacteria, prokaryotes, biochar, soddy-podzolic soil, fertility

During recent decades, the search and development of new types of fertilizers and ameliorants which can maintain a deficiency-free balance of soil humus and ensure high soil fertility are being actively carried out. Biochar is a promising organogenic ameliorant that is actively used in world agricultural production [1-3]. Biochar is produced by pyrolysis of wood or other plant matter in an inert atmosphere. The use of biochar is an opportunity to solve a number of environmental challenges, i.e. the utilization of organic waste [4, 5], carbon sequestration [6], restoration of disturbed soils [5-7] and increase in crop productivity [4, 8, 9].

The composition and properties of various types of biochar, in particular, the feedstock for its production [10], the physical and physicochemical characteristics [11, 12], have been studied in sufficient detail. Its influence on the agronomically valuable properties of some types of soils was studied, including elements of plant mineral nutrition [4, 9, 13], the reaction of soil medium, and water-physical properties [5, 6, 12, 13]. However, the mechanisms responsible for such effects are not fully understood. Changes in microbocenosis in soils at different doses of biochar application [14-16], incubation periods [15, 17] and different biochar quality [18] are described. But this did not reveal clear patterns of modulation of the microbiota profile under the influence of biochar. Information about the associated changes in soil biota and soil organic matter, the most important components of agrocenoses which largely determine their fertility and environmental sustainability, is limited and often contradictory [19-22]. Thus, there is evidence that under the influence of biochar, the microbial biomass and biological activity of soils increase, and dehumification processes begin [21-23]. According to other data, biochar does not stimulate soil microorganisms, so the intensity of mineralization decreases [24-27]. The transformation of humus composition when biochar is incorporated into soils is also poorly investigated. Our recent studies have shown that the introduction of biochar into sod-podzolic soil leads to both negative and positive modifications of humic substances [28, 29]. Intensive mineralization of humus (with losses up to 20%) is accompanied by an increase in the proportion of its stabilized forms which, in turn, increases the stability of humus as a whole [28].

Soil prokaryotes are actively involved in the transformation of organic matter. Therefore, studying the effect of biochar on the prokaryotic community of agro soddy-podzolic soil is of interest both for understanding the fundamental processes of soil fertility formation and for farming practice.

This paper is the first to report about changes in the profile of prokaryotic community of agro soddy-podzolic soil, accompanying its dehumification under the influence of biochar, i.e. an increase in the abundance of oligotrophs and a number of taxa, representatives of which are involved in the decomposition of complex natural biopolymers, and a decrease in the proportion of copiotrophs. In addition, here we present data on the metagenome composition of the sod-podzolic soil microbiota in the northwestern part of European Russia, infor-

mation on which for this region is still extremely limited.

The purpose of the present study was to assess the effect of biochar on parameters of prokaryotic communities of well-cultivated agro soddy-podzolic sandy loam soil of the Leningrad Province.

Materials and methods. Samples of high-humus agro soddy-podzolic sandy loam soil (Menkovsky branch of the Agrophysical Institute, Leningrad Province, Gatchinsky District) were taken from the arable horizon (0-20 cm) in June 2017. Biochar was obtained by rapid pyrolysis from birch and aspen wood at 550 °C. A detailed description of the soil and biochar is given earlier [28, 29].

A short-term incubation experiment was performed at room temperature (20-22 °C). The soil weight in a pot was 300 g dry matter, the content of biochar was 0% (control) and 1.0%. Soil moisture throughout the experiment remained equal to 60% of the total moisture capacity. Soil samples were analyzed on day 7 and day 90, with a 3-fold repetition per variant.

Agrochemical parameters of the soil were assessed by standard methods [30]: pH potentiometrically, soil organic carbon according to Tyurin, organic nitrogen by Tyurin's microchromic method, nitrates with disulfophenolic acid, ammonium with Nessler reagent.

DNA was extracted from 0.25 g portions of soil samples and purified with the NucleoSpin® Soil kit (MACHEREY-NAGEL GmbH & Co. KG, Germany) according to the manufacturer's instructions.

Universal PCR primers to 16S rRNA marker gene variable region V4, F515/R806 (5′-GTGCCAGCMGCCGCGGTAA-3′/5′-GGACTACVSGGGTATCTAAT-3′) modified to contain adapters and unique barcodes were used to construct amplicon libraries (Illumina, Inc., USA). PCR (a T100 Thermal Cycler, Bio-Rad Laboratories, Inc., USA) was carried out in a 15 μl reaction mixture containing Q5® High-Fidelity DNA Polymerase (0.5-1.0 units) and 1× Q5 Reaction Buffer (New England BioLabs Inc., UK), 5 pM of forward and reverse primers, 10 ng of matrix DNA and 2 nM of each dNTP (Thermo Fisher, Inc., USA). The template DNA denaturation (94 °C, 1 min) was followed by 35 cycles of elongation (94 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s) with final elongation at 72 °C for 3 min. The library preparation and sequencing was performed on the Illumina MiSeq platform using MiSeq ReagentKit v3 (600 cycles) with 2×300 nt paired-end reads (Illumina, Inc., USA) as per the Illumina MiSeq Reagent Kit Preparation Guide for metagenome sequencing of 16S amplicon libraries. Illumina software (Illumina, Inc., USA) and Trimmomatic software packages [31], fastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), fastq-join (https://github.com/brwnj/fastq-join) and QIIME software [32] were used for read demultiplexing, removing alien sequences, assessing read quality, trimming, joining paired-end reads, checking for chimeras and homopolymers. Clustering and taxonomic identification of resultant operational taxonomic units (OTU) were performed with QIIME program.

The diversity and evenness of the bacterial communities of agro soddy-podzolic soil was estimated by the number of OTUs (an analogue of species richness) and the Shannon index $H = \Sigma p_i \ln(p_i)$, where p_i is the fraction of the ith species in the community [33].

Statistical processing was performed with the IBM SPSS Statistics, Version 25 software (IBM, USA). The significance of differences was estimated by one-way analysis of variance with the Duncan's test or Student-Newman-Keuls (SNK) test at $p < 0.05$ ($n = 3$). The tables show mean values (M) with a confidence interval at $p < 0.05$ ($t_{0.05} \times$ SEM).

Results. Carbon in biochar, despite the high content, is mainly inert, dif-

ficult to oxidize, and labile C fractions in biochar are small. In the organic matter of biochar, the carbon, determined by wet oxidation method makes less than 1.5%, and the amount of water-soluble carbon compounds is negligible (0.008%) [27]. Bio-char did not affect the content of soil organic carbon (humus), since it is a highly stabilized compound.

Tested agro soddy-podzolic soil was slightly acidic (pH_{H2O} 6.7), with high humus (4.45%), mineral and organic nitrogen content (Table 1).

1. Mineralization of organic matter in agro sod-podzolic soil upon incubation with biochar ($n = 3$, $M \pm t_{0.05} \times$ SEM)

Variant	$C_{org.}$, %	N forms			$C_{org.}:N_{org.}$
		$N_{org.}$, %	$N\text{-}NO_3$, mg/kg	$N\text{-}NH_4$, mg/kg	
		Day 0			
Soil (initial sample)	2.56 ± 0.05^{c}	0.22 ± 0.00^{c}	11.2 ± 0.7^{a}	14.8 ± 0.6^{e}	11.6 ± 0.3^{a}
		Day 7			
Control	2.50 ± 0.05^{bc}	0.21 ± 0.00^{c}	18.9 ± 1.5^{b}	10.4 ± 0.6^{d}	11.9 ± 0.4^{a}
Biochar	2.46 ± 0.04^{b}	0.21 ± 0.01^{c}	17.0 ± 0.6^{b}	9.3 ± 0.04^{c}	11.7 ± 0.3^{a}
		Day 90			
Control	2.48 ± 0.04^{b}	0.19 ± 0.01^{b}	17.9 ± 0.6^{b}	6.8 ± 0.05^{b}	13.1 ± 0.2^{b}
Biochar	2.22 ± 0.04^{a}	0.16 ± 0.01^{a}	12.6 ± 0.7^{a}	4.7 ± 0.07^{a}	13.9 ± 0.4^{c}

Note. Different letters denote mean values that are statistically significantly different from each other at $p < 0.05$ (belonging to different subsets).

During incubation, the soil organic matter mineralization intensified under the influence of biochar. By the end of the experiment (90 days), the content of organic forms of nitrogen and carbon in soil with biochar was lower 16 and 10%, respectively, than in the control. Moreover, the loss of humus in the soil with biochar during the incubation period was 0.57%. These data are consistent with the results of our previous studies on the effect of biochar on the humus content and its fractional group composition in agro soddy-podzolic sandy loam soils [21]. Thus, our short-term (up to 90 days) experiments found out that incubation of sandy-loam agro soddy-podzolic soils with biochar can cause their dehumification.

During the incubation, the mineralization of N-organic compounds was higher compared to C-organic compounds, leading to a significant ($p < 0.05$) increase in C/N upon biochar application, i.e., the soil organic matter humification decreased. In the control soil, the mineralization of organic matter was accompanied by a decrease in the content of ammonium nitrogen and an increase in nitrate nitrogen, i.e., an increase in nitrification occurred. By the end of the experiment (90 days), $N\text{-}NH_4$ concentration decreased sharper in the soil with biochar compared to the control, whereas $N\text{-}NO_3$ content remained unchanged compared to its starting value. The $N\text{-}NO_3$ level in the soil with biochar was higher and comparable to the control only at the beginning of incubation (after 7 days). Apparently, during longer composting, ammonium cations generated due to mineralization of organic matter can be absorbed by negatively charged functional groups of biochar [6]. As a result, the amount of extractable ammonium forms in the soil declined, and nitrification was limited by the amount of substrate (ammonium) available to nitrifying bacteria.

The changes in the soil organic matter occurred together with taxonomic profile modification soil prokaryotes.

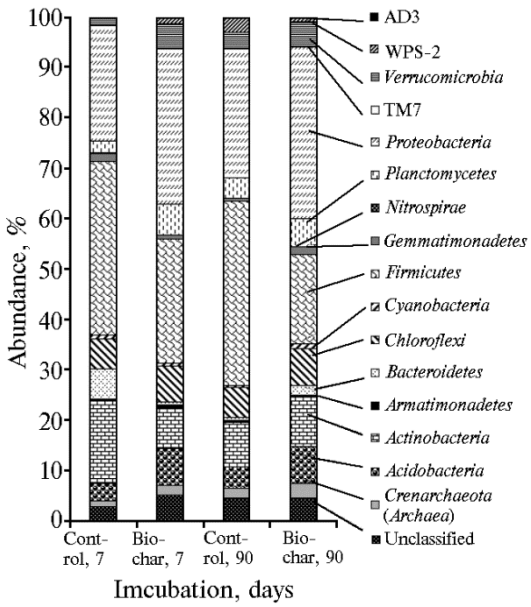
Clustering sequences of the 16S rRNA gene variable region revealed 6392 OTUs for a taxonomic analysis (Table 2). The soil bacterial communities without and with biochar did not significant differed in the OTU number. The Shannon index testified that there were no differences between samples at the beginning of the experiment, but by the end the H value slightly (by 7.5%) but significantly ($p < 0.05$) increased with biochar, which could indicate an increase in

diversity and greater uniformity of the community under the influence of biochar.

2. Operational taxonomic units (OTUs) **and evenness of bacterial community in agro sod-podzolic soil upon incubation with biochar** ($n = 3$, $M \pm t_{0.05} \times$ SEM)

Indicator	Control, 7 days	Biochar, 7 days	Control, 90 days	Biochar, 90 days
OUT number	3211±1085[a]	3710±285[a]	3335±467[a]	3498±185[a]
Shannon index H	9.49±0.35[ab]	9.84±0.45[ab]	9.23±0.20[b]	9.91±0.11[a]

N o t e. Different letters denote mean values that are statistically significantly different from each other at $p < 0.05$ (belonging to different subsets).



Richness of prokaryotic phyla (%) in agro sod-podzolic soil during incubation with biochar ($n = 3$). The figure shows phyla the abundance of which exceeds 0.1% at least in one test variant.

The ratio of *Archaea* and *Bacteria* did not change significantly when biochar was used. There was only a tendency to an increase in the abundance of archaea in the soil with biochar (Fig.). A metagenomic analysis of the prokaryotic community showed that *Actinobacteria*, *Firmicutes*, *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, *Planctomycetes*, *Verrucomicrobia* dominated in all samples (and also *Bacteroidetes* in the soil composted for 7 days without biochar) (see Fig.). The predominance of these phyla is generally characteristic of soddy-podzolic soils, except the phylum *Planctomycetes* which is usually not abundant in these soils [34, 35].

After 7-day incubation, the abundance of *Acidobacteria*, *Verrucomicrobia* and *Planctomycetes* phyla significantly increased ($p < 0.05$), and *Actinobacteria*, *Nitrospirae*, *Fibrobacteres*, *Gemmatimonadetes* phyla decreased in soil with biochar compared to soil without it. We found significant changes in the abundance of a number of classes, orders, families and genera, mainly related to the phyla *Actinobacteria*, *Chloroflexi*, *Planctomycetes*, *Proteobacteria*, and *Firmicutes* (Table 3). After 90-day incubation, the differences in the bacterial community profiles in the soil with and without biochar were much weaker, and changes in abundance were noted mainly for other taxa. Thus, counts of *Proteobacteria*, *Gemmatimonadetes*, and FBP phyla increased, but the abundance of phylum *Firmicutes* decreased.

Currently, no unified regularity has been established for the effect of biochar on the abundance of bacterial phyla in different soils. For example, the decrease in the abundance of *Actinobacteria* phyla we observed as a result of the biochar introduction is consistent with data from other authors [16]. At the same time, some works described an increase in the abundance of this phylum in the presence of biochar [14]. There is also no consensus on the effect of biochar application on the abundance of phyla *Firmicutes* [14, 18, 36], *Verrucomicrobia* [14], *Planctomycetes* [14, 18], *Proteobacteria* [14, 37]. Probably, modification of the prokariotic community is associated indirectly with soil physicochemical changes (pH, sorption ability, and cation exchange capacity) when biochar is applied [4, 37].

3. Richness of prokaryotic taxa (%) in agro sod-podzolic soil during incubation with biochar ($n = 3$)

Taxon	Control, 7 days	Biochar, 7 days	Control, 90 days	Biochar, 90 days
		Phylum		
Actinobacteria	16.29^{a}	8.20^{b}	9.10^{b}	9.90^{b}
Firmicutes	34.35^{a}	24.41^{ab}	29.20^{a}	17.50^{b}
FBP	0.05^{b}	0.05^{b}	0.03^{b}	0. 12^{a}
Fibrobacteres	0.03^{b}	0^{a}	0.01^{b}	0.13^{ab}
Gemmatimonadetes	1.6^{a}	0.84^{b}	0.62^{b}	1.64^{a}
Nitrospirae	0.15^{a}	0.03^{b}	0.01^{b}	0.01^{b}
Planctomycetes	2.28^{b}	5.98^{a}	4.20^{ab}	5.56^{ab}
Proteobacteria	22.81^{b}	30.83^{ab}	26.22^{b}	33.83^{a}
Verrucomicrobia	1.50^{b}	5.00^{a}	3.35^{ab}	4.88^{ab}
		Class		
Sva0725	1.11^{a}	0.18^{b}	0.04^{b}	0.07^{b}
TM1	0.001^{b}	0.01^{a}	0.02^{a}	0.02^{a}
[Chloracidobacteria]	0.90^{a}	0.07^{b}	0.06^{b}	0.02^{b}
DA052	0.007^{b}	1.01^{a}	0.44^{a}	0.91^{a}
Actinobacteria	11.02^{a}	3.29^{c}	3.91^{bc}	4.90^{b}
Nitriliruptoria	0.01^{a}	0^{b}	0^{b}	0^{b}
Sphingobacteriia	1.93^{a}	0.17^{b}	0.15^{b}	0.72^{a}
Chloroflexi	0.85^{a}	0.03^{b}	0.02^{b}	0.01^{b}
Ktedonobacteria	0.07^{b}	4.08^{a}	4.13^{a}	4.5^{a}
TK10	0.41^{c}	1.72^{a}	0.96^{bc}	1.45^{b}
Bacilli	33.38^{a}	21.00^{b}	24.93^{b}	15.05^{c}
Planctomycetia	1.68^{b}	5.08^{a}	3.61^{ab}	4.41^{ab}
		Order		
RB41	0.90^{a}	0.07^{b}	0.0^{b}	0.02^{b}
Actinomycetales	11.01^{a}	3.29^{c}	3.90^{bc}	4.90^{b}
KD8-87	0.5^{a}	0^{b}	0^{b}	0^{b}
Thermogemmatisporales	0.004^{c}	1.04^{b}	1.05^{ab}	2.21^{a}
AKYG1722	0.35^{a}	0.001^{b}	0.004^{b}	0.004^{b}
JG30-KF-AS9	0.01^{b}	0.97^{a}	1.22^{a}	0.56^{a}
Nitrospirales	0.15^{b}	0.03^{a}	0.02^{a}	0.01^{a}
Rhizobiales	4.51^{b}	9.72^{a}	7.90^{ab}	11.79^{a}
Rhodospirillales	1.34^{b}	3.67^{a}	3.18^{ab}	4.37^{a}
Ellin6067	0.06^{b}	0.43^{a}	0.30^{ab}	0.19^{ab}
Myxococcales	2.12^{ab}	2.68^{ab}	1.80^{b}	2.97^{a}
Spirobacillales	0.31^{a}	0.02^{b}	0.04^{b}	0.04^{b}
Pseudomonadales	0.44^{a}	0.04^{b}	0.04^{ab}	0.02^{ab}
Xanthomonadales	2.23^{b}	2.35^{b}	2.02^{b}	3.23^{a}
		Family		
Actinospicaceae	0.001^{b}	0.04^{a}	0.04^{a}	0.04^{a}
Dermabacteraceae	0.05^{a}	0^{b}	0^{b}	0^{b}
Dermacoccaceae	0^{b}	0.01^{b}	0.13^{a}	0.02^{b}
Intrasporangiaceae	0.29^{a}	0.06^{b}	0.04^{b}	0.04^{b}
Microbacteriaceae	1.86^{a}	0.16^{b}	0.43^{b}	0.14^{b}
Nakamurellaceae	0.07^{a}	0.05^{a}	0.03^{b}	0.07^{a}
Propionibacteriaceae	0.02^{a}	0.001^{b}	0.004^{ab}	0.01^{ab}
Chthonomonadaceae	0.01^{b}	0.17^{a}	0.16^{ab}	0.09^{ab}
Gemmataceae	0.07^{b}	1.71^{a}	0.94^{ab}	1.47^{ab}
Burkholderiaceae	0.08^{c}	0.70^{b}	0.49^{b}	1.21^{a}
Comamonadaceae	0.88^{a}	0.23^{b}	0.22^{b}	0.26^{b}
Coxiellaceae	0.08^{b}	0.34^{a}	0.27^{ab}	0.14^{ab}
Xanthomonadaceae	1.80^{a}	0.56^{b}	1.04^{ab}	1.52^{a}
[Chthoniobacteraceae]	0.42^{b}	3.88^{ab}	2.72^{b}	3.96^{a}
		Genus		
Actinotalea	0.22^{a}	0^{b}	0.002^{b}	0.01^{b}
Brachybacterium	0.05^{a}	0^{b}	0^{b}	0^{b}
Agrococcus	0.11^{a}	0.001^{b}	0.004^{b}	0.003^{b}
Actinoplanes	0.15^{a}	0.02^{b}	0.02^{b}	0.05^{ab}
Catellatospora	0.03^{a}	0.001^{b}	0.01^{b}	0.004^{b}
Pontibacter	0.03^{a}	0.001^{b}	0^{b}	0^{b}
Dyadobacter	0.06^{a}	0.001^{b}	0^{b}	0.02^{ab}
Ammoniphilus	1.05^{a}	0.21^{b}	0.27^{b}	0.29^{b}
Coprococcus	0.03^{b}	0.03^{b}	0.10^{a}	0.02^{b}
Symbiobacterium	0.03^{a}	0.01^{b}	0.01^{b}	0.01^{b}
Gemmata	0.02^{b}	0.49^{a}	0.28^{ab}	0.54^{a}
Nostocoida	0^{b}	0.0006^{a}	0.0004^{a}	0.0004^{ab}
Asticcacaulis	0.02^{ab}	0.01^{b}	0.01^{b}	0.06^{a}
Devosia	1.17^{a}	0.10^{b}	0.16^{b}	0.26^{b}

Continued Table 3

Hyphomicrobium	0.11[b]	0.29[a]	0.20[ab]	0.35[a]
Sphingomonas	0.37[a]	0.01[b]	0.13[b]	0.25[a]
Burkholderia	0.02[c]	0.66[b]	0.47[b]	1.16[a]
Methylibium	0.05[a]	0.01[b]	0.02[b]	0.01[b]
Janthinobacterium	0.01[b]	0.02[ab]	0.03[a]	0.01[b]
Pseudomonas	0.07[a]	0.01[b]	0.01[b]	0.01[b]
Rhodanobacter	0.25[a]	0.06[b]	0.25[a]	0.26[ab]
Pedosphaera	0.001[b]	0.05[a]	0.02[ab]	0.03[ab]

N o t e. The table shows taxa for which there were statistically significant difference in abundance at least at least at one period during the experiment. Different letters denote mean values that are statistically significantly different from each other at $p < 0.05$ (belonging to different subsets).

In general, the abundance of oligotrophic bacteria significantly increased on day 7 upon biochar application, the phyla FBP and *Verrucomicrobia* more than 3-fold, and *Planctomycetes* more than 2-fold. Oligotrophic bacteria of the genera *Hyphomicrobium* increased significantly on day 7 of incubation, and *Asticcacaulis* on day 90. At the same time, there was a significant decrease ($p < 0.05$) in the counts of *Actinobacteria* (7 days of incubation) and *Firmicutes* (90 days of incubation). The members of these taxa are copyiotrophs or hydrolytics that can exist under conditions of high concentrations of nutrients. In particular, the abundance of the *Bacilli* class, which includes active soil copyiotrophs, decreased. Thus, biochar provides more favorable conditions for bacteria that are unable to survive at high concentration of available organic compounds. Bacteria that grow well on rich nutrient media (*Actinobacteria*, *Bacilli*), in contrast, were somewhat inhibited. Perhaps the reason is that readily available organic matter is adsorbed on the biochar, decreasing concentration of organic compounds in the soil solution, which creates advantages for more oligotrophic bacteria.

In addition, biochar caused a rapid and significant (5-fold) reduction in the abundance of bacteria of the phylum *Nitrospirae*, namely the nitrification bacteria of the genus *Nitrospira* mainly found in the studied soil, which is consistent with the suppression of nitrification observed in this soil when applying biochar (see Table 1).

A noticeable modification also occurred in the community of soil hydrolytics. The abundance of *Actinobacteria* phylum, the destructors of many difficultly-hydrolyzed organic substances, decreased after a short incubation with biochar as compared to the control, unlike active hydrolytics of difficultly-decomposable polymers from the order *Myxococcales* and cellulolytics of the *Sphingobacteriia* class which, on the contrary, became more abundant [38, 39]. In addition, there was a significant increase in the counts of members of the order *Xanthomonadales*, *Burkholderiaceae* family (almost 10-fold on day 7) and the genus *Asticcacaulis* which was recently shown to be involved in the decomposition of cellulose or products of its degradation [38]. Consequently, the addition of biochar elevates abundance of several bacterial groups responsible for the hydrolysis of difficultly-decomposable organic substances.

So, metagenomic analysis revealed that biochar incorporation into agro soddy-podzolic soil quickly changes the profile of the soil prokaryotic community. We did not observe its fundamental restructuring, nevertheless, the proportion of oligotrophic bacteria increased, copyiotrophs decreased, and in addition, the structure of hydrolytic bacteria community was modified. The latter, probably, explains intensive transformation of organic substances that we identified under the influence of biochar. Of course, profiling modification of microbial community accompanying such a transformation is of interest as a special case of changes in microbiocenosis during soil humification and dehumification. This issue should be be studied, since it is the activity of specific microorganisms that leads to the intensification of these processes and determines their balance which directly affects

soil fertility and sustainability of agrocenoses.

REFERENCES

1. Glaser B., Lehmann J., Zech W. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal — a review. *Biology and Fertility of Soils*, 2002, 35: 219-230 (doi: 10.1007/s00374-002-0466-4).
2. Lehmann J., da Silva Jr. JP., Steiner C., Nehls T., Zech W., Glaser B. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant and Soil*, 2003, 249(7): 343-357 (doi: 10.1023/A:1022833116184).
3. Yamato M., Okimori Y., Wibowo I.F., Anshori S., Ogawa M. Effects of the application of charred bark of *Acacia mangium* on the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia. *Soil Science and Plant Nutrition*, 2006, 52: 489-495 (doi: 10.1111/j.1747-0765.2006.00065.x).
4. *Agricultural and environmental applications of biochar: advances and barriers. SSSA Spec. Publ. 63*. M. Guo, Z. He, S.M. Uchimiya (eds.). SSSA, Madison, WI, 2016 (doi: 10.2136/sssaspecpub63.frontmatter).
5. Zama E.F., Reid B.J., Arp H.P.H., Sun G.-X., Yuan H.-Y., Zhu Y.-G. Advances in research on the use of biochar in soil for remediation: a review. *Journal of Soils Sediments*, 2018, 18(7): 2433-2450 (doi: 10.1007/s11368-018-2000-9).
6. Amonette J.E., Joseph S. Characteristics of biochar: microchemical properties. In: *Biochar for environmental management: science and technology*. J. Lehmann, S. Joseph (eds.). Earthscan, London-Sterling, VA, 2009: 33-52.
7. Liu Y., Wang Y., Lu H., Lonappan L., Brar S.K., He L., Chen J., Yang S. Biochar application as a soil amendment for decreasing cadmium availability in soil and accumulation in *Brassica chinensis*. *J. Soils Sediments,* 2018, 18(7): 2511-2519 (doi: 10.1007/s11368-018-1927-1).
8. Ippolito J.A., Laird D.A., Busscher W.J. Environmental benefits of biochar. *J. Environ. Qual.*, 2012, 41(4): 967-972 (doi: 10.2134/jeq2012.0151).
9. Biederman L.A., Harpole W.S. Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. *GCB Bioenergy*, 2013, 5(2): 202-214 (doi: 10.1111/gcbb.12037).
10. González J.F., Román S., Encinar J.M., Martinéz G. Pyrolysis of various biomass residues and char utilization for the production of activated carbons. *Journal of Analytical and Applied Pyrolysis*, 2009, 85(1-2): 134-141 (doi: 10.1016/j.jaap.2008.11.035).
11. Keiluweit M., Nico P.S., Johnson M.G., Kleber M. Dynamic molecular structure of plant biomass-derived black carbon (biochar). *Environ. Sci. Technol.*, 2010, 44(4): 1247-1253 (doi: 10.1021/es9031419).
12. Lehmann J., Joseph S. Biochar for environmental management: an introduction. In: *Biochar for environmental management: science and technology*. J. Lehmann, S. Joseph (eds.). Earthscan, London-Sterling, VA, 2009: 1-12.
13. Verheijen F., Jeffery S., Bastos A., Velde M., Diafas I. *Biochar application to soils, a critical scientific review of effects on soil properties, processes and functions. EUR 24099—En*. European Commission, Luxembourg, 2010 (doi: 10.2788/472).
14. Xu N., Tan G., Wang H., Gai X. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. *European Journal of Soil Biology*, 2016, 74: 1-8 (doi: 10.1016/j.ejsobi.2016.02.004).
15. Xu M., Xia H., Wu J., Yang G., Zhang X., Peng H., Yu X., Li L., Xiao H., Qi H. Shifts in the relative abundance of bacteria after wine-lees-derived biochar intervention in multi metal-contaminated paddy soil. *Science of the Total Environment*, 2017, 599-600: 1297-1307 (doi: 10.1016/j.scitotenv.2017.05.086).
16. Gao L., Rui W., Shen G., Zhang J., Meng G., Zhang J. Effects of biochar on nutrients and the microbial community structure of tobacco-planting soils. *Journal of Soil Science and Plant Nutrition*, 2017, 17(4): 884-896 (doi: 10.4067/S0718-95162017000400004).
17. Jenkins R.J., Viger M., Arnold E.C., Harris Z.M., Ventura M., Miglietta F., Girardin C., Edwards R.J., Rumpel C., Fornasier F., Zavalloni C., Tonon G., Alberti G., Taylor G. Biochar alters the soil microbiome and soil function: results of next-generation amplicon sequencing across Europe. *GCB Bioenergy*, 2017, 9(3): 591-612 (doi: 10.1111/gcbb.12371).
18. Awasthi M.K., Li J., Kumar S., Awasthi S.K., Wang Q., Chen H., Wang M., Ren X., Zhang Z. Effects of biochar amendment on bacterial and fungal diversity for co-composting of gelatin industry sludge mixed with organic fraction of municipal solid waste. *Bioresource Technology*, 2017, 246: 214-223 (doi: 10.1016/j.biortech.2017.07.068).
19. Mueller C.W., Koegel-Knabner I. Soil organic carbon stocks, distribution, and composition affected by historic land use changes on adjacent sites. *Biol. Fertil. Soils*, 2009, 45(4): 347-359 (doi: 10.1007/s00374-008-0336-9).
20. Sohi S.P., Krull E., Lopez Capel E., Bol R. Chapter 2 — A review of biochar and its use and

function in soil. In: *Advances in Agronomy, Vol. 105*. D.L. Sparks (ed.). Academic Press, 2010: 47-82 (doi: 10.1016/S0065-2113(10)05002-9).
21. Lehmann J., Rillig M.C., Thies J., Masiello C.A., Hockaday W.C., Crowley D. Biochar effects on soil biota — a review. *Soil Biology and Biochemistry*, 2011, 43(9): 1812-1836 (doi: 10.1016/j.soilbio.2011.04.022).
22. Birk J.J., Steiner C., Teixeira W.C., Zech W., Glaser B. Microbial response to charcoal amendments and fertilization of a highly weathered tropical soil. In: *Amazonian dark earths: Wim Sombroek's vision*. W.I. Woods, W.G. Teixeira, J. Lehmann, C. Steiner, A. WinklerPrins, L. Rebellato (eds.). Springer, Dordrecht, 2009: 309-324 (doi: 10.1007/978-1-4020-9031-8_16).
23. Bailey V.L., Fansler S.J., Smith J.L., Bolton H. Jr. Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. *Soil Biology and Biochemistry*, 2011, 43(2): 296-301 (doi: 10.1016/j.soilbio.2010.10.014).
24. Dempster D., Gleeson D., Solaiman Z., Jones D. L., Murphy D. Biochar addition to soil changed microbial community structure and decreased microbial biomass carbon and net inorganic nitrogen mineralised. In: *Soil solutions for a changing world* /R. Gilkes, N. Prakongkep (eds.). Brisbane, 2010, Vol. 2.2.1: 9-12.
25. Lehmann J., Czimczik C., Laird D., Sohi S. Stability of biochar in soil. In: *Biochar for environmental management: science and technology*. J. Lehmann, S. Joseph (eds.). Earthscan, London-Sterling, VA, 2009: 183-206.
26. Kuzyakov Y., Bogomolova I., Glaser B., Biochar stability in soil: decomposition during eight years and transformation as assessed by compound-specific ^{14}C analysis. *Soil Biology and Biochemistry*, 2014, 70: 229-236 (doi: 10.1016/j.soilbio.2013.12.021).
27. Luo Y., Zang H., Yu Z., Chen Z., Gunina A., Kuzyakov Y., Xu J., Zhang K., Brookes P. Priming effects in biochar enriched soils using a three-source-partitioning approach: ^{14}C labelling and ^{13}C natural abundance. *Soil Biology and Biochemistry*, 2017, 106: 28-35 (doi: 10.1016/j.soilbio.2016.12.006).
28. Orlova N., Abakumov E., Orlova E., Yakkonen K., Shahnazarova V. Soil organic matter alteration under biochar amendment practice: study on the Podzol soils of the Leningrad region (Russia). *J. Soils Sediments*, 2019, 19(6): 2708-2716 (doi: 10.1007/s11368-019-02256-z).
29. Orlova N.E., Orlova E.E., Bankina T.A. V sbornike: *Pochvy v biosphere* [In: Soils in the biosphere]. Tomsk, 2018: 113-115 (in Russ.).
30. Kreier K.G., Bankina T.A., Orlova N.E., Yur'eva G.M. *Praktikum po agrokhimicheskomu analizu pochv* [Workshop on agrochemical soil analysis]. St. Petersburg, 2005 (in Russ.).
31. Bolger A.M., Lohse M., Usadel B. Trimmomatic: A flexible trimmer for Illumina Sequence Data. *Bioinformatics*, 2014, 30(15): 2114-2120 (doi: 10.1093/bioinformatics/btu170).
32. Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Gonzalez Peña A., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J., Knight R. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 2010, 7(5): 335-336 (doi: 10.1038/nmeth.f.303).
33. Chernov T.I., Tkhakakhova A.K., Kutovaya O.D. Otsenka razlichnykh indeksov raznoobraziya dlya kharakteristiki pochvennogo prokariotnogo soobshchestva po dannym metagenomnogo analiza. *Pochvovedenie*, 2015, 4: 462-468 (doi: 10.7868/S0032180X15040036).
34. Chernov T.I., Tkhakakhova A.K., Zhelezova A.D., Kutovaya O.D. V sbornike: *Osnovnye dostizheniya i perspektivy pochvennoi metagenomiki* [In: Main achievements and prospects of soil metagenomics]. St. Petersburg, 2017: 68-87 (in Russ.).
35. Filippova V.A., Kruglov Yu.V., Andronov E.E. Phylogenetic structure of community of procariots of soddy-podzolic soil under the cover of winter rye is not influenced by agrotechnics. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2018, 53(5): 994-1003 (doi: 10.15389/agrobiology.2018.5.994eng).
36. Sheng Y., Zhu L. Biochar alters microbial community and carbon sequestration potential across different soil pH. *Science of the Total Environment*, 2018, 622-623: 1391-1399 (doi: 10.1016/j.scitotenv.2017.11.337).
37. Abujabhah I.S., Bound S.A., Doyle R., Bowman J.P. Effects of biochar and compost amendments on soil physico-chemical properties and the total community within a temperate agricultural soil. *Applied Soil Ecology*, 2016, 98: 243-253 (doi: 10.1016/j.apsoil.2015.10.021).
38. Eichorst S.A., Kuske C.R. Identification of cellulose-responsive bacterial and fungal communities in geographically and edaphically different soils by using stable isotope probing. *Applied Environment Microbiology*, 2012, 78(7): 2316-2327 (doi: 10.1128/AEM.07313-11).
39. Prasad S., Manasa B.P., Buddhi S., Pratibha M.S., Begum Z., Bandi S., Tirunagari P., Shivaji S. *Arcticibacter svalbardensis* gen. nov., sp. nov., of the family *Sphingobacteriaceae* in the phylum *Bacteroidetes*, isolated from Arctic soil. *International Journal of Systematic and Evolutionary Microbiology*, 2013, 63(5):1627-1632 (doi: 10.1099/ijs.0.044420-0).