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EFFECT OF BIOCHAR APPLICATION ON VARIABILITY OF THE POLYPHENOLOXIDASE AND PEROXIDASE ACTIVITY OF SOD-PODZOLIC SOIL UNDER LOW AND HIGH FERTILITY

E.Ya. RIZHIYA[1] ✉, L.V. BOITSOVA[1], V.E. VERTEBNIY[1], J. HORAK[2], M.A. MOSKVIN[1], V.I. DUBOVITSKAYA[1], Yu.V. KHOMYAKOV[1]

[1]*Agrophysical Research Institute*, 14, Grazhdanskii prosp., St. Petersburg, 195220 Russia, e-mail alenarizh@yahoo.com (✉ corresponding author), larisa30.05@mail.ru, verteb22@mail.ru, voron27_88@mail.ru, vikot85@mail.ru, himlabafi@yandex.ru;
[2]*Slovak University of Agriculture in Nitra*, Hospodárska 7, 949 76 Nitra, Slovakia, e-mail jan.horak@uniag.sk
ORCID:
Rizhiya E.Ya. orcid.org/0000-0001-7920-867X
Boitsova L.V. orcid.org/0000-0001-7852-3918
Vertebniy V.E. orcid.org/0000-0002-2936-5949
Horak J. orcid.org/0000-0003-0078-9083
Moskvin M.A. orcid.org/0000-0001-7399-7022
Dubovitskaya V.I. orcid.org/0000-0003-2410-5547
Khomyakov Yu.V. orcid.org/0000-0002-9149-3247
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Abstract

Biochars are considered as an attractive tool in agriculture for carbon sequestration and improvement of soil functions. Biochar addition to soils can raise the pH, increase the organic carbon content, enhance nutrient retention, and increase microbial biomass. The introduction of biochar to different soils is an irreversible action. After entering the soil environment, the so called "aging" of biochar begins, due to the water-physical processes occurring in the soil, e.g., moistening, drying, freezing and thawing. Therefore, it is necessary to understand the directions of various changes occurring in the soil when using this ameliorant. A two-year field experiment to study the effect of biochar on the dynamics of some soil enzymes during of biochar aging was performed with the aim to reveal mechanisms of interaction between soil and biochar and to justify the sensitiveness of enzymes on a biochar amendment to the soil. The studied loamy sand Spodosol soil had medium and high soil quality. The experimental design included the soil (control) and the soil with 20 t/ha biochar introduced in the top arable layer (0-10 cm) of 4 m^2 plots in 3 replicates. The impact of birch (*Betula* spp.) biochar produced by fast pyrolysis at 600 °C was studied. Chemical characteristics of the biochar were as follows: $C_{org.}$ — 88.9 %, $N_{tot.}$ — 0.43 %, H — 3.2%, O — 5.1 %, pH_{H_2O} 8.3, water content — 3.1 %, ash content — 1.8 %. In 2019, a seed mixture of oat (*Avena sativa* L.) cv. Borrus and common vetch (*Vicia sativa* L.) L'govskii cv. was cultivated on the plots at the rate of 200 kg/ha (or 85 g per 4 m^2). In 2020, white lupine (*Lupinus albus* L.) cv. Dega was cultivated as green manure for winter wheat at the rate of 200 kg/ha. Soil and biochar samples were collected from a 0-10 cm layer of the humus horizon in May to August (at 14-day intervals) with an Endelman soil drill (Royal Eijkelkamp B.V., the Netherlands). The activity of peroxidase (EC 1.11.1.7) and polyphenoloxidase (EC 1.10.3.1) was assessed by the photocolorimetric method (λ = 440 nm and λ = 590 nm, respectively), and the assessment of temporal changes in the oxidation of the surface of biochar was studied by IR spectrometry (an FSM 2202 Michelson spectrometer, Infraspek, Russia). The biochar was found to increase the activity of the studied enzymes, on average by 12-13 %, as compared to the activity in soils without biochar. The peroxidase activity was on average 1.5 times higher than that of the polyphenoloxidase and significantly ($p < 0.05$) depended on the degree of soil quality. The ratio of polyphenoloxidase to peroxidase in the soil with medium soil quality was approximately 20 % lower than in the soil with high soil quality, where the conditions (temperature, humidity, amount of organic matter) were optimal for humus synthesis. It was found that all treatments showed the soil humification factor less than 1, which indicates the predominance of the processes of mineralization of humic substances in the soil over their immobilization. The biochar increased the mineralization of organic matter by 11.5 % compared to soils without biochar. Over the two-year experiment, IR spectroscopy revealed a tendency to an increase in the amount of hydroxyl, carbonyl, and carboxylate groups compared to the initial biochar, which is consistent with the data on the increase in the activity of polyphenoloxidase and peroxidase. Our findings confirm that biochar introduced into the loamy sand Spodosol remained stable during two years and did not significantly affect the enzymatic activity of soils.

Keywords: soddy-podzolic sandy loam soil, biochar, peroxidase, EC 1.11.1.7, polyphenol

oxidase, EC 1.10.3.1, IR spectra of biochar

The use of biochar as a high-carbon porous ameliorant produced from various biomass wastes is of great interest due to its potential benefits for sustainable agricultural development and reducing the negative impact of agricultural activities on the environment [1-4]. During oxygen-free pyrolysis, aliphatic carbon chains in the initial biomass are transformed into stable aromatic ones and can be preserved in the soil for hundreds of years [5]. After entering the soil environment, the so-called aging of biochar begins due to the water-physical processes occurring in the soil - moistening, drying, freezing and thawing [6-10]. Even before being introduced into the soil, biochar, being hydrophobic in its chemical structure, begins to oxidize in the air and increase its hydrophilicity [11]. The hydrophilicity of coal largely depends on the pyrolysis temperature and the type of raw material. For example, the available water content of grapevine-derived biochar increased with increasing pyrolysis temperature, with optimal results achieved at 700 °C [12]. Analysis of wood and grass biochars obtained at 400 and 650 °C and kept both in soil and directly in the air for 15 months showed a relative increase in the amount of O-containing functional groups, including substituted aryl-, carboxyl- and carbonyl-C as a result of abiotic and microbial oxidation of biochar, as well as the loss of O-alkyl groups due to leaching [13].

In addition, the biochemical process of decomposition of biochar by microorganisms begins to operate in the soil [14, 15]. It is limited by environmental factors, the availability of organic compounds necessary for the life of microorganisms, and manifests itself in a change in functional groups on the surface of biochar [16, 17]. Many aspects of the influence of biochar on the components of the natural environment, in particular the direction of biochemical changes occurring in the soil, are still poorly understood.

In the present work, we have established for the first time that a two-year stay of biochar in soddy-podzolic sandy loamy soil contributed to the variability of functional groups on its surface, which resulted in an increase in the amount of hydroxyl (−OH), carbonyl (C=O), and carboxylate (COO−) groups compared to the original biochar and in a tendency to increase the mineralization of organic matter, based on the activity of polyphenol oxidase and peroxidase.

The aim of this work is to assess the variability of polyphenol oxidase and peroxidase activity in agro-soddy-podzolic soil with different degrees of cultivation when biochar is introduced into it, as well as to determine the oxidation of the biochar surface after incubation in the soil.

Materials and methods. Field trials were carried out during the growing seasons of 2019 and 2020 on the territory of the experimental station of the Agrophysical Research Institute (settlement Menkovo, Gatchina District, Leningrad Province).

Experimental 4 m^2 plots were placed on parcels of the Agrophysical Station on soil with a medium- (MLC) and high-level (HLC) cultivation, as well as on control plots without the application of mineral fertilizers. The content of physical clay in the medium cultivated soil was 12-15%, in the highly cultivated soil it was 17-19%. The experimental design included two options in 3 repetitions: control (without biochar) and experiment (soil with biochar at a dose of 20 t/ha, which was applied to the top layer of 0-10 cm). The medium-level cultivated soil, was 1.53% Corg, 0.17% Ntot, with 16.4 mg/kg $N\text{-}NO_3$, 5.6 mg/kg $N\text{-}NH_4$, mobile P_2O_5 accounted for 255 mg/kg, K_2O for 112 mg/kg (according to Kirsanov), pH_{KCl} = 5.3. The high-level cultivated soil was 2.92%Corg, 0.28% Ntot with 22.3 mg/kg $N\text{-}NO_3$, 6.7 mg/kg $N\text{-}NH_4$; mobile P_2O_5 accounted for 994 mg/kg, K_2O for 542 mg/kg, pH_{KCl} = 6.4.

We used biochar from birch (*Betula pendula* Roth) grade Premium A (fraction with a particle size of 0.5-2 cm), produced by rapid pyrolysis at a temperature of 600 °C. The biochar was 88.9% C_{org}, 0.43% N_{tot}, 3.2% H, 5.1% O, pH_{H2O} = 8.3, W_{hc} was 3.1%, ash content 1.8%.

In 2019, a mixture of spring vetch (*Vicia sativa* L.) cv. Lgovsky and spring oat (*Avena sativa* L.) cv. Borrus (30% + 70%) was grown on the plots; sowing (200 kg/ha, or 85 g per 4 m^2) was carried out on May 21. In 2020, white lupine (*Lupinus albus* L.) cv. Degas was grown as green manure for winter wheat (seeding rate of 200 kg/ha, sowing on May 15, plowing on August 16), winter wheat was sown on September 3, 2020.

Meteorological data were recorded at a weather station located 200 m from the experimental site.

Soil and biochar samples were taken with an Endelman soil drill (Royal Eijkelkamp B.V., the Netherlands) from the 0-10 cm layer of the humus horizon with an interval of 14 days from May to August inclusive. The activity of peroxidase and polyphenol oxidase was studied by the photocolorimetric method according to A.Sh. Galstyan respectively but at λ = 440 and λ = 590 nm [18]. The humification coefficient (K_{hum}) was calculated from the ratio of the activity of polyphenol oxidase (PPO) to the activity of peroxidase (PO). To assess temporal changes in the oxidation of the biochar surface, the content of oxygen-containing groups was determined by IR spectrometry. An attachment was used to measure the multiple frustrated total internal reflection with a ZnSe crystal on an FSM 2202 Michelson-type spectrometer (Infraspek, Russia) with self-compensation (it does not require dynamic adjustment in the wavenumber range 7800-600 cm^{-1}).

Statistical data processing was carried out in Microsoft Excel and Statistics 8.0 programs. Mean values (*M*), standard deviations (±SEM) and linear correlation coefficients were calculated at a significance level of $p \leq 0.05$. The significance of differences in mean values was assessed using analysis of variance (ANOVA).

Results. The experiments were carried out in the Non-Chernozem zone of Russia, which belongs to the temperate cold zone [19]. Comparing the growing seasons of 2019 and 2020, we can note the common features of hydrometeorological indicators by months. May was cold, with an average rainfall of up to 80 mm, June was hot and dry, July was warm and humid, August was warm but also humid in 2020 compared to the moderately humid 2019, September was cold and moderately wet. During the growing season of 2019, 451 mm of precipitation fell, in 2020 - 517 mm of precipitation.

The intensity of enzymatic processes depends on the specific conditions prevailing in the soil and is variable. Horizontal variability depends on temperature, humidity, and the intensity of fresh organic input, while the vertical gradient depends on the quality of organic matter and the physical properties of the soil [20]. Polyphenol oxidase (PPO; o-diphenol:oxygen oxidoreductase, EC 1.10.3.1) is one of the most important enzymes involved in the oxidation of aromatic organic compounds and the formation of humus [21].

In our experiments, there was a significant difference ($p < 0.05$) between the variants with different soil cultivation in terms of PPO activity, while the introduction of biochar only led to an insignificant increase in the indicator by 10-13% (Fig. 1). Despite the level of soil cultivation, polyphenol oxidase activity showed an increase in the initial periods of crop vegetation which, as a rule, is due to the active root growth, low soil temperatures, and optimal moisture. By the second decade of June when the soil temperature began to rise but drought was recorded, PPO decreased. By the end of July, with an increase in the amount of precipitation, a significant ($p < 0.05$) increase in PPO activity was noted, and by the end of the growing season, a decrease. Relatively increased values of polyphenol oxidase activity by the end of July are presented as a total result of plant maturation, an increase in the number of microorganisms, and an intensification of decomposition of plant residues under more favorable hydrothermal conditions [22].

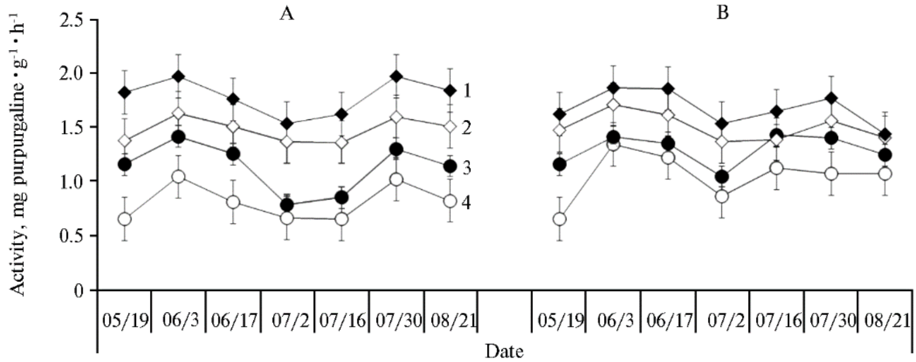


Fig. 1. Activity of polyphenol oxidase (EC 1.10.3.1) **in soddy-podzolic soils with medium- (MLC) and high-level (HLC) cultivation as influenced by biochar from pyrolyzed premium grade birch wood:** A — vetch-oat mixture (2019), B — white lupine (2020); 1 — HLC with biochar, 2 — HLC without biochar, 3 — MLC with biochar, 4 — MLC without biochar (settlement Menkovo, Gatchina District, Leningrad Province).

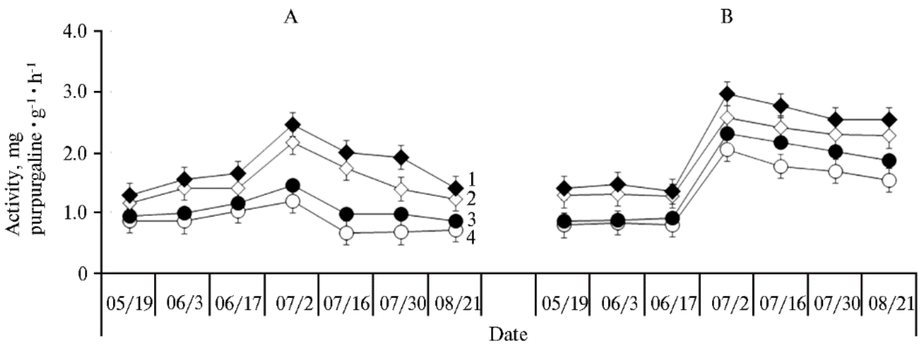
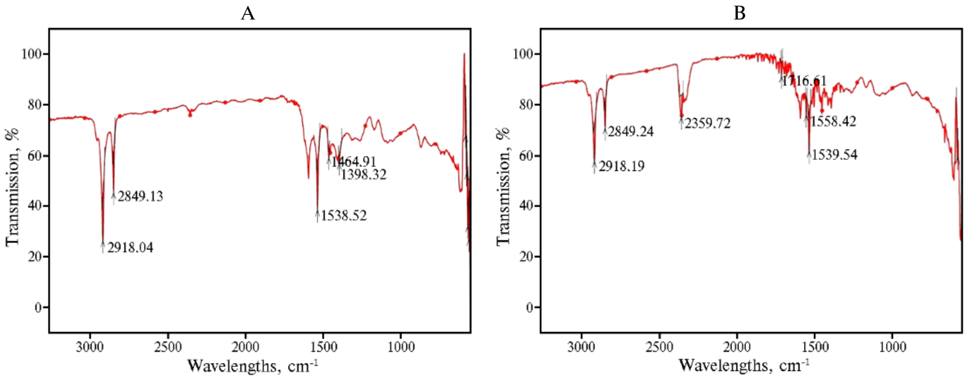


Fig. 2. Activity of peroxidase (EC 1.11.1.7) **in soddy-podzolic soils with medium- (MLC) and high-level (HLC) cultivation as influenced by biochar from pyrolyzed premium grade birch wood:** A —vetch-oat mixture (2019), B — white lupine (2020); 1 — HLC with biochar, 2 — HLC without biochar, 3 — MLC with biochar, 4 — MLC without biochar (settlement Menkovo, Gatchina District, Leningrad Province).

Peroxidase (PO, EC 1.11.1.7) is an enzyme that oxidizes organic matter with the participation of hydrogen peroxide (soil organic matter, including humic substances) [22, 23]. The activity of PO in soddy-podzolic sandy loamy soil was on average 1.5 times higher during the growing season than the activity of PPO, and significantly ($p < 0.05$) depended on the degree of cultivation, that is, the higher the cultivation of the soil, the higher it turned out to be. peroxidase activity (Fig. 2). In addition, in contrast to PPO, the activity of PO increased significantly with increasing temperature.

The introduction of biochar, in addition to influencing the rate of oxygen consumption, had an impact on the production of peroxidase, which increased by 13% both in the soil with MLC and with HLC. The maximum activity of this enzyme in all variants of the experiment was observed in early July, reaching values of 2.3 and 3.0 mg of purpurgalin $\cdot g^{-1} \cdot h^{-1}$ for soil under MLC and HLC, respectively.

The intensity of humus formation is characterized by the humification coefficient (K_{hum}), which is the ratio of polyphenol oxidase activity to peroxidase activity [24]. Calculations showed that in all variants of the experiment $K_{hum} < 1$, which indicated the predominance of the processes of mineralization of humic substances, plant residues, and nonspecific organic compounds in the soil over their synthesis. Kgum. in soil with HLC was about 20% lower than in soil with HLC. The introduction of biochar contributed to the increase in K_{hum}. by an average of 11-12% compared to soils without biochar.



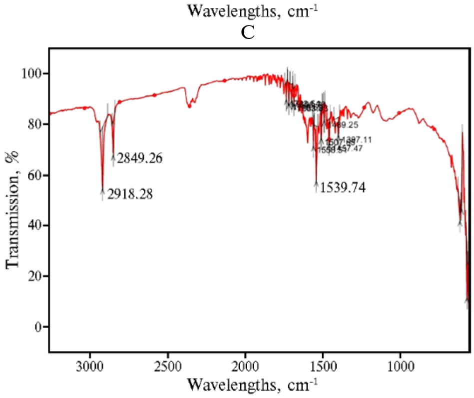


Fig. 3. IR spectra of biochar from pyrolyzed Premium grade birch wood: A — native biochar (control) (May 2019), B — biochar after incubation in soil with a medium-level cultivation (at the end of the growing season, August 2020), C — biochar after incubation in soil with a high-level cultivation (at the end of the growing season, August 2020) (settlement Menkovo, Gatchinsky District, Leningrad Province).

The main changes in the enzymatic activity of soils with biochar can be associated with the transformation of organic functional groups on the surface of biochar (an increase in the number of −COO− and −O− groups), as well as with the ability of biochar particles to absorb H^+ ions. The change in the enzymatic activity of the soil occurs due to the sorption of enzymes on the surface of the biochar with the formation of matrix-enzyme complexes, which are more stable and stable in the soil, have a greater enzymatic activity. Biochar also sorbs various low molecular weight and soluble high molecular weight organic compounds (carbohydrates, organic acids, aromatic compounds, including phenolcarboxylic acids, phenols, etc.) from the soil solution, which can be identified by IR Fourier spectrometry of functional groups. They are localized on the surface of coal and serve as substrates for the activity of microorganisms adsorbed on biochar.

Studies of temporal changes in the oxidation of the surface of biochar made it possible to identify a number of zones in the IR spectra in the absorption mode with an increased intensity of oscillations, which characterize the change in the surface of the coal after it has been in soils. The original coal (Fig. 3, A) had bands in the region of 1500-1100 cm^{-1}, characteristic of the C−O and C=O groups, and bands in the region of 2900 cm^{-1}, which indicated the presence of aliphatic groups, including characteristic of aldehydes (C−H bonds in the state of sp3 hybridization) [25]. A pronounced intensity of the vibrational spectrum was also observed in the region of 1600 cm^{-1}, which is characteristic of the quinoid groups of aromatic compounds.

After incubation of biochar in the soil for 17 months, an increase in the content of various oxygen-containing groups was observed, except for quinoid ones, where the intensity of fluctuations at 1600 cm^{-1} decreased by more than 2 times compared to the control, and the content of aliphatic groups also decreased (bands at 2900 cm^{-1}). The content of hydroxyl (−OH), carbonyl (C=O) and carboxylate (COO−) groups on the biochar surface showed a trend of their increase (1539-1540 cm^{-1}). On the whole, biochar practically remained in a stable state over the two growing seasons, and a significant decrease in the proportion of aliphatic groups with an insignificant dynamics of oxygen-containing groups indicated interaction with the components of the soil solution.

In most studies on the effect of biochar on microbial activity in soils, it has been shown that it increases, but the intensity of the effect depends on the properties of the initial biomass from which biochar is produced, pyrolysis conditions (duration and temperature), and the soil into which biochar is directly applied [1 , 26, 27]. Biomass usually determines the chemical composition, number of macropores, and nutrient content in biochar, while pyrolysis conditions determine changes in the morphology and structure of the surface in the feedstock and the C:H ratio [28].

It should be noted that information on the effect of various biochars on the enzymatic activity and the chemical properties of soils related to it is still contradictory. Since enzymes are proteins, all factors affecting the protein will also affect the activity of the enzyme, and biochar introduced into the soil, as has already been proven, changes gas exchange and the specific surface of the soil, its water-holding capacity and other physical and chemical properties [1, 29]. The large specific surface of biochar helps to adsorb labile substances from the environment, which affects the activity of various enzymes that break down such substances into simple molecules, which are further used by bacteria and fungi in primary and secondary metabolism [30].

In our experiments, we have revealed a general tendency for an increase in the activity of peroxidase and polyphenol oxidase when biochar is introduced into the soil: by an average of 13% (average degree of cultivation) and 12% (high degree of cultivation). The activity of PO was on average 1.5 times higher than the activity of PPO, and significantly ($p < 0.05$) depended on the level of soil cultivation. J. Park et al. [31] and S. Kumar et al. [32] noted the positive effect of biochar on enzymes, as judged by the increased uptake of carbon, nitrogen and phosphorus from the soil. J. Paz-Ferreiro et al. [33], F. Wu et al. [34] and J. Lehman et al. [1] pointed to the negative effects of biochar reducing enzymatic activity compared to soils without biochar. The mechanisms explaining such conflicting observations have not been identified for a long time. It turned out that the sorption of a substance and extracellular enzymes that differ in functional groups on the surface of biochar can enhance or limit the enzymatic reaction, and the characteristics of biochar as a sorbent can change over time [11]. Our work describes the processes that lead to a change in the state of functional groups on the biochar surface over time. Other studies have also shown that the aging of the ameliorant leads to the appearance of more carboxyl functional groups on its surface, a higher oxygen content in biochar and a lower total carbon [35]. F.V.D.N. Tozzi et al. [36] assessed the stability of carbon in biochar and its effect on surfactants when wood biochar was used to sequester carbon in soil. After 120 days of incubation, an increase in the degree of carbon mineralization by 0.4-9.3% (depending on the type of soil) was found, which indicated its high stability. This is also confirmed by our data on a slight change in the carbon content of woody biochar over a two-year stay in the soil.

Oxidation of biochar in soils can lead to an increase in the density of oxygen-containing functional groups on its surface. The high density of oxygen-containing functional groups detected by IR spectroscopy [37] increases the affinity of the biochar surface for the adsorption of water molecules, nutrients, and organic compounds [38-40].

Therefore, for 17 months of the experiment, the activity of polyphenol oxidase (PPO) and peroxidase (PO) in soddy-podzolic sandy loamy soils with the introduction of biochar increased on average by 13% (average degree of cultivation) and 12% (high degree of cultivation) compared with the variants without biochar. The PO activity was on average 1.5 times higher than the PPO activity and significantly ($p < 0.05$) depended on the degree of soil cultivation. The coefficient of humification in the soil with MLC was approximately 20% lower than in the soil with HLC, and in all test variants it turned out to be less than 1, which indicates the predominance of the processes of mineralization of humic substances in the soil over their immobilization. With the introduction of biochar, an increase was observed in the K_{hum} index by 11-12% compared to soils without biochar. In biochar after incubation in the soil, there was a trend towards an increase in the number of hydroxyl (−OH), carbonyl (C=O), and carboxylate (COO−) groups compared to the initial biochar. Based on the results of changes in the values of PPO, PO, and IR spectra, it can be argued that the introduced biochar remained in a stable form and did not significantly affect the enzymatic activity of soils.

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