AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 5, pp. 937-947
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

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

Soil fertility

UDC 631.465;631.48

doi: 10.15389/agrobiology.2023.5.937eng
doi: 10.15389/agrobiology.2023.5.937rus

ENZYME ACTIVITY AND ORGANIC CARBON CONTENT IN AGRICULTURAL SPODOSOL

L.V. BOITSOVA ✉, S.V. NEPRIMEROVA, E.G. ZINCHUK

Agrophysical Research Institute, 14, Grazhdanskii prosp., St. Petersburg, 195220 Russia e-mail: larisa30.05@mail.ru (✉ corresponding author), neprimerova@list.ru, zinchuk-e@yandex.ru
ORCID:
Boitsova L.V. orcid.org/0000-0001-7852-3918
Zinchuk E.G. orcid.org/0000-0001-9208-3306
Neprimerova S.V. orcid.org/0000-0001-7852-9994
The authors declare no conflict of interests
Final revision received June 29, 2023
Accepted July 10, 2023

Abstract

Polyphenol oxidases (PPO) and peroxidases (PO) are soil extracellular enzymes that can be found in free forms and can also be bound in organo-mineral-enzyme complexes. PPO are involved in the catalysis of the humic substances synthesis, while PO are involved in the catalysis of their decomposition. The accumulation of soil humus is associated with the activity of PPO and PO. In this work, the influence of PPO and PO activity on the accumulation of organic carbon in a sandy Spodosol and its clay fraction was studied for the first time. The purpose of the work was to evaluate the seasonal dynamics of the enzymatic activity of PPO and PO as well as its effect on the stabilization of organic carbon in a sandy Spodosol. Field study was carried out during the growing seasons of 2020-2021 at the experimental station of the Agrophysical Research Institute (Menkovo village, Gatchina District, Leningrad Province) on a plot of sandy Spodosol soil without application of mineral fertilizers. A plot of 2×2 m in 2020-2021 was a fallow land while in 2019 a vetch-oat mixture (*Vicia sativa* L., *Avena sativa* L.) was grown there. Soil samples were collected with an Endelman soil drill from 0-10-cm layer using the envelope method, every month (from May to August). The content of total organic carbon (C_{org}) and carbon associated with the soil clay fraction (C_{clay}) was determined by the Tyurin method. Soil moisture was measured gravimetrically, temperature by electronic sensors iButton DS 1921 (Dallas Semiconductor, USA), installed at a depth of 10 cm. The soil carbon accumulation was calculated using the carbon enrichment coefficient of clay soil fractions (E_{soc}). The activity of PO and PPO was assessed using the Galstyan photocolorimetric method at $\lambda = 440$ and $\lambda = 590$ nm. The activity of oxidases in 2021 was, on average, 5 times higher for PPO and 3 times higher for PO compared to 2020. The activity of the PPO was, on average, 1.7 times higher in 2020 and 2.6 times higher in 2021 than the activity of the PO, which was reflected in the value of the humification coefficient (K_{gum}), which is calculated by the ratio of the PPO to the PO. In 2021, when the hydrothermal conditions in the soil were favorable for processes of humus synthesis (average soil temperature –20 °C, 16.4 % humidity), $K_{gum.}$ was 47 % higher than in 2020. With a decrease in soil temperature, the statistically significant ($p < 0.05$) increase in PPO activity occcured throughout the entire observation period. In 2021, with an increase in soil moisture, PO activity significantly increased ($p < 0.05$). The conducted studies indicate that the maximum content of total organic carbon in the soil corresponded to the maximum activity of polyphenol oxidases. In 2021, the total organic carbon content decreased on average by 2 % compared to 2020 ($p < 0.63$). However, the average values of C_{clay} content over the same period increased by 4.78 % ($p < 0.43$). As a result, in 2021 the enrichment coefficient of the clay fraction of the soil increased. The relationship between the activity of oxidases and the content of organic carbon associated with the clay fraction was complex, since the organic matter fixed in the clay fraction is weakly susceptible to the influence of microorganisms and their metabolic products. Temperature and soil moisture had the greatest influence on the accumulation of carbon in the clay fraction of the soil compared to the deposition of total organic carbon in it. At the same time, the relationship between the soil moisture and the accumulation of C_{org} and C_{clay} was stronger ($r = 0.66$ and $r = 0.90$, respectively, $p < 0,05$) than the relationship between the soil temperature and the accumulation of C_{org} and C_{clay} ($r = –0.43$ and $r = –0.73$? $p < 0,05$).

Keywords: Spodosol, polyphenol oxidase, peroxidase, total organic carbon, clay fraction carbon

Soils enzymatic activity is due to the activity of soil biota and its ability to undergo various biochemical transformations [1-3]. Soil extracellular hydrolytic and redox enzymes play an important role in the processes of humus formation. Hydrolytic enzymes are, in particular, proteases, cellulases, amylases, lipases, and redox enzymes are polyphenol oxidases, peroxidases, and dehydrogenases. Polyphenol oxidases (PPO) and peroxidases (PO) belong to the class of oxidoreductases. Soil oxidoreductases may act as free enzymes or as those bound into organo-mineral-enzyme complexes [4, 5]. They enter the soil with intravital secretions of plant roots and microorganisms (bacteria, actinomycetes, fungi) [6, 7]. PPOs are involved in the biosynthesis of humic acids by catalyzing oxidative polymerization reactions of aromatic compounds in the presence of oxygen [8]. Peroxidases are enzymes that catalyze the oxidation of polyphenols and some aromatic amines with the participation of oxygen and hydrogen peroxide [9, 10]. The activity of PPO and PO can serve as indicators of the intensity of humus formation [11] and mainly correlates with abiotic factors. To a lesser extent, it is influenced by the species composition and biomass of soil fungi [12].

The rate of accumulation of soil humus depends on the activity of PPO and PO [13], since the formation and destruction of humic substances occur simultaneously. On the one hand, soil organic matter can influence the activity of extracellular enzymes secreted by microorganisms and plant roots, providing energy and substrate for the secretion of enzymes [14]; on the other hand, increased oxidase activity enhances the decomposition of organic matter, which negatively affects its accumulation [11].

A close connection has been established between the dynamics of the content of organic matter in the soil and the activity of these enzymes [15, 16]. The state in which soil organic matter is found determines the amount of CO_2 released into the atmosphere, in turn, this affects climate change. Soil organic matter is fixed, forming organomineral complexes with clay minerals [17, 18]. In addition, soil carbon content positively correlates with silt content in mineral soil [19].

Humidity and temperature influence seasonal changes in the content of organic matter in the soil and its silt fraction. A number of works [20-23] have established a close correlation between the average annual precipitation, vegetation and the content of organic carbon in the soil. Since net primary production and the input of crop residues to the soil increase as the amount and frequency of precipitation increases, this leads to greater accumulation of organic carbon in the soil. However, the relationship between these parameters is influenced by topography and the quality of plant litter [24].

The soil water regime has a significant impact on soil processes; a direct relationship has been established between precipitation, soil moisture and its enzymatic activity [25]. With an increase in moisture reserves and a decrease in soil temperature, an increase in the activity of the hydrolytic enzymes invertase, urease, phosphatase, ATPase, as well as oxidoreductases, in particular dehydrogenase, occurs. The activity of polyphenol oxidases and peroxidases is associated with soil pH and affected by heavy metals, average annual precipitation and average annual temperature and, thereof, by soil temperature and moisture, and soil and vegetation types [26-28]. S. Liu et al. [29] showed a positive correlation between soil water content and PPO activity. In addition, oxygen availability has been found to limit polyphenol oxidase activity in the substrate [30].

In this work, the influence of PPO and PO activity on the accumulation of organic carbon in agrosoddy-podzolic soil and its silt fraction was established for the first time.

The purpose of the work is to study the seasonal dynamics of the enzymatic activity of polyphenol oxidases and peroxidases and its effect on the stabilization of organic carbon in agrosoddy-podzolic soil.

Materials and methods. Field research was carried out during the growing seasons of 2020-2021 on the territory of the AFI experimental station (Menkovo village, Leningrad Province, Gatchina District) on a plot.

In 2019, on a 2×2 m plot with agro-soddy-podzolic soil, a vetch-oat mixture (*Vicia sativa* L., *Avena sativa* L.) was grown, in 2020-2021, there was not fertilized fallow. The soil was 19% physical clay, and the silt fraction was 4.8%. Agrochemical parameters at the beginning of the experiment were as follows: $C_{org.}$ = 2.92%, $N_{tot.}$ = 0.28%, N-NO_3 = 22.3 mg/kg soil, N-NH_4 = 6.7 mg/kg soil; mobile P_2O_5 = 994 mg/kg soil, mobile K_2O = 542 mg/kg soil (by Kirsanov method), pH_{KCl} 6.4. Soil samples were collected with an Endelman soil drill from a 0-10 cm layer of humus horizon using the envelope method every month from May to August.

The content of total organic carbon ($C_{org.}$) and carbon associated with the clay fraction (C_{silt}) was determined by the Tyurin method [31]. To isolate the silt fraction of the soil (<1 μm), distilled water was added to a 200 g soil sample passed through a sieve with a mesh diameter of 1 mm. The resulting mixture was dispersed for 30 min using a BRANSON 450 ultrasonic unit (Branson Ultrasonics Corporation, USA) at 315 W (70% of maximum). The sample was then transferred to a vessel and subjected to sedimentation. To sediment the silt fraction, a JANETZKI S70 centrifuge (Heinz Janetzki, Poland) (6000 rpm, 15 min) was used. The resulting silt was dried in air at room temperature [32].

Soil moisture was determined by thermostat-weight method, temperature with iButton DS 1921 electronic sensors (Dallas Semiconductor, USA) installed at a 10 cm depth. Carbon accumulation in the soil was assessed using the coefficient of enrichment of carbon in the silt fraction of the soil (E_{soc}) [33]:

$$E_{soc}= C_{f.}/C_{org.},$$

where $C_{f.}$ is carbon content of the fraction, % of the fraction mass, $C_{org.}$ is content of total organic carbon, % of the mass of the soil.

The activity of peroxidases and polyphenol oxidases was studied photocolorimetrically according to Galstyan's method at λ = 440 and λ = 590 nm [34]. To measutr the activity of oxidases, 0.5 ml of toluene and 100 ml of H_2O were added to a 1 g soil sample, shaken for 20 min and filtered. Two rows of flasks were arranged. To determine the activity of PPO, 5 ml of a 1% pyrogallol solution was added to 10 ml of the filtrate; to assess the activity of PO, 5 ml of a 1% pyrogallol solution and 1 ml of 0.5% H_2O_2 were added to 10 ml of the filtrate. All flasks were allowed for 2 h at 37 °C in a thermostat (Smolensk SKTB SPU OJSC, Russia), then at room temperature for 14 h. In the morning, colorimetry was carried out (a PE-3000UF spectrophotometer, PromEcoLab LLC, Russia) with blue light filter. In the control sample, 10 ml of water and 5 ml of pyrogallol were mixed. Enzyme activity was expressed in mg of purpul gallin. For calibration curve, serial dilutions of $K_2Cr_2O_7$ was used. The color of 0.75 g of the reagent dissolved in 1 l of 0.5 N HCl corresponded to the 0.1 mg of purple gallin in 1 ml of solution. Analytical repetition was 3-fold.

To quantify humification, the humification coefficient is used, which is equal to the proportion of carbon in organic residues (%) transformed into humic substances [35]. To assess the intensity of humus formation, A.I. Chunderova [36] proposed the humification coefficient ($C_{hum.}$) which is the ratio of the polyphenol oxidases (PPO) to peroxidases (PO) activities $C_{hum.}$ = PFO/PO. This coefficient may characterize the direction of humification. If $C_{hum.} \geq 1$, then humus synthesis predominates in the soil, if $C_{hum.} < 1$, then the decomposition of humic substances prevail.

Statistical processing was carried out using the Microsoft Excel software package. Means (*M*), standard deviations (±SD), and Pearson correlation coefficients were calculated. The significance of the differences was assessed by one-way analysis of variance at a significance level of $p < 0.05$.

Results. According to the Menkovo weather station (Leningrad Province, Gatchina District), the average air temperature during the growing seasons from May to September 2020 and 2021 was +14.5 and +15.7 °C, respectively, with 517 and 536 mm precipitations (Table 1).

1. Weather conditions of the growing seasons 2020-2021 according to the data of Menkovo weather station (Leningrad Province, Gatchina District)

Parameter	Year, month									
	2020					2021				
	V	VI	VII	VIII	IX	V	VI	VII	VIII	IX
Average daily temperature, °C	+8.8	+18.1	+16.5	+16.4	+12.8	+10.8	+19.7	+21.1	+15.4	+12.4
Precipitation, мм	80.0	38.9	167.7	185.4	45.2	245.5	29.0	36.2	190.4	56.5

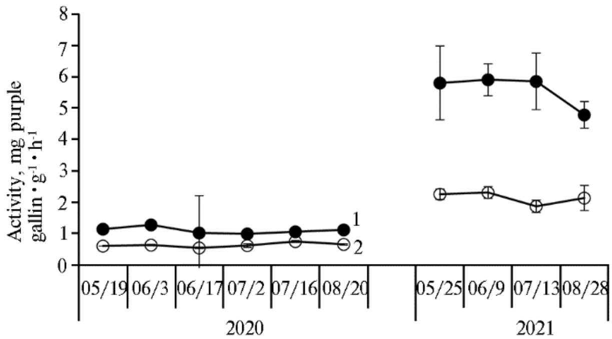

Fig. 1. Seasonal polyphenol oxidase (1) **and peroxidase** (2) **activity of agrosoddy-podzolic soil for two years** (experimental station of the Agrophysical Research Institute, *n* = 3, *M*±SD; Menkovo village, Leningrad Province, Gatchina District).

Over the two year observations, the PPO abd PO activity reached its highest levels in 2021 (Fig. 1). The average PPO activity in 2020 was 1.09 mg of purpur gallin $\cdot g^{-1} \cdot h^{-1}$, in 2021 5.58 mg purple gallin $\cdot g^{-1} \cdot h^{-1}$. The average activity of PO in 2020 was 0.63 mg purple gallin $\cdot g^{-1} \cdot h^{-1}$, in 2021 2.14 mg purple gallin $\cdot g^{-1} \cdot h^{-1}$. The highest PPO activity over the two seasons was observed in early June, PO in early June and mid-July (see Fig. 1). During this period, the most favorable conditions for the microbial community developed, namely the ratio of temperature and soil moisture.

Microbial activity together with abiotic factors (soil temperature, soil moisture, pH) ensure humus formation. According to R.I. Sinsabaugh [15], oxides and hydroxides of Mn, Fe and Al in soil create electron acceptors that catalyze production of reactive species from phenols. These intermediates condense with other phenols or amino acids into humic compounds. Silt and clay fractions of the soil catalyze decarboxylation and deamination, which promote both mineralization of organic matter, and condensation reactions that lead to the formation of humic acids.

The $C_{hum.}$ value for 2 years did not fall below 1, which indicated the predominance of humic substance synthesis over organic substance mineralization (Fig. 2). The average seasonal $C_{hum.}$ amounted to 1.76 in 2020 nad 2.63 in 2021, which indicates an increased formation of humic substances during the growing season of 2021. This was probably due, on the one hand, to favorable soil and wether conditions promoting increased PPO and PO activity. On the other hand, in 2019, a vetch-oat mixture grown on the plot produced large biomass of скщз and root residues, which served as an additional source of organic matter for soil microorganisms and contributed to increased oxidase activity in the second year of the experiment. Small fragments of organic matter probably formed complexes more easily with soil minerals.

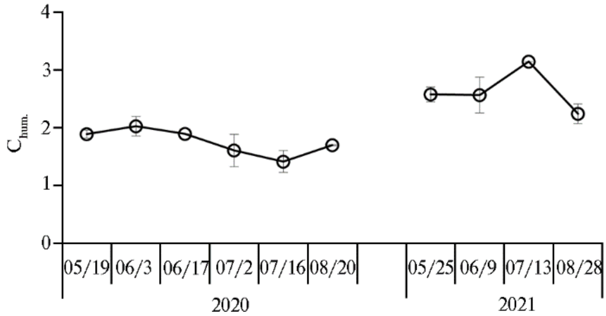

Fig. 2. Humification coefficient (Chum.) of agrosoddy-podzolic soil for two years (experimental station of the Agrophysical Research Institute, n = 3, M±SD; Menkovo village, Leningrad Province, Gatchina District).

Molecules of humates and fulvates, due to their large size and special shape, do not penetrate into the interlayer spaces of clay minerals, but are bound due to adhesion and cohesion. Only low molecular products of organic substance decomposition can penetrate into the interlayer spaces [37]. In general, the binding of humates and fulvates is facilitated by the composition of the surface layers of colloids, in particular the amount of iron and aluminum, the dispersion of minerals, and the presence of non-silicate sesquioxides in the colloids. The adsorption of organic molecules by clay minerals is a complex process in which various interactions, the H-bonding, ion-dipole and van der Waals interactions can occur [38].

The organic compound may also form complexes with clay mineral counterions or undergo ion exchange. The studied soil and its silt fraction contained clay minerals kaolinite, chlorite, hydromicas (mica with a deficiency of interlayer cations, dioctahedral mica, trioctahedral mica) [39].

In our tests, the content of PPO in the soil increased more significantly than PO, which led to an increase in Chum. in 2021 compared to 2020. According to E.Ya. Rijia et al. [40], in the soil of plots where white lupine (*Lupinus albus* L.) was grown in 2020, the activity of PPO was 1.463 mg purple gallin $\cdot$ g^{-1} $\cdot$ h^{-1}, PO 2.074 mg purple gallin $\cdot$ g^{-1} $\cdot$ h^{-1}. In the soil used as pure fallow, the activity of oxidases in 2020 was significantly lower, 1.3-fold for PPO and 3.3-forl for PO. That is, the plants and their species influence the biological activity of soils, which is consistent with the data of other researchers [41, 42].

The large amount of root and above-ground residues that white lupine formed during the growing season increases soil enzymatic activity. According to E.I. Zolkina [43], in soddy-podzolic sandy loam soil, above-ground (stubble and litter) and underground (roots) plant residues of lupine amount to 37-46.7 c/ha of dry matter. Apparently, a small number of groups of microorganisms utilizing organic and mineral nitrogen, e.g., cellulose-degrading micromycetes, micromycetes, actinomycetes, is characteristic of clean fallow due to the lack of fresh organic matter [44, 45]. In turn, green manure fallow (in our case, vetch-oat mixture is the predecessor of pure fallow) enhances the abndance of these groups, especially bacteria. Thus, the number of ammonifying bacteria when using green manure fallow increases 3.3 times compared to pure fallow [46]. This fact can explain the decrease in oxidase activity in soil under pure fallow compared to green manure fallow.

Correlation analysis revealed a significant strong inverse relationship between PPO activity and soil temperature over 2 years of observations (r = –0.73 and r = –0.80, $p < 0.05$) and a significant strong positive relationship in 2021 between PO activity and soil moisture (r = 0.99, $p < 0.05$). In other cases, the relationship between the activity of the studied oxidases, soil temperature and soil moisture was weak and unreliable. In 2020, the relationship of $C_{org.}$ and C_{silt} with PPO was positive (significant for $C_{org.}$, $r = 0.48$, $p < 0.05$ and unreliable for C_{silt}, $r = 0.38$). In 2021, significant positive correlations were recorded between PPO and $C_{org.}$ ($r = 0.84$, $p < 0.05$), and between PO and C_{silt} ($r = 0.90$, $p < 0.05$). I.N. Kurganova et al. [47] noted close positive correlation of PPO activity with $C_{org.}$ ($r = 0.99$, $p < 0.05$) in soddy-podzolic soil. However, enzyme activity cannot be a simple reflection of the amount of soil organic matter, since in arable soils where carbon input is low, enzyme decomposition of organic matter may be inhibited by energy and substrate shortages [14].

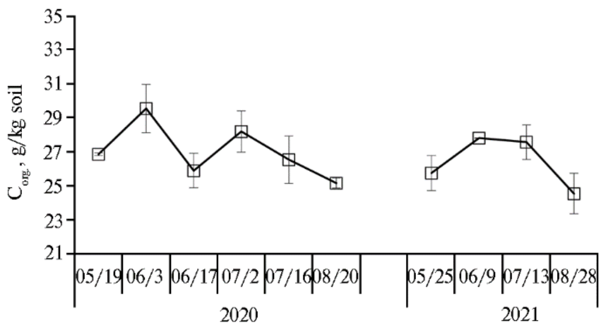

Fig. 3. Seasonal dynamics of total organic carbon content ($C_{org.}$) in agrosoddy-podzolic soil for two years (experimental station of the Agrophysical Research Institute, $n = 3$, *M*±SD; Menkovo village, Leningrad Province, Gatchina District).

In 2020, the amount of total organic carbon in the arable horizon was the largest in early June (29.54 g C/kg of soil) and minimum at the end of August (25.16 C/kg of soil) ($p < 0.70$) (Fig. 3). In mid-June, a 0.37% drop in $C_{org.}$ content occurred compared to the values at the beginning of the month, which is likely due to an increase in mineralization losses of organic carbon. During this period, the soil temperature was 21.5 °C, and the weight humidity was 8.2%.

It is known that the most complete decomposition of organic matter is observed at a soil temperature of 20-25 °C and a humidity of 60-80% of the total moisture capacity (48). However, soil temperature has a greater effect on mineralization losses than moisture. According to A.S. Tulina et al. [49], the potential mineralization of organic matter for gray forest soil, chernozem and dark chestnut soil during a 150-day experiment depended on temperature by 63% and on humidity by only 8%. Perhaps, in our case, in addition to optimal temperature for mineralization, there was also a sharp decrease in the microbial carbon content due to absence of fresh organic matter and low humidity, which affected the $C_{org.}$ content. E.O. Chimitdorzhieva et al. [50] also noted a decrease in the microbial carbon accumulation in chernozem from 0.62 to 0.52, in chestnut soil from 0.39 to 0.31 mg C/g of soil under moisture deficiency in combination with high air and soil temperatures.

In 2021, slightly lower values of the parameters were recorded, probably due to the lack of supply of fresh organic residues and the consumption of the labile part of soil carbon (light fraction, water-soluble organic matter) by soil microorganisms. In soil under pure fallow, the source of carbon for microorganisms is mainly difficult to decompose plant and microbial residues which are subjected to decomposition by natural factors and by the use of tillage tools [51]. In general, in 2021, almost the same trend in the dynamics of $C_{org.}$ remained, as in 2020. Statistically significant ($p < 0.05$) differences were observed between the values of $C_{org.}$ throughout the growing season.

In 2020, the total organic carbon reached its maximum content at a humidity of 16.6-22.3% and soil temperature of 10.6-18.0 °C, and amounted to 26.86-29.51 g C/kg soil. In 2021, the maximum $C_{org.}$, the 27.81 g C/kg of soil, was revealed at 22.3% humidity and 19.5 °C.

In 2020, the largest amount of organic carbon associated with the silt fraction was found in May ($p < 0.04$), 98.31 g C/kg fraction (Fig. 4). Soil samples collected at the end of August were characterized by the lowest average value of C_{silt}, 78.2 g C/kg fraction ($p < 0.04$).

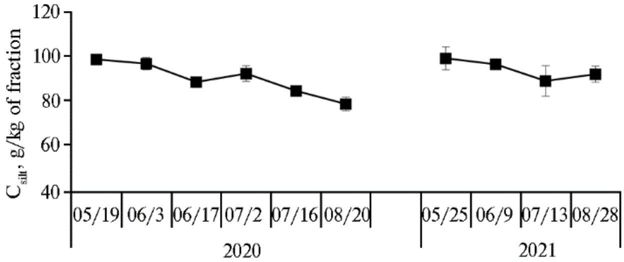

Fig. 4. Seasonal dynamics of silt associated carbon content (C_{silt}) in agrosoddy-podzolic soil for two years (experimental station of the Agrophysical Research Institute, $n = 3$, M±SD; Menkovo village, Leningrad Province, Gatchina District).

During the observation period, C_{silt} gradually decreased. In 2021, almost the same trend was noted, however, the difference between the C_{silt} values during the season was not significant ($p < 0.35$). However, the C_{silt} values in mid-July and at the end of August slightly exceeded the values in 2020 for the same period. Probably, in 2021, the formation of complexes of organic matter with minerals in the silt fraction of the soil occurred to a greater extent than in 2020.

The enrichment factor (E_{soc}) of the silt fraction with organic carbon varied from 3.66 at the beginning of the 2020 period to 3.11 at its end. In 2021, the E_{soc} value ranged from 3.84 to 3.22.

The highest content of silt-associated carbon was found at maximum soil moisture and minimum soil temperature. In 2020, the maximum accumulation of carbon in the silt fraction was recorded at 16.6-22.3% humidity and 10.6-18 °C, from 92 to 98 g C/kg of silt fraction. In 2021, the highest content of C_{silt} was found in soil samples with a moisture content of 16.2-22.3% at 17.3-23.7 °C, the C_{silt} values were comparable to thise in 2020. In 2020, the relationship between the deposited $C_{org.}$ and C_{silt} with soil temperature was significant and negative ($r = -0.43$ and $r = -0.73$, $p < 0.05$, respectively), in 2021 this relationship remained but was unreliable for $C_{org.}$ ($r = 0.38$) and significant for C_{silt} ($r = 0.76$, $p < 0.05$). Correlation analysis of the relationship between soil moisture and $C_{org.}$ and C_{silt} contents revealed significant moderate and strong positive relationships in 2020 ($r = 0.66$ and $r = 0.89$, $p < 0.05$) and a strong positive significant relationship for C_{silt} in 2021 ($r = 0.90$, $p < 0.05$), which is consistent with other reports [52].

Thus, the content of organic matter in agrosoddy-podzolic sandy loam soil and its silt fraction is affected by activity of polyphenol oxidases (PPOs) and peroxidases (POs), and by soil humidity and temperature. The activity of oxidases in 2021 was higher than in 2020, on average 5 times higher for polyphenol oxidases, and 3 times higher for peroxidases. The maximum content of total organic carbon during the entire observation period was recorded at the maximum activity of polyphenol oxidases, in 2020 with PPO activity of 1.27 mg purpur gallin $\cdot g^{-1} \cdot h^{-1}$, in 2021 with 5.9 mg purpur gallin $\cdot g^{-1} \cdot h^{-1}$ ($r = 0.48$ and $r = .84$, $p < 0.05$). In 2021, total organic carbon decreased by an average of 2% compared to 2020 ($p < 0.63$). However, the average values of the C_{silt} content over the same period increased by 4.78% ($p < 0.43$). As a result, in 2021 the enrichment coefficient of the silt fraction of the soil increased. The relationship between the activity of oxidases and the content of organic carbon associated with the silt fraction was complex, since the organic matter of the silt fraction is weakly susceptible to the influence of microorganisms and their metabolic products. Temperature and humidity had the greatest influence on the accumulation of carbon in the silt fraction compared to the deposition of total organic carbon. The relationship between soil moisture and the $C_{org.}$ and C_{silt} accumulation was stronger than the relationship between soil temperature and $C_{org.}$ and C_{silt} accumulation.

REFERENCES

1. Polyak Yu.M., Sukharevich V.I. *Agrokhimiya*, 2020, 3: 83-93 (doi: 10.31857/S0002188120010123) (in Russ.).
2. Bragazza L., Robroek B.J.M., Jassey V.E.J., Arif M.S., Marchesini R., Guglielmin M., Cannone N. Soil microbial community structure and enzymatic activity along a plant cover gradient in Victoria Land (continental Antarctica). *Geoderma*, 2019, 353: 144-151 (doi: 10.1016/j.geoderma.2019.06.033).
3. Raiesi F., Beheshti A. Soil specific enzyme activity shows more clearly soil responses to paddy rice cultivation than absolute enzyme activity in primary forests of northwest Iran. *Applied Soil Ecology*, 2014, 75: 63-70 (doi: 10.1016/j.apsoil.2013.10.012).
4. Fairbridge R.W., Beinroth F.H., Eswaran H., Reich P.F., Campbell G.S., Groenevelt P.H., Quiquampoix N. Enzymes and proteins, interactions with soil-constituent surfaces. In: *Encyclopedia of Soil Science. Encyclopedia of Earth Sciences Series*. Springer, Dordrecht, 2016, 210-216 (doi: 10.1007/978-1-4020-3995-9_189).
5. Hemingway J.D., Rothman D.H., Grant K.E., Rosengard S.Z., Eglinton T.I., Derry L.A., Galy V.V. Mineral protection regulates long-term global preservation of natural organic carbon. *Nature*, 2019, 570: 228-231 (doi: 10.1038/s41586-019-1280-6).
6. Min K., Freeman C., Kang H., Choi S.-U. The regulation by phenolic compounds of soil organic matter dynamics under a changing environment. *BioMed Research International*, 2015, 2015: 825098 (doi: 10.1155/2015/825098).
7. Wu G., Chen Z., Jiang D., Jiang N., Jiang H., Chen L. Oxidases and hydrolases mediate soil organic matter accumulation in chernozem of northeastern China. *Geoderma*, 2021, 403(1): 115206 (doi: 10.1016/j.geoderma.2021.115206).
8. Trubitsina L.I., Lisov A.V., Belova O.V., Trubitsin I.V., Demin V.V., Konstantinov A.I., Zavarzina A.G., Leontievsky A.A. Transformation of low molecular compounds and soil humic acid by two domain laccase of *Streptomyces puniceus* in the presence of ferulic and caffeic acids. *PLoS ONE*, 2020, 15(9): e0239005 (doi: 10.1371/journal.pone.0239005).
9. Bach C.E., Warnock D.D., Horn D.J.V., Weintraub M.N., Sinsabaugh R.L., Allison S.D., German D.P. Measuring phenol oxidase and peroxidase activities with pyrogallol, L-DOPA, and ABTS: Effect of assay conditions and soil type. *Soil Biology & Biochemistry*, 2013, 67: 183-191 (doi: 10.1016/j.soilbio.2013.08.022).
10. Kawana H., Miwa T., Honda Y., Furuya T. Sustainable approach for peroxygenase-catalyzed oxidation reactions using hydrogen peroxide generated from spent coffee grounds and tea leaf residues. *ACS Omega,* 2022, 7(23): 20259-20266 (doi: 10.1021/acsomega.2c02186).
11. Burns R.G., DeForest J.L, Marxsen J., Sinsabaugh R.L, Stromberger M.E., Wallenstein M.D., Weintraub M.N., Zoppini A. Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biology and Biochemistry*, 2013, 58: 216-234 (doi: 10.1016/j.soilbio.2012.11.009).
12. Kivlin S.N., Treseder K.K. Soil extracellular enzyme activities correspond with abiotic factors more than fungal community composition. *Biogeochemistry*, 2014, 117: 23-37 (doi: 10.1007/s10533-013-9852-2).
13. Yang Y., Fang H., Cheng S., Xu L., Lu M., Guo Y., Li Y., Zhou Y. Soil enzyme activity regulates the response of soil C fluxes to N fertilization in a temperate cultivated grassland. *Atmosphere*, 2022, 13(5): 777 (doi: 10.3390/atmos13050777).
14. Cenini V.L., Fornara D.A., McMullan G., Ternan N., Carolan R., Crawley M.J., Clément J.C., Lavorel S. Linkages between extracellular enzyme activities and the carbon and nitrogen content of grassland soils. *Soil Biology and Biochemistry*, 2016, 96: 198-206 (doi: 10.1016/j.soilbio.2016.02.015).
15. Sinsabaugh R.I. Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology & Biochemistry*, 2010, 42(3): 391-404 (doi: 10.1016/j.soilbio.2009.10.014).
16. Zavarzina A.G. Heterophase synthesis of humic acids in soils by immobilized phenol oxidases. In: *Soil enzymology. Soil biology*, vol. 22. G. Shukla, A. Varma (eds.). Springer, Berlin, Heidelberg, 2011: 187-205 (doi: 10.1007/978-3-642-14225-3_10).
17. Hong H., Chen Sh., Fang Q., Algeo T. J., Zhao L. Adsorption of organic matter on clay minerals in the Dajiuhu peat soil chronosequence, South China. *Applied Clay Science*, 2019, 178: 105125 (doi: 10.1016/j.clay.2019.105125).
18. Boytsova L.V., Zinchuk E.G., Neprimerova S.V. *Problemy agrokhimii i ekologii*, 2018, 3: 45-50 (in Russ.).
19. Zhou W., Han G., Li M., Zeng J., Liang B., Liu J., Qu R. Determining the distribution and interaction of soil organic carbon, nitrogen, pH and texture in soil profiles: a case study in the Lancangjiang River Basin, Southwest China. *Forests*, 2020, 11(5): 532 (doi: 10.3390/f11050532).
20. Chang R., Jin T., Lü Y., Liu G., Fu B. Soil carbon and nitrogen changes following afforestation of marginal cropland across a precipitation gradient in loess plateau of China. *PLoS ONE*, 2014, 9(1): e85426 (doi: 10.1371/journal.pone.0085426).
21. Khormali F., Kehl M. Micromorphology and development of loess-derived surface and buried soils along a precipitation gradient in Northern Iran. *Quaternary International*, 2011, 234(1-2): 109-123 (doi: 10.1016/j.quaint.2010.10.022).

22. Klopfenstein S.T., Hirmas D.R., Johnson W.C. Relationships between soil organic carbon and precipitation along a climosequence in loess-derived soils of the Central Great Plains, USA. *Catena*, 2015, 133: 25-34 (doi: 10.1016/j.catena.2015.04.015).
23. Saiz G., Bird M.I., Domingues T., Schrodt F., Schwarz M., Feldpausch T.R., Veenendaal E., Djagbletey G., Hien F., Compaore H., Diallo A., Lloyd J., Variation in soil carbon stocks and their determinants across a precipitation gradient in West Africa. *Global Change Biology*, 2012, 18(5): 1670-1683 (doi: 10.1111/j.1365-2486.2012.02657.x).
24. Zhou T., Lv Y., Xie B., Xu L., Zhou Y., Mei T., Li Y., Yuan N., Shi Y. Topography and soil organic carbon in subtropical forests of China. *Forests*, 2023, 14(5): 1023 (doi: 10.3390/f14051023).
25. Li G., Kim S., Han S. H., Chang H., Dub D., Son Y. Precipitation affects soil microbial and extracellular enzymatic responses to warming. *Soil Biology and Biochemistry*, 2018, 120: 212-221 (doi: 10.1016/j.soilbio.2018.02.014).
26. Mazzon M., Cavani L., Margon A., Sorrenti G., Ciavatta C., Marzadori C. Changes in soil phenol oxidase activities due to long-term application of compost and mineral N in a walnut orchard. *Geoderma*, 2018, 316: 70-77 (doi: 10.1016/j.geoderma.2017.12.009).
27. Nannipieri P., Trasar-Cepeda C., Dick R.P. Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. *Biology and Fertility of Soils*, 2018, 54(1): 11-19 (doi: 10.1007/s00374-017-1245-6).
28. Jia X.Y., Zhong Y.Q.W., Liu J., Zhu G.Y., Shangguan Z.P., Yan W.M. Effects of nitrogen enrichment on soil microbial characteristics: from biomass to enzyme activities. *Geoderma*, 2020, 366: 114256 (doi: 10.1016/j.geoderma.2020.114256).
29. Liu S., Hu R., Zhao J., Brüggemann N., Bol R., Cai G., Shaaban M. Flooding effects on soil phenol oxidase activity and phenol release during rice straw decomposition. *Journal of Plant Nutrition and Soil Science*, 2014, 177(4): 541-547 (doi: 10.1002/jpln.201300356).
30. Esiana B., Coates C.J., Adderley W.P., Berns A.E., Bol R. Phenoloxidase activity and organic carbon dynamics in historic Anthrosols in Scotland, UK. *PLoS ONE*, 2021, 16(10): e0259205 (doi: 10.1371/journal.pone.0259205).
31. Rastvorova O.G., Andreev D.P., Gagarina E.I., Kasatkina G.A., Fedorova N.N. *Khimicheskiy analiz pochv* [Chemical analysis of soils]. St. Petersburg, 1995 (in Russ.).
32. Boytsova L.V., Neprimerova S.V., Zinchuk E.G. *Agrofizika*, 2019, 4: 1-8 (doi: 10.25695/AGRPH.2019.04.01) (in Russ.).
33. Christensen B.T. Physical fractionation of soil and organic matter in primary particle size and density separates. In: *Advances in Soil Science. Advances in Soil Science*, vol. 20. B.A. Stewart (ed.). Springer, New York, NY, 1992, 20(1): 90 (doi: 10.1007/978-1-4612-2930-8_1).
34. *Metodicheskie ukazaniya po provedeniyu issledovaniy v dlitel'nykh opytakh s udobreniyami. ch. 2* /Pod obshchey redaktsiey V.D. Pannikova [Guidelines for conducting research in long-term experiments with fertilizers, part 2. V.D. Pannikov (ed.)]. Moscow, 1983 (in Russ.).
35. Kovrigo V.P., Kaurichev I.S., Burlakova L.M. *Pochvovedenie s osnovami geologii* [Soil science with basic geology]. Moscow, 2000 (in Russ.).
36. Chunderova A.I. *Pochvovedenie*, 1970, 7: 22-26 (in Russ.).
37. Gorbunov N.I. *Mineralogiya i kolloidnaya khimiya pochv* [Mineralogy and colloidal chemistry of soils]. Moscow, 1974 (in Russ.).
38. Fomina M., Skorochod I. Microbial interaction with clay minerals and its environmental and biotechnological implications. *Minerals*, 2020, 10(10): 861 (doi: 10.3390/min10100861).
39. Boytsova L.V., Neprimerova S.V., Zinchuk E.G. *Rossiyskaya sel'skokhozyaystvennaya nauka*, 2023, 1: 23-27 (in Russ.).
40. Rizhiya E.Ya., Boitsova L.V., Vertebniy V.E., Horak J., Moskvin M.A., Dubovitskaya V.I., Khomyakov Yu.V. Effect of biochar application on variability of the polyphenoloxidase and peroxidase activity of sod-podzolic soil under low andhighfertility. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2022, 57(3): 476-485 (doi: 10.15389/agrobiology.2022.3.476eng).
41. Petrova Z.M., Zuev V.S., Boytsova L.V., Rizhiya E.Ya., Bodrov V.A. V sb.: *Fizicheskie, khimicheskie i klimaticheskie faktory produktivnosti poley. AFI — 75 let* /Pod redaktsiey A.M. Globusa [In: Physical, chemical and climatic factors of field productivity. AFI — 75 years. A.M. Globus (ed.)]. St. Petersburg, 2007: 198-203 (in Russ.).
42. Jezierska-Tys S., Wesołowska S., Gałązka A., Joniec J., Bednarz J., Cierpiała R. Biological activity and functional diversity in soil in diferent cultivation systems *International Journal of Environmental Science and Technology*, 2020, 17: 4189-4204 (doi: 10.1007/s13762-020-02762-5).
43. Zolkina E.I. *Vladimirskiy zemledelets*, 2020, 4: 7-13 (doi: 10.24411/2225-2584-2020-10138) (in Russ.).
44. Semenov M.V., Nikitin D.A., Stepanov A.L., Semenov V.M. *Pochvovedenie*, 2019, 3: 355-369 (doi: 10.1134/S0032180X19010131) (in Russ.).
45. Dehtiarova Z. The effect of short-term crop rotation with different proportions of sunfl ower on cellulolytic activity of the soil. *Soil Science Annual*, 2022, 73(4): 156097 (doi: 10.37501/soilsa/156097).
46. Gordeeva T.K., Novoselov S.I. *Sel'skokhozyaystvennye nauki i APK na rubezhe vekov*, 2014, 5: 81-84 (in Russ.).
47. Kurganova I.N., Telesnina V.M., Lopes de Gerenyu V.O., Lichko V. I., Ovsepyan L.A. Changes in

the carbon stocks, microbial and enzyme activities of Retic Albic Podzol in southern taiga during postagrogenic evolution. *Eurasian Soil Science*, 2022, 55: 895-910 (doi: 10.1134/S1064229322070079).
48. Marchik T.P. Efremov A.L. *Pochvovedenie s osnovami rastenievodstva: uchebnoe posobie* [Soil science with basics of crop production: textbook]. Grodno, 2006 (in Russ.).
49. Tulina A.S., Semenov V.M. *Pochvovedenie*, 2015, 8: 952-962 (doi: 10.7868/S0032180X15080109) (in Russ.).
50. Chimitdorzhieva E.O., Chimitdorzhieva G.D. *Vestnik KGU*, 2012, 18(3): 16-20 (in Russ.).
51. Semenov V.M., Kogut B.M. *Pochvennoe organicheskoe veshchestvo* [Soil organic matter]. Moscow, 2015 (in Russ.).
52. Kerr D.D., Ochsner T.E. Soil organic carbon more strongly related to soil moisture than soil temperature in temperate grasslands. *Soil Sci. Soc. Am. J.*, 2020, 84(2): 587-596 (doi: 10.1002/saj2.20018).