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SOIL BIOLOGY

Temperature Dependence of the Activity of Polyphenol Peroxidases and Polyphenol Oxidases in Modern and Buried Soils

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Abstract—Under conditions of the global climate warming, the changes in the reserves of soil humus depend on the temperature sensitivities of polyphenol peroxidases (PPPOs) and polyphenol oxidases (PPOs). They play an important role in lignin decomposition, mineralization, and humus formation. The temperature dependence of the potential enzyme activity in modern and buried soils has been studied during incubation at 10 or 20°C. The experimental results indicate that it depends on the availability of the substrate and the presence of oxygen. The activity of PPOs during incubation in the absence of oxygen for two months decreases by 2–2.5 times, which is balanced by an increase in the activity of PPPOs by 2–3 times. The increase in the incubation temperature to 20°C and the addition of glucose accelerates this transition due to the more abrupt decrease in the activity of PPOs. The preincubation of the soil with glucose doubles the activity of PPPOs but has no significant effect on the activity of PPOs. The different effects of temperature on two groups of the studied oxidases and the possibility of substituting enzymes by those of another type under changing aeration conditions should be taken into consideration in predicting the effect of the climate warming on the mineralization of the soil organic matter. The absence of statistically significant differences in the enzymatic activity between the buried and modern soil horizons indicates the retention by the buried soil of some of its properties (soil memory) and the rapid restoration of high enzymatic activity during the preincubation.

Keywords: global climate change, anaerobic conditions, soil temperature, enzymatic activity of soil
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INTRODUCTION

Extracellular soil enzymes perform the oxidation and hydrolysis of complex organic compounds and control the balance between the decomposition of plant residues arriving into the soil and the formation of stable humic compounds. The activity of extracellular enzymes determines the formation rate of dissolved organic compounds available to soil microorganisms. It is believed that this is the limiting step in the chain of biochemical transformations resulting in the mineralization of soil organic matter to CO_2 [5, 9].

The extracellular hydrolytic enzymes participating in the degradation, transformation, and mineralization of soil organic matter have been studied in considerable detail. In particular, thorough studies deal with the activities of cellulases, phosphatases, and other hydrolases [6], and the response of the soil enzyme system to changes in the physicochemical properties of soils and temperature is known in many cases [7, 13]. On the contrary, phenol oxidases and peroxidases, whose activities frequently do not correlate with the activity of hydrolases, were studied in few works [12]. An important role in the formation of humic substances resistant to degradation is assigned to polyphenol peroxidases (PPPOs) and polyphenol oxidases (PPOs) involved in the transformations of aromatic compounds. They catalyze the oxidation of mono-, di-, and triphenols to quinones in the presence of air oxygen or hydrogen peroxide [3]. Under the corresponding conditions, the condensation of quinones with amino acids and peptides yields primary molecules of protohumic acids; this immobilizes carbon in the soil humus and hampers carbon accumulation in the form of carbon dioxide in the atmosphere. Fungi, some bacteria, and archaea form laccases and lignin peroxidases, which participate in the decomposition of lignin in the soil. The synthesis of organic molecules participating in humus formation (melanin-like substances in fungi and lignin in plants) involves phenol oxidases. Thus, phenol oxidases and peroxidases are responsible for several important processes at the ecosystem level: the mineralization of lignin and the synthesis of secondary compounds participating in humification. At the same time, the high activity of oxidases

Table 1. Chemical properties of the gray forest soil

Horizon	Depth, cm	Humus	N	pH_{water}
		%		
A	1–11	4.06	0.14	5.75
Bt1	24–38	0.73	0.02	5.44
[A]	83–96	0.62	0.15	7.17
[Bt1]	109–130	0.33	0.07	7.14

increases the mineralization rate of organic matter and decreases its content in the soil.

Phenol oxidases and peroxidases are less stable in the environment than extracellular hydrolases [12]. The high variability of these enzymes in time and space hampers the formation of direct connections of its activity with the soil properties and environmental factors. Therefore, the determination of the temperature sensitivity of these enzymes is an important problem in terms of the forhtcoming climate changes and the increasing risk of the accelerated mineralization of soil organic matter, which results in the emission of CO_2. For the justified assessment of the changes in the mineralization of soil organic matter under increasing temperature, the significant amount of carbon (roughly estimated as more than 50% [4]) contained in the soil horizons below 20–30 cm should be considered. Of special interest is the comparison of modern and fossil (buried) soils. Some buried horizons formed in the 19th century at the latter end of the cooling period (Little Ice Age) in Europe are characterized by their high content of organic matter, which can be mineralized under climate change [1, 11]. The determination of the temperature sensitivity of enzymes participating in the mineralization and transformation of organic substances in different horizons of the soil profile is necessary for the simulation and prediction of these processes under global climate warming. It is important to determine the activity of the studied enzymes in microzones rich in organic matter (rhizosphere, invertebrate intestines), where it can reach the maximum levels, as well as its temperature sensitivity. The contribution of the enzyme activity from these hot spots to the total CO_2 flux from the soil is crucial for the assessment of the warming effect on the mineralization of the organic matter. Therefore, this work involved experimental treatments with the addition of glucose and the incubation of samples in sealed vials for simulating the microaerophilic conditions developed in animal intestines and the plant rhizosphere with intensive root respiration.

The aim of the work was to study the activity of PPPOs and PPOs in modern and buried soils at different temperatures during an incubation experiment with a native soil without addition of a substrate and a soil with glucose added.

MATERIALS AND METHODS

The enzymatic activity and its temperature sensitivity were determined in samples taken from a soil profile established on a reclamation mound near the Experimental Field Station of the Institute of Physicochemical and Biological Problems of Soil Science of the Russian Academy of Sciences (Serpukhov district, Moscow oblast) built up by A.T. Bolotov about 200 years ago (S.V. Gubin, personal communication). The profile includes a modern gray forest soil (A and Bt1 horizons) and a gray forest soil buried since 100 years ([A] and [Bt1] horizons) (Table 1). The dynamics of the enzymatic activity under incubation conditions after the soil wetting to 60% of the field capacity were studied in a laboratory experiment at two temperatures: 10°C (the present mean annual soil temperature) and 20°C. An increase of the temperature by 10°C was used to obtain a contrasting effect under the model experimental conditions. To simulate the input of available organic matter into the soil (e.g., with root exudates and from invertebrate intestines), a treatment with the addition of glucose was used.

Soil samples (5 g) were sieved (<2-mm) and placed in glass beakers of 15 mL and preincubated at 10 or 20°C for 7 days; water or glucose at a rate of 4 mg/g soil was then added for the A horizon of the modern soil and at a rate of 2 mg/g soil for the B horizon and buried soils, and the incubation was continued. The vials with the soil were covered with rubber stoppers and incubated without ventilation. Thus, the experimental conditions corresponded to the aeration features in the lower soil horizons (including the buried horizons), where the content of oxygen in the soil air is lower than in the atmospheric air by an order of magnitude. On the first, third, and 60th days, the soil from several vials was used for destructive analysis and the determination of the enzymatic activity after incubation at 20 or 10°C.

The activities of the PPPOs and PPOs in the soils were determined at 20°C according to the described procedure [8]. Tetramethylbenzidine was used as a substrate for the enzymatic reactions. The formation of the reaction product was detected colorimetrically at 620 nm on a Tecan Sunrise ELISA reader.

Data analysis and statistics. The input and significance of each studied parameter (the soil preincubation temperature, the profile distribution of the samples, the soil incubation time, and the addition of available organic matter) to the variability of enzy-

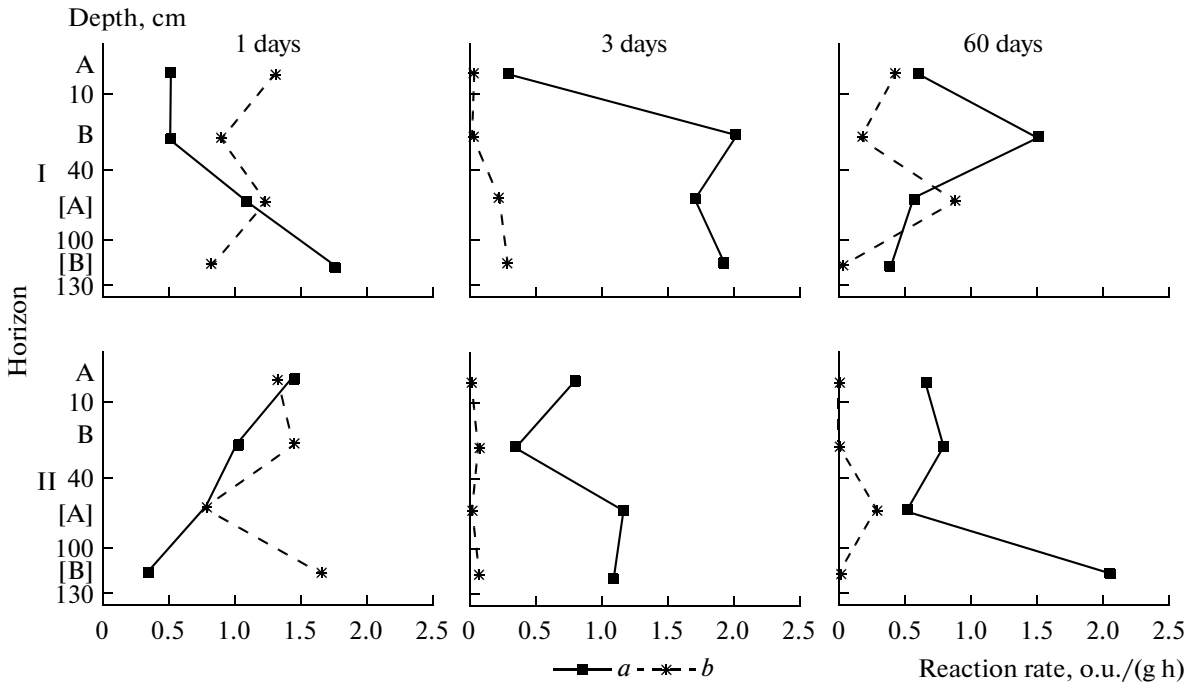

Fig. 1. Dynamics of the PPO activity in the horizons of the gray forest soils during incubation under laboratory conditions (I) without glucose and (II) with glucose. Here and below, the preincubation temperature is as follows: (*a*) 10°C; (*b*) 20°C. The reaction rate is given in optical units per g of soil per hour (o.u./(g h)).

matic activity was examined using multifactor analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The activity of the PPPOs exceeded that of the PPOs in almost all the soil horizons throughout the experiment (Figs. 1 and 2). The significance of the differences is confirmed by the four-factor analysis of the variance (Figs. 3 and 4): the total contribution of the considered factors to the general variance of the oxidation rates was 76% for the PPOs and 79% for the PPPOs (Table 2). This tendency observed under the given experimental conditions can indicate the predominance of anaerobic polycondensation of aromatic compounds to humic substances, because the functioning of the PPPOs in the soil does not require aerobic conditions.

Activity of polyphenol oxidases. The statistically significant effect of the preincubation temperature was observed for the PPOs from the third day of the experiment and was manifested as a significant decrease in the activity: the increase of the preincubation temperature from 10 to 20°C resulted in the reduction of the potential rate of the enzymatic reaction by 10 times on the average (Fig. 3). For the PPOs, the preincubation temperature was the most significant factor, which made the largest contribution (18%) to the general variance of the enzymatic reaction rates (Table 2). It can be supposed that, at the low preincubation temperature (10°C), the substrate available to the microorganisms is slowly consumed; therefore the level of the microbial biomass and the activity of the PPOs remain at a high level. The preincubation of the soil at 20°C apparently results in the more rapid exhaustion of the available substrates and oxygen and a decrease in the PPO pool; therefore, the potential activity of the enzymes determined after the incubation is significantly lower than that for the soil incubated at 10°C. The incubation time is the second significant factor controlling the activity of the enzymes. The contribution of this factor to the total variability is 14%. The enzymatic activity of the PPOs in the soil incubated at 20°C statistically significantly decreased by 10 times for 2 months (Fig. 3). The interaction between the temperature and time factors was also significant (Table 2): the differences in the temperature sensitivity of the PPOs were remarkable already on the third day of the incubation. The increase of the soil preincubation temperature from 10 to 20°C decreased the activity of the PPOs by 10 times already on the third day. The preincubation at 20°C could result in the rapid exhaustion of the available substrates and oxygen and a decrease in the activity of the microorganisms producing this type of enzymes.

The allocation of the sample in the profile (depth) and the addition of glucose has no statistically signifi-

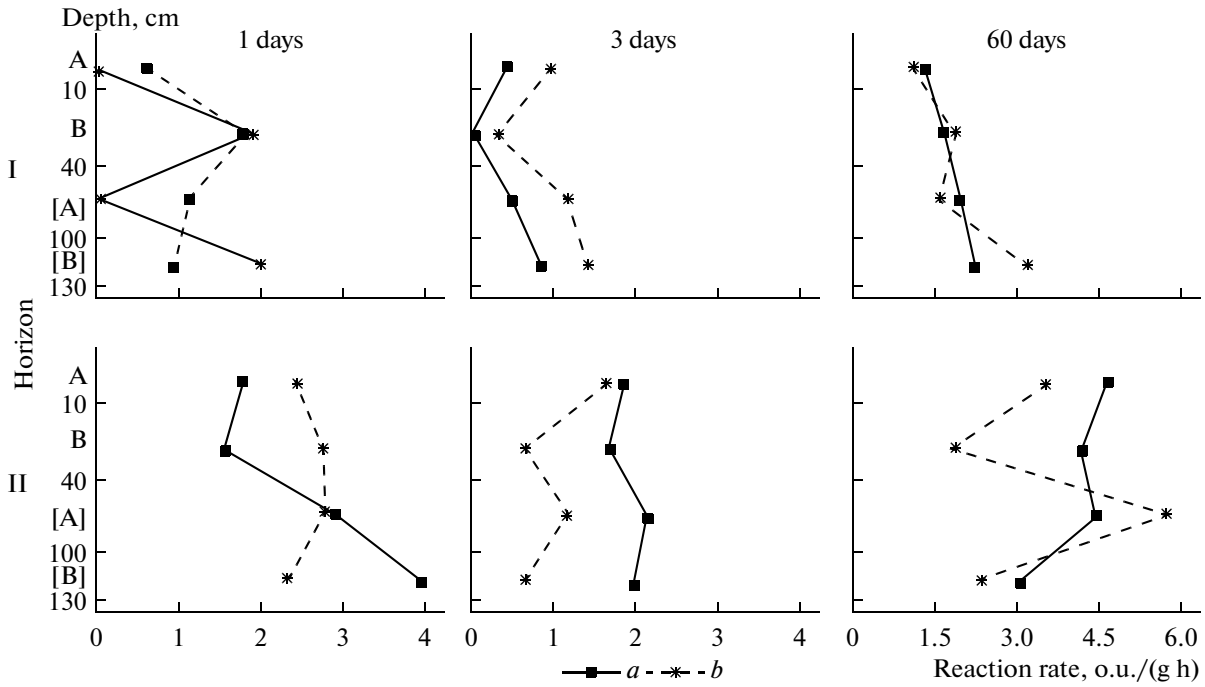

Fig. 2. Dynamics of the PPPO activity in the horizons of gray forest soils during incubation under laboratory conditions (I) without glucose and (II) with glucose.

cant effect on the enzymatic activity of the PPOs (Table 2).

Activity of polyphenol peroxidases. The effect of the temperature on the activity of the PPPOs was insignificant, unlike the activity of the PPOs. The incubation time was the most significant factor for the PPPOs; its contribution to the total variance was 31% (Table 2). Unlike the activity of the PPOs decreasing with time, the activity of the PPPOs increased by 2–3 times on the average during two months of incubation both in the presence and absence of glucose. The addition of glucose (an available substance) was a significant factor determining the activity of the PPPOs; its contribution to the total variance was 31%. The effect of the glucose addition was observed on the first and 60th days of the incuba-

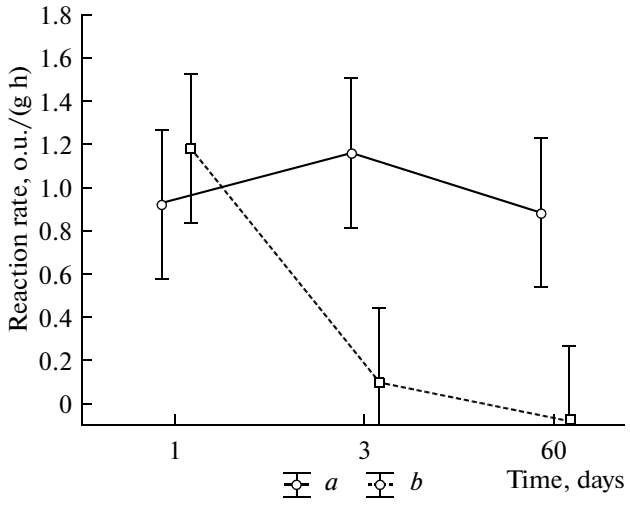

Fig. 3. Effect of the temperature and incubation time on the activity of the PPOs from the analysis of the variance (the confidence intervals are given).

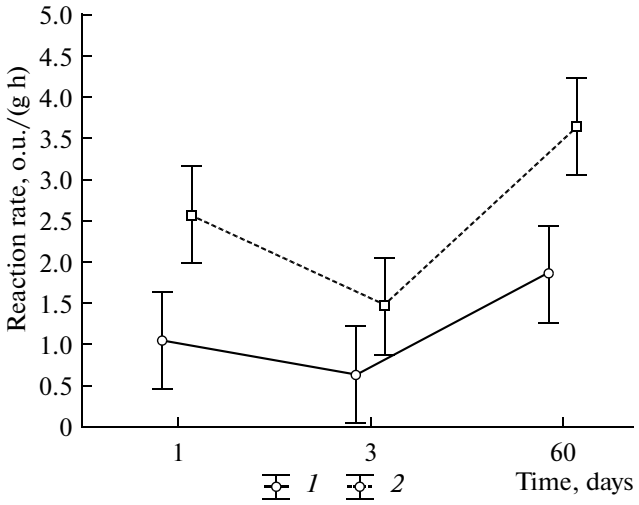

Fig. 4. Effect of glucose ((*1*) without glucose; (*2*) with glucose) and incubation time on the activity of the PPPOs (confidence intervals are given) from the analysis of the variance.

Table 2. Contributions of the studied factors and their combinations to the total variance of the enzymatic reaction rates for the PPOs and PPPOs from the analysis of the variance

Factor	Sum of the squares, *SS*	Degrees of freedom, *df*	Contribution to the variance, %	Fischer *t*-test, *F*	Statistical significance level, *p*
PPOs					
Total	23.31	47	100		
Time	**3.47**	**2**	**14.91**	**7.60**	**0,00**
Burial	0.43	1	1.85	1.75	0,20
Glucose	0.28	1	1.21	1.24	0.28
Temperature	**4.21**	**1**	**18.06**	**18.48**	**0.00**
Time + burial	0.27	2	1.14	0.58	0.57
Time + glucose	0.38	2	1.65	0.84	0.44
Burial + glucose	0.09	1	0.39	0.02	0.89
Time + temperature	**4.34**	**2**	**18.63**	**9.53**	**0.00**
Burial + temperature	0.06	1	0.26	0.16	0.69
Glucose + temperature	0.05	1	0.21	0.00	1.00
Time + burial + glucose	**1.94**	**2**	**8.31**	**4.25**	**0.03**
Time + burial + temperature	0.44	2	1.87	0.96	0.40
Time + glucose + temperature	1.14	2	4.90	2.51	0.10
Burial + glucose + temperature	0.13	1	0.56	0.57	0.46
Time + burial + glucose + temperature	0.42	2	1.79	0.92	0.41
Fraction of the variance unexplained by all the selected factors	5.66	24	24.29		
PPPOs					
Total	74.23	47	100.00		
Time	**22.80**	**2**	**30.72**	**17.17**	**0.00**
Burial	0.84	1	1.13	1.26	0.27
Glucose	**22.86**	**1**	**30.80**	**34.42**	**0.00**
Temperature	0.28	1	0.38	0.43	0.52
Time + burial	1.00	2	1.35	0.75	0.48
Time + glucose	1.95	2	2.62	1.47	0.25
Burial + glucose	0.02	1	0.03	0.04	0.85
Time + temperature	0.11	2	0.15	0.08	0.92
Burial + temperature	0,39	1	0.53	0.59	0.45
Glucose + temperature	1.54	1	2.08	2.32	0.14
Time + burial + glucose	0.90	2	1.21	0.68	0.52
Time + burial + temperature	1.88	2	2.53	1.41	0.26
Time + glucose + temperature	1.56	2	2.10	1.17	0.33
Burial + glucose + temperature	0.22	1	0.30	0.33	0.57
Time + burial + glucose + temperature	1.93	2	2.60	1.45	0.25
Fraction of the variance unexplained by all the selected factors	15.94	24	21.47		

The significant factors at $p < 0.05$ are shown in bold.

tion (Fig. 4). The rate of the enzymatic reaction increased by about 2–2.5 times.

According to the obtained results, the following mechanism can be proposed for the changes in the activity of the PPPOs in the soil during the incubation. The addition of glucose activated the respiration of the microorganisms and the consumption of oxygen. The vials were not ventilated, which created anaerobic or microaerophilic conditions in microcosms. The anaerobic conditions and the presence of an available substrate (glucose) could favor the synthesis of PPPOs by microorganisms. The addition of a carbon source, along with the effect on the oxygen consumption rate, could increase the biomass of the microorganisms producing PPPOs. This phenomenon can be observed both in an undisturbed soil after the input of an available substrate under field conditions and in invertebrate intestines. An abrupt change of the aerobic conditions to anaerobic conditions can occur in some microzones because of the high respiration activity and the limitation of oxygen diffusion. This can result in changes in the type of phenol oxidase activity.

Redundancy of the activity of oxidative enzymes under varying incubation conditions. Under the conditions of our experiment, the incubation time was the most significant factor determining the activity of the oxidative enzymes. It apparently affected the development of the microbial succession and the activity of the produced enzymes. For the PPOs, the long-term incubation and the conjugated succession of microorganisms decreased the activity of the enzyme by an order of magnitude, while the air tight incubation, especially at the addition of glucose, stimulated the microbial production of PPPOs, whose activity significantly increased during the experiment. The experimental design did not included the measurements of the concentration of oxygen in the vials; however, we observed a decrease in the soil pH from 8 to 4 in samples from the plow horizon of a calcareous ordinary chernozem at the addition of glucose under similar incubation conditions, which indicated the active formation of organic acids because of fermentation under an oxygen deficiency.

Thus, the differences in the enzyme activity dynamics between the PPOs and PPPOs are apparently related to the changes in the aerobes/anaerobes ratio in the microbial community with time. The rise of the temperature by 10°C increased the observed effect due to the intensified oxygen consumption by the more active microorganisms. The comparison of the dynamic changes in the activities of the PPOs and PPPOs showed that the transition from aerobic to anaerobic conditions during the experiment resulted in the substitution of the activity of an oxidizing enzyme type (PPO) by the activity of another enzyme type (PPPO) acting under an oxygen deficiency in the soil. In other words, the general principle of redundancy inherent to the microbial community of the soil was confirmed experimentally [10, 14]. According to the principle of redundancy, when the biomass and activity of a part of the microbial (or enzymatic) pool performing an ecologically significant function (in our case, the oxidation and transformation of phenolic compounds) decrease, other microorganisms and enzymes produced by them occupy the place left empty by increasing their biomass (activity). As a result, the total homeostasis of the soil (process equilibrium) persists, because the oxidative activity is realized under changed conditions due to the substitution of one enzyme type for another.

Changes in the phenol oxidase activity along the soil profile. Assessing the general effect of the location of the soil sample on the enzymatic activity, we should note that no significant differences in the activity of both phenol oxidase types were found between the humus-accumulative and the illuvial horizons of the studied soils (Table 2). However, it can be seen (Figs. 1 and 2) that, for some incubation times, the activity of the enzymes in both the modern and buried humus-accumulative horizons is higher than in the illuvial horizons. The analysis of the variance also shows a reliable effect of the interacting factors (the soil horizon and incubation time) for the PPOs (Table 2). The absence of significant differences in the enzymatic activity between the buried and modern horizons of the soil indicates the rapid restoration of high enzymatic activity during the preincubation. Another interpretation of the revealed tendency is also possible: the long-term storage of dry samples and their preparation for the experiment (grinding and sieving) could level the initial differences in their enzymatic activities, as was shown, e.g., by Zhuravleva et al. [2].

CONCLUSIONS

The temperature sensitivity of the potential activities of the PPOs and PPPOs is determined by the physicochemical conditions, i.e., the availability of a substrate and the presence of oxygen, which dynamically change during the incubation experiment. The activity of the PPOs, which decreased by 2–2.5 times during the two-month incubation, was balanced by the activity of the PPPOs, which increased by 2–3 times. The increase of the incubation temperature by 10°C accelerated this transition due to the more abrupt decrease in the activity of the PPOs. The change in the PPPO activity with the temperature was statistically insignificant. These results emphasize the importance of the phenol oxidase activity under climate warming. The increase of the temperature in combination with improved aeration, e.g., at the lowering of the water table in peat soils, can abruptly accelerate the oxida-

tion of organic substances and result in the loss of soil humus.

The observed absence of a significant effect of the burial on the enzymatic activity of PPOs and PPPOs indicates the partial retention of biological activity in the buried soil (the soil memory). This confirms the hazard of decreasing the soil humus reserves not only in the upper but also in the lower and buried horizons under the global climate warming.

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