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EXPERIMENTAL ARTICLES

The Effect of Pyrogenically Modified Substrates on Mineralizing Activity and Growth Strategies of Microorganisms of Grey Forest Soil

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Abstract—The rates of the mineralization processes initiated by the input of plant residues and pyrogenically modified plant material into gray forest soil under forests and meadows were assayed. While meadow plant residues was mineralized more rapidly than the forest floor, decomposition of the pyrogenic material resulted in disproportional changes in CO_2 emission from soils. Statistical treatment showed that the respiratory activity of CO_2 emission by heterotrophic microorganisms, which is a physiological characteristic of microbial communities, is 89% determined by the substrate quality. The maximal specific growth rate, which reflects the functional changes in microbial communities, was affected by the cenosis (36%) and the substrate (30%). Most of the carbon of the original plant material (up to 90%) was removed during the burning of plant substrates. The remaining compounds in the pyrogenically transformed material changed the process of mineralization in soil compared both to the control variant and to soil enriched with plant residues. Input of plant residues and ash into the soil resulted in increased total and active biomass, while the maximal specific growth rate decreased and the generation time for the active biomass increased. In the case of soils with plant residues, these changes in the state of microbial communities were brief and occurred during the period of intense mineralization (0–5 days), while, in soils with plant ash, stable changes were revealed after more prolonged incubation. Experimental determination of the microbial biomass turnover time (MTT) by means of two methods (from the ratio between the microbial biomass and respiration and from microbial specific growth rates) made it possible to determine the economical coefficient Y for microbial communities metabolizing the substrates of different availability. Depending on the experimental variant, the Y values varied from 0.22 to 0.51. Decreased maximal specific growth rate and increased values of Y (the coefficient of efficiency of substrate utilization) showed the predominant contribution of K-strategists in the mineralization of low available substrates in soil. The balance calculations and physiological characteristics of the microbial community suggested that the priming effect was most probable in soils enriched with plant ash.

Keywords: CO_2 emission, microbial growth kinetics, mineralization of organic matter, microbial biomass.

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In the last few decades, fires have become one of the major factors disrupting soil ecosystems [1]. An anthropogenic or natural pyrogenic factor results in a disrupted structure of terrestrial biocenoses [2]. The ecological consequences of fires in biocenoses depend on their type (crown or ground fire), intensity, frequency, season, etc.

In many regions of Russia, seasonal grass burning is carried out in order to remove last year's plant residues and stimulate plant growth on pastures. Mass burnings begin in early spring after melting of the snow and continue for three to four weeks prior to plant vegetation. In early spring, grass burnings cover greater areas than do peat and forest fires and spread more rapidly. Since fires of high and medium intensity cause the most pronounced changes in microbial communities [3], little attention is paid to the effect of grass burnings, which do not result in heat treatment of the soil; partially remove (burn) vegetation, plant residues, and litter; and enrich the soil with pyrogenically transformed plant material. However, the effect of low-intensity fires changing the state of mineral nutrients in the upper soil horizons may be significant. Independently of the intensity and duration of the burnings, during the first year thereafter, negative changes in the structure and biochemical and functional activity of soil microbial communities were found to be aftereffects [2]. The activity of soil microorganisms metabolizing the carbon of soil organic matter (SOM) is among the major factors determining CO_2 balance in the atmosphere [4, 5]. The microbial mineralizing activity depends, in turn, on the quantity and availability of organic compounds arriving into soil with plant residues. An increased rate of microbial respira-

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tion in after-fire soils results from active mineralization of pyrogenically transformed organic matter of plant, animal, and microbial origin, together with leaching of pyrolysis products into the deeper soil horizons [2]. The effect of fire causes transformations in the plant material, changing its quality and availability. After a low-intensity fire, the structure of the compounds in plants that were killed by fire but did not burn was shown to have a higher aromatics content. Other authors have shown that pyrogenic coal after low-intensity fires resulting in incomplete combustion of organic matter contains compounds with functional groups originating from polysaccharides, lignin, aliphatic, or heterocyclic compounds [6, 7].

The input of the products of incomplete combustion of plant residues into soil may enhance the mineralization of soil organic matter, causing the priming effect [8]. This intensification of mineralization results in increased CO_2 levels in the atmosphere. Formation of the negative carbon balance in the soil and rejuvenation of soil humus are among the possible results of the priming interactions [10].

Grass burnings often affect adjacent forest areas, causing burnings out of the forest litter. The pyrogenically transformed material from forest vegetation is different from the postfire material of meadow plants. The differences in the quality of pyrogenic substrates may cause differences in microbial transformation of incompletely burnt forest and meadow plant residues. Input of pyrogenically transformed substrates may initiate changes in microbial communities resulting in changed growth characteristics. Microorganisms with the predominant *r* growth strategy exhibit high growth rates in the presence of excessive substrate and in the absence of limitation by the substrate and mineral elements. They are less efficient substrate utilizers than the K-strategists [10]. Microorganisms with the K strategy are highly competitive, have lower growth rates, and benefit upon decomposition of low-availability substrates [11]. The maximal specific growth rate of soil microorganisms on an easily available substrate is one of the criteria used to characterize the ecological strategy [9]. This value is usually higher for *r*-strategists than for K-strategists [12, 13]. Few works have dealt either with the characterization of the ecological strategies of microbial cenoses of terrestrial ecosystems, or with comparison of the growth strategies of microbial communities of pyrogenically modified soils. The rate of transformation of postpyrogenic substrate depends, among other factors, on the initial value of microbial biomass. Investigation of the functional structure of microbial communities at different stages of the transformation of pyrogenically modified plant material of forest and meadow cenoses is therefore relevant.

Determination of the kinetic parameters of the growth and mineralizing activity of soil microorganisms will reveal the changes in the ecological strategy of soil microbial community resulting from the indirect effect of fire.

The goal of the present work was to determine the mineralizing activity in soils of forest and meadow cenoses in the course of incubation experiments with input of plant residues and plant ash and reveal the effect of the stage of substrate transformation on the growth characteristics of soil microorganisms.

MATERIALS AND METHODS

Objects. Aftereffects of low-intensity grass burning were investigated on a nonmowed grassland established in 1980 by A.M. Ermolaev at the experimental station of the Institute of Physicochemical and Biological Problems of Soil Science, Russian Academy of Sciences, Pushchino, Russia. Another experimental site was a plot of mixed forest that had survived a short fire caused by accidental burnings of the previous year's plant residues in early spring. Similar sites unaffected by fire (C_{total} 1.4–1.5%, N_{total} 0.11–0.12% [14], microbial biomass content in soil under forest 1024 μg C g^{-1} and under meadow 804 μg C g^{-1}) were used as controls. The pH of the soil before and after the fire was 5.7 and 5.8, respectively. The samples of gray forest soil were collected by the composite sample method from the 0–5 cm layer, and plant roots and large detritus particles were carefully removed. Soil samples were sieved through a 3-mm sieve, moistened to 60% of water holding capacity, and stored for one month in a refrigerator at 4–6°C. Prior to the experiments, the soil was preincubated at room temperature for 3 days. Last-year plant residues of meadow and forest floor (from the control plots) and plant ash, i.e., pyrogenically transformed plant residues (from the plots after burning) were used as substrates. Prior to the application into the soil, plant residues (meadow grasses and coarse forest tree residues as needles, branches, and seeds) was homogenized to 1–5 mm. Soil samples (25 g of air-dry soil) were placed in 250-ml vials and supplemented with plant residues (120 mg g^{-1}) of ash (40 mg g^{-1}). For the forest and the meadow, the inputs of carbon with plant material were 58.6 and 65.4 mg C g^{-1}, respectively; carbon input with ash was 12.2 and 19.0 mg C g^{-1}, respectively. Incubation was carried out for 40 days at 22°C. The following designations were used in the experiments: "forest PR" and "meadow PR" for addition of plant residues, "forest A" and "meadow A" for addition of plant ash, and "forest C" and "meadow C" for the controls.

In order to determine the mineralization activity of soil microorganisms, gas samples were collected during the incubation and the amount of CO_2 was determined by gas chromatography.

Kinetic characteristics of microbial growth were used to visualize the changes in the functional structure of microbial communities at different stages of the transformation of plant residues. For this goal, 10 g of

soil was mixed with talcum–glucose mixture (4 mg C g soil^{-1}) supplemented with mineral salts (mg g^{-1}): $(NH_4)_2SO_4$, 1.9; K_2HPO_4, 2.25; and $MgSO_4 \times 7H_2O$, 3.8. The rate of CO_2 emission was then determined on an ADC-2250 continuous flow infrared Gas analyzer (BioScientific Ltd., Germany) [15].

Maximal specific growth rate (μ_m, h^{-1}) was determined by approximating the experimental values of CO_2 emission rates (μg C g^{-1} soil^{-1}) according to the equation [16]:

$$v = v_0^{coupled} \exp(\mu_m t) + v_0^{uncoupled}, \quad (1)$$

where $v_0^{coupled}$ is the initial rate of productive substrate oxidation coupled to ATP synthesis, $v_0^{uncoupled}$ is the respiration uncoupled from growth, and t is time. The parameters of Eq. (1) for the best fitting of the calculated and experimental values were adjusted by the least square method using the Model Maker 3.1 software package (Cherwell Scientific Publishing Ltd.). The part of the growth curve providing the highest values of the Q and r statistical criteria was used for approximation.

Total microbial biomass was calculated from the values of the rate of initial respiratory response ($v_0^{uncoupled} + v_0^{coupled}$) obtained by adjustment of the parameters of Eq. (1) and expressed in μg C g soil^{-1} h^{-1} by the method of substrate-induced respiration (SIR), C_{micr} (μg/g^{-1} soil) using, according to [17], the ratio

$$C_{mirc} = 1.89 \times 40.04(v_0^{uncoupled} + v_0^{coupled}). \quad (2)$$

To calculate the ratio of the biomass of active soil microorganisms capable of growth on added glucose, the parameters determined for Eq. (1) were used. The coefficient of the physiological state of the microorganisms (r_0) was calculated from the ratio of the rates of idle and productive respiration:

$$r_0 = v_0^{pr} 0.1/(v_0^{uncoupled} + v_0^{coupled} 0.1). \quad (3)$$

The percentage of active microorganisms is r $r_0 \times 100$. A detailed derivation and justification of these calculations were published earlier [17, 18].

Lag phase duration (t_{lag}, h) in the respiratory response characterizes the time interval in which the respiration rate remains at the background level after addition of glucose, i.e., when no active microbial growth occurs. The moment when the rate of growth (productive) respiration v_0^{pr} became equal to the rate of idle respiration v_0^{idle} (1), according to [15], was considered the beginning of growth:

$$t_{lag} = \ln(v_0^{uncoupled}/v_0^{coupled})/\mu_m. \quad (4)$$

Generation time of active microorganisms ((T_g^a, h) as the doubling period for active biomass was calculated based on the values of the maximal specific microbial growth rate:

$$T_g^a = \ln(2)/\mu_m. \quad (5)$$

Generation time of total microbial biomass (T_g^{total}, day) was calculated from the generation time of active microorganisms and the coefficient of physiological state r_0:

$$T_g^{total} = T_g^a/r_0/24. \quad (6)$$

Microbial mass turnover time (MTT, T_t) in soil as the time interval required for complete renewal of microbial biomass was calculated for the period of termination of the mineralization processes characterized by stable values of microbial biomass and respiration (steady-state conditions) which is a necessary prerequisite for MTT determination. MTT and T_t were calculated by two methods.

(1) Using the values for microbial biomass (MB) and respiration (R_s) [19], accepting the economical coefficient for substrate consumption Y = 0.45 and the coefficient for maintenance of microbial respiration $R_m = 0.08\%$ of the biomass per day:

$$MTT = (MB(1 - Y)/Y)/(R_s - MB \times R_m), \quad (7)$$

(2) Using the values for the generation time for the total microbial biomass ((T_g^{total}),) and the equation for turnover time of the active biomass: $T_g^a = \ln(2)/\mu$; $T_t^a = 1/\mu$:

$$T_t = T_g^{total}/\ln(2). \quad (8)$$

Statistical treatment of the results was carried out by one- and two-way ANOVA with the "cenosis" (forest, meadow) and "substrate" (plant residues, ash) chosen as independent factors. Cumulative emission of CO_2 from soil and the maximal specific microbial growth rate were considered as resulting characteristics. The reliability of the differences was assayed using the Duncan criterion.

RESULTS AND DISCUSSION

Effect of added substrates on mineralization processes in soil. During incubation of soil with plant material, three periods were observed with different rates of decomposition of organic matter (Fig. 1): the period of intense mineralization (0–5 days), retardation (5–25 days), and termination (25–40 days) of the processes of mineralization. During all these periods, CO_2 emission from soil samples with plant residues exceeded the control values for forest and meadow soil by five- and sixfold, respectively. In general, CO_2 emission from soil with the pyrogenically transformed substrate was 1.5–2 times higher than in the control soils from both cenoses (Fig. 1).

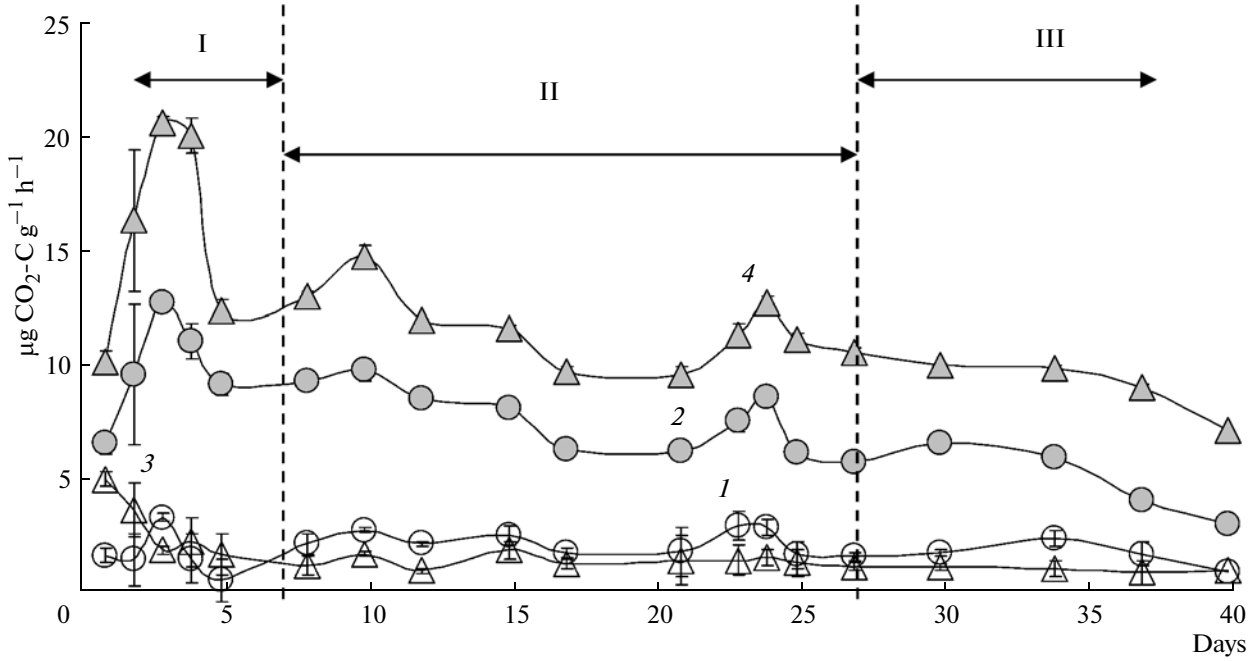

Fig. 1. Rate of CO_2 emission from soils with plant residues after subtraction of the control values. Intense mineralization (I), retardation of mineralization (II), and termination of mineralization (III). Forest A (*1*), forest PR (*2*), meadow A (*3*), and meadow PR (*4*).

Two-way ANOVA statistical analysis revealed that the differences in the net CO_2 emission depended on both the type of plant cenosis and the amount of added substrates (Table 1). The substrate had the greatest effect on CO_2 emission (88.7% of the total variability of this parameter). Thus, CO_2 emission reflects mainly the physiological reaction of microorganisms, which is manifested as enhanced respiratory activity in the course of decomposition the substrates of different availability. Independently of the period of transformation of plant residues in soil, the cumulative CO_2 emission was higher in meadow soil than in forest soil (Table 2). The previous year's meadow residues appears to be a more available substrate than forest floor. However, in variants with input of plant ash, total CO_2 emission was higher in the forest soil, due to higher microbial biomass in this cenosis (see Materials and Methods). Pyrogenically transformed material is a substrate containing mineral elements and organic compounds of the incompletely combusted waste. In the course of combustion, the polysaccharides, proteins, and heterocyclic nitrogen compounds of plant residues are transformed to aromatic compounds [19, 20]. During weak fires, the coarser forest waste is mineralized less intensely than are meadow mats. Organic compounds of the forest ash are thus more susceptible

Table 1. The effect of various factors on the physiological parameters of microbial communities in the variants with plant residues and pyrogenically transformed material at the end of incubation

Cumulative CO_2 emission, mg CO_2-C g^{-1} soil			Maximal specific growth rate, h^{-1}		
Factor	% of total variability	*P*	Factor	% of total variability	*P*
Cenosis	0.8	0.0017**	Cenosis	36	0.0014**
Substrate	89	0.0000***	Substrate	30	0.0102*
Cenosis + substrate	10	0.0000***	Cenosis + substrate	8	0.1886 ns
Residue	0.3		Residue	25	

Notes: The differences are significant at the significance level:
* 5%;
** 1–5%;
*** 0.1–1.0%.
ns indicates insignificant differences.

Table 2. Cumulative CO_2 emission from soils at different stages of mineralization of plant substrates

Variant	Intense mineralization (0–5 days)	Retardation of mineralization (5–25 days)	Termination of mineralization (25–40 days)	Total emission during days 0–40 of incubation
Cumulative CO_2 emission, μg CO_2-C g^{-1} soil				
Forest PR	1192	3024	2862	7078[b]*
Forest A	178	663	1189	2030[c]
Meadow PR	1911	4561	3487	11870[a]
Meadow A	314	595	630	1539[d]

* The values marked by different letters differ significantly at $P < 0.05$.

to microbial decomposition than those of the meadow ash. Transformation of the forest ash was therefore more intense than mineralization of the ash collected in the meadow.

Changes in the functional structure of microbial communities and the content of microbial biomass in soil in the course of decomposition of plant residues and pyrogenically transformed material. During intense mineralization, the curves characterizing microbial growth in the controls were below those for microbial growth in soils supplemented with plant substrates (Fig. 2a). The period required for transition to exponential growth (t_{lag}) in the "forest PR" variant and in the variant with plant ash was 9 and 4 h shorter, respectively, than in the control (Table 3). In the soil of the meadow cenosis, the same tendency was observed, with lag phase duration for the "meadow PR" variant being 12 h shorter than in the control. Thus, prior to addition of the glucose–mineral mixture, soil "activated" by the introduction of plant residues contained significant numbers of active microorganisms capable of immediate rapid growth. This was confirmed by a sharp increase in the total and active microbial biomass compared to the control (Fig. 3).

Thus, decomposition of plant substrates initiated transition of microorganisms to the actively growing state and the microbial community was able to utilize glucose with almost no lag phase. A prolonged period of suppressed microbial growth was observed in soil with plant ash. In this variant, the shape of the respiration curves was less steep than in the control. The differences between the variants with plant residues and the control were especially pronounced at the stage of intense mineralization (Figs. 4a and 4b). For soil enriched with plant ash, the greatest differences from the control were observed during the slowing down of mineralization (Fig. 4c). During the subsequent periods, the respiratory response in soils with plant residues or ash decreased, with less steep curves (Fig. 4d).

Content of total and active microbial biomass and rates of CO_2 emission. In both cenoses, addition of the substrates resulted in an increase in microbial biomass compared to the control. It increased 1.5- and 2.5-fold in soils with plant ash and 3- and 5-fold in soils

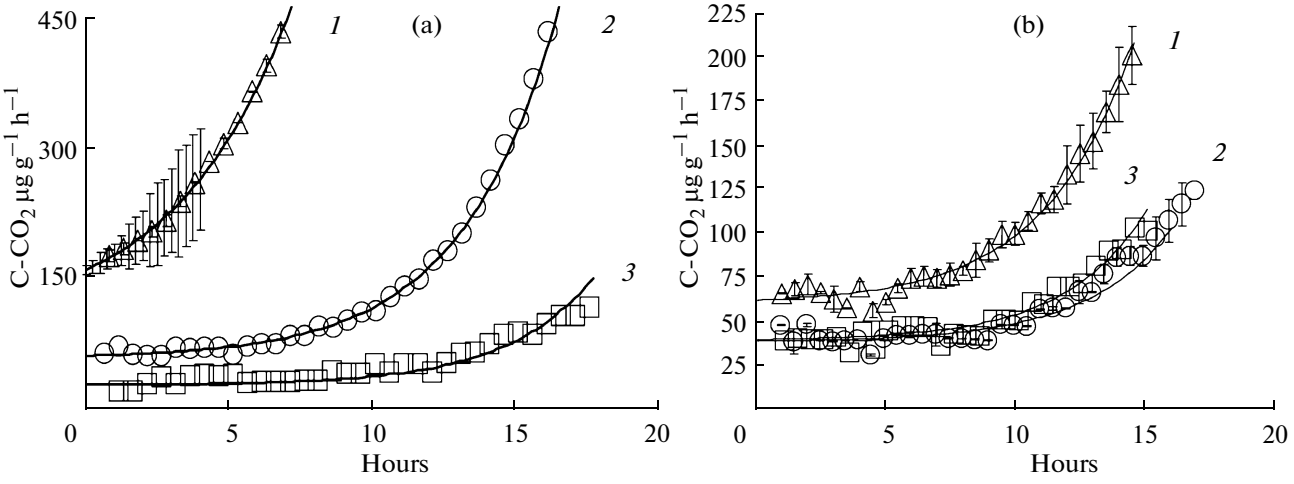

Fig. 2. Kinetics of microbial respiratory response to addition of glucose during intense (a) and decelerated (b) mineralization of plant residues and ash for the forest (a) and meadow (b) cenoses: soil with plant residues (*1*), soil with ash (*2*), and control (*3*). The symbols represent experimental values for the respiration rates; the curves represent the values calculated using Eq. (1).

Table 3. The effect of mineralization of plant substrates on lag phase duration (t_{lag}), generation time for the total (T_g^{total}) and active (T_g^a) microbial biomass, and the economic coefficient of substrate utilization (Y)

Variant	Intense mineralization (0–5 days)			Retardation of mineralization (5–25 days)			Termination of mineralization (25–40 days)					
	t_{lag}, h	T_g^a, h	T_g^{total}, days	t_{lag}, h	T_g^a, h	T_g^{total}, days	t_{lag}, h	T_g^a, h	T_g^{total}, days	MTT* days	T_t, days	**Y
Forest C	13.9	2.16 ± 0.03	79 ± 2	17.5	2.3 ± 0.42	191 ± 1.5	12.7	2.1 ± 0.09	56 ± 1.76	20	80	0.17
Forest PR	4.4	2.88 ± 0.02	4 ± 3	11.4	2.5 ± 0.11	25 ± 2.3	10.8	2.3 ± 0.08	24 ± 2.15	12	35	0.22
Forest A	9.5	2.28 ± 0.21	17 ± 2.12	13.9	2.6 ± 0.17	46 ± 2.2	12.0	2.5 ± 0.05	31 ± 2.3	23	44	0.30
Meadow C	13.0	2.12 ± 0.23	63 ± 1.6	12.9	2.2 ± 0.23	58 ± 2	15.8	2.5 ± 0.36	87 ± 1.7	34	125	0.18
Meadow PR	1.6	2.86 ± 0.22	2 ± 3	11.4	2.4 ± 0.17	29 ± 2.13	9.8	2.5 ± 0.1	17 ± 2.3	9	24	0.24
Meadow A	10	2.34 ± 0.05	19 ± 2.2	13.7	2.8 ± 0.22	32 ± 2.5	10.3	2.6 ± 0.1	17 ± 2.45	30	24	0.51

Notes: * The MTT values calculated from experimental data using the value Y = 0.45.
** The Y values calculated using Eq. (9).

enriched with plant residues for the forest and meadow, respectively. Microbial biomass in soil incubated with ash was 1.6–2 times lower than in soil with plant residues. In meadow soil, the biomass increase was greater than in forest soil (Fig. 3a).

Dynamics of active biomass in the experiments reflected the availability of the added substrates. Addition of plant residues and ash resulted in a 100- to 200- and 9- to 10-fold, respectively, increase of active biomass in the soils of both cenoses (Fig. 3b). Thus, the content of available compounds in plant residues was higher than in plant ash. Although exhaustion of these compounds resulted in decreased ratios of active microorganisms, by the end of incubation they were still five to ten times higher than in the control, reflecting the stage of decomposition of the difficultly available fractions of plant substrates.

The maximal specific growth rate of microorganisms (μ_m) obtained by approximation of the curves according to model (1) showed no reliable increase during incubation of control samples of forest and meadow soil. This finding indicates permanent growth of soil microorganisms. Addition of plant residues to the soil resulted in a reliable drastic decrease in μ_m values during intense mineralization (Fig. 5), indicating the activation of slowly growing microorganisms with the K strategy. The change in the dominating strategy in soils enriched with plant residues was sharp but brief. The difference in growth rates between the experimental and control samples decreased in the course of incubation, so that, by the end of incubation, this difference remained significant only for forest soils.

During intense mineralization, no significant differences were found between the maximal specific growth rate values for the variant with plant ash and the control. However, during slowing down of mineralization, the maximal specific growth rate decreased relative to the control, suggesting that, in the soil with ash, microorganisms with the K strategy dominated only at the later stages of mineralization. In the course of exhaustion of available compounds of plant ash, K-strategists, i.e., microorganisms possessing the enzyme systems for the degradation of difficultly available substrates [19], gained an advantage. Thus, the changes in growth strategy associated with exhaustion of available substrates made it possible for the microorganisms to make up for their limitation by decomposition of the polymers of ash and in soil organic matter (SOM). Structural and functional changes in soil with ash occurred more slowly than in the variant with plant residues, while the difference between the control and experimental samples were more pronounced in this case. Two-factor dispersion analysis confirmed that both the quality of the substrate and the type of plant cenosis affected the maximal specific

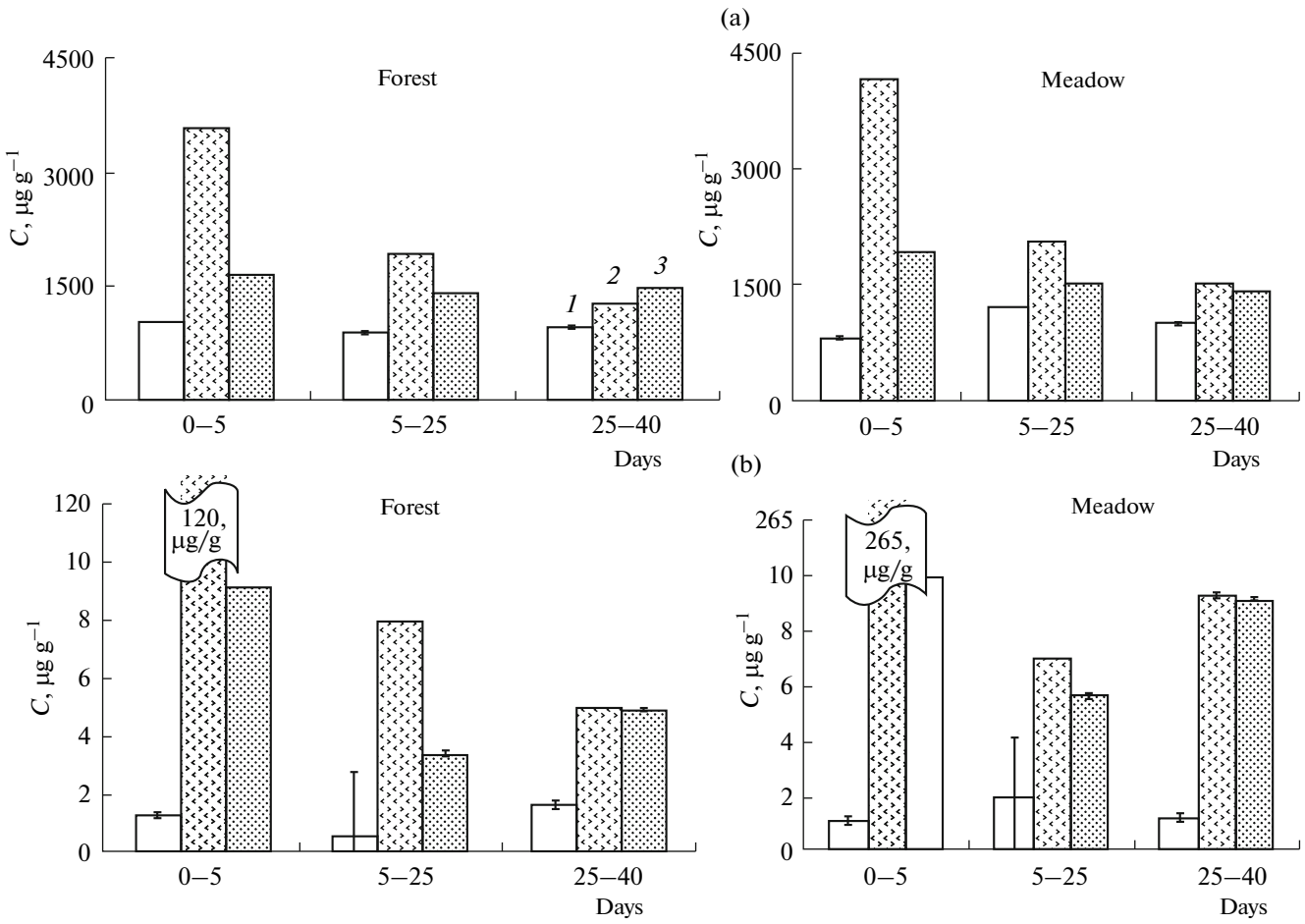

Fig. 3. Content of total (a) and active (b) microbial biomass in the control (*1*), soil with plant residues (*2*), and soil with ash (*3*).

growth rate at the end of incubation. The contribution of the type of vegetation (meadow and forest cenoses) and of the quality of the substrate were 36 and 30%, respectively, of the total variability for μm values. Thus, the maximal specific growth rate is a more sensitive indicator than the cumulative CO_2 emission and reflects the functional changes in microbial communities.

The microbial community formed on the added substrates had a higher biomass, a shorter lag period, and lower growth rates than the control one. Our experiments revealed no positive correlation between lag phase duration and microbial growth rate. Thus, similarly to previous works [21], the present investigation demonstrated that lag phase reflects the time required for the microorganisms to restructure their metabolism for transition from the dormant state to active growth. It is therefore an additional characteristic of a microbial community, not necessarily related to growth rate. The specific growth rate is the main parameter characterizing the relative dominance of the microorganisms with a specific survival strategy for each ecological setting.

Enrichment of soil with *plant substrates* decreased the generation times for the total microbial biomass T_g^{total}. In the variants with addition of plant residues, a 19- and 32-fold decrease, compared to the control, was observed for the forest and meadow, respectively. In the soils with ash, the decrease was four- and threefold for the forest and meadow, respectively (Table 3). Addition of the substrates, apart from an increase in the total microbial biomass, resulted in its more rapid turnover due to a drastic increase of the ratio of active microorganisms. Characteristically, decreased generation time for the total biomass was accompanied by an increase (up to 1.3-fold compared to the control) in the generation time for the active biomass. This finding suggests that slowly growing microorganisms were the most active decomposers of the added substrates. By the end of the incubation, due to continued mineralization in soils supplemented with plant residues or ash, the generation time for the total microbial biom-

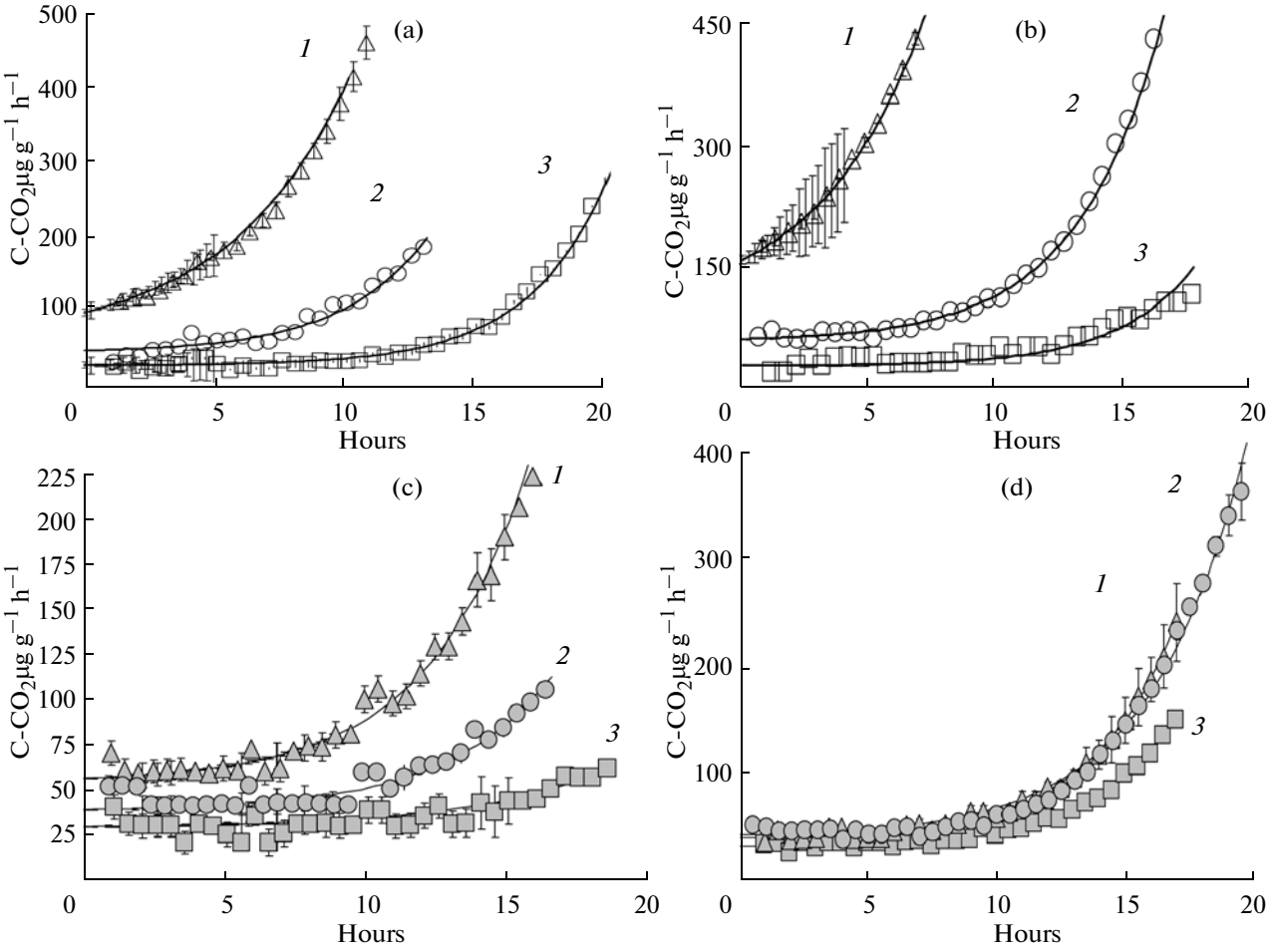

Fig. 4. Dynamics of microbial respiratory response to addition of glucose in soils enriched with plant residues and pyrogenically transformed organic material during intense mineralization for forest (a) and meadow (b) soil, during retardation for forest soil (c), and during termination of mineralization for meadow soil (d). Soil with plant residues (*1*), soil with pyrogenically transformed material (*2*), and control (*3*).

ass was less in the variants with these substrates than in the control.

The MTT values (24–125 days in the present study) correlate with the turnover time values for the soils of agrocenoses (40–80 days) [18]. The experimentally obtained values of turnover time for microbial biomass, MTT and T_t, suggest the same pattern of their changes in soils supplemented with plant residues and ash (Table 3). However, a discrepancy between the absolute values of MTT and T_t was observed. This possibly resulted from incorrect use for MTT calculation of the theoretical values for the economic coefficient Y = 0.45, which have been determined for pure cultures. Unlike MTT, T_t calculation involves only experimental data with no theoretical assumptions. Since the MTT and T_t values, both characterizing the turnover of microbial biomass, should be the same, the true values of the economic coefficient Y can be calculated:

$$Y = MB/(T_t(R_s - MB \times R_m) + MB). \quad (9)$$

The calculated values at MTT = T_t are presented in Table 3. The increasing Y values in the row control ≪ soil with plant residues ≪ soil with ash shows that the microorganisms of soils enriched with plant residues or ash used the substrate more efficiently than did those of the control soil. This finding confirms our conclusion that K-strategists predominate in soils enriched with plant residues and ash.

Priming effect (PE) in soils enriched with plant residues and pyrogenically transformed plant material. In our experiment, two types of substrates were used, namely, plant residues, which are relatively rich in carbon and poor in minerals, and the pyrogenically transformed material, containing low available compounds of organic origin and relatively enriched with mineral elements [22], particularly with nitrogen [23–25]. Plant residues and the pyrogenically transformed substrate may initiate microbial decomposition of soil

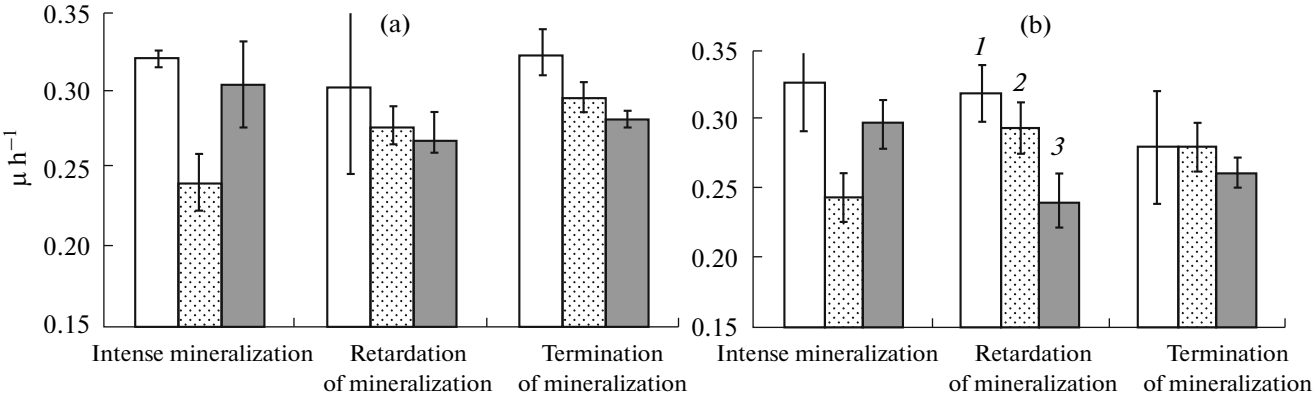

Fig. 5. Dynamics of the maximal specific growth rate in the forest (a) and meadow (b) soil: control (*1*), soil with plant residues (*2*), and soil with ash (*3*).

organic matter (i.e., cause the priming effect) in order to compensate for nutrient deficiency. However, the design of our experiment, for which labeled substrates were not used, did not allow direct assessment of the priming effect. The balance calculations give the value of about 10% of added carbon in plant ash and about 16.2% of the plant residues carbon mineralized by the end of incubation for the forest and meadow, respectively. Our calculations considering weight losses of the plant material during the fire demonstrated that, during burning, about 90% of carbon contained in the plant residues evolved to the atmosphere. Assuming the efficiency of microbial utilization of organic carbon (calculated by equation 9) $Y = 0.2–0.5$, it may be concluded that no more than 20% of the added substrates were mineralized. This impedes direct assessment of the priming effect. However, changes in microbial strategies may provide indirect information concerning the priming effect. The amount of organic substrates applied with plant residues or with the pyrogenically transformed material was sufficient to cause structural changes of microbial communities resulting in predomination of the microorganisms using the K strategy. K-strategists are known to possess enzyme systems with a high affinity to low-availability substrates [4]. The exoenzymes produced by such microorganisms may affect, apart from the added organic compounds, similar compounds present in the soil organic matter [26]; i.e., they may cause the priming effect, i.e., accelerated decomposition of soil organic matter.

The probability of the PE development was especially high when microbial specific growth rates exhibited the maximal differences from the control, i.e., during the period of intense mineralization for soils supplemented with plant residues and during the period of deceleration of mineralization for soils enriched with ash. In ash-enriched soil, the priming interactions were probably more pronounced than in soil with plant residues, since the structural and functional changes in its microbial community were more stable and the microorganisms using the K strategy remained active for a longer period than in soil enriched with plant residues.

Thus, it may be concluded that three periods with different rates of decomposition processes were clearly observed in the course of mineralization of added plant substrates: (1) the period of intense mineralization (0–5 days), (2) retardation (5–25 days), and (3) termination of the processes of mineralization (25–40 days). Independently of the period of mineralization, the cumulative CO_2 emission was higher in the meadow soil than in the forest soil. In the variants with addition of plant ash, on the contrary, the forest soil produced the highest total amount of CO_2. The type of the added substrate exhibited the highest effect on the cumulative CO_2 emission (86% of the total variability). On average, the variants with plant residues yielded twice the amount of CO_2 produced by the variants with ash. Characteristically, mineralization of plant residues was especially intense in the soils of the meadow cenosis, while decomposition of the pyrogenic material was higher in the forest soil. Thus, pyrogenic treatment had a different effect on the quality and availability of plant material in the forest and meadow cenoses. This results in disproportionate variations of CO_2 emission from soil and in the structural changes of microbial communities.

The changes in the structure of microbial communities in soils incubated with plant residues were most pronounced during the period of intense mineralization as a 1.3- to 1.4-fold decrease (for the forest and meadow, respectively) of the maximal specific growth rate, compared to the controls. This is an indication of the relative dominance of the K-strategists. Short lag and the generation times for the total and active biomass confirm that microbial K-strategists decomposing the plant residues were in an active state at the time of μ measurements.

In soils with pyrogenically transformed material, the domination of K-strategists was observed from the

period of deceleration of mineralization to the end of the experiment. The maximal growth rate was statistically demonstrated to depend on both the type of the cenosis and the quality of the added substrate. It was a more sensitive indicator of the state of microbial communities than physiological characterization of microorganisms by their respiratory activity.

The experimentally determined values for the economic coefficient Y were generally lower than the theoretically accepted Y = 0.45 and varied within the range Y = 0.17–0.51. The highest Y value, i.e., the highest efficiency of substrate utilization, was observed for the microbial community from soils supplemented with plant ash. This finding confirms the domination of the microorganisms using the K strategy.

Taking into consideration the values for efficiency of substrate utilization Y, decomposition of plant residues and pyrogenically transformed plant material to CO_2 did not exceed 20% of the added amount. Since distinguishing CO_2 sources in the course of ongoing mineralization was impossible, we can draw no direct conclusions concerning the origin of the priming interactions. However, changes in microbial growth strategy provide indirect evidence for the priming effect. The relative domination of K-strategists in the structure of microbial communities indicates the possibility of developing the priming effect in the soils of both cenoses. The probability of priming interactions was higher in the variants with plant ash; this was confirmed by the low μ values and high efficiency of substrate utilization, which remained stable until the end of incubation.

Thus, introduction of ash to soil after plant burning affects the carbon turnover in the soil–atmosphere system by changing the functional structure of soil microbial communities.

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