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EXPERIMENTAL ARTICLES

The Effect of Earthworms on the Physiological State of the Microbial Community at Vermicomposting

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Abstract—The effect of earthworms on the microbial community of composts and vermicomposts was assayed by the following parameters: mineralization activity, the levels of physiologically active and growing microbial biomass, the requirement for growth factors, and the spectrum of assimilation of organic substrates by the microbial community. The substrate affinities of microbial enzyme systems in vermicompost were found to be lower than in compost without earthworms, which is evidence of a higher amount of *r*-strategists in the microbial community of vermicomposts. Physiologically active biomass of microorganisms is higher in peat-based vermicompost than in compost. The microorganisms of vermicomposts and composts experience deficiency in growth factors to a lesser extent than the microorganisms in soil. The presence of earthworms influences the physiological diversity: the Shannon index increases or decreases depending on the type of composted substrate and incubation time. The growth rate of microorganisms increases on various test substrates in the presence of worms.

Key words: vermicompost, microbial biomass, growth strategy, functional biodiversity.

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When earthworms are used for composting, the resultant vermicomposts have higher fertility and biological activity towards plants and microorganisms than composts. It is believed that the fertility of vermicomposts is determined, apart from their agrophysical properties, by the presence of microorganisms useful to plants [1]. However, rather few works are devoted to microorganisms in composts. Microbial composition and enzyme activity in vermicomposts and composts were shown to be reliably different. At the same time, there is a tendency to an increase of microbial diversity [2, 3] and activity of some enzymes [4] in vermicomposts. Up to now, there have been no attempts to characterize the trophic characteristics and growth strategies of the microorganisms of vermicompost in comparison with conventional composts. There is no information for the assessment of the changes in the mineralization activity of microorganisms and for determination of the physiological groups of microorganisms responsible for the increase of this activity. Clear insight into the effect of earthworms on the process of composting will contribute to the development of microbiological standards of vermicomposting and the control of microbial populations in vermicomposts.

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It is known that earthworms facilitate the transformation of nutrients into available forms and increase microbial activity [5]. We believe that the presence of earthworms will influence primarily the trophic spectrum and growth strategy of the microbial community. The previously established patterns of growth kinetics and changes in the activity of soil microorganisms in continuous and batch cultures permit quantitative characterization of the growth of individual groups of microorganisms and microbial communities as a whole directly in the habitat [6]. The kinetic approach has not yet been used for the study of microbial communities of vermicomposts, though its usefulness in the study of microorganisms directly in the habitat has been clearly demonstrated. For example, some of the works compare soils under monoculture and crop rotation [7], soils with different systems of fertilizers [8], and rhizospheric and nonrhizospheric soils [9]. Thus, there are prerequisites to the study of microorganisms involved in composting from the standpoint of synecology, i.e., at the community level. Analysis of the oxidation kinetics of a substrate introduced into composts makes it possible to determine the maximal specific growth rate, physiological state of microorganisms, and the requirement for growth factors. Hence, it will be possible to characterize the patterns of the functioning of a micro-

bial community depending on the environment-forming factor such as the presence of worms.

The goal of the work was to determine the physiological states of microbial communities in vermicomposts by measuring the functionally active and growing microbial biomass and to characterize the growth strategies of microorganisms and the functional (trophic) diversity of the microbial community.

MATERIALS AND METHODS

Composts and vermicomposts

Vermicomposting and composting model substrates were as follows: cattle manure that had rotted for 6 months (from May to November) in a pile out-of-doors; the mixture of freshly fallen leaves of black alder (*Alnus glutinosa* (L.) Gaertn.), goat willow (*Salix caprea* L.), and white birch (*Betula pubescens* Enrh.) in parts equal in weight; and eutrophic peat. The initial leaf litter-based compost mixture was obtained by adding bank sand (36%) to the leaves. The weight of the dry substance was 200 g for manure and leaf composts and 400 g for peat. In the control, the substrates were composted without worms. The microcosms were incubated in three repeats at 16–19°C; humidity was maintained at 150% for manure and leaf composts, and at 100% for peat composts. The assimilation spectrum was analyzed in dynamics for 6 months of vermicomposting. Respirometric investigations were carried out with 6-month vermicomposts for manure and peat substrates, and 1-month vermicomposts for leaves. Abbreviations for the composts accepted in the work are as follows: PVC, peat vermicompost; CP, composted peat; MVC, manure vermicompost; MC, manure compost; LVC, leaf vermicompost; and LC, leaf compost.

Earthworms

Red Californian earthworms *Eisenia foetida andrei* var. "Russky Moskovsky gibrid" (Russian Moscow hybrid) were introduced into leaf and manure vermicomposts in the quantity of 100 individuals/kg of dry substance corresponding to the production norm [10]. The worms *Aporrectodea caliginosa* and *A. rosea* collected on drained valley peat were introduced into peat (1:1) in the quantity of 75 individuals/kg of dry substance.

Determination of the parameters of mineralization activity and growth strategies of microbial communities

The composts were analyzed by respirometric methods based on determination of CO_2 emission rate: (1) the modified Wright–Hobbie method for determination of the parameters of mineralization activity of microorganisms and (2) the kinetic method for determination of microbial biomass (parameters of microbial growth were determined by the dynamics of the rate of CO_2 release from glucose-amended soil). CO_2 concentration was measured on an LHM-80 gas chromatograph with a conductivity detector; columns (2.7 m in length) were filled with Polysorb-1; the thermostat temperature was 30°C; and the flow rate of the carrier gas (helium) was 40 ml/min. The volume of the gas sample was 1.0 cm^3.

The parameters of mineralization activity of microorganisms were determined by measuring the initial rates of peat respiration after application of increasing amounts of easily available substrate, using the modified Wright–Hobbie method [8, 11]. Soil samples (6 g) were put into glass vials (10 ml) and aerated for 1 h, followed by application of glucose solutions in the concentration range of 0–6 mg C/g. The volume of applied solutions was adjusted so that the humidity of the resultant soil was 60–70% of the field water capacity. The flasks were then hermetically sealed and incubated at room temperature (20–22°C) for 30–40 min; the initial respiratory rate was determined by the amount of accumulated CO_2. The resultant dependence was described by the Monod equation modified for the concentration of natural substrate [12] available to microorganisms and analogous by accessibility to applied glucose (S_n):

$$V = \frac{V_{\max}(S + S_n)}{K_s + S + S_n}, \quad (1)$$

where $V_{\max}$ is the maximum theoretical rate of CO_2 release or heterotrophic potential of the soil microbial community; S is the amount of glucose added into compost; V is CO_2 release rate at glucose concentration S; and K_s is a saturation constant equal to the amount of glucose needed to achieve the respiration rate corresponding to the half of $V_{\max}$.

The time of turnover of an accessible natural substrate (T_t) analogous by availability to glucose was determined by the formula:

$$T_t = \frac{K_s + S_n}{V_{\max}}. \quad (2)$$

Metabolic coefficient qCO_2 characterizing the specific respiration activity of microbial biomass:

$$qCO_2 = \frac{V_{\text{basal}}}{C_{\text{micr}}}, \quad (3)$$

where V_{basal} is the rate of CO_2 release from soil without glucose application and C_{micr} is the microbial biomass determined by the SIR method.

Calculation of the microbial biomass by the method of substrate-induced respiration (SIR). Biomass C_{micr} was calculated using the value of initial respiration after the application of excess glucose [13], using the value of the heterotrophic potential ($V_{\max}$) determined by the modified Wright–Hobbie method described in the previous section. The calculation was performed according to the formula:

$$C_{\text{micr}} = 40.04V_{\max} + 0.37. \quad (4)$$

Kinetic parameters of microbial growth were determined by the dynamics of the rate of CO_2 release from glucose-enriched soil [14]. The solution containing glucose (10 mg/g of soil), NH_4SO_4 (1.91 mg/g), and K_2HPO_4 (2.25 mg/g) was added to 6-g weighed portions. Yeast extract, 4 mg/g, was introduced instead of glucose to determine the maximum specific growth rate on yeast extract [15].

The obtained experimental data on the change in the release rate of CO_2 with time were described by equation:

$$v = v_0^{pr} \, exp(\mu_m t) + v_0^{bl}, \quad (5)$$

where v is the rate of CO_2 release, v_0^{pr} is the initial respiration rate uncoupled from ATP production, v_0^{bl} is the initial rate of the growing fraction of total respiration coupled with ATP generation and cell growth, t is time, h, and μ_m is the maximum specific rate of microbial growth on glucose [14].

Auxotrophicity index (I_a) [15] was determined by the formula:

$$I_a = \frac{\mu_m}{\mu_{yeast}}, \quad (6)$$

where μ_m and μ_{yeast} are the maximum specific growth rates of microorganisms on glucose and yeast extract, respectively. This index reflects the microbial requirement for growth factors. The higher the I_a values, the lower the requirement for growth factors.

The biomass of the growing part of the microbial community in soil was calculated by the formula:

$$X_0' = \frac{v_0^{pr} Y_{x/p} \lambda}{\mu_m}, \quad (7)$$

where $Y_{x/p}$ is a stoichiometric coefficient characterizing the yield of biomass per unit of substrate carbon oxidized to CO_2 and equal to 1.5, and λ is the basal stoichiometric constant characterizing the relation between the total and cyanide-resistant respiration activity equal to 0.9 [14].

Coefficient of the physiological state of microorganisms (r_0) was calculated by the ratio of productive and total respiration using the formula:

$$r_0 = \frac{v_0^{pr}(1-\lambda)}{v_0^{bl} + v_0^{pr}(1-\lambda)}. \quad (8)$$

Total (responding to glucose application) microbial biomass was calculated by the formula:

$$X_0 = \frac{X_0'}{r_0}. \quad (9)$$

Determination of the spectrum of assimilation of organic substrates by the microbial community

The assimilation spectrum was determined by the Gorlenko and Kozhevin method of multisubstrate testing [16] in our modification [17]. Substrate consumption was assessed not by an end point measurement but by the kinetic parameters of microbial growth in the wells of the test plate. The optical density in the wells (D) containing a suspension of microbial cells and a stain depends on the presence of formazane formed at respiration of microorganisms. Growth was described by a logistic equation:

$$D = \frac{D_{max}}{1 + \frac{D_{max} - D_0}{D_0} e^{-\mu_{max} t}}, \quad (10)$$

where t is time (h), D is optical density at the moment of time t, D_0 is optical density at the initial moment, μ_{max} is the maximum specific growth rate, and D_{max} is the maximum optical density in a cell for 50 hours of incubation [17].

Hydrolase activity.

Hydrolase activity was determined by the reaction of fluorescein diacetate (FDA) hydrolysis [18]. The procedure was modified for organic substrates: a wet weighed portion equivalent to 1 g of dry substance was covered with 0.1 M Serensen's phosphate buffer, pH 7.6 (0.35 g/200 ml of KH_2PO_4, 6.2 g/200 ml of $Na_2HPO_4 \cdot 12H_2O$), with the buffer/compost ratio of 1:10. FDA predissolved in acetone was added, and the test tube was shaken. The suspension was incubated for 1 h at 30°C and centrifuged, and the optical density of the supernatant was determined at 490 nm. The maximum reaction rate (V_{max} *FDA*) and the Michaelis constant (K_m) were determined as a result of approximation of experimental data by the Michaelis–Menten equation. The equation parameters were determined by measuring the reaction rate V at 5 FDA concentrations (S): 4.80; 19.23; 33.65; 48.07; and 96.15 μmol/l.

Specific hydrolase activity [19] was determined as:

$$q\text{FDA} = \frac{V_{maxFDA}}{C_{micr}}. \quad (11)$$

Analysis of the chemical properties of substrates

The contents of total carbon and nitrogen in compost samples were determined in a Vario EL III (Elementar) HCNS Mode elemental analyzer.

Table 1. The values of microbial biomass and activity of microorganisms

Parameter	PVC	PC	MVC	MC
Biomass, SIR method (C_{micr}), μg C/g	3104 ± 396	1130 ± 52	672 ± 65	657 ± 68
Biomass, kinetic method (X_0), μg C/g	1151 ± 117	218 ± 97.0	223 ± 78	235 ± 93
Growing biomass (X'_0), μg C/g	3.48 ± 1.15	0.028 ± 0.008	0.16 ± 1.2	2.21 ± 1.3
CO_2 production rate (V_{basal}), μg C–CO_2/(g h)	10.5 ± 2.4	14.1 ± 0.5	5.3 ± 1.2	5.4 ± 0.9
Glucose mineralization potential (V_{max}), μg C–CO_2/(g h)	64.0 ± 9.9	23.0 ± 1.3	8.9 ± 1.85	8.7 ± 1.2
Metabolic coefficient (qCO_2), μg C–CO_2/(μg C_{micr} h)	$(3.4 \pm 2.4) \times 10^{-3}$	$(12.5 \pm 3.3) \times 10^{-3}$	$(7.9 \pm 2.3) \times 10^{-3}$	$(8.2 \pm 2.7) \times 10^{-3}$
Specific hydrolase activity (qFDA), μmol fluorescein/(μg C_{micr} h)	$(1.6 \pm 1.2) \times 10^{-5}$	$(3.0 \pm 0.2) \times 10^{-5}$	$(8.6 \pm 0.2) \times 10^{-5}$	$(21.3 \pm 0.2) \times 10^{-5}$
Affinity of enzymes to glucose (K_s), μg C–glucose/g	400.0 ± 295	33.2 ± 32	176 ± 342	54 ± 102
V_{max}/K_s, μg C–CO_2/(μg C glucose h)	0.16 ± 0.11	0.70 ± 0.6	0.05 ± 0.6	0.16 ± 0.7
Coefficient of the physiological state of microorganisms (r_0),	$(30 \pm 6.4) \times 10^{-4}$	$(1 \pm 0.3) \times 10^{-4}$	$(7.2 \pm 2.4) \times 10^{-4}$	$(93.7 \pm 12.3) \times 10^{-4}$

Note: ± is the confidence interval at $p = 0.95$; PVC, peat vermicompost; PC, composted peat; MVC, manure vermicompost; MC, manure compost.

RESULTS AND DISCUSSION

Respirometric investigations of peat and manure composts

Microbial biomass. According to the physiological method (C_{micr}), the total biomass of microorganisms in peat vermicompost was 2.7 times greater than in peat compost without the earthworms (Table 1). The biomass of microorganisms determined by the kinetic method X_0 (Table 1) was 5.3 times different, which was in agreement with the previous data on the increase of microbial biomass in soil in the presence of earthworms [20]. The growing biomass (X'_0) determined by the kinetic method was 124 times greater in vermicompost as compared with conventional compost, and its portion in the total biomass (the coefficient of physiological state of microorganisms, r_0) increased 30-fold. Consequently, the activity of earthworms in peat-based vermicompost creates conditions for the growth of microorganisms, which may be a result of enrichment with the accessible substrate supporting microbial growth (Table 2). In manure composts, biomass values are similar in the variants with and without the worms (Table 1), and this is in agreement with the previous data on microbial biomass at the final stage of vermicomposting [21]. In manure-based vermicompost, the value of growing biomass (X'_0) determined by the kinetic method was less than in the compost without the worms. However, this difference was insignificant and could be attributed to the decreased content of the community members capable of growth on glucose with mineral salts. This assumption is also supported by our data on the auxotrophicity of microorganisms presented below.

Mineralization activity of microorganisms. The potential of glucose mineralization V_{max} is higher in vermicompost (PVC) than in peat compost without the earthworms (CP), but the specific respiration activity of microbial biomass estimated by the value of metabolic coefficient (qCO_2) is higher in the compost (Table 1). The higher value of the metabolic coefficient in this case indicates that the organic substance in the compost is less accessible to microorganisms, and they consume more energy for oxidation of natural substrates. This fact is confirmed by the value of the coefficient of specific hydrolase activity of microbial biomass qFDA,which is higher in the compost than in the vermicompost. In manure composts (MVC and MC), the potentials of glucose mineralization are similar; the qFDA values, however, are relatively higher in the vermicompost. As concerns absolute values, qFDA is

Table 2. Trophic characterization of the habitat

Parameter	PVC	PC	MVC	MC	LVC	LC
Organic carbon (C_{org}), %;	28.2 ± 4.4	28.6 ± 3.8	21.0 ± 2.0	21.6 ± 0.9	20.7 ± 5.0	28.6 ± 5.5
Enrichment of organic matter with nitrogen (C/N), molar ratio	17.5 ± 0.6	18.3 ± 1.0	15.4 ± 1.7	16.5 ± 0.8	20.5 ± 3.0	19.2 ± 0.2
Portion of microbial biomass carbon in the total organic carbon (C_{micr}/C_{org}), %	1.1 ± 0.8	0.4 ± 0.2	0.3 ± 0.5	0.3 ± 0.6	N.d.	N.d.
Quantity of easily available natural substrate (S_n), μg C_{org}/g	59.0 ± 160	52.6 ± 52	273 ± 433	84.7 ± 148	N.d.	N.d.
Time of turnover of the easily available organic substance (T_t), h	7.2 ± 1.1	3.7 ± 0.6	206 ± 12	15 ± 3	N.d.	N.d.

Note: ± is the confidence interval at $p = 0.95$; n.d., not determined; PVC, peat vermicompost; CP, composted peat; MVC, manure vermicompost; MC, manure compost; LVC, leaf vermicompost; LC, leaf compost.

higher in manure composts, which results from the lower accessibility of organic matter to microorganisms (Table 1).

The microorganisms utilizing the *r*-strategy are less efficient in substrate assimilation than the K-strategists whose enzyme transport systems exhibit high affinity to the substrate [9]. At a lower substrate concentration per biomass unit, the K-strategists gain an advantage over the *r*-strategists in competition for the substrate. The value of the saturation constant K_s determining the affinity of the enzyme systems of soil microorganisms to a substrate is more than 12-fold higher in PVC than in CP and 3-fold higher in MVC than in MC (Table 1). Thus, the presence of earthworms increases the portion of *r*-strategists with less selective enzyme systems.

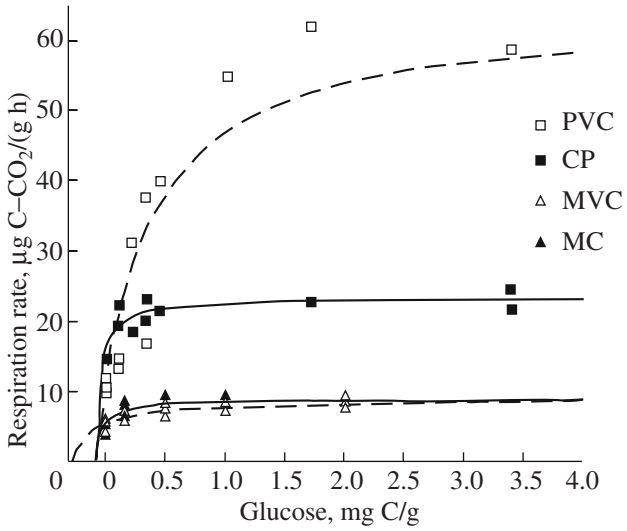

Fig. 1. Dependence of the respiration rate on the quantity of added glucose. PVC, peat vermicompost; CP, composted peat; MVC, manure vermicompost; MC, manure compost. The curves were calculated according to Equation 1 with the parameter values presented in Tables 1 and 2.

The competitive abilities of microorganisms should be compared more precisely by the values of the V_{max}/K_s ratio [22]. The ratio of the Monod equation (1), parameters equal to the slope of a curve (Fig. 1) at the lowest concentration of introduced substrate better reflects the competitive abilities of a microbial community, because it accounts for both indices: the maximum rate of substrate oxidation and the affinity of enzyme systems to the substrate. The higher ratio of these parameters in compost as compared with vermicompost (Table 1) demonstrates the relative dominance of K-strategists providing higher rates of substrate oxidation at lower substrate concentrations. Hence it follows that the pressure of K-selection in vermicompost decreases, probably due to a greater amount of accessible substrate per unit of microbial biomass.

Growth characteristics of microbial communities. Figure 2 shows the curves of the growth of the microbial community on glucose (Fig. 2a) and on yeast extract (Fig. 2b) calculated according to Equation 5. The optimized values of the parameters of Equations 5 and 6 for composts and vermicomposts are presented in Table 3. The specific growth rate in MVC was 1.4-fold higher than in MC, while in peat compost, however, specific growth rate decreased in the presence of earthworms. Different results may indicate additional

growth limitation for PVC after addition of glucose with mineral salts. The μ_m/K_s ratio, which takes into account both the specific growth rate (μm) and the value of the half-saturation constant (K_s) [23], seems to be a more informative growth index. This ratio (Table 3), as well as the V_{max}/K_s ratio, demonstrates a relative increase in the amount of r-strategists in vermicomposts (MPC and PVC) as compared with composts (MC and CP).

The auxotrophicity indices (Equation 6) for compost and vermicompost were close: 0.69–0.85 (Table 3); their highest values were found for manure vermicompost. This index (I_a) for soil conditions is usually below 1 [15, 24], indicating the limitation of the rate of microbial growth by growth factors missing in the glucose–mineral mixture, which is added to soil to estimate the respiratory response. Our results showed that microorganisms in vermicomposts and composts also lacked growth factors, but this limitation proved to be less pronounced than for the microbial communities of chernozem soil, where the auxotrophicity index was lower and varied in the range of 0.4 to 0.63 [24].

Trophic characterization of the habitat of microorganisms. PVC and CP do not differ in the total content of organic carbon, C/N ratio in organic matter, and the amount of substrate readily available to microorganisms (Table 2). The turnover time for the easily available substrate (T_t) in PVC is as much again as in the compost (7.2 h); this results mainly from the higher K_s value for the vermicompost. However, the portion of microbial biomass in total soil carbon, C_{micr}/C_{org}, is higher in vermicompost, which is indicative of stimulation of decomposition of organic matter by worms in peat vermicompost. Introduction of the earthworms into the manure compost also did not change reliably the total carbon content and the C/N ratio; however, MVC contained 13-fold more readily available organic substrate than MC. At the same time, the C_{micr}/C_{org} value was the same in the variants of manure composts with and without the earthworms.

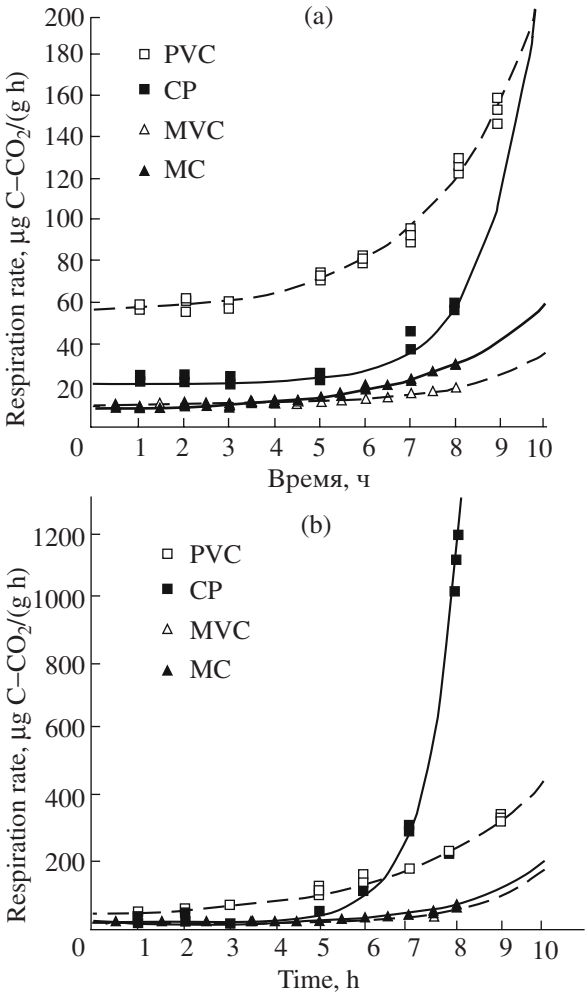

Fig. 2. Kinetics of the respiratory response of microorganisms grown on glucose (a) and yeast extract (b). PVC, peat vermicompost; CP, composted peat; MVC, manure vermicompost; MC, manure compost. The curves were calculated according to Equation 5 with the parameter values presented in Table 2.

Respirometric investigations of leaf composts

The respiratory response to substrate application should be considered particularly for leaf composts (LVC and LC). Since the studies were carried out a month after introduction of the earthworms on a substrate that had not been composted completely, a high value of mineralization activity was revealed in comparison with manure and peat substrates. For LVC,

Table 3. Growth characteristics of the microbial community

Parameter	PVC	CP	MVC	MC
Maximum specific growth rate on glucose (μ_m), l/h	0.46 ± 0.045	0.9 ± 0.17	0.59 ± 0.16	0.42 ± 0.10
Maximum specific growth rate on yeast extract (μ_{yeast}), l/h	0.62 ± 0.23	1.31 ± 0.03	0.69 ± 0.10	0.61 ± 0.09
Auxotrophicity index (I_a)	0.73 ± 0.26	0.69 ± 0.16	0.85 ± 0.19	0.69 ± 0.12
μ_m/K_s g/(μg C-glucose h)	$(1.1 \pm 0.4) \times 10^{-3}$	$(27.0 \pm 2.3) \times 10^{-3}$	$(3.4 \pm 0.8) \times 10^{-3}$	$(7.8 \pm 1.3) \times 10^{-3}$

Note: ± is the confidence interval at $p = 0.95$; the variants are designated as in Table 1.

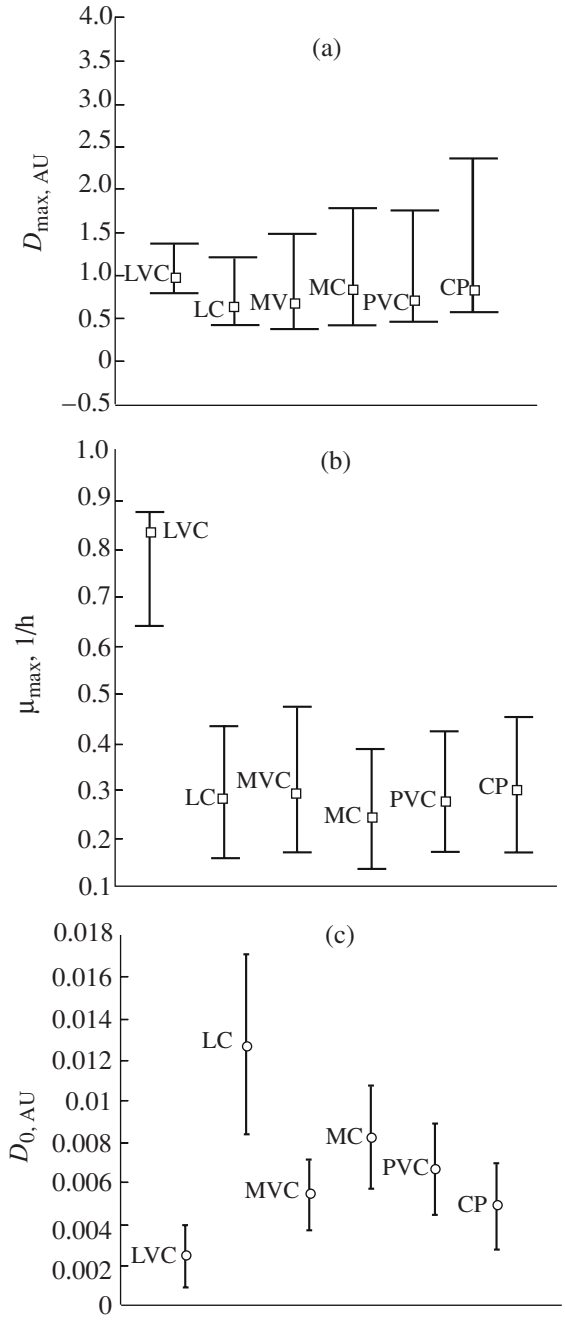

Fig. 3. Parameters characterizing the growth of microbial community on a set of substrates: a, maximum optical density in test wells, D_{max}; b, maximum specific rate of optical density increase μ_{max} (median, quartiles); c, initial optical density of tested samples, D_0 (arithmetical average and confidence intervals at $p = 0.95$). CP, composted peat; PVC, peat vermicompost; LC, leaf compost; LVC, leaf vermicompost; MN, manure compost; MVC, manure vermicompost.

$V_{basal} = 61.3 \pm 14.8$ μg C–CO_2/(g h); for LC, $V_{basal} = 45.9 \pm 9.0$ μg C–CO_2/(g h). Glucose application (10 mg/g) did not result in increased CO_2 emission. For LVC, $V_{max} = 60.0 \pm 11.3$ μg C–CO_2/(g h); for LC, $V_{max} = 47.2 \pm 12.7$ μg C–CO_2/(g h). Thus, a high level of mineralization activity was observed at the early stage of decomposition of leaf substrates, associated with the high content of relatively easily available nutrients. The presence of earthworms probably activates the mineralization process. Since the environments so rich in microorganisms have not been used for the calibration of the SIR method, recalculation of V_{max} to C_{micr} is incompetent without additional calibration experiments.

Assimilation spectrum of organic substrates

The spectrum of assimilation of different organic substrates by a microbial community supplements the information about the mineralization potential of microorganisms obtained by respirometric methods. Results of the analysis of the kinetics of the variation of optical density in the wells of the test plate are presented in Figure 3. Assuming direct proportionality between the respiratory rate of microorganisms and the biomass, one can consider the measured optical density D as an equivalent of microbial biomass. In this case, D_{max} (Fig. 3a) reflects the maximum microbial biomass obtained on a given test medium, μ_{max} (Fig. 3b) is the maximum specific growth rate of microorganisms on test media, and D_0 (Fig. 3c) is identical to the initial biomass of microorganisms growing on a given medium.

The D_{max} value averaged by all the substrates used for the microbial community of vermicomposts growing on test media does not differ from the value obtained for the microbial community of composts (Fig. 3a). At the same time, the specific growth rate of microorganisms (μ_{max}) on the test media in leaf vermicompost is much higher than in compost (Fig. 3b). For peat and manure substrates, the composts do not differ in this parameter from vermicomposts. The increase in the growth rate revealed for leaf composts supports the conclusion based on the analysis of respiration curves (Table 1): the presence of earthworms at composting may result in an increase of the portion of r-strategists in the microbial community. It may be associated with the fact that vermicomposting of leaves results in a more pronounced increase of their specific surface than in the case of manure and peat [17], while the total carbon content, on the contrary, decreases (Table 2).

The averaged values of the parameter D_0 (Fig. 3c) are lower in manure and leaf vermicomposts than composts. This fact can be explained by a lesser content of microorganisms capable of growth on the media for prototrophic microorganisms, because none of the test media contained the growth factors present, e.g., in peptone or yeast extract. D_0 values for peat compost and peat vermicompost are not reliably different, but the tendency to a higher D_0 value for the vermicompost is in agreement with the revealed difference for values of the microbial biomass determined by the method of substrate-induced respiration (Table 1).

The functional (trophic) diversity of microbial communities was assessed by calculation of the Shannon index for the parameter D_0. It was established (Fig. 4) that the biodiversity values of composts and vermicomposts were most different for the manure and peat substrates on day 44 of composting and in the end of composting (on day 112) for the leaf compost. The effect of worms on the functional biodiversity depended on the type of composted substrate. In vermicomposts of manure and leaves, the Shannon index was observed to decrease as compared with the composts without earthworms. The decrease of the functional Shannon index in manure vermicompost was recorded earlier [25], while the presence of earthworms in peat vermicomposts, on the contrary, enhanced the functional diversity of microorganisms. Thus, the greatest effect on biodiversity in more mature substrates (half-rotten manure, lowland peat) was observed after 1.5 months of incubation, while the differences in poorly decomposed substrate (leaves) increased abruptly at the very end of the experiment.

The effect of earthworms on microbial communities of soils [26] and vermicomposts [25, 27] has been investigated previously using the method of BIOLOG EcoPlates. However, the physiological profile at the community level obtained by this method was difficult for microbiological interpretation. In these works, the initial quantity of microorganisms forming the spectrum was not taken into account, because the optical density was analyzed at the end of incubation of the test plate. The fixed incubation time did not permit the measurement of color intensity, i.e., of the physiological response, at the same stage of growth for different substrates. At the moment of determination, the quantity of microorganisms in a certain well probably did not reach its maximum values, while in the other one, the maximum quantity had already been reached, or dying-off of the microorganisms had already begun. Single measurement is possibly too rough an instrument for community characterization and the obtained result is difficult for interpretation. The BIOLOG EcoPlates method does not take into account the growth rate of microorganisms, so the important information characterizing the microbial community is lost. The modification of this method used in the present work yields characteristics which are unambiguously interpreted in terms of microbiology: D_0, D_{max}, and μ_{max}.

In conclusion, reliable changes in the physiological characteristics of the microbial community of composts under the influence of worms can be stated. First of all, the total and growing biomass increases in peat-based vermicomposts. The portion of copiotrophs and *r*-strategists in vermicomposts increases apparently due to an increased flow of organic matter easily available to microorganisms, which is contained in earthworm coprolites and surface excretions. This conclusion was made on the basis of a decrease of the affinity of enzyme systems to a substrate by the example of glucose oxidation.

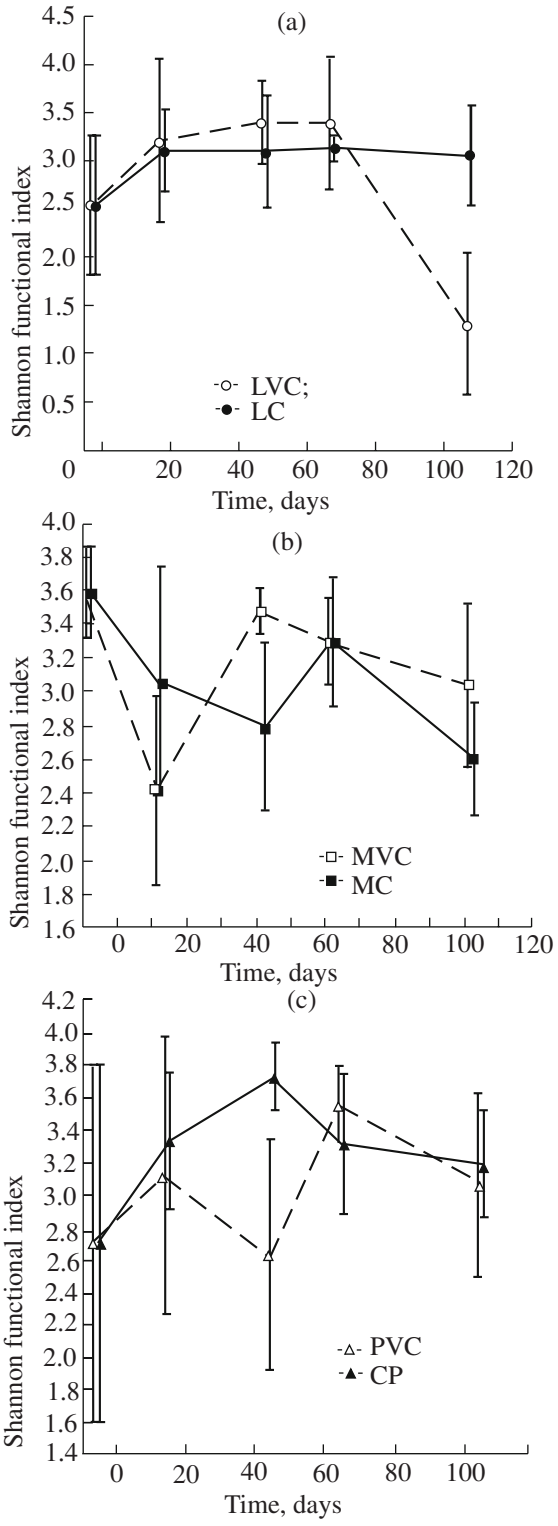

Fig. 4. The values of the Shannon functional index calculated by the spectrum (D_0): a, leaf substrate; b, peat substrate; c, manure substrate. CP, composted peat; PVC, peat vermicompost; LC, leaf compost; LVC, leaf vermicompost; MN, manure compost; MVC, manure vermicompost. Error bars show confidence intervals ($p = 0.95$).

The analysis of the assimilation spectrum demonstrated that the direction of the changes in the functional biodiversity of microbial communities, of specific growth rate, and of microbial abundance under the influence of earthworms depended significantly on the type of composted substrate. The results obtained by respirometric methods and by assimilation spectra give different information. The Wright–Hobbie method and the kinetic method were more suitable for deep ecological analysis of the objects (ecological strategies, physiological state of the biomass, and operation of enzyme systems). Determination of assimilation spectra is a universal method applicable for the carbonate-containing substrates and for the substrates with the high content of easily available organic matter (in these cases, respirometric methods cannot be used). Although the data were obtained for model objects, we believe that the relationships revealed are of universal character and the knowledge gained may be used for standardization of vermicomposting.

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