Effects of *Eisenia fetida* population size on *Lactuca sativa* growth and productivity in model ecosystems

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Artificial ecosystems are a convenient tool for studying the environmental effect on the functioning and inter-relation between different elements in a model biosphere. An increase in the productivity of artificial ecosystems can be associated with the introduction of natural decomposers (earthworms), which alter the qualitative and quantitative composition of the soil microbiota, accelerate the processes of organic matter mineralization, increase the suppressive activity of substrates, and positively affect plant growth and development. In laboratory experiments, we evaluated the effect of a biotic factor (*Eisenia fetida* population) on model system productivity (*Lactuca sativa* plants). Earthworms were introduced at 6, 12, 25, 37, and 62 individuals per kg substrate (peat and 10 % of cattle manure). At the experiment's finish, each plant's height, leaf area, biomass (fresh and dry), photosynthetic pigment content, and photosystem II photochemical activity parameters were measured; earthworms and cocoons were counted; and the earthworms were weighed as well as worm cast. It was found that earthworms reproduced successfully in all microcosms, increasing their population. The earthworm population's size in the microcosm affected the lettuce plants' vegetative growth and physiological and biochemical parameters. The earthworms also significantly affected lettuce productivity and photochemical quenching coefficients. The effect of earthworm population size had a classical ecological form and could be described by an optimum curve (Shelford's law of tolerance). Maximum plant productivity and vegetative growth were achieved with 25 and 37 earthworms per kg substrate. These data can be extrapolated to the biosphere's natural elements and can be used to develop artificial ecosystems and increase greenhouse system productivity.

Keywords: earthworm, lettuce, microcosm, photochemical activity of photosystem, photosynthesis, productivity, Shelford's law of tolerance.

УДК 57.02

Влияние численности популяции *Eisenia fetida* на рост и продуктивность *Lactuca sativa* в модельных экосистемах

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Использование искусственных экосистем является удобным инструментом для изучения влияния факторов окружающей среды на функционирование различных элементов в модельной системе и взаимосвязи между ними. Повышение их продуктивности может быть связано с введением в них естественных редуцентов (дождевых червей), которые изменяют качественный и количественный состав почвенной микробиоты, ускоряют процессы минерализации органических отходов, увеличивают супрессивную активность субстратов, положительно влияют на рост и развитие растений. В ходе лабораторных экспериментов установлено влияние биотического фактора (популяции дождевых червей *Eisenia fetida*) на продуктивность модельной системы (биомасса растений салата). Дождевых червей вносили в количестве 6, 12, 25, 37 и 62 особей/кг субстрата (торф и 10 % навоза крупного рогатого скота). В конце эксперимента измерены высота каждого растения, площадь листьев, биомасса (свежая и сухая), содержание фотосинтетических пигментов и параметры фотохимической активности фотосистемы II; учтено количество и масса дождевых червей, количество коконов, а также выход копролита. Установлено, что дождевые черви успешно размножались во всех микрокосмах, увеличивая свою популяцию. Численность популяции дождевых червей в микрокосме влияла на вегетативный рост и физиологические и биохимические параметры и продуктивность растений салата, а также коэффициенты фотохимического тушения. Влияние численности популяции дождевых червей на продуктивность модельной системы имело классическую экологическую форму и описано кривой оптимума (закон толерантности Шелфорда). Максимальная продуктивность растений и вегетативный рост достигались при 25 и 37 дождевых червях/кг субстрата. Эти данные могут быть экстраполированы на естественные элементы биосферы и использованы для создания искусственных экосистем и повышения продуктивности тепличных систем.

Ключевые слова: дождевой червь, латук, микрокосм, фотохимическая активность фотосистемы, фотосинтез, продуктивность, закон толерантности Шелфорда.

Model ecosystems differing in closure levels, size, complexity, components, and temporal boundaries are of particular interest to researchers because they provide information on ecological processes and biogeochemical cycles; interactions between species and populations; and the impact of abiotic, biotic, and anthropogenic factors [1–3].

Such systems can comprise different numbers of elements: from the simplest, comprising single producers, consumers, and decomposers, to complex closed multi-component ecosystems, such as controlled closed ecological life support systems [1]. Their use overcomes the problem of developing working ecosystem models to study ecological processes, create life support systems for space purposes, ensure a high quality of human life in extreme environmental conditions, solve pollution problems, and develop highly productive sustainable agriculture [3, 4]. Closed artificial ecosystems used to create autonomous high-tech biocomplexes for use in far-north regions and space expeditions deserve special attention [1].

Artificial ecosystems enable the study of interactions between the basic elements of any agro-system and solving of applied problems: increasing the productivity of plants included in these systems, controlling plant pathogens, and organic waste recycling [3, 5]. The solutions to these problems can be associated with introducing earthworms into model systems, natural decomposers that also change the qualitative and quantitative compositions of the soil microbiota and plant rhizosphere zone, increasing the antagonist species of soil phytopathogens and plant growth promoters. Therefore, introducing earthworms changes the system. Earthworms act as catalysts for two major 'supporting services', soil formation and nutrient cycling, which are prerequisites for other services [6]. Earthworms affect plant growth positively, play a major role in soil function, and are responsible for many ecosystem services [5–8]. The presence of earthworms or vermicompost alters plant morphology and pathogen resistance [6] and increases plant biomass by 23 % and yield by 25 % [8]. However, some reports show neutral or adverse effects of earthworms on plants [6, 7, 9]. Factors such as soil characteristics, plant species and physiology, and earthworms play important roles in this process [5]. Studies on earthworm-plant interactions may contribute significantly to a more comprehensive understanding of terrestrial ecosystems and the development of more environmentally friendly agricultural practices [7].

However, understanding how all these components function is necessary to optimise the functioning of the whole system, from photosynthesis (creating organic substances) to recycling. This knowledge will enhance understanding of the mechanisms and consequences of earthworm introduction, the size of their introduced population, and the time necessary for ecosystem productivity. Moreover, well-designed laboratory experiments with closed ecosystems and field experiments, coupling basic biological and soil science measurements, enable us to better understand how the system's mechanisms function and fill knowledge gaps.

There is much experimental research on the effect of worms such as *Aporrectodea caliginosa*, *Pontoscolex corethrurus*, *Metaphire guillelmi*, *Octolasion tyrtaeum*, and *Allolobophora chlorotica*.

However, there is insufficient information about the effects of *Eisenia fetida* on plants and their productivity in closed artificial systems.

Therefore, this study aimed to investigate the effect of initial *Eisenia fetida* populations introduced into an artificial model ecosystem on lettuce vegetative growth, productivity, and physiological parameters and the earthworms' characteristics and productivity.

Materials and research methods

The effect of earthworm population size (individuals per kg substrate) was investigated in laboratory experiments. The model ecosystems (microcosms) comprised a peat substrate into which the earthworms were introduced and lettuce plants were planted. Experiments were set up in plastic opaque experimental pots (2 L volume) and used deoxidised high-moor peat (80 % moisture) supplemented with 10 % cattle manure (80–90 % moisture; by weight) with a total substrate mass of 800 g. Substrate moisture was maintained at 80–85 % and temperature at 15–22 °C during the experiment [10].

The peat used in the experiments was obtained from the Ust-Bakcharsky peat deposit (Chainsky district, Tomsk region, Russia). Its substrate agrochemical characteristics were the following: pH (KCl) 6.9 ± 0.1, 0.65 ± 0.07 N-NH$_4$ g kg^{-1}, 0.16 ± 0.02 N-NO$_3$ g kg^{-1}, 1.06 ± 0.17 P$_2$O g kg^{-1}, and 0.35 ± 0.08 K$_2$O g kg^{-1}.

Adult *E. fetida* (Sav.) earthworms were collected from a laboratory where they were reared in controlled conditions (16 ± 2 °C, in the dark, and ~80 % moisture by mass) on a mixture of peat, vegetable rests, and cattle manure. The earthworm *E. fetida* is often used in vermicomposting due to its ability to decompose agricultural organic residues, adaptability to different environmental conditions, rapid reproduction, and effects on soil nutrient availability and plant growth [11, 12].

Earthworms were chosen for the experiment and test preparation as described in the paper [5]. Sub-adult individuals of similar size and developmental stage were selected. Earthworm weight (combined weight per treatment) was recorded at the beginning (m_0) and end (m_t) of the 61-day exposure. After seven days of substrate preparation, in all earthworm treatments, earthworms were introduced at 6, 12, 25, 37, or 62 individuals per kg substrate. At the experiment's end worm fecundity and worm cast were determined. Worm fecundity was calculated as the sum of hatching and non-hatching worms (cocoons) per adult. The substrate in all microcosms was sieved (2 mm), and worm cast was calculated as its percentage weight difference in microcosms with and without worms [10].

Control microcosms (without earthworms) were prepared and incubated simultaneously.

After seven days, five pre-germinated lettuce (*Lactuca sativa* L. var. *longifolia* variety 'Adamant') seeds were transferred to each microcosm. Plant growth was performed under controlled conditions: 8 kLq fluorescent lamps (110 μmol m^{-2}·s^{-1} photosynthetic photon flux density) for 12 h per day, and 20–22 °C (day) and 15–17 °C (night) temperatures.

The experiments comprised 64 days of growing plants (68 days from substrate preparation). Plant heights were repeatedly measured during the experiment to determine their growth rate. At the experiment's end (54 days after transplanting), each plant height, leaf area, and biomass (fresh and dry) were measured. The photochemical activity parameters of photosystem II, PS II, were measured on a dark-adapted plant leaf with a Junior-PAM fluorimeter (Heinz-Walz, Germany). A special leaf clip holder was placed on each leaf to maintain dark conditions for at least 30 min before taking a reading. The maximum potential quantum efficiency of PS II, Fv/Fm, the PS II effective quantum yield, Y(II), and the quantum yields of light-induced, Y(NPQ), and non-light-induced, Y(NO) non-photochemical fluorescence quenching (NPQ) were calculated with the WinControl-3 software for PAM fluorometers. After determining the photochemical activity parameters primary photosynthetic pigment content was also measured on the same leaf spectrophotometrically (Eppendorf BioSpectrometer fluorescence, Germany) [13].

The peat substrate in the pots was poured out, earthworms were carefully collected, and the substrate on their surface was removed after determining the plant characteristics. The numbers of earthworms (adults and juveniles) and cocoons were counted, and the earthworms were weighed (m_t was the weight of worms at the experiment's end).

All experiments were performed in three independent biological replicates of five plants per microcosm. All data were tested for homogeneity of variance and normality with the Kolmogorov-Smirnov test. The experimental data are presented in tables as medians ($M\bar{e}$) and figures as means with standard errors of the mean ($M \pm mM$). Differences between groups were assessed using nonparametric Mann-Whitney U tests, considering $p < 0.05$ as statistically significant.

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Results and discussion

The main characteristics of *Eisenia fetida* populations in the microcosms. The effect of *E. fetida* earthworm density in a closed system on lettuce growth and development on a peat-dung substrate without amended mineral nutrition was studied for the first time. Another original feature of this study was investigating the main characteristics and the initial population of earthworms in microcosms.

In our experiments, all introduced earthworms survived. We did not find earthworms in microcosms that did not have *E. fetida* introduced at the beginning of the experiment, indicating no contamination among microcosms. Earthworm abundance increased in all microcosms (Table 1). Earthworms show excellent survival, fecundity, and substrate processing in laboratory experiments. Adult earthworm biomass increased by 36.6 and 27.0 %, respectively, in microcosms with 6 and 12 earthworms, while it decreased by 9.5–28.0 % in the other microcosms.

The number of earthworms (adult and juvenile) at the experiment's end was higher in microcosms with larger initial population sizes. In microcosms with 62 earthworms, there was only a small increase in their final population size. However, in microcosms with 37 earthworms, their numbers doubled. Similarly, in microcosms with 67 earthworms, their numbers increased 1.2-fold. Impressively, in microcosms with 12 earthworms, their final numbers increased 4.6-fold. The earthworms laid similar numbers of cocoons during the experiments with initial 12–37 earthworms. However, the number of cocoons was two-fold lower in microcosms with 67 earthworms at the end of the experiment.

Previous studies focused on the effects worms had on plant development, heavy metal absorption, soil nitrogen content, and emission when introduced into the substrate. However, studying worm population and productivity parameters and vermicomposting processes is insufficient. Researchers usually counted the number and weight of worms at the beginning and end of the experiment and noted the presence of cocoons or worm casts. The choice of the number of worms for the laboratory experiment was not always justified and was calculated based on their population density in nature [5, 14, 15].

In our experiments, one main focus was studying the effect of the initial earthworm population on substrate volume. We also studied the size of the earthworm population for optimal coexistence in microcosms comprising earthworms and plants. Moreover, the efficiency of vermicomposting depends on earthworm biomass and population structure [12].

In addition, we included a seven-day rest period between substrate preparation and earthworm introduction and lettuce seedlings planting. This rest period was necessary for the preliminary adaptation of earthworms to the microcosm's conditions. Therefore, we attempted to create close to natural conditions for the system's functioning and optimal living conditions for all its components. Other studies planted plants before adding earthworms [15–17] and mineral nutrition to improve plant growth during the experiment [15]. In addition, they used natural soils as a substrate [9, 14–16, 18].

Almost all studies noted a relatively high survival rate of earthworms in experiments. However, many noted lower per-earthworm weights [10, 14–16], especially when using a poor soil [9]. Some studies have shown that adding artificial nitrogen to the substrate or couple cultivation of earthworms and nitrogen-fixing

Table 1

The main characteristics of *Eisenia fetida* population in the microcosms ($M\bar{e}$)

Microcosm	Worm weight of one individual, g		Worm number, psc.	Cocoon number, psc.	Fecundity, psc./worm	Worm cast, %
	m_0	m_t				
0 worms (Control)	0	0	0	0	0	0
6 worms	0.19	0.25	20.0	25	6.2	3.44
12 worms	0.19	0.24	55.2	34	6.1	8.04
25 worms	0.21	0.19	65.6	36	3.1	8.50
37 worms	0.19	0.16	76.5	34	1.9	2.33
62 worms	0.18	0.13	77.5	16	0.5	8.27

Note: m_0 – earthworm weight (combined weight per treatment) at the beginning of the experiment; m_t – earthworm weight at the end of the 61-day exposure.

Table 2

Effect of *Eisenia fetida* abundance on *Lactuca sativa* vegetative growth parameters ($M\bar{e}$)

Microcosm	Height, cm	Growth rate, cm per day	Leaf area, cm²	Productivity, kg/m²
0 worms (Control)	21.60	0.080	264.62	2.82
6 worms	20.90	0.082	306.94*	2.48*
12 worms	21.40	0.091*	331.78*	3.06*
25 worms	21.80	0.095*	351.70*	3.11*
37 worms	22.10	0.070*	385.96*	2.85*
62 worms	19.95	0.081	293.18*	1.58*

*Note (here and further in tables 3, 4): * – differences from a microcosm without earthworms are significant at $p < 0.05$.*

plants increased earthworm biomass, survival, and reproduction [12]. In our study, decreases in *E. fetida* reproductive activity with increasing initial abundance in these ecosystems were likely due to a lack of food resources (10 % manure in a peat substrate), which acted as a limiting factor. Therefore, according to our results, the experimental conditions (microcosms) favoured *E. fetida* development and reproduction. In addition, this earthworm species is well-suited and adapted to microcosms with lettuce maintained under abiotic conditions.

Effect of *Eisenia fetida* on *Lactuca sativa* vegetative growth parameters. According to our results, introducing *E. fetida* earthworms in peat substrate stimulated *L. sativa* productivity in a pot laboratory experiment (Table 2). The plant growth rate in microcosms with earthworms was significantly higher than in those without earthworms ($p < 0.05$). Plant leaf areas were 11–45 % larger in microcosms with earthworms than without them. The earthworms also significantly affected lettuce productivity.

Earthworm introduction (12–37 earthworms per microcosm) tended to increase plant height. The more earthworms there are in the microcosms, the greater stimulating effect on the lettuce plants, up to a certain limit: 37 individuals per kg substrate (2102 individuals per m²). Further increases in earthworm initial population size had adverse effects, inhibiting lettuce plant growth and productivity. In our opinion, this effect may be due to the competition of earthworms and their microflora with plants for the limited nutrient content in the substrate and damage to their root system associated with earthworm movements in the root zone. Possible damage to plant roots due to earthworm movement in the soil was found in *Arabidopsis thaliana* plants grown in closed-space microcosm. Multiple root injuries were caused by earthworms, which led to wounding stress [9].

Few studies have examined the effects of earthworm population size on plant growth in artificial model systems in the laboratory. It was reported that worms had a more significant effect on plant growth and development at a population density of >400 individuals per m², corresponding to ≥7 individuals per kg [19]. Many reports on the beneficial roles of earthworms in model systems support our results and the positive effects of earthworms and vermicompost on plants and their productivity [9, 8, 15, 16, 18, 20]. Due to earthworm activity, plants received more nutrients, and soil structure improved [7, 10, 20, 21].

Therefore, our data show that the beneficial role of earthworms on plants depends on population size. An increase in the number of earthworms above the established limit (>37 individuals per kg substrate) had adverse effects. This limit must be accounted for when developing terrestrial model systems comprising producers (plants) and decomposers (earthworms), where the population size of the latter should be regulated both when functioning systems are established and during their long-term maintenance.

Effects of *Eisenia fetida* on *Lactuca sativa* photosynthesis. Photosynthesis is an essential process on which the productivity of systems ultimately depends. Maintaining high photosynthetic pigment levels is an important indicator of the plant's physiological state under stress [22]. Our experiments showed that introducing *E. fetida* earthworms into the microcosms increased photosynthetic pigments in lettuce plants (Table 3). Their chlorophyll (*a* and *b*) and carotenoid contents were higher in microcosms with than without earthworms. The chlorophyll *a* content in lettuce leaves increased by 26–82 %, chlorophyll *b* – by 32–80 %, and carotenoid – by 6–65 %, depending on the population size of earthworms in the substrate. Increases in the earthworm population size in the microcosm up to 37 earthworms per kg substrate significantly increased pigment content. The introduction of >37 earthworms decreases pigment content

Table 3

Effect of *Eisenia fetida* abundance on *Lactuca sativa* leaves'
photosynthetic pigment content (mg/g fresh weight) (*Mē*)

Microcosm	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
0 worms (Control)	0.57	0.25	0.17
6 worms	0.72*	0.37*	0.18
12 worms	0.83*	0.38*	0.23*
25 worms	0.86*	0.40*	0.25*
37 worms	1.04*	0.45*	0.28*
62 worms	0.72*	0.33	0.22*

Table 4

Effect of *Eisenia fetida* population on *Lactuca sativa* leaf fluorescence values (*Mē*)

Microcosm	Maximum PS II efficiency, Fv/Fm	Effective PS II quantum yield, Y(II)	Quantum yield of non-regulated non-photochemical fluorescence quenching, Y(NO)	Quantum yield of regulated non-photochemical fluorescence quenching, Y(NPQ)
0 worms (Control)	0.836	0.723	0.165	0.104
6 worms	0.825	0.718	0.163	0.112
12 worms	0.838	0.737	0.157	0.101
25 worms	0.848	0.734	0.163	0.098
37 worms	0.850	0.742	0.153	0.104
62 worms	0.835	0.715	0.167	0.115

below the maximum values, making these microcosms less effective in increasing plant productivity.

The chlorophyll content in plant tissues is determined primarily by the presence and ratio of nutrients in the substrate on which plants grow. Therefore, it can be concluded that worms beneficially increase or change the ratio of the nutrients and trace elements in the substrate for plant cultivation [8, 10, 20]. Increases in photosynthetic pigments in the presence of earthworms are likely due to the enhanced availability of nutrients such as N, P, K, Mg, Fe, and Cu. These are required to produce chlorophyll, which is essential for light harvesting and its conversion into chemical energy via photo assimilation [8, 23].

Our findings are supported by the fact that earthworm supplementation (*E. fetida*) increased carotenoid, anthocyanin, flavonoids and xanthophyll content in 30- and 60-day-old *Brassica juncea* L. plants [24]. The presence of earthworms in the substrate positively affects plants and their pigment content and the release of small amounts of vermicompost, vermiwash, and humic acids from vermicast into the substrate [11, 20, 24]. During vermicomposting, earthworm-mediated decomposition releases plant-available forms of N, P, and K from organic residues as they ingest organic material,

grind it with a gizzard, and pass it through their gut [12]. Vermicomposting is a biological decomposition process involving the interaction of earthworms and microorganisms to convert different organic waste types into nutrient-rich manure.

In our experiments in microcosms with earthworms (12–62 individuals per kg substrate), we observed a carotenoid content of 29–65 %, depending on the number of earthworms. The highest carotenoid content was in microcosms with 27 earthworms. This outcome can lead to partial infections and greater stress resistance in plants associated with the activation of nonspecific antioxidant systems. Since carotenoids are involved in quenching reactive oxygen species and reducing oxidative stress intensity, maintaining their levels in plants is one factor in plant stress resistance [22]. Carotenoids also contribute to PS II complex formation in the membrane [25]. Therefore, our result may partially indicate greater stress resistance in plants associated with the activation of nonspecific antioxidant systems.

Photosynthesis is an essential process in plants. Most stressors change the course of photosynthetic processes, increasing NPQ and affecting other photosynthetic parameters [26]. The PS II photochemical activity parameters were determined to assess the effect of earth-

worms on lettuce plant photosynthetic function. The Fv/Fm value reflects the potential PS II quantum yield and is a sensitive indicator of the photosynthetic system's functional state [22]. The PS II photochemical activity parameters were studied to assess the effects of introducing earthworms into the substrate and their population size on the lettuce leaf photosynthetic function (Table 4).

In our experiments, introducing earthworms into microcosms caused an increase in Fv/Fm compared to microcosms without earthworms, indicating that the substrate's increased earthworm population did not damage PS II in chloroplasts in lettuce leaves. Similarly, the absence of significant changes in Y(II), Y(NPQ), and Y(NO) values indicates no pronounced stress response of plants to the presence of earthworms. Lettuce plants grown in microcosms with different numbers of earthworms showed similar maximum efficiency and effective PS II quantum yield, with slightly higher values at 12–37 worms per kg substrate. A significant increase in the photochemical quenching coefficient in the experimental microcosms was observed, indicating more open PS II reaction centres. The non-photochemical quenching index tended to be higher in plants grown with earthworms than without them. However, this index was significantly ($p < 0.05$) lower in microcosms with 25 earthworms (0.418) than without earthworms.

Therefore, introducing earthworms into the microcosm did not adversely affect the photosynthetic function of lettuce leaves. It even had a protective effect on the PS II photochemical activity, except with 25 earthworms in the microcosm.

The overall pattern of effects from introducing earthworms, depending on the initial population size, on the lettuce development in microcosms. We noted that the effects of introducing earthworms into the substrate on lettuce generally obeyed Shelford's law of tolerance. Its optimal range is determined by the organism's tolerance to that factor.

A convex curve with an extremum (optimum curve) describes the influence of environmental factors on an organism. This pattern is demonstrated in Figure using the relationship between the mass distribution of lettuce plants and the number of earthworms in the substrate of model ecosystems as an example. This figure shows that the convex curve's extremum corresponds to the number of worms in the substrate at which the maximum lettuce masses are observed (20–40 individuals per kg substrate). Including 37 individuals in the substrate affected the biomass of lettuce plants the most, with a 24 % increase in this microcosm compared to plants in microcosms without earthworms.

Other plant vegetative growth parameters, pigment content, and fluorescence indices can
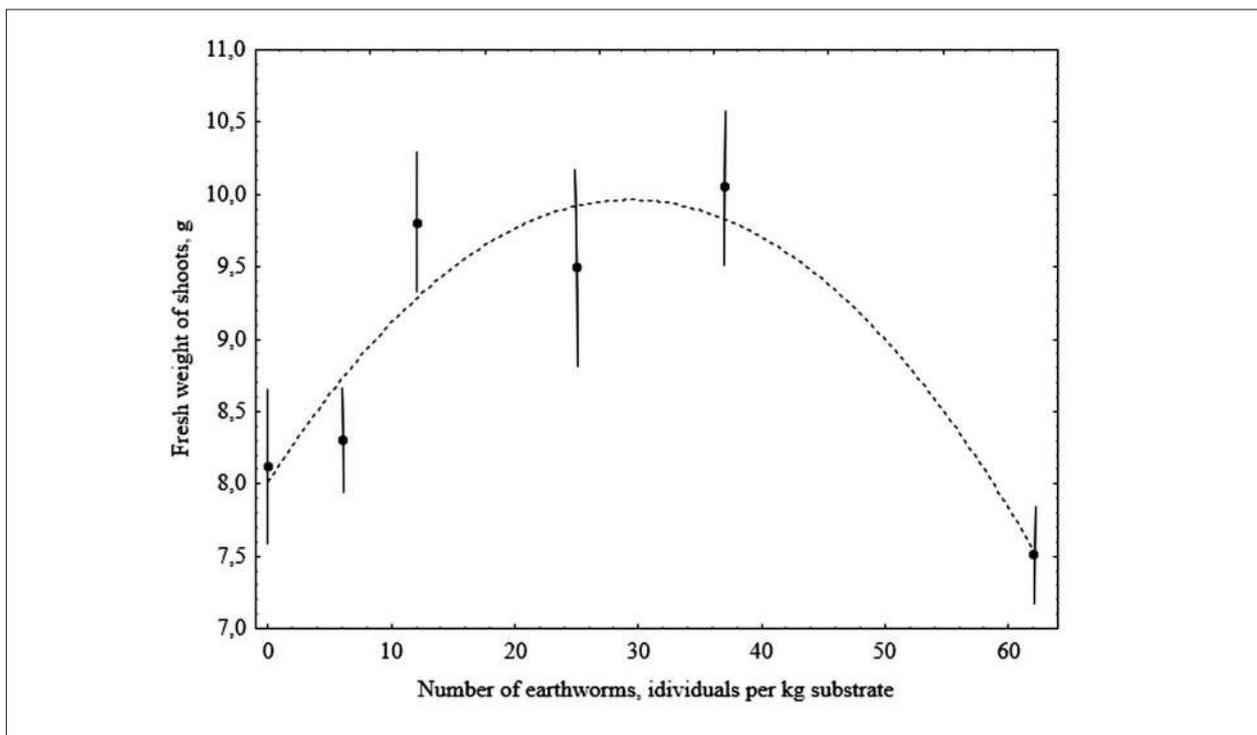

Fig. Effect of *Eisenia fetida* population size on *Lactuca sativa* fresh weight in the microcosm ($M \pm mM$)

be similarly described. According to the data presented, the optimum zone also includes earthworm numbers in the indicated range. The maximum productivity and plant development values were found with 25 and 37 earthworms per kg substrate. Therefore, the effect of the number of earthworms introduced into the model system's substrate had a classical ecological form. It can be described using formulae for optimum (tolerance) curves, the estimation of which can significantly affect the stabilisation and increase in plant productivity in artificial ecosystems.

Conclusion

Successful coexistence of all ecological factors was observed in artificial model systems (microcosms) comprising a substrate (peat and manure), producers (lettuce), and decomposers (earthworms). With all initial population sizes (6–62 earthworms per kg substrate), earthworms reproduced successfully, increasing their population. The size of the earthworm population in the microcosm affected the development and physiological and biochemical parameters of lettuce plants. The classic Shelford's tolerance curve generally characterises population size dependence for earthworms. Plants showed maximum productivity and their highest physiological and biochemical parameters in microcosms with 12–37 earthworms per kg substrate. This finding shows that in artificial biosphere-like systems combining production and reduction processes it is necessary to study the optimal densities of reducers and regulate them during long-term operation for the successful coexistence of all model system components.

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