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The first data regarding the effect of the exotic Eisenia ventripapillata (Oligochaeta, Lumbricidae) on the cation composition of soils in Western Siberia

Kirill A. Babiy¹, Stanislav Yu. Kniazev², Anna S. Abramenko³, Elena V. Golovanova⁴

1, 2, 3, 4 Omsk State Pedagogical University, Omsk, Russian Federation

1, 2 labinvert@omgpu.ru

3 abramenko_97@mail.ru

4nilseb@omgpu.ru

Summary. We have performed laboratory experiments to determine the impact of earthworms (exotic Eisenia ventripapillata and peregrine Aporrectodea caliginosa) on the water-soluble forms of ammonium, potassium, sodium, magnesium, calcium in Calcic Chernozem, Greyic Phaeozem, and Haplic Chernozem, which are widespread in the south of Western Siberia. The differences between the impacts made by the two above-mentioned species have been estimated according to the changes they make in the initial cation concentrations in soil. For E. ventripapillata, the obtained evidence regarding the influence of this species on soil characteristics is first-ever data. We found that E. ventripapillata and A. caliginosa changed the content of all cations in the soils under this study, but the nature of these changes varied from one soil type to another. In contrast to A. caliginosa, E. ventripapillata reliably increased the content of potassium and calcium ions in Calcic Chernozem. In comparison with the A. caliginosa influence, the E. ventripapillata variants reliably differed in the content of ammonium, potassium, and sodium ions in Greyic Phaeozem. In Haplic Chernozem, the ammonium, magnesium, and sodium ions content in the E. ventripapillata variants was reliably lower than in the A. caliginosa variants. Thus, the observed difference between native and exotic species in the effects on the content of available cations can trigger changes in the mineral nutrition of plants growing in the examined soils.

The article contains 2 Figures, 1 Table and 49 References.

Keywords: earthworms, exotic species, *Eisenia ventripapillata*, soil cations, laboratory microcosms; capillary electrophoresis

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Первые данные о влиянии экзотического Eisenia ventripapillata (Oligochaeta, Lumbricidae) на катионный состав почв Западной Сибири

Кирилл Анатольевич Бабий¹, Станислав Юрьевич Князев², Анна Сергеевна Абраменко³, Елена Васильевна Голованова⁴

1, 2, 3, 4 Омский государственный педагогический университет, Омск, Российская Федерация

1, 2 labinvert@omgpu.ru

3 abramenko_97@mail.ru

4 nilseb@omgpu.ru

Аннотация. В лабораторном эксперименте исследовано влияние дождевых червей: экзотического Eisenia ventripapillata и перегринного Aporrectodea caliginosa на водорастворимые формы аммония, калия, натрия, магния, кальция в Calcic Chernozem, Grevic Phaeozem и Haplic Chernozem, распространенных на юге Западной Сибири. Различия во влиянии между видами оценивалось на основании изменения ими начальных концентраций катионов в почве. Для E. ventripapillata полученные данные о воздействии вида на характеристики почвы приводятся впервые. Установлено, что E. ventripapillata и A. caliginosa изменяли содержание всех катионов в исследуемых почвах. При этом в каждом типе почв изменения носили специфический характер. В Calcic Chernozem E. ventripapillata достоверно увеличивал содержание ионов калия и кальция в отличие от A. caliginosa. В Greyic Phaeozem варианты с E. ventripapillata достоверно отличались по содержанию ионов аммония, калия и натрия. В Haplic Chernozem содержание аммония, магния и кальция в вариантах с E. ventripapillata было достоверно ниже, чем в вариантах с A. caliginosa. Таким образом, наблюдаемая разница между перегринным и экзотическим видами во влиянии на содержание доступных катионов может спровоцировать изменения в минеральном питании растений, произрастающих на исследованных почвах.

Ключевые слова: дождевые черви, экзотические виды, *Eisenia ventripapillata*, катионы почвы, лабораторные микрокосмы, капиллярный электрофорез

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Introduction

The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) identified five major global drivers of environmental change. One such factor is invasive species [1]. Invasive plant, insect, and vertebrate invasions into terrestrial ecosystems are thoroughly studied [2]. At the same time, soil invasions are invisible and hard to detect, so these processes are less highlighted in the relevant literature on the subject. Nonetheless, these invasions result in ecosystem shifts that are as serious as those caused by terrestrial invasions [3-4]. Earthworm invasions are a striking example of this process [5].

Changes in climatic conditions [6], including soil temperature rise [7], facilitate the range expansion of earthworm species [3, 8-9] and enhance the intensity of exotic earthworm species' invasions of new territories [9].

It has been reported that the increase of the average winter temperatures allowed earthworms of the genus Amynthas to expand their range into new areas [6, 9]. The appearance of exotic Lumbricus terrestris Linnaeus, 1758 in Romania is also considered to be related to climatic changes [10]. Likewise, the soil temperature rise is common in Russia [11]; Western Siberia is characterized as a region of accelerated warming [12]. For example, the soil freezing depth has decreased by 40 cm in the south of Western Siberia [13, 14]. This factor also contributed to the changes in the earthworm species composition in the area. According to Vsevolodova-Perel's data [15], ten species and two subspecies of Lumbricidae are distributed in the lowland territory of Western Siberia. The most recent survey, however, [13] lists 18 species and 4 subspecies for the same area. Earthworms are classified as ecosystem engineering organisms because they modify soil system functioning [16]. This impact can be both direct and indirect [17]. One effect caused by earthworms is shifting the chemical composition of the soil. The knowledge of this effect is important from several perspectives, especially when earthworms invade new territories, and their effects on soil differ from the impact made by the native earthworm species inhabiting the area.

The invasions of endogeic earthworms can most drastically modify soil chemical composition [18-19], nutrient redistribution [20], and the availability of nutrients (Ca, K, Mg, Na, NH₄) for plants [21-22] as earthworms ingest and transform a large amount of soil [23]. The exotic endogeic *Pontoscolex corethrurus* (Müller, 1857) changed the Mg, Ca, K, NH₄ content and pH in Colombian soils compared to the endogeic earthworms already inhabiting the area [24]. In addition, the exotic endogeic *Aporrectodea caliginosa* (Savigny, 1826) and *Octolasion lacteum* Örley, 1881 changed the ammonium content and pH in New Zealand soils, whereas the native species did not [25]. The directions of change caused by exotic species invasions in both cases depended on the soil type [24-25].

A. caliginosa is one of the most common endogeic earthworm species in the south of Western Siberia. At present, this species is widely distributed in the

natural habitats throughout Omsk Oblast [13], in the southern taiga of Tyumen Oblast [26], and the forest-steppe of the Ob region [27]. The endogeic *Eisenia ventripapillata* Perel 1985 was first recorded in Omsk Oblast in 2018. Its range in Omsk Oblast is similar to that of *A. caliginosa*, and in several habitats the two species are found in sympatry [13, 14]. According to the Russian earthworm checklist, this species has not been registered in the country yet [15]. *E. ventripapillata* was registered in the Rudny Altai Mts, East Kazakhstan [28], and it was considered endemic to that area. Currently, no relevant publications describing the biology, ecology, and environmental impact of *E. ventripapillata* are available.

The impact of *E. ventripapillata* on the availability of soil cations can differ from that of *A. caliginosa*. The ground for such assumption is the outcomes of invasions of exotic endogeic earthworms in Colombia [24] and New Zealand [25], where soils had already been inhabited by endogeic earthworms before the invasions. As a result, the nutrient availability changes can give a competitive advantage to certain plant species [20] and reduce plant species diversity in communities [4].

This research aims to investigate the changes in the availability of cations $(NH_4^+, K^+, Na^+, Mg^{2+}, Ca^{2+})$ in three soil types of Western Siberia colonized by *E. ventripapillata* and to perform a comparative study of the impact of the exotic *E. ventripapillata* and peregrine *A. caliginosa* on the availability of soil cations.

Materials and methods

Two endogeic earthworm species were used in the experiment: exotic E. ventripapillata and peregrine A. caliginosa. Mature individuals were collected in the vicinity of Chernoluchie village located in Omsk District, Omsk Oblast (55°16'33" N, E 73°02'35" E). Three soil types were used in the study: Calcic **Chernozem** (Cal) (Sand 20%; clay 34%; silt 27%; humus 6.0%; pH 7.52; NH₄⁺ 0.33 mg/kg; K+ 4.55 mg/kg; Na+ 88.8 mg/kg; Mg²⁺ 8.84 mg/kg, Ca²⁺ 39.3 mg/kg), Greyic Phaeozem (Gre) (Sand 11%; clay 61%; silt 17%; humus 3.5%; pH 6.03; NH₄⁺ 3.9 mg/kg; K⁺ 41.9 mg/kg; Na⁺ 4.48 mg/kg; Mg²⁺ 4.35 mg/kg, Ca²⁺ 26.2 mg/kg), Haplic Chernozem (Hap) (Sand 18%; clay 44%; silt 18%; humus 5.2%; pH 6.86; NH₄⁺ 1.07 mg/kg; K⁺ 17.2 mg/kg; Na⁺ 8.63 mg/kg; Mg²⁺ 8.63 mg/kg, Ca²⁺ 47.8 mg/kg). The sampling sites for the above-mentioned soil types were as follows: Hap – the research field station of the Omsk Ecological Station of Young Naturalists, Omsk City (54°58'50" N, 73°18'10" E); Cal – the OmSPU research field station, Omsk (55°02'38" N, 73°22'52" E); Gre – the vicinity of Chernoluchie village, Omsk District, Omsk Oblast (55°16'33" N, 73°02'35" E). The soil was dried and sifted using a 4-mm mesh sieve to remove macrofauna.

Two-liter plastic containers with perforated lids were employed for the experiment. Each container was filled with 1.6 liters of soil. Leaf litter of *Populus tremula* L., 1753 dry leaves weighing 8.00±0,05 g was placed on the soil surface for the approximation to natural conditions. Five individuals of a

single earthworm species were placed into each container. The average earthworm biomass in the microcosms was 4.10 ± 0.20 g/vessel for *A. caliginosa* and 3.99 ± 0.26 g/vessel for *E. ventripapillata*. Before the start of the experiment, the earthworms were kept for two days in the containers with the same soil which was added to the microcosms. Microcosms without earthworm treatment were used as the control. Nine experiment variants were performed; five replicates of each variant were analyzed. All microcosms were kept in constant climate chambers at 12°C. The soil moisture content was maintained at 40% with deionized water. In 90 days, the soil samples were taken and dried at 60 °C.

The NH₄⁺, K⁺, Na⁺, Mg²⁺, Ca²⁺ content in the soil samples was determined with a capillary electrophoresis system "Capel 104T" (Lumex Instruments, Russia). The preparation of soil samples and aqueous soil extracts, the analysis of cation content were conducted following the Lumex Method M 03-08-2011 standardized technique. The conditions for conducting the analysis were as follows: a capillary with an effective length of 60 cm and a diameter of 75 μm; sample injection by a pressure of 30 mbar×sec; electric power: 25kW; temperature: 20 °C; photometric detector wavelength: 254 nm. The leading electrolyte for cations contained benzimidazole, tartaric acid, and 18-crown-6. The pH values of the aqueous extracts were measured using an "ANION-4100" pH-meter (SPE Infraspak-Analit, Russia) with an "ESK-10301" glass combination electrode (soil to water ratio was 1:5, ISO 10390). The experimental data were processed with the Chrom&Spec software for Windows.

All data were converted using the natural logarithm response ratio (LnRR) where LnRR (variable) = ln (coprolite property / control soil property). LnRR < 0 implies a negative effect of earthworms on nutrient availability in each soil type, whereas LnRR> 0 implies their positive impact on nutrient availability. The normality of the data was evaluated by applying the Shapiro-Wilk test with Statistica 13.0 (StatSoft Inc., United States). The principal component analysis (PCA) was performed using the PAST 4.05 statistical software package [29] with a matrix of 45 samples and 6 variables. This method permits visualizing the data of a smaller set of variables, but at the same time, it retains maximum information from the original set of variables. The PCA results were presented as two-dimensional plots using the first two principal components with higher data variability. A two-factor ANOVA was employed to evaluate the effect of earthworms and the initial soil characteristics on the changes to cation availability. The reliability of the influence exerted by earthworms on the shifts in cation availability in comparison with LnRR = 0 and the differences in the effects caused by the species in each soil type was checked using Student's t-test. ANOVA and t-test were performed utilizing the Statistica 13.0 software package (StatSoft Inc., United States).

Results and discussion

Changes in the chemical composition of soil driven by exotic earthworms are registered in various regions of the world [18-19, 25]. This is a threat to the

stability of ecosystem functioning [9, 25]. In Russia, this problem remains underexplored, though it is reliably known that exotic earthworm species [30] are presented in Western Siberia [31], the European part of Russia [8], and in the Kamchatka Peninsula [32].

According to the results of this research, *E. ventripapillata* is able to change the availability of cations in all three studied soil types. This species lowered the soil pH and the content of accessible ammonium, potassium, and sodium in most soils, as well as magnesium in Gre. However, an increase in potassium and calcium accessibility was identified in Cal. Additionally, an increase in sodium availability was observed in Hap. The nature of the changes in cation availability depended on the soil type (Fig. 1).

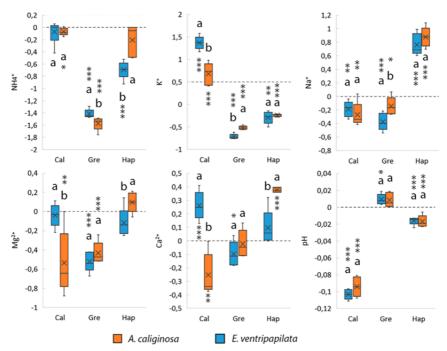


Fig. 1. Box-plot of the intensity of earthworm species effects (LnRR = Ln (variant / control) on the soil cation composition. The comparisons within the earthworm species are in lower-case letters. The variants with different letters are reliably dissimilar (p ≤ 0.05). The dashed line represents the zero impact of earthworms (LnRR = 0). The significant variation from 0 was verified with a one-sample t-test, and it is indicated with asterisks («*»: p <0.05; «**»: p <0.01; «**»: p <0.001). Cal - Calcic Chernozem, Gre - Greyic Phaeozem, Hap - Haplic Chernozem.

The PCA shows that *E. ventripapillata* and *A. caliginosa* modified the soil composition differently. The nature of these changes depended on the soil type for all cations (Fig. 2). Axis 1 explains 62.4% of the total inertia. This axis is positively correlated with NH_4^+ (r=0.92), K^+ (r=0.95) and, to a lesser extent, with Mg^{2+} (r=0.40) and Ca^{2+} (r=0.28). It is also negatively correlated with pH

(r=-0.93). Axis 2 explains 29.6% of the total inertia. It is positively correlated with Na $^+$ (r=0.99), Mg $^{2+}$ (r=0.72), and Ca $^{2+}$ (r=0.65), but it is negatively correlated with K $^+$ (r=0.27).

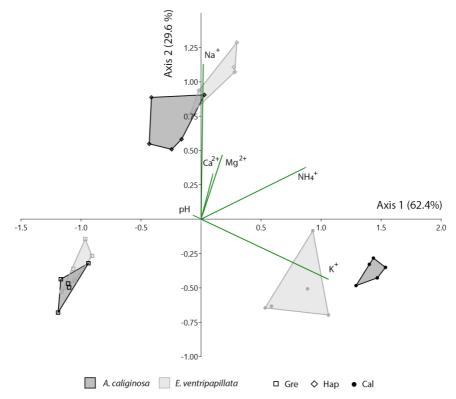


Fig. 2. PCA biplot of five different soil cations and pH. The factorial planes and the corresponding correlation vectors (superimposed on the plane) of the two earthworm species' influence on the changes in the soil cation composition were explored using the PCA method. Cal - Calcic Chernozem,

Gre - Greyic Phaeozem, Hap - Haplic Chernozem.

According to the multivariate ANOVA (Table), the soil type factor affected changes in all cations (p<0.001), and the earthworm species influenced the concentration change of K⁺ (p<0.01). The factor interaction was associated with changes in NH₄⁺, K⁺, Mg²⁺, Ca²⁺ (p<0.001). Our results correspond to the conclusions drawn by other researchers stating that both soil type and the original content of elements determine the processes related to elemental soil composition changes caused by earthworms [21, 33]. This holds true for exotic endogeic *A. caliginosa* and *O. lacteum* in New Zealand [25] as the nature of their impact on the cation content also depends on the properties of soil.

ANOVA for each soil type (Fig. 1) demonstrates that the impact of the exotic *E. ventripapillata* on the water-soluble NH_4^+ , K^+ , Mg^{2+} , Ca^{2+} content differed from the effects caused by *A. caliginosa* (p<0.05) in most soils. The difference in the change of the Na^+ availability was only in Gre (p<0.05).

Multivariate ANOVA for testing the influence of the factors on the changes in the ion composition of three soil types with the LnRR index

	NH ₄ ⁺	Na ⁺	K^{+}	Mg^{2+}	Ca ²⁺	рН
Species	4.03	9.31**	2.09	1.02	1.73	0.74
Soil	220.00***	422.70***	180.20***	18.00***	20.60***	533.60***
Type×Species	10.80***	31.80***	2.45	11.70***	35.78***	1.40

Note. «*»: p <0.05; «**»: p <0.01; «***»: p <0.001.

The specific biological features of earthworm species can explain the observed differences in the Ca²⁺, Mg²⁺ content, and pH in the soil. Calcium slowly returns to the soil in a form available to plants [34]. Earthworms contribute to an increase in the availability of calcium ions by producing calcium carbonate granules [34-35]. It is a species-specific process [36], which explain the difference in the changes of the Ca²⁺ content in the soil caused by *E. ventripapillata* and *A. caliginosa* in our research. The change in the magnesium content shows a pattern similar to calcium because earthworms' gut can be the major route for the release of these cations [37].

In our study, the soil pH after earthworm treatment differed from the pH of the original soil (Figure 1), but there is no significant difference between *E. ventripapillata* and *A. caliginosa*. Calcium carbonate production by earthworms can account for the pH increase in Gre (*A. caliginosa* p<0.05) which was less alkaline than the other soil types. *E. ventripapillata* and *A. caliginosa* decreased the soil pH in Cal and Hap (p<0.001) due to the activation of the nitrification process which leads to the release of protons [38]. Earthworm-driven pH decrease is rarely observed [33]; more often, earthworms contribute to soil alkalinization [39].

There is an evidence that earthworms stimulate the development of several functional groups of soil bacteria, including Proteobacteria, Actinobacteria, Firmicutes u Acidobacteria [40]. Consequently, it is correct to assume that the change in the availability of nutrients is related to the metabolic activity of bacteria and not the direct impact of earthworms [40-41]. Several authors note that the soil K⁺ u Na⁺ content is formed by microbial processes, including those occurring in earthworm gut [42]. In this research, the change in the K⁺ content caused by E. ventripapillata differed from the effects produced by A. caliginosa (p<0.001) (Fig. 1). It can be explained by the specific features of the earthworm gut microflora and the soil aggregation process because they actively modify the microbial composition of soil and depend on the earthworm species identity [43-44]. It is also known that the availability of K⁺ decreases when pH increases and vice versa [45], which was also confirmed by our data (Fig. 1). The availability of Na⁺ also negatively correlates with the pH value [45]. However, our study did not reveal such dependence. Supposedly, the impact of microbiota is more significant, and it forms the species specificity (Fig. 2) [46]. At the same time, the increase in the water-soluble Na⁺ form in the Hap soil (p<0.001) coincides with the data obtained by Wu [37] while studying the P. corethrurus impact on soil.

It is reported that a rise in the NH₄⁺ content is associated with earthworm presence due to the increased mineralization of organic nitrogen [47]. In our study, earthworms either didn't change the NH₄⁺ content (Fig. 1), which conforms to the results obtained by Bohlen & Edwards [48] or decreased the NH₄⁺ content due to bacterial oxidation. The difference between *A. caliginosa* and *E. ventripapillata* (Fig. 1, 2) confirms the previously revealed dependence of soil nitrogen dynamics on the earthworm species [41]. This can be related to the species-specific difference of the gut bacterial composition and the earthworms casts responsible for nitrogen transformation [49].

Conclusions

The effects of E. ventripapillata mainly contribute to the lower availability of the water-soluble forms of ammonium, potassium, and sodium in soils. The content of available water-soluble cation forms in the soil after E. ventripapillata treatment differs from the impact made by A. caliginosa; the nature of these dissimilarities depends on soil characteristics. The potential replacement of A. caliginosa, a species that is widespread in Western Siberia, by the exotic E. ventripapillata species can cause a shift in the content of water-soluble K^+ , Na^+ , Mg^{2^+} , Ca^{2^+} μ NH_4^+ in soils.

As the soil environment characteristics are much more diverse under natural condition than in laboratory settings, to fully understand the changes in nutrient availability related to *E. ventripapillata* invasion, it is necessary to carry out field research using various soil types and consider not only specific earthworm species but also earthworm communities.

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Information about the authors:

Babiy Kirill A, Junior researcher, Research laboratory of systematics and ecology of invertebrates, Omsk State Pedagogical University (Omsk, Russian Federation).

E-mail: labinvert@omgpu.ru

ORCID ID: https://orcid.org/0000-0001-7735-9052

Kniazev Stanislav Yu, Researcher, Research laboratory of systematics and ecology of invertebrates, Omsk State Pedagogical University (Omsk, Russian Federation).

E-mail: labinvert@omgpu.ru

ORCID ID: https://orcid.org/0000-0003-0031-0618

Abramenko Anna S, Junior researcher, Research laboratory of systematics and ecology of invertebrates, Omsk State Pedagogical University (Omsk, Russian Federation).

E-mail: abramenko 97@mail.ru

Golovanova Elena V, Cand. Sci. (Biol.), Senior Researcher, Research laboratory of systematics and ecology of invertebrates, Omsk State Pedagogical University (Omsk, Russian Federation).

E-mail: nilseb@omgpu.ru

ORCID ID: https://orcid.org/0000-0003-0871-9274

The Authors declare no conflict of interest.

Информация об авторах:

Бабий Кирилл Анатольевич, м.н.с., Научно-исследовательская лаборатория систематики и экологии беспозвоночных, Омский государственный педагогический университет (Омск, Россия).

E-mail: labinvert@omgpu.ru

ORCID ID: https://orcid.org/0000-0001-7735-9052

Князев Станислав Юрьевич, научный сотрудник, Научно-исследовательская лаборатория систематики и экологии беспозвоночных, Омский государственный педагогический университет (Омск, Россия).

E-mail: labinvert@omgpu.ru

ORCID ID: https://orcid.org/0000-0003-0031-0618

Абраменко Анна Сергеевна, м.н.с., Научно-исследовательская лаборатория систематики и экологии беспозвоночных, Омский государственный педагогический университет (Омск, Россия).

E-mail: abramenko 97@mail.ru

Голованова Елена Васильевна, с.н.с., Научно-исследовательская лаборатория систематики и экологии беспозвоночных, Омский государственный педагогический университет (Омск, Россия).

E-mail: nilseb@omgpu.ru

ORCID ID: https://orcid.org/0000-0003-0871-9274

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