Supplementary Materials for

Modification of the bait-lamina test to estimate the soil macrofauna and mesofauna feeding activity

Evgenii L. Vorobeichik* and Igor E. Bergman

Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences, 8 Marta Str. 202, 620144, Yekaterinburg, Russia

* Corresponding author. Email: ev@ipae.uran.ru

Description of the study site



Fig. S1. Location of the study area. The red rectangles represent the area shown in detail at the smaller scale, while the red dots denote the sampling plots. The scheme is based on the data from Open Street Map (www.openstreetmap.org and www.opentopomap.ru).



Fig. S2. Photo of a pine forest (*Pinus sylvestris*) at the study site.

Table S1. Characteristics of the study site

Landscape description	Pine forest on the gentle southeastern midslope of a small mountain Krasnaya (412 m a.s.l.)
Altitude, m a.s.l.	310–316
Stand composition *	90–100% of <i>Pinus sylvestris</i> with up to 10% of <i>Betula</i> spp., <i>Abies sibirica</i> , and <i>Picea obovata</i>
Stand age, years	70–90
Dominant species of herbaceous layer **	Oxalis acetosella L. (60–75%), Calamagrostis obtusata Trin. (25–45%), Dryopteris carthusiana (Vill.) H.P.Fuchs (15– 40%), Aegopodium podagraria L. (10–25%)
Minor species of herbaceous layer **	Aconitum septenrionale Koelle (2–20%), Carex montana L. (1–15%), Fragaria vesca L. (5–10%), Asarum europaeum L. (1–5%), Lathyrus vernus (L.) Bernh. (1–5%), Maianthemum bifolium (L.) F.W.Schmidt. (1–5%), Betonica officinalis L. (1–3%), Geum rivale L. (1–4%), Ajuga reptans L. (1–3%), Veronica chamaedrys L. (1%), Viola selkirkii Pursh ex Goldie (0.5–1.5%)
Height of herbaceous layer, cm	45–75
Projective cover of moss layer, %	60–85
Soil description	Soddy-podzolic soil (Stagnic Retisol). Soil texture is medium loam in the upper part of the soil profile and heavy loam in the lower one. pH (water) is 5.3–5.5 and 4.7–4.9 in the forest litter and A1.
Humus forms	Dysmull, Hemimoder, and Eumoder

* Fractions of the species in the total number of individuals (for trees of not less than

4 cm in diameter at breast height).

** Projective cover is in parentheses.

Main macro-detritivores taxa in	Earthworms enchytraeids (occupy an intermediate		
regional fauna	position between macrofauna and masofauna)		
Tegional Tauna	millingdes Nemetogeren lemas** Coloopteren		
	larvae (Elateridae***), mollusks**		
Epigeic earthworms	Dendrobaena octaedra (2–4 cm long),		
	Dendrodrilus rubidus (2–4 cm)		
Epi-endogeic earthworms	Rhiphaeodrilus diplotetratheca (5–10 cm),		
	Lumbricus rubellus (5–10 cm),		
	Eisenia atlavinyteae (up to 20 cm)		
Endogeic earthworms	Aporrectodea rosea (5–7 cm),		
	Perelia tuberosa (7–12 cm),		
	Octolasion lacteum (7–12 cm),		
	Aporrectodea caliginosa caliginosa (up to 15 cm)		
Anecic earthworms****	Absent		
Nematoceran larvae	Tipulidae and Limoniidae (3–4 cm long);		
	Bibionidae, Sciaridae, Chironomidae,		
	Cecidomyiidae, and some others (about 0.5–1 cm		
	long)		
Woodlice (Oniscoidea)	Absent or occasional		
Wood cockroaches (<i>Ectobius</i>)	Absent or occasional		
Millipedes	Only <i>Polyzonium germanicum</i> (1.0–1.5 cm long),		
	low abundance		
Dominant species of Elateridae	Athous subfuscus, Dalopius marginatus		
Dominant species of mollusks	Perpolita hammonis, Discus ruderatus, Euconulus		
	fulva, Cochlicopa spp.		

Table S2. Regional features of soil fauna *

* Based on (Korkina and Vorobeichik, 2021; Vorobeichik et al., 2022; Vorobeichik et al., 2021; Vorobeichik et al., 2019)

** Phytosaprophages

*** Omnivores

**** Like Lumbricus terrestris or Allolobophora longa

Taxon	Density	Homoptera, Coccodea, i.+l.	32 ± 10
		Lepidoptera, l.+p.	8 ± 5
Mermithidae	45 ± 12		2 . 1
Lumbricidae worms	322 + 78	Hymenoptera phytophaga, 1.+p.	3 ± 1
	522 _ 70	Carabidae, i.	12 ± 1
Lumbricidae, cocoons	195 ± 73		
· · · · · · ·		Carabidae, l.	7 ± 1
Lumbricidae, cocoon exuvium	660 ± 74	Stanhylinidaa i	115 + 16
Enchytraeidae	357 + 65	Staphynnidae, I.	113 ± 10
		Staphylinidae, l.+p.	32 ± 8
Aranei	247 ± 24		
	7 . 1	Cantharidae, l.	22 ± 3
Opiliones	1 ± 1	Elateridae 1 + p	108 ± 14
Lithobiidae	195 ± 18	Elatendae, I.+p.	108 ± 14
		Curculionidae, l.+p.	25 ± 7
Geophilomorpha	148 ± 19		
Dinlonada	0	Coleoptera varia, i.	15 ± 4
Dipiopoda	0	Coleontera varia 1+n	58 + 6
Diptera, Nematocera, l.+p.	997 ± 402		50 ± 0
		Mollusca	743 ± 117
Diptera, Brachycera, l.+p.	147 ± 12		4510 455
Hemintera phytophaga i 1	12 + 5	Total	4510 ± 456
i iemipiera priviopilaga, i.+i.	12 ± 3		

Table S3. Density (ind. m^{-2}) of macrofauna in the study site (\pm SE)

The sampling plot $(10\times10 \text{ m})$ was a statistical unit (n = 3); the arithmetic mean for five soil monoliths was calculated previously for each plot. Soil macrofauna (including enchytraeids) were hand-sorted out of soil monoliths 20×20 cm in area and 25-30 cm in depth, depending on the occurrence of macroinvertebrates. Census was taken in July of 2019. Developmental stages: (i) imago, (l) larva, (p) pupa or pseudopupa.

List of earthworm species (percentage is in parentheses): *Rhiphaeodrilus diplotetratheca* (77.2%), *Dendrobaena octaedra* (15.5%), *Eisenia atlavinyteae* (0.5%), *Dendrodrilus rubidus* (1.6%), *Aporrectodea rosea* (1.0%), *Perelia tuberosa* (1.0%), *Octolasion lacteum* (2.1), *Aporrectodea caliginosa caliginosa* (1.0%).



Fig. S3. Feeding activity (% per 10 days) in the control boxes. Strip was a statistical unit (n = 11). Note the different scale of the Y-axis compared to Fig. 2 in the main text.



Fig. S4. Frequency of slight damage to the bait surface (%) in the boxes with earthworms (I) and control boxes (II).



Fig. S5. Schematic view of the strip for the differential BLT. Dimensions are in millimeters. The drawing is based on ISO 18311.

References

- Korkina, I.N., Vorobeichik, E.L., 2021. Non-typical degraded and regraded humus forms in metalcontaminated areas, or there and back again. Geoderma 404, 115390. https://doi.org/10.1016/j.geoderma.2021.115390
- Vorobeichik, E., Nesterkov, A., Ermakov, A., Zolotarev, M., Grebennikov, M., 2022. Diversity and abundance of soil macroinvertebrates along a contamination gradient in the Central Urals, Russia. Biodivers. Data J. 10, e76968. https://doi.org/10.3897/BDJ.10.e76968
- Vorobeichik, E., Nesterkov, A., Golovanova, E., Nesterkova, D., Ermakov, A., Grebennikov, M., 2021. Long-term dynamics of the abundance of earthworms and enchytraeids (Annelida, Clitellata: Lumbricidae, Enchytraeidae) in forests of the Central Urals, Russia. Biodivers. Data J. 9, e75466. https://doi.org/10.3897/BDJ.9.e75466
- Vorobeichik, E.L., Ermakov, A.I., Grebennikov, M.E., 2019. Initial stages of recovery of soil macrofauna communities after reduction of emissions from a copper smelter. Rus. J. Ecol. 50, 146–160. https://doi.org/10.1134/S1067413619020115