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RESEARCH ARTICLE

Soil saprophages as an emerging global source of micronutrients

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Abstract

'Hidden hunger' occurs in humans and livestock and stems from deficiencies in microelements, essential amino acids, and vitamins. Triggered by insufficient intake of micronutrients in food and feed, even when macronutrients are abundant, hidden hunger can result in the development of serious diseases and pathological conditions. Finding sufficient micronutrients is often challenging because they are either obtained from limited external natural sources or synthesised *de novo*. Soil-dwelling saprophages comprise one of the largest proportions of zoomasses on Earth but remain surprisingly overlooked as a potential micronutrient source. To assess their nutritional content concerning micronutrients, we selected 31 invertebrate species obtained from natural ecosystems of European Russia or widely cultivated species originating mainly from tropical regions. They belong to major soil saprophage taxa: cockroaches (Blattodea), beetle (Coleoptera) larvae and imagoes, springtails (Collembola), millipedes (Diplopoda), fly (Diptera) larvae, earthworms (Haplotaxida), woodlice (Isopoda), crickets (Orthoptera). We assessed their proteinogenic amino acid, microelement, and vitamin composition. Taxonomic differences in the composition and ratio of micronutrients were determined and we identified specific taxa naturally enriched with micronutrients for future consideration as potential candidates for incorporation into food and feed supplements to alleviate hidden hunger in livestock and humans.

Keywords

soil-dwelling saprophage – food supplements – fodder

1 Introduction

'Hidden hunger' promotes the development of many human diseases and pathological conditions (Lowe, 2021). While the physiologically required ratios of basic macronutrients are provided, a human being or livestock animal might suffer from the deficiency of trace elements and substances required for the proper organismal development, physiology, and reproduction (Burchi *et al.*, 2011; Denton-Thompson and Sayer, 2022; Gödecke *et al.*, 2018). Micronutrients vital for humans include vitamins, microelements, and essential amino acids, and their deficiencies annually lead to approximately 7% of the total sickness cases worldwide (Ezzati

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et al., 2004; Muthayya *et al.*, 2013). The concentrations of micronutrients in healthy food have been standardised by FAO (Lupien, 1996). Besides humans, 'asymptomatic diseases associated with lack of micronutrients' occur among livestock (Fisher, 2008). The range of micronutrients required for livestock depends on the type of feed they receive (Gupta *et al.*, 2008) and rearing conditions, for example, if an element is present in the soil, then the livestock may obtain it while grazing. However, many micronutrients, namely essential proteinogenic amino acids, vitamins, and microelements (WHO, 2009) are deficient in both food and livestock feed (Barabasi *et al.*, 2020).

Amino acid composition of proteins is an important structural characteristic of the nutritional value of dietary supplements. If one of the essential amino acids is rare or completely absent in the protein content of the feed, it can lead to disruption of metabolic processes in the body of a mammal or bird, as well as incomplete assimilation of other amino acids. If a protein is characterised by low biological value (contains amino acids in improper proportion), it should be in the diet in larger quantities to meet the minimal physiological requirements for essential amino acids. Simultaneously, the other amino acids will enter the body in excessive volume. Excessive amino acids undergo deamination in the liver and are converted into glycogen or fat (Evans and Heather, 2019). In semi-industrial meat and milk production, cattle mainly require vitamins A and E as supplements (Gupta *et al.*, 2008; Smith and Akinbamijo, 2000), while vitamin D is needed for livestock kept in stables (McDowell, 2006). Among microelements, copper, iron, manganese, zinc, and selenium are especially important for livestock (Gupta, 2008; Smith and Akinbamijo, 2000), while calcium, magnesium, cobalt, iodine, and phosphorus are also noted as being among the microelements in demand (Fisher, 2008). For example, cobalt allows cattle to independently synthesise vitamins of the B-group and participates in sustaining immunity. However, copper, cobalt, selenium, manganese, and zinc are toxic to livestock in large concentrations, and too high of a dose can lead to disease (Fisher, 2008). Selenium is shown to be extremely important for livestock breeding but is often absent in sufficient quantities in the feed (Smith and Akinbamijo, 2000). Thus, primary microelement deficiency for livestock occurs for selenium, copper, iron, zinc, and manganese as well as vitamins A, E, and D, and finally the amino acids lysine, methionine, histidine, tyrosine, and leucine.

The livestock industry has substantially increased demand for micronutrients in the form of feed supplements, most of which are produced chemically (Miller and Welch, 2013). The largest market share of the supplemented livestock feed products includes products with added zinc, iron, manganese, and copper. The same is true for food supplements aggressively marketed to humans. Due to the controversial effects of the quality and applicability of these micronutrients in the form of artificial food and feed supplements, there is a great and constantly growing demand for naturally produced micronutrients (Denton-Thompson and Sayer, 2022). Recently, the demand for purchasing environmentally safe and naturally-sourced micronutrients has skyrocketed, resulting in an urgent search for alternative sources and raw materials. However, their availability in the market is still insufficient and prices are far from being sustainable for most of the agricultural producers in the world.

In light of the large gap between demand and availability, insects, marine organisms and microorganisms are becoming increasingly important as sources of essential micronutrients (Tzachor *et al.*, 2021; Willer *et al.*, 2021). Recently, a substantial part of the world's micronutrient market has been saturated with aquaculture and insect biomass products. It is predicted that with the growing human population, these sources will play an increasingly vital role in both macro- and micronutrient supply (Churchward-Venne *et al.*, 2017; Rumpold and Schlüter, 2013).

At the same time, soil biota remains an unlikely candidate for modern food and feed science, despite being potentially the most significant and cheapest source of microelements. Therefore, there exists a tremendous potential for belowground animals, which have previously been generally overlooked as a source of nutrients (but see Tzachor *et al.*, 2021 for some exceptions). However, entomophagy among humans has been already suggested as a possible solution for counteracting excessive environmental loads associated with conventional agriculture and especially with meat production. Insects emit lesser greenhouse gases per unit of zoomass than conventional farm animals and can be grown on regular organic wastes (Premalatha *et al.*, 2011).

Soil is a globally distributed habitat hosting up to 0.63 Gt carbon in zoomass (calculation based on the data by Bar-On *et al.*, 2018; Fierer *et al.*, 2009). Soil-living saprophages are important participants of biogeochemical cycles, supporting several ecosystem functions such as maintaining humus pool regeneration in the soil, water balance, and others (Crowther *et al.*, 2019;

FAO *et al.*, 2020; Lal, 2009). They also play a key role in the organic matter decomposition and carbon stabilisation in soil. Earthworms alone may constitute up to 90% of the total terrestrial animal biomass (Fierer *et al.*, 2009) which results in biomass estimates as high as 2 kg/m^2 (Phillips *et al.*, 2019). Due to nearly unlimited resources and the renewable nature of the dead organic matter in the soil, the abundance and biomass of belowground saprophages available worldwide are enormous. The diversity of saprophages in soil includes not only earthworms, but also larvae and adult insects, millipedes, woodlice, and many other taxa belonging predominantly to soil macrofauna (Gongalsky, 2021).

There is scant information on the distribution of micronutrients among different soil saprophagous taxa depending on their phylogeny or ecology. Several invertebrates are known to accumulate microelements (such as calcium, zinc, molybdenum, etc.) in their bodies, while other species are capable of concentrating heavy metals (lead, mercury, cadmium) for possible use in bioremediation (Ardestani *et al.*, 2014; Janssen and Hogervorst, 1993). There are also species known for being rich in select amino acids (Pokarzhevskii *et al.*, 1997).

In this study, we addressed the knowledge gap of nutrient availability and analysed soil saprophages as a potential source of micronutrients concerning their ability to concentrate vitamins, microelements, and amino acids. The choice of saprophages was determined by the wide availability of detritus in the world, which serves as their food. Such an approach will help to contribute to the problem of agricultural waste disposal and valorisation of agricultural plant residues. This study represents the first attempt to measure the nutritional value of soil macroinvertebrates, and while the possible toxicity of selected species is beyond the scope of the study, therefore, we only evaluated their micronutrient concentration ability.

2 Methods

Selection of species for analysis

The original data for micronutrient composition of selected soil saprophages were obtained from natural ecosystems of European Russia. In addition, we have collected widely-distributed species of saprophages that live locally in stacks of straw, dung heaps, and along the banks of water bodies. In addition, we selected widely cultivated species of soil saprophages that were included in the set of test objects and comprised mainly species from tropical and subtropical regions. This study represents the first attempt to measure the micronutrient value of soil saprophages and we decided to choose a variety of species. We have collected widely-distributed species and widely cultivated species of soil saprophages. In total, 30 taxa were used for further analysis (Table 1).

Identification of micronutrients in soil saprophages

The animals were killed by freezing, washed in distilled water, dried in a freeze dry system (FreeZone Freeze Dryer, Labconco Corporation, Kansas City, KS, USA) at −52 °C at a low vacuum level (0.002 mBar), and homogenised using a laboratory mill (Retsch GmbH, Haan, Germany) in the Joint Usage Center 'Instrumental methods in ecology' at the Institute of Ecology and Evolution RAS. The resulting dry milled powder was used for all further laboratory analyses.

The study of the composition of micronutrients in soil invertebrates included the determination of microelements, amino acid composition, and the content of the selected vitamins. We used different dry weights of animals' samples to study the composition of micronutrients: 1 mg was needed for amino acid composition assessment, 10 g for vitamins, and 100 mg for microelements. Due to this weight restriction, vitamins were measured in 14 species only, and microelements in 28 species (Supplementary Table S1) due to the lack of sufficient dry biomass for some of the collected invertebrate species. Laboratories work in accordance with ISO 17025:2017 protocol.

Amino acids

Preliminary acid hydrolysis was carried out to determine the amino acid composition of the samples. A mixture of concentrated hydrochloric acid and a strong organic acid, trifluoroacetic acid, was used, which makes it possible to determine all amino acids with a high yield (Tsugita and Scheffler, 1982). Total protein was calculated as the sum of the mass fractions of all individual proteinogenic amino acids. For total acid hydrolysis, 300 µl (0.3 ml) of a freshly prepared hydrolysing mixture (concentrated hydrochloric and trifluoroacetic acids in a ratio of 2:1 with the addition of 0.1% β-mercaptoethanol) was added to a sample weighed in a molybdenum glass ampoule. The sample was frozen by placing the ampoule into liquid nitrogen, evacuated, and melted. Hydrolysis was carried-out at 155 °C for 1 hour. At the end of the hydrolysis, the ampoule was opened (after cooling), the contents were quantitatively transferred into a plastic tube (Eppendorf, Ger-

TABLE 1 Soil invertebrate taxa used in the analysis of selected amino acids, vitamins, and microelements. Species with maximal values of each micronutrient are indicated.[1]

		Amino acid	Vitamins	Microelements
	Oligochaeta			
1	*Aporrectodea caliginosa* (Savigny, 1826)		B1	Se, Nb, Hg
2	*Aporrectodea rosea* (Savigny, 1826)			Al, Fe
3	*Dendrobaena veneta* (Rosa, 1886)			
4	*Eisenia fetida* (Savigny, 1826)			Cd, Sb
5	*Lumbricus rubellus* Hoffmeister, 1843			V, Cr, Zn, Mo
6	*Lumbricus terrestis* L., 1758			
7	*Octolasion lacteum* Örley, 1881	Asp, Thr, Ser, Glu, Cys, Phe, Lys	A, D3	U
	Isopoda			
8	*Armadillidium versicolor* Stein, 1859			Li
9	*Armadillidium vulgare* Latreille, 1804			
10	*Cylisticus convexus* (De Geer, 1778)			
11	*Ligia cinerascens* Budde-Lund, 1885			Na, Mn, As, Sr
12	*Porcellio scaber* Latreille, 1804			Pb
13	*Protracheoniscus major* (Dolfuss, 1903)		B2	
14	*Trachelipus rathkii* (Brandt, 1833)			
	Diplopoda			
15	*Cylindroiulus latestriatus* (Curtis, 1845)			Mg, P, Ca, Co, Ni, Cr
16	Julida gen. sp			
17	*Pachyiulus krivolutskyi* Golovatch, 1977			
18	*Rossiulus kessleri* (Lochmander, 1927)			
19	Spirostreptidae gen. sp.			
	Collembola			
20	*Orchesella flavescens* (Bourlet, 1839)			
21	*Tomocerus vulgaris* (Tullberg, 1871)			
	Blattoptera			
22	*Shelfordella lateralis* Walker, 1868			
23	*Nauphoeta cinerea* (Olivier, 1789)			
	Orthoptera[2]			
24	*Acheta domesticus* L., 1758	Pro, Gly, Ala, Val, Ile, Leu, Arg		
25	*Gryllus bimaculatus* De Geer, 1773			Bi
	Coleoptera			
26	*Alphitobius diaperinus* Panzer, 1797, ad			
27	*Cetonia aurata* (L., 1758), l			Rb
28	*Oryctes nasicornis* (L., 1758), l		E	
29	*Tenebrio molitor* L., 1758, l	Tyr, His		Ag
	Diptera			
30	*Hermetia illucens* (L., 1758), l	Met		
	Gastropoda			
31	*Helix pomatia* L., 1758			B, Ba

1 Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartic acid; Cys = cysteine; Gly = glycine; Gln = glutamine; Glu = glutamic acid; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Pro = proline; Ser = serine; Thr = threonine; Trp = tryptophan; Tyr = tyrosine; Val = valine.

2 Ad = adults; l = larvae.

many), and the hydrolysis mixture was evaporated to dryness. Traces of acids were removed by twice repeating the procedure for evaporating small portions of water added to the dry residue on a CentriVap Concentrator (Labconco Corporation, Kansas City, KS, USA). The samples were hydrolysed as described by Tsugita and Scheffler (1982), and amino acid analysis was carried out on an Amino Acid Analyzer Hitachi L-8800 system (Hitachi, Tokyo, Japan) in the standard mode for protein hydrolysate analysis with cation-exchange separation and ninhydrin postcolumn derivatisation (Moore *et al.*, 1958; Trofimova *et al.*, 2016). The number of analytical replicates is 5.

Vitamins

The quantitative analysis of vitamins A and E was carried out according to a modified method by López-Cervantes *et al.* (2006). Vitamins A and E. A sample (2-5 g) was dissolved in an aqueous-alcoholic solution of potassium hydroxide (50%). Vitamins were extracted with diethyl ether, separated on a column with aluminium oxide, and their concentrations quantitatively determined by photometrically either directly for vitamin A, or indirectly, after colour staining for vitamin E. Alpha-tocopherol (vitamin E) was eluted with a mixture of diethyl and petroleum ether, monitoring the progress of the vitamin extraction with UV light (alpha-tocopherol has a bluish glow). Retinol (vitamin A) was eluted with an increasing gradient of diethyl ether, also monitoring the extraction progress of the vitamin with UV light (retinol has a yellow-green glow). The eluate was evaporated and re-dissolved in ethanol. The optical density of the resulting retinol solution was measured on a Lambda 650 spectrophotometer (Perkin Elmer) at a wavelength of 326 nm against a cuvette with absolute ethanol. The concentration was determined from the calibration curve.

The alpha-tocopherol eluate was also evaporated and dissolved in ethanol. A solution of phenanthroline and ferric chloride was added to the sample and incubated for 5 min for colour development. The optical density of the sample was measured on a KFK-3-01 photoelectrocolorimeter ('ZOMZ' JSC, Sergiev-Posad, Russia) at a wavelength of 520 nm relative to absolute ethanol. A sample without the addition of alpha-tocopherol was used as a control.

Vitamin D3. The competitive reaction of biotin-labelled D3 and unlabelled D3 (standard and sample, respectively) with immobilised antibodies specific to D3 was used. We used monoclonal antibodies (Enzyme-linked immunosorbent assay kit for vitamin D3, Cloud-Clone Corp., Katy, TX, USA). The unbound conjugate was washed off, then peroxidase-bound ovidin was added to the sample. The bound biotin-ovidin-peroxidase conjugate reflects an inverse proportion depending on the concentration of D3 in the test sample. The determination was carried out in accordance with the instructions of the kit manufacturer.

Vitamins B1 and B2. At the initial stage of the analysis, hydrolysis was carried out with hydrochloric acid (ISO 21470:2020). The pH of the solution was adjusted to 4.5-4.6 using sodium acetate. Enzymatic hydrolysis was carried out first with pepsin (37 °C for 4 hours) and then with fungamyl (37 °C for 12-16 hours). The analyte was purified by filtration, then isobutanol was used to purify the filtrate. The lower aqueous layer was used for analysis during phase separation. Vitamin B1 was oxidised to thiochrome. For this, a 1% solution of potassium ferricyanide and 30% sodium hydroxide solution were used. Extraction was carried out with isobutanol. A control sample (solvent blank of isobutanol) was prepared. After oxidation, the organic layer was taken and placed in a cuvette, and the mass concentration (fluorescence optical density) of vitamin B1 was measured using a Fluorat-02 liquid analyser (LUMEX Group, St. Petersburg, Russia). For quantitative calculations, we used the graduation for vitamin B1. For vitamin B2, the sample filtrate (lower water layer purified by isobutanol) was placed in a cell of a Fluorat-02 liquid analyser and the concentration of vitamin B2 was measured. Then, thiourea was added to the same cuvette, and after stirring, the fluorescence was measured again. The addition of thiourea was continued until the smallest value of the measurement result was obtained. The determination was carried out in accordance with the instructions of the analyser manufacturer. The number of analytical replicates is 2.

Microelements

The content of trace elements in soil invertebrates was analysed by ICP mass spectrometry (Kokot and Matysiak, 2008). A MARS-6 sample digestion unit (CEM Corporation, Matthews, NC, USA) was used, which makes it possible to create a temperature of up to 250 °C in an acid-resistant fluoroplastic vessel for decomposition, while the pressure in the vessel can reach up to 5.166 MPa. For analysis, a weighed portion of the sample was placed in a decomposition vessel, filled with nitric acid, and held until the evolution of gases ceased. Control samples were used for each batch of six test samples – only acid was poured into one of the vessels to establish the background signal. The decomposition process was carried out in a microwave oven using a special temperature-time profile, including heating, incubation, and cooling. After cooling, the vessels were opened, the required volume of an aliquot was taken, and measurements were taken on a Perkin-Elmer ELAN-DRC-e ICP mass spectrometer (PerkinElmer Inc., Waltham, MA, USA). The number of analytical replicates is 5.

Comparison of invertebrates with conventional meat products

A comparison of invertebrates with basic protein products of animal origin (beef, pork, chicken breast, horse meat) was done based on the mean nutritional values of these products. The nutritional composition of different types of meat was sourced from literature (Supplementary Tables S2, S3, S4). Soil saprophages were allocated to higher-rank taxa of class or order (Blattodea, Coleoptera, Collembola, Diplopoda, Diptera, Haplotaxida, Isopoda, Orthoptera) to achieve sufficient replication and reduce individual sample variance.

Comparison of invertebrates with feed supplements

The content of micronutrients in dry invertebrate biomass of the tested saprophage species was compared with commercial feed supplements. We calculated a nutritional value index as the sum of the ratios between the mean concentration of each analysed micronutrient in a species and the mean concentration of the respective micronutrient in commercial feed supplements taken for our comparative analysis. Nominal values of micronutrients were recorded from the official information sheets accompanying respective feed supplement products. At least five types of supplements were selected for each micronutrient type, resulting in a list of 16 commercial supplements (Supplementary Table S1). The mean value of each soil saprophagous taxa (Blattodea, Coleoptera, Diplopoda, Diptera, Haplotaxida, Isopoda, Orthoptera) was divided by the mean value of each micronutrient (Se, Cu, Fe, Zn, Mn, Lys, Met, Tyr, Leu, His, Vitamins A, D3, E) across five supplements. The resulting ratios higher than 1.0 indicated taxa richer in the measured micronutrients than the averaged values in supplements.

Data analyses

The similarity of micronutrient composition between main groups of soil saprophages (Blattodea, Coleoptera, Diplopoda, Diptera, Haplotaxida, Isopoda, Lepidoptera, Orthoptera, Stylommatophora) and basic protein products of animal origin (beef, pork, horse meat, chicken breast, for more information, see Supplementary Table S5) was estimated using values of 4 amino acids (Lys, Met, Tyr, Leu) and 5 microelements (Se, Cu, Fe, Zn, Mo). For each micronutrient, content values were normalised using min-max feature scaling to the range $[0, 1]$. Accumulation indices of the samples were calculated as sums of normalised values of all 9 micronutrients. Pearson correlation values between samples were used as distances. To visualise correlations between micronutrient composition of different tested invertebrate saprophages versus different types of basic protein products of animal origin, we built a network of associations using *graph* R package in R-4.2.1 (R Core Team, 2022); only links with correlation values >0.5 were shown. The degree of similarity between the micronutrient composition of invertebrates and animal protein products is shown as a Pearson correlation matrix. The similarity of micronutrient composition between various species of soil saprophages from different groups was estimated using values of 48 micronutrients. The network was built using the method described above, only links with correlation values >0.5 are represented.

3 Results and discussion

Invertebrate micronutrient composition relative to conventional meat products and food supplements

The micronutrient composition of many soil saprophages approximates the values found in meat products. For example, the micronutrient composition of fly larvae (Diptera) was closely related to the basic protein composition in both pork and beef (Figure 1). Composition of earthworms (Haplotaxida) and cockroaches (Blattodea) was also quite similar to beef and pork, respectively, while none of the analysed invertebrate taxa was similar to basic protein signature of chicken and horse meat (Figure 1b) in terms of the composition of micronutrients. The lowest similarity with conventional meat products was found in woodlice (Isopoda) and millipedes (Diplopoda). However, representatives of the latter two taxa additionally contained high concentrations of calcium in their bodies (Figure 1).

We also compared soil invertebrate micronutrient composition with those found in commercially produced feed supplements for cattle. Based on the nutritional value index, we found several soil macrofauna taxa that demonstrated higher nutritional value than the mean value of the tested feed supplements. Beetle larvae and cockroaches were indeed richer in several valuable micronutrients (Figure 2) while earthworms

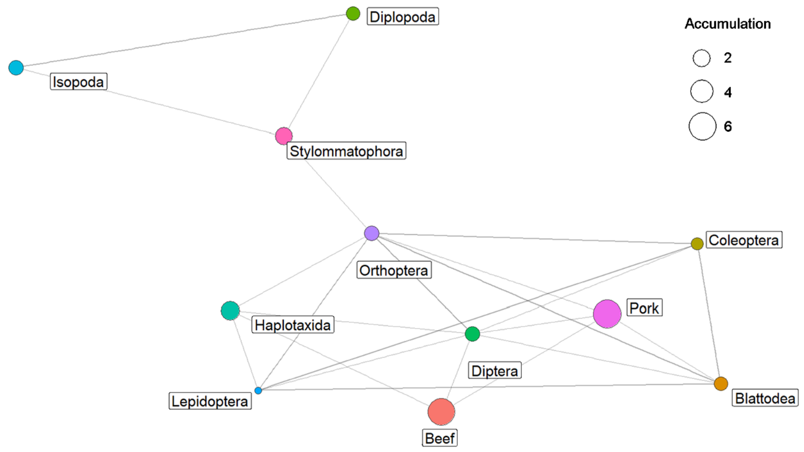

FIGURE 1A Network of associations between composition of micronutrients of soil saprophages main groups and basic protein products of animal origin (beef, pork, chicken breast, horse meat) according to published data. Node colours correspond to soil saprophage group/protein product; node sizes are proportional to accumulation indices.

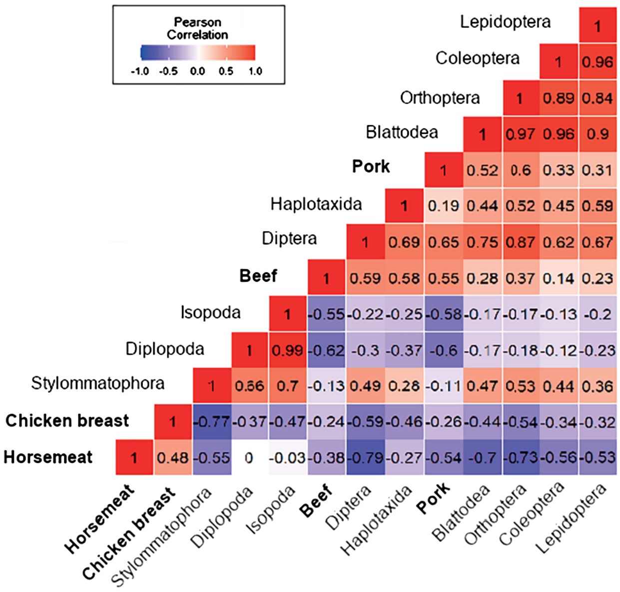

FIGURE 1B A Pearson correlation matrix between micronutrient content of invertebrate taxa and basic protein products of animal origin.

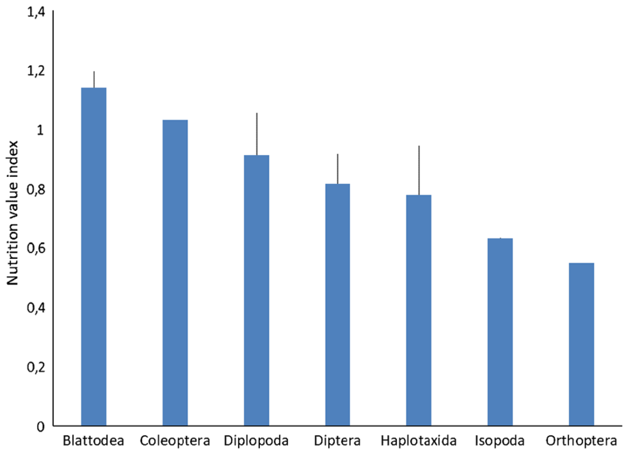

FIGURE 2 The integral content of micronutrients in dry invertebrate biomass compared with the mean content of the same set of nutrients in commercial feed supplements (mean ± S.E., n = 5). (Ratio of an index value in each taxonomic group to the mean index of additives). The micronutrient content of supplements is shown in Supplementary Table S1.

were particularly rich in amino acids. This makes these soil macrofauna taxa highly attractive as a raw material for future feed supplement development and production.

The material we obtained may have several shortcomings. As we studied several contrasting biomes within the European part of Russia when searching for suitable test taxa and the fact that about a quarter of taxa were obtained from cultures which contained tropical species (e.g. cockroaches and Diptera larvae) we are positive that results obtained have a global significance. Another argument substantiating this judgement is dependence of phylogenetic similarity and similarity of their micronutrient composition. This means that different but taxonomically close species to those, covered in our work will demonstrate similar micronutrient content patterns. On the other hand, if the recommended species are used as a laboratory culture that is kept on plant litter, then changing the composition of micronutrients will not depend on the biome of their origin. Soil saprophages inhabit different horizons of the soil, for example, in the litter and in the humus horizon. Due to the different resources they feed on, they may differ in chemical composition. For example, there is data demonstrating, that the isotopic composition of earthworms (Hsu *et al.*, 2023) and smaller invertebrates (Zhang *et al.*, 2021) may differ in different soil strata.

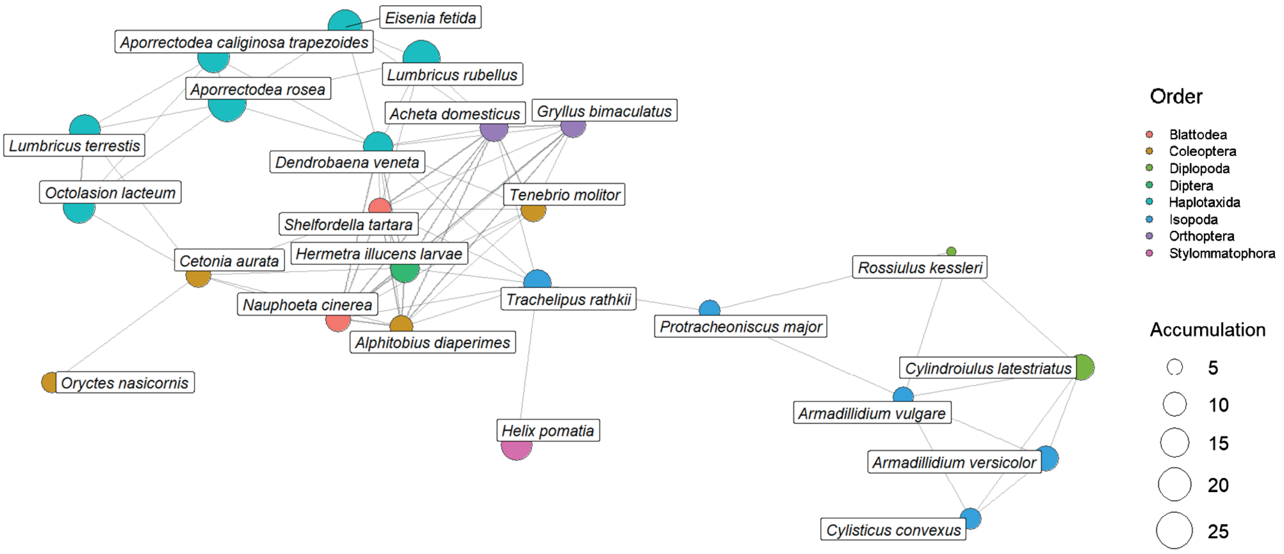

FIGURE 3 Similarity of the micronutrient composition among the main soil saprophagous species. Node colours correspond to soil saprophage group; node sizes are proportional to accumulation indices.

Though, this aspect was not in the focus of our current research, vertical habitat preferences of each species and actual feeding on substrates of different origin and consequently of different micronutrient composition in soil represent an important perspective to be addressed in the future.

Comparison of invertebrate micronutrient composition with each other

Soil saprophages were most similar to each other in amino acid composition. The variability of concentrations of each amino acid was low, according to both our data (Supplementary Table S5) and the literature (Evans *et al.*, 2015; Finke, 2002; Pokarzhevskii *et al.*, 1997). Dipteran larvae and springtails (Collembola) were characterised by a slightly higher content of Asp and Glu, while the crickets bore notably higher content of Ala. Collembolans had a low Cys content and a slightly higher Met content. The content of Asp and Glu in insects was lower than those in the representatives of other taxa, while Pro, Ala, and Val, on the contrary, were found in relatively higher concentrations. The content of Leu and Lys in all studied earthworm species compared to other invertebrates was relatively high (Pokarzhevskii *et al.*, 1997), as well as Tyr in the earthworms and the migratory locust, *Locusta migratoria* (Bednářová *et al.*, 2013).

Our results show that earthworms represent a potentially micronutrient-rich source of biomass among the studied saprophages in terms of amino acid composition. Moreover, among representative terrestrial invertebrates, the absence of chemical defence against predators in earthworms, like those present in many species of beetles or diplopods (Eisner and Meinwald, 1966; Shear, 2015), also indicates a low probability of toxicological risks arising during the production of food or feed supplements derived from earthworm zoomass.

Overall, we found that micronutrient composition similarity is higher in evolutionary closer taxa. In general, the closest micronutrient composition and content were found between species within the higher rank of taxa (e.g. class or order) (Figure 3). This helps to make predictions on the micronutrient composition of taxa that are yet to be studied.

The ground invertebrate material has not been additionally refined. Additional refinery may actually lead to even higher concentration of the target micronutrients. However, this aspect was left beyond the scope of the current study as relevant techniques have not been developed yet. Another aspect is that soil saprophage powder should not be directly fed to livestock. Some of the invertebrates bear toxic substances (Eisner and Meinwald, 1966; Shear, 2015) and may poison animals when fed with large amounts of such powder. Most known examples are diplopods which bear hydrocyanic acid. Investigations on the toxicological status of the resulting powder from different soil saprophage taxa should be carried out in the future.

4 Conclusions

We found that several taxa of soil saprophages (especially insect larvae, millipedes, and earthworms) are rich in micronutrients (amino acids, vitamins, and microelements), yet, with few exceptions, are largely ignored by humans as naturally-sourced raw materials to manufacture potentially valuable food and feed supplements. Our comparison of the micronutrient composition of the major soil saprophage taxa has demonstrated that many are similar in composition to conventional meat products. Moreover, for several micronutrients, the high content in some taxa may even offer superior quality enhancement to the currently available nutritional supplements. If the direct consumption of these invertebrates appears bizarre nowadays due to social stereotypes, taboos, and sanitary regulations, their indirect ingestion as supplements for livestock diets is promising as some soil invertebrate taxa have micronutrient concentrations far higher than in the commercial products currently available on the feed supplement market. This will, however, require further large-scale research on their toxicity safety, flavour profiles, and technological scalability during the commercialisation and marketing process.

The major predictor determining the content of micronutrients in a given soil saprophage species is its evolutionary and taxonomic position. This opens the ground for intensifying research efforts and making further extrapolations on the nutritional values of local taxa with a limited geographic range based on the studies of the relatively widespread species. This finding will aid local communities, including low-income and/or nature-dependent communities, to more efficiently search for local, sustainable resources to rapidly counteract the problem of hidden hunger.

Based on our results, we recommend focusing further research using species with the following characteristics: micronutrient richness, ease of cultivation, and scalability of production (e.g. can produce a large amount of biomass within a short time). The most promising candidates are the earthworms *Lumbricus terrestris* and *Octolasion lacteum*, whose tissues are extremely rich in amino acids, and rhinoceros beetle larvae (*Oryctes nasicornis*), whose tissues are rich in vitamins. Moreover, none of these species are known to possess chemical defences which can be problematic in toxicological and sanitary safety assessments as constituents of feed supplements. In the case of the rhinoceros beetle, one additional challenge is that this particular species is considered endangered in many European countries.

Supplementary Material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.23913024

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Conflict of interest

The authors declare no conflict of interest.

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