Possibility of studying earthworm feeding ecology using mid-range infrared spectrometry

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Abstract

Earthworms in forests ensure the return of nutrients to the soil by recycling litter. Climate warming promotes changes in the feeding ecology of earthworms, which leads to changes in soil nutrient cycling due to the saturation of biotopes with invasive plants and lumbricids. This study explored the use of mid-infrared (IR) spectrometry as a technique for identifying earthworm dietary preferences. In a controlled laboratory-based experiment, we examined how consumption of three different types of leaf litter — *Populus tremula*, *P. sibirica*, and *Acer negundo* — affects the IR spectra of three earthworm species — *Eisenia nana*, *E. nordenskioldi*, and *E. ventripapillata*. The results demonstrated that body composition changes in each earthworm species indicated the specific type of leaf litter ingested. Therefore, mid-infrared spectrometry is promising as a method for investigating the feeding preferences of earthworms.

Keywords: earthworms, forest litter, food preferences, IR spectrometry.

Introduction

Litter decomposition is one of the most important ecological processes that influences nutrient release for plant growth and CO_2 emissions into the atmosphere (Krishna and Mohan, 2017). Earthworms play a key role in this process (Patoine et al., 2020), with a preference for leaf litter (Bohlen et al., 2004b; Kim, Kim, Kwon, and Son, 2022; Song, Yanghua, Yitong, and Mingfeng, 2023), which accounts for over 70 % of the total forest detritus (Song, Yanghua, Yitong, and Mingfeng, 2023).

Climate warming has contributed to the increased forest coverage and density (Devi et al., 2020), an increase in the proportion of invasive woody plant species (Langmaier and Lapin, 2020), and the active introduction of exotic earthworm species into forest ecosystems (Bohlen et al., 2004a; Bohlen et al., 2004b; Berg et al., 2010; Golovanova et al., 2021). The result will be new types of relationships between plants and earthworms, leading to changes in nutrient cycling in forest soils (Resner et al., 2015; Craven et al., 2016; Richardson, Johnston, and Herrick, 2022). Therefore, it is necessary to determine whether native earthworm species can adapt to new plant resources and whether introduced earthworm species can modify the intensity and characteristics of litter decomposition.

To study earthworm feeding habits, researchers have traditionally relied on methods such as direct observation (Briones and Schmidt, 2004), intestines content analysis (Striganova, 1980), preference tests (Striganova, 1980; Curry and Schmidt, 2007), the bait-lamina test (BLT) (Vorobeichik and Bergman, 2023), and litter bags (Patoine et al., 2020). Although these methods are informative, they are also labor-intensive and yield limited insights into feeding strategies

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and digestible food components. Stable isotope analysis (^{13}C and ^{15}N) has offered new perspectives on nutritional ecology (Kim, Kim, Kwon, and Son, 2022; Potapov et al., 2022) and has been particularly effective in distinguishing the diets of cohabiting earthworms that consume litter or soil organic matter (Potapov et al., 2022). However, the high costs and limited availability of the necessary equipment make this method less accessible for widespread use. Consequently, there is a demand for more accessible methods to explore the feeding ecology of earthworms.

Infrared spectroscopy is increasingly used in ecology to determine the origin (Zangerle, Hissler, McKey, and Lavelle, 2016; Domínguez-Haydar et al., 2020) and biogeochemical changes (Bottinelli et al., 2020) of soil biogenic earthworm aggregates, as well as for species identification of lumbricids (Pham et al., 2021). An infrared (IR) spectrum provides a chemical profile of organic material, revealing crucial biochemical information about functional groups such as –CH, –OH, and –NH bonds (Jouquet, Capowiez, Bottinelli, and Traoré, 2014). This results in a biochemical "fingerprint" of the earthworm's body composition (Pham et al., 2021). The principal benefits of IR analysis are its affordability, reproducibility, speed, and widespread availability. Most universities and research institutions are equipped with IR spectrometers. Pham et al. (2021) found that the IR signature of an earthworm is influenced by the type of organic matter ingested and the soil type; a similar relationship was also observed in termites (Jouquet et al., 2018). Thus, IR spectrometry may be an appropriate method for investigating earthworm feeding ecology.

The aim of this study was to evaluate the impact of different types of litter on the mid-IR signature of earthworms and to determine whether these signatures (a collection of absorption bands) can be used to identify the types of litter consumed by earthworms.

Materials and methods

Earthworms and leaf litter sampling

The study was conducted in the laboratory of systematic and ecology of invertebrates at Omsk State Pedagogical University. Three species of earthworms were collected for the study: *Eisenia nana* (Perel, 1985); *E. nordenskioldi nordenskioldi* (Eisen, 1879); and *E. ventripapillata* (Perel, 1985); and three types of leaf litter of: *Populus tremula* L., 1753; *P. sibirica* (G. V. Krylov & G. V. Griffin, ex A. K. Skvortsov, 2007); and *Acer negundo* L., 1753. Earthworms and leaf litter were collected from three locations: the Omsk region, Omsk district, near the village of Chernoluchye, in birch (*Betula pendula*) small-grass forests and maple (Acer negundo) thicket communities at coordinates N55°16′33.6″, E73°02′35.3″; the Omsk region, Omsk, within the Park of the 30th Anniversary of the Victory, in a forest protection belt of *A. negundo* and *P. sibirica* at coordinates N54°57′08.6″, E73°21′18.1″; and the Omsk region, Omsk district, near the village of Ust-Zaostrovka, in birch (*Betula pendula*)-aspen (*P. tremula*) small-grass forests at coordinates N54°45′49.4″, E73°36′44.0″.

Incubation experiment

To study the body composition of the earthworms, they were placed into 27 two-liter plastic containers with perforated lids (3 types of earthworms × 3 types of leaf litter × 3 microcosms for each variant). The microcosms were filled with 1.5 liters of identical soil, which was cleared of macrofauna using sieves with a 4 mm mesh diameter. The soil type is calcic chernozem (IUSS, 2006). In each microcosm, one of the three types of dry leaf litter, weighing 5.00 ± 0.01 g, was added according to the experimental design. Soil moisture in the microcosms was monitored using a TR 46908 soil moisture meter (TR di Turoni & Snc, Italy) and maintained at 45–50 % with distilled water. Five earthworms were introduced into each microcosm and incubated for 90 days in climate chambers at 13 °C. The average biomass of an individual earthworm in a microcosm was 0.68 ± 0.02 g for *E. n. nordenskioldi*, 0.70 ± 0.03 g for *E. nana*, and 0.33 ± 0.01 g for *E. ventripapillata*.

MIR spectra acquisition and pre-processing

Following the exposure period, earthworms were removed from the soil, rinsed with distilled water, and then placed in containers with replaceable filter paper for five days at 13 °C to clear their intestines. The next step involved freezing the worms for 24 hours, followed by drying at 60 °C for 48 hours and grinding them into a fine powder using a mortar. Six samples (two individuals per sample) were prepared for each earthworm species on each type of leaf litter.

To conduct the analysis, tablets of a mixture with potassium bromide in a ratio of 1 : 200 were prepared from the sample. The spectrum of pure potassium bromide was used as a reference. The measurements were performed on an FT-801 IR Fourier spectrometer (SIMEX) in the range of 500–4000 cm^{-1} (number of scans: 32, resolution: 4 cm^{-1}). The spectra were processed using ZaIR3.5 software (SIMEKS). The position, intensity, and area of absorption bands (AB) were determined on all spectra.

Statistical analysis

Data normality was checked using the Shapiro-Wilk test in the Statistica 13.0 software package. Principal component analysis (PCA) of the spectral profiles of earthworm bodies for each type of leaf litter was conducted using the R software package (with the "FactoMinerR" package) (Le, Josse, Husson, 2008). The influence of litter type on the absorption band spectrum was evaluated using two-way and one-way ANOVA, as well as the paired Tukey HSD test in the Statistica 13.0 software package (StatSoft).

Results and discussion

Our study reveals variations in the composition and ratios of infrared (IR) absorption bands (ABs) in the bodies of earthworms, which are contingent on the type of leaf litter they consume, as outlined in the accompanying table. Significant differences in the body composition of earthworms fed on *Acer negundo* or *Populus tremula* were noted by AB of 613, 1338, 1619, 3547 cm^{-1} specifically for *Eisenia nana*; by AB 1355, 2856, 2855 cm^{-1} specifically for *Eisenia nordenskioldi nordenskioldi*; and by AB 1029, 1052, 1153 cm^{-1} specifically for *Eisenia ventripapillata*. When comparing *A. negundo* with *Populus sibirica*, significant differences were clear in the IR spectra by AB 1315, 1405 cm^{-1} for *E. nana*, by AB 1153, 1239, 2874 cm^{-1} for *E. n. nordenskioldi*, and by AB 930, 1029 cm^{-1} for *E. ventripapillata*. Additionally, significant differences were observed between the closely related *P. tremula* and *P. sibirica* by AB 3547 cm^{-1} for *E. nana*, by AB 1355, 1536, 2928 cm^{-1} for *E. n. nordenskioldi*, and by AB 613, 1030, 1053, 1080, 1657, 2855 cm^{-1} for *E. ventripapillata*.

According to a two-factor analysis of variance (ANOVA), the "leaf litter" factor influenced 72 % of the AB characteristics in the earthworm body ($p < 0.05$), while the "species" factor impacted 65 % of the AB characteristics ($p < 0.05$). The interaction between these two factors also affected 72 % of the absorption bands.

Our findings align with those of a previous study by Pham et al. (2021), which indicated that the absorption band spectrum is dependent on the substrate ingested by earthworms. Both soil and leaf litter (Table) types affect the biochemical composition of earthworm bodies. In an experiment, the IR spectral characteristics of *Lumbricus terrestris* (Linnaeus, 1758), *Allolobophora chlorotica* (Savigny, 1826), and *Aporrectodea icterica* (Savigny, 1826) were influenced by the soil composition where they resided, absorbing their organic and mineral components (Pham et al., 2021). A similar impact on the spectral composition was observed in the termite *Odontotermes obesus* (Rambur, 1842), with variations attributed to habitat, food sources, and contrasting physicochemical conditions (Jouquet et al., 2018).

PCA further distinguishes *E. nana*, *E. n. nordenskioldi*, and *E. ventripapillata* by their characteristic absorption band spectra associated with their body chemistry. Axis 1 accounts for 18.7 % of the total variance, with positive correlations to AB cm^{-1}: 2929 ($r = 0.75$), 1405 ($r = 0.72$), 930 ($r = 0.71$), 2959 ($r = 0.64$), 1153 ($r = 0.58$), 1237 ($r = 0.54$), 2874 ($r = 0.50$), 2856 ($r = 0.48$), 3417 ($r = 0.39$), 1657 ($r = 0.32$), and 1536 ($r = 0.28$). It negatively correlates with ABs cm^{-1}: at 1620 ($r = 0.50$), 1338 ($r = 0.43$), 613 ($r = 0.43$), 1080 ($r = 0.39$), and 3547 ($r = 0.34$). Axis 2 explains 13.6 % of the total variance and is positively correlated with ABs cm^{-1}: at 1547 ($r = 0.85$), 1454 ($r = 0.84$), 1237 ($r = 0.66$), 1315 ($r = 0.63$), 1405 ($r = 0.41$), 1338 ($r = 0.36$), 1080 ($r = 0.32$), and 930 ($r = 0.29$), while showing a negative correlation with the AB at 2856 cm^{-1} ($r = 0.45$). The PCA also indicates differentiation based on the type of leaf litter consumed by the earthworms, as depicted in the Fig. 1.

The dependence of the chemical composition of the body of earthworms on the consumed litter, obtained by

Differences in the spectrum of the absorption bands of earthworm bodies depending on the type of leaf litter consumed

AB, cm^{-1}	*A. negundo / P. tremula*			*A. negundo / P. sibirica*			*P. tremula / P. sibirica*		
	Ena	Eno	Eve	Ena	Eno	Eve	Ena	Eno	Eve
613	**			*		**			*
930	*		***			*		*	***
1029			**			*			**
1053			*						*
1080									**
1153			**		*		*	*	***
1237	*	**			**		**	**	*
1315		***	*	*				***	**
1338	***						***		*
1356		**						**	
1405	**		**	**			*	*	***
1454					***	*		***	*
1536								**	
1547					***	**		***	***
1620	***			***		*	**		*
1657									*
2874		**			*				
2856		***		**	**	**			*
2929	*	***		*	**			*	
2959	**	***		*	***				
3547	**						**		

Note: *Significance levels are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, empty graphs — no significant differences. Ena — *E. nana*; Eno — *E. nordenskioldi*; Eve — *E. ventripapillata*.

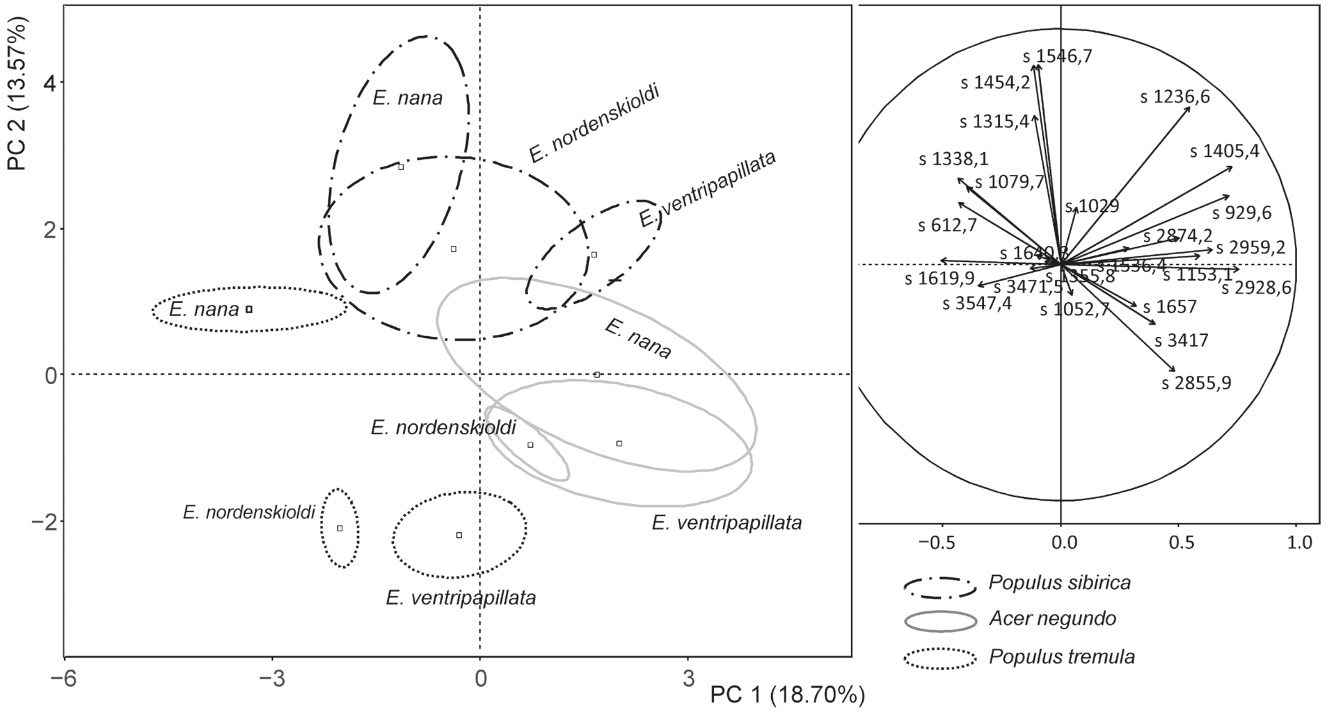

Fig. 1. Factorial plane and its corresponding correlation circle (PCA) for correlating earthworm species with leaf litter consumed. The vector shows the direction of the correlation, and its length indicates the strength of the variable.

us (Fig. 1 and Table) is also known from several studies. Thus, Pokarzhevsky, Zaboyev, Ganin, and Gordienko (1997), while investigating the amino acid profile of *E. nordenskioldi*, observed that the body composition varied with the habitat, soil type, and organic matter ingested. Schmidt, Scrimgeour, and Handley (1997) also assessed the natural isotopic content of carbon and nitrogen and discovered that the mean $\delta^{15}N$ values in the bodies of *Allolobophora chlorotica* (Savigny, 1826), *Aporrectodea caliginosa* (Savigny, 1826), *A. longa* (Ude, 1885), *A. rosea* (Savigny, 1826), *Lumbricus rubellus* (Hoffmeister, 1843), *L. terrestris* were markedly lower in wheat and clover fields than in wheat-only fields by 3.5–4.4 ‰. Schmidt, Scrimgeour, and Curry (1999) studied the carbon cycle within *Lumbricus festivus* (Savigny, 1826) using $\delta^{13}C$ and found notable changes in isotope tissue levels when earthworms transitioned from clover (a C3 plant) to corn (a C4 plant).

Our study examined two species of earthworms, which, according to (Shekhovtsov et al., 2020a; 2020b) form two isolated genetic lines of the *E. nordenskioldi* complex: *E. nana* (L5) and *E. n. nordenskioldi* (L6). We found that the body composition of these lines was dependent on the type of litter they fed on. Xiao et al. (2024) also confirmed the impact of habitat conditions, including litter type in forest biotopes, on the body composition of these *E. nordenskioldi* genetic lines by examining the ratios of carbon, nitrogen, and phosphorus.

IR spectrometry is a proven technique for identifying the species origin of earthworm coprolites, as described by Zangerle, Hissler, McKey, and Lavelle (2016) and Domínguez-Haydar et al. (2020). Casts collected in the field were identified by comparing their spectral signatures with those of macroaggregates produced by the same earthworm species living in the same soil under laboratory conditions. The casts of each earthworm species were characterized by a specific NIR spectral signature, which resulted from quantitative and qualitative differences in the organic matter in casts among the species. This method allows the evaluation of the contribution of each earthworm to the macroaggregate composition of the soil matrix. This algorithm can also be used to study earthworm food preferences in the field; however, we offer the incubation of earthworms in microcosms within the natural habitat where their food preferences are being analyzed, rather than in standard laboratory settings laboratory-based experiment.

This recommendation is based on Pham et al. (2021), who found that the IR spectrum signatures of earthworms incubated in natural habitats differed from those in laboratory microcosms on the same soil. The authors attributed this discrepancy to altered soil conditions and potential stress factors.

In addition, to more accurately simulate natural environmental conditions, it is necessary to incubate earthworms with not only a single type of leaf litter but also with combinations of various leaf litters.

The data that support the findings of this study are openly available at: http://doi.org/10.6084/m9.figshare.25486606.

Conclusion

Our results show that the specific type of leaf litter ingested by earthworms can affect the IR spectral characteristics of their bodies. Experimental data show that body composition changes in each of the three earthworm species are linked to the distinct types of litter consumed. Therefore, IR spectrometry could be an effective approach for analyzing dietary habits. Although IR spectrometry is not a standalone replacement for modern methods such as stable isotope analysis in the study of earthworm feeding ecology, it certainly has a role in augmenting these methods. Its ability to reflect the influence of habitat on an earthworm's biochemical composition adds significant value.

The proposed method could become the initial step in assessing the trophic preferences of earthworms because it is more accessible and allows for the determination of which type of litter is preferred by a particular lumbricid species.

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