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EARTHWORM CASTS AS INDICATORS OF SOIL SUSTAINABILITY IN A SILVOARABLE ECOSYSTEM FROM WESTERN ROMANIA

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Abstract

The research aimed to characterize a silvoarable ecosystem from western Romania through an indirect biological indicator of soil sustainability: the earthworms' casts. The ecosystem consisted of two components: a no-tilled woody perennial plant - Euro-American hybrid poplar trees (Populus deltoides x Populus nigra) and a conventional tilled agricultural crop – the rapeseed (Brassica napus L.) - hybrid LG Architect. The earthworms' casts have been identified as belonging to two ecological groups: epigeic and anecic earthworms in the poplar plantation and anecic earthworms in the rapeseed plantation. For both categories, the analyzed parameters of the earthworm casts were pH, organic matter (humus), total nitrogen, plant-available phosphorus, and plant-available potassium. The values of these chemical parameters were higher for the earthworm casts collected from the rapeseed crop than those collected from the poplar plantation. The results showed statistically significant differences ($p<0.05$) between certain analyzed cast characteristics (total N, plant-available P, plant-available K) of the two plant sub-systems and the same characteristics of the surrounding soil, suggesting that the differences arise from the microhabitat ecological conditions provided by the establishing of the silvoarable system.

Key words*: agroforestry, intercropping, canola, Oligochaeta, Lumbricidae.*

INTRODUCTION

The classical indicators of soil sustainability that are generally more often addressed in studies regarding the sustainability of human ecosystems, and especially the sustainability of agricultural ecosystems are those physical (soil texture, bulk density, porosity, water infiltration, soil erosion etc.), chemical (pH, organic matter content, nutrient concentrations etc.) and biological (soil biodiversity and activity at different taxonomical units) (Phillips et al., 2020). Earthworms are largely monitored in the assessment of the agricultural ecosystems, including in the agroforestry ecosystems, predominantly by counting, biomass weighing, and species diversity (Iordache, 2012; Phillips et al., 2020; Chaudhary and Ghaley, 2025; Reis et al., 2025). The earthworm casts are less approached in the studies regarding the sustainability of the anthropogenic ecosystems, and in Romania these are newly proposed in this field (Iordache et al., 2021; Iordache, 2023; Iordache et al., 2023). Earthworm casts are indirect biological indicator of earthworms activity in soil which are recommended based on solid and concrete scientific arguments (Norgrove and Hauser, 2016; Iordache et al., 2024; Lejoly et al., 2024; Dinh et al., 2025). Even so, this indicator remains insufficiently addressed both for the conventional and widely practiced agricultural systems and for the alternative ones. Thus, in the non-conventional agricultural ecosystems and systems, this indicator is addressed even less often, and therefore there is a lack of data at this level. In Romania, the silvoarable ecosystems are rarely and less researched, and the contribution of earthworms within this type of ecosystem remained unstudied until present. In the last years, several attempts to understand the earthworm contribution in several types of anthropic ecosystems of Romania have been addressed, and also the study of earthworm casts (Iordache et al., 2021; Iordache, 2023; Iordache et al., 2023). Nevertheless, this indicator remains insufficiently studied within Romanian agricultural ecosystems, despite the globally acknowledged role of earthworms in such human-influenced environments, as highlighted by other research in the field (Lalthanzara et al., 2011; Hmar and Ramanujam, 2014; Mulia et al., 2021; Reis et

al., 2025). This study intended to chemically characterize and compare the earthworm casts sampled from two component systems (a herbaceous crop and a woody plantation) of a silvoarable ecosystem from Romania, but also aimed to compare the chemical composition of the earthworm casts with the chemical composition of the adjacent soil, in order to reveal the chemical parameters which show differences, and thus to emphasize the contribution of earthworm casts to the assessment of the studied silvoarable ecosystem.

MATERIALS AND METHODS

The study was conducted in the year 2023 and the study site was a silvoarable ecosystem located in the Western side of Romania, in Timiș County (45.45418°N, 20.90334°E). Several chemical soil parameters widely used as indicators for soil sustainability were studied, namely: pH, the content of organic matter (soil humus), the content of total nitrogen, the content of plant-available phosphorous and the content of plant-available potassium. These parameters have been pursued both in earthworm casts and in the adjacent soil, as earthworm casts are soil transformed by these organisms by ingestion and digestion processes and afterwards excreted. According to earthworm ecological classification using the criteria of their feeding and activity ecology (Bouché, 1977), in this research, only the casts found at the soil surface, susceptible to be produced by the epigeic and anecic earthworm species were chosen to be studied. The susceptibility resides from the exploitation specificity of the researched silvoarable system and its components: a woody plantation of Euro-American hybrid poplar tree (*Populus deltoides* × *Populus nigra*) and a rapeseed crop (*Brassica napus* L.), LG Architect hybrid intercropped by the poplar plantation. These ecosystems were subjected to varying levels of exploitation at the soil level: the poplar plantation remained undisturbed for a continuous period of eight years, while the rapeseed crop underwent conventional tillage practices. Thus, in the poplar plantation, the analyze of the earthworm cast depositions at soil surface, revealed the predominance of epigeic earthworms, which are characteristic to undisturbed soils and live in the topsoil organic layer, mostly in the first 10 cm, covered by the litter and other decaying vegetal matter. In contrast, the rapeseed crop is characterized by tilled and disturbed soil, which creates unfavorable living conditions for epigeic species. However, the earthworms presence has been noted in this agroecosystem too, based on the casts deposited at soil surface and identified, by their shape and size, as belonging to anecic species. The anecic earthworms burrow deeply into the soil profile, but the great part of their feeding and casting activity takes place in the first 30 cm of soil profile and at the soil surface. These reasons led to the decision to collect the earthworm casts from the soil surface in both rapeseed crop and in the poplar plantation. In the present study, only the chemical composition of earthworm casts is presented, along with a comparison to the chemical composition of the adjacent soil. The latter has been previously reported for the top 30 cm of soil in the poplar plantation and the top 10 cm in the rapeseed crop, with both depth ranges being justified in the same prior study (Mazăre and Iordache, 2023). The earthworm casts analyses have been performed by OSPA Timiș County (Office for Pedological and Agrochemical Studies) using the following methodology: for pH (SR 7184-13:2001-PS-03); for humus (STAS 7184/21-82-PS-01); for the total N (STAS 7184/2-85-PS-08); for the plant-available P (STAS 7184/19-82-PS-02); for the plant-available K (STAS 7184/18-80-PS-06). The soil from the studied silvoarable ecosystem is vertisol (World Reference Base for Soil Resources, 2022). The statistical analysis has been performed using the IBM SPSS 28.0.0.0. software.

RESULTS AND DISCUSSIONS

The chemical compositions of earthworm casts sampled within the studied silvoarable ecosystem are presented in Table 1, alongside the chemical composition of the adjacent soil for the two subsystems (hybrid poplar plantation and rapeseed crop) of the silvoarable ecosystem (Mazăre and Iordache, 2023).

Table 1. Chemical composition of earthworm casts in the two sub-systems of the silvoarable ecosystem

Values of soil and earthworm casts parameters in *Populus* spp. (hybrid poplar) plantation (mean values)					
Substrate type	pH	Humus (%)	Total N (%)	Plant available P (ppm)	Plant available K (ppm)
Topsoil (0-30 cm) (Mazăre and Iordache, 2023)	6.36 ±0.16	1.71 ±0.63	0.13 ±0.10	4.72 ±0.81	104.66 ±18.71
Earthworm casts	6.53 ±0.27	1.93 ±0.87	0.15 ±0.017	17.26 ±3.56	131.33 ±13.61
Values of soil and earthworm casts parameters in *Brassica napus* (rapeseed) crop (mean values)					
Substrate type	pH	Humus (%)	Total N (%)	Plant available P (ppm)	Plant available K (ppm)
Topsoil (0-10 cm) (Mazăre and Iordache, 2023)	6.42 ±0.24	2.07 ±0.65	0.12 ±0.0057	18.78 ±15.61	124 ±15.87
Earthworm casts	5.91 ±0.22	2.16 ±0.20	0.17 ±0.03	101.56 ±52.64	237 ±91.06

It could be observed that the values of the analyzed chemical parameters of the earthworm casts are higher in the rapeseed crop than in the poplar plantation (Figure 1). This situation could be due to several factors, but any interpretation should consider that earthworm casts are in fact soil ingested (even not solely) and excreted, so the initial composition and fertility of the ingested soil is a direct factor which influences the final excreted cast. In our study, the rapeseed crop underwent conventional tillage practices, so it was chemically fertilized, and this could be a reason why higher values of the chemical factors were found in the earthworm casts sampled here than in the poplar casts. Another factor susceptible to be responsible for the mentioned result may be the different rate of decomposition of the organic matter input in the rapeseed crop versus in the poplar plantation (Heyn et al., 2019). Thus, in the poplar plantation, although the input of organic matter (leaf litter contribution, root decaying)is higher than in the rapeseed crop, its decomposition and turnover into mineral nutrients may be slower. Therefore, the biogeochemical cycle in the rapeseed soil may be faster than in the poplar soil. Another important factor which may differentiate the chemical composition of earthworm casts is the food type (C/N ratio) ingested by earthworms which ends in excreted casts, because in the rapeseed crop there were collected the casts of the anecic earthworm species, while in the poplar plantation the great part of the collected casts belonged to the epigeic species. The ratio C/N is higher in the casts of epigeic earthworms than in the casts of the anecic species (Zi et al., 2024), and the same in our study (Table 1).

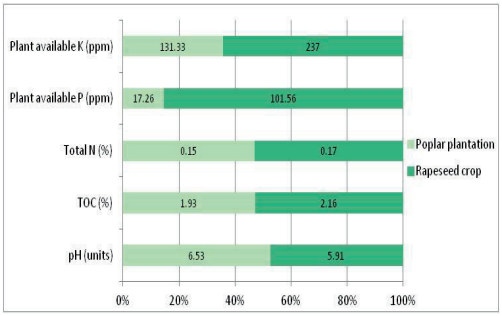

Figure 1. Chemical composition of earthworm casts in the two sub-systems of the studied silvoarable ecosystem

The statistical analysis (Paired Samples *t*-test) showed significant differences only for the soil pH and soil content of plant-available P between the earthworm casts of the two plant sub-systems (Table 2). However, higher values in the chemical composition are observed in the rapeseed casts compared to the poplar casts (for instance, the plant-available K content in the rapeseed casts is 80.46% higher than that in the poplar casts). Therefore, the lack of statistical significance is considered to be due to other factors that may influence the statistics, without undermining the validity of the results obtained (Botinelli et al., 2010; Chen et al., 2021; Iordache et al., 2024).

Table 2. Comparison (Paired Samples *t*-test) of the chemical composition of earthworm casts in the two sub-systems of the silvoarable ecosystem

Crt.no.	Comparison of chemical composition of earthworm casts (poplar versus rapeseed)	t	df	Significance ($p<0.05$)
1.	$pH_{poplar} - pH_{rapeseed}$	10.169	2	0.005
2.	$Humus_{poplar} - Humus_{rapeseed}$	-0.447	2	0.349
3.	$Total\ N_{poplar} - Total\ N_{rapeseed}$	-2.000	2	0.092
4.	$P_{poplar} - P_{rapeseed}$	-2.654	2	0.059
5.	$K_{poplar} - K_{rapeseed}$	-2.315	2	0.073

An interesting situation can be observed for the content of plant-available P both in the soil and in the earthworm casts sampled from the poplar plantation as compared to the same parameters analyzed in rapeseed. The content of this nutrient was very low in the poplar soil as compared to the rapeseed soil. According to the Egner-Riehm method (1960), this content corresponds to a medium supply of soil with

plant-available P, while the content found in the rapeseed soil is considered to correspond to a very well supplied soil. This consistent difference may be a result of the fertilization practices applied in the agricultural plot or even the result of crop rotations during the cultivation technologies, but it could also be the effect of some possible consequences resulted from the silvoarable establishment. A similar situation was previously reported by Yemadje et al. (2023) in an intercropped oil-palm agroforestry system, where levels of phosphorus (P) and potassium (K) were higher in soils from cropped fields compared to palm fallow soils. These results were attributed both to fertilization practices and to the substantial nutrient uptake by the woody oil palm plants. However, the demands of Euro-American hybrid poplar tree (*Populus deltoides* × *Populus nigra*) for P content in soil are met, because it requires moderate to high values of P in soil, and the necessities are high especially in the earlier stages of its development and growth (Singh and Sharma, 2007; Salehi et al., 2022).

For the comparative analysis of earthworm casts (poplar versus rapeseed), the differences between the chemical component parameters were statistically significant only for pH and plant-available P which suggest the contribution of the epigeic and anecic earthworms in the nutrient dynamics of the silvoarable ecosystem of these elements when the rapeseed and the poplar are associated as sub-systems of an silvoarable ecosystem. Thus, the pH slightly decreased in the rapeseed casts as compared to the poplar casts, and the plant-available P increased in the rapeseed cast by approximately 5.88 times than in the poplar casts (Figure 1). The higher value of the pH of earthworm casts sampled from the poplar soil could be an effect of the higher amount of decaying litter in the poplar plantation which determine changes in the pH of the topsoil and also is consumed by the epigeic and anecic earthworms as food together with topsoil particles. The genus *Populus* was earlier shown to increase the topsoil (0-40 cm) pH as effect of litter contribution (Ciadamidaro et al., 2014) as provider of organic matter.

The comparison of the chemical composition of earthworm casts collected from the rapeseed agroecosystem related to chemical composition of the surrounding (adjacent) soil showed higher values of the parameters in the earthworm casts (Figure 2), excepting the value of cast pH, which is lower than the pH of the adjacent soil. The slight decrease of the pH of earthworm casts related to the surrounding soil has been previously reported in other studies as result of microbiological complex interactions occurred during the food processing in earthworm gut (Yang et al., 2024).

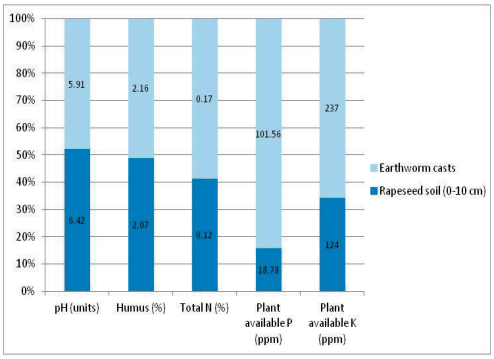

Figure 2. Chemical composition of earthworm casts related to the adjacent soil in the rapeseed crop

The differences between casts and the adjacent soil regarding the chemical composition are consistent especially for the contents of plant-available P and K in the rapeseed crop, respectively by 5.4 and 1.9 times greater in earthworm casts than in the adjacent soil, but these differences are not entirely supported by the statistical analysis (Paired Samples *t*-test), which showed significant differences only for the content of total N (Table 3). Thus, the content of total N was found to be 41.66% higher in earthworm casts than in the surrounding soil.

Table 3. Comparison (Paired Samples *t*-test) of the chemical composition of earthworm casts related to the adjacent soil in the rapeseed crop

Crt.no	Comparison of chemical parameters (adjacent soil vs earthworm cast)	t	df	Significance (p<0.05)
1.	$pH_{soil} - pH_{cast}$	1.952	2	0.095
2.	$Humus_{soil} - Humus_{cast}$	-0.190	2	0.434
3.	$Total\ N_{soil} - Total\ N_{cast}$	-2.982	2	0.048
4.	$P_{soil} - P_{cast}$	-2.485	2	0.065
5.	$K_{soil} - K_{cast}$	-2.442	2	0.067

Although the statistical analysis does not provide conclusive support for the increases observed in other nutrients, it does not

undermine the clear potential of earthworm casts as a source of nutrient enhancement within the studied silvoarable system, benefiting both its components (rapeseed and poplar) (Figures 2 and 3). However, when the earthworm casts and the adjacent soil are compared, the variability of their nutritive elements is high, and therefore there is no generality and consistency regarding certain nutrients that would always be increasing in the earthworm casts as compared to the adjacent soil, while others would be decreasing, because the variability of factors that can lead to very heterogeneous results is high, as well as their range. For example, other studies on agroforestry systems showed significant differences between earthworm casts and its adjacent soil for K and P, while no significant differences were found for N (Hmar & Ramanujam, 2014).The absence of statistical significance in the achieved results does not diminish their relevance, as the variability of soil chemical parameters within anthropogenic ecosystems is influenced by multiple factors. These include the environmental heterogeneity, sampling limitations and inherent soil variability, all of which can contribute to the lack of statistical assurance (Iordache, 2023).
A similar situation was found in poplar: significant differences (Paired Samples *t*-test) between the chemical composition of earthworm cast and the chemical composition of the adjacent soil were found only for plant-available P and K (Figure 3 and Table 4).

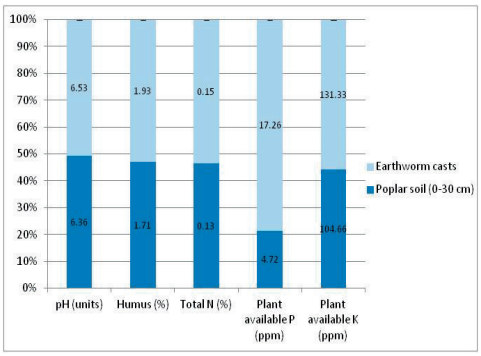

Figure 3. Chemical composition of earthworm casts related to the adjacent soil in the poplar soil

In the case of poplar soil, all the analyzed chemical parameters of earthworm casts were higher than the same parameters recorded for the adjacent soil, including the pH (Figure 3). Higher pH values of the earthworm casts than those of the adjacent soil has been previously found in the agroforestry systems (Hmar and Ramanujam, 2014) and could be the result of the *Populus* litter ingestion by earthworms, which accelerates the turnover process of nutrients within the biogeochemical cycles than does the litter decomposition (mineralization) unmediated by earthworms, by fragmenting the litter and enhancing thus complex microbial interactions with its ingestion (Yang et al., 2023).

Table 4. Comparison (Paired Samples *t*-test) of the chemical composition of earthworm casts related to the adjacent soil in the poplar plantation

Crt. no	Parameter comparison (soil vs earthworm cast)	t	df	Significance (p<0.05)
1.	$pH_{soil} - pH_{cast}$	0.695	2	0.279
2.	$Humus_{soil} - Humus_{cast}$	0.552	2	0.318
3.	Total N_{soil} – Total N_{cast}	2.646	2	0.059
4.	$P_{soil} - P_{cast}$	7.621	2	0.008
5.	$K_{soil} - K_{cast}$	2.915	2	0.050

In order to understand the relationship between the chemical parameters characterising the earthworm casts, statistical correlations (Pearson, Kendall, Spearman, p<0.05) were performed both for the casts sampled in poplar plantation and in the rapeseed crop (Table 5).

Table 5. Relationship (Pearson, Kendall and Spearman correlations, p<0.05) between chemical parameters of earthworm casts

Earthworm casts - Rapeseed crop					
			Nt	P	K
Pearson Correlation	pH	Correlation Coefficient	-	0.998	-
		Significance (p<0.05)	-	0.038	-
Kendall Correlation, Spearman Correlation	pH	Correlation Coefficient	1,000	1,000	1,000
		Significance (p<0.05)	.	.	.
	Nt	Correlation Coefficient	-	1,000	1,000
		Significance(p<0.05)	-	.	.
	K	Correlation Coefficient	-	1,000	-
		Significance (p<0.05)	-	.	-
Earthworm casts - Poplar plantation					
			Humus		K
Kendall Correlation, Spearman Correlation	pH	Correlation Coefficient	1,000		1,000
		Significance (p<0.05)	.		.
	Humus	Correlation Coefficient	-		1,000
		Significance (p<0.05)	-		.

There were found positive correlations between cast pH and casts' humus, total N, plant-available P, and plant-available K. These types of relationship have been earlier identified within earthworm casts, although in other anthropogenic ecosystems (Iordache et al., 2023). For the earthworm casts sampled in the

rapeseed crop, the soil pH was statistically significant correlated with the content of total N, plant-available P and plant-available K. Also, the content of total N was statistically significant correlated with the content of plant-available P and plant-available K, and the content of plant-available K was statistically significant correlated with the content of plant-available P. For the earthworm casts sampled in the poplar plantation, the soil pH was statistically significant correlated with the content of organic matter (humus) and with the content of plant-available K. Also, the content of organic matter (humus) was statistically significant correlated with the content of plant-available K.

There are many factors which can influence the great variability of earthworm casts pH, emphasized in various types of anthropogenic ecosystems, and its correlations with other chemical parameters of their composition: the most studied and frequent are the bioturbation of soil layers and its biochemical related factors determined by the native ecological group of earthworms and by the microbial interactions which characterize the earthworm activity within drilosphere (Wang et al., 2021; Ferlian et al., 2022). Interesting correlations were established by the plant-available K with two factors: plant-available P and total N (in rapeseed casts), but also with the humus in the poplar casts, which are consistent with other findings regarding the earthworm casts in the Romanian anthropogenic ecosystems (Iordache, 2023), revealing the complexity of the chemical interactions occurred inside the earthworm casts and their importance in the ecosystem biogeochemical cycles, and thus as indicator of soil sustainability.

CONCLUSIONS

The contents of the organic matter (humus), total nitrogen, plant-available phosphorus and plant-available potassium were higher in the earthworm casts collected from the rapeseed crop than those collected from the poplar plantation.

The chemical composition of earthworm casts related to chemical composition of the surrounding (adjacent) soil showed higher values of the parameters in the earthworm casts.

The results suggest the contribution of the epigeic and anecic earthworms to the nutrient dynamics in the studied silvoarable ecosystem established by associating the rapeseed crop and the hybrid poplar plantation.

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