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Effects of Selected Annual and Perennial Energy Crops on Lumbricidae Community Assemblages

Anna Mazur-Pączka^{1*}, Kevin R. Butt², Mariola Garczyńska¹, Joanna Kostecka¹, Grzegorz Pączka¹

- ¹ Department of the Basis of Agriculture and Waste Management, Institute of Agricultural Sciences, Land Management and Environmental Protection, College of Natural Sciences, University of Rzeszow, Cwiklinska 1a, 35-601 Rzeszow, Poland
- ² School of Natural Sciences, University of Central Lancashire, Natural Sciences, Preston PR 1 2HE, UK
- * Corresponding author's e-mail: anamazur@ur.edu.pl

ABSTRACT

An increase in demand for energy from renewable sources has increased the hectareage of crops grown for energy purposes. The impact of large-scale energy crop monocultures on soil biodiversity is poorly understood and requires long-term monitoring. Due to their specific lifestyle, Lumbricidae, known as "ecosystem engineers", have found application in biomonitoring of the soil environment. This study aimed to evaluate the qualitative and quantitative structure of Lumbricidae in annual rapeseed (Brassica napus L.) and perennial willow (Salix viminalis L.) crops for energy purposes, with a permanent grassland as a control site. The research was conducted on the territory of the Podkarpackie Agricultural Advisory Center in Boguchwała (southeastern Poland). Earthworms were obtained by hand sorting soil blocks of 25×25×25 cm and a 0.4% formalin solution was used to extract individuals from deeper soil layers. There were no differences in the species composition of Lumbricidae between the analyzed crops. Five species of earthworms, Dendrodrilus rubidus tenius, Lumbricus rubelllus, Aporrectodea caliginosa, A. rosea, and L. terrestris, were found at each study site. Rapeseed had the lowest density (17.26 ± 9.16 ind m⁻²) and biomass $(5.93 \pm 2.42 \text{ g/m}^{-2})$ of Lumbricidae (p < 0.05). On sites with willow and permanent grassland, density and biomass of Lumbricidae were similar (69.15 ± 28.99 ind m⁻²; 26.55 ± 9.67 g m⁻² and $54.04 \pm$ 22.93 ind m^{-2} ; 20.03 \pm 7.99 g m⁻², respectively (p > 0.05). The study demonstrated the beneficial effect of perennial willow cultivation on the quantitative structure of earthworm communities. Only long-term biomonitoring will make it possible to determine the real impact of energy crops on invertebrate assemblages and their appropriate management to promote biodiversity.

Keywords: earthworms, biodiversity, monocultural cultivation, annual and perennial plants, *Brassica napus* L., *Salix viminalis* L.

INTRODUCTION

The increase in global energy demand is due to societies' civilization and economic development. Burning fossil fuels is estimated to provide 80% of the energy humanity needs [Barchanski, 2010]. The extraction of non-renewable resources has become less available and more expensive, and resources have been significantly reduced. Projections show that oil will last for about 40 years, natural gas about 60 years, and coal deposits will be depleted in 220 years [Stankiewicz, 2010]. Use of fossil fuels contribute to global warming, environmental pollution, and a decline in biodiversity. Their exploitation also has a major negative impact on human health. Therefore, a search has begun for alternative, renewable energy sources, including wind, solar, geothermal, sea currents, wave energy, biomass, biogas, and biofuels [Law of April 10, 1997, Energy Law]. In the European Union in 2021, about 22% of energy came from renewable sources. The oldest and most significant source of energy in the world is biomass. One hectare of agricultural land is estimated to yield 10-12 tons of biomass per year, equivalent to about 5-10 tons of coal [Wyszomierski et al. 2017]. Due to the agricultural nature of Poland, it has great potential for biomass production and use for energy purposes. However, the impact of energy crops on the diversity of soil organisms is poorly understood. It depends mainly on the plant species, agricultural systems, and agrotechnical treatments [Verdade et al. 2015, Feledyn-Szewczyk et al. 2019 b, Bourke et al. 2014]. Some of the most well-studied perennial plants and their impact on soil biodiversity include Miscanthus (e.g., Miscanthus x giganteus) [Semere and Slater 2009, Rola et al. 2009], honeysuckle willow (Salix viminalis L.) [Mola-Yudego et al. 2015, Mazur-Paczka et al. 2023], Pennsylvania honeylocust (Sida hermaphrodita (L.) Rusby) [Feledyn-Szewczyk et al. 2019 b], overgrown coneflower (Silphium perfoliatum L.) [Emmerling at al. 2021, Schorpp and Schrader 2016, Ruf and Emmerling 2022], wheatgrass (Agropyron elongatum) [Emmerling et al. 2021]. Among annual crops, studies have been conducted chiefly on cereals and corn [Felten and Emmerling 2011, Smith et al. 2008, Ruf and Emmerling 2022]. From previous research on perennial crops for energy purposes, it can be concluded that they show positive effects on biodiversity compared to annual crops [Sage 1998, Felten and Emmerling 2011, Pedroli et al. 2013, Feledyn-Szewczyk et al. 2019a]. Under Polish conditions, there are few studies on the juxtaposition of the effects of annual and perennial plants on Lumbricidae assemblages in a single publication. Due to the little-understood impact of energy crop species on the soil environment and biodiversity, monitoring of these crops is necessary. It is crucial in decision-making processes regarding competition from large-scale monocultures of energy crops versus the need to support biodiversity [Verdade et al. 2015].

Due to the specific mode of life, representatives of the edaphic Lumbreidae [Lavelle *et al.* 2007] are applicable for biomonitoring of the soil environment. These organisms provide all categories of ecosystem services. They take part in forming soil structure, humifying organic matter, nutrient cycling, participating in carbon sequestration, reducing pollution, and providing a source of protein-rich food for animals [Blouin et al. 2013]. Species composition, ecological group membership, and abundance indicate soil fertility [Van Groenigen et al. 2014]. This study aimed to evaluate the effects of popular energy crops *Brassica napus* (annual plant) and *Salix viminalis* (perennial plant) on earthworm community patterns (qualitative and quantitative structure) concerning a grassland habitat (control site) in southeastern Poland (Central Europe). Specific objectives were to measure: i) species present; ii) community attributes (density, biomass, diversity); iii) individual population attributes (density, biomass, dominance).

MATERIAL AND METHODS

Study site and soil conditions

The research was conducted on the experimental plots of the Podkarpackie Agricultural Advisory Center in Boguchwała (PODR 49°98.906'N 21°93.584'E) in Podkarpackie province (southeastern Poland). The experimental field area covers 18.83 hectares, and arable land covers 17.94 hectares. Two test sites were designated in the area with winter rapeseed Brassica napus L. (BN) and willow Salix viminalis L. (SV). The control site was a permanent grassland (PG) about 0.6 kilometers from the PODR. The area under oilseed rape was about 1 hectare. Cultivation consisted of disking, seed plowing, and cultivation with an aggregate. Annual mineral fertilization was applied at 70 kg $P \cdot ha^{-1}$ (polyphoska 6), 105 kg K·ha⁻¹ (polyphoska 6). For nitrogen fertilization before sowing, i.e., in August each year, 21 kg N·ha⁻¹ (polyphoska 6) and 34 kg N·ha⁻¹ (34% ammonium nitrate) were applied. In the spring (March), 65 kg N·ha⁻¹ (26% saltpeter) and 33.5 kg N·ha⁻¹ (33.5% ammonium nitrate) were applied annually. The fifteen-year-old S. viminalis crop was 0.5 hectares in size and consisted of eleven clones of energy willow. The meadow stand was established ten years before the start of the experiment, with the following plant species present: Poa pratensis L., Festuca pratensis Huds., Dactylis glomerata L., Trifolium pratense L., Plantago major L., Taraxacum officinale F.H. Wigg.

The soils in the study area belong to classes I, II, and III a and are classified as good wheat complex. In the study area, soil temperature and moisture were measured at a depth of up to 20 cm and below during each sampling. Moisture content was determined gravimetrically at 105 °C [ISO PN 11465:1999]. Soil samples (3n) were also taken from each study

Soil property ^{b)}	BN	SV	PG			
Soil temperature (°C)						
0–0 cm	10.4 ± 1.7	10.2 ± 1.8	9.7±2.5			
10–5 cm	9.3 ± 2.8	9.0 ± 2.2	8.9±1.7			
Soil moisture (%)						
0–0 cm	32.7 ± 4.1	38.8.6 ± 4.9	37.3 ± 3.9			
10–5 cm	31.8 ± 4.9	39.2 ± 4.4	37.8 ± 4.1			
pH (KCl)	5.66 ± 0.183	6.34 ± 0.179	6.29 ± 0.188			
Organic C (g·kg ⁻¹)	38335.2 ± 477.4	46107.4 ± 559.2	41794.1 ± 869.3			
Organic N (g·kg ⁻¹)	5228.2 ± 118.3	3288.4 ± 96.1	2688.3 ± 91.4			
Available P (mg P·kg ⁻¹)	198.5 ± 13.4	224.3 ± 16.1	179.9 ± 14.5			
Available K (mg K·kg ⁻¹)	315.2 ± 22.8	318.1 ± 19.6	277.4 ± 21.3			
Available Mg (mg·kg ⁻¹)	45.9 ± 12.7	45.7 ± 9.8	36.3 ± 3.2			

Table 1. Characteristics of soil properties in cultivated rapeseed (BN), willow (SV), and permanent grassland (PG)

area (annually) to determine the physical properties and the content of selected macronutrients and trace elements. pH was analyzed in KCl, organic carbon (C) using the Tiurin method modified by Nikitin and Fishman (1969), total nitrogen (N) using a modified Kjeldahl method [ISO 11261:1995], and the content of the assimilable forms of phosphorus (P), potassium (K), and magnesium (Mg) using the methods described by Mehlich (1984).

Sampling of earthworms

According to ISO [23611-1:2006], a mixed method was used to collect earthworms. Lumbricidae were searched for by hand sorting soil blocks of 25×25×25 cm. To extract individuals from deeper soil levels, 10 liters of 0.4% formalin solution (HCHO by CHEM-PUR) was used to flood each hole created after digging out the soil. Five test samples (5n) were randomly taken at each site. Lumbricidae sampling was conducted in April and September in three annual cycles (2020–2022). The earthworms found were euthanised in 30% ethanol and then preserved in a 4% formalin solution (HCHO by CHEM-PUR). Collected specimens were identified, counted, and weight (with gastrointestinal contents) was determined using a RADLAB WAS 160/X balance. Species identification was carried out at the University of Rzeszow using keys for the determination of terrestrial angiosperms of Poland [Kasprzak, 1986].

Biocenotic indicators and data analysis

Biocenotic indices were used to determine the groupings of earthworms: D – dominance, D = na/n, where: na – is the number of individuals belonging to species a in all samples, n – the number of individuals of the studied species group in all samples. Dominance classes were adopted according to Górny and Grüm [1981], Shannon-Wiener species richness index:

$$(H'), H' = \Sigma p_i \times \log p_{n_i} \tag{1}$$

where: p_i is the ratio of the number of the ith species to the total number of all the species [Southwood, 1978].

The results are presented as mean \pm standard deviation (SD) and were statistically analyzed using STATISTICA version 12.5. One-way analysis of variance ANOVA with Tukey's test was used. If assumptions for parametric tests were not met, non-parametric equivalents of the Kruskall-Wallis test with multiple comparisons of mean ranks for all samples were used.

RESULTS

A total of five species of earthworm: *Dendrodrilus rubidus tenius* (Savigny 1826), *Lumbricus rubelllus* (Hoffmeister 1843), *Aporrectodea caliginosa* (Savigny 1826), *A. rosea* (Savigny 1826), *L. terrestris* (L. 1758) were found at the surveyed sites, with all species occurring at all surveyed sites (Table 2).

The earthworms represented all three ecological groups, i.e., epigeic, endogeic, and anecic (Table 2). Pfiffner and Mäder [1997] stated that one to eleven earthworm species are primarily found in arable fields in Central Europe. However, some authors suggest that only one to two species can be found on arable land [Makulec, 2005, Shmith et al. 2008]. The same number of earthworm species (5) as on the PODR was found by Mazur-Pączka et al. [2023] in willow crops fertilized with and without sewage sludge in the village of Trzciana (Podkarpackie Province, southeastern Poland) 20 km from the current sampling site. In a study by Feledyn-Szewczyk et al. [2019b], they found the most earthworm species (10) in willow cultivation in Osiny (Poland, Lublin voivodeship), while they found the fewest species (7) in a high-input wheat monoculture. Previous studies on perennial crops for energy purposes show beneficial effects on biodiversity [Emmerling 2014, Feledyn-Szewczyk et al. 2019a]. In the present study, there were no differences in the qualitative structure of Lumbricidae between the study sites, as evidenced by a similar Shannon-Wiener diversity index, which raised in rape $1.24a \pm 0.11$, in willow 1.46a \pm 0.07 and in the meadow 1.26a \pm 0.11 (p > 0.05) (Table 3). This may be due to all earthworms found in Boguchwała being common in Poland, eurytypic species with high adaptability [Kasprzak, 1986].

The rapeseed crop had the lowest density of Lumbricidae, 17.26 ± 9.16 ind \cdot m⁻² (p < 0.05). In the willow and permanent grassland sites, the density did not differ and was 69.15 ± 28.99 ind \cdot m⁻² and 54.04 ± 22.93 ind \cdot m⁻² (p > 0.05), respectively (Table 3). Similar trends were observed in the studied crops for earthworm biomass (Table 3). According to research, mineral fertilizers, including ammonium sulfate, harm earthworm populations mainly by acidifying the soil [Pfiffner, 2014], possibly explaining the lowest earthworm density and biomass in the rapeseed crop. Mineral fertilization is estimated to reduce earthworm density and biomass by about 40%

compared to crops where mineral-organic fertilization was applied [Pfiffner and Mäder 1997]. A study by Emmerling et al. [2021] also found the lowest density and biomass of Lumbricidae in annual silage corn, which were 43.3 ind m⁻² and 21.1 g·m⁻². This study found intermediate earthworm density values for perennial energy crops (cup plant, tall wheatgrass, giant knotweed) 86.7 ind·m⁻², the highest for permanent grassland 114.4 ind m-2. However, perennial energy crops' highest biomass was 40.1 g \cdot m⁻². It has also been shown that intensive cultivation of annual crops for energy purposes leads to soil compaction, nitrogen loss, and a decrease in biodiversity. Feledyn-Szewczyk et al. [2019b] found similar earthworm densities in willow, as in the present study, which was 74 ind \cdot m⁻² and was the highest among the energy crop species studied. Field experiments conducted in Wales [Fry et al. 2008], among others, confirmed the beneficial effects of willow on invertebrate assemblages compared to intensive conventional cropping on arable land. According to Ruf and Emmerling [2020], the value of soil organic matter, aggregate stability, and soil microbial activity improved under perennial plantings for energy purposes compared to high-input corn cultivation. In addition, perennial crops affect soil carbon deposition [Chimento et al. 2016, Ruf et al. 2018], protect against erosion, reduce leaching of minerals from the soil, and increase organic matter and microbial activity over time [Don et al. 2012, Hargreaves and Hofmockel 2014, Schmidt et al. 2018].

The highest density and biomass of Lumbricidae among the studied sites were recorded in willow cultivation for the litter-dwelling (*D. rubidus*) and epi-endogeic species *L. rubellus* and the deepburrowing (epi-anecic) *L. terrestris* (p < 0.05) (Table 4). Willow created a favorable food base for

Species of earthworms ecological group	Research sites*					
	BN	SV	PG			
Epigeic						
D. rubidus tenius	+	+	+			
L. rubellus	+	+	+			
Endogeic						
A. caliginosa	+	+	+			
A. rosea	+	+	+			
Anecic						
L. terrestris	+	+	+			

 Table 2. Qualitative structure of earthworms at study sites

* Note: Table 1 presents a detailed description of the three sampling sites; + indicates the presence.

Ecological parameters	BN	SV	PG
Density [ind·m ⁻²]	17.26ª ± 9.16	69.15 ^b ± 28.99	54.04 ^b ± 22.93
Biomass [g·m⁻²]	5.93ª ± 2.42	26.55 ^b ± 9.67	20.03 ^b ± 7.99
Shannon-Wiener index (H').	1.24ª ± 0.11	1.46ª ± 0.07	1.26ª ± 0.11

Table 3. Ecological parameters of earthworms in research sites*

* Note: Table 1 presents a detailed description of the three sampling sites.

earthworms of the groups mentioned above, as it provided mulch, on which the occurrence of earthworms of the epigeic and anecic groups is mainly dependent. The lowest density and biomass were recorded for all species found in the rapeseed crop (p < 0.05). (Table 4). This could have been due to use of nitrogen mineral fertilizers and plowing, which negatively affects deep-digging species [Monroy, 2006]. In annual crops, the soil beneath the plants is bare, resulting in moisture loss, direct exposure of individuals to desiccation and UV exposure, and lack of protection from predators [Smith et al. 2008, Kanianska et al. 2016].

The subdominants on the site with oilseed rape cultivation included *D. rubidus tenius* and *L. rubellus*. The most considerable contribution to the assemblage was *A. caliginosa*, *A. rosea*, and *L. terrestris*, which were eudominants (Table 4). In willow, for energy purposes, *D. rubidus* (subdominant) and *L. rubellus* (dominant) achieved the smallest share in the assemblage. Eudominants similar to as rapeseed included *A. caliginosa*, *A. rosea*, and *L. terrestris*. In permanent grassland, the dominants were *D. rubidus* tenius and L. terrestris, and the subdominant was L. rubellus, the largest share of the grouping, similar to the other sites were A. caliginosa and A. rosea (Table 4). Earthworms digging shallow branching burrows A. caliginosa and A. rosea are common species in various habitats. They show high tolerance in terms of acidity and organic matter content. They also tolerate tillage well, often found in meadows, pastures, orchards, and farmland. A. caliginosa was the most abundant species in various crops [Ivask et al. 2007, Kanianska et al. 2016].

CONCLUSIONS

No differences in the species composition of Lumbricidae were found in soils below the analyzed energy crops, as evidenced by the similar biodiversity index H'. All species found are common, typical of the land use, and characteristic of the region.

The rapeseed crop had the lowest density and biomass of Lumbricidae due to the high-input

Table 4. Characteristic of earthworms community in oilseed rape (BN), willow (SV), and permanent grassland(PG) crops

Earthworm sp	pecies	Density [ind·m ⁻²]	Biomass [g·m ⁻²]	Dominance [%]
D. rubidus tenuis	BN	3.44ª ± 1.62	$0.85^{a} \pm 0.36$	3.09 subdominant
	SV	42.30 ^b ± 22.04	13.62 ^b ± 5.13	12.21 eudominant
	PG	24.17° ± 10.62	7.20° ± 2.89	8.96 dominant
L. rubellus	BN	4.15ª ± 2.30	2.30ª ± 0.76	4.72 subdominant
	SV	24.31 ^b ± 7.35	10.06 ^b ± 3.14	6.98 dominant
	PG	9.36° ± 5.61	5.02° ± 1.79	3.36 subdominant
A.caliginosa	BN	42.34ª ± 22.08	13.12ª ± 5.36	49.09 eudominant
	SV	132.55 ^b ± 56.89	44.81 ^b ± 15.33	38.37 eudominant
	PG	127.34 ^b ± 47.89	45.34 ^b ± 17.67	1.39 eudominant
A. rosea	BN	25.80ª ± 15.30	7.56° ± 3.55	29.94 eudominant
	SV	90.58 ^b ± 35.64	30.01 ^b ± 12.38	26.16 eudominant
	PG	85.67 ^b ± 39.03	27.54 ^b ± 11.45	31.72 eudominant
L. terrestris	BN	10.60° ± 4.52	5.86ª ± 2.05	12.30 eudominant
	SV	56.03 ^b ± 23.02	34.27 ^b ± 12.34	16.28 eudominant
	PG	23.64° ± 11.50	15.04° ± 6.16	8.58 dominant

cropping system, which adversely affects Lumbricidae groupings. The perennial cultivation of willow created favorable conditions for Lumbricidae due to less environmental interference than in annual crops. The quantitative structure of earthworms in energy willow was similar to that in permanent grassland (control site). The highest density and biomass of earthworms from the litter dwelling and deep-burrowing groups were found in the willow crop, where the litter present is particularly important for earthworms of the epigeic and anecic groups.

Research on the impact of annual and perennial energy crops on earthworm assemblages would usefully be expanded to analyze more plant species. Groups of other invertebrate ecosystem service providers from within the soil could also be assessed. Long-term monitoring would also allow appropriate management of large-scale energy crops to help promote soil biodiversity.

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