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Effects of Energy Crop Monocultures and Sewage Sludge Fertiliser on Soils and Earthworm Community Attributes

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Abstract: Biomass is one of the most significant renewable energy sources. Doubts arise from large-area plantations of energy monocultures, which can lead to the depletion of, and a decrease in, soil biodiversity. Community association analyses of Lumbricidae may help to indicate environmental change. Therefore, the study objectives were to determine the qualitative and quantitative diversity of Lumbricids in plantations of energy crops—basket willow (*Salix viminalis* L.), foxglove tree (*Paulownia tomentosa* Steud.), and black locust (*Robinia pseudoacacia* L.)—by investigating the following cultivation treatments: SV and SVSS—*S. viminalis* without (w/o) and with the addition of sewage sludge to the soil (+SS); PT and PTSS—*P. tomentosa* w/o and + SS; RP and RPSS—*R. pseudoacacia* w/o and +SS; and MW—meadow community. A significantly higher density ($p < 0.05$) and biomass ($p < 0.05$) of earthworms were found in the SV and SVSS plantations than in other sites. The application of sewage sludge contributed to a significant increase in the mean number and biomass of all Lumbricids within cultivations of *S. viminalis* and *R. pseudoacacia*, which were significantly higher than those in the control site. This work suggests that woody energy crop production with an appropriate selection of plants and fertilisation can be a favourable habitat for the development of earthworm populations.

Keywords: earthworms; biodiversity; monoculture of energy plants; *S. viminalis*; *P. tomentosa*; *R. pseudoacacia*; sewage sludge

Citation: Mazur-Pączka, A.; Pączka, G.; Garczyńska, M.; Jaromin, M.; Hajduk, E.; Kostecka, J.; Butt, K.R. Effects of Energy Crop Monocultures and Sewage Sludge Fertiliser on Soils and Earthworm Community Attributes. *Agriculture* **2023**, *13*, 323. https://doi.org/10.3390/agriculture13020323

Academic Editor: Mirna Velki

Received: 12 December 2022
Revised: 24 January 2023
Accepted: 26 January 2023
Published: 28 January 2023

1. Introduction

A need to combat climate change and become independent of excessive fossil fuel consumption has increased the use of renewable energy sources. Biomass is one of the most significant and common renewable energy sources in agricultural countries. It can be obtained from agricultural and forest waste, urban greenery, the wood industry and plantations intended for energy purposes [1]. Despite the many benefits of biomass energy processing, doubts are raised by large-area plantations of energy plant monocultures, which are often the cause of the reduced production of food crops and can lead to soil sterilisation and a decrease in soil biodiversity [2]. Preserving essential functions of soil ecosystems, such as a habitat for plants and animals, the circulation of water and nutrients, and a buffer that neutralises numerous anthropogenic pollutants strongly related to soil biodiversity, has been a priority in the implementation of the principles of sustainable agriculture for many years [3].

Due to the prolonged process of pedogenesis, soil is considered a non-renewable or potentially renewable resource. Soil is a complex of abiotic and biotic components characterised by the complexity of various ecological processes, where soil organisms play a fundamental role. However, some current agricultural practices may not only negatively affect physical and chemical properties but may also cause adverse changes in soil invertebrate associations [4]. One practice used in plant cultivation is fertilisation with sewage sludge, as the macronutrients present may be a good source of nutrients for plants and may also have a positive effect on the structure and condition of the soil [5]. However, studies also report on the risks associated with sewage sludge from the potentially high concentration of toxic elements that may accumulate in the soil after applying large doses of this fertiliser [6]. For example, heavy metals such as lead or cadmium often permanently accumulate in the upper layers of soil and can seriously threaten the biodiversity of soil ecosystems [7]. Recently, much attention has been paid to the contamination of the soil environment with microplastics [8] and persistent organic compounds (POPs), including per- and polyfluoroalkyl substances (PFAS) that disrupt the endocrine economy of organisms [9]. Therefore, one of the priorities in the adequate protection of soil ecosystems should be the constant monitoring of changes in groups of organisms that significantly affect soil functioning, of which the Lumbricidae is undoubtedly one [10].

Due to their specific lifestyle, earthworms play a crucial role in the soil environment as "ecosystem engineers" [11–13]. The life functions of earthworms are also vital in all categories of services provided by ecosystems. They participate in forming soil structures, nutrient cycling, primary production, climate regulation, pollution reduction, and cultural services [14]. Earthworm burrows improve the physical properties of soils, e.g., humidity, aeration, density, and porosity [10,15–18]. In turn, the formation of coprolites positively affects soil structure and its chemical and biological properties [10,19–21]. Earthworms are also responsible for the compaction and decompaction of soil. These dynamic processes affect the maintenance of soil structure through the conversion of loose and compacted soils to an intermediate mechanical state that is more favourable for maintaining structural stability [16,22]. The beneficial effects of Lumbricids on plant production have also been confirmed [23,24] with the most significant effects on perennial plants and trees and the least on annuals [25–27]. Studies on the impact of annual energy crops such as maize or cereals on the qualitative and quantitative structure of earthworm communities have been presented [28–30], with similar information provided by Curry et al. [31] and Smith et al. [32] concerning grasslands and pastures. The research also concerned the impact of perennial energy plants such as cup plant, tall wheatgrass, and giant knotweed on the qualitative structure, density, biomass and morphological groups of Lumbricids [33,34]. The few studies available concern comparisons of the impact of different agricultural systems on arable land and the impact of some energy crops (Miscanthus, Virginia mal-low, willow) on selected features of Lumbricid associations [35,36]. Other than the aforementioned willow, there are few studies on the effects of long-term woody monoculture energy crops on Lumbricid communities. Understanding the impact of woody monoculture energy crops on biodiversity and the soil environment is of particular importance, as these species are preferred for the production of solid fuel due to their higher calorific value, low humidity, and low ash content compared to other energy crops.

There are indications that long-term monoculture cultivation may cause a decrease in biodiversity [37]. By contrast, Rowe et al. [3] and Cunningham et al. [38] reported that long-term monoculture crops can contribute to an increase in biodiversity due to the lack of annual mechanical interference in the vertical structure of the soil. Although earthworms are generally considered to be a critical element in the functioning of ecosystems [14], there is still a deficit of information to sufficiently understand the distribution of Lumbricid associations in various agricultural ecosystems [39] and to recognise the impact of plant monocultures on these invertebrates.

Therefore, the main aim of this study was to analyse selected features of Lumbricid associations at sites with long-term woody monoculture energy crops either fertilised with or without sewage sludge and compare these with nearby uncultivated meadows. The objectives were to record the earthworm species richness and diversity, population density and biomass at each site and seek to relate these findings to soil physio-chemical characteristics brought about through species of energy crops and fertiliser treatments.

2. Materials and Methods

2.1. Study Site and Soil Condition

The research was conducted in southeast Poland near the city of Rzeszów (50°07.808′ N 21°84.677′ E) on experimental plots of the Cooperative Group of Producers of Energy Plants "Agroenergia". It is a flat area located at an altitude of 221 m above sea level and soils are classified as loamy sand (fraction content <0.02 mm from 16.17% to 18.24%) [40]. Mean temperature and soil moisture results during the experimental period (2019–2021) are presented in Table 1. For research purposes, the following monoculture experimental stands were distinguished: SV—the cultivation of *S. viminalis* without the addition of sewage sludge in the soil (w/o SS); SVSS—the cultivation of *S. viminalis* + SS; PT—the cultivation of *P. tomentosa* w/o SS; PTSS—the cultivation of *P. tomentosa* + SS; RP—the cultivation of *R. pseudoacacia* w/o SS; RPSS—the cultivation of *R. pseudoacacia* + SS; and MW—meadow community (a control site, for comparative purposes). The 10 ha plantation of energy crops was located in one area. Each of the experimental sites was 1.5 ha in size. The test sites were separated from each other by buffer zones with a width of about 4 metres. The control site (meadow community) was about 50 metres away from the experimental sites. Stabilised municipal sewage sludge, which contained 16.4 ± 3.3% dry matter (pH < 10.0) and by dry weight contained organic matter 48.5 ± 9.8%, total nitrogen 3.76 ± 0.77, Ca 16.6 ± 3.4%, Mg 0.35 ± 0.08%, Cd 0.55 ± 0.11 mg kg^{-1}, and Pb 8.81 ± 1.79 mg kg^{-1} [41] came from the mechanical and biological sewage treatment plant in Świlcza (SE Poland) and was applied (30 tonnes dry matter ha^{-1}) to the soil. For application, a slurry tanker equipped with an adapter for introducing fertiliser directly below the soil surface was used [41]. Fertilisation with sewage sludge and the establishment of energy plantations in 2010 was undertaken by the Cooperative Group of Producers of Energy Plants "Agroenergia". Contents of selected macro-elements and trace elements in the soils of the studied sites are presented in Table 1.

Table 1. Content of macro-nutrients and trace elements in the given cultivation treatment soils (mean ± standard deviation based on five samples).

		Cultivation Treatments						
Parameter	**Units**	**SV**	**SVSS**	**RP**	**RPSS**	**PT**	**PTSS**	**MW**
C_{org}	mg kg^{-1}(d.m.)	147,611.4 ± 1442.5 ^{a}	163,809.2 ± 1518.3 ^{b}	64,741.3 ± 788.7 ^{c}	75,184.1 ± 894.5 ^{d}	47,622.5 ± 605.9 ^{e}	53,814.8 ± 537.8 ^{f}	62,337.3 ± 704.2 ^{g}
TN		13,376.1 ± 202.1 ^{a}	14,624.5 ± 187.3 ^{b}	7141.2 ± 146.9 ^{c}	7215.8 ± 159.8 ^{c}	5122.4 ± 104.6 ^{d}	5338.9 ± 116.5 ^{d}	6462.2 ± 128.2 ^{e}
P		100.1 ± 19.7 ^{a}	123.6 ± 19.9 ^{b}	58.0 ± 10.5 ^{d,e}	77.6 ± 12.1 ^{c,d}	46.2 ± 8.3 ^{e}	94.3 ± 17.1 ^{a,c}	53.8 ± 11.2 ^{e}
K		37.8 ± 4.4 ^{a,b}	49.1 ± 5.1 ^{c}	21.5 ± 2.9 ^{d}	23.3 ± 2.7 ^{d}	35.3 ± 3.9 ^{b}	41.3 ± 4.9 ^{a}	21.9 ± 2.7 ^{d}
Ca		4433.4 ± 99.2 ^{a}	5787.2 ± 116.9 ^{b}	3297.7 ± 87.1 ^{c}	4792.9 ± 97.5 ^{d}	3089.1 ± 83.9 ^{e}	4899.3 ± 110.6 ^{d}	2882.2 ± 79.9 ^{f}
Mg		207.6 ± 24.4 ^{a,b}	274.9 ± 26.1 ^{c}	162.1 ± 19.9 ^{d}	202.6 ± 21.3 ^{b}	186.0 ± 19.6 ^{b,d}	238.4 ± 22.7 ^{a}	170.7 ± 16.4 ^{d}
Cd		0.5 ± 0.0 ^{a}	0.8 ± 0.0 ^{b}	0.6 ± 0.0 ^{a}	1.1 ± 0.1 ^{c}	1.2 ± 0.1 ^{c,d}	2.9 ± 0.2 ^{e}	1.3 ± 0.1 ^{d}
Pb		11.5 ± 2.5 ^{a}	14.7 ± 2.7 ^{a}	24.9 ± 3.9 ^{b}	31.7 ± 4.4 ^{c}	34.0 ± 4.3 ^{c}	56.9 ± 8.1 ^{d}	37.2 ± 4.5 ^{c}
C/N ratio	-	11.03 ± 0.5 ^{a}	11.20 ± 0.4 ^{a}	9.07 ± 0.3 ^{b}	10.42 ± 0.4 ^{d}	9.29 ± 0.3 ^{b}	10.08 ± 0.5 ^{c,d}	9.64 ± 0.4 ^{b,c}
pH in H_20	-	7.48 ± 0.2 ^{a,b}	7.94 ± 0.2 ^{e}	7.21 ± 0.1 ^{c}	7.77 ± 0.2 ^{d,e}	7.13 ± 0.1 ^{c}	7.64 ± 0.2 ^{a,d}	7.25 ± 0.2 ^{c}
Electrical conductivity	mS·cm^{-1}	0.310 ± 0.01 ^{a}	0.351 ± 0.01 ^{b}	0.152 ± 0.02 ^{c}	0.262 ± 0.02 ^{d}	0.191 ± 0.01 ^{e}	0.323 ± 0.01 ^{a}	0.149 ± 0.02 ^{c}
Temp.	°C	13.6 ± 1.4 ^{a}	13.7 ± 1.3 ^{a}	13.8 ± 1.3 ^{a}	13.9 ± 1.4 ^{a}	13.6 ± 1.3 ^{a}	13.7 ± 1.2 ^{a}	13.6 ± 1.2 ^{a}
Moisture	%	28.5 ± 6.5 ^{a}	28.8 ± 6.6 ^{a}	28.3 ± 6.4 ^{a}	28.7 ± 6.7 ^{a}	28.9 ± 6.4 ^{a}	28.9 ± 6.5 ^{a}	28.8 ± 6.3 ^{a}

Mean values in a row followed by different letters are significantly different ($p < 0.05$). (SV—*S. viminalis*; PT—*P. tomentosa*; RP—*R. pseudoacacia*; SS with sewage sludge added; MW—meadow community—control site).

2.2. Earthworm and Soil Sampling

Five randomly selected areas (5 × 5 m) were designated within each cultivation treatment and following the recommendations of Zisci [42], within each of these areas, n = 5 random samples of 25 × 25 × 25 cm soil blocks were extracted and manually sorted for earthworms. In addition, ten litres of 0.4% formalin solution (vermifuge) was poured into each hole created after digging the soil to extract individuals from the deeper soil levels [43]. Sampling for earthworms occurred three times each year (May, July, and September) in three annual cycles (2019–2021). Collected earthworms were placed in a 15 min water bath, anaesthetised by immersion in 30% ethyl alcohol, and then transferred to a preservative solution (4% formalin). After 24 h, the formalin solution was replaced with a fresh solution. Earthworms were identified, counted, and weighed with gastrointestinal contents present. The density and biomass of earthworms from soil monoliths were calculated per square metre. Species were identified using keys for determining terrestrial oligochaetes in Poland [44]. Soil samples (n = 3) were also collected annually from each cultivation treatment to determine selected physicochemical properties.

2.3. Physicochemical Analysis of Soil

The total content of macro elements (N, P, K, Ca, Mg) and trace elements (Cd, Pb) in treatment soils was determined using the procedures described by Ostrowska et al. [45]. Nitrogen was determined by the Kjeldahl method using a Kjeltec 8100 distillation unit and a 2006 Foss Tecator Digestor apparatus (Foss Tecator AB, Hoeganaes, Sweden). To determine the remaining elements, the soil was mineralised in pure concentrated $HClO_4$. Phosphorus was colourimetrically determined using the vanadium–molybdenum method on a UV-VIS spectrophotometer (Shimadzu UV 2600, Kyoto, Japan), and K, Mg, Ca, and trace elements were determined using flame atomic absorption spectrophotometry [45] with a Hitachi Z-2000 (Hitachi Inc., Tokyo, Japan). In addition, the carbon content in the soil was determined using a Vario EL-CUBE elemental analyser (Elemental Analysensysteme GmbH, Langenselbold, Germany). The C/N ratio was calculated. Soil pH was determined by potentiometry with a soil:water ratio of 1:2.5 using an HI 4221 pH meter (HANNA Instruments Inc., Woonsocket, RI, USA). The conductivity method determined salt concentration with a HI 2316 Conductivity/Resistivity Meter (HANNA Instruments, Woonsocket, RI, USA). At sampling sites, temperature and soil moisture were measured at a depth of 10 cm. Soil moisture was determined by oven drying at 105 °C [46].

2.4. Data Analysis

To assess Lumbricid associations, the Shannon–Wiener species richness index (H') and dominance index (D) were used:

$$H' = \Sigma p_i \times \log_n p_i$$

where p_i is the ratio of the number of organisms of a given species to the total number of all organisms [47];

$$D = N_a/n$$

where N_a is the number of individuals belonging to the species in all tested samples and n is the number of individuals of the studied group of species in all samples [48]. Dominance classes were adopted according to Górny and Grüm [49]: eudominants >10%, dominants 5.1–10%, subdominants 2.1–5%, recedents 1.1–2%, and subrecedents <1% of the total number of individuals in the group.

The results were analysed statistically by Statistica software v. 13.3. To assess the differences between the means of density (ind. m^{-2}), biomass of earthworms (g m^{-2}), and Shannon–Wiener species richness index (H') per cultivation treatment and species, a two-way analysis of variance and Tukey HSD (*post hoc*) test were used. Correlation analysis was conducted using the Pearson correlation coefficients to identify the relationship between earthworms and selected soil variables per location. The significance of the correlation coefficients was tested by a t-test ($p > 0.05$).

3. Results and Discussion

3.1. Species of Earthworms in the Study Area

A total of five species of earthworm were found in the study area: *Dendrodrilus rubidus* (Savigny 1826), *Lumbricus castaneus* (Savigny 1826), *Allolobophora caliginosa* (Savigny 1826), *Allolobophora rosea* (Savigny 1826), and *Lumbricus terrestris* (Linnaeus 1758) (Table 2). Each species occurred at each of the examined treatment sites. Most species of Lumbricid in the studied sites were classified as eudominants, except for *D. rubidus* in the unfertilised cultivation of *R. pseudoacacia* (RP) and in the meadow site (MW), where it was a dominant (Table 2).

Table 2. Mean abundance (ind. m^{-2}), mean sum of biomass (g m^{-2}), and dominance of earthworm species found in the cultivation treatments.

Species/Ecological Group *	Features	Cultivation Treatments **						
		SV	SVSS	RP	RPSS	PT	PTSS	MW
Epigees								
	Abundance	51.9 ± 10.1 [a]	130.4 ± 12.2 [b]	24.0 ± 4.8 [d]	80.2 ± 13.1 [c]	24.1 ± 6.9 [d]	24.1 ± 4.4 [d]	24.7 ± 4.8 [d]
Dendrodrilus rubidus	Biomass	4.63 ± 1.22 [a]	12.38 ± 2.13 [b]	1.73 ± 0.38 [d]	5.99 ± 1.09 [c]	2.88 ± 1.01 [d]	2.33 ± 0.52 [d]	2.12 ± 0.48 [d]
	Dominance %	11.9	17.9	9.5	16.1	11.5	11.5	9.5
	Abundance	91.2 ± 3.1 [a]	149.0 ± 8.7 [b]	56.0 ± 7.1 [d]	93.6 ± 4.5 [a]	56.9 ± 12.1 [d]	56.6 ± 17.4 [d]	56.9 ± 9.1 [d]
Lumbricus castaneus	Biomass	77.87 ± 2.96 [a]	126.44 ± 8.08 [b]	48.06 ± 6.11 [d]	79.82 ± 3.79 [a]	47.62 ± 11.33 [d]	44.28 ± 12.71 [d]	46.88 ± 8.33 [d]
	Dominance %	20.8	20.5	22.2	18.8	27.2	27.2	21.9
Endogees								
	Abundance	115.9 ± 20.4 [a]	164.8 ± 41.8 [b]	61.1 ± 15.6 [d]	135.4 ± 26.2 [c]	49.6 ± 13.6 [d]	48.7 ± 19.4 [d]	60.9 ± 17.2 [d]
Allolobophora caliginosa	Biomass	58.58 ± 10.28 [a]	84.57 ± 21.96 [b]	27.27 ± 7.56 [d]	61.48 ± 12.41 [a]	24.09 ± 6.81 [d]	21.53 ± 8.11 [d]	28.31 ± 8.03 [d]
	Dominance %	26.5	22.7	24.2	27.2	23.7	23.4	23.5
	Abundance	85.6 ± 5.7 [a]	176.0 ± 16.4 [b]	48.9 ± 11.3 [d]	103.4 ± 20.3 [c]	48.5 ± 10.1 [d]	48.6 ± 13.9 [d]	51.1 ± 12.2 [d]
Allolobophora rosea	Biomass	43.56 ± 3.85 [a]	92.24 ± 10.21 [b]	25.81 ± 5.92 [d]	55.45 ± 11.24 [c]	23.69 ± 5.31 [d]	21.92 ± 5.31 [d]	26.47 ± 6.17 [d]
	Dominance %	19.6	24.3	19.4	20.8	23.2	23.3	19.7
Aneciques								
	Abundance	93.1 ± 18.4 [a]	105.1 ± 19.2 [b]	62.8 ± 16.3 [c]	85.2 ± 24.5 [d]	30.2 ± 7.7 [e]	30.3 ± 10.6 [e]	65.9 ± 17.4 [c]
Lumbricus terrestris	Biomass	230.91 ± 46.89 [a]	248.92 ± 46.99 [b]	136.95 ± 39.99 [c]	172.94 ± 50.84 [d]	77.19 ± 20.58 [e]	71.92 ± 25.81 [e]	162.66 ± 44.17 [d]
	Dominance %	21.3	14.5	24.8	17.1	14.4	14.6	25.4

* Ecological group of Bouché [50]; ** abbreviations of cultivation treatments are as given in Table 1. Different letters in a row indicate statistically significant differences ($p < 0.05$).

Close to this study area (some 20 km away in the village of Krasne), on arable land and permanent grassland, six and seven species of earthworms, respectively, had previously been found [51]. The number of earthworm species found in sites for the cultivation of energy crops described here is smaller by comparison to the data obtained by Felendyn-Szewczyk et al. [35], who found eight to ten Lumbricid species in Miscanthus, mallow, and willow crops. In turn, Felten and Emmerling [36], in a long-term cultivation of Miscanthus, found a similar number of five species of earthworms, while in the cultivation of rape and maize, the aforementioned authors showed four and three species of Lumbricid, respectively. Studies have shown that earthworm populations are more abundant and species-rich in less disturbed habitats and soils subjected to reduced tillage [52], supporting the cultivation of perennial crops for energy purposes.

From the mean density and mean sum of earthworm biomass, a significant increase in values was found at the SVSS and RPSS sites compared directly with unfertilised SV and RP treatments. In most cases, they were also significantly higher than those recorded at the MW control site (RP was here the exception). No significant differences in mean density and biomass between PT and PTSS were found at sites with *P. tomentosa* cultivation. The highest values of analysed earthworm traits were observed in treatments of *S. viminalis* and *R. pseudoacacia* with additions of sewage sludge (Figure 1a,b), tendencies also illustrated by the Shannon–Wiener species richness index (H') (Figure 1c).

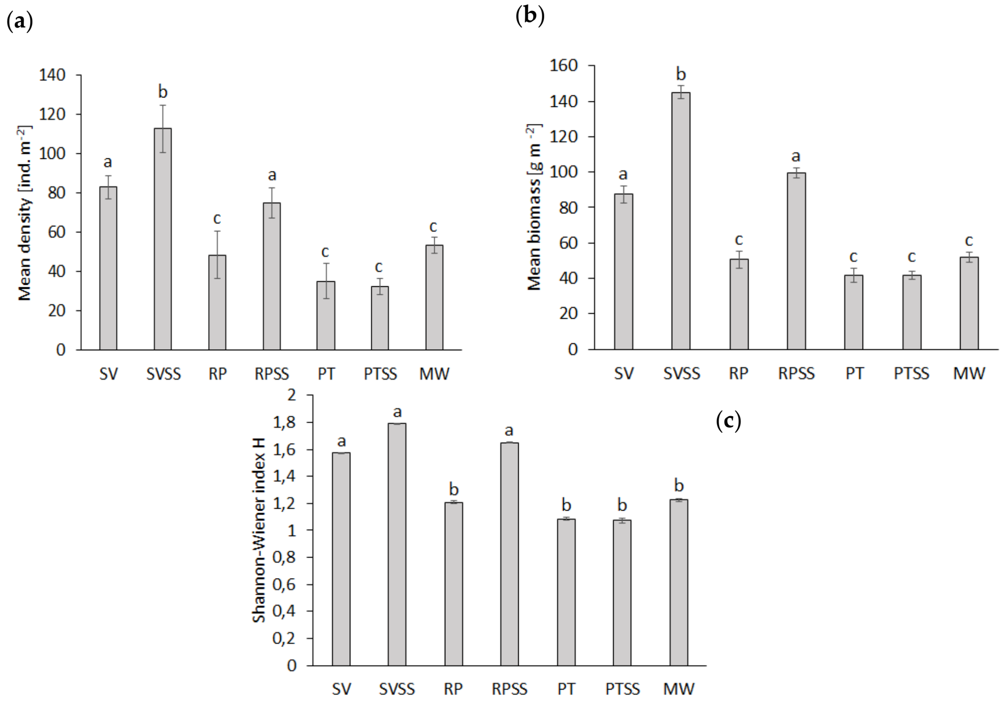

Figure 1. Bar plot of (**a**) the mean density of earthworms (ind · m^{-2}); (**b**) mean biomass (g m^{-2}); and (**c**) Shannon–Wiener species richness index (H') per cultivation treatment with the standard error of the mean (SEM) as error bars. Bars with different letters are significantly different at $p < 0.05$ (N = 45). (Abbreviations of cultivation treatments are as given in Table 1).

The earthworms found represented three ecological groups (Table 2 and Figure 2). The epigeic group (litter dwelling) was represented by two species, *D. rubidus* and *L. castaneus*. Horizontally burrowing species (endogees) were *A. caliginosa* and *A. rosea*. *L. terrestris* belonged to the aneciques group of earthworms, which are large and dig the deepest burrows.

Considering individual earthworm ecological categories across all cultivation treatments, the highest values occurred in the SV (willow) and were significantly different (w/o SS additions) from comparable treatments (RP, PT, and MW), where SV > RP = MW > PT. A similar trend was found for the sites (with SS) where SVSS > RPSS > PTSS ($p < 0.05$) (Figure 2a,b). Analysing the mean density and biomass of Lumbricids belonging to individual morpho-ecological groups, SV < SVSS and RP < RPSS ($p < 0.05$). No significant differences were found in the PT = PTSS treatments (Figure 2a,b).

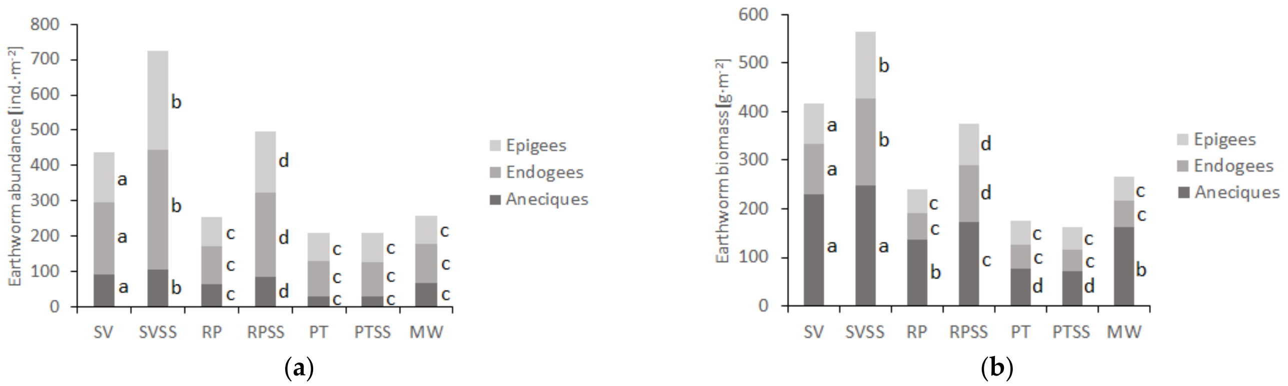

Figure 2. Density (**a**) and biomass (**b**) of earthworms in the cultivation treatments based on ecological groups. (Abbreviations of cultivation treatments are as given in Table 1). Different letters within individual ecomorphological groups of Lumbricids indicate statistically significant differences ($p < 0.05$).

3.2. The Relationship between Plant Monocultures and the Number and Biomass of Earthworms

As shown by Edwards et al. [53], long-term grasslands not subjected to agrotechnical treatments that change the structure of the soil have a beneficial effect on earthworms. This was confirmed in this study, as it was found that the cultivations of *S. viminalis* (SV) and *R. pseudoacacia* (PR) were characterised by higher density, biomass, and Shannon–Wiener species richness index compared with the meadow site (MW $p < 0.05$) (Figure 3). The exception was the cultivation of *P. tomentosa*, which was characterised by the lowest values of the analysed traits ($p < 0.05$) (Figure 3). Due to its sensitivity to frost and low temperatures, this tree species is not popularly grown in Poland for energy purposes. Very rapid annual shoot growth may be associated with the uptake of large amounts of water and nutrients from the soil necessary for development. However, further research on this species in Poland is required.

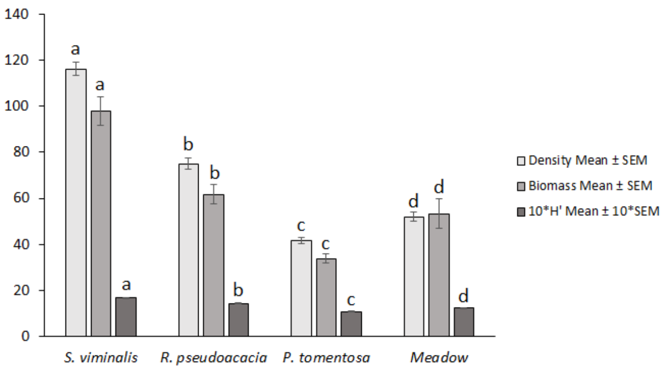

Figure 3. Effects of plant monocultures on density (ind. m^{-2}), biomass (g m^{-2}), and H' index without consideration of sewage sludge influence (H' index was multiplied by a factor of ten for visibility). Different letters for a particular trait in different plant monocultures indicate statistically significant differences at $p < 0.05$.

In this study, the qualitative structure of earthworms was not characterised by high values, but species number was high (Table 2) compared to other soils, where plant monocultures may be an important factor limiting the density of species, in particular those from the epigeic group [53]. Hedde et al. [54] showed that a change in the cultivation system from annuals to perennials did not affect the changes in the qualitative structure of soil invertebrates but contributed to an increase in the number of these organisms. From the present study of Lumbricids, a significantly higher number and biomass of earthworms were present in SV willow compared with the other cultivation treatments (SV > RP, PT, and MW; higher on average by 83%; $p< 0.05$, biomass increased by an average of 89%, $p < 0.05$) (Table 2). The SV site, due to the specificity of the willow, was characterised by a significant remnant of fallen leaf litter, which probably contributed to the significantly higher average number and average total biomass of epigeic earthworms *D. rubidus* and *L. castaneus* (SV > RP = PT = MW) (Table 2). Singh et al. [55] showed that the abundance of fallen leaf litter promoted the growth of earthworm numbers, as it is a source of readily available nutrients. In turn, Curry [52] found that the factor limiting the number and biomass of Lumbricids is the quality of the litter, not its actual quantity. Bostrom and Lofs-Holmin [56] showed that the amount of plant matter left in the litter significantly influenced the growth rate of *A. caliginosa*. The observations of these authors are supported by the results of the present study because *A. caliginosa* had the highest abundance and biomass at the SV site (SV > RP = MW > PT) (Table 2), where litter material consisted of small leaves. At the remaining sites, plant remains were scarce (PT) or consisted of leaves with smaller or larger remnants of branches (RP). A similar trend was observed for *L. terrestris* (SV > RP = MW > PT). Kohli et al. [57] found a decrease in the number of *L. terrestris* (from 55 to 26 ind. m^{-2}) after a four-year transformation of a meadow into a monoculture of *Miscanthus sinensis* and showed an increase in density (from 28 to 46 ind. m^{-2}) of *L. terrestris* after converting a monoculture maize crop into a wildflower meadow. This tendency partly correlates with the results obtained here, where the mean number of *L. terrestris* was significantly higher in MW compared with PT (difference by 118%) and similar to RP monoculture (PT < MW = RP) (Table 2). By contrast, the highest mean number of *L. terrestris* was found at the site with the SV monoculture, 41% higher than individuals found in the MW meadow (Table 2).

3.3. Influence of Selected Physicochemical Soil Parameters on the Number and Biomass of Earthworms

The number of biotic and abiotic factors that may affect earthworm communities is vast. Current knowledge of these numerous relationships does not provide satisfactory answers to fully understand, for example, the relationship between soils and earthworms. On the other hand, it can be said with a high probability that certain soil characteristics modify Lumbricid populations, e.g., organic matter content [58], pH and moisture [59], and heavy metal content [60,61]. As shown in Table 1, the selected physical and chemical properties of the soil were different at all experimental sites. The highest content of analysed macro-elements was found in the soils of SV and SVSS, plus RP and RPSS, which was probably related to the specificity of the plants themselves, as the cultivation of *S. viminalis* and *R. pseudoacacia* provides the soil with a large amount of organic matter in the form of fallen leaves and small branches. There was a strong relationship between the carbon content in the soil and the density of earthworms (r was 0.94, 0.95, 1.0 and 0.98, respectively; $p < 0.05$) and biomass ($r = 0.94$, 0.95, 0.94 and 0.98; $p < 0.05$). In the production of *P. tomentosa*, less waste biomass was available, as shoots with large leaves were removed entirely from the field and utilised. The lowest content of analysed heavy metals in the soil was found at the sites with willow, where Pb content was: SV 11.5 ± 2.5 and SVSS 14.7 ± 2.7 ($p < 0.05$), while the concentration of Cd was: SV 0.5 ± 0.0 and SVSS 0.8 ± 0.0 ($p < 0.05$).

The pH values differed significantly between sites within individual cultivation treatments (Table 1) but probably had little effect on the analysed earthworm characteristics (the coefficient r between soil pH and Lumbricid density and biomass were statistically insignificant for all sites). De Wanderer et al. [62] found a link between an increase in species diversity and abundance of Lumbricids with a change in soil pH from acidic to neutral, with the highest values of earthworm characteristics observed at a pH of approximately 7. Here, soil pH ranged from 7.13 (PT) to 7.94 (SVSS). Similar soil moisture values (presented in Section 2.1) were also found at all examined sites, which, like pH, probably had little influence on the abundance and biomass of Lumbricids. An increase in the number and biomass of earthworms (Table 2) was associated with an increase in the content of organic carbon in the soil and a decrease in the concentration of analysed heavy metals (Table 1). An exception was observed at the cultivation treatments of PT and PTSS, where, despite an increase in the content of organic C (PTSS > PT by 13%), a decrease in both the mean abundance and mean total biomass was found in most species of earthworm (the exception was a negligible increase in the abundance of *A. rosea* and *L. terrestris* by 0.2 and 0.3%, respectively). This could have been caused by a significant increase in the content of cadmium (by 142%) and lead (by 67%) in the PTSS soil (Table 1).

According to Spurgeon and Hopkin [63,64], even high concentrations of heavy metals in the soil may not affect the earthworm community structure, while a much lower content of these stressors may have a significant impact on the number and biomass of individual species of Lumbricid. This was demonstrated in this study, as earthworm density and biomass were negatively correlated with soil lead content (r coefficient for site density was SV r = −0.91, SVSS r = −0.94, RP r = −1, RPSS r = −0.98, PT r = −0.92, PTSS r = −0.96, and MW r = −0.92, and for biomass SV r = −0.92, SVSS r = −0.94, RP r = −0.94, RPSS r = −0.98, PT r = −0.92, PTSS r = −0.91, and MW r = −0.94). As shown by Jänsch et al. [65], there is also a close relationship between the content of organic carbon in the soil and the distribution and abundance of earthworms. These authors showed that this increase was related to an increase in the total number of Lumbricid species and vice versa. Alternatively, an increase in soil organic carbon may be influenced by the abundant presence of litter from fallen leaves, which favours an increase in earthworm numbers by acting as a source of food [55]. Here, the highest abundance and biomass of Lumbricid were found in SV (437.7 ± 23.1 ind. m^{-2} and 415.55 ± 86.89 g m^{-2}, respectively) and SVSS (725.3 ± 28.2 ind. m^{-2} and 564.55 ± 86.63 g m^{-2}) (Table 2), where the highest content of organic carbon in the soil was also recorded (SV 147,611 ± 1442.5 and SVSS 163,809 ± 1518.3 mg kg^{-1}; $p < 0.05$) (Table 1).

The use of organic fertilisation in the form of sewage sludge at the SVSS and RPSS sites not only increased the content of selected macronutrients in the soil but could have also contributed to the increase in the mean number and mean total biomass of individual species of earthworm compared with the SV and RP sites (Table 2). The most significant increases were found for the epigeic species *D. rubidus* in RP and RPSS (by 234 and 246%, respectively) and SV and SVSS (by 151 and 167%, respectively) and the shallow-burrowing *A. rosea* (RPSS > RP, respectively, by 111 and 115% and SVSS > SV by 106 and 112%, respectively). According to Solomou et al. [66] and Singh et al. [67], the use of organic fertilisation not only improves the quality of the soil but also contributes to the enrichment of the qualitative and quantitative structure of Lumbricids. Singh et al. [68] showed that using sheep manure to cultivate grass plants could contribute to an approximately fourfold increase in local earthworm communities.

In this study, significantly higher density, biomass and *H′* index were found in the sites with *S. viminalis* and *R. pseudoacacia* with the addition of sewage sludge (w/SS) than in the sites with the same plants not fertilised with sewage sludge (w/o SS, $p < 0.05$) (Table 3).

Table 3. Effect of sewage sludge on density (ind. m^{-2}), biomass (g m^{-2}), and *H′* index for Lumbricidae.

Features	***S. viminalis***		***R. pseudoacacia***		***P. tomentosa***		***Meadow***
	w/o SS	**w/SS**	**w/o SS**	**w/SS**	**w/o SS**	**w/SS**	**w/o SS**
Density	83.11 ^{a} ± 12.11	112.91 ^{b} ± 12.17	48.47 ^{a} ± 7.68	75.06 ^{b} ± 8.92	35.10 ^{a} ± 4.14	32.40 ^{a} ± 4.08	53.29 ± 9.01
Biomass	87.53 ^{a} ± 3.64	145.06 ^{b} ± 5.00	50.57 ^{a} ± 5.00	99.55 ^{b} ± 4.04	41.86 ^{a} ± 2.39	41.64 ^{a} ± 2.74	51.94 ± 2.87
S-W index *H′*	1.575 ^{a} ± 2.87	1.793 ^{b} ± 2.87	1.209 ^{a} ± 0.009	1.651 ^{b} ± 0.006	1.086 ^{a} ± 0.014	1.0726 ^{a} ± 0.019	1.225 ± 0.012

w/o SS—site without sewage sludge fertilisation. w/SS—site fertilised with sewage sludge. Different letters within individual plant crops for energy purposes indicate statistically significant differences at $p < 0.05$.

This agrees with the findings, for example, of Emmerling and Paulsch [69], who reported that the application of fresh and composted sewage sludge to the soil stimulated the growth of the density and fresh mass of earthworms. In turn, Xie et al. [70] showed that the use of sewage sludge as the sole food source for *Eisenia fetida* earthworms positively increased their population size. Pallant and Hilster [71] found a 26% increase in the average biomass of *L. terrestris* after a 10-week exposure of earthworms in a mixture of reclaimed postmining substrate with the addition of sewage sludge.

4. Conclusions

The results presented in the paper show that monoculture cultivations (*S. viminalis*, *R. pseudoacacia* and *P. tomentosa*) with or without fertilisation do not negatively affect local communities of earthworms. At the study sites, the qualitative structure of earthworm communities was not characterised by high values, but the density of individual species was high, with a relatively even distribution of individuals of specific species within individual cultivation treatments. A significantly higher density and biomass of earthworms were found in SV and SVSS willow plantations than in the other sites. The application of fertilisation in the form of sewage sludge contributed to a significant increase in the mean number and biomass of all Lumbricid species found with the cultivation of *S. viminalis* and *R. pseudoacacia*, with significantly higher values than those in a meadow (MW) constituting the control site. Lower density, biomass, and *H'* index were found with the cultivation of *P. tomentosa* compared to the control meadow site. Lumbricidae research under *P. tometosa* needs further exploration, especially in different soil types. The results show that woody monoculture crops with an appropriate selection of plants and adequate fertilisation can be a favourable habitat for the development of earthworm populations, contributing to the maintenance of required soil functions in agroecosystems. This work has added to the understanding of energy crop production systems on ecosystem engineering earthworms, and the role of sewage sludge as a fertiliser. Further research could usefully examine the effects of sewage sludge application on energy crop production in tandem with soil health assessment over longer time scales. In addition, other than earthworms, further elements of the functional soil fauna could be assessed.

Author Contributions: Conceptualisation, A.M.-P.; methodology, A.M.-P.; investigation, A.M.-P., G.P., M.G., M.J., E.H., J.K. and K.R.B.; data curation, M.J. and M.G.; writing—original draft preparation, A.M.-P.; writing—review and editing, A.M.-P., G.P., J.K. and K.R.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical review and approval were waived for this study due to reason (the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes requires the approval of the Ethics Committee on Animal Experiments for vertebrate species and cephalopods (representatives of invertebrates). The approval of the Ethics Committee is not required for other species of invertebrates (not under protection), which include earthworms).

Informed Consent Statement:

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgements: The authors would like to thank Konieczny for the relevant information on the research area.

Conflicts of Interest: The authors declare no conflict of interest.

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