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Fertilization Effects of Solid Digestate Treatments on Earthworm Community Parameters and Selected Soil Attributes

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Abstract

An increasing number of soils, including those in EU countries, are affected by organic matter deficiency and the deterioration of nutrients, and using mineral fertilizers is often associated with negative environmental impacts. One of the basic recommendations for sustainable agriculture is to increase the proportion of organic fertilizers in crop production and preserve soil biodiversity. An increasingly common organic fertilizer is biogas plant digestate, the physical and chemical properties of which depend primarily on the waste material used in biogas production. However, the fertilizer value of this additive and its effects on the soil environment, including beneficial organisms, remain insufficiently studied. Soil macrofauna, particularly earthworms, play a crucial role in soil ecosystems, because they significantly impact the presence of plant nutrients, actively participate in forming soil structures, and strongly influence organic matter dynamics. The present study was undertaken to determine the effects of fertilizing a silt loam soil with the solid fraction of digestate in monoculture crop production on earthworm community characteristics and the resulting changes in selected soil physicochemical properties. The research was conducted at a single site, so the original soil characteristics across the experimental plots were identical. Plots were treated annually (for 3 years; 2021–2023) with different levels of digestate: DG100 (100% of the recommended rate; 30 t ha^{-1}), DG75 (75% of the recommended rate; 22.5 t ha^{-1}), DG50 (15 t ha^{-1}), DG25 (7.5 t ha^{-1}), and CL (a control plot without fertilizer). An electrical method was used to extract earthworms. Those found at the study site belonged to seven species representing three ecological groups: *Dendrodrilus rubidus* (Sav.), *Lumbricus rubellus* (Hoff.), and *Dendrobaena octaedra* (Sav.) (epigeics); *Aporrectodea caliginosa* (Sav.), *Aporrectodea rosea* (Sav.), and *Octolasion lacteum* (Örley) (endogeics); and *Lumbricus terrestris* (L.) (anecics). Significant differences in the abundance and biomass of earthworms were found between the higher level treatments (DG100, DG75, and DG50), and the lowest level of fertilization and the control plot (DG25 and CL). The DG25 and CL plots showed an average of 24.7% lower earthworm abundance and 22.8% lower biomass than the other plots. There were no significant differences in the earthworm metrics between the plots within each of the two groups (DG100, DG75, and DG50; and DG25 and CL). The most significant influence on the average abundance and average biomass of Lumbricidae was probably exerted by soil moisture and the annual dosage of digestate. A significant increase

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in the abundance and biomass of Lumbricidae was shown at plots DG100, DG75, and DG50 in the three successive years of the experiment. The different fertilizer treatments were found to have different effects on selected soil parameters. No significant differences were found among the values of the analyzed soil traits within each plot in the successive years of the study.

Keywords: digestate; fertilization; earthworms; biodiversity; soil condition; sustainable agriculture

1. Introduction

With renewable energy sources in high demand, biogas plants have grown intensively in recent years. In addition to energy, biogas plants produce a nutrient-rich waste material called digestate, but they also make it possible to manage often troublesome organic waste, reduce greenhouse gas emissions, and reduce the degradation of water resources [1]. According to Dziedzic et al. [2], Poland's annual production of the solid fraction of digestate in recent years has amounted to about 172,000 tons. One of the most important current challenges for many countries is the implementation of a closed-loop economy, and recycling the nutrients necessary for plant growth is an example of one approach to this type of management [3]. As more and more soils, including those in EU countries, are characterized by low organic matter content and the continuous loss of nutrients, and the use of mineral fertilizers is often associated with negative environmental impacts, one of the priorities of sustainable agriculture is to maximize the use of organic fertilizers in plant cultivation [4] and protect the biodiversity of soil ecosystems [5]. Natural fertilizers, such as manure and slurry, have been used in agriculture for a long time [6]. A relatively common organic fertilizer is vermicompost, which is characterized by a slow release of macro and micronutrients into the soil environment [7]. However, biogas plant digestate is increasingly used as an organic fertilizer, the physical and chemical properties of which depend on the waste mass used for biogas production and the technological processes involved [8]. The most important characteristics of biogas digestate as a fertilizer are dry matter content, nitrogen, phosphorus and potassium levels, and the permissible content of heavy metals [9]. According to the Directive of the European Parliament and of the Council (EU) 2018/851, it is possible to add biogas plant digestate to the soil for fertilizing purposes [10]. However, the use of this additive and its impact on soil environments are still insufficiently studied, and the effectiveness of this biomass as an organic fertilizer additive requires further investigation [11]. Alburquerque et al. [12] studied the effect of digestate addition on soil fertility and plant yields, comparing its fertilizing capacity with mineral fertilizers and cattle manure. These authors showed that digestate can be a good source of nitrogen and phosphorus, which can have a significant effect on soil fertility and plant yields. Its use can be beneficial in agricultural practice, but when determining the applied dose, both short-term and long-term effects on the soil should be considered. In turn, Clements et al. [13] showed that digestate is a good fertilizer for use in organic farming, because it improved nitrogen availability, which led to increased plant growth. However, it had a lower organic matter content compared to slurry. Therefore, the authors propose drying or separating digestate to increase the content of organic matter, which will make its properties similar to those of slurry.

Little work is dedicated to understanding the use of liquid and solid digestate fractions on living soil organisms, including Lumbricidae (earthworms) [14]. Lumbricidae are sensitive to different tillage systems and are an important bio-indicator used to assess soil health [15]. There is also a lack of sufficient information on the effect of methods of digestate application (spreading, strip application, or plowing) to fields on earthworm mortality. Johansen et al. [16] found lower earthworm mortality using a lower volume of 25 t ha^{-1} of digestate than applying a 50 t ha^{-1} volume delivering 170 kg N/ha. Soil fertilization is vital for earthworm activity, as both organic and mineral fertilizers provide a direct or indirect food source (by increasing the proportion of plant residues) for these invertebrates [17]. Since earthworms perform essential functions (i.e., ecosystem services) in the agricultural ecosystem (e.g., influencing the availability of nutrients for plants, participating in the formation of soil structure, and significantly impacting the content of organic matter) [18], there is an urgent need for extensive long-term research on the effects of fertilization with digestate on the groupings of Lumbricidae in different soil types and cropping systems, etc. Accordingly, the present study attempted to analyze the effect of solid fraction fertilization of silt loam soil with monoculture cultivation of *Cucurbita pepo* L. (pumpkin) on the characteristics of earthworm communities and selected parameters of the soil environment. The aim was to assess the effects of digestate application rate on (i) earthworm abundance, (ii) earthworm biomass, (iii) selected features of Lumbricidae ecological groups, and (iv) selected physicochemical soil properties.

2. Materials and Methods

2.1. Digestate

The solid digestate used in the experiment came from an agricultural biogas plant, a byproduct of the anaerobic digestion of a grain, corn, and cattle manure waste mixture. The solid form of the digestate was created through a separation process, and pasteurization was carried out at 80 °C; the pasteurization process of the fertilizer substrate aimed to eliminate pathogenic bacteria and parasite eggs. A local farm made solid digestate available for research purposes for the experiment. Characteristics of the digestate are shown in Table 1.

Table 1. Content of macronutrients, trace elements, and selected features of digestate applied annually to the experimental plots (mean ± standard deviation).

Parameter	Units	Digestate
OC	mg kg^{-1} (d.m.)	99,086.4 ± 1283.7
TN		5621.3 ± 33.2
P		1741.2 ± 82.5
K		2557.2 ± 121.8
Ca		2736.8 ± 163.1
Mg		1659.4 ± 75.9
Cd		0.4 ± 0.1
Pb		1.4 ± 0.2
C/N ratio	-	17.63 ± 1.3
pH in H_2O	-	6.89 ± 0.2
Electrical conductivity	mS-cm^{-1}	1.25 ± 0.06
Temp.	°C	-
Moisture	%	-

Abbreviations: OC—organic carbon; TN—total nitrogen.

2.2. Experimental Design

The field research was conducted on a private farm in southeastern Poland (Podkarpackie province) near Rzeszów (50°05′08′′ N 21°59′08′′ E). The experiment was conducted on flat terrain located at an altitude of about 195 m above sea level. According to the

World Reference Base for Soil Resources, based on grain size, the soil was classified as silt loam [19]. Average soil temperature and moisture content for the experiment (2021–2023) are shown in Table 3. In this area of about 0.2 hectares, experimental sites differing in the rate of applied fertilizer substrate were separated for the experiment as follows:

DG100—digestate dose of 30 t ha^{-1} (100% of the recommended dose [20]);

DG75—digestate 22.5 t ha^{-1} (75% of the recommended dose);

DG50—digestate 15 t ha^{-1} (50% of the recommended dose);

DG25—digestate 7.5 t ha^{-1} (25% of the recommended dose);

CL—control (without fertilization).

Each experimental and control plot was a single 10 × 10 m square (area 100 m^2), located in the same area (same soil characteristics) and approximately 5 m apart.

Every year (2021–2023), in the second half of April (the date depended on weather conditions), appropriate digestate doses were applied manually to the designated test plots and then mixed to a depth of 15 cm by plowing (a two-furrow reversible plough was used). In mid-May, pumpkin seeds (Junona variety, producer: W. Legutko Breeding and Seed Company, Poland) were sown. The preparation of the control plot followed the same procedures but was completed without fertilizer application. Annual applications of specific doses of digestate in each treatment were made in the same designated initial plots throughout the experiment. Common pumpkin was grown annually in the DG and CL monocultures; no pesticides or other forms of fertilization were applied, and emerging weeds were pulled out by hand (fortnightly).

2.3. Earthworm and Soil Sampling

One year prior to the experiment, i.e., before the application of different digestate treatments, earthworm qualitative and quantitative structures were analyzed in the designated plots using the procedures later used during the experiment (the results obtained were homogeneous in all plots, very similar to those shown in Table 2, under the CL column). During the study, earthworm sampling at the experimental and control plots was conducted three times per year (in June, August, and October) over three annual cycles (2021–2023). Five randomly determined samples were taken each time within the five plots (CL, DG25, DG50, DG75, and DG100, with an area of 100 m^2 each), but in such a way as not to duplicate the places where earthworms were extracted on subsequent dates. A method using the action of electric current was used to collect earthworms [21]. The octet apparatus consists of eight soil probes in an octagonal arrangement (6 mm in diameter and 65 cm in active length), with insulated handles around a circle (52 cm in diameter, covering an area of 0.22 m^2). Adjacent probes are spaced 20 cm apart, and opposite probes are spaced 52 cm apart [22]. The time selected for each sequence was 2.5 min. Earthworm individuals were obtained for analysis from the central portion defined by the ring and probes. When collecting earthworms, five samples were taken from each site (during each of the three months, annually), in order to obtain the total values of the analyzed features of Lumbricidae species per m^2 (5 samples × 0.22 m^2).

The collected earthworms were washed in water for 15 min and then killed by immersion in 30% ethanol, followed by preservation in 4% formalin. They were identified and counted, and the biomass of each individual was determined. Species identification was carried out using the key of Kasprzak [23]. During the ongoing study of Lumbricidae, soil temperature was measured (at a depth of 0–15 cm; always about 10 a.m.), and soil samples (n = 3; sampling replications) were also taken (from a depth of 0–15 cm) from each plot (three times each year for the three years of research) to determine physical properties and the content of selected macroelements and heavy metals (Table 3).

2.4. Soil Analysis

The total content of macroelements (N, P, K, Ca, and Mg) and microelements (Cd and Pb) was determined using the procedures of Ostrowska et al. [24]. Nitrogen was determined by the Kjeldhal method using Kjeltec 8100 and 2006 Foss Tecator Digestor apparatus (Foss Tecator AB, Hoeganaes, Sweden) [24]. The test material was digested in pure concentrated $HClO_4$ to determine other elements. Phosphorus was determined colorimetrically by the vanadium–molybdenum method with a Shimadzu UV-2600 (Kyoto, Japan) spectrophotometer, and potassium, magnesium, calcium, and trace elements by an atomic absorption spectrophotometry technique using Hitachi Z-2000 (Hitachi Inc., Tokyo, Japan) [24]. Carbon was determined using a Vario EL-CUBE elemental analyzer (from Elementar Analysensysteme GmbH, Langenselbold, Germany). Soil pH was determined by a potentiometric method, with a soil:water ratio of 1:2.5, using a HI 4221 pH meter (HANNA Instruments Inc., Woonsocket, RI, USA). Salt concentration was determined by a conductometric method using a HI 2316 conductivity/resistivity meter (HANNA Instruments Inc. Woonsocket, RI, USA). The C/N ratio of the analyzed cultivated soils was also calculated. At the sampling plots, soil temperature and moisture were measured at a depth of 20 cm. Soil moisture content was determined by oven drying at 105 °C [25].

2.5. Data Analysis

To assess the Lumbricidae associations, the Shannon–Wiener diversity index (H') and dominance index (D) were used:

$$H' = \Sigma \mathrm{p_i} \times \log_{(\mathrm{n})} \mathrm{p_i}$$

where pi—the ratio of the number of organisms of a given species to the total number of all organisms [26];

$$D = \mathrm{N_a}/\mathrm{n}$$

where N_a—the number of individuals belonging to the species in all samples, and n—the number of individuals of the studied species group in all samples [27]. Classes of dominance were adopted according to Górny and Grüm [28]: 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 assemblage.

The results were analyzed statistically using Statistica software v. 13.3 and R 4.5.1. The effects of experimental treatments (digestate application rates) on earthworm abundance and biomass were assessed using two-way analysis of variance (ANOVA), followed by Tukey-adjusted pairwise comparisons on marginal means as a post-hoc test after confirming assumptions of homogeneity of variances (Levene's test) and normality of distribution (Shapiro–Wilk test). A linear increasing effect of cumulative digestate doses (across successive years) on earthworm abundance and biomass was confirmed using multiple regression analysis. Preliminary relationships between soil physicochemical parameters and earthworm abundance, biomass, and Shannon–Wiener diversity index were explored using Partial Least Squares (PLS) regression. The effects of fertilization on soil parameters were evaluated using two-way ANOVA when assumptions were met; otherwise, the Kruskal–Wallis test was applied (e.g., for soil moisture).

3. Results and Discussion

3.1. Earthworm Species Found in the Studied Plots

Conducting the present study in a monoculturally managed agroecosystem, seven earthworm species were found: *Dendrodrilus rubidus* (Savigny 1826), *Lumbricus rubellus* (Hoffmeister 1843), *Dendrobaena octaedra* (Savigny 1826), *Aporrectodea caliginosa* (Savigny

1826), *Aporrectodea rosea* (Savigny 1826), *Octolasion lacteum* (Örley 1885), and *Lumbricus terrestris* (Linnaeus 1758) (Table 2). All of the species found were present in each of the analyzed study plots. A similar species composition in a nearby area was shown by Mazur-Pączka et al. [7], but no epigeic *D. octaedra* were found.

Table 2. Mean (±sd) abundance [ind. m^{-2}], mean (±sd) biomass [g m^{-2}], and dominance of earthworm species found in the digestate treatments. The values in the table are mean for 2021–2023.

Species/Ecological Group *	Features	Digestate Treatments ** CL	DG25	DG50	DG75	DG100
Epigeics						
Dendrodrilus rubidus	Abundance	8.44 ± 1.21 d	10.06 ± 1.46 c	11.59 ± 2.33 b,c	12.54 ± 3.02 a,b	13.55 ± 4.12 a
	Biomass	1.05 ± 0.17 d	1.23 ± 0.21 c	1.43 ± 0.31 b,c	1.53 ± 0.39 a,b	1.66 ± 0.51 a
	Dominance %	3.18	3.71	3.32	3.52	3.73
Lumbricus rubellus	Abundance	10.98 ± 2.00 d	12.12 ± 1.93 c	13.22 ± 2.28 b,c	15.64 ± 3.90 a,b	16.47 ± 4.95 a
	Biomass	5.79 ± 1.16 d	6.40 ± 1.06 c	7.01 ± 1.22 b,c	8.31 ± 2.09 a,b	8.78 ± 2.69 a
	Dominance %	4.14	4.47	4.79	4.39	4.53
Dendrobaena octaedra	Abundance	15.26 ± 1.74 c	16.52 ± 1.03 c	18.21 ± 1.86 b,c	20.19 ± 3.41 a,b	21.99 ± 5.42 a
	Biomass	8.12 ± 0.93 c	8.86 ± 0.54 c	9.74 ± 0.98 b,c	10.91 ± 1.83 a,b	11.87 ± 2.95 a
	Dominance %	5.76	6.09	5.22	5.67	6.06
Endogeics						
Aporrectodea caliginosa	Abundance	122.96 ± 9.18 b	122.76 ± 9.04 b	157.58 ± 32.16 a	158.16 ± 36.44 a	159.75 ± 37.16 a
	Biomass	55.78 ± 5.69 b	55.24 ± 5.33 b	71.65 ± 15.71 a	71.89 ± 17.68 a	71.20 ± 18.34 a
	Dominance %	46.37	45.29	45.13	44.39	44.01
Aporrectodea rosea	Abundance	85.47 ± 7.57 b	87.07 ± 9.94 b	119.50 ± 29.61 a	119.89 ± 28.85 a	121.16 ± 31.59 a
	Biomass	40.23 ± 4.77 b	41.22 ± 5.92 b	56.47 ± 14.61 a	56.93 ± 14.29 a	57.49 ± 15.59 a
	Dominance %	32.23	32.13	34.23	33.65	33.38
Octolasion lacteum	Abundance	15.67 ± 1.61 b	15.87 ± 1.53 b	22.38 ± 5.92 a	23.11 ± 6.55 a	23.27 ± 6.02 a
	Biomass	7.88 ± 1.09 b	8.01 ± 1.12 b	11.35 ± 3.25 a	11.66 ± 3.53 a	11.76 ± 3.19 a
	Dominance %	5.91	5.86	6.41	6.48	6.41
Anecics						
Lumbricus terrestris	Abundance	6.39 ± 2.24 a	6.63 ± 1.95 a	6.65 ± 1.84 a	6.79 ± 2.02 a	6.81 ± 1.62 a
	Biomass	20.09 ± 7.11 b	20.82 ± 6.21 b	20.87 ± 5.85 b	21.32 ± 6.44 b	21.39 ± 5.16 b
	Dominance %	2.41	2.45	1.90	1.90	1.88

* Ecological group of Bouché [29]. ** Abbreviations: CL—control; DG25—digestate 7.5 t·ha^{-1}; DG50—digestate 15 t·ha^{-1}; DG75—digestate 22.5 t·ha^{-1}; DG100—digestate 30 t·ha^{-1}; Different letters in a row indicate statistically significant differences ($p < 0.05$).

A characteristic feature of most of the species found was homogeneity in membership of the dominant class, regardless of the plot. The only exception was *L. terrestris*, which, in the plots where the highest doses of biofertilizer were applied (DG100, DG75, and DG50), constituted the recedent group. In contrast, in the plot where the lowest level of biofertilizer was applied (DG25) and the unfertilized control plot (CL), it was classified as subdominant (Table 2). In addition, in the other treatments, two species were included in the dominant group (*A. caliginosa* and *A. rosea*), two species in the dominant group (*D. octaedra* and *O. lacteum*), and two species in the subdominant group (*D. rubidus* and *L. rubellus*) (Table 2).

3.2. Effect of Applied Fertilization on Earthworm Abundance and Biomass

A two-way analysis of variance revealed the significant effect of both fertilizer dose ($F(4, 30) = 18.36$, $p < 0.05$) and year ($F(2, 30) = 39.23$, $p < 0.05$) on earthworm abundance, as well as on biomass ($F(4, 30) = 7.52$, $p < 0.05$; $F(2, 30) = 18.94$, $p < 0.05$, respectively). For earthworm abundance, a significant interaction between fertilizer dose and year was also observed ($F(8, 30) = 5.24$, $p < 0.05$), indicating that the effect of fertilization varied depending on the year. The study found significant differences in the average abundance and average biomass of earthworms between plots where higher doses of digestate were applied (DG100, DG75, and DG50), and the plots with the lowest fertilization levels and the control plot (DG25 and CL) (Figure 1a,b). Treatments DG25 and CL showed an average of 24.7% lower abundance and 22.8% lower biomass than the other treatments. There were no significant differences in the values of the traits in question between treatments within each of the two groups (DG100, DG75, and DG50; and DG25 and CL) (Figure 1a,b).

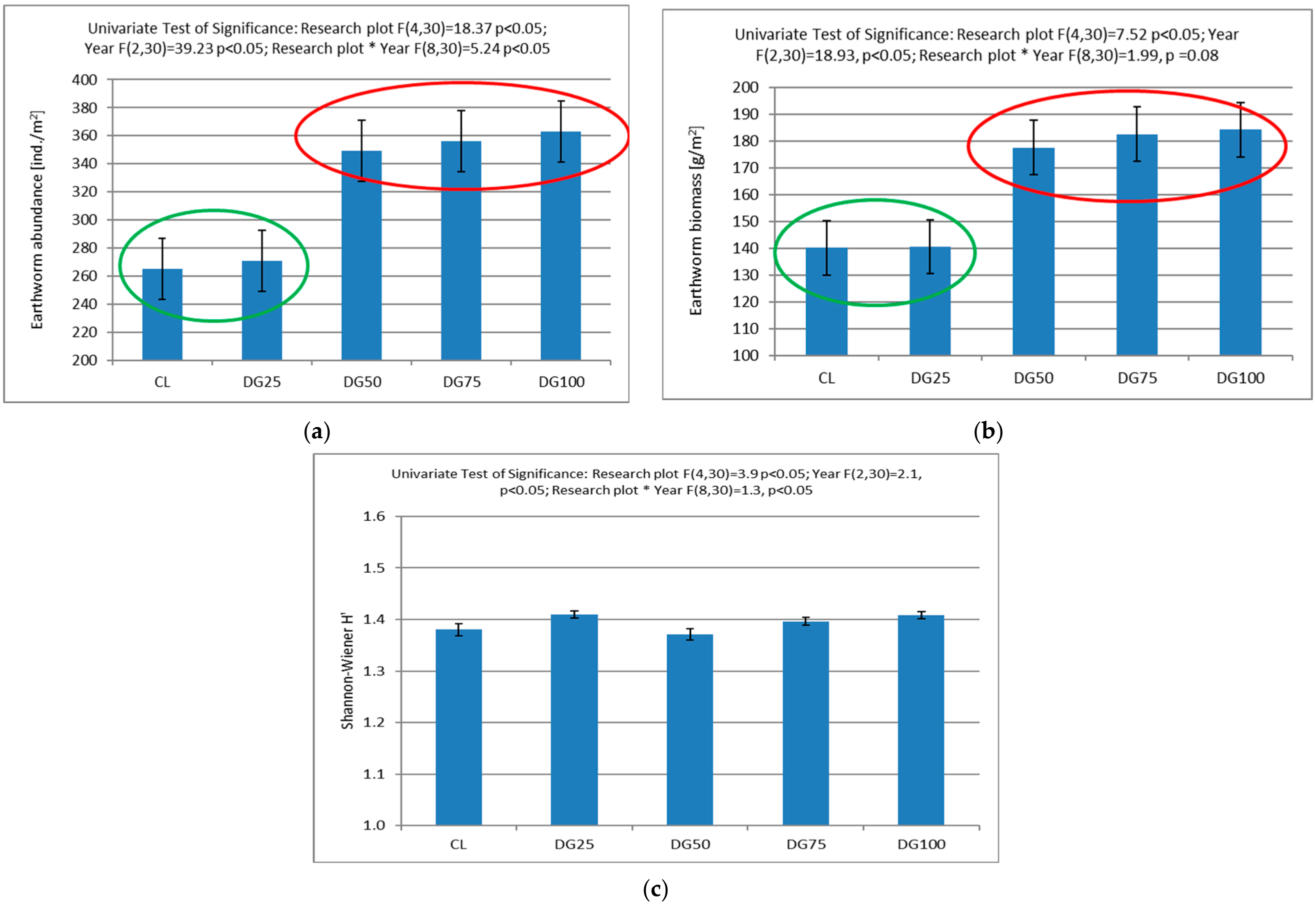


Figure 1. Mean abundance [ind. m^{-2}] (**a**), mean biomass [g m^{-2}] (**b**) and Schannon–Wiener index (H') (**c**) of Lumbricidae in the experimental sites. Error bars are the standard error of the mean. The values in different frames show statistically significant differences ($p < 0.05$). Abbreviations of treatments are given in Table 2.

Koblenz et al. [30] found significantly higher abundance and biomass of earthworms in crops fertilized with digestate or manure than in chemically fertilized or controlled unfertilized soils. Butt and Putwain [31] also observed higher abundance and biomass of earthworms in restored colliery soils fertilized with digestate compared to unfertilized soils. Similar positive results were also obtained by Leroy et al. [32], who observed an increase in the abundance of Lumbricidae after applying organic fertilization in the form of manure, slurry, and composts compared to unfertilized sites. These observations coincide with the results obtained here, which show that the application of higher organic fertilizer treatments, such as digestate, has, in effect, a positive effect on selected characteristics of earthworm populations since, according to Timmerman et al. [33], organic fertilizers in the right quality and quantity provide earthworms with an immediate, sufficient supply of organic matter. The significant difference in abundance and biomass values between the plots where higher doses of digestate were applied and the plot with the lowest fertilization and the control plot, in addition to differences in the amount of organic matter available to earthworms, could also be due to differences in soil structure (influenced by the different applications of digestate), which translates into differences in soil moisture (DG100, DG75, and DG50) > (DG25 and CL) by 11% (Figure 2a,b, Table 3). Tripathi and Bhardwaj [34] and Adigun et al. [35] showed that the distribution of earthworms and their abundance and biomass are significantly influenced by soil moisture, organic carbon, and nitrogen content.

Analyzing the obtained results regarding the effect of the applied doses of digestate on the Shannon–Wiener diversity index (H'), it can be concluded that the values of earthworm metrics were similar in all experimental and control plots (Figure 1c). In the year 2023, significant differences in the Shannon–Wiener diversity index (H') were observed between fertilization treatments. Specifically, plots with DG50 exhibited significant power values of H compared to both DG100 ($p = 0.0199$) and DG25 ($p = 0.0170$), based on Tukey-adjusted pairwise comparisons. No significant differences were detected between treatments in 2021 or 2022. Different observations were presented by Rollett et al. [36], who showed a significant decrease in the qualitative and quantitative structures of Lumbricidae in densely populated fields after the application of digestate. In contrast, Burmaister et al. [37] showed dead earthworm individuals on the soil surface immediately after applying liquid digest. On the other hand, Moinard et al. [38] found that liquid organic products of the digestate can be toxic upon direct contact. The ammonia contained in the digestate, lack of oxygen, excessive salinity, and heavy metals are the leading causes of the digestate's toxic effects. However, there is insufficient research on this issue.

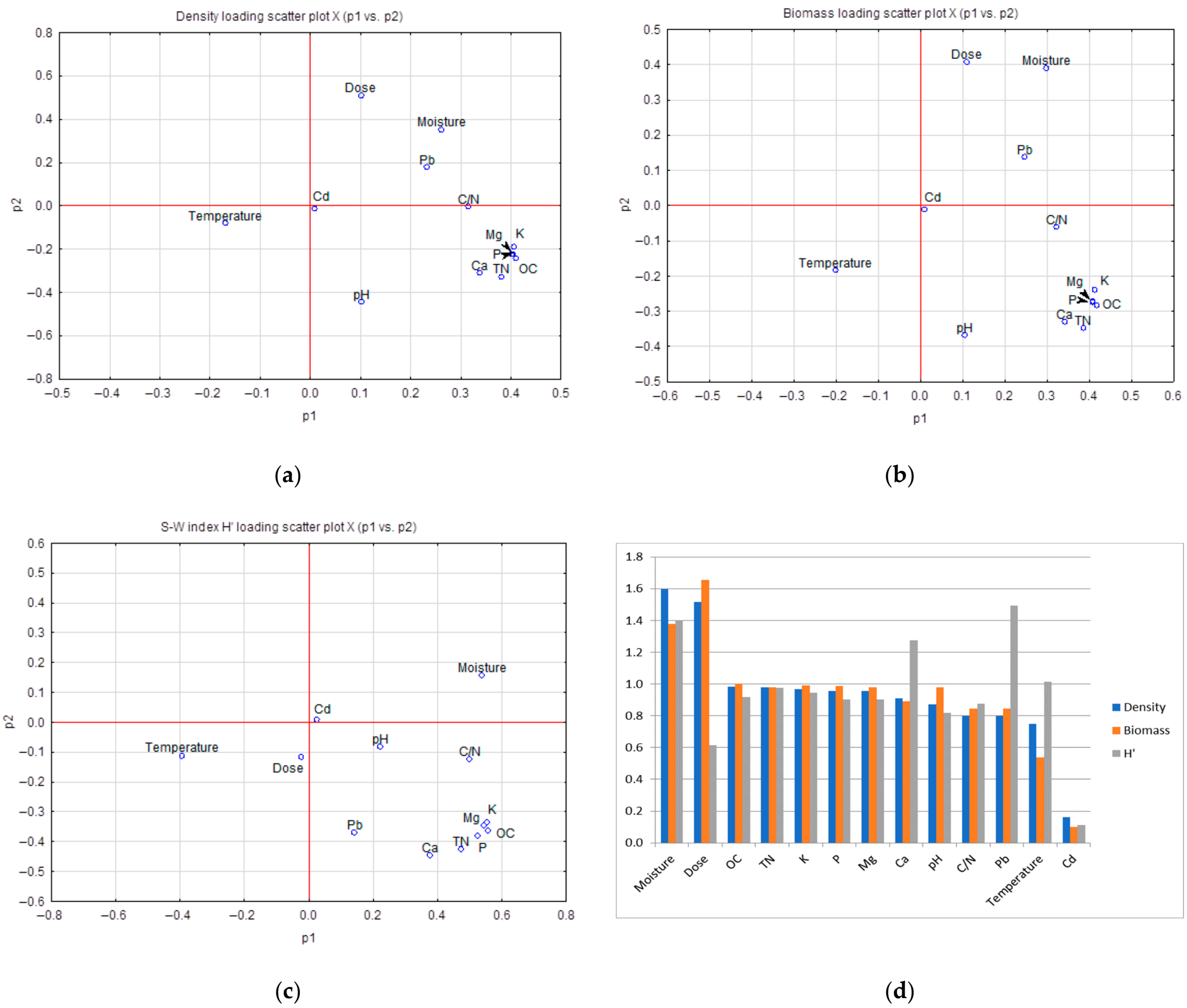


Figure 2. Loading scatter plot for the two most essential components (p1 and p2) of PLS models for (**a**) density, (**b**), biomass, (**c**) H' index, and (**d**) variability importance in the projection.

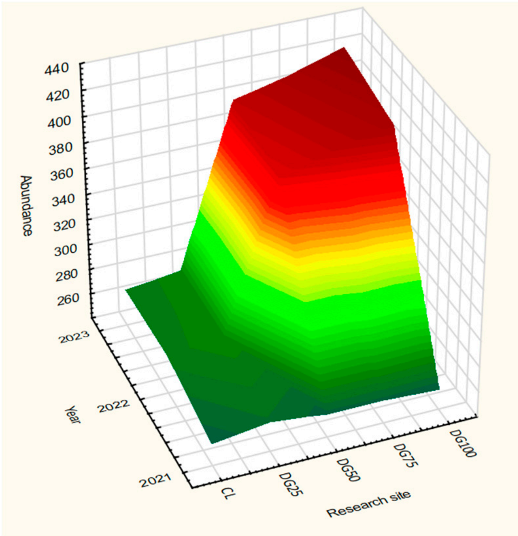
Table 3. Content of macronutrients, trace elements, and selected features in the soils at the research sites (mean ± standard deviation based on 45 samples). The values in the table are means for 2021–2023.

Substrate Characteristics						
Parameter	**Units**	**CL**	**DG25**	**DG50**	**DG75**	**DG100**
OC	$mg\ kg^{-1}$ (d.m.)	13,119.6 ± 19.2 e	14,104 ± 833.6 d	14,990.3 ± 833.8 c	15,867.3 ± 762.6 b	16,797.7 ± 713.3 a
TN		1303.2 ± 13.1 d	1349.1 ± 88.9 c,d	1391.0 ± 87.5 b,c	1449.4 ± 86.7 a,b	1486.1 ± 80.9 a
P		223.7 ± 13.5 e	249.7 ± 14.2 d	264.3 ± 14.0 c	279.6 ± 16.9 b	299.5 ± 16.7 a
K		238.4 ± 11.6 e	260.3 ± 13.3 d	273.1 ± 13.9 c	288.8 ± 13.1 b	320.7 ± 12.8 a
Ca		1491.7 ± 24.0 b	1492.6 ± 71.3 b	1528.3 ± 72.9 a	1552.8 ± 71.5 a	1587.8 ± 71.1 a
Mg		83.1 ± 6.7 d	97.0 ± 11.2 c	103.9 ± 9.9 c	124.8 ± 11.9 b	139.1 ± 12.4 a
Cd		0.6 ± 0.1 a	0.6 ± 0.0 a	0.6 ± 0.0 a	0.6 ± 0.0 a	0.6 ± 0.1 a
Pb		18.9 ± 0.2 a	18.9 ± 0.1 a	19.2 ± 0.1 a	19.3 ± 0.2 a	19.0 ± 0.2 a
C/N ratio	-	10.07 ± 0.1 e	10.46 ± 0.2 d	10.78 ± 0.1 c	10.95 ± 0.1 b	11.30 ± 0.2 a
pH in $H\ 0_2$	-	7.21 ± 0.1 a	7.26 ± 0.0 a	7.18 ± 0.1 a	7.33 ± 0.1 a	7.20 ± 0.1 a
Electrical conductivity	mS-cm^{-1}	0.28 ± 0.04 b	0.29 ± 0.00 b	0.30 ± 0.01 a	0.35 ± 0.02 a	0.39 ± 0.03 a
Temp.	°C	13.7 ± 1.6 a	13.7 ± 1.5 a	13.8 ± 1.4 a	13.8 ± 1.9 a	13.6 ± 1.5 a
Moisture	%	24.6 ± 2.9 b	24.9 ± 2.8 b	27.3 ± 2.7 a	27.9 ± 2.7 a	28.3 ± 2.7 a

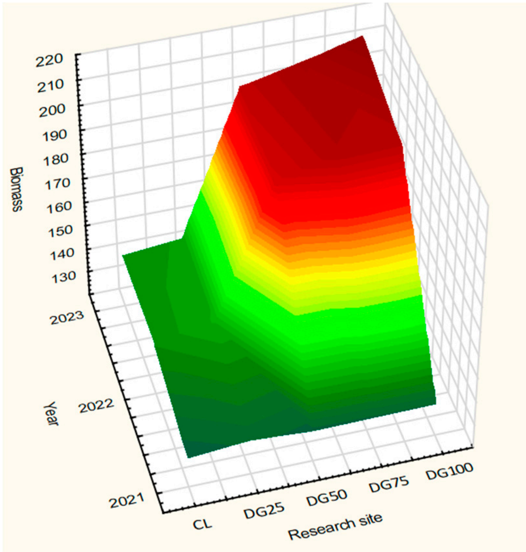
Abbreviations: CL—control; DG25—digestate 7.5 t·ha^{-1}; DG50—digestate 15 t·ha^{-1}; DG75—digestate 22.5 t·ha^{-1}; DG100—digestate 30 t·ha^{-1}; OC—organic carbon; TN—total nitrogen. Different letters in a row indicate statistically significant differences ($p < 0.05$).

In addition, digestate treatments had a differential effect on the habitats of Lumbricidae, thus allowing invertebrates to migrate to more favorable ones. Hoogerkamp et al. [39] reported that earthworms colonizing habitats with more favorable living conditions spread at a rate of 2 to 15 m per year. From our results, it seems that soil moisture and the annual dosage of digestate (Figure 2a,b,d) had the most significant influence on the mean abundance and mean biomass of Lumbricidae. In contrast, soil moisture significantly affected the H' index (Figure 2c,d). According to Edwards and Bohlen [40] and Edwards and Lofty [41], earthworm abundance and biomass are strongly influenced by soil moisture, pH, and organic matter content. Hendrix et al. [42] showed that these Lumbricidae metrics were strongly influenced by increased soil organic carbon content.

The present study shows a significant increase in the abundance and biomass of Lumbricidae at plots DG100, DG75, and DG50 in the successive years of the experiment (Figure 3a,b). An average increase in the values of the traits in question was shown between 2021 (the first digestate application) and in subsequent years, 2022 and 2023 (a difference of 56% for abundance and 49% for biomass, respectively) (Figure 3a,b). There were no significant differences in abundance and biomass in the subsequent years of the study, conducted at the plots where the lowest digestate treatment was applied annually (DG25) and the control plot (CL). The significant difference in the earthworm metrics between the first year of application of the digestate and subsequent years in DG100, DG75, and DG50 treatments may have been due to the synergistic effect of numerous factors. The toxic effects of digestates were reported by Pivato et al. [43] for the earthworm *Eisenia fetida* and by Ross et al. [44] for the species *Aporrectodea caliginosa*, while no specific explanation was given for the stressful effects of these biopreparations.



(**a**)

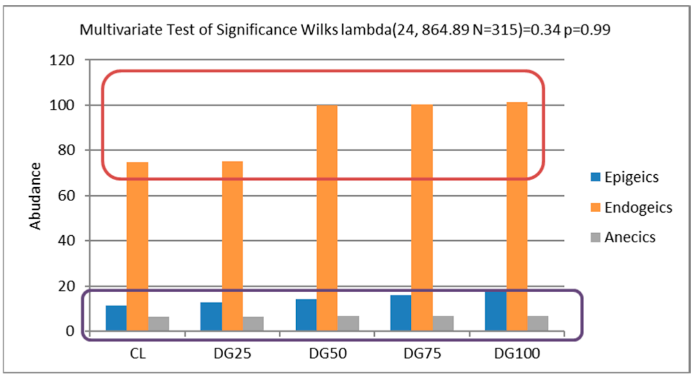


(**b**)

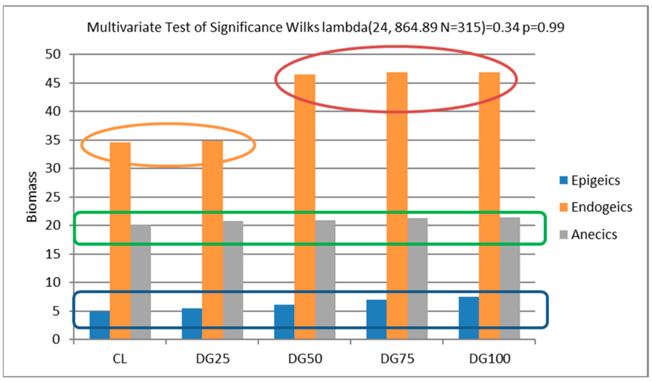
Figure 3. (**a**) Mean abundance [ind. m^{-2}] and (**b**) mean biomass [g m^{-2}] of Lumbricidae at the experimental sites in the individual years of the study. Abbreviations of treatments are given in Table 2.

According to Paoletti [15], the various agricultural treatments applied in agriculture, including fertilization, have strongly impacted selected Lumbricidae species. Particularly negative impacts were found decades ago, concerning species of small size (mainly epigeics) and large species (anecics), whose abundance was decreasing in intensively cultivated areas. Admittedly, determining the abundance and biomass of Lumbricidae may be sufficient for a general assessment of soil health. However, only a species analysis that allows the characterization of changes in the ecological groups of these invertebrates will allow more detailed assessments and the formulation of specific conclusions.

Lumbricidae species found at the study plots were classified into three ecological groups (Table 2 and Figures 4 and 5). The epigeics group was represented by three species: *D. rubidus*, *L. rubellus*, and *D. octaedra*, for which the primary habitat is the litter layer. The endogeics group of horizontally burrowing species was represented by *A. caligionosa*, *A. rosea*, and *O. lacteum*. Meanwhile, the group of anecics, or earthworms that live in deeper soil layers, was represented by *L. terrestris*, one of the largest species found in Poland.

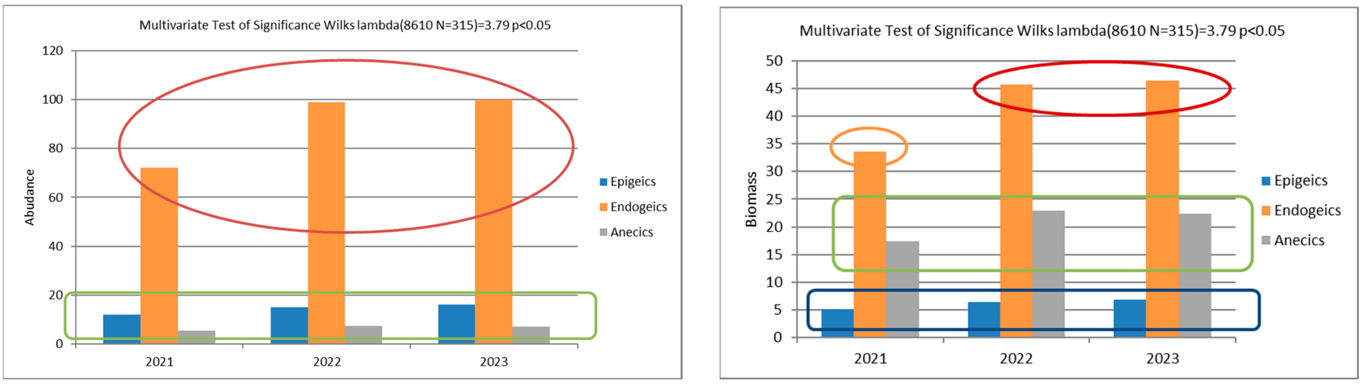


(**a**)



(**b**)

Figure 4. (**a**) Mean abundance and (**b**) mean biomass of Lumbricidae in the research sites based on ecological groups (Abbreviations of treatments are given in Table 2). The values in different frames show statistically significant differences ($p < 0.05$).



(**a**) (**b**)

Figure 5. (**a**) Mean abundance and (**b**) mean biomass of Lumbricidae based on ecological groups in the individual years of the research. The values in different frames show statistically significant differences ($p < 0.05$).

As shown here, the highest mean abundance was found in the group of horizontally burrowing endogeic earthworms, where the mean values were similar in treatments DG100, DG75, and DG50, and were 34% higher than the mean value in treatment DG25 and CL. However, this difference was not statistically significant due to the large difference in values between the two groups (Figure 4a). However, a significant difference was found within the endogeics relative to mean biomass, with DG100, DG75, and DG50 > DG25 and CL by an average of 35% (Figure 4b). These observations do not coincide with the results of Ernst et al. [45], who showed that, although they did not avoid plots with digestate, Lumbricidae belonging to the endogeic group (those digging branching burrows in the surface soil layer) showed a decrease in biomass after its application. In the present study, there were no significant differences in mean abundance between the epigeic and anecic groups at the treatment and control plots (Figure 4a). However, a significant difference was found in the average biomass of epigeics and anecics (Figure 4b). As reported by Ross et al. [44], earthworms of the litter-dwelling epigeics group actively avoided soil with added digestate, which does not coincide with the results of the present study, as this ecological group had the highest values for both abundance and biomass of earthworms at the DG100 plot, where the highest doses of digestate were applied (by 19% and 49% for abundance and biomass of DG100 > CL, respectively; Figure 4a,b). Moinard et al. [38] showed that deep-burrowing earthworms (anecics) responded positively to the addition of digestate to the soil, as they likely avoided the toxic effects of digestate due to digging deep vertical burrows into the soil profile. In the present study, Lumbricidae of the anecics group—represented by a single species, *L. terrestris*—had similar abundance and biomass values at all plots (Figure 4a,b). In contrast, a significant increase in the mean biomass of *L. terrestris* was observed between 2021 (when fertilization with digestate was first applied) and the following years of the study (2022 and 2023 > 2021 by 31%) (Figure 5b). Similar results were found concerning endogeics, with an increase in mean abundance and a significant increase in mean biomass observed between 2021 and subsequent years (increases of 31 and 33%, respectively) (Figure 5a,b). There were no significant differences in mean abundance in groups of epigeics and anecics in the subsequent years of the study (Figure 5a).

In general, the variation in the metrics that occurred in earthworm populations under different digestate treatments may confirm the results of other studies, which show that organic fertilization has a much more positive effect on earthworms than no application of organic matter to the soil [46,47].

3.3. Effect of Different Doses of Digestate on Selected Soil Physicochemical Parameters

Digestate is a complex fertilizer material, so its application significantly impacts the soil's physical, chemical, and biological properties, depending on its type [48]. We found that the different digestate treatments applied affected selected soil parameters to a different extent. Significant differences were found in selected mean values of soil characteristics (mean from three years) between individual plots (Table 3). No significant differences were found in the values of all analyzed soil characteristics within individual plots in the successive years of the study (Figure 6a–h).

Significant differences in the mean organic carbon content of DG100 > DG75 > DG50 > DG25 > CL were found between all plots (Table 3, Figure 6a), but there were no significant differences within individual plots in successive years of digestate application (OC content within specific plots remained at similar levels) (Figure 6a). Another consequence was a significant difference in the C/N ratio between all plots (Figure 6g). Similar observations were made by Odlare et al. [49], and these authors confirmed a significantly higher OC content in soils fertilized with digestate than in the control soil. Villario et al. [50] showed that the increase in OC content in soil is directly related to the supply of this material with digestate. In contrast, Johansen et al. [51] report that long-term digestate application can reduce soil OC content. In contrast, Triwari et al. [52] report that maintaining adequate soil organic carbon content over several years of fertilizer application using digestate requires an additional source of OC. It should be noted here that the high availability of perishable organic carbon in the soil, due to microbial activity, can lead to oxygen deficiency and facilitate denitrification, resulting in nitrogen loss [53]. As previously reported by Alburquerque [54], and Tambone and Adani [55], nitrogen delivered to the soil with digestate has a short-term fertilizing effect and resembles the fertilizing properties of urea. This regularity is independent of soil characteristics and the amount of fertilizer biomass applied. These authors concluded that the nitrogen in the digestate, both because of the chemical form in which it occurs and the mechanism by which it is released, can be compared to nitrogen mineral fertilizer. Similarly, Šimon et al. [56] showed that digestate is similar to mineral nitrogen fertilizer in terms of the form of nitrogen contained and the rate of N utilization by plants. They also reported that digestate affects yield growth but does not significantly improve the level of organic matter in the soil, so it is necessary to add organic matter from other sources. In the present experiment, an increase in nitrogen content was found in successive years of testing at each test plot (excluding the CL control plot), but the differences were not statistically significant. However, significant differences were found in the mean value of the analyzed trait between each plot (DG100 > DG50, DG25, CL, and DG75 > DG25, CL) (Table 3, Figure 6b).

As a result of the conducted studies, a similar trend to OC was shown concerning phosphorus and potassium, where DG100 > DG75 > DG50 > DG25 > CL, while there were no differences in the values of the analyzed traits within individual plots in the successive years of the study (Figure 6c,d). Alburquerque et al. [54] reported that digestate can significantly increase soil phosphorus content and alter the biogeochemistry of this element in the soil. Vanden Nest et al. [57] showed that applying solid digestate obtained from waste organic matter and manure increased the phosphorus content of the soil. Möller and Muller [58] reported that digestate has a similar effect on the soil environment and plants as mineral fertilizers, regarding potassium and nitrogen.

Figure 6. Mean value of selected soil attributes: (**a**) OC, (**b**) TN, (**c**) P, (**d**) K, (**e**) Ca, (**f**) Mg, (**g**) C/N, (**h**) Moisture at the analyzed research sites in the individual years of the experiment (2021–2023). Soil attributes from (**a**–**f**) in [mg kg^{-1} (d.m.)] and (**h**) in [%]. The values in different frames show statistically significant differences ($p < 0.05$). Abbreviations are given in Table 3.

Analyzing the effect of different digestate treatments on the soil's calcium (Ca) content, significantly higher values were observed at plots DG100, DG75, and DG50 compared to DG25 and the unfertilized control plot. The most considerable difference was shown between DG100 > DG25 and CL (6% difference; Figure 6e). In addition, significant differences between plots were observed concerning magnesium (Mg) content. There was a close relationship between the amount of applied biofertilizer and the value of the analyzed trait at the analyzed plots (DG100 > DG75 > DG50 = DG25 > CL) (Table 3, Figure 6f). Due to the low content of cadmium (Cd) and lead (Pb) (0.4 ± 0.1 and 1.4 ± 0.2 mg kg^{-1}) in the applied biosolids, respectively), there were no significant differences in the content of these elements among the analyzed plots (Table 3).

In the study described here, there was also a significant difference in soil moisture between the plots with the highest addition of digestate (DG100, DG75, and DG 50) and the plot with the lowest applied fertilizer rate of DG25 and the CL control plot (Figure 6h). As reported by Zeng et al. [59], the application of digestate improved soil water retention in sandy loam areas. Nevertheless, Xin et al. [60] showed that adding organic matter, such as compost and digestate, improved the water absorption capacity of the soil. Schomburg et al. [61] reported that earthworms, along with plants, are organisms that improve the structural stability of soil by incorporating mineral and organic matter into soil aggregates. This, in turn, has a beneficial effect on water storage. It can be noted that the results of the study also indicate better development of earthworm populations in variants with a higher amount of digestate (DG100, DG75, and DG50). In turn, Jasa et al. [62] claim that fertilizer application of digestate reduced soil porosity and minimum air capacity. Although these authors indicate that fertilization with digestate contributed to better crop yields, at the same time, there is a risk of deterioration of the physical properties of the soil, which can lead to irreversible soil degradation. Therefore, appropriate application of digestate can enable its use in crop production with minimal changes in soil productivity. Garcia-Lopez et al. [63] reported that applied digestates showed high fertilizer value, but their effect on soil functionality depended on the applied rate. These authors suggest that further research on the use of digestate as a fertilizer in soils with different properties is needed to better understand its effect on soil functionality.

4. Conclusions

Digestate fertilizer treatments applied to a silt loam soil, in monoculture plant cultivation, had no effect on the qualitative structure of earthworm communities, but they did affect their mean abundance and biomass. The greatest values were found in plots with higher digestate applications from all years of the experiment. Rates of application had no adverse effect on earthworm diversity but did affect the earthworm ecological groups, e.g., endogeics showed an increase in mean abundance and a significant increase in mean biomass. No significant differences were found between most soil characteristics within treatments during the study, except for the mean values of OC, P, K, and C/N between all analyzed plots. A significant difference in soil moisture content was also present. Use of solid digestate as a fertilizer is justified; however, its impact on soil environments has still not been sufficiently studied, and the effectiveness of this biomass as an organic fertilizer additive warrants further investigation.

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Institutional Review Board Statement: Ethical review and approval were waived for this study. The European Parliament and of the Council Directive 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.

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

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

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