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Article

Earthworm Community Metrics and Soil Attributes Are Driven by the Addition of Cattle Horn Shavings Fertilizer

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Abstract

One of the fundamental recommendations for sustainable agricultural practices is protecting soil biodiversity by increasing the use of organic fertilizers and substrates. According to EU regulations, certain animal by-products (including horn shavings) may be used as crop fertilizers; however, insufficient information is available on the impact of this fertilizer substrate on the soil environment. This study was conducted to determine the effects of annual soil application of horn shavings on selected characteristics of Lumbricidae communities and physicochemical properties of the soil. Experimental plots had the following treatments of cattle horn shavings (CHS): CHS100 (100%; 1.3 t·ha^{-1}; equivalent to 161 kg N/ha), CHS75 (75%; 0.98 t·ha^{-1}), CHS50 (50%; 0.65 t·ha^{-1}), and SL (control without fertilization). After 2 years of application, an electrical method was used to collect earthworms over the following 3 years. Earthworms found belonged to five species representing three ecological groups: *Dendrobaena octaedra*, *Dendrodrilus rubidus tenuis*, *Lumbricus rubellus*, *Aporrectodea caliginosa*, and *Lumbricus terrestris*. Significantly higher values of earthworm metrics were demonstrated between the plot with the highest fertilization (CHS100) and the plots with lower horn shavings additions (abundance: CHS100 > CHS75 and CHS50 by a mean of 43.2%; biomass: CHS100 > CHS75 and CHS50 by a mean of 43%). Species richness was not affected but an increase in CHS application led to a greater biodiversity index. CHS treatments affected selected soil parameters to varying degrees, with soil moisture having the greatest influence on the given earthworm traits. Cattle horn shavings used as a fertilizer are a positive promoter of earthworms in soils and further research in this area may be warranted.

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

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1. Introduction

The ongoing degradation of environmental resources, including soil ecosystems, environmental pollution, and the loss of biodiversity, is currently a key topic of discussion at the local and global levels [1]. Soil ecosystems are considered a fundamental environmental resource; they regulate many ecosystem processes, serve as a habitat for a large proportion of our planet's biodiversity, and are largely responsible for production of plant matter that forms the basis of life on Earth [2]. Research shows that a recurring feature of soils across many regions of Europe is a progressive decline in fertility, driven by decreases in organic matter, nutrient depletion, and reduced biodiversity. There is evidence that if preventive measures are not implemented, these adverse processes will continue to intensify [3]. Therefore, for several years, one of the main recommendations of sustainable agriculture has been the protection of soil biodiversity [4], by including increased use of organic fertilizers and substrates in the cultivation of economically important crops [5]. For many years, various organic materials have been used to fertilize cultivated soils: manure and slurry [6], compost and vermicompost [7], and digestate [8]. Horn shavings—properly processed, finely ground cattle horns and hooves—are becoming an increasingly popular organic fertilizer worldwide. According to EU regulations, certain animal by-products (including cattle horn shavings) may be used as fertilizer substrates in agriculture [9]. Given that 6.4 million tons of beef were produced in the European Union in 2023 [10], the volume of horn and hoof waste is significant. As a result, thousands of tons of horn waste are produced and accumulated each year, which can be utilized as fertilizer in agriculture, particularly in organic farming. While studies on the use of horn shavings as fertilizers in crop production demonstrate their high efficacy [11,12], information on their impact on the soil environment is very limited. Studies have shown that while animal-based fertilizers at appropriate doses increased soil nitrogen content, higher doses increased ammonium ion concentration, harming soil microorganisms [13]. There are no reports on the impact of using horn shavings as fertilizer on soil mesofauna, particularly earthworms. Lumbricidae, given that they constitute approximately 90% of the invertebrate biomass in the soil [14], are undoubtedly among the most important engineers of soil ecosystems. They perform key functions in the soil, providing numerous ecosystem services [15]. Lumbricidae participate in the decomposition and mineralization of organic matter [14], play a significant role in the nutrient cycle for plants, and improve soil structure, chemical composition, and biological activity [16]. These invertebrates produce excrement known as coprolites, which are rich in organic compounds that play a vital role in soil physical properties and SOM dynamics. Through the production of coprolites, earthworms stimulate the growth of various groups of microorganisms that play a key role in decomposing dead organic matter in the soil [17]. Therefore, multifaceted, long-term research is essential on the impact of relatively "new" fertilizer substrates, such as horn shavings, on earthworm populations–organisms that determine soil fertility. This research is of paramount importance because a key challenge in agroecosystems today is the transition from intensive agricultural production—which poses a significant threat to global biodiversity—to a conservation-oriented approach that revitalizes ecosystem services [18]. Considering the above, this study sought to assess the impact of fertilizing clay loam soil with a long-standing monoculture of *Cucurbita pepo* L. (pumpkin) and horn shavings on the characteristics of earthworm communities and selected physicochemical soil parameters. The objective was to assess the effect of horn shavings treatment on (i) Lumbricidae abundance, (ii) Lumbricidae biomass, (iii) selected characteristics of earthworm ecological groups, and (iv) selected physicochemical properties of the soil.

2. Materials and Methods

2.1. Fertilizer Material–Cattle Horn Shavings

The fertilizer material (producer: Natureum companies, Gdańsk, Poland) used in the experiment was purchased from a retail source. It consisted of shavings measuring

5–10 mm, produced by grinding waste material from cattle hooves and horns. The characteristics of cattle horn shavings are presented in Table 1.

Table 1. Content of macronutrients, trace elements, and selected features in the cattle horn shavings (mean ± standard deviation).

Parameter	Units	CHS
OC	mg kg^{-1} (dry matter)	354,335.6 ± 6450.8
TN		124,530.5 ± 3615.5
P		2326.2 ± 168.6
K		1259.6 ± 113.3
Ca		5351.4 ± 283.5
Mg		216.2 ± 27.8
Cd		0.1 ± 0.0
Pb		1.8 ± 0.1
C/N ratio	-	2.85 ± 0.3
pH in H_2O	-	7.1 ± 0.1

Abbreviations: CHS—cattle horn shavings; OC—organic carbon; TN—total nitrogen.

2.2. Experimental Design

Field studies were conducted in southeastern Poland (Podkarpackie Province) near the town of Tyczyn (49°57′16″ N 22°02′31″ E), on the grounds of a private farm. The experimental area, which was unshaded and flat, was located at an elevation of approximately 232 m above sea level. According to the World Reference Base for Soil Resources, the soil texture was classified as clay loam (<0.02 mm fraction content–37.9%) [19]. For research purposes, experimental plots were established with varying doses of the applied fertilizer substrate:

CHS100—cattle horn shavings 1.3 t·ha^{-1} (100% recommended usage; 161 kg N/ha) [20]
CHS75—cattle horn shavings 0.98 t·ha^{-1} (75% of the recommended usage); (121 kg N/ha)
CHS50—cattle horn shavings 0.65 t·ha^{-1} (50% of the recommended usage); (80.5 kg N/ha)
SL—unfertilized soil (control)

The experimental plots and the control plot, each measuring 200 m^2 (10 × 20 m), were located approximately 5 m apart.

The experimental plots using the specified treatments of horn shavings were prepared in late April 2019, while monitoring (earthworm collection and soil sampling) began in June 2021. The decision to include a two-year grace period before analysis of earthworm communities and soil characteristics was made considering that the impact of horn shavings on the soil environment is gradual and takes time. Additionally, earthworms, when colonizing habitats with more favorable conditions, move at a rate of 2 to 15 m per year [21].

Each year of the experiment, during the last week of April, the specified horn shavings treatments were applied manually to each experimental plot and then mixed to a depth of 15 cm using a two-furrow reversible plow. In mid-May, pumpkin seeds (Junona variety, producer: W. Legutko Breeding and Seed Company, Jutrosin, Poland) were sown annually. The control plot was prepared using the same procedures, but without fertilization. Annual applications of appropriate cattle horn shavings were carried out on the same originally designated plots throughout the duration of the experiment. No plant protection products or other fertilizing substrates were used. At each plot, emerging weeds were manually removed monthly.

2.3. Earthworm and Soil Sampling

In the year preceding the application of the cattle horn shavings fertilizer substrate, earthworm populations were analyzed in designated plots using the same procedures later employed during the experiment. This pilot analysis showed very similar results across all plots, comparable to those reported for the control plot SL in Table 2. The experimental applications were conducted over three annual cycles (2021–2023). During each year, earthworm samples were collected three times per year (June, August, October) at each treatment and control site. At each of the four sites (each with an area of 200 m^2), three random samples were collected on each occasion. Earthworms were extracted using an electric-current method known as the octet method [22]. The octet consists of eight soil probes arranged in an octagonal pattern (6 mm in diameter and 65 cm in active length) with insulated handles arranged in 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 [23]. The duration selected for each sequence was 2.5 min. Lumbricidae specimens were collected for analysis from the central area defined by the ring and the probes.

Table 2. Mean (±sd) abundance [ind. m^{-2}], mean (±sd) biomass [g m^{-2}], and dominance of earthworm species found in the digestate treatments (derived from a combination of all sampling periods over all years).

Species/Ecological Group *	Features	Cattle Horn Shaving Treatments **			
		SL	CHS50	CHS75	CHS100
Epigeics					
Dendrobaena octaedra	Abundance	12.98 ± 4.49 a	17.69 ± 5.55 b	22.41 ± 4.88 b	29.83 ± 6.23 c
	Biomass	2.92 ± 1.09 a	4.18 ± 1.36 b	5.33 ± 1.31 b	7.16 ± 1.76 c
	Dominance %	7.19	7.72	8.49	8.46
Dendrodrilus rubidus tenuis	Abundance	13.48 ± 5.57 a	18.37 ± 6.73 b	24.44 ± 6.69 c	34.38 ± 9.88 d
	Biomass	3.11 ± 1.37 a	4.38 ± 1.80 b	5.82 ± 1.77 c	8.01 ± 2.54 d
	Dominance %	7.47	8.01	9.27	9.74
Lumbricus rubellus	Abundance	25.95 ± 11.96 a	37.75 ± 12.67 b	41.96 ± 13.19 b	53.08 ± 16.31 c
	Biomass	11.37 ± 5.44 a	16.51 ± 5.88 b	18.23 ± 6.41 b	23.63 ± 7.82 c
	Dominance %	14.38	16.47	15.91	15.04
Endogeics					
Aporrectodea caliginosa	Abundance	84.42 ± 22.86 a	100.27 ± 19.61 ab	113.08 ± 23.02 b	155.04 ± 24.30 c
	Biomass	38.04 ± 10.93 a	45.82 ± 10.22 ab	51.83 ± 11.85 b	71.62 ± 13.24 c
	Dominance %	46.77	43.75	42.88	43.93
Anecics					
Lumbricus terrestris	Abundance	43.65 ± 9.96 a	55.11 ± 12.70 b	61.85 ± 11.31 b	80.55 ± 10.84 c
	Biomass	31.16 ± 7.64 a	40.46 ± 9.63 b	45.44 ± 8.75 b	59.77 ± 8.45 c
	Dominance %	24.19	24.05	23.45	22.83

* Ecological group of Bouché [24]. ** Abbreviations: SL—control; CHS50—cattle horn shavings 0.65 t·ha^{-1}; CHS75—cattle horn shavings 0.98 t·ha^{-1}; CHS100—cattle horn shavings 1.3 t·ha^{-1}. Different letters in a row indicate statistically significant differences ($p < 0.05$).

All collected earthworm specimens were rinsed for 15 min in tap water, then anesthetized by immersion in 30% ethanol, and subsequently preserved in 4% formalin. Earthworms were identified to species, counted, and their biomass determined using a Radwag AS 160 electronic scale. Kasprzak's [25] key was used for species identification. During the collection of Lumbricidae samples, soil temperature was also measured (at a depth of 0–15 cm, always around 10:00 a.m.), and soil samples were also collected (n = 3) (from a depth of 0–15 cm) were also collected from each experimental and control site to determine selected physical properties and the content of macronutrients and clay metals listed in Table 3.

Table 3. Content of macronutrients, trace elements, and selected features in the soils at the research sites (mean ± standard deviation based on twenty-seven samples).

Parameter	Units	Soil Characteristics *			
		SL	CHS50	CHS75	CHS100
OC	$mg\ kg^{-1}$ (d.m.)	10,138.7 ± 51.5 a	14,273.1 ± 877.5 b	16,686.8 ± 1135.0 c	17,908.1 ± 1361.9 d
TN		880.7 ± 37.4 a	1412.9 ± 161.5 b	1754.9 ± 257.5 c	1973.3 ± 257.9 d
P		153.9 ± 3.3 a	174.1 ± 10.3 b	196.9 ± 17.5 c	219.1 ± 17.7 d
K		155.9 ± 2.7 a	165.5 ± 5.4 b	175.6 ± 9.4 c	186.7 ± 9.0 d
Ca		698.1 ± 5.4 a	726.1 ± 9.6 b	746.1 ± 19.8 c	766.9 ± 19.8 d
Mg		169.2 ± 2.1 a	171.8 ± 2.0 b	177.2 ± 3.6 c	179.7 ± 3.6 d
Cd		1.3 ± 0.1 a	1.3 ± 0.2 a	1.29 ± 0.1 a	1.3 ± 0.2 a
Pb		12.5 ± 0.2 a	12.5 ± 0.2 a	12.5 ± 0.1 a	12.5 ± 0.2 a
C/N ratio	-	11.5 ± 0.5 a	10.2 ± 0.6 b	9.63 ± 0.9 c	9.15 ± 0.7 d
pH in $H_2$0	-	6.34 ± 0.1 a	6.4 ± 0.1 a	6.45 ± 0.1 a	6.52 ± 0.1 a
Electrical conductivity	$mS{\cdot}cm^{-1}$	0.21 ± 0.00 a	0.29 ± 0.01 b	0.35 ± 0.01 c	0.37 ± 0.02 d
Temp.	°C	13.8 ± 1.0 a	13.6 ± 1.0 a	13.6 ± 1.1 a	13.7 ± 1.1 a
Moisture	%	26.9 ± 3.0 a	30.7 ± 2.9 b	33.8 ± 2.7 c	37.6 ± 2.5 d

* Abbreviations: SL—control; CHS50—cattle horn shavings 0.65 $t{\cdot}ha^{-1}$; CHS75—cattle horn shavings 0.98 $t{\cdot}ha^{-1}$; CHS100—cattle horn shavings 1.3 $t{\cdot}ha^{-1}$; OC—organic carbon; TN—total nitrogen. Different letters in a row indicate statistically significant differences ($p < 0.05$).

2.4. Physicochemical Analysis

At the sampling points, soil temperature and moisture were measured at a depth of 20 cm. Soil moisture was determined by oven-drying at 105 °C [26]. The soil-substrate material was analyzed to determine the total content of selected macro- and microelements and other properties, using the procedures described by Ostrowska et al. [27]. Soil reaction was determined potentiometrically using a HI 4221 pH meter (HANNA Instruments Inc., Woonsocket, RI, USA), at a soil-to-water ratio of 1:2.5. Salt concentration was determined conductometrically using a HI 2316 conductometer (HANNA Instruments Inc., Woonsocket, RI, USA). The contents of K, Ca, Mg, Cd, and Pb were determined by atomic absorption spectrophotometry (using a Hitachi Z-2000, Hitachi Inc., Tokyo, Japan) after mineralization of the test material in concentrated hot HClO4 (at a maximum temperature of 210 °C) using a Tecator digestion system heating block. Phosphorus was determined in the same solution after substrate mineralization using the vanadium–molybdenum method (using a Shimadzu UV-2600 spectrophotometer, Kyoto, Japan). Furthermore, nitrogen content in the test material was determined using the Kjeldahl method with a Foss Tecator Digestor 2006 heating block (for sample digestion) and a Kjeltec 8100 distillation unit. Organic carbon content was determined using a Vario EL-CUBE elemental analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). Results obtained were used to calculate the carbon-to-nitrogen (C/N) ratio.

2.5. Data Analysis

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

$$H' = -\sum_{i=1}^{S} p_i * log_{10} p_i$$

where p_i is the ratio of the number of organisms of a given species to the total number of all organisms [28];

$$D = Na/n$$

where Na is the number of individuals belonging to the species in all samples and n is the number of individuals of the studied species group in all samples [29]. Dominance classes were adopted from Górny and Grüm [30]: eudominants >10%, dominants 5.1–10%, subdominants 2.1–5%, recedents 1.1–2%, subrecedents <1% of the total number of individuals in the assemblage.

The results were analyzed statistically using Statistica software v. 13.3 and R 4.5.2. The effects of experimental treatments (research sites) on earthworm abundance, biomass, and the Shannon–Wiener diversity index (H') were analyzed using mixed-effects models. For abundance and biomass, generalized linear mixed models (GLMMs) with a Gamma distribution and log link function were applied using the *glmmTMB* R package [31,32]. The Shannon–Wiener index (H') was analyzed using a linear mixed-effects model (LMM) fitted with the lme4 R package [33]. In all models, the research site was treated as a fixed effect, while year was included as a random factor to account for temporal variability and repeated measurements within sites. The potential effect of year as a fixed factor was initially tested but was not statistically significant for any of the response variables ($p > 0.05$); therefore, it was retained only as a random effect in the final models. Estimated marginal means (EMMs) were calculated using the *emmeans* package, and pairwise comparisons between sites were performed with Tukey-adjusted post hoc tests. For GLMMs, results were back-transformed to the response scale. Homogeneous groups were identified using compact letter display (CLD). Preliminary relationships between soil physicochemical parameters and earthworm abundance, biomass, and the Shannon–Wiener diversity index were explored using Partial Least Squares (PLS) regression.

3. Results and Discussion

3.1. Earthworm Species in the Study Area

Five species of Lumbricidae were found at each experimental site: *Dendrobaena octaedra* (Savigny 1826), *Dendrodrilus rubidus tenuis* (Savigny 1826), *Lumbricus rubellus* (Hoffmeister 1843), *Aporrectodea caliginosa* (Savigny 1826), and *Lumbricus terrestris* (Linnaeus 1758) (Table 2). A similar number of five species was reported by Koblenz et al. [34] in studies on the impact of digestate fertilization on earthworm communities in loamy soils.

A characteristic feature of all identified species was uniformity in their dominance class across experimental sites. At each experimental and control site, three species were classified as eudominants (*L. rubellus*, *A. caliginosa*, and *L. terrestris*), while two species were classified as dominants (*D. octaedra* and *D. rubidus*) (Table 2). Slightly different results were obtained by Mazur-Pączka et al. [35] in an area fertilized with various levels of solid digestate, where they demonstrated that *L. terrestris* constituted a recessive group in plots fertilized with the highest doses of digestate. In contrast, the unfertilized control was classified as subdominant. The number of Lumbricidae species identified in this study is small compared to that reported by Rodriguez et al. [36], who identified 12 species in studies conducted in areas with varying levels of crop intensification.

3.2. Effect of Fertilization Technologies on Earthworm Abundance and Biomass

Analysis of the results of this study revealed significant differences in the average number and average biomass of earthworms both between plots where cattle horn shavings (CHS) were applied and the control plot, and within plots receiving different doses of CHS (Figure 1a,b). Significantly higher values of the analyzed traits were observed between the site with the highest application rate (CHS100) and sites with lower horn shavings additions (abundance: CHS100 > CHS75 and CHS50 by an average of 43.2%; biomass: CHS100 > CHS75 and CHS50 by an average of 43%) (Table 2, Figure 1a,b). No significant differences were found in the discussed traits between the CHS50 and CHS75 plots. Taking into account the significantly higher average biomass and abundance in the sites with CHS application compared to the plot without SL fertilization in these studies, then similar observations were reported by Leroy et al. [37], who found a significant increase in earthworm abundance when using organic fertilizers, such as manure and compost, compared with unfertilized plots. Ngosong et al. [38] also demonstrated several times higher earthworm abundance in plots where organic fertilization with poultry manure was applied than in inorganically fertilized areas and the unfertilized control. Singh et al. [39] reported that the soil application of organic fertilizers in the form of sheep manure can contribute to an approximately fourfold increase in the abundance of local earthworm communities. The general tendencies presented above result from the observations of these authors align with those obtained in the present study, which indicate that an increase in the soil-applied dose of organic substrate in the form of horn shavings contributes to an increase in both the average abundance and average biomass of Lumbricidae. Although the studies cited by other authors used organic fertilizers other than CHS, it can probably be assumed that horn shavings, as a natural substrate, may have a positive effect, similar to the manures and compost presented above, on selected traits of Lumbricidae. As a result of the conducted research, a positive effect of the applied doses of cattle horn shavings (CHS) on the Shannon–Wiener index (H') was also observed. The values of the analyzed trait differed significantly at all study sites and the control site. An increase in earthworm biodiversity was observed with increasing CHS dose (Figure 1c). However, it should be noted that not all organic fertilizers may have a positive effect on selected characteristics of the Lumbricidae population, as Rollet et al. [40] demonstrated a negative impact of fertilization with organic substrate in the form of digestate on the quantitative and qualitative structure of the earthworm population.

In addition to the amount of organic matter available to earthworms, soil structure (which varied with the amount of CHS added) may also have contributed to the significant differences in the abundance and biomass of Lumbricidae between sites. According to Pekarskas et al. [11], horn shavings can significantly contribute to increasing soil water retention capacity and improving soil structure, which in the present study may have influenced the significant differences in soil moisture across sites (CHS100 > CHS75 > CHS50 > SL) (Table 3). Tripathi and Bhardwaj [41] and Adigun et al. [42] demonstrated that soil moisture, along with its OC and N content, can significantly influence the distribution of earthworms, as well as their abundance and biomass. In turn, Capowiez et al. [43] reported that favorable moisture and temperature conditions, among other factors, have a significant impact on the abundance and biomass of Lumbricidae. As reported by Singh et al. [44], Lumbricidae species are sensitive to varying degrees to excess water in the soil environment, as well as to drought, which can degrade earthworm populations and reduce burrowing activity due to overly hard soil. The general trend given by the above authors may have been reflected in the present study at the SL control plot, as clay loam soil is prone to prolonged waterlogging from rainfall and excessive drying during drought, resulting in the formation of a hard crust in the topsoil layers. When analyzing these results, soil moisture and temperature likely had the greatest influence on the values of the analyzed earthworm traits at the study sites (Figure 2a,b). Although no significant differences in soil temperature were found at the study sites, given the potential synergistic effects of various factors on the traits of Lumbricidae discussed here, a significant influence of temperature cannot be ruled out.

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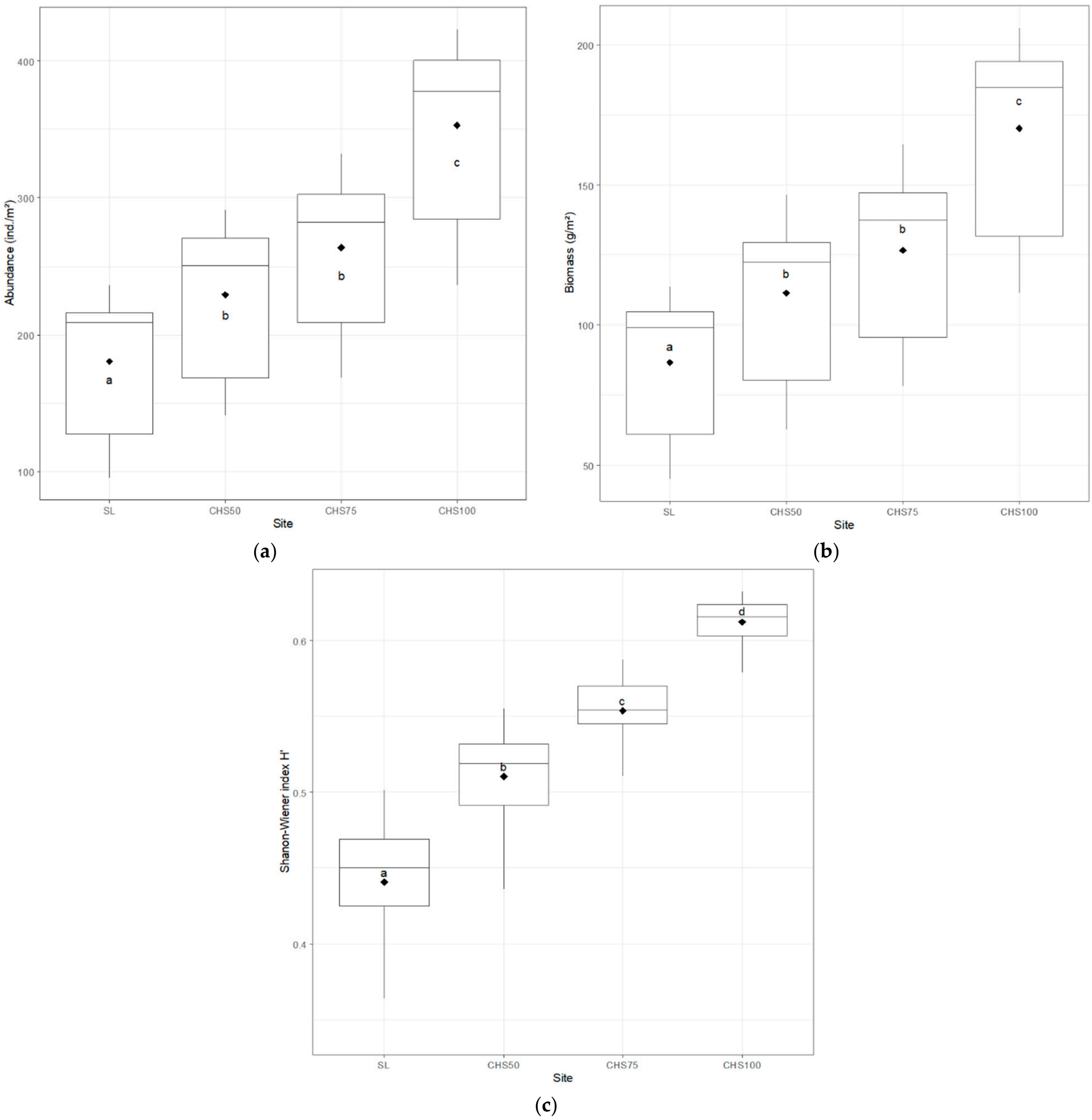
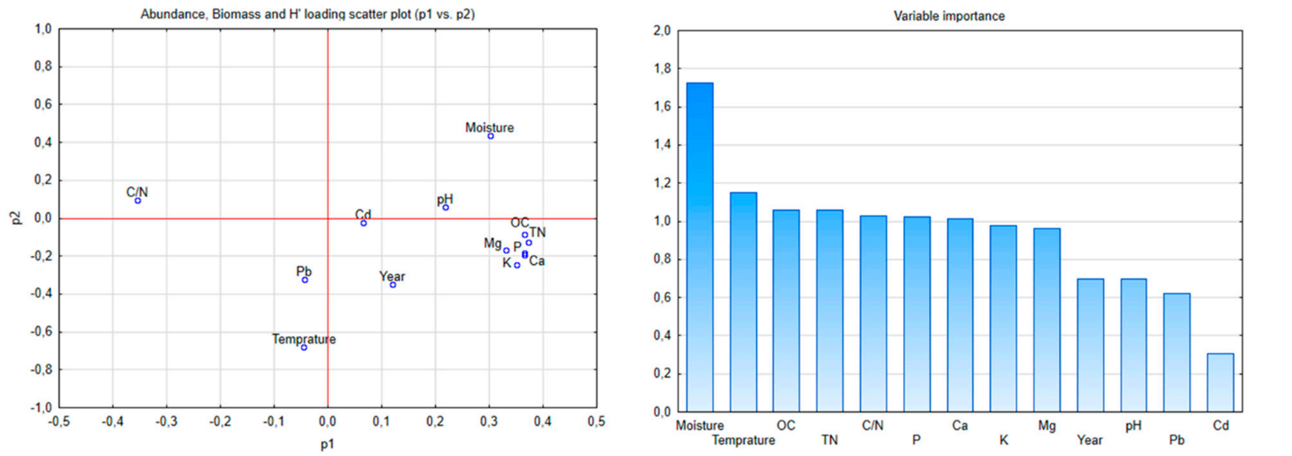


Figure 1. The boxplots show the distribution of earthworm abundance [ind. m^{-2}] (**a**), biomass [$g\ m^{-2}$] (**b**), and the Shannon–Wiener index (H') (**c**), with boxes representing the interquartile range and the central line indicating the median. Points represent mean values. Different letters indicate significant differences between sites (GLMM, $p < 0.05$).

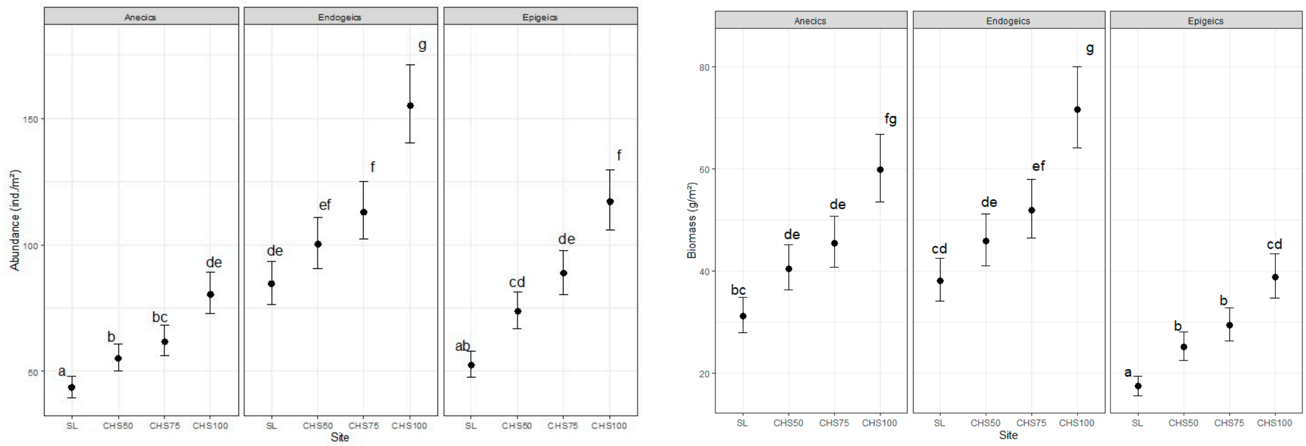
Depending on their ecological group, members of the Lumbricidae family occupy distinct ecological niches—such as burrow type in the soil or their feeding habits—thereby providing a range of important ecosystem services specific to the ecomorphological group of these invertebrates. Earthworms participate in the mineralization of organic matter, improve soil structure, and increase microbial diversity, which, in turn, leads to greater ecosystem stability [45–47].



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

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

As a result of the study, representatives of three ecological groups of Lumbricidae were identified at the analyzed sites (Table 2 and Figure 3a,b). The most abundant group in terms of species richness was the epigeics (earthworms inhabiting litter), which included three species: *D. octaedra*, *D. rubidus tenuis*, and *L. rubellus*. The endogeics group, comprising horizontally burrowing earthworms, was represented by a single species, *A. caligionosa*. Similarly, the group of deep-burrowing earthworms—anecics—was represented by a single species, *L. terrestris*.



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

Figure 3. Estimated marginal means (±95% CI) of earthworm abundance [ind. m^{-2}] (**a**) and biomass [g m^{-2}] (**b**) across sites and ecological groups. Different letters indicate significant differences between sites within each group (Tukey-adjusted, $p < 0.05$). The interaction between site and ecological group was not significant; however, it was retained in the model to allow visualization of group-specific responses.

When analyzing average abundance and average biomass of ecological groups, significant differences in the analyzed traits were found in most cases between plots with CHS biofertilizer and the control plot (SL). The only exception was the absence of significant differences in Lumbricidae abundance and biomass between the control plot (SL) and the plot fertilized with the lowest treatment of horn shavings (CHS50) in the endogeics group (Figure 3a,b). However, within the plots where CHS was applied at different levels, significant differences in earthworm metrics were observed across ecological groups in the

CHS100, CHS75, and CHS50 plots. The greatest difference in abundance and biomass was observed in the endogeics group between CHS100 > CHS75 and CHS50 (on average 45.3% for abundance; on average 46.8% for biomass). No significant differences regarding the discussed traits were found between CHS50 and CHS75 in all ecological groups (Figure 3a,b). Slightly different observations were obtained by Mazur-Pączka et al. [35], who used solid digestate fertilization in relation to earthworm abundance. No significant differences were found in earthworm abundance between the experimental sites and the control site (without fertilization). Although these authors applied a different organic substrate compared to CHS, it is important to note that the use of organic fertilization (potentially environmentally friendly) does not always influence the developmental characteristics of Lumbricidae communities. However, the aforementioned authors demonstrated a similar general trend in earthworm biomass in epigeic and anecic ecological groups compared to the present study. In this study, the endogeic group (*A. caliginosa*) showed the highest mean abundance and biomass in all sites, which could be due to its relatively high ecological tolerance and adaptability in agroecosystems [24]. Furthermore, as stated by McDaniel et al. [48], *A. caliginosa* can survive in soils with low organic matter content and low moisture levels. It should also be noted that this species often plays a very important role in agroecosystems, as it is responsible, among other things, for increasing nitrogen bioavailability and lowering the C/N ratio, as well as increasing the availability of nutrients for plants and microorganisms [48,49]. Boyle et al. [50] demonstrated that earthworm cocoons can remain in the soil for extended periods in diapause until habitat conditions become favorable. By contrast, the application of an appropriate dose of horn shavings could provide these conditions, as seen particularly with CHS100, where the abundance and biomass of all ecological groups increased (Figure 3a,b). The greatest species richness of the epigeic group at the studied sites may have been due to the greater tolerance of Lumbricidae species in this group to adverse environmental conditions (common in clay soils). As reported by Novak et al. [51], *D. rubidus tenuis*, classified as an epigeic, is well-adapted to scarce food resources, as it can survive several months without food with no serious harm. By contrast, *L. terrestris*, (the sole anecic) in this study, showed a significant increase in both abundance and biomass at the site with the highest addition of CHS100 horn shavings, which may have been due, among other factors, to improved soil structure and more favorable moisture conditions. According to Berry and Jordan [52] and Fournier et al. [53], high soil moisture may be beneficial for the development of *L. terrestris* populations, whereas prolonged waterlogging is harmful. Grigoropoulou and Butt [54] reported that the spread of *L. terrestris* may be influenced by population density and resource availability.

According to Lemtiri et al. [55], earthworms, due to their sensitivity to farming techniques and systems, can be used as bioindicators of soil health. These authors suggest that earthworms could serve as indicators of sustainable agricultural practices that farmers can use to optimize various agricultural systems. However, in this regard, it is essential to conduct extensive long-term research on the subject to fully understand the responses of Lumbricidae species within specific ecological groups to changes in the soil environment resulting from the application of various agrotechnical treatments, including potentially pro-environmental ones [56,57].

3.3. Effect of Fertilization Technologies on Selected Soil Physicochemical Parameters

Fertilizer substrates, such as horn shavings or horn powder, can be used as slow-release nitrogen fertilizers. This type of fertilizer is characterized by the gradual release of plant nutrients, improving the efficiency of nitrogen and other elements utilization. The release of biogenic elements, primarily nitrogen, is complex and depends on numerous factors [12]. The results of the study showed that different horn meal application rates had varying effects on selected soil parameters (Table 3, Figure 4a–h).

The organic carbon (OC) content in the soil was significantly influenced by the treatment, the year, and their interaction ($p < 0.05$). Post hoc comparisons revealed a steady increase in organic carbon concentration from the control plot (SL) to the plot with the highest CHS application rate, with the magnitude of the difference varying across years (Figure 4a). The greatest difference in the average content of the analyzed element among the plots where fertilization was applied was observed between CHS100 and CHS50 (25%), while the largest difference among all plots was recorded between CHS100 and SL (77%) (Table 3). Ouattara et al. [58], in studies on the use of compost enriched with horn, bone, and hoof meal as fertilizer, demonstrated a positive effect of this substrate on the content of organic carbon, total nitrogen, and total phosphorus in soils; however, the intensity of this effect depended on soil properties. These authors also demonstrated that enriching composts with a 30% addition of powder from horns, bones, and hooves not only optimally affects soil OC content but also represents a promising option for environmentally friendly management of slaughterhouse waste. As demonstrated by Mazuolyte-Miskine et al. [59], 120 days after application, the mass of horn shavings decreased by 36%. During the biodegradation of horn shavings under field conditions after 40 days, the organic matter content in the soil increased from 2.53% to 3.20%, and the soil pH decreased from 8.0 to 7.1. Analysis of the results of this study revealed no significant differences in soil pH at the study sites (Table 3).

The highest mean values of the analyzed trait were observed at the CHS100 > CHS75 > CHS50 > SL treatment, although it is worth noting the large difference in mean nitrogen content between the site with the lowest fertilizer doses and the control site (CHS50 > SL by 60%; Table 3). Differences between plots in terms of the analyzed trait became more pronounced in the later years of the experiment (Figure 4b). Lanauskas et al. [60] demonstrated in their studies that the use of horn shavings to fertilize apple trees at doses of 50 and 100 kg·ha^{-1} of N equivalent increased soil nitrogen content similar to that obtained with the application of 50 kg·ha^{-1} of nitrate nitrogen. However, these authors clearly note that horn shavings are a slow-release nitrogen fertilizer substrate. In the present study, the largest significant increase in soil nitrogen was observed after applying a dose of horn shavings with an N equivalent of 161 kg/ha (CHS100). The increase in soil nitrogen content at sites where higher shavings (CHS) treatments may have been associated with higher soil moisture (Figure 4h). Horn shavings have a high capacity to absorb and store water in the soil, whereas biological processes significantly influence the mineralization of organic nitrogen compounds, the intensity of which depends on soil moisture and temperature [61]. As demonstrated by Zibutis et al. [12], higher soil moisture and temperature had a significant effect on the decomposition efficiency of horn waste fertilizers, resulting in greater amounts of nitrogen accumulating in the 0–30 cm soil layer. In the present study, no significant differences in soil temperature were observed, whereas significant differences in soil moisture were found depending on the applied CHS treatments (Table 3; Figure 4h). By contrast, Zumaeta-Barbarán and Arévalo-Hernández [62] demonstrated that the application of horn and hoof meal as an unconventional organic fertilizer derived from cattle by-products, due to its high N content, positively influenced soil nitrogen content and, consequently, the growth of Theobroma cacao plants. A study by Cayuela et al. [13] showed that while animal-based fertilizers increased soil nitrogen content compared to plant-based fertilizers, higher doses of animal-based fertilizers increased soil ammonium ion concentrations, which negatively impacted microorganisms.

The fertilization effects of applied additives may be determined by the mineralization rate, particularly the nitrogen release rate [63]. Based on an analysis of numerous reports,

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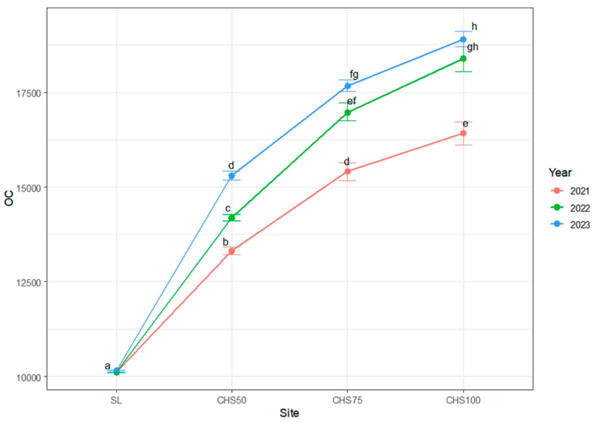
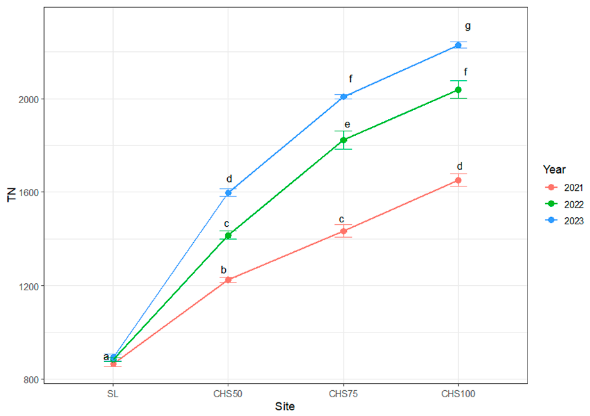
Sradnick and Feller [64] note that the C to N ratio in organic fertilizers appears to be a good predictor of N availability from organic substances, but it also depends on many other factors. Additionally, plant root exudates can stimulate the development of beneficial microbiota, chelate nutrients in the root zone, modulate root zone pH, and increase the availability of specific nutrients [65]. Generally, nitrogen availability from mineral fertilizers is much higher than the availability of organic nitrogen, but organic fertilization increases nitrogen use efficiency [66]. Lohr et al. [67] examined the kinetics of N release from various organic fertilizers and found that the daily mineralization rates of coarse horn meal were among the highest among various organic fertilizers. Furthermore, they note that the nitrogen released from organic fertilizers constitutes only about half of the total N applied. However, Lanauskas et al. [60] found in their study of apple orchard fertilization that the effect of horn shavings was in many cases equivalent to that of ammonium nitrate, despite differences in the dynamics of changes in soil mineral nitrogen content.

A similar trend to that observed for OC and TN was found for phosphorus, potassium, and calcium, with the content of the analyzed elements (P, K, and Ca) in the soil significantly influenced by the experimental plot, the year, and their interaction ($p < 0.05$). Post hoc comparisons showed a steady increase in P, K, and Ca content from the control to the highest treatment of CHS100 horn shavings was applied, with the magnitude of the differences also determined by the year of the experiment (Figure 4c–e). It should be noted here that although there was a steady increase in P, K, and Ca content at every plot where CHS was applied, only the increase in phosphorus and calcium concentrations was significant in every year of the study within each of the fertilized plots (Figure 4c,e). Significant differences in potassium content within each CHS plot were observed only between 2021 and 2023 (Figure 4d). Genisel et al. [68], in their studies on the use of bonemeal in wheat cultivation, demonstrated that the addition of this fertilizer substrate positively influenced selected plant parameters, in part due to its phosphorus and potassium content. According to these researchers, phosphorus plays an important role in photosynthesis, glycolysis, respiration, and fatty acid biosynthesis in plants, while potassium performs regulatory functions in photosynthesis, starch synthesis, and protein synthesis.

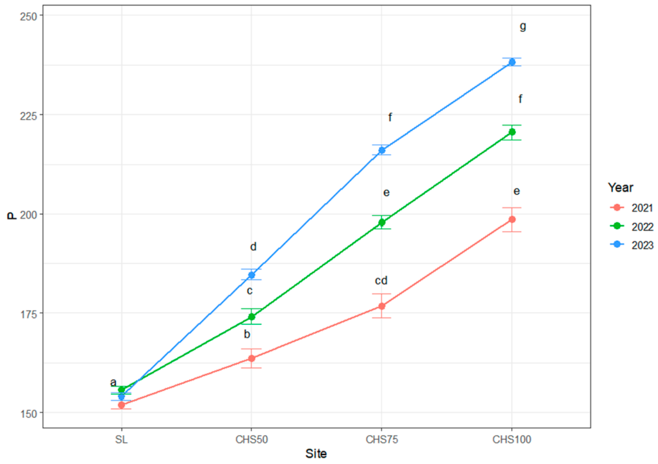


(**a**)

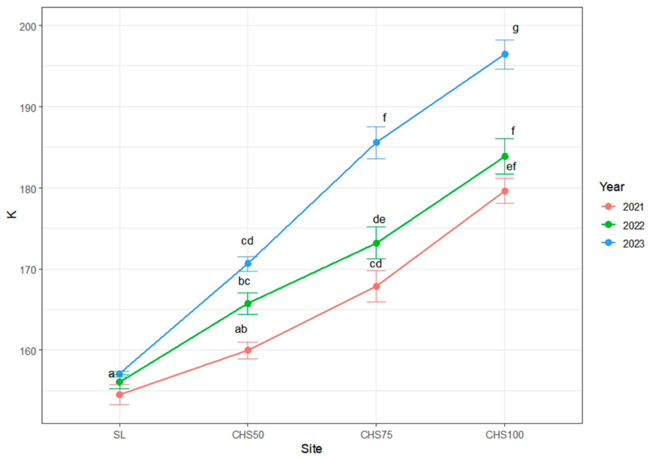


(**b**)

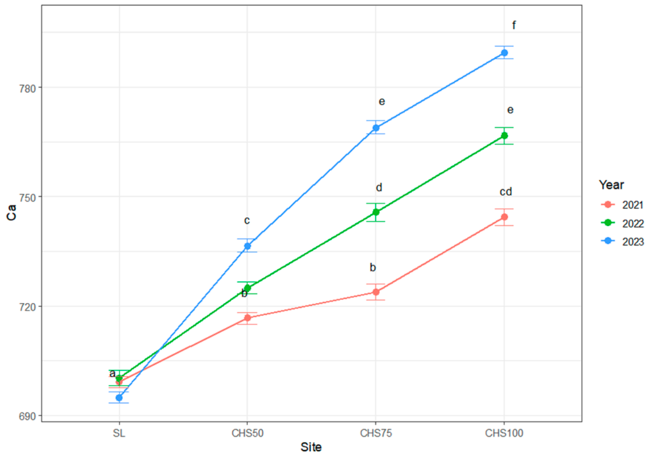
Figure 4. *Cont.*



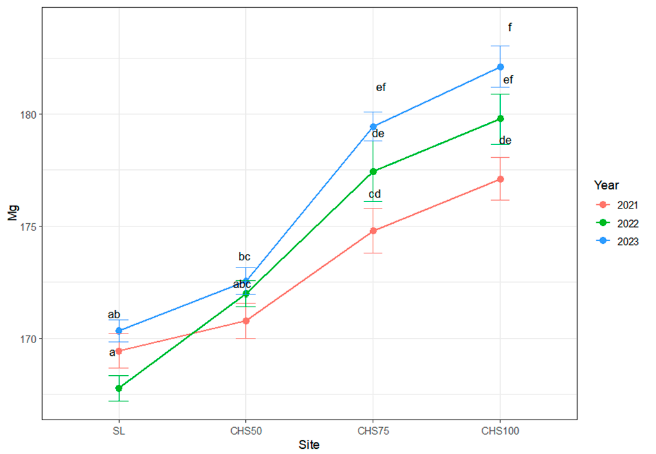
(**c**)



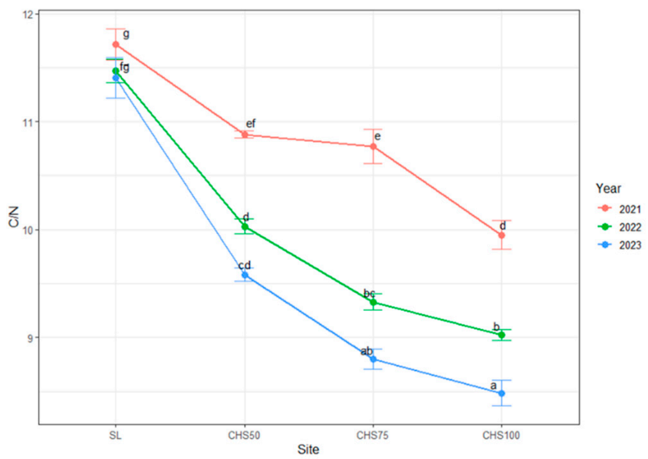
(**d**)



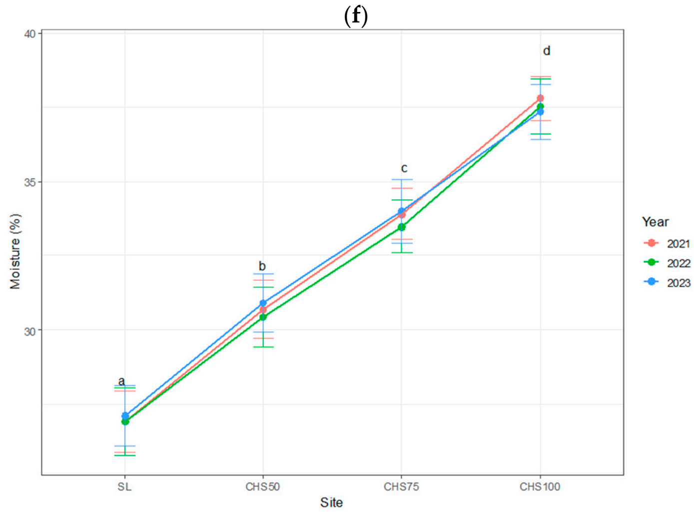
(**e**)



(**f**)



(**g**)



(**h**)

Figure 4. Mean values of selected soil parameters: OC (**a**), TN (**b**), P (**c**), K (**d**), Ca (**e**), Mg (**f**), C/N (**g**), and moisture (**h**) at the treatment plots during years (2021–2023). Soil parameters from (**a**) to (**f**) in [mg kg^{-1} (dry weight)] and (**h**) in [%]. Different letters indicate statistically significant differences ($p < 0.05$).

The soil magnesium content was significantly dependent on both the experimental site and the year, with the site effect clearly stronger. No significant interaction between the experimental site and year was observed, indicating consistent differences between sites across years (Figure 4f). The C/N ratio was significantly influenced by the experimental site, the year, and their interaction ($p < 0.05$). The highest C/N values were observed at the control (SL), and the lowest at the highest treatment of horn shavings applied (CHS100), with intermediate values at sites CHS50 and CHS75. Differences between experimental treatments became more pronounced in later years of fertilization application (Figure 4g).

Soil moisture differed significantly among treatments ($p < 0.05$), whereas neither the year nor the interaction between treatment and year had a significant effect. Post hoc comparisons showed an increase in the soil moisture from the control to the highest treatment of CHS (Figure 4h). Both horn shavings and other organic fertilizers applied to the soil may increase water-holding capacity and improve structure [11]. Xin et al. [69] demonstrated that adding organic matter to soil improves soil water absorption capacity. In turn, Schomburg et al. [70] reported that earthworms can improve soil structural stability by incorporating organic matter into soil aggregates, thereby potentially enhancing water storage. The present results show significantly better earthworm community development in treatments where horn shavings were applied.

4. Conclusions

- Cattle horn shavings (CHS) treatments applied to a clay loam soil did not affect the species richness of the earthworm community but increased application (as tested) had a positive effect on mean earthworm abundance and biomass.
- Earthworm biodiversity (Shannon–Wiener H' index) increased with increased level of CHS application.
- Soil moisture and temperature had the greatest influence on earthworm traits (after accounting for synergistic effects).
- Content of OC, TN, P, K, and Ca in the soil was significantly influenced by treatment, year, and their interaction.
- As use of horn shavings enhanced earthworm communities, further multifaceted studies are warranted to assess the impact of this fertilizer on additional soil parameters.

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