Research Article

Pedro R. Soares*, Rosa Guilherme, Antónia Conceição, Cristina Galhano

Soil macrofauna under laying hens' grazed fields in two different agroecosystems in Portugal

https://doi.org/10.1515/opag-2022-0172
received May 18, 2022; accepted January 15, 2023

Abstract: Although chickens can improve the chemical properties of soil through the deposition of excreta, their effects on soil macrofauna are poorly known. This work assessed the effects of grazing indigenous laying hens on soil macrofauna of two agroecosystems in Portugal: an organic horticultural field and a conventional orchard. At the horticultural field, laying hens were used to control weeds and the results were compared with those of two other weed control treatments: mechanical (rototiller) and thermal (flame weeding). At the orchard, the effects of hens were compared to that of the orchard understory vegetation, as a control. Soil epigeic macrofauna was collected in both locations, and earthworms were only collected in the horticultural field. Relative to the other treatments, grazing in the horticultural field increased the density of earthworms in the medium term (ranging from 150 to 625 earthworms/m^2), without harming the density and diversity of epigeic macrofauna. However, at the orchard, the grazed soils presented lower soil epigeic macrofauna diversity, as well as significantly lower density of spiders than the control (4.67 vs 8.67 individuals/sample, respectively). These results suggest that the grazing effects can be affected by several factors, including the type of agroecosystem and farm management. Further research is required to optimize grazing management in different farming systems, considering animal density and grazing duration, thus ensuring the best contributions of chickens to soil fertility.

Keywords: earthworms, insects, laying hens, soil biology, spiders, weed control

1 Introduction

Chickens are multipurpose animals that can provide additional sources of income through the production of meat and/or eggs, while providing natural services such as pest and weed control and soil fertilization [1]. The general effects of chickens on the chemical properties of soil, due to the deposition of excreta, have been reported by several authors [2–5]. However, little is understood regarding their influence on soil ecological dynamics and their effects on the biological properties of soil, such as soil macrofauna. It has been reported that in free-range systems, indigenous chickens can take advantage of several insects, worms, and larvae, due to their scavenging behavior. According to previous studies, this intake may vary between 0.99 and 12% of their total intake [6,7]. However, these results were obtained through the crop content analysis, disregarding other effects of grazing on soil organisms, such as trampling and the deposition of droppings. Clark and Gage [8] evaluated the effects of free-range chickens on the abundance of beneficial soil macroinvertebrates in a nonchemical apple orchard. According to the authors, the presence of chicken resulted in a reduction in spider (Araneae) and harvestmen (Opiliones) activity based on pitfall trap catches, but no reduction in ground beetle (Carabidae) or rove beetle (Staphylinidae) activity. Earthworms, sampled through hand-sorting, were also unaffected.

Chickens can probably contribute to improvements in soil macrofauna through the deposition of excreta,

* **Corresponding author: Pedro R. Soares,** Polytechnic Institute of Coimbra, Coimbra Agriculture School, Bencanta, 3045-601 Coimbra, Portugal; Research Centre for Natural Resources, Environment and Society (CERNAS), Polytechnic Institute of Coimbra, Bencanta, 3045-601 Coimbra, Portugal, e-mail: pedro.soares@esac.pt
Rosa Guilherme: Research Centre for Natural Resources, Environment and Society (CERNAS), Polytechnic Institute of Coimbra, Bencanta, 3045-601 Coimbra, Portugal; Direção Regional de Agricultura e Pescas do Centro, Av. Fernão Magalhães, 465, 3000-177 Coimbra, Portugal
Antónia Conceição: Polytechnic Institute of Coimbra, Coimbra Agriculture School, Bencanta, 3045-601 Coimbra, Portugal; Research Centre for Natural Resources, Environment and Society (CERNAS), Polytechnic Institute of Coimbra, Bencanta, 3045-601 Coimbra, Portugal
Cristina Galhano: Polytechnic Institute of Coimbra, Coimbra Agriculture School, Bencanta, 3045-601 Coimbra, Portugal; Centre for Functional Ecology – Science for People & the Planet (CFE), TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra (UC), 3000-456 Coimbra, Portugal

stimulating the activity of organisms. But they can also have negative effects, through ingestion and trampling. Considering the multiplicity and infinity of scenarios, which include breeds, regions, altitudes, and season variations, obtaining more knowledge in this matter may enlighten farmers to better manage their grazing chickens. Finding a balance in which the animals feed on the pasture resources, without eradicating it, and understanding the effects of these animals on the aforementioned subject are particularly important since macrofauna plays a key role in soil fertility, which is sometimes overlooked, especially in the agricultural sphere [9,10]. Soil macrofauna significantly contributes to regulating the important physical and chemical properties of soil, as well as influencing the activities of soil microorganisms, such as fungi and bacteria [11–15]. However, despite the relevant role these organisms play in soil fertility, and the perception that integrating chickens with crops can significantly contribute to farm sustainability, to the best of our knowledge, the effects of grazing chickens on soil macrofauna are still largely unknown, taking into account the limited amount of literature that is currently available. Thus, the main objective of this study was to evaluate the effects of grazing Portuguese indigenous laying hens on soil macrofauna abundance and diversity in two agroecosystems: an organic horticultural field and a conventional orchard.

2 Materials and methods

2.1 Study region

The research took place in the central region of Portugal, which has a Mediterranean climate temperate by the Atlantic influence. The historical annual total precipitation is 905.1 mm, with most rain falling in autumn and spring, and the historical annual average temperature is 15.5°C [5]. The assessments were made in two different locations, 0.5 km apart, both belonging to Coimbra Agriculture School (Figure 1). The soils of both locations are Eutric Fluvisols, according to the World Reference Base for Soil Resources [16], with sandy loam texture (sand: 73%; clay: 7%; and silt: 20%).

2.2 Description of the conducted experiments

2.2.1 Horticultural field

This experiment was carried out in a certified organic farming field focused on horticultural production. The latitude, longitude, and altitude are, respectively, 40°13′ N, 8°26′ W, and 12 m above the sea level. The soil characteristics were measured before the beginning of the experiment and were as follows: organic matter 2.0%, pH (H_2O) 6.7, total N 1.2 g/kg, NH_4^+–N 21.4 mg/kg, NO_3^-–N 9.8 mg/kg, and plant available nutrients P_2O_5 50.0 mg/kg and K_2O 171.8 mg/kg.

In this location, we compared the effects of endangered Portuguese indigenous laying hens (breeds *Preta Lusitânica* and *Amarela*) on soil epigeic macrofauna and abundance of earthworms with two other weed control treatments allowed in organic farming and usually used in the research field: mechanical (rototiller) and thermal (flame weeding). We hypothesized that the use of laying hens could be a viable alternative to other weed control treatments, causing less impact on soil epigeic macrofauna and/or earthworms (data regarding the effects on weeds are not published here). The treatments were applied between rows of horticultural crops. Four between rows of crops were studied per treatment ($n = 4$), 0.75 m wide and 30 m long (a total area of 22.5 m^2). Each row was occupied by three organic certified crops, planted at random in each one-third of the row, namely, sweet pepper (*Capsicum annuum* L., var. Entinas), kale (*Brassica oleracea* L., var. Winterbor), and red onion (*Allium cepa* L., var. Red Bull). Before plantation, the soil was tilled with a hammer shredder, a disk harrow, a spading machine, and a power harrow, by this order. Crops were planted on May 6, 2020, and thereafter irrigated by a drip system. The most abundant weeds found during the experiment period are as follows: *Cyperus rotundus* L., *Digitaria sanguinalis* L. (Scop.), *Amaranthus* spp. L., *Portulaca oleracea* L., *Raphanus raphanistrum* L., *Panicum repens* L., and *Oxalis pes-caprae* L.

The animals grazed for 84 days (entrance on June 3, 2020; and exit on August 26, 2020), in fixed tunnels, 30 m along the between-vegetable crops rows. The animal management was made according to the European regulations for organic production, specifically Regulation (EU) 2018/848 and Commission Regulation (EC) No 889/2008. Each tunnel was occupied by five laying hens, respecting the outdoor minimum density of 4 m^2 per hen. The tunnels were provided with two shelter structures, one at each end. Each shelter structure supplied a nest, 0.8 m of perch, and *ad libitum* water. Furthermore, each hen was fed with 80 g day^{-1} of certified organic compound feed.

The mechanical and thermal treatments were intermittent and intervened six and nine times, respectively, between May and August 2020, according to the growth of weeds. The last interventions of these treatments were approximately coincident with the end of the grazing chickens (difference of *ca.* 50 h) to allow comparisons among treatments in time.

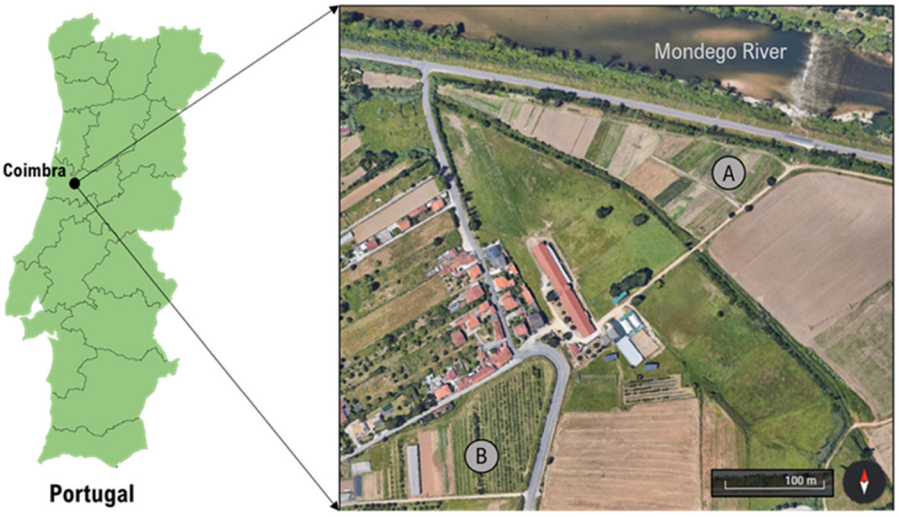

Figure 1: Geographic location of the study site (A: horticultural field and B: orchard).

Ethical approval: The research related to animal use has complied with all the relevant national regulations and institutional policies for the care and use of animals. The experiment was approved by the Animal Welfare Board (ORBEA) of Coimbra Agriculture School, in agreement with Directive 2010/63/EU and with the Portuguese Decree Law No. 113/2013 of August 7, 2013, on the protection of animals used for scientific purposes.

2.2.2 Orchard

This experiment was carried out in a conventional farming plot focused on fruit production. The latitude, longitude, and altitude are 40°12′ N, 8°27′ W, and 20 m above the sea level, respectively. The soil characteristics were measured before the experiment and are as follows: organic matter 2.6%, pH (H_2O) 6.7, total N 1.4 g/kg, NH_4^+–N 64.7 mg/kg, NO_3^-–N 1.8 mg/kg, and plant available nutrients P_2O_5 254.17 mg/kg and K_2O 570.00 mg/kg.

In this location, we compared the effects of indigenous laying hens (breed *Preta Lusitânica*) on soil epigeic macrofauna with the orchard understory vegetation without treatments, as a control, to evaluate the positive and/or negative effects of hens on the natural density of the existing fauna. The experiment was installed on a row of persimmons (*Diospyros kaki* L., var. Fuyu), 35 m long. The understory of the orchard was composed of a mixture of several herbaceous species, such as *Bromus rigidus* Roth, *Poa annua* L., *Lolium rigidum* Gaud., *Avena* spp. L., *Vicia sativa* L., and *Medicago nigra* L. We randomly chose three fruit trees per treatment (n = 3), which were wire-fenced to obtain plots of 3.5 m long and 2 m wide (total area of 7 m^2). Each plot of the chicken treatment was occupied by one shelter structure and two laying hens, with an animal outdoor density of 3.5 m^2 per chicken. Each shelter structure provided a nest, 0.8 m of perch, *ad libitum* water, and certified organic compound feed.

The chicken treatment was intermittent, with two grazings, in the same fixed plots. The first grazing lasted 34 days (entrance on November 11, 2020, and exit on December 15, 2020), and the second grazing lasted 33 days (entrance on February 4, 2021, and exit on March 9, 2021). On November 20, 2020, a laying hen went missing and was replaced on November 24, 2020, by another hen of the same breed. During the experiment period, no fertilizers or plant protection products were applied in the field.

2.3 Data collection

2.3.1 Horticultural field

Soil epigeic macrofauna was sampled with pitfall traps (7 cm in diameter, with 145 mL of antifreeze solution) before and after grazing in 5 points per treatment (n = 5), randomly chosen, within rows of crops. Each sampling event took place for 7 days. The biological material was then sorted and identified to the morphospecies level.

Earthworms were sampled on November 4, 2020, in 5 points per treatment (n = 5), randomly chosen, in between rows of horticultural crops. Soil moisture and soil temperature were assessed before sampling, to confirm the existence of good living conditions for earthworms and, thus,

ensure a successful collection. Soil moisture was measured at five points per treatment (n = 5), following the gravimetric method [17], and soil temperature was measured on-site at three points per sample (n = 3). Earthworms were hand-sorted and extracted according to Lavelle et al. [18], with adaptations. One soil block per point, with a volume of 20 × 20 × 30 cm^3, was dug out and hand-sorted. Then, 4.44 L of 0.33% mustard flour suspension was applied into the dug-out hole [19,20]. Collected earthworms were weighed and counted within 8 h after the extraction to avoid weight loss. Individuals were classified into juveniles and adults. Adults were then classified into their ecological distribution in soil, as defined by Bouché [21].

2.3.2 Orchard

Soil epigeic macrofauna was sampled with pitfall traps (7 cm in diameter, with 145 mL of antifreeze solution) after the last grazing, in 6 points per treatment (n = 6), randomly chosen. The sampling event took place for 7 days. The biological material was then sorted and identified to the morphospecies level.

2.4 Data analysis

Results are presented as mean values (±standard deviation). In the horticultural field, data were compared by the Kruskal–Wallis test combined with Dunn's multiple comparisons test. At the orchard, comparisons were made using the Mann–Whitney t-test for discrete variables (individuals and morphospecies per sample) and an unpaired t-test for continuous variables (exponent of Shannon index). The normality of continuous variables was tested (Shapiro–Wilk test) and data with non-normal distribution were transformed to meet assumptions of normality, through the logarithmic transformation. Statistical analysis was performed using GraphPad Prism software version 8.0.2 (GraphPad Software, Inc. San Diego, USA), with the alpha level set at 0.05.

Morphospecies diversity was quantified using Hill numbers, differing from each other in the parameter q, which controls the weight of the relative abundance of species and includes advantageous diversity indexes, such as species richness (q = 0), the exponent of Shannon entropy (q = 1), the inverse of the Simpson concentration index (q = 2), and the inverse of Berger–Parker index (q = 3). Additional consecutive Hill numbers (q = 4 and q = 5) were calculated and plotted as a continuous function of the q parameter.

3 Results

3.1 Horticultural field

3.1.1 Soil epigeic macrofauna

A total of 1,767 individuals of soil epigeic macrofauna were caught and identified into 88 different morphospecies. The dominant groups belong to four Arthropod orders, namely, spiders (Arachnidae: Araneae) and insects (Hexapoda: Coleoptera, Hymenoptera, and Diptera), representing 48.16, 20.09, 18.96, and 8.15% of the total population, respectively (Table 1). The remaining individuals, making up the other 4.64% of the population, belong to five Arthropod orders, namely, insects (Uniramia: Hemiptera, Lepidoptera, and Orthoptera), myriapods (Myriapoda: Scolopendromorpha), and crustaceans (Crustacea: Isopoda), and one Annelida order, namely, worms (Annelida: Haplotaxida).

After the intervention of treatments, no significant differences were found between treatments regarding the number of individuals per sample, morphospecies richness, and the exponent of the Shannon index in any of the dominant orders, nor the set of all groups (p > 0.05; Table 1). Moreover, considering the whole diversity profiles (Hill numbers; Figure 2), the chicken treatment and the mechanical treatment have virtually identical profiles, suggesting identical diversities between these two treatments. In contrast, the thermal treatment presented values slightly lower than the other treatments in the first Hill numbers (q = 0, q = 1, and q = 2), with matching profiles thereafter, suggesting a slightly lower diversity than the other studied treatments. Figure 3 presents the diversity profiles before and after the intervention of each treatment, showing a general increase in diversity in all treatments over time, despite the performance of the treatments.

3.1.2 Earthworms

During the sampling event, soil moisture contents in the chicken, mechanical, and thermal treatments were 19.8, 18.5, and 16.3%, and the soil temperatures were 15.4, 15.0, and 15.1°C, respectively.

In general, almost all the collected earthworms were juveniles (98%). Very few adult individuals were found, and none of them belong to the ecological category of epigeic earthworms. In the chicken treatment, five adults/m^2 of endogeic species were found (biomass of 4.9 g/m^2). In the mechanical treatment, five adults/m^2 of anecic species were found (biomass of 3.8 g/m^2). No adults were found in the thermal treatment.

Table 1: Density of soil epigeic macrofauna and diversity (exponent of Shannon index) of the dominant groups and all groups at the horticultural field (mean ± standard deviation)

	Period regarding grazing	Treatments			*p*-value		
		Chicken (C)	Mechanical (M)	Thermal (T)	C vs M	C vs T	M vs T
Araneae							
Individuals/sample	Before	4.80 ± 2.39[a]	23.40 ± 41.36[a]	39.00 ± 24.57[a]	>0.9999	0.0532	0.1822
	After	38.20 ± 19.61[a]	33.00 ± 7.97[a]	31.80 ± 26.28[a]	>0.9999	>0.9999	0.9627
Morphospecies/sample	Before	4.60 ± 2.07[a]	3.60 ± 2.41[a]	4.80 ± 0.84[a]	>0.9999	>0.9999	>0.9999
	After	6.80 ± 1.64[a]	8.20 ± 2.05[a]	5.60 ± 1.14[a]	0.7689	>0.9999	0.1209
Exponent of Shannon Ind.	Before	4.55 ± 2.00[a]	2.38 ± 1.09[a]	2.81 ± 1.07[a]	0.1965	0.7711	>0.9999
	After	3.72 ± 1.03[a]	5.61 ± 1.91[a]	3.81 ± 0.45[a]	0.1980	>0.9999	0.3594
Coleoptera							
Individuals/sample	Before	11.80 ± 7.12[a]	11.20 ± 4.82[a]	10.00 ± 3.00[a]	>0.9999	>0.9999	>0.9999
	After	12.80 ± 5.02[a]	12.60 ± 7.99[a]	15.75 ± 7.80[a]	>0.9999	>0.9999	>0.9999
Morphospecies/sample	Before	2.80 ± 0.84[a]	3.20 ± 1.64[a]	2.80 ± 0.84[a]	>0.9999	>0.9999	>0.9999
	After	2.80 ± 0.84[a]	2.40 ± 1.14[a]	2.50 ± 0.58[a]	>0.9999	0.9429	>0.9999
Exponent of Shannon Ind.	Before	2.14 ± 0.49[a]	1.98 ± 0.71[a]	2.05 ± 0.82[a]	>0.9999	>0.9999	>0.9999
	After	1.95 ± 0.32[a]	1.81 ± 0.85[a]	1.51 ± 0.33[a]	0.8665	0.1017	0.8665
Hymenoptera							
Individuals/sample	Before	8.80 ± 5.45[a]	4.25 ± 2.99[a]	9.80 ± 6.87[a]	0.3554	>0.9999	0.1530
	After	12.00 ± 3.94[a]	13.80 ± 3.42[a]	19.20 ± 6.06[a]	>0.9999	0.1808	0.4686
Morphospecies/sample	Before	2.60 ± 0.55[a]	2.25 ± 1.50[a]	2.20 ± 0.45[a]	0.6991	>0.9999	>0.9999
	After	3.80 ± 1.10[a]	3.60 ± 1.14[a]	3.00 ± 1.00[a]	>0.9999	0.6397	>0.9999
Exponent of Shannon Ind.	Before	2.23 ± 0.41[a]	2.19 ± 1.40[a]	1.94 ± 0.63[a]	0.9627	>0.9999	>0.9999
	After	3.16 ± 1.14[a]	2.81 ± 0.84[a]	2.36 ± 0.64[a]	>0.9999	0.5015	>0.9999
Diptera							
Individuals/sample	Before	4.25 ± 2.75[a]	1.33 ± 0.58[a]	1.33 ± 0.58[a]	0.4562	0.4562	>0.9999
	After	8.60 ± 4.72[a]	6.60 ± 2.88[a]	10.75 ± 8.14[a]	>0.9999	>0.9999	>0.9999
Morphospecies/sample	Before	3.25 ± 1.71[a]	1.33 ± 0.58[a]	1.33 ± 0.58[a]	0.4562	0.4562	>0.9999
	After	4.60 ± 1.52[a]	3.00 ± 0.71[a]	4.25 ± 2.50[a]	0.3136	>0.9999	>0.9999
Exponent of Shannon Ind.	Before	3.09 ± 1.57[a]	1.33 ± 0.58[a]	1.33 ± 0.58[a]	0.4562	0.4562	>0.9999
	After	4.06 ± 1.28[a]	2.70 ± 0.94[a]	3.28 ± 2.11[a]	0.5015	0.5015	>0.9999
All groups							
Total individuals	Before	167	197	309	—	—	—
	After	374	343	377	—	—	—
Individuals/sample	Before	33.40 ± 16.10[a]	39.40 ± 45.95[a]	61.80 ± 25.32[a]	>0.9999	0.5373	0.1980
	After	74.80 ± 22.24[a]	68.60 ± 10.06[a]	75.40 ± 28.64[a]	>0.9999	>0.9999	>0.9999
Total morphospecies	Before	43	31	29	—	—	—
	After	45	39	41	—	—	—
Morphospecies/sample	Before	14.60 ± 3.58[a]	10.00 ± 4.85[a]	12.40[a] ± 2.07	0.3465	>0.9999	>0.9999
	After	20.00 ± 4.24[a]	18.60 ± 3.13[a]	16.20 ± 3.96[a]	>0.9999	0.4339	0.8102
Exponent of Shannon Ind.	Before	10.74 ± 2.76[a]	5.41 ± 2.38[a]	6.84 ± 2.84[a]	**0.0486***	0.3116	>0.9999
	After	11.35 ± 2.62[a]	11.44 ± 2.78[a]	9.22 ± 0.95[a]	>0.9999	0.4127	0.8665

Regarding biomass, the results in the chicken treatment varied between 84.0 and 367.9 g/m^2, with a mean value of 183.5 g/m^2. Although the ranges of variation in the mechanical treatment (between 6.8 and 135.8 g/m^2) and the thermal treatment (between 12.1 and 163.3 g/m^2) were lower than those found in the treatment with chickens, no statistical differences were found between treatments concerning this parameter ($p > 0.05$; Figure 4).

However, the density of earthworms was significantly higher in the treatment with chickens ($p < 0.05$; Figure 4), compared to the other treatments. The population of earthworms in this treatment ranged from 150 to 625 earthworms/m^2, with a mean value of 320 earthworms/m^2, while in both other treatments, it did not exceed 175 earthworms/m^2 with a minimum density of 25 earthworms/m^2.

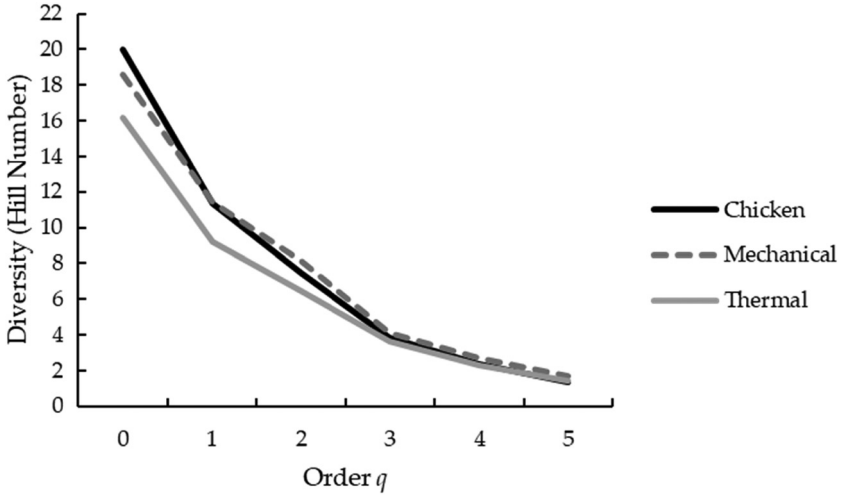

Figure 2: Diversity profiles (Hill numbers) of the different treatments after the intervention of treatments at the horticultural field.

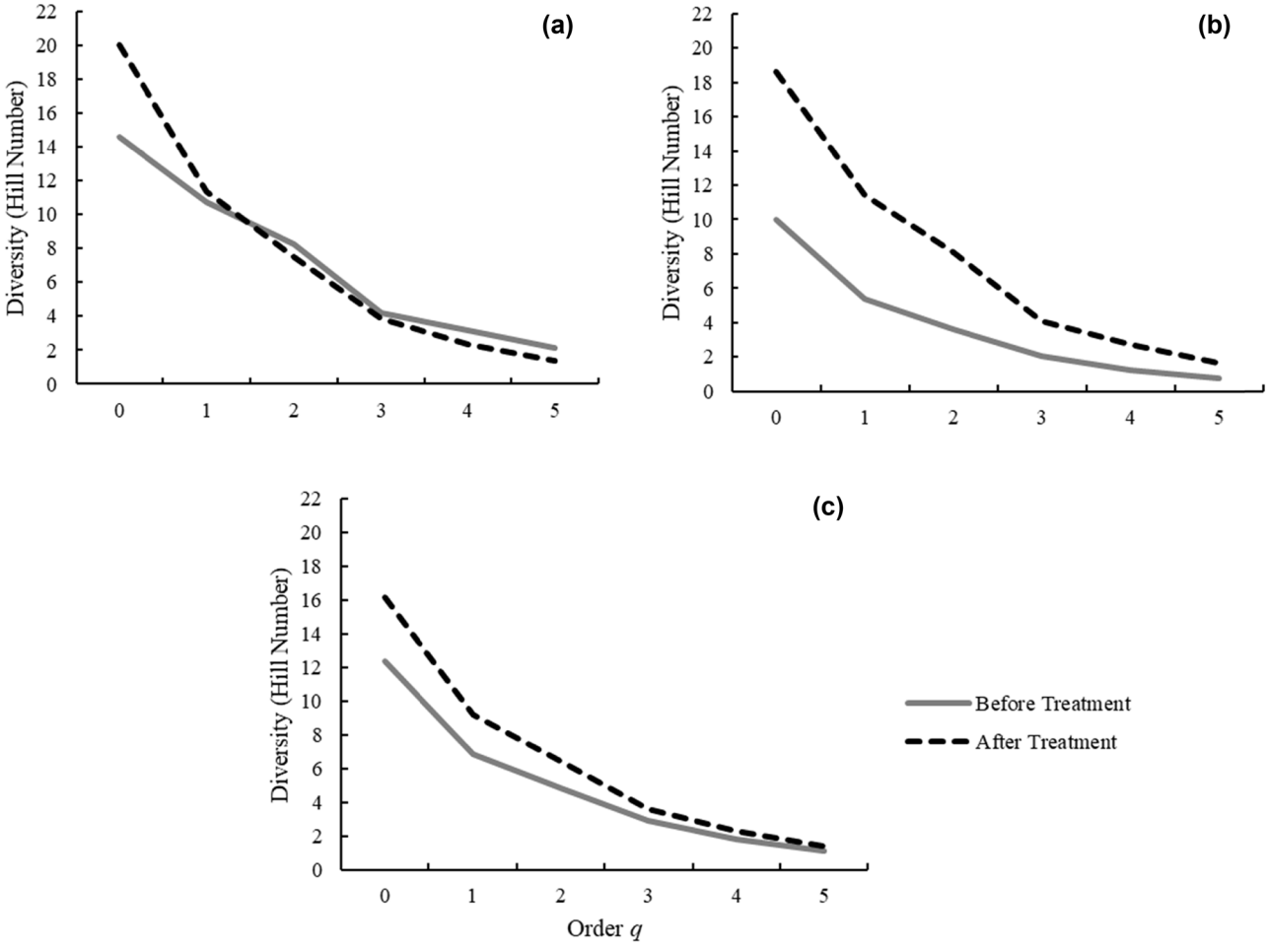

Figure 3: Diversity profile (Hill numbers), at the horticultural field, in the two studied periods, for the different treatments: (a) chicken treatment, (b) mechanical treatment, and (c) thermal treatment.

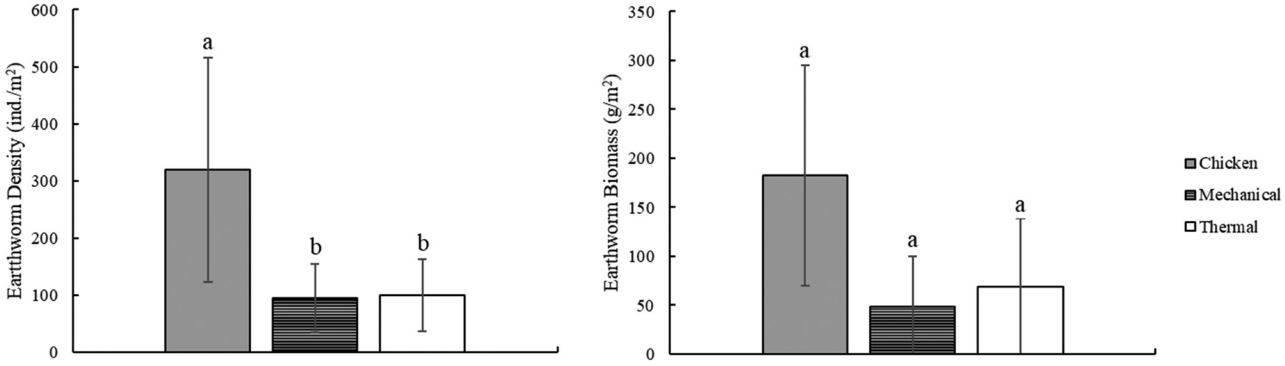

Figure 4: Earthworms' density (individuals/m^2) and biomass (g/m^2), for each treatment, at the horticultural field (mean ± standard deviation). Different small letters mean statistical differences between treatments (Kruskal–Wallis test combined with Dunn's multiple comparisons test; p-value < 0.05).

3.2 Orchard

3.2.1 Soil epigeic macrofauna

A total of 275 individuals of soil epigeic macrofauna were caught and identified into 50 different morphospecies. The dominant groups belong to five Arthropod orders, namely, spiders (Arachnidae: Araneae), insects (Hexapoda: Hymenoptera, Coleoptera), crustaceans (Crustacea: Isopoda), and millipedes (Diplopoda: Julida), representing 29.09, 32.73, 9.09, 10.91 and 9.09% of the total population, respectively (Table 2). The remaining individuals, making up the other 9.09% of the population, belong to five Arthropod orders, namely, insects (Hexapoda: Diptera; Uniramia: Hemiptera and Orthoptera), millipedes (Diplopoda: Polydesmida), myriapods (Myriapoda: Scolopendromorpha), and one Mollusca order, namely, terrestrial mollusks (Gastropoda: Pulmonata).

After grazing, no significant differences were observed between the chicken treatment and the control regarding the number of individuals per sample, the morphospecies richness, and the exponent of the Shannon index in the dominant orders Hymenoptera, Isopoda, Coleoptera, and Julida ($p > 0.05$; Table 2). However, the results indicate that the chicken treatment presented significantly fewer spiders per sample than the control ($p = 0.0325$), suggesting a negative effect of hens regarding these individuals, at the studied animal density (3.5 m^2 per hen). Furthermore, also regarding this group, the exponent of the Shannon index was 4.39 (mean value) in the control and approximately half in the chicken treatment (2.58). Although there are no statistically significant differences between the treatment and the control regarding this index ($p = 0.0592$), we emphasize that the found p-value is very close to the limit that takes into account significant differences, suggesting that the hens can also eventually affect the diversity of this group.

Moreover, the results from comparing the diversity profiles (Hill numbers) between the chicken treatment and the control showed substantially less diversity in the chicken treatment (Figure 5), suggesting that the hens can also negatively affect the general epigeic macrofauna diversity of the system. Although grazing was intermittent, the use of fixed structures, the grazing duration, and the applied density (3.5 m^2 per hen) may have contributed to a more aggressive impact on the system's fauna diversity.

4 Discussion

4.1 Horticultural field

In the horticultural field, our results suggest that the thermal treatment, using flame weeding, may have a slightly negative effect on the diversity of epigeic macrofauna, when compared to the other studied treatments (chicken and mechanical), which presented identical diversity profiles. However, no significant differences were found between treatments in the various analyzed parameters, suggesting that, despite the lower diversity of the thermal treatment, in general, the effects of the studied treatments on soil macrofauna are quite identical.

Regarding the increase in both abundance and diversity in all treatments over time, it is reasonable to consider that this common increase was probably not because of the effects of treatment, but due to external factors common to all treatments. Since soil tillage significantly affects this kind of fauna [22], and since the first sampling was done before treatments and after the tillage which prepared the field for crop production, it is realistic to consider this tillage as a possible factor that contributed to the much lower number of individuals found before the intervention of treatments.

Table 2: Density of soil epigeic macrofauna and diversity (exponent of Shannon index) of the dominant groups and all groups, after grazing, at the orchard (mean ± standard deviation)

	Treatments		*p*-value
	Control	Chicken	
Hymenoptera			
Individuals/sample	8.83 ± 9.66[a]	6.17 ± 3.66[a]	0.9675
Morphospecies/sample	2.33 ± 1.21[a]	1.67 ± 1.03[a]	0.4372
Exponent of Shannon Ind.	2.07 ± 0.93[a]	1.39 ± 0.62[a]	0.1977
Araneae			
Individuals/sample	8.67 ± 3.27[a]	4.67 ± 2.42[b]	**0.0325***
Morphospecies/sample	4.67 ± 1.51[a]	2.83 ± 1.72[a]	0.1190
Exponent of Shannon Ind.	4.39 ± 1.49[a]	2.58 ± 1.46[a]	0.0592
Isopoda			
Individuals/sample	3.17 ± 2.14[a]	1.83 ± 1.17[a]	0.2965
Morphospecies/sample	1.00 ± 0.63[a]	1.00 ± 0.63[a]	>0.9999
Exponent of Shannon Ind.	0.93 ± 0.51[a]	1.00 ± 0.63[a]	0.7772
Coleoptera			
Individuals/sample	1.83 ± 1.41[a]	2.33 ± 1.26[a]	0.6667
Morphospecies/sample	1.50 ± 1.52[a]	2.00 ± 1.10[a]	0.5628
Exponent of Shannon Ind.	1.48 ± 1.51[a]	1.98 ± 1.10[a]	0.5264
Julida			
Individuals/sample	1.67 ± 2.25[a]	2.50 ± 3.33[a]	0.6688
Morphospecies/sample	0.67 ± 0.52[a]	0.83 ± 0.41[a]	>0.9999
Exponent of Shannon Ind.	0.67 ± 0.52[a]	0.83 ± 0.41[a]	>0.9999
All groups			
Total individuals	160	115	
Individuals/sample	26.67 ± 9.83[a]	19.17 ± 7.44[a]	0.2576
Total morphospecies	40	30	
Morphospecies/sample	12.33 ± 1.51[a]	9.50 ± 4.85[a]	0.4784
Exponent of Shannon Ind.	10.21 ± 2.15[a]	7.28 ± 3.43[a]	0.0962

Different small letters mean statistical differences between treatments (Mann–Whitney *t*-test for discrete variables and unpaired *t*-test for continuous variables; *p*-value <0.05).

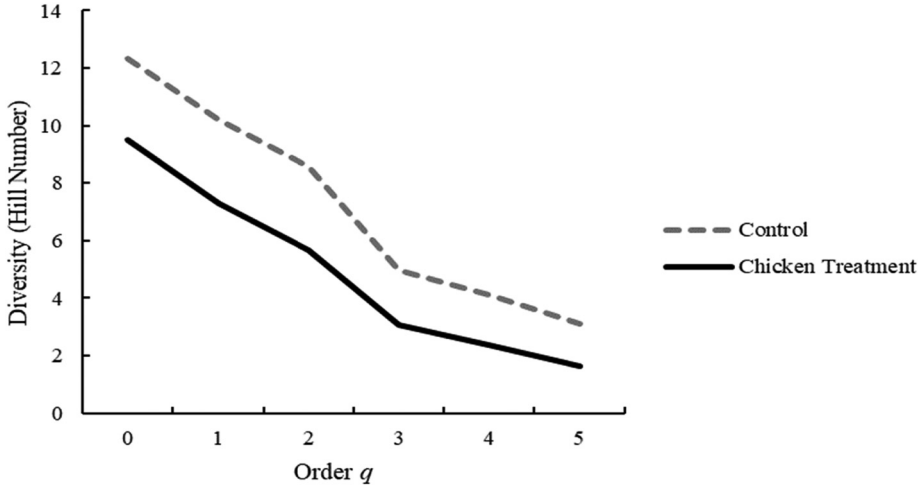

Figure 5: Diversity profiles (Hill numbers) of the chicken treatment and the control, after grazing, at the orchard.

Despite the absence of significant differences between the studied treatments regarding soil epigeic macrofauna, we found a significantly higher earthworm density in the chicken treatment, after grazing. These results disagree with the results of Clark and Gage [8] with foraging chickens but are in agreement with the results found by

other authors regarding grazed cattle and grazed sheep [23,24]. Several authors have reported increases in both earthworm density and biomass in organically fertilized fields [25,26]. Nitrogen-rich materials are highly preferred by earthworms [27], which justifies their higher abundance and higher biomass in nitrogen-rich soils, even when the nitrogen source is based on mineral fertilizers [28]. Grazing chickens at the horticultural field for 84 continuous days significantly contributed to an increase in the amounts of both nitric and ammoniacal nitrogen in the grazed soils [5]. This high and significant increase in the amounts of nitrogen in the soil, due to the deposition of dropping, may justify the higher density of earthworms found in the chicken treatment. Although chickens are likely to eat these macrofauna [29], since grazing took place during the dry season, when earthworms are less active, the intake may have been reduced. In these circumstances, the earthworms would have benefited from the accumulation of droppings, without being negatively affected by the scavenging behavior of hens. These results are particularly relevant since earthworms play a key role in soil fertility, contributing to the humification of organic materials and the formation of soil aggregates, which enhances soil stability and water infiltration [30]. However, more research is needed for a better understanding of the scavenging effects of hens on populations of earthworms, especially considering different meteorological conditions.

4.2 Orchard

Grazing chickens at the orchard significantly changed the usual soil macrofauna of this agroecosystem. In this location, the chicken treatment presented significantly fewer spiders per sample than the control treatment (orchard understory vegetation, without treatments). This finding agrees with the results found by Clark and Gage [8], with foraging chickens, reinforcing the negative effects of chickens on these soil organisms. Although the biological control of spiders strongly depends on several factors, such as phenotype, pests, alternative preys, and environmental conditions, spiders are dominant nonvertebrate predators in most terrestrial ecosystems and have high potential as pest control agents [31,32]. Accordingly, the lower number of spiders in the grazed soils requires further attention. This result can arise from the chickens' intake of these individuals since arachnids have high contents of nitrogen, minerals, and trace elements that can contribute to the nutrition of chickens [33,34]. However, this result can also arise from the habitat disturbance in the grazed plots, due to the presence of the animals. The grazing chickens led to the eradication of vegetation (bare soil) and superficial soil disturbance, due to the scavenging behavior, which may have led to the loss and/or escape of the spiders from the grazed soils.

Furthermore, our results suggest that grazing chickens at the orchard contributed to a significant reduction in the natural epigeic macrofauna diversity of this agroecosystem, according to the found diversity profiles (Hill numbers). Particular attention is needed to this disturbance, since it can compromise the system's resilience, decreasing its capacity to resist external disturbances. This loss of diversity reinforces the need to practice good grazing management, applying pasture rest periods and/or lower animal outdoor densities. We emphasize that more research into the effects of chickens on soil biology is needed to find a grazing balance that benefits animal production without harming soil biological properties.

4.3 Research limitations

The findings reported herein should be considered in light of some limitations. This work is limited by the use of endangered native breeds in both experiments. The use of such breeds in scientific studies contributes to the conservation of their genetic heritage, in addition to proving their genetic value in systems with outdoor access, such as organic systems. However, the use of these endangered breeds in both experiments and the small number of animals available made it difficult to carry out larger and several experiments, in different locations during the same season. Furthermore, our research could have been improved by identifying organisms at the species level, rather than identifying morphospecies; by increasing the number of samples; and by evaluating earthworms at the orchard, which was not assessed. This work is part of a broad project, which aims to evaluate the integration of laying hens with crops considering several scientific areas, such as soil fertility, pest and weed control, and crop and animal productivities. In this context, it became difficult to collect and analyze more biological samples than those previously mentioned, due to logistical difficulties, such as a high volume of samples from other scientific areas, and short available time and human resources. Moreover, due to the high volume of samples not related to soil macrofauna and the size of each plot (which was conditioned by the low number of available animals), it was particularly difficult to obtain volumes of undisturbed soils for earthworm collection at the orchard, which was not assessed in this location.

5 Conclusions

Soil macrofauna plays a key role in soil fertility. This study demonstrates that chickens can contribute positively or negatively to soil epigeic macrofauna, depending on the agroecosystem. In the horticultural field, where we used chickens as weed control agents and compared their effects to two other weed control treatments (thermal and mechanical), we found that the effects of chickens on soil epigeic macrofauna are quite identical to the other studied treatments. On the other hand, the chicken treatment contributed positively to the density of earthworms in the medium term after grazing, probably due to the accumulation of droppings. At the orchard, the chicken treatment contributed negatively to the spiders' natural density of that agroecosystem, and it also contributed to a decrease in the general soil epigeic macrofauna diversity, which requires special attention.

Considering the lack of previous research studies on this topic and the importance of soil biology to sustainable soil management, further research is recommended regarding the effects of grazing chickens on soil macrofauna. Future research should address the ways to optimize the management of these animals in the pasture. It is important to identify a combination of animal density and a grazing duration that allows reaching a balance between the chickens' use of the pasture and the damage caused to soil macrofauna, although this is highly variable and depends on other factors, such as weather conditions and breeds. Chickens can be valuable elements in integrated crop–livestock systems, but more research is recommended regarding their impacts to enhance the sustainability of such agroecosystems.

Acknowledgments: The authors are deeply thankful to Rui Amaro, Daniela Santos, Alexandra Oliveira, Rosinda Leonor Pato, Isabel Costa, Fernando Amaral, Jorge Bandeira, Filipe Melo, Luís Miguel Valério, and Rui Ferreira for their knowledge, and field and laboratory support; to the Azorean Biodiversity Group Members, Paulo Borges, Rosalina Gabriel, and Alejandra Roz Prieto for all the suggestions; to Lúcio Paiva for his field contributions; to Clarinda Paixão from Américo Duarte Paixão Lda for kindly providing the necessary amount of mustard to the extraction of earthworms; to Ana Bela Lopes and Noémia Bárbara for their diligent proofreading and English editing; and to the anonymous reviewers, whose suggestions improved this manuscript.

Funding information: This work received financial support from the Polytechnic Institute of Coimbra within the scope of Regulamento de Apoio à Publicação Científica dos Professores e Investigadores do IPC (Despacho no. 12598/2020). The work was also partially supported by Grant IIA_PDR2020_GMÓVEL_LIC1 and Project Grant PDR2020-101-031353, through the European Regional Development Fund, the PDR 2020 Programs, and FCT Portuguese Foundation for Science and Technology.

Author contributions: Conceptualization, P.R.S., R.G., and A.C.; methodology, P.R.S., R.G., and A.C.; formal analysis, P.R.S.; investigation, P.R.S. and C.G.; resources, P.R.S., R.G., and A.C; writing – original draft preparation, P.R.S.; writing – review and editing, P.R.S., R.G., A.C., and C.G.; supervision, C.G. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: All data generated or analyzed during this study are included in this published article.

References

[1] Soares PR, Lopes MAR, Conceição MA, Santos DVS, Oliveira MA. Sustainable integration of laying hens with crops in organic farming. A review. Agroecol Sustain Food Syst. 2022;46(7):1–33. doi: 10.1080/21683565.2022.2073509.

[2] Yu C, Jiang X, Du H, Li Q, Zhang Z, Qiu M, et al. Effects of free-range broiler production on vegetation characteristic and soil physicochemical property of the stocking areas in China. Agrofor Syst. 2020;94(3):675–85. doi: 10.1007/s10457-019-00438-9.

[3] Kratz S, Rogasik J, Schnug E. Changes in soil nitrogen and phosphorus under different broiler production systems. J Env Qual. 2004;33:1662–74. doi: 10.2134/jeq2004.1662.

[4] Hilimire K, Gliessman SR, Muramoto J. Soil fertility and crop growth under poultry/crop integration. Renew Agriculture Food Syst. 2012;28(2):173–82. doi: 10.1017/S174217051200021X.

[5] Soares PR, Pato RL, Dias S, Santos D. Effects of grazing indigenous laying hens on soil properties: Benefits and challenges to achieving soil fertility. Sustainability. 2022;14(6):3407. doi: 10.3390/SU14063407.

[6] Admasu S, Solomon D, Meseret M. Poultry feed resources and chemical composition of crop content of scavenging indigenous chicken. Online J Anim Feed Res. 2019;9(6):247–55.

[7] Mohammed KAF, Sarmiento-Franco L, Santos-Ricalde R, Solorio-Sanchez JF. Egg production, egg quality and crop content of Rhode Island Red hens grazing on natural tropical vegetation. Trop Anim Health Prod. 2012;45(1):367–72. doi: 10.1007/s11250-012-0225-y.

[8] Clark MS, Gage SH. The effects of free-range domestic birds on the abundance of epigeic predators and earthworms. Appl Soil Ecol. 1997;5(3):255–60.
[9] Bottinelli N, Jouquet P, Capowiez Y, Podwojewski P, Grimaldi M, Peng X. Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? Soil Tillage Res. 2015;146(PA):118–24.
[10] Velasquez E, Lavelle P. Soil macrofauna as an indicator for evaluating soil based ecosystem services in agricultural landscapes. Acta Oecol. 2019;100:103446. https://doi.org/10.1016/j.actao.2019.103446.
[11] Anderson JM. Spatiotemporal effects of invertebrates on soil processes. Biol Fertil Soils. 1988;6:216–27.
[12] Chapuis-Lardy L, le Bayon R-C, Brossard M, López-Hernández D, Blanchart E. Role of soil macrofauna in phosphorus cycling. In Bünemann E, Oberson A, Frossard E, editors. Phosphorus in action. Berlin, Heidelberg: Springer; 2011. p. 199–213. doi: 10.1007/978-3-642-15271-9_8.
[13] Sofo A, Mininni AN, Ricciuti P. Soil macrofauna: A key factor for increasing soil fertility and promoting sustainable soil use in fruit orchard agrosystems. Agronomy. 2020;10(4):456.
[14] Lal R. Effects of macrofauna on soil properties in tropical ecosystems. Agric Ecosyst Env. 1988;24(1–3):101–16. doi: 10.1016/0167-8809(88)90059-X.
[15] Frouz J, Elhottová D, Kuráž V, Šourková M. Effects of soil macrofauna on other soil biota and soil formation in reclaimed and unreclaimed post mining sites: Results of a field microcosm experiment. Appl Soil Ecol. 2006;33(3):308–20. doi: 10.1016/J.APSOIL.2005.11.001.
[16] Anjos L, Gaistardo CC, Deckers J, Dondeyne S, Eberhardt E, Gerasimova M, et al. World reference base for soil resources 2014 - International soil classification system for naming soils and creating legends for soil maps. In Schad P, van Huyssteen C, Micheli E, editors. Rome, Italy: Food and Agriculture Organization; 2015.
[17] ISO 11461: 2001. Soil quality — Determination of soil water content as a volume fraction using coring sleeves — Gravimetric method. International Standard Organization; 2001.
[18] Lavelle P, Maury M, Serrano V. Estudio cuantitativo de la fauna del suelo en la region de Laguna Verde, Veracruz. Epoca de lluvias. In Reyes-Castillo, editor. Estudios Ecológicos en el Trópico Mexicano. México: Instituto De Ecologia; 1981. p. 75–105.
[19] Högger CH. Mustard flour instead of formalin for the extraction of earthworms in the field. Bull Bodenkundl Ges Schweiz. 1993;17:5–8.
[20] Gunn A. The use of mustard to estimate earthworm populations. Pedobiologia (Jena). 1992;36(2):65–7.
[21] Bouché MB. Strategies lombriciennes. Ecol Bull. 1977;25:122–32.
[22] Robertson LN, Kettle BA, Simpson GB. The influence of tillage practices on soil macrofauna in a semi-arid agroecosystem in northeastern Australia. Agric Ecosyst Env. 1994;48(2):149–56.
[23] Curry JP, Doherty P, Purvis G, Schmidt O. Relationships between earthworm populations and management intensity in cattle-grazed pastures in Ireland. Appl Soil Ecol. 2008;39(1):58–64.
[24] Schon NL, Mackay AD, Minor MA, Yeates GW, Hedley MJ. Soil fauna in grazed New Zealand hill country pastures at two management intensities. Appl Soil Ecol. 2008;40(2):218–28.
[25] Guo L, Wu G, Li Y, Li C, Liu W, Meng J, et al. Effects of cattle manure compost combined with chemical fertilizer on topsoil organic matter, bulk density and earthworm activity in a wheat–maize rotation system in Eastern China. Soil Tillage Res. 2016;156:140–7.
[26] Leroy BLM, van den Bossche A, de Neve S, Reheul D, Moens M. The quality of exogenous organic matter: Short-term influence on earthworm abundance. Eur J Soil Biol. 2007;43(Supplement 1):S196–200.
[27] Curry JP, Schmidt O. The feeding ecology of earthworms – A review. Pedobiologia (Jena). 2007;50(6):463–77.
[28] Iordache M, Borza I. Relation between chemical indices of soil and earthworm abundance under chemical fertilization. Plant Soil Env. 2010;56(9):401–7. doi: 10.17221/234/2009-PSE.
[29] Ncobela CN, Chimonyo M. Nutritional quality and amino acid composition of diets consumed by scavenging hens and cocks across seasons. Trop Anim Health Prod. 2016;48:769–77.
[30] Orgiazzi A, Bardgett RD, Barrios E, Behan-Pelletier V, Briones MJI, Chotte J-L, et al. Global Soil Biodiversity Atlas. Luxembourg: European Commission, Publications Office of the European Union; 2016. doi: 10.2788/799182.
[31] Michalko R, Pekár S, Entling MH. An updated perspective on spiders as generalist predators in biological control. Oecologia. 2019;189:21–36. doi: 10.1007/s00442-018-4313-1.
[32] Nyffeler M, Benz G. Spiders in natural pest control: A review. J Appl Entomol. 1987;103(1–5):321–39. doi: 10.1111/J.1439-0418.1987.TB00992.X.
[33] Punzo F. Nutrient composition of some insects and arachnids. Fla Sci. 2003;66(2):84–98.
[34] Razeng E, Watson D. Nutritional composition of the preferred prey of insectivorous birds: Popularity reflects quality. J Avian Biol. 2015;46:89–96.