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Soil Invertebrate Communities as Indicator of Ecological Conservation Status of Some Fertilised Grasslands from Romania

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Abstract: Quantification of soil biological status, through investigation of edaphic communities' composition, constitutes an important factor for the assessment of the grassland ecosystems, including their protection. The structure of soil invertebrate communities was investigated for five grasslands under different chemical and organic treatments, for the first time in Romania. In order to accomplish this task, some structural parameters were quantified: numerical abundance, taxa richness, Shannon diversity index of taxa and equitability. We demonstrated the relationship between five environmental factors (vegetation coverage, soil temperature, soil acidity, soil resistance at penetration, soil moisture content) and the community structures of soil fauna. In total, 17 invertebrate groups were identified with a total numerical abundance of 14,953 individuals. Considering the numerical abundance, the dominant taxa were Acaridae, Collembola, Oribatida and Mesostigmata, the least dominant being Coleoptera, Opiliones and Araneae. In spatial dynamics the investigated plots were characterised specifically by soil invertebrates' communities' structures, highlighted by the varied values of structural parameters: by indicator taxa and by the characteristic average values of environmental parameters. Multivariate statistical analysis revealed that the most important environment parameters influencing the soil taxa were vegetation coverage (especially on Acaridae, Glycyphagidae and Formicoidea) and soil resistance at penetration (Nematoda and Coleoptera). This study constitutes a scientific argument for the usage of soil invertebrate communities as indicators of the ecological conservation status of some fertilised grasslands.

Keywords: abiotic; arthropods; biotic; environment; fertilisation; soil

1. Introduction

Soil is one of the most complex ecosystems in nature. Many drivers influence its genesis and quality, including biotic (presence of flora and fauna) and abiotic factors (chemical parameters). Some of the most important soil functions are the decomposing of organic matter and the nutrient cycling processes, which are strongly correlated with the presence of soil fauna [1–3]. In order to evaluate the soil quality, the soil invertebrates' communities have been used as valuable biological indicators [4–8]. Numerical abundance, community structure/composition, taxa diversity and relations between biotic–abiotic factors are some important parameters which can be used to evaluate the role of soil fauna as bioindicators. All these parameters are used as a measure of soil health [9]. According to van Straalen, 1998 [10] we must consider two characteristics of these biological indicators:

specificity and resolution. Specificity relates to the response of soil fauna at different values of abiotic and biotic factors, which in many cases are specific for a few taxa. Resolution is connected with the dynamics of the environmental parameters and to the capacity of the soil fauna to indicate these modifications.

The soil fauna is classified mainly after its body size and it comprises microfauna (as Protozoa, Nematoda, Rotifera), mesofauna (as Acari, Collembola, Diplura, Enchytraeidae), macrofauna (as Coleoptera, Aranea, Diplopoda, Isopoda, Chilopoda, Gastropoda, Oligocheta, Formicidae, etc.) and megafauna (Mammalia, Reptilia, Amphibia). They are more abundant in the first 20 cm of soil [2,11,12]. The relationship between the body size of soil functional groups and the abiotic factors (soil temperature, precipitation, soil moisture, pH, soil organic carbon, total soil N, total soil P, soil C:N, soil N:P, soil C:P) at the global scale was highlighted by Johnston and Sibly in 2020 [13]. They demonstrated that temperature influences soil community composition through temperature- and size-dependent metabolic demands, and soil pH and soil organic carbon reflect the availability of multiple nutrients that drive resource availability.

In many ecological studies, the soil fauna taxa have been used as biological indicators. Higher taxa are widely used as surrogates for species-level identification in invertebrates, being a cost-effective approach to monitoring the impacts of anthropogenic disturbance [14,15]. However, many studies revealed that soil invertebrate communities have a differential response to the environmental factors [1,15–20]. According to Pryke and Samways, 2012 [21], using a multi-taxon approach is scientifically approved, due to the wide range of feeding guilds and the mobility of soil invertebrates.

Soil nematodes are poikilothermic organisms and this is the reason for their sensitivity to the temperature and to soil moisture fluctuations [22]. Their biological responses to these fluctuations are: short generation time, more time for development, capacity to adapt to dry habitats. The soil pH influences the community organisation, their favourable environment having a pH between five and seven [22,23]. An input of organic manure and inorganic fertilisers modify soil features such as porosity, texture and pH, increasing the species diversity and the numerical abundance of free-living nematodes. They are also very sensitive to heavy metals pollution, from Pb, Cd, Zn, As, Cr, Cu [24].

Another soil fauna group bioindicator of chemical stress and agricultural practices is enchytraeids. Practices such as soil tillage, pesticide applications, grazing, liming or fertilisation will influence their community structures in terms of abundance, biomass, spatial distribution or dominance [7,25]. According to Briones et al., 2007 [26], a threshold of 16 °C of the maximum mean annual temperature could be a critical limit for enchytraeids distribution. Considering the earthworms, their abundance was influenced by some soil properties such as soil texture, pH value, moisture, C/N ratio, and organic matter [27]. Other studies revealed that their populations are regulated by both climate and land management, being bioaccumulators of Cd, Hg, and Zn especially [5,28,29]. In different types of ecosystems, under the influence of disturbance factors as pesticides, hydrocarburans, heavy metals or other airborne pollutants, Collembola, Acari, Araneae, Formicidae and Diplopoda fauna show spatial variation or modification in community structure [13,30–33].

Coleoptera are very sensitive to temperature and humidity, the presence of competitors, vegetation coverage and to the availability of food. There are studies demonstrating that the main management practices to which the carabids respond quickly are: fertilisation, grazing, habitat fragmentation and forest cutting [4,34].

In wider Europe, as well as in Romania, this multi-taxa approach in the soil biodiversity assessment of the experimental grasslands, was not used as much. In Europe, most research is focused on one or a few taxonomic groups, without taking into consideration the complex interrelations among edaphic communities and their relations with soil abiotic and biotic parameters, in experimental grasslands, organically and chemical fertilised, situated on high altitude, in a mountain area [4,7,22,26,34,35]. In Romania, only a small amount of research has used soil invertebrate communities as bioindicators for the conservation status of some protected areas or some anthropized ecosystems (abandoned railway tunnels, vineyards) [19,20,36,37]. None of the above presented studies took into consideration fertilised experimental grasslands and their relationship with soil invertebrate taxa. Thus, the objectives of the present paper are: (1) to investigate the structural communities' differences between soil fauna from five fertilised experimental plots and (2) to demonstrate the relationship between five environmental factors and the community structures of soil invertebrates.

Using a multi-taxa approach, rather than individual species, provides a novel examination of the study. With individual species, there is a possibility that one might obscure or confuse the ecological trends, whilst the use of functional groups emphasises the main changes and impacts in the system. The present study provides valuable information regarding the influence of grasslands management practices (fertilisation) on the soil invertebrate communities. This study can constitute an assessment instrument for the conservation status of grasslands, using soil fauna as bioindicators.

2. Materials and Methods

2.1. Study Area

The present study was carried out in five grasslands in Romania, in July 2017. The study area is located in the Bucegi Mountains (45°26′44″ N; 25°27′22″ E) [35]. The Bucegi Mountains are part of the Southern Carpathians, one group of the Carpathian Mountains. This research was carried out in five experimental grasslands (with an area of 0.75 hectar/plot), located at 1785 m average altitude. A more detailed description of the investigated grasslands is presented in the Supplementary Material (Table S1).

The type of management was described after Blaj et al., 2017 [38] and the type of soil after Stănilă and Dumitru, 2016 [39]. The control plot (CG) is a semi-natural pasture, unimproved, grazed in summer by cows; grassland A is a semi-natural pasture, fertilised with chemical fertilisers in the periods 2000–2002, 2010–2012, 2014–2016, with an average 100 kg ha^{-1} N + 50 kg ha^{-1} P_2O_5 + 50 kg ha^{-1} K_2O; grassland B is a semi-natural pasture, chemically fertilized in 1996–1998 with 150 kg ha^{-1} N + 75 kg ha^{-1} P_2O_5 + 75 kg ha^{-1} K_2O and organically fertilised in 2004, 2010 and 2016 by paddocking with dairy cows; grassland C is a semi-natural pasture, calcium limed in 1995, chemically fertilised similar to B variant and paddocked in 2003, 2009 and 2015; and grassland D is a pasture reseeded in late summer of 1995, after herbicide Roundup at 5 L ha^{-1}, calcium limed and chemically fertilised similar to C variant and paddocked in 2002, 2008 and 2014 [38].

2.2. Soil Fauna Samples

The present study was accomplished in July 2017. The soil fauna samples were collected using the MacFadyen soil core, with a length of 10 cm and a diameter of 5 cm. The samples were collected randomly. A total of 50 soil samples were taken from each grassland. In total, 250 samples from five grasslands samples were collected (50 samples/grassland). Soil fauna was extracted for 10–14 days using the Berlese–Tullgren method, with natural light and heat [40]. The samples were kept in a refrigerator until the next sorting process (14 days). Taxa identification and counting were undertaken using a Zeiss stereomicroscope and an Axioscope A1 Zeiss microscope.

The taxa identification was made using the published identification keys [3,41–44]. The classification of the soil functional groups was made, taking into account the trophic niche [3,45,46].

2.3. Environmental Variables

The biotic factor of vegetation cover (VegCovr) (%) was investigated and abiotic factors were investigated: soil penetration resistance (RPs) (Map), soil temperature (Ts) (°C), soil moisture content (Rhs) (%) and soil acidity (pH). A more detailed description of the methodology and the used equipment was provided in [35]. The measured values of these environmental parameters are present in Table 1.

Table 1. Mean (standard deviation) and range of biotic, i.e., vegetation cover (VegCovr) (%) and the abiotic factors, i.e., soil penetration resistance (RPs) (Map), soil temperature (Ts) (°C), soil moisture content (Rhs) (%) and soil acidity (pH) and the *p* values of Kruskal–Wallis test for each grassland and control group.

Factor	CG (*N* = 50)	A (*N* = 50)	B (*N* = 50)	C (*N* = 50)	D (*N* = 50)	Total (*N* = 250)	*p* Value
VegCovr	70.360 (5.992) 60.000–80.000	75.960 (8.748) 50.000–85.000	60.540 (10.514) 35.000–70.000	57.280 (8.593) 50.000–72.000	66.160 (8.665) 50.000–80.000	66.060 (10.871) 35.000–85.000	<0.001
RPs	1.352 (0.163) 1.034–1.861	1.309 (0.139) 0.965–1.723	1.394 (0.149) 1.034–1.723	1.327 (0.119) 1.034–1.585	1.340 (0.173) 1.034–1.723	1.344 (0.151) 0.965–1.861	0.06
Ts	15.842 (2.124) 12.400–19.600	16.746 (1.918) 14.300–21.700	16.868 (2.917) 13.200–26.500	16.980 (1.624) 14.000–22.000	14.656 (1.745) 14.000–22.000	16.218 (2.276) 11.800–26.500	<0.001
Rhs	65.260 (8.741) 47.900–82.400	68.976 (6.820) 52.000–83.500	63.964 (7.683) 43.400–80.500	61.490 (5.902) 49.800–76.900	63.230 (5.578) 49.800–75.000	64.584 (7.424) 43.400–83.500	<0.001
pH	4.654 (0.139) 4.480–4.990	4.980 (0.266) 4.520–5.260	4.553 (0.219) 4.220–5.180	5.053 (0.421) 4.670–6.200	4.975 (0.300) 4.420–5.450	4.843 (0.346) 4.220–6.200	<0.001

2.4. Data Analysis

The soil invertebrate assemblages were characterised in terms of composition, abundance and taxon richness. To describe the composition of the taxonomic assemblage and its relationship with the environmental variables we applied Constrained Correspondence Analyses (CCA) to the taxa abundance matrix. CCA can provide useful insights on the multivariate response of taxonomic assemblages to a matrix of environmental variables [47,48]. In order to avoid the "arch effect" in CCA and maintain normal distribution we used ln (x + 1) transformed abundance [49]. All environmental variables were scaled to mean zero and unit variance. The CCA were conducted with Vegan package version 2.5-2 (R package version 2.5-2, vegan: Community Ecology Package, Jari Oksanen, Boston, MA, USA) [50]. We used the function "ordistep" in the Vegan package to select the variables of the environmental matrix that best explain taxa matrix (i.e., constraints). For each of the constraint and contrast in factor constrains from the environmental matrix we calculated the Variance Inflation Factors (VIF) using the function "vif.cca". VIF values above 10 indicate redundant constraints. The stepwise regression model involved a selection of independent variables (VIF < 10) to be used in the CCA model. We conducted a permutation test (based on 9999 cycles) for CCA to test the significance of explanatory variables, of explained variance by the CCA axes, and of the model [50].

We used the Indicator Value method (IndVal) [51], which determines the specificity (uniqueness to a particular treatment) and fidelity (frequency of occurrence) of a taxon, to identify taxa characteristic to the four grasslands and control plot. A high IndVal (%) indicates that a taxon can be considered characteristic to particular grassland. To calculate the IndVal measures for each taxon we used the taxa abundance matrix, and to test them we used Dufrêne and Legendre's (1997) [51] random reallocation procedure of sites among site groups. A taxon is considered characteristic to a grassland type or control plot if its IndVal measure is >25% and significant at $p < 0.05$ [51].

Abundance was calculated as the total number of individuals per sample point. General linear mixed models (GLMM), with a Poisson error distribution and a log function, were used to examine how environmental variables (i.e., predictors) affect abundance and taxon richness (i.e., response variables). In all GLMMs we included sample points as a random effect. We designed a set of 26 candidate models, out of which the first five candidate models included a single predictor. If there were no collinearity problems, we also considered models with two, three and four predictors. To test for collinearity between two or more predictors we used VIF. VIF values over 3 suggest collinearity. All highly collinear variables were removed through a stepwise procedure using the "vifstep" function in the "usdm" package.

To evaluate the performance of the models we conducted model selection based on Akaike's information criterion corrected for sample size (AICc) [52,53], using the "aictab" function of the package AICcmodavg [54]. We ranked the models according to their AICc values and considered the model with the lowest AICc value as the reference for calculating the AICc difference (Δi). Then we calculated the Akaike weights (w_i), which give the relative evidence of each model and are interpreted as the likelihood of a model given the data and the model. Models in which the difference in AICc relative to AICcmin is < 2 were considered competitive and more plausible than others [52]. We chose the most parsimonious model for obtaining parameter estimates and predictions of abundance and taxon richness. To plot the GLMM results we used the "effects" package. Tukey tests were performed for multiple pairwise comparisons. All the statistical procedures were implemented in R 4.0.2. [55].

3. Results

In grassland A, the vegetation coverage and soil moisture content recorded the highest values. The soil from grassland B was characterised by the highest resistance at penetration and the lowest pH. In grassland C, the soil was characterised by the lowest percentage of vegetation cover, moisture content and by the highest values of pH and temperature. In grassland D, the soil recorded the lowest value of temperature, and in control grassland CG all parameters recorded medium values. With one exception, soil resistance at penetration, all the other environmental variables differentiated significantly between the five investigated plots ($p < 0.001$) (Table 1).

In total, 17 invertebrate groups were identified with 14,953 individuals as the total numerical abundance. Analysing the numerical abundance of the each identified taxa, we observed a better representation of Acaridae, Collembola, Oribatida and Mesostigmata taxa. The opposite was true for Coleoptera, Opiliones and Araneae (Table 2).

Table 2. The numerical abundance of the identified taxa from the five grasslands, Romania, in 2017 (CG = control plot; A, B, C, D = fertilised experimental grasslands; Nr. Ab. = numerical abundance).

Taxon	Short Name	CG	A	B	C	D	Total Nr. Ab.
Nematoda	Nematoda		8			14	22
Enchytraeidae	Enchytrd	37	54	13	37	21	162
Lumbricidae	Lumbricd	22	105	19	22	11	179
Collembola	Collembl	1176	889	124	1180	1207	4576
Diplura	Diplura		5	8		3	16
Mesostigmata	Messtgmt	273	204	52	273	584	1386
Acaridae	Acaridae	756	2250	357	756	679	4798
Glycyphagidae	Glycyphg					8	8
Oribatida	Oribatid	693	1110	447	693	716	3659
Trombidiidae	Trombidd	3			3		6
Opiliones	Opiliond	1			1		2
Araneae	Araneae			3			3
Isopoda	Isopoda					45	45
Formicoidae	Formicod			3		3	6
Coleoptera	Coleoptr			1			1
Staphylinidae	Stphlnd	4	5		4	22	35
Insect larva	Inselarv	9	8	6	9	17	49
Total no. taxa		10	10	11	10	13	17
Total nr. ab.		2974	4638	1033	2978	3330	14,953
Shannon_H		1.401	1.321	1.371	1.4	1.547	
Equitability_J		0.608	0.573	0.571	0.608	0.603	

The highest number of identified taxa was recorded in plot D, and the lowest in CG, A and C. The highest value in the Shannon diversity index was recorded in plot D (Table 2). The highest values of the abundance were recorded in grasslands A and D, the lowest in plot B. In the grasslands CG and C the values of numerical abundance were quite close, recording a medium level (Table 2). The lower values of the equitability index were obtained in the grasslands A and B, showing better numerical representations of Collemobala, Acaridae and Oribatida taxa. In grasslands CG, C and D the equitability index recorded higher values, demonstrating that the numerical representation of the taxa is more equilibrate (Table 2).

The global CCA model was significant ($p < 0.001$) and selected VegCovr and RPs as meaningful explanatory variables. The majority of explained variation was attributed to the first axis, with only the first canonical axis being significant (first axis $F = 6.573$, $p < 0.001$; second axis $F = 1.632$, $p = 0.128$). The first axis accounted for 80.115% and the second for 19.885% of the total variation, respectively, and both explained 9.652% of the total inertia in the overall data. The biplot graphical representation indicated that the Glycyphagidae, Isopoda and Opiliones were the most negatively correlated taxa with the VegCovr, while the Formicoidae and Acaridae were the most positively correlated taxa with VegCovr. The Diplura, Lumbricidae and Aranea were the most negatively correlated taxa with RPs and the Coleoptera, Formicoidae and Staphylinidae were the most positively correlated taxa with RPs (Figure 1).

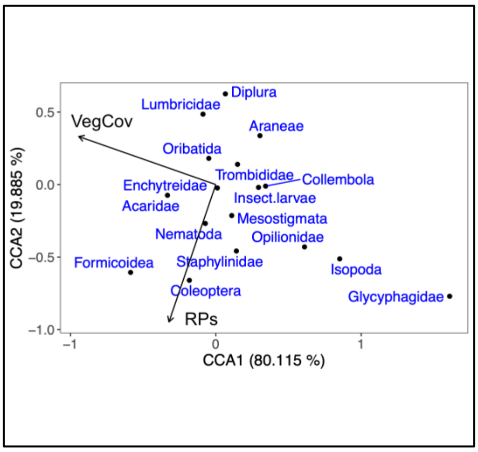

Figure 1. Biplots of the CCA model of the taxon abundance matrix in relation to environmental variables; VegCovr—Vegetation cover (%); RPs—soil penetration resistance (Map). Abbreviations for the taxa are shown in Table 2.

Indicator taxon analysis identified three characteristic taxa for grassland A, one taxon for grassland B, and two taxa for grassland D (Table 3).

Table 3. The identified taxon characteristics of grasslands.

Grassland	Taxon	IndVal	*p*
A	*Lumbricidae*	32.1	<0.001
	Acaridae	27.0	<0.01
	Oribatida	22.0	<0.01
B	*Araneae*	18.6	<0.05
D	*Staphylinidae*	35.1	<0.001
	Mesostigmata	27.2	<0.001

IndVal = Indicator Value (%).

The model selection indicated that for taxon richness the best model supported by the data included only pH, whereas for the abundance the best model included both vegetation cover and pH (Table S2).

The abundance increased significantly both with vegetation cover ($df = 1$, Chi square = 13.406, $p < 0.001$; Figure 2) and soil pH ($df = 1$, Chi square = 10.065, $p = < 0.001$; Figure 3). Furthermore, taxon richness significantly increased with soil pH ($df = 1$, Chi square = 4.054, $p = 0.044$; Figure 4).

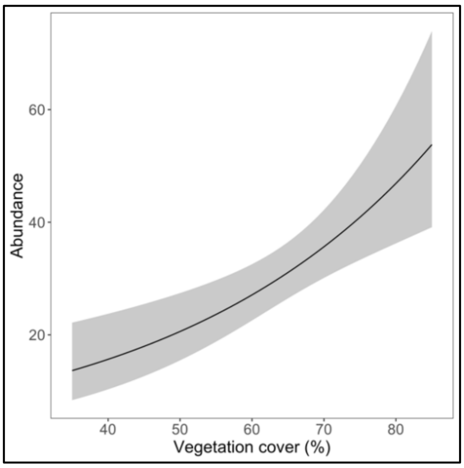

Figure 2. The effect of vegetation cover on abundance. In grey are the confidence intervals.

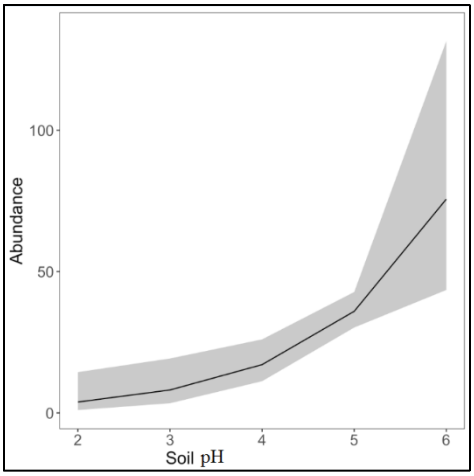

Figure 3. The effect of soil pH on abundance. In grey are the confidence intervals.

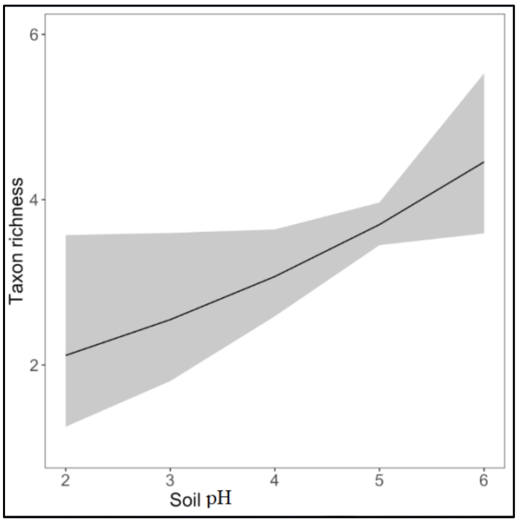

Figure 4. The effect of soil pH on treatment on taxon richness. In grey are the confidence intervals.

4. Discussion

To highlight the similarities or differences between Romanian and European data, we compared the results of studies developed on soil fauna from different types of grassland ecosystems from temperate regions of Europe. Thus, we stressed the importance of soil fauna as bioindicators for the conservation status of this type of ecosystem in Europe, considering the geographical position and the climate. In view of these considerations, many European studies focused on soil invertebrate communities from grasslands, revealing the high diversity and abundance of soil fauna taxa.

In 1987, Curry [1] made an inventory of soil functional groups of different types of meadows from the world, especially from humid temperate regions of Europe: old grasslands and permanent pasture from Ireland; moorland limestone grasslands, moorland alluvial grasslands, and permanent pastures from England; permanent pastures from France; mown and natural meadows from Poland; old grassland fen soil from Sweden; dry mountain meadows from Norway. All these terrestrial ecosystems recorded a common feature: the numerical dominance of few soil invertebrates' taxa, as Lumbricidae, Enchytraeidae, Collembola and Acari. In Germany, studies in grazed, fertilised and mown grasslands, revealed the presence of 5–8 soil functional groups, with variated abundance in concordance with the type of ecosystem's management as: Collembola, Oribatida and Mesostigmata dominant taxa, followed by Diptera, Lumbricidae, Coleoptera and Araneae and at the last Chilopoda and Diplopoda–Julida [6,56]. In the United Kindom, 18 soil functional groups have been identified in controlled and warm grasslands, dominant being Thysanoptera, Isopoda, Hemiptera, Homoptera, Coleoptera and Formicidae [57]. In Ireland and the United Kingdom, in limestone grasslands, acid upland and lowland grasslands, the dominant soil fauna taxa were Nematoda, Enchytraeidae, Collembola and Acari [11]. In Spain, studies on grazed grasslands with an oceanic climate revealed the presence of 11 ground-dwelling functional groups, dominated by Araneae, Coleoptera and Formicidae [58]. Diplopoda, Coleoptera larvae and Diptera larvae were the most abundant soil taxa from managed (mown and fertilised) or abandoned pastures from Austria [59]. Studying the indirect short- and long-term effects of above-ground invertebrate and vertebrate herbivores on soil microarthropod communities, in subalpine grasslands in Switzerland, Vandegehuchte et al., in 2015 [60], highlighted that Prostigmata was one of the most abundant soil groups (from 7 identified soil invertebrate groups), followed by Collembola and Oribatida. In grasslands from Italy, 17 soil taxa were identified, dominant being Acari, Coleoptera and insect larvae [61]. Recent studies revealed that in temperate grasslands the most numerically abundant were Lumbricidae, Acari, Collembola, Enchytraeidae and macrofauna [62]. Based on the present knowledge, we consider that the data obtained from our study were comparable with those from Germany, Poland and Switzerland, due to the similar climate or to the similar type of grassland management. Furthermore, we can affirm that the soil fauna from Romania is more diverse that those from Spain and Austria.

Our research teams up with similar studies, confirming that the soil fauna groups of Enchyraeidae, Lumbricidae, Collembola, Mesostigmata, Acaridae, Oribatida and insect larva are bioindicators for different types of grasslands from Europe, being used in monitoring programs [1,6,10,11,21,56,60–62]. We also identified these seven edaphic communities in all five investigated experimental plots (control plot, organically and chemical fertilised grasslands) in the Bucegi Mountains, Romania.

Analysing the invertebrates' fauna from the five investigated plots, we found different patterns in soil community structure and composition, shaped by the biotic and abiotic factors. Plot A was characterised by the highest percentage of vegetation coverage, the highest average value of soil moisture content and the lowest soil resistance at penetration, which means higher porosity. These environmental conditions created favourable habitats for Lumbricidae, Acaridae and Oribatida, which recorded the highest values of numerical abundance. It was recorded that earthworms had the greatest number and biomass in the soil of the northern exposition with a highest soil moisture content, but a significant decrease in their population density (63%) was recorded as a response to the reduction of plant species richness. Other studies revealed that a low abundance and diversity of Oribatida reflects low soil porosity [19,63–65].

Plot B was characterised by the presence of Araneae and Coleoptera taxa (with an exception—family Spaphylinidae). In this experimental plot the soil resistance at penetration was the highest, the soil being more compact. Laška et al., in 2011 [66], studied the vertical distribution of spiders in soil and they assumed that it was correlated with the spaces from the edaphon. Some of these spiders are exclusively surface-dwelling species. The same ecological preferences were reported at Coleoptera, its presence in a certain habitat being correlated with some soil proprieties, as macro and microporosity [17,67]. These two taxa being mainly predators and very mobile, it is possible that their presence in plot B was only accidental.

Plot D was characterised by an increased abundance of Mesostigmata and Staphylinidae taxa. This grassland was characterised by the lowest average value of soil temperature and optimum average soil moisture content (63.2%) for Mesostigmata. There are studies demonstrating that these two parameters have an impact on mite taxa [8,19,57,68]. Isopoda, which was identified only in this plot, preferred the habitat with increased soil moisture content (about 70%) and a low soil temperature. A reduction of 20–25% in soil moisture content and an increase of 5 °C in soil temperature had a negative influence on the aggregation, growth and survivorship of species [69]. Glychyphagidae are generally associated with the nests of rodents, insectivores [43].

Considering the numerical abundance, significant differences were recorded between plots B and CG (control plot). This parameter was lower in Plot B, in comparison with plot CG. This experimental plot (B) was characterised by the highest average values of soil resistance at penetration (1.39 Map) and by the most acidic soil (4.55), in comparison with the other investigated grasslands. Soil resistance at penetration is directly correlated with soil compaction or porosity [70]. A higher resistance at soil penetration means a lower porosity and the lower capacity of invertebrates to migrate in soil, this phenomenon being an efficient method of edaphic fauna to adapt to rough environmental conditions (as dryness). The porosity of the soil is one of the most important factors which determined the vertical distributions of soil organisms, migrating on both a daily and a seasonal basis [2,57,61,66]. Soil pH is another important edaphic factor that influenced the invertebrate communities. A drastic reduction of the soil pH will decrease the abundance of the soil fauna. On more basic soil, some species of bacteria develop, bacterial community development constituting a favourable factor for the edaphic invertebrates that represent the food source for predator taxa [2,20,22,23].

Plot B was the area which was chemically fertilised with the highest quantity of fertiliser (an average application rate of 150 kg ha^{-1} N + 75 kg ha^{-1} P_2O_5 + 75 kg ha^{-1} K_2O), in period 1996–1998, the same period as plots C and D, but with some major differences: plot C was limed in 1995, and in the same year in plot D herbicide Roundup at 5 l ha^{-1} and calcium liming [35,38] were used. Even though the application of the chemical fertilisers was made 24 years ago, in plot B it is possible that a higher quantity of these chemicals modified the heterogeneity of the habitat, influencing the invertebrates' communities in terms of numerical abundance. Increased nutrient additions have direct and indirect effects on the abundance and structure of soil faunal communities [31,64]. Intensification of fertilisation reduced the association between the diversities of plant–plant and plant-primary consumer taxa, decreasing the habitat heterogeneity [18,63].

Other studies revealed that controlled and rational nutrient additions increased plant biomass and productivity (which are directly correlated with the vegetation coverage), which constitutes a reservoir of organic matter for soil fauna, demonstrating that soil biotic communities are predominately regulated by bottom-up forces [6,22,25,31,64]. This could explain the more increased abundance of soil invertebrates from plot A, C and D in comparison with plot B, where the most acidic soil was recorded (pH = 4.55), and the lowest percent of vegetation coverage (60.54%). On the other hand, the lime addition from plots C and D could affect the soil invertebrate communities. According to Cole et al., 2006 [31], the liming especially affected the Lumbricidae and Enchytraeidae communities.

Roundup herbicide, which was applied in 1995 on plot D, is the brand name of a systemic, broad-spectrum glyphosate-based herbicide. According to data presented in the literature and provided by the glyphosate manufactures, the glyphosate is not considered to be a persistent organic pollutant (POP), persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) chemical. On the other hand, excessive glyphosate use induces stress on crops and on non-target plants, and is toxic for mammalians, microorganisms and invertebrates [71]. Its half-life depends on environmental conditions, such as soil moisture content, temperature, content of soil organic matter, nutrients and biological properties (especially the presence of microbiota). It has an average half-life of more than two months [71–73]. Phosphorus present in glyphosate is a driving force for its microbial degradation, and microorganisms using this phosphorus for their metabolic functions. At the same time, the microorganisms realise enzymes that cleave to the C–P bond of the glyphosate molecule. The rate of glyphosate degradation in soil is directly correlated with the type of microbial community. Glyphosate is virtually biologically inactive due to its strong binding to soil components [71–73].

Studies demonstrated that the usage of Roundup in recommended limits has minor and transient effects on the structure and functioning of food webs, not affecting the number and composition of soil fauna. Good agricultural practices, such as nutrient restoration, management of pest, weeds and disease and the rational use of agrochemicals (especially in no-till fields), influence the plant biomass, increasing the quality and quantity of litter. This first soil layer constitutes a favourable habitat and an important trophic source for soil invertebrates. These responsible practices could modify the soil faunal composition and abundance by improving its resilience against chemicals (herbicide and insecticide) application [6,11,74–76]. This could be a good explanation of the increased number of taxa and their numerical abundance identified in plot D. On the other hand, the higher number of taxa from plot D, in comparison with plots CG, A and C, could be explained through the high mobility of predator invertebrates (as Mesostigmata, Acaridae, Diplura and Staphylinidae), always searching for the food, and due to the small distance between investigated areas [3,43].

According to the canonical correspondence analysis, the most important environmental parameters which influence the taxa were vegetation coverage and soil resistance at penetration. According to Menta, 2012 [2] the taxa composition and abundance may be correlated with abiotic and biotic factors such as vegetation cover, the pH of the soil and the content of organic matter, etc. The richness of the animal taxa is indicative of the maturity of the community of vegetation (or of the habitat type) in the recovered area [20,60,61,63,77]. The same statistical analysis revealed that the Acaridae, Glycyphagidae and Formicoidea communities were influenced by the vegetation coverage and the Nematoda, Coleoptera by the soil resistance at penetration. Mites have frequently been used as indicators of diversity and habitat quality, which is strongly correlated with the vegetation coverage [17,60]. Direct correlations between mite communities and the vegetation coverage have been found by much of the research, revealing that the vegetation constitutes a source of future organic matter, a favourite trophic source for these invertebrates [3,78]. In 2000, Spehn et al. [63] demonstrated that a lower abundance and activity in decomposers with reduced plant species richness were related to altered substrate quantity.

The same multivariate statistical analysis revealed that Coleoptera, Staphylinidae and Nematoda communities were influenced by the soil resistance at penetration, which is a proper indicator of soil compaction or porosity in grasslands [70]. These results are in concordance with other studies, which revealed that some soil properties such as biopores influenced the spatial distribution of Coleoptera families [4,67,79]. It is very well known that the soil resistance at penetration is highly dependent on soil moisture, a lower value of the first parameter meaning a higher value of the second one [70]. This is in accordance with the preferences of Nematoda for more humid soil habitats [2,3,22].

5. Conclusions

In this study, we gained detailed knowledge about the soil fauna communities in experimental grasslands in Romania. The communities' structure of soil fauna, evaluated through some parameters (such as numerical abundance, taxa richness, the Shannon diversity index of taxa and equitability) was influenced by the type of fertilisation and by the local biotic and abiotic drivers (such as vegetation coverage, soil temperature, soil acidity, soil resistance at penetration and soil moisture content). The composition of investigated grasslands revealed the presence of seventeen soil invertebrate groups, with different spatial distribution. Natural grassland (control plot) was characterised by an equilibrate distribution of the numerical abundance of the soil fauna. Chemical fertilisation has a positive effect on the numerical abundance of soil taxa, with a major contribution of Collembola, Acaridae and Oribatida. Organic and chemical fertilisation, together with calcium liming, has positive effects on taxa diversity. At the same time, this study revealed that soil fauna groups are sensitive to the abiotic and biotic factors, confirming their quality as bioindicators. Acaridae, Glycyphagidae and Formicoidea were sensitive to the vegetation coverage, and Nematoda, Coleoptera and Staphylinidae to the soil resistance at penetration.

A responsible usage of fertilisers, in recommended limits, could have a positive influence on the soil fauna groups. The present study demonstrates that soil invertebrate communities could be precious indicators of the ecological conservation status of some fertilised grasslands.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14121031/s1, Table S1: A detailed description of investigated grasslands from the Bucegi Mountains, Romania (CG = control plot; A, B, C, D = experimental plots) and Table S2: Model selection results. Models are ranked in a decreasing order of the Akaike weights (w_i). For clarity, models with $w_i < 0.02$ are not shown. Statistics include: LL—log likelihood; K—number of parameters; the second-order Akaike information criterion corrected for small sample sizes AICc; Δi—AICc differences; w_i—Akaike weights.

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References

1. Curry, J.P. The invertebrate fauna of grassland and its influence on productivity. The composition of the fauna. *Grass Forage Sci.* **1987**, *42*, 103–120. [CrossRef]
2. Menta, C. Soil Fauna Diversity—Function, Soil Degradation, Biological Indices, Soil Restoration. In *Biodiversity Conservation and Utilization in a Diverse World*; Lameed, G.A., Ed.; IntechOpen: London, UK, 2012; pp. 59–94.
3. Coleman, D.C.; Wall, D.H. *Soil Fauna: Occurrence, Biodiversity, and Roles in Ecosystem Function, In Soil Microbiology, Ecology and Biochemistry*, 4th ed.; Eldor, A.P., Ed.; Academic Press: Cambridge, MA, USA, 2015; pp. 1–41.

4. Rainio, J.; Niemelä, J. Ground beetles (Coleoptera: Carabidae) as bioindicators. *Biodivers. Conserv.* **2003**, *12*, 487–506. [CrossRef]
5. Fischer, S. Earthworms (Lumbricidae) as bioindicators: The relationship between in-soil and in-tissue heavy metal content. *Pol. J. Ecol.* **2009**, *57*, 531–541.
6. Lemanski, K.; Scheu, S. The influence of fertilizer addition, cutting frequency and herbicide application on soil organisms in grassland. *Biol. Fertil. Soils* **2014**, *51*, 197–205. [CrossRef]
7. Pelosi, C.; Römbke, J. Enchytraeids as bioindicators of land use and management. *Appl. Soil Ecol.* **2018**, *123*, 775–779. [CrossRef]
8. Meehan, M.L.; Song, Z.; Lumley, L.M.; Cobb, T.P.; Proctor, H. Soil mites as bioindicators of disturbance in the boreal forest in northern Alberta, Canada: Testing taxonomic sufficiency at multiple taxonomic levels. *Ecol. Indic.* **2019**, *102*, 349–365. [CrossRef]
9. Crotty, F. Assessing soil health by measuring fauna. In *Advances in Measuring Soil Health*, 1st ed.; Otten, W., Ed.; Burleigh Dodds Science Publishing: Cambridge, UK, 2021; pp. 1–22.
10. Van Straalen, N.M. Evaluation of bioindicator systems derived from soil arthropod communities. *Appl. Soil Ecol.* **1998**, *9*, 429–437. [CrossRef]
11. Bardgett, R.D.; Cook, R. Functional aspects of soil animal diversity in agricultural grasslands. *Appl. Soil Ecol.* **1998**, *10*, 263–276. [CrossRef]
12. Tajik, S.; Ayoubi, S.; Shirani, H.; Zeraatpisheh, M. Digital mapping of soil invertebrates using environmental attributes in a deciduous forest ecosystem. *Geoderma* **2019**, *353*, 252–263. [CrossRef]
13. Johnston, A.S.A.; Sibly, R.M. Multiple environmental controls explain global patterns in soil animal communities. *Oecologia* **2020**, *192*, 1047–1056. [CrossRef]
14. Samways, M.J.; McGeoch, M.A.; New, T.R. *Insect Conservation: A Handbook of Approaches and Methods*; Oxford University Press: Oxford, UK, 2010; 458p.
15. Báldi, A. Using higher taxa as surrogates of species richness: A study based on Coleoptera, Diptera, and Acari species in Central-Hungarian reserves. *Basic Appl. Ecol.* **2003**, *4*, 589–593. [CrossRef]
16. Ekschmitt, K.; Stierhof, T.; Dauber, J.; Kreimes, K.; Wolters, V. On the quality of soil biodiversity indicators: Abiotic and biotic parameters as predictors of soil faunal richness at different spatial scales. *Agric. Ecosyst. Environ.* **2003**, *98*, 273–283. [CrossRef]
17. Gerlach, J.; Samways, M.; Pryke, J. Terrestrial invertebrates as bioindicators: An overview of available taxonomic groups. *J. Insect Conserv.* **2013**, *17*, 831–850. [CrossRef]
18. Manning, P.; Gossner, M.M.; Bossdorf, O.; Allan, E.; Zhang, Y.-Y.; Prati, D.; Blüthgen, N.; Boch, S.; Böhm, S.; Börschig, C.; et al. Grassland management intensification weakens the associations among the diversities of multiple plant and animal taxa. *Ecology* **2015**, *96*, 1492–1501. [CrossRef]
19. Manu, M.; Bîrsan, C.C.; Mountford, O.; Lăcătuşu, A.R.; Onete, M. Preliminary study on soil fauna as a tool for monitoring of the "Springs Complex of Corbii Ciungi" protected area—Romania. *Sci. Pap. Ser. D Anim. Sci.* **2020**, *63*, 272–280.
20. Manu, M.; Chiriac, L.S.; Onete, M. Structural characteristics of the soil invertebrate communities from two fragmented Natura 2000 sites from Romania. *Sci. Pap. Ser. D Anim. Sci.* **2021**, *64*, 529–535.
21. Pryke, J.S.; Samways, M.J. Importance of using many taxa and having adequate controls for monitoring impacts of fire for arthropod conservation. *J. Insect Conserv.* **2012**, *16*, 177–185. [CrossRef]
22. Nisa, R.U.; Tantray, A.Y.; Kaisar, N.; Allie, A.K.; Wania, S.M.; Alamri, S.A.; Alyemeni, M.N.; Wijaya, L.; Shah, A.A. Influence of ecological and edaphic factors on biodiversity of soil nematodes. *Saudi J. Biol. Sci.* **2021**, *28*, 3049–3059. [CrossRef]
23. Warner, F. Soil fertility, pH, texture and nematodes. *Diagn. Serv.* **2009**, *13*, 21.
24. Šalamún, P.; Renčo, M.; Kucanová, E.; Brázová, T.; Papajová, I.; Miklisová, D.; Hanzelová, V. Nematodes as bioindicators of soil degradation due to heavy metals. *Ecotoxicology* **2012**, *21*, 2319–2330. [CrossRef]
25. Van der Wal, A.; Geerts, R.H.E.M.; Korevaar, H.; Schouten, A.J.; Jagers op Akkerhuis, G.A.J.M.; Rutgers, M.; Mulder, C. Dissimilar response of plant and soil biota communities to long-term nutrient addition in grasslands. *Biol. Fertil. Soils* **2009**, *45*, 663–667. [CrossRef]
26. Briones, M.J.I.; Ineson, P.; Heinemeyer, A. Predicting potential impacts of climate change on the geographical distribution of enchytraeids: A meta-analysis approach. *Glob. Chang. Biol.* **2007**, *13*, 2252–2269. [CrossRef]
27. Rőmbke, J.; Jänsch, S.; Didden, W.A.M. The use of earthworms in ecological soil classification and assessment concepts. *Ecotoxicol. Environ. Saf.* **2005**, *62*, 249–265. [CrossRef] [PubMed]
28. Nahmani, J.; Hodson, M.E.; Devin, S.; Vijver, M.G. Uptake kinetics of metals by the earthworm *Eisenia fetida* exposed to field-contaminated soils. *Environ. Pollut.* **2009**, *157*, 2622–2628. [CrossRef] [PubMed]
29. Fründ, H.C.; Graefe, U.; Tischer, S. Earthworms as Bioindicators of Soil Quality. In *Biology of Earthworms, Soil Biology*; Karaca, A., Ed.; Springer: Berlin/Heidelberg, Germany, 2011; Volume 24, pp. 261–278.
30. Frampton, G.W. Spatial variation in non-target effects of the insecticides chlorpyrifos, cypermethrin and pirimicarb on Collembola in winter wheat. *Pesticide Sci.* **1999**, *55*, 875–886. [CrossRef]
31. Cole, L.; Bradford, M.A.; Shaw, P.J.A.; Bardgett, R.D. The abundance, richness and functional role of soil meso- and macrofauna in temperate grassland—A case study. *Appl. Soil Ecol.* **2006**, *33*, 186–198. [CrossRef]
32. García-Segura, D.; Castillo-Murrieta, I.M.; Martínez-Rabelo, F.; Gomez-Anaya, A.; Rodríguez-Campos, J.; Hernández-Castellanos, B.; Contreras-Ramos, S.M.; Barois, I. Macrofauna and mesofauna from soil contaminated by oil extraction. *Geoderma* **2018**, *332*, 180–189. [CrossRef]

33. Manu, M.; Honciuc, V.; Neagoe, A.; Băncilă, R.I.; Iordache, V.; Onete, M. Soil mite communities (Acari: Mesostigmata, Oribatida) as bioindicators for environmental conditions from polluted soils. *Sci. Rep.* **2019**, *9*, 20250. [CrossRef]
34. Pearce, J.L.; Venier, L.A. The use of ground beetles (Coleoptera: Carabidae) and spiders (Araneae) as bioindicators of sustainable forest management: A review. *Ecol. Indic.* **2006**, *6*, 780–793. [CrossRef]
35. Manu, M.; Băncilă, R.I.; Mountford, O.J.; Maruşca, T.; Blaj, V.A.; Onete, M. Soil Mite (Acari: Mesostigmata) Communities and Their Relationship with Some Environmental Variables in Experimental Grasslands from Bucegi Mountains in Romania. *Insects* **2022**, *13*, 285. [CrossRef]
36. Covaciu-Marcov, S.-D.; Ferenţi, S.; Urák, I.; Sas-Kovács, É.-H.; Cicort-Lucaciu, A.-Ş.; Sas-Kovács, I. After the Last Train Passes: Data on the Fauna from Abandoned Railway Tunnels in Romania. *Ann. Zool. Fenn.* **2017**, *54*, 335–346. [CrossRef]
37. Fiera, C.; Ulrich, W.; Popescu, D.; Bunea, C.I.; Manu, M.; Nae, I.; Stan, M.; Markó, B.; Urák, I.; Giurgincă, A.; et al. Effects of vineyard inter-row management on the diversity and abundance of plants and surface-dwelling invertebrates in Central Romania. *J. Insect Conserv.* **2020**, *24*, 175–185. [CrossRef]
38. Blaj, V.A.; Maruşca, T.; Andreoiu, A.C.; Mocanu, V. The long-term effect on cow's milk quality of methods for improving subalpine grasslands. In *Grassland Resources for Extensive Farming Systems in Marginal Lands: Major Drivers and Future Scenarios, Grassland Science of Europe*; Porqueddu, C., Franca, A., Lombardi, G., Molle, G., Peratoner, G., Hopkins, A., Eds.; Wageningen Academic Publishers: Wageningen, The Netherlands, 2017; Volume 22, pp. 109–111.
39. Stănilă, A.L.; Dumitru, M. Soil zones in Romania and pedogenetic processes. *Agric. Agric. Sci. Procedia* **2016**, *10*, 135–139. [CrossRef]
40. Balogh, J. *The Oribatid Genera of the World*; Akademiai Kiado: Budapest, Hungary, 1972; 188p.
41. Gîdei, P.; Popescu, I.E. *Îndrumător Pentru Cunoașterea Coleopterelor*; Editura Pim: Iași, Romania, 2009; pp. 5–419.
42. Krantz, G.W.; Walter, D.E. *A Manual of Acarology*, 3rd ed.; Texas Tech University Press: Lubbock, TX, USA, 2009; 807p.
43. Ceuca, T. Diplopoda. In *Determinatorul Ilustrat Al Florei Si Faunei Romaniei—Mediul Terestru*; Godeanu, S.P., Ed.; Vasile Goldiş University Press: Arad, România, 2010; Volume 3, pp. 290–300.
44. Platnick, N.I.; World Spider Catalog. World Spider Catalog. Version 19.5. Natural History Museum Bern. 2018. Available online: http://wsc.nmbe.ch (accessed on 5 February 2022).
45. Brussaard, L.; Behan-Pelletier, V.M.; Bignell, D.E.; Brown, V.K.; Didde, W.; Folgaria, P.; Fragoso, C.; Freckman, D.W.; Gupta, V.V.S.R.; Hattori, T.; et al. Biodiversity and ecosystem functioning in soil. *Ambio. J. Hum. Environ.* **1997**, *26*, 563–570.
46. Chahartaghi, M.; Langel, R.; Scheu, S.; Ruess, L. Feeding guilds in Collembola based on nitrogen stable isotope ratios. *Soil Biol. Biochem.* **2005**, *37*, 1718–1725. [CrossRef]
47. Borcard, D.; Legendre, P.; Drapeau, P. Partialling out the Spatial Component of Ecological Variation. *Ecology* **1992**, *73*, 1045–1055. [CrossRef]
48. Legendre, P.; Legendre, L. *Numerical Ecology*, 2nd ed.; Elsevier: Amsterdam, The Netherlands, 1998; 1006p.
49. Ter Braak, J.F.C. Canonical Correspondence Analysis: A new eigenvector technique for Multivariate Direct Gradient Analysis. *Ecology* **1986**, *67*, 1167–1179. [CrossRef]
50. Oksanen, J.; Blanchet, F.G.; Kindt, R.; Legendre, L.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; Stevens, M.H.H.; Wagner, H. Community Ecology Package. R Package Version 2.5-2. October 2018. Available online: https://cran.r-project.org/web/packages/vegan/index.html (accessed on 12 May 2022).
51. Dufrêne, M.; Legendre, P. Species assemblages and indicator species: The need for a flexible asymmetrical approach. *Ecol. Monogr.* **1997**, *67*, 345–366. [CrossRef]
52. Burnham, K.P.; Anderson, D.R. *Model Selection and Multimodel Interference. A Practical Information—Theoretic Approach*, 2nd ed.; Springer: New York, NY, USA, 2002; 515p.
53. Johnson, J.B.; Omland, K.S. Model selection in ecology and evolution. *Trends Ecol. Evol.* **2004**, *19*, 101–108. [CrossRef]
54. Mazerolle, M.J. AICcmodavg: Model Selection and Multimodel Inference Based on (Q)AIC(c). R Package Version 2.0-3. Available online: http://CRAN.R-project.org/package=AICcmodavg (accessed on 10 May 2022).
55. R Development Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2018; Available online: http://www.R-project.org/ (accessed on 15 May 2022).
56. Birkhofer, K.; Dietrich, C.; John, K.; Schorpp, Q.; Zaitsev, A.S.; Wolters, V. Regional Conditions and Land-Use Alter the Potential Contribution of Soil Arthropods to Ecosystem Services in Grasslands. *Front. Ecol. Evol.* **2016**, *3*, 150. [CrossRef]
57. Briones, M.J.I.; Ostle, N.J.; McNamara, N.P.; Poskitt, J. Functional shifts of grassland soil communities in response to soil warming. *Soil Biol. Biochem.* **2009**, *41*, 315–322. [CrossRef]
58. García, R.R.; Ocharan, F.J.; García, U.; Osoro, K.; Celaya, R. Arthropod fauna on grassland–heathland associations under different grazing managements with domestic ruminants. *Comptes Rendus Biol.* **2010**, *333*, 226–234. [CrossRef] [PubMed]
59. Seeber, J.; Seeber, G.U.H.; Kössler, W.; Langel, R.; Scheu, S.; Meyer, E. Abundance and trophic structure of macro-decomposers on alpine pastureland (Central Alps, Tyrol): Effects of abandonment of pasturing. *Pedobiologia* **2005**, *49*, 221–228. [CrossRef]
60. Vandegehuchte, M.L.; Raschein, U.; Schütz, M.; Gwiazdowicz, D.J.; Risch, A.C. Indirect Short- and Long-Term Effects of Aboveground Invertebrate and Vertebrate Herbivores on Soil Microarthropod Communities. *PLoS ONE* **2015**, *10*, e0118679. [CrossRef] [PubMed]
61. Menta, C.; Leoni, A.; Gardi, C.; Conti, F.D. Are grasslands important habitats for soil microarthropod conservation? *Biodivers. Conserv.* **2011**, *20*, 1073–1087. [CrossRef]

62. Gongalsky, K.B. Soil macrofauna: Study problems and perspectives. *Soil Biol. Biochem.* **2021**, *159*, 108281. [CrossRef]
63. Spehn, E.M.; Joshi, J.; Schmid, B.; Alphei, J.; Körner, C. Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. *Plant Soil* **2000**, *224*, 217–230. [CrossRef]
64. Schon, N.L.; Mackay, A.D.; Minor, M.A. Effects of dairy cow treading pressures and food resources on invertebrates in two contrasting and co-occurring soils. *Soil Res.* **2011**, *49*, 703–714. [CrossRef]
65. Skuodiene, R.; Tomchuk, D.; Kinderiene, I. The influence of natural and anthropogenic conditions on the earthworm population in different grassland ecosystems. *Agriculture* **2019**, *106*, 19–226. [CrossRef]
66. Laška, V.; Kopecký, O.; Růžička, V.; Mikula, J.; Véle, A.; Šarapatka, B.; Tuf, I.H. Vertical distribution of spiders in soil. *J. Arachnol.* **2011**, *39*, 393–398. [CrossRef]
67. Pompeo, P.N.; Filho, L.C.I.O.; Klauberg Filho, O.; Mafra, Á.L.; Baretta, D. Coleoptera Diversity and Soil Properties in Land Use Systems. *Floresta Ambiente* **2020**, *27*, e20180068. [CrossRef]
68. Qodri, A.; Raffiudin, R.; Noerdjito, W.A. Diversity and Abundance of Carabidae and Staphylinidae (Insecta: Coleoptera) in Four Montane Habitat Types on Mt. Bawakaraeng, South Sulawesi. *Hayati J. Biosci.* **2016**, *23*, 22–28. [CrossRef]
69. Dixie, B.; White, H.; Hassall, M. Effects of microclimate on behavioural and life history traits of terrestrial isopods: Implications for responses to climate change. *Zookeys* **2015**, *515*, 145–157. [CrossRef]
70. Benevenute, P.A.N.; Morais, E.G.; de Souza, A.A.; Vasques, I.C.F.; Cardoso, D.P.; Sales, F.R.; Severiano, E.C.; Homem, B.C.G.; Casagrande, D.R.; Silva, B.M. Penetration resistance: An effective indicator for monitoring soil compaction in pastures. *Ecol. Indic.* **2020**, *117*, 106647. [CrossRef]
71. Singh, S.; Kumar, V.; Datta, S.; Wani, A.B.; Dhanjal, D.S.; Romero, R.; Singh, J. Glyphosate uptake, translocation, resistance emergence in crops, analytical monitoring, toxicity and degradation: A review. *Environ. Chem. Lett.* **2020**, *18*, 663–702. [CrossRef]
72. Duke, S.O. Glyphosate: Environmental fate and impact. *Weed Sci.* **2020**, *68*, 201–207. [CrossRef]
73. Zaller, J.G.; Weber, M.; Maderthaner, M.; Gruber, E.; Takács, E.; Mörtl, M.; Klátyik, S.; Győri, J.; Römbke, J.; Leisch, F.; et al. Effects of glyphosate-based herbicides and their active ingredients on earthworms, water infiltration and glyphosate leaching are influenced by soil properties. *Environ. Sci. Eur.* **2021**, *33*, 51. [CrossRef]
74. Santos, M.J.G.; Morgado, R.; Ferreira, N.G.C.; Soares, A.M.V.M.; Loureiro, S. Evaluation of the joint effect of glyphosate and dimethoate using a small-scale terrestrial ecosystem. *Ecotoxicol. Environ. Saf.* **2011**, *74*, 1994–2001. [CrossRef]
75. Hagner, M.; Mikola, J.; Saloniemi, I.; Saikkonen, K.; Helander, M. Effects of a glyphosate-based herbicide on soil animal trophic groups and associated ecosystem functioning in a northern agricultural field. *Sci. Rep.* **2019**, *9*, 8540. [CrossRef]
76. Lemic, D.; Pajač Živković, I.; Posarić, M.; Bažok, R. Influence of Pre-Sowing Operations on Soil-Dwelling Fauna in Soybean Cultivation. *Agriculture* **2021**, *11*, 474. [CrossRef]
77. Chiriac, L.S.; Murariu, D. Plant–soil fauna interaction—Bioindicators of soil properties in agroecosystems. *Sci. Pap. Ser. A Agron.* **2021**, *64*, 39–49.
78. Ponge, J.F. Food resources and diets of soil animals in a small area of Scots pine litter. *Geoderma* **1991**, *49*, 33–62. [CrossRef]
79. Bernardes, A.C.C.; Oliveira, O.C.C.; Silva, R.A.; Albuquerque, P.M.C.; Rebêlo, J.M.M.; Viana, J.H.; Siqueira, G.M. Abundance and diversity of beetles (Insecta: Coleoptera) in land use and management systems. *Rev. Bras. Cienc. Solo.* **2020**, *44*, e0190183. [CrossRef]