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The Effect of Biochar Used as Soil Amendment on Morphological Diversity of Collembola

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Abstract: Biochar was reported to improve the chemical and physical properties of soil. The use of biochar as a soil amendment have been found to improve the soil structure, increase the porosity, decrease bulk density, as well increase aggregation and water retention. Knowing that springtails (Collembola) are closely related to soil properties, the effect of biochar on morphological diversity of these organisms was evaluated. The main concept was the classification of springtails to the life-form groups and estimation of QBS-c index (biological quality index based on Collembola species). We conducted the field experiment where biochar was used as soil amendment in oilseed rape and maize crops. Wood-chip biochar from low-temperature (300 °C) flash pyrolysis was free from PAH (polycyclic aromatic hydrocarbon) and other toxic components. Results showed that all springtail life-form groups (epedaphic, hemiedaphic, and euedaphic) were positively affected after biochar application. The QBS-c index, which relates to springtails' adaptation to living in the soil, was higher in treatments where biochar was applied. We can recommend the use of Collembola's morphological diversity as a good tool for the bioindication of soil health.

Keywords: biochar; biological soil quality; Collembola life-form groups; QBS-c index

1. Introduction

One of the major threats to global agriculture is soil degradation, including decreased fertility and increased erosion [1]. The common problem is acidification and soil organic matter depletion, which decreases soil aggregate stability [2]. Therefore, the development of methods is needed to sustain soil resources by different remediation strategies. The application of organic materials like manure, compost, and biomass waste seems very promising, but a lot of attention has been paid to stable forms of organic carbon like biochars [3,4]. The main feature of biochar is the porous carbonaceous structure, which can contain amounts of extractable humic-like and fluvic-like substances [5]. Biochar was reported to improve the chemical and physical properties of soil [6]. The use of biochar as a soil amendment has been found to improve the soil structure, increase the porosity, decrease bulk density, as well increase aggregation and water retention [7–9]. On the other hand, the main concerns with respect to biochar use as a soil amendment is its potential contamination with heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs) [10].

Springtails (Hexapoda: Collembola) are a key group of soil arthropods with densities often reaching thousands of individuals per square meter [11,12]. They contribute mainly in substrate decomposition and nutrient cycling [12,13]. Moreover, these organisms are sensitive to environmental changes in soil and are therefore often used as indicators of soil quality [14]. For bioindication, Collembola species diversity is used [15,16]. The disadvantage of this method is the difficulty in the determination to the species level. An alternative could be the QBS-c index (biological quality index based on Collembola), which responds to the morphological diversity of springtails [17]. Using this index, each individual is evaluated in terms of different morphological traits, e.g., for antennal length, size of furca, presence of ocelli pigmentation, and the presence of hairs and/or scales along the body. The principle of this index is that the presence of individuals with better adaptation to live in soil (with reduced appendages or less pigmented) indicates better soil quality [17,18]. Also, on the basis of morphological traits, springtails can be divided into three main life-forms [19]. First, epedaphic Collembola are adapted to live on the soil surface. The major features of this group are a pigmented body, well developed eyes and appendages, as well a fast dispersal ability. In contrast to them, soil dwelling species (euedaphic), with a relatively small, less pigmented body and reduced eyes. Their dispersal ability is limited. Species showing adaptations between epedaphic and euedaphic species are classified as belonging to the hemiedaphic group [20]. The vertical stratification of springtails reflects their function in the ecosystem. For instance, only epedaphic springtails contribute in the early stages of organic matter decomposition [19]. Ponge et al. [21] suggested that Collembola living in the soil characterized by limited active dispersal, may suffer more from land use intensification, than species living on the soil surface. In contrast, Ellers et al. [22] showed stronger effects of intensive land use on epedaphic than on euedaphic Collembola. The majority of studies on biochar effect on springtails were conducted in laboratory conditions on one model species [23–25]. Considering the impact of biochar under field conditions some experiments have been made also on nematodes [26] and earthworms [27].

The potential of biochar for pH and nutrient availability changes or improvement of some physical properties like porosity, water retention, or temperature and impact on soil microbial life, are well documented [28–30]. Therefore, springtails can be affected directly by the changes in soil chemical properties [31,32] or indirectly from biochar-induced changes in microorganisms' biomass [33]. It has been reported that many Collembola species feed on bacteria or fungi [34,35]. Biochar particles might be considered analogous to soil aggregates in that their large internal surface areas and pores could be important for biological processes [36].

Within the presented study we aimed to estimate the effect of biochar on the morphological diversity of Collembola species and evaluate its potential for field application.

It was hypothesized that:

1. Biochar will increase the soil biological quality mainly through improvement of the physical and chemical soil properties.
2. From the analyzed life-form groups of springtails, the response of euedaphic assemblages will be most distinct after biochar amendment. On the other hand, springtails living on the soil surface and in the litter layer (epigeic and hemiedaphic) will be more sensitive to cover plants.
3. The QBS-c index will show higher values in crops where biochar was applied.

2. Materials and Methods

2.1. Experimental Design

The field experiment was set up in mid-April 2014 in the south of Poland (50.5740 N, 17.8908 E) and continued until October 2016. The soil type was poor (sandy and weakly acidic) agricultural soil [37]. The climate of the area is moderately warm with an average annual temperature of 8.4 °C and an average annual rainfall of 611 mm. The biochar effect on soil dwelling springtails was explored in two crops (oilseed rape and maize) compared to control (two crops with no biochar application). Within each treatment (plot), three replicates (subplots) were established. The area of each subplot was 3 × 3 m. The research area was previously (before 2014) used as conventionally agricultural field. The forecrop was maize. Biochar was applied up to a depth of 30 cm at a rate of 50 t/ha and was mixed by ploughing. No chemical protection was applied before or during experiment period. Weeds were removed manually upon occurrence. Weeds were harvested manually a few times during the vegetation season. The maize variety in two years of the study was P8745 (FAO 250, Pioneer Company) and oilseed rape variety Monolit. The only fertilizer used in oilseed rape was ammonium sulfate 34% in a dose of 300 kg/ha, and in maize ammonium phosphate (Polydap) in a dose of 25 kg/ha. The same amount of fertilizers was applied in biochar and control treatments.

2.2. Biochar Characteristic and Soil Properties

The biochar used in the experiment was industrial produced by Fluid S.A. Company (Poland). It was produced in the low-temperature flash pyrolysis (300 °C) of pine and spruce wood chips. Its heating value was 25 MJ/kg. During the experiment selected properties of biochar (pH, organic carbon content, cation exchange capacity, heavy metal content and total PAH's) were analyzed according to International Biochar Initiative (IBI) Standard Product Definition and Product Testing Guidelines [38]. The particle size fraction of biochar applied on the field was more than 2 mm (sieve method).

The tested biochar was alkaline (pH 8.2) and had 52.3% of carbon (Table 1). The surface area of the tested biochar was low (only 16.5 m^2/g) and cation exchange capacity was also lower—39.5 cmol/kg, compared with biochars produced at higher temperatures and from other feedstock, like wheat straw, giant miscanthus, rice husk, or sewage sludge [39,40]. It was free from polycyclic aromatic hydrocarbon and the concentration of all tested toxic compounds was very low or even under the level of detection, passing fixed recommendations for acceptable levels [38].

Table 1. The chemical characteristic of biochar properties used in the experiment (sourced from Gruss et al. [41]).

Parameter	Value
pH H_2O	8.2
CEC (cmol/kg)	16.8
Carbon content (% of DM)	52.3
H/Corg ratio	0.026
Pb (g/t DM)	1.57
Mn (g/t DM)	29.7
Cu (g/t DM)	0.50
Hg (g/t DM)	0.32
Zn (g/t DM)	13.04

For physicochemical analysis, soil samples were collected twice a year from topsoil, before each crop in rotation in five replicates from each plot. The pH, total organic carbon, CEC, exchangeable acidity, and water properties were measured. Soil was classified as Cambisol [38], with a typical sandy loam texture with the addition of medium fine gravel. Application of biochar significantly increased CEC values in both trials, due to the increase of exchangeable Ca2+, Mg2+, and H+ + Al3+ (exchangeable acidity) in the soil sorption complex and total organic carbon in biochar trials (Table 2).

Table 2. Soil properties after biochar application in oilseed rape and maize (sourced from Gruss et al. [41]).

Parameter	Oilseed Rape		Maize	
	Biochar	Control	Biochar	Control
C_{org} (%)	0.94	0.92	0.94	0.78
pH H_2O	6.88	7.26	6.49	7.22
Na^+ (cmol/kg)	0.20 *	0.12	0.12	0.18
Mg^{2+} (cmol/kg)	3.14	1.13	2.76	0.86
K^+ (cmol/kg)	0.30	0.19	0.34	0.31
Ca^{2+} (cmol/kg)	5.12	2.29	5.72	2.28
CEC (cmol/kg)	8.76	3.74	8.98	4.73

* The values in bold font differ significantly between treatments.

2.3. Collembola Studies

Soil samples for Collembola analysis were taken three times during each of the vegetation season (from May to July) in 2015 and 2016. The growth stages according to the BBCH (growth stages of plants) scale [42] in the sampling dates were: maize: 10–15, 32–37, and 61–67; oilseed rape: 60–69, 72–79, and 83–89. On each date, 12 samples were taken from each subplot (36 samples from one plot), and transported to the laboratory. The samples were taken with the use of a soil sampler (diameter 5 cm and depth 10 cm). The volume of one sample was 196 cm^2. Collembola were extracted over 24 h from the soil samples with the use of Tullgren funnels modified by Murphy [43]. After the extraction the springtails were kept in 75% ethyl alcohol.

Springtails from each sample were counted and identified to the species or genus. Each individual was placed on permanent microscope slide and determined to the species level with the use of following keys [44–46]. Springtails were classified to three life-form groups (euedaphic, hemiedaphic and epigeic) according to Karaban [20]. Epedaphic forms have strong pigmentation, fully developed furca and other appendages, and pigmented eyes (8 + 8). Hemiedaphic have reduced body pigmentation, eye numbers, and a reduced furca. Euedaphic forms are characterized by an unpigmented body (or eyes' pigmentation) with eyes and furca not developed. The QBS-c (biological quality index based on Collembola species) is calculated as the sum of EMI values in each sample (Table 3). The springtails species were evaluated for seven morphometric traits according to the scale. The results were the sums of scores (EMI) obtained for each trait. Species which are well adopted to live in soil obtain more EMI scores in comparison to those with adaptation to live on the soil layer [17].

Table 3. Description of the Collembola species identified in the experiment including their morphological description.

Species	Abbr. on the CCA Biplot	Life-form Group	Size *	Pigmentation	Structures on Cuticle	Ocelli	Antennae	Legs	Furcula	EMI Scores (QBS-c)
Bourietiella hortensis (Fitch)	Bou_hor	Epedaphic	4	0	1	0	0	0	0	5
Brachystomella parvula (Schaffer)	Bra_par	Hemiedaphic	4	0	1	0	3	0	3	14
Caprainea marginata (Schoett)	Cap_mar	Epedaphic	4	0	0	0	0	0	0	4
Desoria multisetis (Carpenter & Phillips)	Des_mul	Epedaphic	4	3	0	0	0	0	0	7
Desoria tigrina (Nicolet)	Des_tig	Hemiedaphic	4	6	0	0	0	0	0	10
Folsomia sexuolata (Tullberg)	Fol_sex	Hemiedaphic	4	6	3	6	2	2	2	25
Folsomides angularis (Axelson)	Fol_ang	Hemiedaphic	4	6	3	6	3	2	3	27
Folsomides parvulus (Stach)	Fol_par	Hemiedaphic	4	6	3	3	3	3	3	25
Friesea mirabilis (Tullberg)	Fri_mir	Hemiedaphic	4	3	1	0	2	2	2	14
Hypogastrura spp.	Hopogast	Hemiedaphic	4	0	3	0	2	2	2	13
Isotoma anglicana (Lubbock)	Iso_ang	Epedaphic	4	3	1	0	0	0	0	8
Isotoma antennalis (Bagnall)	Iso_ant	Epedaphic	4	1	0	0	0	0	0	5
Isotoma viridis (Bourlet)	Iso_vir	Epedaphic	4	3	0	0	0	0	0	7
Isotomiella minor (Schaeffer)	Iso_min	Hemiedaphic	4	6	3	3	3	3	3	25
Isotomodes productus (Axelson)	Iso_pro	Hemiedaphic	4	6	3	6	2	3	2	26
Isotomurus palustris (Mueller)	Iso_pal	Epedaphic	4	1	0	0	0	0	0	5
Isotomutus gallicus (Carapelli et al.)	Iso_gal	Epedaphic	4	1	0	0	0	0	0	5
Lepidocyrtus violaceus (Fourcroy)	Lep_vio	Epedaphic	4	0	0	0	0	0	0	4
Mesaphorura spp.	Mesaphor	Euedaphic	4	6	3	6	3	3	6	31
Parisotoma notabilis (Schaeffer)	Par_not	Hemiedaphic	4	6	1	3	0	2	0	16
Proisotoma minima (Absolon)	Pro_mini	Hemiedaphic	4	3	1	3	2	2	2	17
Proisotoma minuta (Tullberg)	Pro_minu	Hemiedaphic	4	3	1	0	2	2	2	14
Proisotoma tenella (Reuter)	Pro_ten	Hemiedaphic	4	3	1	0	2	2	2	14
Protaphorura spp.	Protapho	Euedaphic	4	6	3	6	3	3	6	31
Pseudosinnela sexoculata (Schott)	Pse_sex	Hemiedaphic	4	6	1	3	0	0	0	14
Sminthurides parvulus (Krausbauer)	Smi_par	Epedaphic	4	0	0	0	0	0	0	4
Sminthurinus alpinus (Gisin)	Smi_alp	Epedaphic	4	0	1	0	2	0	0	7
Sphaeridia pumilis (Krausbauer)	Sph_pum	Epedaphic	4	0	0	0	0	0	0	4
Stenacidia violacea (Reuter)	Ste_vio	Epedaphic	4	0	0	0	0	0	0	4
Stenaphorura spp.	Stenapho	Euedaphic	4	6	3	6	3	3	6	31

* Size: >3 mm = 0; 2–3 mm = 2; <2 mm = 4.

Pigmentation: Fully pigmented=0; only strips on the body = 1; r = Reduced to appendages = 3; none = 6.

Structures on cuticle: Well developed chaeta or scales, present trichobothria = 0; relatively low number of structures on cuticle=1; Reduced number of chaetae, presence of PSO (pseudocelli) on cuticle = 3; Low number of chaeteae, other structures present only in selected parts of the body = 6.

Number of ocelli in the eye spot: 8 + 8 = 0; 6 + 6 = 2; form 5 + 5 to 1 + 1 = 3; absence of ocelli = 6.

Antennae: antennae longer than the head = 0; antennae more or less the same length as the head = 2; antennae shorter than the head = 3; antennae much shorter than the head = 6.

Legs: Well developed = 0, Medium developed = 2; Short = 3; Reduced or with reduced claw and mucro =6.

Furcula: Well developed = 0; Medium developed = 2; Short with reduced number of chaetae = 3; the absence of mucro and modification of manubrium = 5; Furcula reduced in residual form = 6.

2.4. Data Analysis

The effect on springtails life-form groups and QBS-c index was analyzed with the mixed model in SAS University Edition (proc Mixed). In the analysis, the effect of crop and treatment, as well their interaction, were included. The year and term of the study were the random factors. The abundance of springtails per sample was relatively low (with the number of individuals of 10.5 per sample). Therefore, the abundance of springtails was calculated for 1 m^2, knowing that the area of one sample was 0.000785 m^2 (5 cm diameter).

The springtails abundance as well morphometric trails in relation to experimental treatments was analyzed using canonical correspondence analysis (CCA) in Canoco, Version 4.5 (Ithaca, New York, USA). Significance of the first canonical axis and all axes together was calculated with Monte Carlo Test.

3. Results

The abundance of Collembola per m^2 and the QBS-c index differed significantly between all tested factors (Table 4). Generally, the most abundant group was epedaphic, then hemiedaphic, and the least, euedaphic Collembola (Figure 1). Within the epedaphic and hemiedaphic groups, both the plant ($p < 0.0001$) and biochar ($p = 0.0009$, 0.0058) significantly differed with respect to springtails abundance. Springtails were significantly more abundant in oilseed rape in comparison to maize crop. At the same time more Collembola were found in biochar, but only in oilseed rape. The abundance of euedaphic Collembola differed between biochar and control plots ($p = 0.003$). In both crops, significantly more individuals were found in biochar treated soil.

Table 4. Results of repeated ANOVA (GML, $p \leq 0.05$) considering effects on treatment, plant and year, and its interactive effects on Collembola life-form groups and QBS-c index.

Dependent Variable	Treatment		Plant		Treatment × Plant	
	F *	p	F *	p	F	p
Epedaphic	11.10	0.0009	20.53	<0.0001	12.43	0.0004
Hemiedaphic	7.65	0.0058	22.04	<0.0001	11.53	0.0007
Euedaphic	13.09	0.0003	0.04	0.8501	2.15	0.1425
QBS-c	6.16	0.01132	4.77	0.0292	0.02	0.8943

* F = ratio of two different measure of variance for the data.

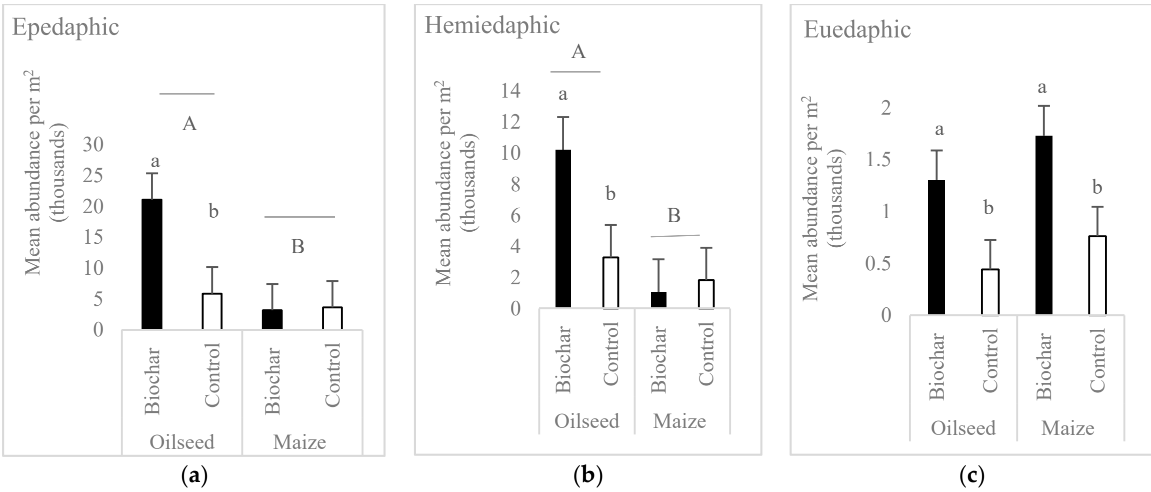

Figure 1. Effect of crop and biochar application on different Collembola life-form groups. Note: (**a**), (**b**), (**c**); indicate significant differences ($p \leq 0.05$) between biochar and control; A, B indicate significant differences ($p \leq 0.05$) between plants; the results of repeated ANOVA used for data analysis are given in the Table 4.

Considering the QBS-c index, it was significantly higher in oilseed rape crop in comparison to maize (Figure 2) ($p = 0.0292$). In both plants, the index was significantly higher in treatments, where biochar was applied ($p = 0.01132$). As shown by the life-form groups, significantly higher QBS-c index was found in oilseed rape only.

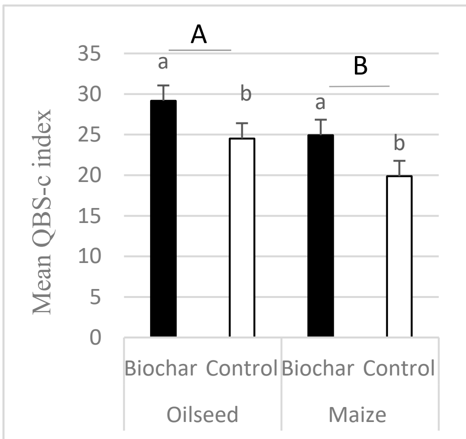

Figure 2. Effect of crop and biochar application on the QBS-c index. Note: (a), (b), (c); indicate significant differences ($p \leq 0.05$) between biochar and control; A, B indicate significant differences ($p \leq 0.05$) between plants; the results of repeated ANOVA used for data analysis are given in the Table 4.

The morphometric traits used for the calculation of QBS-C index were correlated with experimental treatments (Figure 3). The significance of the first canonical axis (CCA1), as well all axes together was $p = 0.002$ (Table 5). Biochar (in oilseed rape) was positively correlated with reduced legs, antennae and furcula, as well absence of specific structures on cuticle. In maize, where biochar was applied, springtails were characterized by a reduced number of ocelli. Size and pigmentation were positively correlated with oilseed rape and maize, both without biochar.

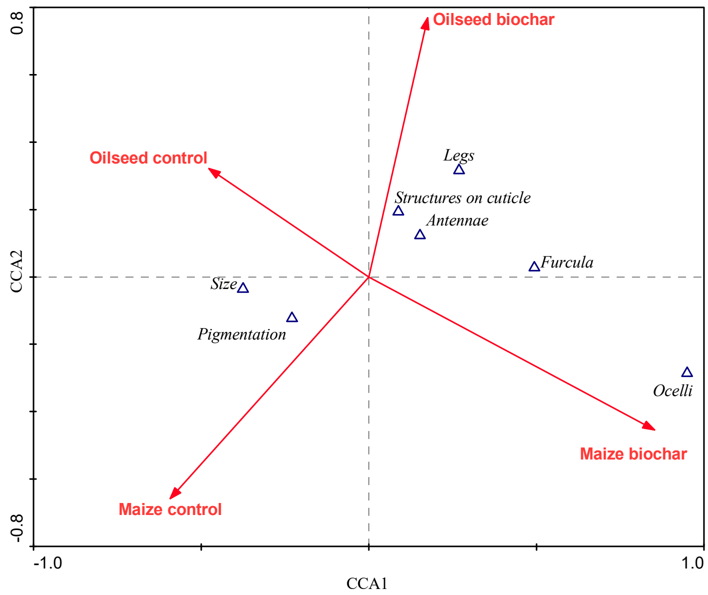

Figure 3. Canonical correspondence analysis (CCA) biplot on Collembola morphomentric traits in relation to experimental treatments. Notes: The morphometric traits are described in more detail in Table 1.

Table 5. Results of Canonical correspondence analysis (CCA) of Collembola morphometric traits correlated with experimental treatments.

CCA Axes	**1**	**2**	**3**	**4**
Eigenvalues	0.013	0.002	0.000	0.103
Morphometric traits-environment correlations	0.013	0.002	0.000	0.103
Significance of the first canonical axis	F = 26.362, p = 0.002			
Significance of all canonical axes	F = 10.296, p = 0.002			

The eigenvalues of the first two CCA axes were 0.0102 and 0.041, respectively (Table 6). Both the first canonical axis (CCA1), as well all axes were significant (p = 0.002, Monte Carlo test). As shown on the CCA biplot (Figure 4), the Collembola community was affected more by crop than by biochar. There was only minor effect of biochar in oilseed. In maize the group of species related to the control site (e.g., *Desoria tigrina* and *Pseusinella sexoculata*) differed from the species which preferred biochar (e.g., *Stenaphorura* spp., *Isotoma antenalis*). Considering life-form groups, most of the hemiedaphic species were found in the oilseed crops to oil seed (with similar effect for biochar and control). The epedaphic species were frequently found in all of the treatments, while euedaphic mostly in maize with biochar.

Table 6. Results of Canonical correspondence analysis (CCA) of Collembola species in relation to experimental treatment.

CCA Axes	**1**	**2**	**3**	**4**
Eigenvalues	0.102	0.041	0.013	0.476
Species-environment correlations	0.563	0.388	0.270	0.000
Significance of the first canonical axis	F = 7.754, p = 0.002			
Significance of all canonical axes	F = 4.016, p = 0.002			

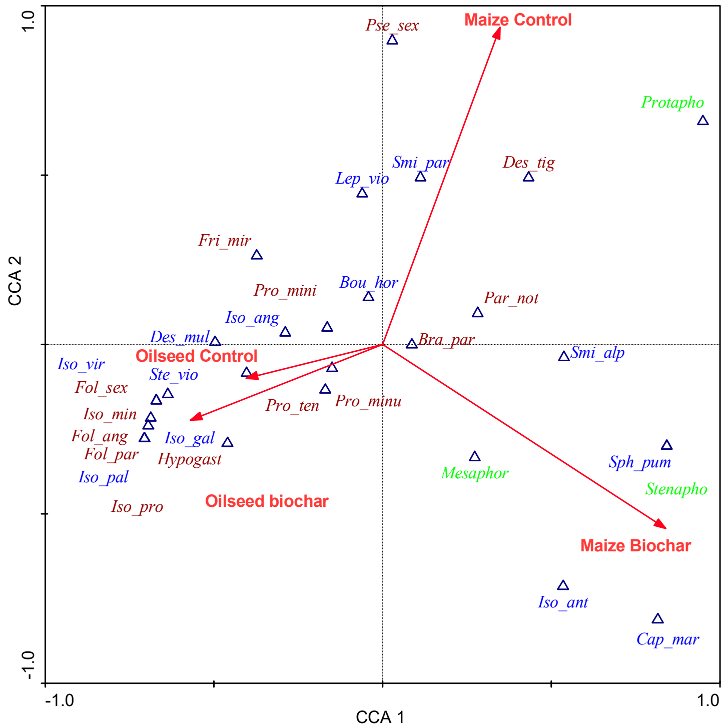

Figure 4. Canonical correspondence analysis (CCA) biplot on Collembola species in relations to the crop and biochar application. * Designation of the life-form groups: epedaphic, hemiedaphic, euedaphic. Note: the detailed description of the species is included in Table 1.

4. Discussion

The low temperature pine/wood chip biochar used in the experiment is characterized by the high carbon content but low surface area, and ability for nutrient storage, therefore the effect of its application to soil had little effect on soil properties. Tested pined wood biochar, was free from PAH and the concentration of all tested toxic compounds was very low or even under the level of detection. The release of toxic compounds like PAH's and heavy metals after biochar application seems to be crucial for survival and reproduction of soil fauna [47,48]. Some of the soil properties were improved, like total organic carbon content (only in maize trials) or CEC, as reported by other authors [49–53]. The liming effect, which is mostly expected [54,55], when biochar is applied to soil was not determined in our experimental trials.

Our estimations showed a higher QBS-c index value and higher individual number of particular life-form groups in biochar amended soils, confirming the hypothesis of soil biological properties improvement. We state that the positive response of Collembola to biochar addition was the result of improved soil properties. Some authors have found that soil mesofauna abundances are closely related to soil conditions, especially soil pH and organic matter content [56,57]. To compare, the significant increase of fungivorous nematodes was found after biochar application in the rates from 12 to 48 t/ha [26]. In contrast, no response of soil faunal feeding activity to biochar addition was found in the rates range from 0 to 30 t/ha [58]. Also, Castracani et al. [59] did not find any interaction between biochar and the abundance of epigeic macroarthropods in the rate of 14 t/ha.

The response of euedaphic springtails was predicted to be most distinct after biochar amendment. This would result from low dispersal ability and living in deeper soil layers [60]. Therefore, euedaphic springtails would be more sensitive to changes in the soil environment [61]. In our experiment the abundance of euedaphic springtails was relatively low compared to hemiedaphic and epedaphic groups. However, in both analyzed crops, its number increased after biochar application. Considering the two other groups living on upper soil layers, the effect was significant only for oilseed.

The main concept of the QBS-c index is that soil quality is positively correlated with the number of Collembola species that are well adapted to soil habitats [17]. Otherwise, numerous occurrences of euedaphic forms of springtails in a given habitat can indicate the better biological quality of the soil [43]. Based on the results, we can agree with the hypothesis that the QBS-c index will have higher values in crops where biochar was applied. For instance, in the study of Twardowski et al. [61], the QBS-c showed higher soil quality in the potato crop rotation in comparison to monoculture. Jacomini et al. [62] found decreased QBS-c index values in degraded soils. To confirm our last hypothesis, two springtails life-form groups (epigeic and hemiedaphic) differed significantly between crops. More springtails were found in oilseed rape in comparison to maize crop. Considering that maize and oilseed rape are plants which differ in their development, this should also affect organisms living on the soil surface. Similarly, Op Akkerhuis [63] found differences in mesofauna abundance between crops, i.e., specifically the mesofauna groups were much more abundant in cereals than in root and tuber crops. We state also that cover plant might modify the effect of biochar on soil fauna.

5. Conclusions

The main findings of the present study are that:

(1) Crop affected more Collembola community than the biochar application. More springtails occurred in oilseed rape.
(2) In each of the life-form groups, biochar caused a significant increase in individual number of Collembola in comparison to the no-biochar treatment. The effect was significant mainly for the oilseed rape crop.
(3) The QBS-C index (biological quality index based on Collembola species) was higher in treatments where biochar was applied.
(4) Collembola related to biochar were characterized by reduced appendages and the absence of specific structures on the cuticle, what indicates better adaptation to live in soil.

To conclude, biochar was found to increase springtails abundance and diversity in field conditions. A greater occurrence of species better adopted to life in the soil with biochar use indicated better soil quality. Thus, we can recommend the use of the morphological diversity of Collembola as a good tool for the bioindication of soil health.

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

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