

Article

The Change of pH Value and *Octolasion cyaneum* Savigny Earthworms' Activity under Stubble Crops after Spring Triticale Continuous Cultivation

Angelika Kliszcz * D and Joanna Puła

Department of Agroecology and Crop Production, University of Agriculture in Cracow Mickiewicz Ave. 21, 31-120 Cracow, Poland; rrpula@cyf-kr.edu.pl

* Correspondence: angelika.kliszcz@student.urk.edu.pl

Received: 1 June 2020; Accepted: 30 June 2020; Published: 4 July 2020



Abstract: The changes of pH value followed by various agricultural practices are crucial for biotic components of soil, along with other environmental factors, like temperature and moisture content. In this paper, the earthworms population in triticale continuous cultivation was monitored. Their presence associated with various agrotechnical methods (e.g., stubble crops maintained as mulch, mineral fertilization without pesticides) were assessed twice by the handsorting method, and their presence during the triticale vegetation season (occurrence of coprolites) were monitored too. The aim of the study was to analyse the distribution of earthworm populations in cereal continuous cultivation fields, and whether they prefer any of the stubble crop species (Sinapis alba L., Phacelia tanacetifolia Benth., Fagopyrum esculentum Moench.), which were sown after triticale harvest. The results reveal the most abundant earthworms occurence in the *F. esculentum* objects (43.1% of all sampled earthworms, which consists of 42% and 47.2% of all sampled matured and juveniles individuals, respectively), and species homogeneity (dominate Octolasion cyaneum Savigny, 1826). The changes of pH varied through the triticale vegetation season, but didn't exhibit severe variation between sampling sites. Using earthworm services in cropping systems after having enticed them to the field through stubble crop has the potential to boost agricultural sustainability. Their ecological preferences, along with their trophic behaviour, have already been put in place to complete a case study of the autecology of the O. cyaneum Savigny 1826 species.

Keywords: *Octolasion cyaneum* Savigny; mustard; buckwheat; phacelia; triticale continuous crop; soil pH; earthworm abundance

1. Introduction

Earthworms are a key factor in supporting soil system services. They improve soil structural stability, modify soil organic matter and nutrient cycling or induce the production of hormone-like substances that improve plant growth [1,2]. According to Paoletti [3] and Curry et al. [4], earthworm populations in cultivated land are generally lower than those found in undisturbed habitats. The presence, activity and biodiversity of earthworms in the cultivated field may be limited by the intensification of cultivation treatments [5] or the use of herbicides [6,7]. However, the introduction of additional plant biomass into fields, e.g., in the form of stubble crops, serves as an additional food source and can contribute to an increase in the earthworm population in cereal cultivation after itself. This positive relation of additional biomass for earthworms was found by Riley et al. [8], Froseth et al. [9], Buck et al. [10] when earthworm abundance in lupine stands, grasses, and legumes was analysed.

During vegetation season, any agrotechnical treatments shaped the soil system in particular ways and influenced the value of soil parameters, like pH, moisture content, temperature. Tillage may



saturate soil, mix the layers of aerobic and anaerobic microorganisms, and destroy persistent vertical galleries of anecic earthworm species (as well as horizontal endogeic-derived biopores, but they could be restored faster during crop vegetation). Ploughing the aboveground biomass causes the immediate supplying, in soil, of living forms (mainly microorganisms and mesofauna) with proteins, sugars, fat-like substances, and ions, which also initiate POM (*particulate organic matter*) formation in the soil, the first form of SOM (*soil organic matter*).

Applying any substances (fertilizers, pesticides, soil and plant enhancers) influences the hot-spot mosaic in the soil. Kuzyakov and Blagodatskaya [11] defined hot-spots as a local clusters of microorganisms that cooperate in common area and shape it in particular way, varied from adjacent bulk soil. They are the crucial and basic organizational form of microbial activity in the macroscale (field) perspective. Their presence in the soil is sought by the earthworms, probably with strength proportional to the palatability of these microorganisms for earthworms. They can spread the microbial strains throughout the area they occupy, and that function seems to be one of the most prominent skills of earthworms sought in future agriculture. Moreover, the "fertilization only" variant is tested in agriculture along with agrotechnical methods (cover, stubble crops, intercrops and mulches) avoiding negative effects of pesticides. Many indicators of soil health also include the welfare level of earthworms' field occurrence [3,12–14].

Stubble crop has many positive functions in the soil system, mainly through enhancing the soil C pool, accelerating microbial life, positively shaping soil structure. Fast growing species cover stand with pronounced biomass and prevent soil moisture from escaping. These features include white mustard (*Sinapis alba* L.), tansy phacelia (*Phacelia tanacetifolia* Benth.), and buckwheat (*Fagopyrum esculentum* Moench.). But even application of milled (<3mm) cereal straw may result in enhanced earthworm growth rates more than farmyard manure, due to their much higher calorific value [15].

The aim of the study was to assess earthworms' activity in continuous cereal tillage cultivation as well as their preferences for tested stubble crops along with recorded environmental features.

2. Material and Methods

2.1. Description of Experimental Set Up

The background for earthworm samplings and soil analyses was a field experiment located in the Experimental Station of the University of Agriculture in Cracow conducted in 2018–2019 (50.085264 N, 19.833110 E). Before 2017, the field was maintained as an ecological system for 10 years (tillage occurred). The present experiment consists of three factors arranged as split-split-plot design, where the spring triticale (xTriticosecale Wittm. ex A. Camus var Mamut) was cultivated after itself for two years (2018–2019). The following stubble crop species: white mustard Sinapis alba L. var. Borowska, tansy phacelia *Phacelia tanacetifolia* Benth. var. Stala, buckwheat *Fagopyrum esculentum* Moench. var. Kora, were sown twice, in 2018 and 2019 (first factor), although earthworm sampling procedure was performed in 2019 only. The second factor of experimental design, stubble crop termination term, had two levels: autumn and spring (mulch). In addition, the cultivation of spring triticale was managed in three ways (third experimental factor): Natural-without fertilization and pesticides (NAT), mineral fertilization only (NPK 80:80:120) (MF), and mineral fertilization (NPK 80:80:120) with subsequent pesticides application (PEST). Graphical description of experimental design is presented in Figure S1 (Supplementary Material). The pesticides were applied twice during the vegetation season of triticale (in the tillering phase—BBCH 21–22: herbicide Puma 1 l·ha⁻¹ (a.i. *fenoxaprop-P-ethyl* 69 g·l⁻¹ (6.54%)) and Mustang 306 0.6 l·ha⁻¹ (a.i. *florasulam* 6.25 g·l⁻¹ and 2,4-D EHE 300 g·l⁻¹), and in the heading phase—BBCH 59: fungicide (a.i. protiokonazol 53 g·l⁻¹ (5,4 %), spiroksamine 224 g·l⁻¹ (22,9 %), tebukonazol 148 g·l⁻¹ (15,1 %)) and insecticide Dursban 480EC (a.i. chloropiryfos 44,86%). The P and K mineral fertilizers were applied before the sowing of triticale (along with the first, bigger part of N fertilization); the second N fertilization dose was applied in BBCH 33–37 of the triticale phases.

2.2. Soil Analysis

Measurement series of pH value (Figure 1) were collected in situ with the IJ44A IONODE pH electrode (ELMETRON, Australia) equipped with a CP 401 pH meter (ELMETRON), once a week (March 2019–July 2019) in the following phenological terms: 1—before spring tillage (mulch), 2—after tillage, 3—two weeks after tillage, 4—one day before sowing triticale, 5—sowing and mineral fertilization, 6—BBCH 5 (start of triticale germination), 7—BBCH 10 (development of leaves), 8—BBCH 11, 9—BBCH 12, 10—BBCH 13 and herbicide application, 11—BBCH 22, 12—BBCH 30–37 (stem elongation and flag leaf appearance) and second dose of N fertilization, 13—BBCH 41–43 (booting phase), 14—BBCH 59 (heading phase), 15—BBCH 71 (seed development), 16—BBCH 89–92 (triticale harvest). The time between terms was named in this article as subsequent 'periods' and the last measurement series ('after triticale harvest') was collected three weeks after the previous one. Chemical and physical features of soil are depicted in Table 1. Soil samples were taken from topsoil (to 0.3 m). C org (%), N tot (%) and S tot (%) were analysed using Vario Elementar and the averaged content of CaCO₃ (%) according to Scheibler's method [16]. Granulometric fractions were assessed using Casagrande's areometric method with modification by Prószyński [17].

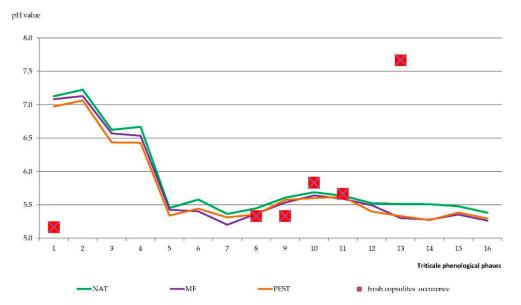


Figure 1. Continuous measurements of pH value in earthworm sampling sites (March 2019–July 2019) according to different triticale management methods. * The distance of red dots from the *x*-axis represents abundance of monitored sites where fresh coprolites were occurred in the particular terms; the lack of red dots means no fresh coprolites at the monitored sites.

Value
0.70
0.63
0.01
0.05
81.0:15.5:3.5

Table 1. Soil characteristics description.

2.3. Earthworm Sampling and Identification

Activity of earthworms (presence of fresh coprolites) was monitored in the field every pH measurement series (three times within each plot, $n = 54 \times 3 = 162$), and showed as red dots in Figure 1. Then, after harvest of triticale (July 2019) the samplings from each plot (one per plot, assumed to be

three samplings per object) was conducted within a frame ($0.5 \times 0.5 \text{ m}$; 0.4 m in depth); the vegetation cover was excavated. The distance between sampling sites was several meters. The handsorting method was chosen only, due to dryness of soil (many individuals were in diapause) and problems with proper infiltration of mustard suspension. Moreover, in this field, abundance of anecic species are not expected due to tillage occurrence. So, the best sampling procedure in these conditions was handsorting. The sampled earthworms were kept in plastic boxes, transported to the laboratory, maintained for clearing the gut content in cold conditions, and then manipulated to obtain fresh biomass of all life stages groups according to their number (cocoons, hatchlings, juveniles, pre-matured individuals, and matured adult individuals with clitellum well developed). All results for 0.25 m² were adjusted for 1 m² area. Among all caught individuals, *O. cyaneum* Savigny, 1826 totally dominated (there were a few unrecognized endogeic or anecic ones and only one individual from the epigeic group). The species identification was performed according to Pilsko [18] and Kasprzak [19].

This whole procedure was performed twice, after harvest of triticale (before sowing of stubble crops) and during the vegetation of stubble crops (10 weeks after the previous sampling procedure). The second sampling site within the plot (12 m^2) was designed 5 m from the previous sampling site. There were three replications of earthworm sampling within the plot in each series. The whole experimental area was 2025 square meters.

2.4. Weather Conditions and Soil Moisture

Air temperature (T*a*, °C) and precipitation (P, mm), as well as soil temperature (T*s*, °C) and soil moisture (M*s*, %) were monitored during vegetation season. Figure 2. shows environmental conditions for sampling sites of earthworm occurrence in the field. T*a* and P were obtained from Kraków-Balice Meteorological Station, IMGW-PIB (no. 350190566, 4.7 km far from experimental area) and computed as periods average (T*a*, °C) and periods sum (P, mm). Soil measurements were recorded in earthworm sampling sites precisely. They were taken in the triticale rhizosphere layer (5 cm in depth) with the SM150 Kit soil moisture sensor (Delta-T Devices), and CT2B-121 temperature sensor (ELMETRON) attached to a pH meter; recordings were taken once a week (except the last one, which was collected three weeks after the previous one). The additional soil moisture measurements were recorded at the bottom of soil monoliths handsorted in the second sampling time (in stubble crops), at the depth of 40 cm.

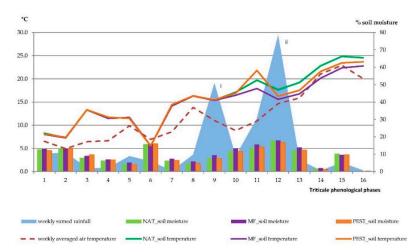


Figure 2. Above- and underground thermal-humidity conditions of sampling sites. ⁱ P = 50.69 mm, ⁱⁱ P = 78.37 mm.

2.5. Statistical Analyses

For earthworm biomass (g·m⁻²) parameters and two sampling terms, a Wilcoxon matched-pairs test ($\alpha = 0.05$) was performed using Statistica PL ver. 13.1, StatSoft, Krakow, Poland. Coefficients of

variation were also computed to illustrate the degree of homogeneity of the results (Table 2.) All of the graphical outputs were performed with MS Excel Tools (including a radar chart).

Stubble Crop Species ⁱⁱ	Tillage Term	Triticale Management ⁱ	Earthworm Biomass (g·m ⁻²) After Triticale Harvest ⁱⁱⁱ	Earthworm Biomass (g·m ⁻²) in Stubble Crops
White mustard	autumn	NAT	0.00 (0)	0.00 (0)
		MF	0.00 (0)	2.43 (173)
		PEST	0.00 (0)	1.87 (173)
	spring	NAT	0.56 (151)	4.72 (28)
	1 0	MF	4.15 (87)	9.96 (108)
		PEST	0.15 (173)	1.42 (173)
Buckwheat	autumn	NAT	0.03 (173)	0.13 (173)
		MF	1.24 (173)	9.45 (77)
		PEST	5.65 (172)	2.35 (162)
	spring	NAT	0.49 (173)	2.06 (173)
	1 0	MF	0.02 (173)	8.15 (87)
		PEST	0.75 (118)	5.97 (63)
Tansy phacelia	autumn	NAT	2.27 (161)	2.05 (151)
5 1		MF	1.35 (173)	6.89 (119)
		PEST	1.34 (88)	3.66 (107)
	spring	NAT	0.00 (0)	2.29 (173)
		MF	3.25 (82)	2.59 (94)
		PEST	1.33 (167)	1.41 (173)

Table 2. The changes in earthworm presence as a fresh biomass parameter $(g \cdot m^{-2})$ within 10 weeks in various stands.

ⁱ NAT = natural; MF = Mineral fertilization; PEST = mineral fertilization + pesticide; n = 3, ⁱⁱ Wilcoxon matched-pairs test $\alpha = 0.05$, p = 0.005618, ⁱⁱⁱ coefficient of variation is presented in brackets, high values occurred when no t all replicates reported the earthworms' presence.

3. Results

As the results of samplings were obtained from the tillage system area, the output of earthworm individuals is relatively low, contrary to those acquired from pasture [20] or other permanent crops [9]. But even in agricultural lands, where ploughing treatments were employed, the presence of earthworms is visible. The ploughed continuous cereal stands belong to the poorest stands for soil organisms due to the necessity of plant protection treatments, soil fatigue, losses of nutrients. The fresh biomass of earthworms ($g \cdot m^{-2}$) collected from triticale stands varied correspondingly to the management method of triticale (Table 2). Immediately after the terminating of triticale plants, the highest amount of this parameter occurred in MF objects (on average 1.67 g·m⁻²). The lowest earthworm fresh biomass was obtained from NAT objects (on average 0.56 g·m⁻²). The introduction of stubble crops on these stands enhanced the average biomass sampled within each square meter, although the tendency remained the same, i.e., the highest value occurred in MF objects 6.58 g·m⁻², and the lowest in the NAT objects, on average 1.87 g·m⁻². The earthworm preference of particular stubble crops species is presented in detail on the random chart (Figure 3).

The most preferred species was buckwheat (*Fagopyrum esculentum*) (Figure 3B) due to 43.1% of earthworms of all life stages being collected in plots covered by buckwheat. Tansy phacelia rhizosphere enticed 30.3% of all earthworms, and mustard plants only 26.6%. Cocoons were observed only in the first sampling term (after triticale harvest) (Figure 3A). The plots prepared for buckwheat contain 53.3% of all cocoons (Figure 3A), which gives, after 10 weeks, 42.0% of all juveniles in buckwheat stands as the hatchlings constituted a negligible part of the population in stubble crops' rhizosphere (eight individuals in total). The abundance of earthworm's life cycle structure reveal two main directions (increases and decreases of particular life stages). During 10 weeks, matured earthworms statistically significantly enhanced four times in all objects (Wilcoxon matched-pairs test $\alpha = 0.05$, p = 0.005618), of which the most grew in buckwheat stands (by 55%). Then, the juveniles increased almost two fold, giving again the most abundant group penetrating the buckwheat area (by 49%). The negative

changes were recorded in cocoons (as above), hatchlings, and pre-matured individuals. Taking into consideration the type of stand (stubble crop species), it could be stated that hatchlings dropped the most in tansy phacelia sites (by 58%), and pre-matured earthworms have developed into matured forms rather than escaped from buckwheat stands (57% decrease in this group in buckwheat stands compared to 55% increase in matured forms in buckwheat) (Figure 3A,B).

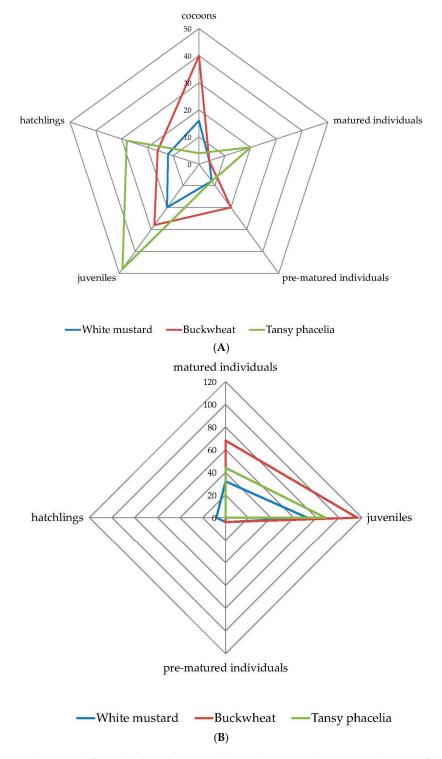


Figure 3. Earthworm's life cycle abundance and their changes within 10 weeks, (**A**) after triticale harvest, (**B**) in stubble crops.

The changes of soil pH during the experiment is depicted in Figure 1. Stands without either fertilization or pesticides had a little higher pH value with no significant difference between objects. During the whole vegetation season (after sowing till harvest) pH values oscillated between 5.20 and 5.69 and seem to be a constant parameter for developing earthworm individuals. It is worth noting that values from a higher range have always been taken from objects' NAT. The median pH value for NAT, MF, and PEST objects were, respectively, 5.51, 5.39, and 5.37 (Figure 1).

During the whole vegetation season, the occurrence of fresh coprolites was monitored. Figure 1 contains the overall results of field inspection (the bigger the distance of red dots from the *x*-axis, the more frequent the occurrence of coprolites). The most frequent occurrence of coprolites during all seasons on buckwheat stands (51%) were recorded (unpublished data). The other two stubble crop species contained within their area 39% of the fresh coprolites (tansy phacelia) and 10% (white mustard). The peak of produced coprolites coincided with the booting phase of triticale (BBCH 41–43) (Figure 1) and bigger rainfalls before (Figure 2).

Comparing the values of soil moisture recorded in triticale (Figure 2) to those obtained in stubble crops (Table 3), the more preferable environment for earthworms in more dense cover (stubble crops) is highlighted (in the range between 10.53 and 15.10%; median equal to 12.84%). The 'bottom' moisture (see: '2.4 Weather conditions and soil moisture') range was between 14.10 and 16.57% (16.19% value of median) and those recordings suggest the earthworms were forced to choose between fresh organic matter in the plant rhizosphere and higher humidity in the deeper layers of the soil profile. The depth of arable layer in all sites was on average 34.2 cm (±3.97).

Stubble Crop Species	Tillage Term		Moisture (%)			
			Triticale Management	at the Top	in the Bottom	 Depth of Arable Layer (cm)
		8	of Sampled Soil N	Monoliths in Stubble Crops		
White mustard	autumn	NAT	11.97 ± 0.404	14.10 ± 0.361	28	
		MF	12.03 ± 2.303	14.27 ± 2.203	35	
		PEST	14.63 ± 3.493	16.37 ± 1.457	40	
	spring	NAT	14.30 ± 0.954	16.20 ± 2.778	40	
	1 0	MF	14.90 ± 0.608	16.53 ± 0.611	34	
		PEST	15.10 ± 0.458	15.83 ± 1.002	34	
Buckwheat	autumn	NAT	12.50 ± 1.389	15.03 ± 1.159	33	
		MF	11.50 ± 3.005	14.40 ± 2.600	36	
		PEST	12.97 ± 3.386	15.60 ± 0.400	39	
	spring	NAT	12.70 ± 1.015	16.03 ± 1.210	28	
		MF	13.23 ± 2.470	16.27 ± 1.206	29	
		PEST	11.47 ± 2.403	16.33 ± 1.724	35	
Tansy phacelia	autumn	NAT	12.80 ± 2.718	16.17 ± 1.739	31	
		MF	12.87 ± 1.890	16.37 ± 1.582	31	
		PEST	14.43 ± 0.981	16.37 ± 0.379	38	
	spring	NAT	12.57±1.457	16.57 ± 1.747	37	
	- 0	MF	13.47 ± 1.943	15.87 ± 3.009	30	
		PEST	10.53 ± 2.483	16.20 ± 0.529	37	
average			13.00 ± 1.283	15.81 ± 0.802	34.2	

Table 3. Soil moisture and depth of arable layer in sampling sites in the second sampling series (in stubble crop stands).

* n = 3, \pm standard deviation (*sd*); objects' descriptions as in Table 2.

4. Discussion

Determining ecological preferences of particular earthworm species, along with their trophic behaviour, is assumed as the complete earthworm autecology approach. In the field conditions, where tillage occurred, their abundance is minimalized along with agrotechnical intensifications and frequency of plant protection treatments [5,6,21]. Moreover, cereal monocultures do not promote considerable amounts of earthworms [15]. The results revealed low amounts of earthworm biomass

(average of all objects equals 1.25 g·m^{-2}) and strong domination of the *O. cyaneum* Savigny species (above 95% of all sampled earthworm individuals; unpublished data). However, anthropogenic factor may have also enhanced soil fertility, even in difficult stands, i.e., monoculture. The introduction of stubble crops is a common good practice, which supplies soil systems with additional portions of fresh organic material [22], mobilizes rhizospheric organisms through root exudates, interrupts soil fatigue (phytosanitary plants) and can limit agricultural pests through allelopathic substances. According to studied stubble crop species, all of them act as weed suppressors; additionally, buckwheat and white mustard limit development of *Elateridae* larvae (wireworm) and nematodes, respectively [23].

Among the tested stubble crops, buckwheat enticed earthworms (particularly *O. cyaneum* Savigny) the most (Figure 3B). With regard to Froseth et al. [9], earthworm density was increased when grass and *Trifolium pratense* were left in the field as a mulch. The authors demonstrated that the number of earthworms increased 1.4 to 2.6 times in the first year after mulching. The earthworm biomass increased 1.2 to 3.3 times relative to the control (without mulches). In the present study, the term of ploughed stubble crops (2nd factor) didn't differentiate the earthworm harvest in the first year of mulch occurrence. The obtained results didn't reveal considerable variations in moisture content (Table 3), which could be interpreted as a stable water consumption within the studied stubble crop species.

Agricultural environment shaped by various abiotic factors is the second crucial factor of earthworms' development. Sanchez et al. [24] tried to determine the autecology of 28 earthworm species along with 22 soil factors. The ecological profiles technique revealed a preference of *O. cyaneum* for high values of porosity and C content, as well as lower pH values, which could be partly in line with our results due to the sandy medium of experimental area (Table 1). Palm et al. [20] constructed boosted regression tree models (BRTs) for predicting the distribution of ecological groups of earthworms (i.e., anecic, endogeic, and epigeic *sensu* Bouché) and they assumed that management practices, soil conditions and biotic interactions with other earthworm groups are the most important predictors for spatial distribution patterns. They also found little positive relationship between ploughing and endogeic frequency.

On the other hand, various species of endogeic earthworms can prefer the areas of particular plant species. This mechanism is, however, not yet thoroughly understood. First of all, earthworms communicate with the surrounding environment through the skin, which serves mainly as a breathing medium for them and their neuroreceptor-embedded surface. The mechanism of how the earthworms decided to consume encountered particles remains unrecognized. With regard to this issue, a few studies were performed. The vast majority of studies were carried out in laboratory [25,26]. Kliszcz and Puła [26] studied the ratio of food intake per 24 h to the average weight of the *L. terrestris* L. individuals, and the results revealed white mustard and buckwheat were more preferable (0.24 and 0.27, respectively) plants than tansy phacelia (0.14).

Field experiments are not so common due to many technical constraints and environmental factors. Ernst et al. [27] found the strongest reduction (by 36%) in biomass of *Octolasion tyrtaeum* in the occurrence of winter rape biomass (*Brassicaceae* family). This corresponds to the obtained results; the lowest amount of sampled individuals (and lower biomass $g \cdot m^{-2}$) revealed white mustard stand (the same plant family). It could be explained by the fact that the *Brassicaceae* family contains thioglycosides of volatile mustard oils in their tissues, which after being subjected to mechanical grinding and under the influence of the enzyme myrosinase, may decompose in white mustard to the toxic and irritating soft tissues *p*-hydroxybenzyl isothiocyanate [28].

Beside the type and chemical content of plant-derived organic matter, earthworms can choose the substrate by microbial species presence [29]. The palatability of microorganisms was studied by Dominguez et al. [30], Doube et al. [31], Neilson and Boag [32], Bonkowski et al. [33]. Jayasinghe et al. [34] found that *O. tyrtaeum* Savigny preferred organic matter inoculated with actinomycetes (*Streptomyces* sp.) in comparison with control (without inoculation).

O. cyaneum Savigny, the species found in the studied area, is an European omnipresent endogeic earthworm [20], which means that plant rhizosphere and horizontal galleries are the main habitat area

in the soil. For sure, it encounter many plant species, but it always has to make a choice between plants covered in a particular area. During unfavourable conditions (e.g., drought), however, its maintenance depends on diapause and burrowing down ability. They feed on fresh organic matter, microorganisms and consume large quantities of soil and accompanied organic residues [32], as well as can produced 1.88 mg dry wt·g⁻¹·fresh body of *Octolasion lacteum* wt·d⁻¹ [35]. Bonkowski et al. [33] found that the presence of *Cladosporium cladosporioides* attracts *O. cyaneum* 3 fold more than other fungal species. The obtained results indicated that the population of *O. cyaneum* may not develop the next generation (the lack of cocoons in stubble crops) before winter, when its development was limited by the moisture content during the triticale vegetation season.

5. Conclusions

A key biological component of the soil, earthworms, could be enticed by fresh root exudates derived from additional biomass sources in the field, like stubble crops in cereals, and they will also afterward benefit from stubble crop residues. After the first year of stubble crops, the presented results are slightly varied, but the tendencies could be seen: (i) During the whole vegetation season of triticale, the MF objects contain (after the triticale harvest) the highest earthworm biomass, (ii) After 10 weeks of recovery, the whole population of earthworms increased two fold (juveniles) and four fold (matured earthworms); (iii) The buckwheat rhizosphere seems to be the best stand for *O. cyaneum* Savigny, which chose these stands the most frequently (55% of all matured earthworms and 49% of all juveniles).

Stubble crops can serve as an enticement for particular earthworm species in agricultural intensively-managed fields, as well as being scrutinized in terms of their phytosanitary and pollinator function thus far.

Supplementary Materials: The following are available online at http://www.mdpi.com/2571-8789/4/3/39/s1, Figure S1: Graphic scheme of experimental design.

Author Contributions: Conceptualization, A.K.; Methodology, A.K., and J.P.; Writing—Original Draft Preparation, A.K.; Writing—Review & Editing, A.K.; Supervision, A.K. and J.P.; Funding acquisition, J.P. and A.K. All authors have read and agreed to the published version of the manuscript.

Funding: The research was financed by the Ministry of Science and Higher Education of the Republic of Poland.

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

References

- Mudrák, O.; Frouz, J. Earthworms increase plant biomass more in soil with no earthworm legacy than in earthworm-mediated soil, and favour late successional species in competition. *Func. Ecol.* 2018, *32*, 626–635. [CrossRef]
- 2. Angst, G.; Mueller, C.W.; Prater, I.; Angst, Š.; Frouz, J.; Jílková, V.; Peterse, F.; Nierop, K.G.J. Earthworms act as biochemical reactors to convert labile plant compounds into stabilized soil microbial necromass. *Commun. Biol.* **2019**, *2*, 441. [CrossRef] [PubMed]
- 3. Paoletti, M.G. The role of earthworms for assessment of sustainability and as bioindicators. *Agric. Ecosyst. Environ.* **1999**, *74*, 137–155. [CrossRef]
- 4. Curry, J.; Byrne, D.; Schmidt, O. Intensive cultivation can drastically reduce earthworm populations in arable land. *Eur. J. Soil Biol.* **2002**, *38*, 127–130. [CrossRef]
- 5. Briones, M.J.I.; Schmidt, O. Conventional tillage decreases the abundance and biomass of earthworms and alters their community structure in a global meta-analysis. *Glob. Chang. Biol.* **2017**, *23*, 4396–4419. [CrossRef]
- Pelosi, C.; Toutousa, L.; Chiron, F.; Dubs, F.; Hedde, M.; Muratet, A.; Ponge, J.-F.; Salmon, S.; Makowski, D. Reduction of pesticide use can increase earthworm populations in wheat crops in a European temperate region. *Agric. Ecosyst. Environ.* 2013, 181, 223–230. [CrossRef]
- 7. Pelosi, C.; Barot, S.; Capowiez, Y.; Hedde, M.; Vandenbulcke, F. Pesticides and earthworms. A review. *Agron. Sustain. Dev.* **2014**, *34*, 199–228. [CrossRef]

- 8. Riley, H.; Pommeresche, R.; Eltun, R.; Hansen, S.; Korsaeth, A. Soil structure, organic matter and earthworm activity in a comparison of cropping systems with contrasting tillage, rotations, fertilizer levels and manure use. *Agric. Ecosyst. Environ.* **2008**, *124*, 275–284. [CrossRef]
- 9. Froseth, R.B.; Bakken, A.K.; Bleken, M.A.; Riley, H. Effects of green manure herbage management and its digestate from biogas production on barley yield, N recovery, soil structure and earthworm populations. *Euro J. Agron.* **2014**, *52*, 90–102. [CrossRef]
- 10. Buck, C.; Langmaack, M.; Schrader, S. Influence of mulch and soil compaction on earthworm cast properties. *Appl. Soil Ecol.* **2000**, *14*, 223–229. [CrossRef]
- 11. Kuzyakov, Y.; Blagodatskaya, E. Microbial hotspots and hot moments in soil: Concept & review. *Soil Biol. Biochem.* **2015**, *83*, 184–199.
- 12. Rochfort, S.J.; Ezernieks, V.; Yen, A.L. NMR-based metabolomics using earthworms as potential indicators for soil health. *Metabolomics* **2009**, *5*, 95–107. [CrossRef]
- 13. Fründ, H.C.; Graefe, U.; Tischer, S. Earthworms as Bioindicators of Soil Quality. In *Biology of Earthworms*; Springer: Berlin/Heidelberg, Germany, 2011; pp. 261–278.
- 14. De Lima, A.C.R.; Brussaard, L. Earthworms as soil quality indicators: Local and scientific knowledge in rice management systems. *Acta Zool. Mex.* **2010**, *2*, 109–116.
- 15. Sizmur, T.; Martin, E.; Wagner, K.; Parmentier, E.; Watts, C.; Whitmore, A.P. Milled cereal straw accelerates earthworm (Lumbricus terrestris) growth more than selected organic amendments. *Appl. Soil Ecol.* **2017**, *113*, 166–177. [CrossRef] [PubMed]
- 16. Bauer, H.P.; Beckett, P.H.T.; Bite, W. A rapid gravimetric method for estimating calcium carbonate in soils. *Plant. Soil* **1972**, *37*, 689. [CrossRef]
- 17. Warzyński, H.; Sosnowska, A.; Harasimiuk, A. Effect of variable content of organic matter and carbonates on results of determination of granulometric composition by means of Casagrande's areometric method in modification by Prószyński. *Soil Sci. Annu.* **2018**, *69*, 39–48. [CrossRef]
- 18. Pilsko, J.D. Fauna Poloniae. Lumbricidae; PWN Publisher: Warsaw, Poland, 1973; pp. 88–98.
- Kasprzak, K. Skąposzczety Glebowe III. Rodzina Dźdżownice: Lumbricidae; Polish Academy of Sciences-Institute of Zoology, PWN: Warsaw, Poland, 1986; pp. 97–100.
- 20. Palm, J.; Van Schaik, N.L.M.B.; Schroder, B. Modelling distribution patterns of anecic, epigeic, and endogeic earthworms at catchement-scale in agroecosystems. *Pedobiologia* **2013**, *56*, 23–31. [CrossRef]
- 21. Ivask, M.; Kuu, A.; Sizov, E. Abundant of earthworm species in Estonian arable soils. *Eur. J. Soil Biol.* 2007, 43, S39–S42. [CrossRef]
- 22. Schmidt, O.; Curry, J.P.; Hackett, R.A.; Purvis, G.; Clements, R.O. Earthworm communities in conventional wheat monocropping and low-input wheat-clover intercropping systems. *Ann. Appl. Biol.* **2001**, *138*, 377–388. [CrossRef]
- 23. Noronha, C. Crop rotation as a management tool for wireworms in potatoes. *IOBC/wprs Bull.* 2011, 66, 467–471.
- 24. Sanchez, E.G.; Munoz, B.; Garvin, M.H.; Briones, M.J.I.; Cosin, D.J.D. Ecological preferences of some earthworm species in southwest Spain. *Soil Biol. Biochem.* **1997**, *29*, 313–316. [CrossRef]
- 25. Lowe, C.N.; Butt, K.R. Influence of organic matter on earthworm production and behaviour: A laboratory-based approach with applications for soil restoration. *Eur. J. Soil Biol.* **2002**, *38*, 173–176. [CrossRef]
- 26. Kliszcz, A.; Puła, J. Assessment of earthworms activity based on eaten biomass from selected catch crops. *Anna. Univ. Paedagog. Crac. Stud. Nat.* **2019**, *4*, 81–90. [CrossRef]
- 27. Ernst, G.; Henseler, I.; Felten, D.; Emmerling, C. Decomposition and mineralization of energy crop residues governed by earthworms. *Soil Biol. Biochem.* **2009**, *41*, 1548–1554. [CrossRef]
- 28. Sawicka, B.; Kotiuk, E. Gorczyce jako rośliny wielofunkcyjne. Acta Sci. Pol. Agric. 2007, 6, 17–27.
- 29. Zirbes, L.; Thonart, P.; Haubruge, E. Microscale interactions between earthworms and microorganisms: A review. *Biotechnol. Agron. Soc. Environ.* **2012**, *16*, 125–131.
- 30. Dominguez, J.; Briones, M.J.I.; Mato, S. Effect of the diet on growth and reproduction of Eisenia andrei (Oligochaeta, Lumbricidae). *Pedobiologia* **1997**, *41*, 566–576.
- Doube, B.M.; Schmidt, O.; Killham, K.; Correll, R. Influence of mineral soil on the palatability of organic matter for Lumbricid earthworms: A simple food preference study. *Soil Biol. Biochem.* 1997, 29, 569–575. [CrossRef]

- 33. Bonkowski, M.; Griffths, B.S.; Ritz, K. Food preferences of earthworms for soil fungi. *Pedobiologia* **2000**, 44, 666–676. [CrossRef]
- 34. Jayasinghe, B.A.T.D.; Parkinson, D. Earthworms as the vectors of actinomycetes antagonistic to litter decomposer fungi. *Appl. Soil Ecol.* **2009**, *43*, 1–10. [CrossRef]
- 35. Scheu, S. The role of substrate feeding earthworms (Lumbricidae) for bioturbation in a beechwood Soil. *Oecologia* **1987**, 72, 192–196. [CrossRef] [PubMed]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).