

Original Research

Effects of Irrigation and Organic Mulching on the Abundance and Biomass of Earthworms

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Abstract

Soil is an important medium of biomass production due to storing, transforming, and harmonizing natural resources. This experimental site (Kungyalu, Central-East Hungary) on Chernozem soil was examined based on: a) irrigation (linear – LI; drum irrigation – DI; no irrigation – NI); and b) mulching (mulched – M; unmulched – U). The objective was to investigate the effect of irrigation and organic mulching (wheat straw) on earthworm abundance, biomass and species composition. The earthworm abundance and biomass under U (60.7 ind m⁻², 16.8 g m⁻²) and M (68.1 ind m⁻², 17.3 g m⁻²) were greater in autumn compared to summer (U: 16.6 ind m⁻², 3.14 g m⁻²; M: 29.9 ind m⁻², 6.6 g m⁻²), but the difference was only significant in summer. Significantly greater earthworm abundance and biomass were found under NI in autumn (92.5 ind m⁻², 23.9 g m⁻²). Five earthworm species were detected (*Aporrectodea caliginosa*, *Aporrectodea rosea*, *Aporrectodea georgii*, *Proctodrilus opisthoductus*, *Octolasion lacteum*), mostly juveniles. Soil mulching significantly increased earthworm abundance and biomass in summer, while irrigation significantly decreased it.

Keywords: irrigation, organic mulching, earthworm, tillage

Introduction

Soil is a medium for human life, space for flora and fauna, and in relation to the human life cycle it is considered as a non-renewable natural resource [1]. Additional important functions of the soil are storing rainwater and providing space for food production. Hungary has a large agricultural area (4,334,000 ha), which accounts for about 46.6% of the total land area

[2], where continental climate dominates. Due to lack of infrastructure, farmers currently irrigate approximately 100,000 hectares out of the possible 200,000 hectares [3].

In the last few decades tillage practice has changed, and currently, in addition to moldboard ploughing, conservation agriculture (CA) has also increasingly been implemented [4]. One of the main fundamental principles of CA is the retention of crop residues on the surface with minimal soil disturbance and proper crop rotation [5], thus, by this requirement, soil quality condition may be improved and preserved [6]. There is a positive correlation between straw mulching and

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water use efficiency [7], it plays a prominent role in water conservation and fertility enhancement [8]. Organic mulch enhances soil microbial properties and enzyme activity [9], and at the same time it reduces soil penetration resistance [10] and weed infestation [11]. Soil tillage reflected in the various environmental factors in soil [12], furthermore it affects the level of soil organic matter (SOM) [13].

Invertebrates living in the soil influence the linkage between microbial activity and crop yield [14]. Jouquet et al. 2006 [15] proved that earthworms can improve the physico-chemical and biological properties of soil. The activities of earthworm affect soil water content [16], usually improve soil structural stability and soil porosity [17]. Since the earthworm-fragmented litter was mechanically mixed into the soil [18], earthworms significantly affected soil organic carbon content through physical conditions [19].

Moreover, various stressors by human activities, and among those tillage and its intensification, and especially moldboard ploughing, have strong negative effects on earthworm abundance and biomass. Johnston et al. 2018 [20] stressed that tillage as mechanical manipulation can affect earthworms directly (injuring or killing them mechanically) or indirectly (destroying the living environment by changing the physical conditions of the soil - primarily water content, temperature and reducing food supply) [21]. Negative effects of tillage on the abundance and biomass of anecic earthworms was detected, which lasted up to two years after treatments [22]. In central Hungary, a field experiment with six different tillage methods investigated the impact on soil moisture content and earthworm population dynamics in detail, concerning climatic and soil conditions [23]. The lowest abundance or complete absence of earthworms was observed during the summer months (June to August), while the highest abundance was observed during spring (April and May) and late fall (October and November).

Climate extremes and their consequences are of great interest both globally [24], and locally [25]. One of the key elements in global and regional climate change is water and its resources. The potential for reducing water use in crop production varies significantly, from soil mulching to reducing soil evaporation [26]. In addition, the suitable application of controlled irrigation and drainage can save fresh water and reduce nutrient losses [27]. In areas where certain yields are failed to be achieved even through rational soil water management, irrigation as an agrotechnical measure may be improved.

In this study, the objective was to investigate the effects of irrigation (drum, linear irrigation and no irrigation) and organic mulching on earthworm abundance, biomass and species composition. We hypothesized that a) those areas which receive organic mulching would have greater earthworm abundance and biomass compared to the control plots, which did not receive mulch treatment; b) non-irrigated

area would have lower earthworm abundance compared to the irrigated ones.

Material and Methods

Experimental Site, Irrigation Methods and Soil Mulching

The experimental site is located near Kungyalu (GPS coordinates: 46°54'47.2"N-20°15'46.7"E), in Jász-Nagykun-Szolnok County, Hungary (Fig. 1). The topography of the area is flat and homogenous, and the soil type is Endocalcic Chernozem (Loamic) according to the World Reference Base for Soil Resources [28], with loam texture.

The experiment was set in the summer of 2015 and lasted till the summer of 2018. The applied tillage methods and the produced crops were the same on the whole area throughout the research period. Soybean (*Glycine max*, L.; in 2015), seed corn (*Zea mays*, L.; in 2016), maize (*Zea mays*, L.; in 2017) and sunflower (*Helianthus annuus*, L.; in 2018) were sown in the experimental field. The details of the agricultural management are shown in Table 1.

Regarding the irrigation methods, the area was divided into three main parts as shown in Fig. 1: linear irrigation (LI), drum irrigation (DI), and no irrigation (NI). The irrigation water was provided by the West branch of the „Main Channel of Nagykunság”. The physical and chemical parameters of the irrigation water met the requirements set by „FVM regulations” (90/2008 (VII. 18.)), and the quality of the irrigation water was in the first-class category. The irrigation schedule was adjusted to the climatic conditions, i.e. to air temperature and precipitation in case of both irrigation systems. Irrigation with two methods (DI and LI) was applied on the same day (three times in 2015; six times in 2016 and 2017; once in 2018). The irrigation intensity was 20 mm ha⁻¹ in all cases.

The soil mulching was carried out manually by using wheat straw in a 10×10m area on each treatment (M – mulched treatments) after sowing in about 6 cm thick layer (30.000 kg ha⁻¹). The areas that did not receive straw mulching were called “unmulched treatments” (U).

Meteorological Data

The mean annual precipitation of the area is 520 mm (based on the 1965-1995 average). During the examination period, the precipitation was very erratic (i.e. the driest was 2015 with only 453.8 mm and the rainiest was 2017 with 610.3 mm). This was found below the thirty-year average, i.e. there was only 517 mm precipitation measured (Szolnok). In 2015 and 2016, the precipitation was average (453.8 and 583.4 mm), while in 2017, there was greater amount of precipitation measured in this area, i.e. 120% of the

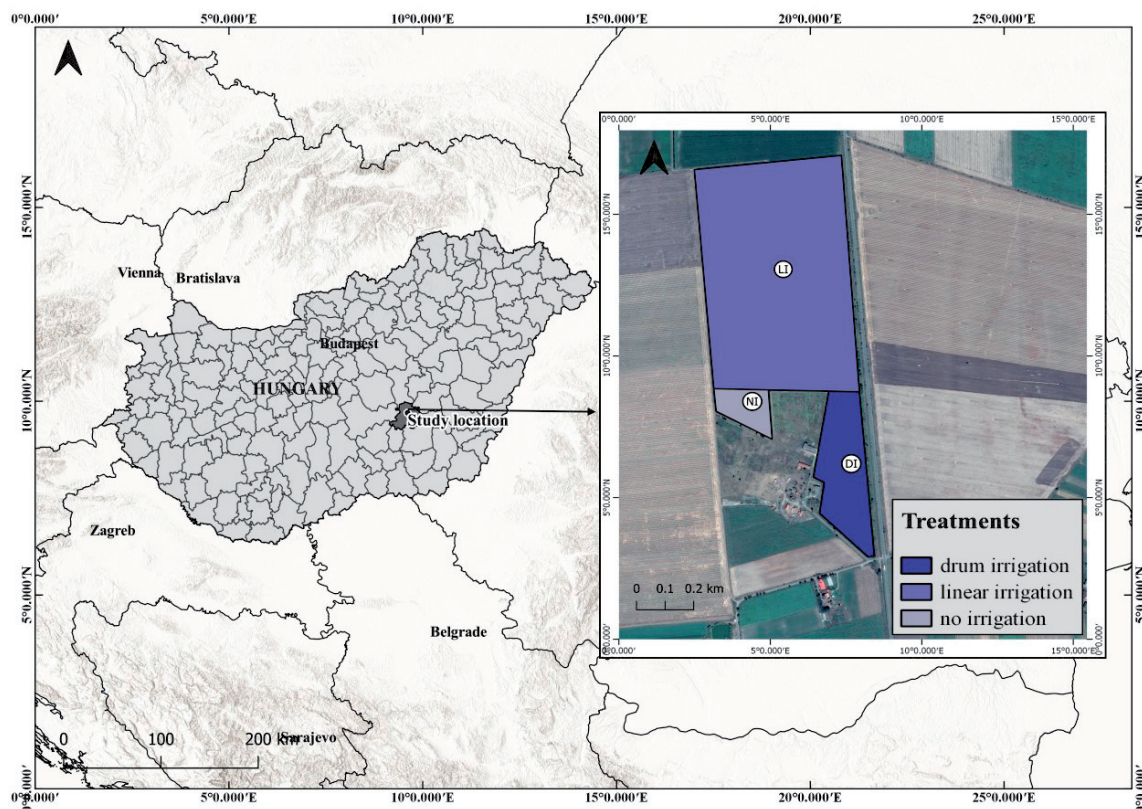


Fig. 1. The experimental area (Kungyalu) in Jász-Nagykun-Szolnok County, Hungary. Drum irrigation (DI), linear irrigation (LI), and with no irrigation (NI).

normal amount. During 2018, precipitation amounts in February, March, June, and July were above average (66.2; 87.0; 66.4; 94.9 mm), while during sunflower sowing and germination (April and May) they were below average (10.8 and 11.5 mm). Given the climatic conditions during 2018, with the aim of encouraging better germination, irrigation was used once.

Soil Chemical Parameters

The soil samples for chemical analyses were taken from the top 30 cm layer in five repetitions in all treatments on 9th April, 2016. The soil chemical parameters were examined according to the methods by the Official Hungarian Standards (MSZ) [29-31]. The pH(KCl) was determined in 1:2.5 soil to 1 N KCl solution ratio with digital pH meter (HACH-LANGE, HQ411D) potentiometrically [29]. The humus content was determined by the [31] 2.1 ("Testing organic carbon content in soils") by spectrophotometer. The CaCO₃ content was examined by volumetric method with 10% HCl by Scheibler calcimeter [29]. The available nutrient content of the soil samples was determined by the Official Hungarian Standards [31]. The NO₃⁻-NO₂⁻-N content (MSZ-20135:1999, 5.4.5) was determined by Contiflo Analyzer from KCl extract. The P₂O₅ content (MSZ-20135:1999, 5.4.2) was also determined by Contiflo Analyzer. The K₂O content

(MSZ-20135:1999, 5.3) was determined by atomic absorption spectroscopy (Table 2).

Soil Physical Parameters

The soil texture was determined by the upper limit of liquid limit according to Arany [32].

The soil moisture content (SMC) values were measured by PCE SMM1 analyzer in the topsoil (0-7.5 cm) at all treatments, nearby the SPR points (% v/v).

The soil penetration resistance (SPR) values were measured in three replicates per treatments at random locations, 5-10 m away from each other nearby the soil mulching treatments. Measurements were taken twice a year (in autumn and spring) considering the weather conditions. For the SPR measuring a handheld Szarvas-type penetrometer (Mobitech, Hungary) was used which was installed with 1 cm² conical point in area with 60° angle. Data were grouped in five soil layers (0-7.5; 7.5-15; 15-22.5; 22.5-30 and 30-37.5 cm) and presented in MPa.

Earthworm Sampling

The earthworm abundance and biomass were assessed twice a year from 2015 to 2018 according to the ISO Standards [33]. The sampling was carried out from

Table 1. Schedule for the applied agricultural management methods.

Year/Crop	Management	Depth	Seeding rate	Doses	Date
2014/2015 <i>Soybean</i> (SG Eider)	Fertilization (PK 10-28) Disking Seedbed preparation Sowing Fertilization (NP starter) Plant protection (Agrichem Bentazon)* Desiccation (Reglone)** Harvest Disking	8 cm 5 cm 4 cm 8cm	420 000 ha ⁻¹ 2416 kg ha ⁻¹	250 kg ha ⁻¹ 370 kg ha ⁻¹ 2 l ha ⁻¹ 2.5 l ha ⁻¹	28 th October 2014 29 th October 2014 5 th and 15 th April 2015 18 th April 2015 18 th April 2015 10 th May 2015 25 th August 2015 17 th September 2015 20 th September, 2015
2015/2016 <i>Seed maize</i> (PR38A24)	Fertilization (NPK 5-10-30+3S+5CaO+3MgO) Subsoiling Seedbed preparation Sowing Fertilization (N 34) Plant protection (Lumax)*** Inter row cultivation Harvest Stubble residue mulching	35 cm 6 cm 6 cm 8 cm 10 cm	66 000 ha ⁻¹ 770 kg ha ⁻¹	230 kg ha ⁻¹ 100 kg ha ⁻¹ 4.5 l ha ⁻¹	12 th October 2015 20 th October 2015 14 th April 2016 20 th April 2016 20 th April 2016 30 th April 2016 16 th May 2016 1 st September 2016 6 th September 2016
2016/2017 <i>Maize</i> (P9486)	Fertilization (NPK 5-10-30 +3S+5CaO+3MgO) Ploughing Seedbed preparation Sowing Fertilization (N 34) Plant protection (Lumax)*** Inter row cultivation Harvest Stubble residue mulching	30 cm 6 cm 5 cm 8 cm 10 cm	72 000 ha ⁻¹ 8500 kg ha ⁻¹	250 kg ha ⁻¹ 100 kg ha ⁻¹ 4.5 l ha ⁻¹	27 th October 2016 3 rd November 2016 10 th April 2017 18 th April 2017 18 th April 2017 4 th May 2017 24 th May 2017 9 th October 2017 12 th October 2017
2017/2018 <i>Sunflower</i> (P64LE25)	Fertilization (NPK 5-10-30) Disking Subsoiling Seedbed preparation Sowing Fertilizing (NP 15-25) Plant protection (Express 50 SX)**** Harvest Disking	12 cm 25 cm 5 cm 5 cm 10 cm	50 000 ha ⁻¹ 2960 kg ha ⁻¹	250 kg ha ⁻¹ 100 kg ha ⁻¹ 45 g ha ⁻¹	3 rd November 2017 5 th November 2017 17 th November 2017 7 th April 2018 15 th April 2018 20 th April 2018 29 th April 2018 17 th September 2018 20 th September, 2018

*Active ingredients in Bentazone (480 g/l bentazone); **Active ingredients in Reglone (37,3% diquat-dibromide + pyrazinedium dibromide); ***Active ingredients in Lumax (37,5 g/l mesotrione + 375,0 g/l S-metolachlor + 125,0 g/l terbuthylazine); **** Active ingredients in Express 50 SX (500g/kg tribenuron-methyl)

Table 2. Results of soil chemical parameters.

Treat-ments	pH (KCl)	Humus (%)	CaCO ₃ (%)	NO ₃ ⁻ + NO ₂ ⁻ (mg kg ⁻¹)	NSL-N	P ₂ O ₅ (mg kg ⁻¹)	NSL-P	K ₂ O (mg kg ⁻¹)	NSL-K
NI-M	7.5	2.6	4.3	16.8	Medium	178	Medium	335	Appropriate
LI-M	7.4	2.5	3.6	11.3	Medium	182	Medium	264	Medium
DI-M	7.4	2.5	4.3	14.3	Medium	188	Medium	260	Medium
NI-U	7.4	2.6	3.6	14.8	Medium	166	Medium	323	Appropriate
LI-U	7.5	2.7	4.4	13.9	Medium	126	Weak	240	Weak
DI-U	7.4	2.4	2.1	16.1	Medium	241	Good	313	Appropriate

Abbreviations: NI: non-irrigation; LI: linear irrigation; DI: drum irrigation; M: mulched soil; U: unmulched soil; NSL - nutrient supply level (N - nitrogen; P - phosphorus; K – potassium, respectively)

the autumn of 2015 till the summer of 2018; i.e. a total of six times during the three years, with the following sampling times: 12th September 2015, 10th June and 20th September 2016, 19th July and 10th October 2017, and 24th June 2018. The earthworms were sampled in 24 soil blocks (50×50×20cm) in one sampling season, i.e. eight samples per treatment. In each irrigation treatment, four samples were taken from the mulched (M) and four from the unmulched (U) treatments. Hand-sorting was applied *in situ* in all treatments. Earthworm were killed in 70% ethanol, then fixed in 4% formalin solution. The abundance was calculated to m² area (ind m⁻²). The biomass of earthworms was measured in the laboratory and expressed in g m⁻². The earthworm species were determined based on the external and internal characteristics of earthworms by the identification key offered by [34, 35].

Statistical Analyses

One-way analysis of variance (ANOVA) was used to examine the effect of irrigation (non-irrigation, linear, drum) on the abundance and biomass of earthworms. In the case of a significant result of the ANOVA, the groups with significant difference were determined by Tukey HSD (Honestly Significant Difference) post hoc test. The Tukey HSD test can also be used for multiple pairwise comparisons on samples of different sizes. Previously to the ANOVA analysis, all parameters were checked for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test).

Since the two-sample t-test may be sensitive to the impairment of normality, and the result of the Kolmogorov-Smirnov test was significant in most cases, for comparing the mean values of soil penetration resistance, abundance and biomass of earthworms, for the two groups based on the treatments (mulched, unmulched) the F-test was used. The IBM SPSS Statistics 25 statistical software package was used for all the statistical analyses.

Results and Discussion

The obtained *pH(KCl)* values were between 7.4 and 7.5, while the *humus content* was between 2.4 and 2.7%. The highest was in case of linear irrigation, unmulched (LI-U) treatment. The *CaCO₃ content* was between 2.1 (DI-U) and 4.4% (LI-U). These basic soil chemical parameters were relatively homogenous throughout the areas under the different treatments. Regarding the *plant available nitrogen content* (NO₃⁻ and NO₂⁻), it was between 11.3 and 16.8 mg kg⁻¹. Concerning the nutrient supply levels (NSL) of the soils based on the Hungarian standards, the plant available nitrogen content was considered "medium" in all cases, under M and U treatments as well. The *P₂O₅ content* was between 178 and 188 mg kg⁻¹ under M treatments, thus, they fell in the "medium" category. However, under

U treatments they were between 126 and 241 mg kg⁻¹, which means the following nutrient supply levels were gained: „weak” (LI-U: 126 mg kg⁻¹); „medium” (NI-U: 166 mg kg⁻¹); and „good” (DI-U: 241 mg kg⁻¹). As for the *available K₂O content*, it was between 240 and 335 mg kg⁻¹. The K₂O content was considered „weak” (LI-U: 240 mg kg⁻¹); „medium” in two cases (DI-M: 260 and LI-M: 264 mg kg⁻¹); and all the others were „appropriate”.

Soil physical parameters, the texture of the examined soils determined by the upper limit of liquid limit according to Arany was between 40-43, which means that the textural classes were loam and clay loam.

According to the soil moisture content (SMC) measured at 7.5 cm, significantly greater values were found in the summer compared to the autumn sampling period under mulched (M) and unmulched (U) treatments as well. The SMC was 25.3±1.4 % v/v in U and 32±0.6 % v/v in M treatments in the summer (*p*<0.05), while only 22.3±2.1 % v/v in U and 23.1±1.6 % v/v in M in the autumn sampling periods. The SMC of the M was almost 7% greater than the U treatments in summer, which is probably due to the positive effect of mulching, i.e. lower evaporation and sun exposure, thus less moisture loss. In autumn there was no difference between the two treatments. In our region, also in summer Kalmár et al. (2013) [36] achieved 8-11% more moisture in undisturbed soil with 55-65% soil surface cover. According to the above, with higher soil coverage we can reduce water consumption during the season of irrigation. Li et al. (2020) [37] examined the effect of organic mulch on SMC and found increased SMC values under greater mulch treatment (100%) compared to the control and the lower mulch cover (20, 40, and 60%).

According to the results of soil penetration resistance (SPR) (measured at depths of 7.5; 15.0; 22.5; 30.0 and 37.5 cm), the values generally increased with depth (Table 3). The highest value (3.73 MPa) was measured in drum irrigated, unmulched plots (DI-U) at 37.5 cm depth (in autumn). Significant differences were detected in all cases between the two measurement times (summers and autumns), within the same depths and treatments (a, b). However, in NI-U treatments significant difference was only detected in one case, i.e. NI-U autumn (2.62 MPa) was significantly greater than NI-U summer (1.99 MPa) at 22.5 cm depth. Furthermore, between the two measurement times, the summer data were more favorable (lower values) compared to the autumn data, probably due to irrigation in the summer, which resulted higher SMC.

During the summer measurements, more favorable SPR values were measured in the unmulched and irrigated areas compared to the NI areas; there were significant differences detected between NI-U and LI-U (*p*<0.05). However, in the autumn measurements there were greater SPR values measured under the irrigated areas (LI and DI), thus there was no significant difference found as compared to NI sites,

Table 3. Results of soil penetration resistance.

Measuring time	Depth (cm)	Treatments									
		NI-U	LI-U	DI-U	Mean	SD	NI-M	LI-M	DI-M	Mean	SD
Summers	-7.5	1.51 ^{aA}	0.64 ^{aB}	0.98 ^{aAB}	1.04	0.44	0.89 ^{aA}	0.60 ^{aA}	0.60 ^{aA}	0.70	0.17
	-15.0	1.75 ^{aA}	0.83 ^{aB}	1.30 ^{aA}	1.29	0.46	1.26 ^{aA}	0.99 ^{aA}	1.05 ^{aA}	1.10	0.14
	-22.5	1.99 ^{aA}	0.89 ^{aB}	1.75 ^{aA}	1.54	0.58	1.39 ^{aA}	1.34 ^{aA}	1.34 ^{aA}	1.36	0.03
	-30.0	2.64 ^{aA}	1.17 ^{aB}	2.15 ^{aA}	1.99	0.75	1.64 ^{aA}	1.61 ^{aA}	1.70 ^{aA}	1.65	0.05
	-37.5	3.21 ^{aA}	1.75 ^{aB}	2.63 ^{aAB}	2.53	0.74	1.93 ^{aA}	1.85 ^{aA}	2.03 ^{aA}	1.94	0.09
	Mean	2.22	1.06	1.76	1.68	0.59	1.42	1.28	1.34	1.51	0.07
	SD	0.70	0.44	0.67	0.59		0.40	0.50	0.56	0.48	
Autumns	-7.5	1.81 ^{aA}	1.90 ^{bA}	2.02 ^{bA}	1.91	0.11	1.27 ^{aA}	1.46 ^{bA}	1.56 ^{bA}	1.43	0.15
	-15.0	2.25 ^{aA}	2.31 ^{bA}	2.07 ^{bA}	2.21	0.12	1.84 ^{bA}	1.80 ^{bA}	1.82 ^{bA}	1.82	0.02
	-22.5	2.62 ^{bA}	3.03 ^{bA}	2.98 ^{bA}	2.88	0.22	2.12 ^{bA}	2.42 ^{bA}	2.37 ^{bA}	2.30	0.16
	-30.0	3.02 ^{aA}	3.35 ^{bA}	3.34 ^{bA}	3.24	0.19	2.54 ^{bA}	2.79 ^{bA}	2.86 ^{bA}	2.73	0.17
	-37.5	3.32 ^{aA}	3.68 ^{bA}	3.73 ^{bA}	3.58	0.22	2.77 ^{bA}	3.12 ^{bA}	3.39 ^{bA}	3.09	0.31
	Mean	2.60	2.85	2.83	2.76	0.14	2.11	2.32	2.40	2.49	0.31
	SD	0.62	0.74	0.77	0.70		0.59	0.68	0.75	0.67	

The different lowercase letters (a, b) in the columns indicate significant differences among the measured depths between summers and autumns, and different uppercase letters (A, B) in rows indicate significant differences among type of irrigation treatments at $p < 0.05$ (Tukey HSD post hoc test); treatments: NI: non-irrigation; LI: linear irrigation; DI: drum irrigation; M: mulched soil; U: unmulched soil.

since at this time irrigation was not carried out and the effect of soil settling was more noticeable. There was no significant difference found between irrigated and NI sites under M treatments (A, B) during the summer measurements, however, the irrigated sites were more favorable compared to the NI sites, except for DI-M (1.70MPa at -30 cm) and DI-M (2.03MPa at -37.5 cm).

The summer SPR values were more favorable (lower) compared to the autumn data. Since irrigation was carried out during the summer months, under these plots greater SMC was achieved in the summer time, thus probably due to this fact the SPR values were lower in the soil with moister soil condition. Within the summer measurement times, when the U and M were compared, lower SPR, thus more favorable values, were obtained in the U, irrigated areas (LI-U, DI-U) compared to the NI-U areas, and several significant differences were detected between NI-U and LI-U. Souza et al. (2021) [38] in water-controlled environments under different SMC observed that in the wet conditions average SPR was 2.8MPa, while during the dry period it increased to 14.4 MPa, however some SPR values were higher than 34MPa.

The most favorable SPR values were detected in the NI-M treatments in the autumn measurements among the M treatments, and the least favorable one was measured on average at DI-M (average: 2.40MPa) sites. Generally, when the autumn measurements were analyzed, greater SPR values (less favorable) were measured under the irrigated areas (LI, DI), thus in autumn, there was no significant difference found

as compared to NI sites. This time of the year, irrigation was not carried out any more and the effect of soil settling due to the kinetic force of the irrigation water applied during summer times was more and more noticeable, thus greater SPR values were gained. The kinetic force of rain in contribution to the stronger soil settling was confirmed by Nanko et al. (2015) [39]. Recent research further confirms that the phenomenon was strongly dependent on soil quality [4]. When the effect of mulching was concerned in our experiment in the autumn samples, lower SPR values were detected under M than under U treatments. In this case, the mulching probably provided physical protection on the soil surface against evapotranspiration, thus by the greater SMC content, lower SPR values were obtained. Our results are in agreement with Dean and Merry (2015) [40] who found that mulched treatments provided lower SPR values when compared to unmulched treatments.

Furthermore, the difference between the two non-irrigated treatments in the summer and autumn samplings was significant, too; however, mulched plots (NI-M) showed more favorable results (summer: 1.42 MPa and autumn: 2.11 MPa on average) as compared to unmulched plots (NI-U) (summer: 2.22 MPa and autumn: 2.60 MPa on average). Thus, the application of the organic mulching material caused measurable positive effect on SPR values in our experiment. Most of the authors found that SPR values over 3 MPa in slightly moist soil harmfully influence plant development [41]. In our case, this value (>3 MPa) was

only reached in one case during the summer sampling period (NI-U: 3.21 MPa at -37.5 cm), and only in deeper layers during the autumn measurement period (-30 and -37.5 cm mostly in U treatments).

During the cropping season, the climatic conditions, such as precipitation, both higher temperature and evapotranspiration had strong influence on SPR fluctuation.

Earthworm Abundance, Biomass, and Species Composition

When the effect of irrigation was analyzed, significantly greater earthworm abundance and biomass values were found under the non-irrigated sites in autumn samplings (92.5 ind m⁻², 23.9 g m⁻², respectively) (Fig. 2a-b), however, there was no significant differences detected in the summer sampling periods. Agricultural practices and their intensification, such as tillage, irrigation, the use of pesticides, crop rotation as well as SMC can affect the biomass and abundance

of earthworms [42, 43]. While tillage, in most cases, negatively affects earthworm abundance and biomass [44], irrigation usually has a positive effect [45]. In our experiment, however, significantly greater earthworm abundance and biomass values were found under the NI sites in autumn. The values were almost twice as much as the abundance and biomass under LI (55.5 ind m⁻², 14.7 g m⁻²) and DI (45.3 ind m⁻², 12.6 g m⁻²). This finding was opposite to our first hypothesis. In our opinion, our results can be related to the greater SPR values measured under LI and DI sites in autumn. The SPR values (Table 3) were almost always greater in LI and DI as compared to NI sites, however, the difference was not significant. The greatest SPR values (>3MPa) were usually gained in lower layers (-30 and -37.5 cm), especially under U treatments. This greater compaction could result in greater SMC in the upper soil layer, thus, the soil habitat in this layer is moister and colder which was apparently not preferred by earthworms. These higher soil compaction values probably did not let the precipitation infiltrate through the soil profile quickly and easily. Thus, the negative effect of irrigation was proved by our findings.

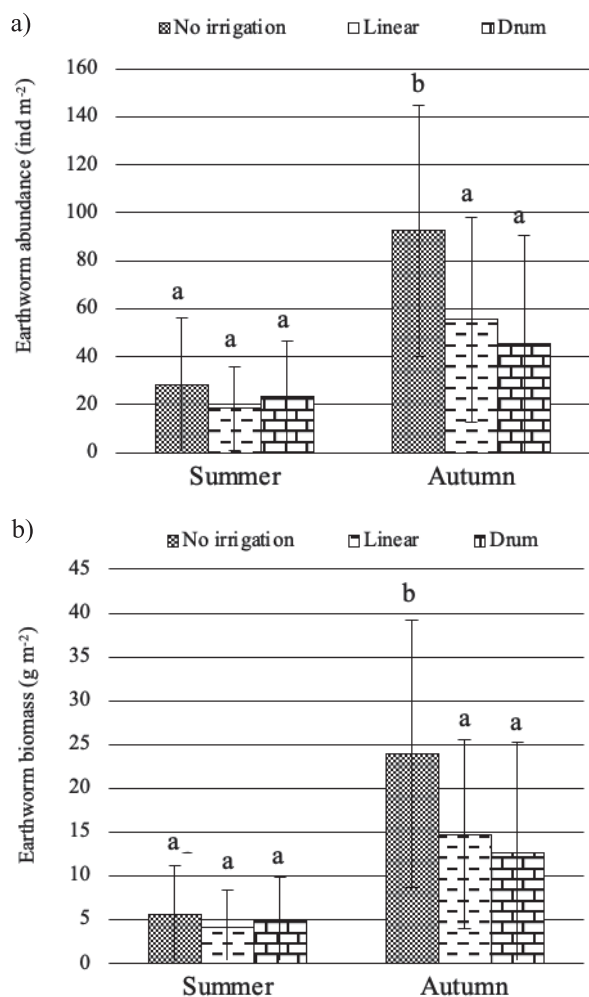


Fig. 2a) Abundance of earthworms (ind m⁻²) The lowercase letters (a, b) indicate significant difference between the irrigation treatments (Tukey HSD post hoc test); b) biomass of earthworms (g m⁻²) The lowercase letters (a, b) indicate significant difference between the irrigation treatments (Tukey HSD post hoc test).

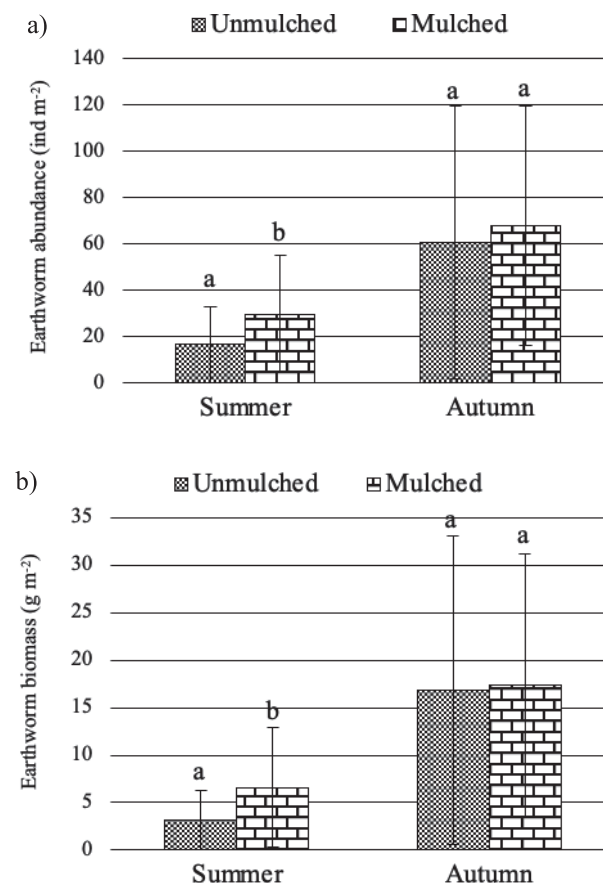


Fig. 3a) Abundance of earthworms (ind m⁻²) The lowercase letters (a, b) indicate significant difference between mulched and unmulched treatments (Tukey HSD post hoc test); b) biomass of earthworms (g m⁻²) The lowercase letters (a, b) indicate significant difference between mulched and unmulched treatments (Tukey HSD post hoc test).

Table 4. Ratio of earthworm species (%) under mulched and unmulched treatments.

Treatments	Sampling time	<i>A. caliginosa</i>	<i>A. rosea</i>	<i>A. georgii</i>	<i>P. opisthoductus</i>	<i>O. lacteum</i>
Mulched (M)	Autumn 2015	92.3	5.7	0	1.9	0
	Summer 2016	68.75	31.25	0	0	0
	Autumn 2016	30	70	0	0	0
	Summer 2017	50	50	0	0	0
	Autumn 2017	46.67	26.67	26.67	0	0
	Summer 2018	60	24	8	8	0
Unmulched (U)	Autumn 2015	64	28	8	0	0
	Summer 2016	33.33	16.67	50	0	0
	Autumn 2016	36.36	54.55	9.09	0	0
	Summer 2017	0	0	0	0	0
	Autumn 2017	53.33	23.33	20	3.33	0
	Summer 2018	66.67	22.22	0	0	11.11

Greater earthworm abundance and biomass values were found in the autumn sampling periods under both mulched (M) and unmulched (U) treatments (Fig. 3a-b). The earthworm abundance and biomass under U (60.7 ind m⁻²; 16.8 g m⁻², respectively) and M (68.1 ind m⁻²; 17.3 g m⁻², respectively) treatments were higher in the autumn samples compared to the summer samples (U: 16.6 ind m⁻²; 3.14 g m⁻², respectively; M: 29.9 ind m⁻²; 6.6 g m⁻², respectively). The difference of earthworm abundance and biomass between M and U treatments was only significant in case of the summer sampling, showing greater values under the M treatments. In our first hypothesis, we assumed that the areas which receive organic mulching (M) would have greater earthworm abundance and biomass compared to the control plots (U), was justified. The organic mulching material that was applied on these areas might have served as food source for earthworms, protection against wind and water erosion, and probably helped in decreasing evaporation. Results from other researchers [46, 47] showed that soil covering has an extremely important role in soil structure protection and moisture conservation. However, the positive effect of mulching in our experiment was only significantly different in the summer periods, and the abundance and biomass were almost the same in the autumn periods. Based on a two-year study (2014 and 2015) Abail and Whalen (2018) [48] found that earthworm species composition was similar among treatments. There were 2.3 times more earthworms under higher plant residues and almost twice as much biomass compared to lower amount of plant residues. The number of earthworms and biomass were positively correlated with the weight of the surface cover (number of individuals: $r_s = 0.74$, $p < 0.001$, $n = 36$; biomass: $r_s = 0.55$, $p < 0.001$, $n = 36$).

Greater earthworm abundance and biomass values were found in all cases in the autumn sampling periods compared to the summer sampling times. The earthworm abundance values were almost four times greater, while the biomass values were five times greater on average in autumn compared to summer samplings. The soil environmental conditions, especially SMC and soil temperature, are usually better during spring and autumn seasons [23], and earthworm sampling should be carried out in these seasons. However, in this research, mulching and irrigation were also applied, thus one might think that the sampling season in this case becomes a little more flexible. Apparently, we measured lower earthworm abundance and biomass values in the summer samplings, which were probably compensated to some extent by mulching, i.e. the loss of soil moisture was probably lower under these treatments.

Five earthworm species were detected in our study. These were the following in decreasing order: *Aporrectodea caliginosa* (Savigny, 1826), *Aporrectodea rosea* (Savigny, 1826), *Aporrectodea georgii* (Michaelsen, 1890), *Proctodrilus opisthoductus* (Zicsi, 1985), *Octolasion lacteum* (Örley, 1881) (Table 4). The two of the most dominant earthworm species were *A. caliginosa* and *A. rosea*. These two species were present in all samples except for the summer of 2017 under unmulched (U) treatment, when only juveniles were detected. The majority of the earthworms was juvenile.

Conclusions

Based on the two main aspects of our examinations (organic mulching and irrigation) it can be stated that greater earthworm abundance and biomass were found in autumn samplings under M and U treatments as

well. The organic mulching (M) helped in retaining SMC during the summer period, which resulted in significantly greater earthworm abundance and biomass values. This finding agreed with our first hypothesis. On the other hand, earthworm abundance and biomass detected under NI sites in autumn samplings were significantly greater than under DI and LI. Our original hypothesis was that irrigation treatment would have positive effect on earthworm abundance and biomass, however, the opposite was proved. We concluded that the reason for this might be that the kinetic energy of the irrigation water could destroy the soil structure in the topsoil drastically, which might have resulted in plugging the earthworm burrows. This way the positive effect of earthworms, i.e. soil aeration, loosening activity, etc. could not be expressed. Soil compaction, thus, was more pronounced under DI and LI sites and it probably resulted in higher SMC and colder habitable space for prolonged time for earthworms in the upper layers, which are not favored by these animals. The abundance and also the biomass were twice as much under the NI sites, which means that in this case the irrigation, regardless of which type (LI or DI), had strict negative effect on earthworm abundance and biomass.

Abbreviations

CA – conservation agriculture; SOM – soil organic matter; NI – no irrigation; LI – linear irrigation; DI – drum irrigation; M – mulched soil; U – unmulched soil; SPR – soil penetration resistance; SMC – soil moisture content; NSL – nutrient supply level.

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Conflict of Interest

The authors declare no conflict of interest.

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