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Earthworm Abundance Increased by Mob-Grazing Zero-Tilled Arable Land in South-East England

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Abstract: Regenerative agriculture is a potential alternative to conventional agricultural systems. It integrates the components of zero-tillage, permanent soil cover, diverse crop rotations and rotational or mob-grazing by ruminant livestock. Earthworms are beneficial soil macrofauna and function as indicators of soil health. A need exists to identify how earthworm populations are affected when all four regenerative agriculture components are implemented simultaneously. This study investigates earthworm abundance in three split-plot treatments located on adjacent land within the same farm: (1) ungrazed permanent grassland, (2) a three-year grass-clover ley within an arable zero tillage system without grazing and (3) identical to treatment 2 but with mob-grazing. Earthworms were sampled using soil pits and classified into four functional groups: epigeic (surface dwellers), endogeic (sub-surface), anecic (deep soil) and juveniles. The total earthworm count, epigeic and juvenile functional groups were significantly ($p \leq 0.05$) higher in treatment (3), the arable zero tillage system with mob-grazing. Mob-grazing increases the diversity of carbon sources available to earthworms and has a positive impact on earthworm abundance and functional group diversity within the arable rotation under evaluation.



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Keywords: adaptive paddock grazing; anecic; earthworm; endogeic; epigeic; mob-grazing; regenerative agriculture; zero-tillage

1. Introduction

‘Conservation Agriculture’ (CA) is comprised of three elements: zero/no-till or direct seeding, permanent cover cropping to prevent soil exposure and a diverse crop rotation of at least three different crops [1]. Together these are credited with increased levels of soil carbon (C) sequestration, improved soil structure and reduced soil erosion and increased biodiversity. A second system, Regenerative Agriculture (RA), adopts the same three tenants but adds a fourth, the grazing of livestock within the crop rotation [2–4]. This fourth element is also termed multi-paddock, adaptive paddock or mob-grazing.

Mob-grazing is a rotational grazing system that utilises a high stocking density and frequent movement of animals. It aims to simulate the movement of large herds of herbivorous animals that graze areas for short periods then move on to locate new forage [5–7]. It differs to more traditional rotational grazing systems in that it grazes a smaller area (known as compartments) with a greater number of animals for a shorter period of time [5], typically hours rather than days [6,7]. Crucially, after removal of the livestock the grass and herb species are permitted to grow and accumulate biomass to a stage beyond that present before the onset of the original grazing period [8]. The animals are then returned at a date in the future, typically after a minimum period of eight weeks although a mob-grazed compartment may only be grazed once or twice per annum. Grazing should not proceed to a level where the plant is grazed excessively such that either root growth is diminished or

that the lowest growing point is removed preventing regrowth [9]. Ideally only the upper part of the plants are consumed. These components will then be recycled as urine and faeces. Under a mob-grazing system foraged areas receive an extended period of recovery before livestock are allowed to return, resulting in plants that are taller with well-developed root systems and hardy above-ground biomass [10]. A proportion of the plant material not eaten will sustain physical damage by livestock trampling and remain on the soil surface [11]. This increases the diversity of the organic C molecules available to micro-organisms and detritivores within the soil. Further, the flattened vegetation affords a layer of protection as a mulch on the soil surface. All four RA components complement each other, but all must be adopted in combination in order to derive maximum benefit. The critical factor with this approach is that the root systems and lower growing points are maintained [9] and that overgrazing the area through retaining the livestock in a given compartment too long or providing insufficient recovery time is avoided [12]. Farming systems in the UK have become more specialised, tending towards either arable or livestock, with a decline in the total area of mixed farms [13]. Present grazing tends to be continuous for a period of several weeks, with limited rotation of stock between fields or compartments. A lack of livestock within predominantly arable areas or excessive grazing rates where present results in the implementation of only selected aspects of the RA system and the failure to realise all potential benefits. Enhanced earthworm abundance is one such potential benefit.

Earthworms are macrofauna, acting as 'eco-system engineers' that improve the movement of water, air and nutrients through the soil [14]. They are associated with improved crop yield and above-ground biomass [15] and function as indicators of enhanced soil health [16]. A key question to address is how does mob-grazing within an arable system impact on earthworm abundance overall, and do individual functional groups respond differently? The impact of tillage is well documented and depends on the earthworm functional group. The deep burrowing anecic species are affected adversely by conventional soil inversion tillage systems [17–20]. The deep, permanent vertical burrows they construct are destroyed by the inversion process while the worms themselves sustain physical damage due to their large size [21]. Endogeic species are less susceptible, burrowing within the horizontal soil plane and constructing temporary burrows in the upper (10–30 cm soil layer). Epigeic species do not construct burrows, rather they are active in, and consume plant litter on the soil surface. In their meta-analysis, [17] outline that providing a food source for earthworms, either through crop residues, mulch or cover crops as part of a diverse crop rotation also has positive results. Direct reference to the impact of the mob-grazing of grass leys within arable rotations and earthworm abundance in the published literature is however sparse. This study aims to identify how earthworm populations and individual functional groups are affected when all four components of RA, including mob-grazing, are implemented.

2. Materials and Methods

2.1. Site Description

Sampling was completed at a farm practicing mob grazing located near Stevenage, Hertfordshire, UK (nearest climate station Rothamsted) mean 30 year annual rainfall 712.3 mm, mean minimum and maximum annual temperature 6.0–13.7 °C [22]. The dominant soil texture at each sample location in all treatments is silty-clay-loam (SiCL) stagnic luvisol (LVj), pH 6.5.

2.2. Field Treatments

Fields were selected in consultation with the participating land manager. Plots were identified adjacent to one another where the recent crop history was identical for each, except for the presence or absence of mob grazing. The cropped area had been managed commercially under the current zero-tillage regime for 11 years at the time of sampling. Direct seed sowing was implemented by a John Deere® (Langar, Nottinghamshire, UK) 750A direct drill. Further soil samples were taken from a permanent ungrazed grass

margin immediately adjacent to the crop perimeter to provide a non-cropped control that eliminated all crop production operations. Three land use and management treatments 15 hectares (ha) in total were evaluated as a split-plot design:

1. zero tillage + grass-clover ley (ZT)
2. zero tillage + grass-clover ley + mob-grazing (ZTMG)
3. permanent grassland (PG)

Key variables within the three treatments are summarised in Table 1. The ley was sown three years previously and included perennial ryegrass (*Lolium perenne*), red and white clover (*Trifolium pratense* L. and *Trifolium repens* Walter.), chicory (*Cichorium intybus* L.) and plantain (*Plantago* spp.). Self-seeding annually forms the fertility-building component within an arable zero-tillage crop rotation with winter wheat (*Triticum aestivum* L.), spring barley (*Hordeum vulgare* L.) and field beans (*Vicia faba* L.). No supplementary nutrients or crop protection products were applied to the grass ley or permanent grassland. Treatment (2) was grazed with a minimum of 70 yearling Beef Shorthorn Cross cattle (British Cattle Movement Service breed code: BSHX [23]) confined to a 0.4 ha cell for a period of 12 or 24 h every eight weeks.

Table 1. Summary of management for the three treatments.

Management	Treatment		
	ZT	ZTMG	PG
Permanent grass	-	-	✓
Previous soil inversion	11 years	11 years	-
Annual cropping	✓	✓	-
3-year grass-clover ley	✓	✓	-
Mob-grazing ley with cattle	-	✓	-

2.3. Earthworm Sampling

Earthworms were sampled in the autumn (October) 2021 when the soil moisture content was stipulated as optimal by [24], and using the approach described by [24], in which ten sampling pits 20 cm × 20 cm × 20 cm were dug in each of the three treatments. The locations were determined by handheld GPS (Garmin® eTrex 10 (Garmin (Europe) Ltd., Southampton, UK)). The soil from each pit was decanted onto a plastic sheet and hand sorted for earthworms. All individuals without a saddle (clitellum) were recorded as ‘juvenile’. Adult specimens were sub-divided by ecological functional group according to [24] and counted: epigeic (small red), endogeic (pale/green) and anecic (large heavily pigmented). After counting, all earthworms were returned to the sampling pit and sprayed with water when necessary to prevent desiccation.

2.4. Soil Analysis

Soil texture was determined according to ([25], (Annex 4)). A silty-clay-loam (SiCL) Stagnic Luvisol was present at each sample location [25]. Soil samples of 100 g were transferred to a labelled air-tight sealable plastic bag and stored at 4 °C before the analysis of moisture content and soil organic matter (SOM) in the laboratory. Soil moisture and SOM were determined according to the approach of [26]. Soil organic matter contained 58% C [26].

2.5. Statistical Analysis

The total count, mean count and standard error of the mean (SEM) were determined for the three adult earthworm functional groups and juvenile individuals. Data normality was determined by the Kolmogorov-Smirnov test and, in the absence of a normal distribution, statistical analysis proceeded with the Kruskal–Wallis test. Where a significant relationship existed (Asymp. Sig (or p) ≤ 0.05) a further analysis was conducted with the Mann–Whitney U test to determine which treatments were significantly different. Only two treatments

could be compared at any time, three tests were run per category (treatment 1 + 2, 1 + 3, 2 + 3) to cover all combinations. All statistical tests were run using the IBM® SPSS version 27 statistical package. A Canonical Correspondence Analysis (CCA) and Hierarchical Cluster Analysis (HCA) were completed using the PAleontological STatistics (PAST) version 4.09 (January 2022) statistical software [27].

3. Results

Total earthworm counts were highest in the ZTMG treatment, with counts consisting mainly of endogeic species and juveniles (Figure 1). Similar overall counts were observed for ZT and PG, there were however proportionally greater numbers of juveniles in the PG.

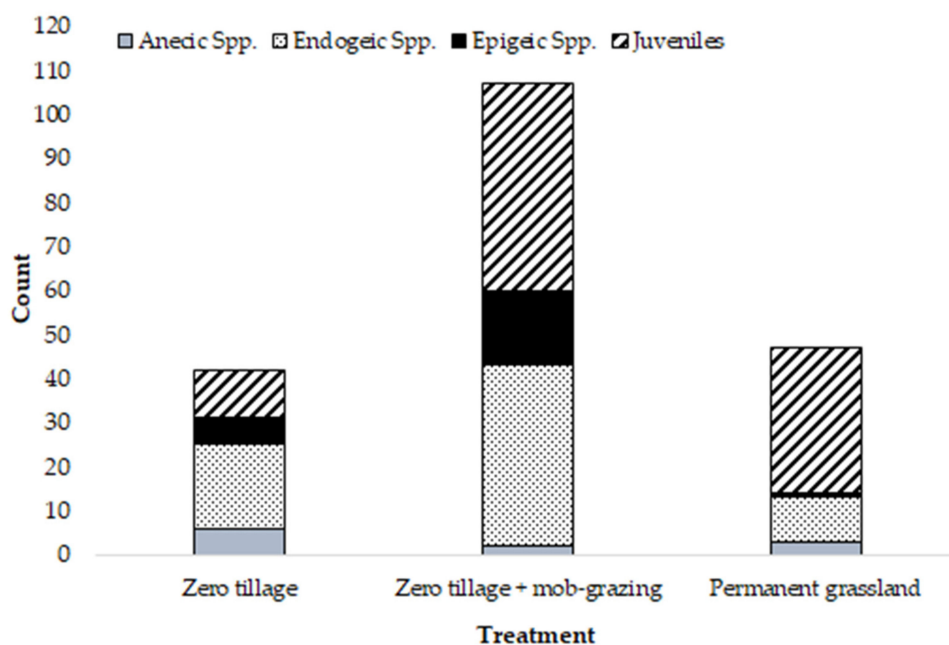


Figure 1. Counts of earthworm functional group for three treatments (ZT: zero tillage; ZTMG: zero tillage + mob-grazing; PG: permanent grassland), *n* = 10 per treatment.

All data were analysed for normality with the Kolmogorov–Smirnov test. The Kruskal–Wallis test indicated that a significant difference existed between total earthworm count, juveniles and epigeic species, and the soil physical parameters of soil moisture and SOM (Table 2). There was no significant difference between the three treatments for counts of anecic and endogeic species.

Table 2. Kruskal–Wallis test statistical output for three treatments (* *p* (Asymp. sig) ≤ 0.05; ** *p* (Asymp. sig) ≤ 0.01).

	Total Count	Anecic spp.	Endogeic spp.	Epigeic spp.	Juveniles	% Moisture	% SOM
Kruskal-Wallis	9.89	0.412	5.774	11.026	8.902	13.025	7.884
H							
df	2	2	2	2	2	2	2
Asymp. Sig.	0.007 **	0.814	0.056	0.004 **	0.012 *	0.001 **	0.019 *

Where a significant difference was identified by the Kruskal–Wallis test, a further analysis using the Mann–Whitney test determined where a significant relationship [*p* (Asymp.sig) ≤ 0.05] existed between individual treatments. Although numbers of endogeic species were greater in the ZTMG treatment (Figure 2), this was not significant (Table 3). Epigeic species were however present in significantly (*p* ≤ 0.05) higher

numbers in the ZTMG treatment relative to the ZT and PG treatments (Table 2), although lower in abundance compared to endogeic species. This was also applicable to juvenile specimens. The greater proportion of juveniles in the PG samples resulted in significantly higher [p (Asymp.sig) ≤ 0.05] numbers of juveniles in this treatment relative to the ZT treatment but not the ZTMG treatment. The total number for all functional groups and juveniles combined was significantly higher in the ZTMG treatment; the other treatments were not significantly different.

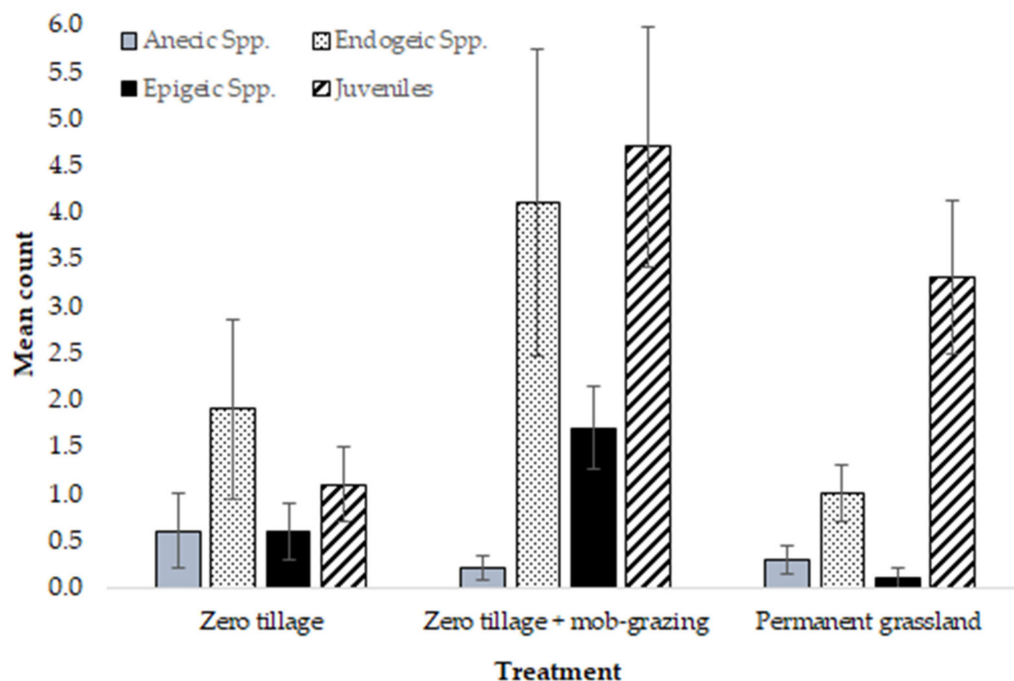


Figure 2. Mean count of earthworm functional group for three treatments (ZT: zero tillage; ZTMG: zero tillage + mob-grazing; PG: permanent grassland), $n = 10$ per treatment. Error bars \pm one standard error of the mean.

Table 3. Mann–Whitney test statistical output for three treatments [$* p$ (Asymp.sig) ≤ 0.05 ; $** p$ (Asymp.sig) ≤ 0.01].

	Total Count	Epigeic spp.	Juveniles	% Moisture	% SOM
zero tillage + mob grazing compared to zero tillage					
Mann–Whitney U	15.5	24.5	14.5	17	33
Wilcoxon W	70.5	79.5	69.5	72	88
Z	−2.625	−2.024	−2.727	−2.495	−1.287
Asymp. Sig. (2-tailed)	0.009 **	0.043 *	0.006 **	0.013 *	0.198
zero tillage compared to permanent grassland					
Mann–Whitney U	29.5	34.5	21	19	24.5
Wilcoxon W	84.5	89.5	76	74	79.5
Z	−1.564	−1.55	−2.25	−2.346	−1.928
Asymp. Sig. (2-tailed)	0.118	0.121	0.024 *	0.019 *	0.054
zero tillage + mob grazing compared to permanent grassland					
Mann–Whitney U	18	12.5	38	11	16.5
Wilcoxon W	73	67.5	93	66	71.5
Z	−2.443	−3.124	−0.92	−2.949	−2.534
Asymp. Sig. (2-tailed)	0.015 *	0.002 **	0.358	0.003 **	0.011 *

Soil moisture (Figure 3) was significantly different between all treatments (Table 2), ranging from a mean of 10.6% and 15.2% in the ZTMG and PG treatments, respectively.

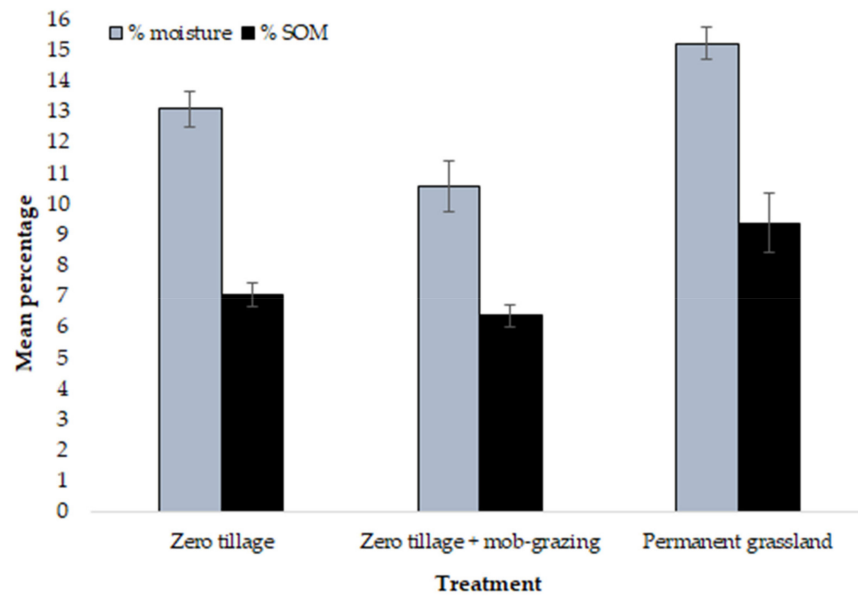


Figure 3. Mean percent soil moisture and soil organic matter (SOM) for three treatments (ZT: zero tillage; ZTMG: zero tillage + mob-grazing; PG: permanent grassland), $n = 10$ per treatment. Error bars \pm one standard error of the mean.

A CCA showing similarity between individual sample sites for functional group counts and the environmental variables of % SOM and % moisture distinguishes the three treatments (Figure 4). The zero tillage + mob-grazing treatment is characterised mainly by counts of epigeic species, PG by juvenile individuals, and the ZT sample sites by endogeic species.

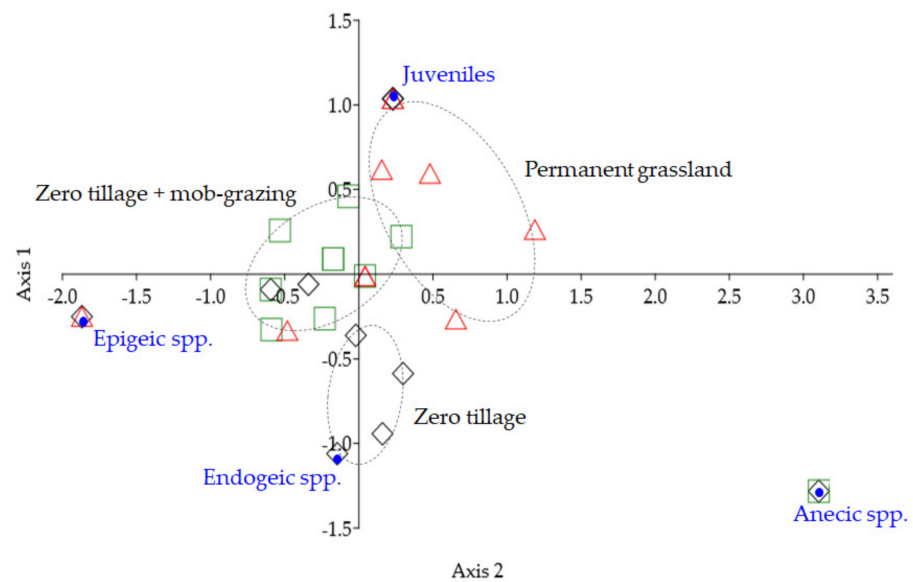


Figure 4. Canonical Correspondence Analysis of earthworm functional group, % soil organic matter and % soil moisture for three treatments (\diamond : zero tillage; \square : zero tillage + mob-grazing; \triangle : permanent grassland), $n = 10$ per treatment. Axis 1: Eigenvalue 0.0075, 96.6% variation; Axis 2: Eigenvalue 0.0003, 3.4% variation.

4. Discussion

Three variables have been evaluated within a split-plot design: ZT compared to PG (treatments 1 and 2 vs. treatment 3) and mob-grazing a grass ley within a ZT arable rotation (treatment 1 vs. treatment 2). Total earthworm abundance and the number of epigeic species were significantly higher in the arable rotation with mob-grazing relative to the treatment without grazing and the permanent grassland treatment. Each is discussed in turn.

4.1. Cultivation Legacy

None of the treatments currently apply soil inversion by ploughing. Zero tillage has been implemented in the ZT and ZTMG treatments for 11 years. The first question to consider is whether there is the potential presence of a legacy or historic management impact due to the previous cultivation of the land 11 years earlier. The overall impact of soil inversion on earthworm populations is reported as being varied, determined in part by their functional group and corresponding differences in life strategy and behaviour. Although an overall increase in numbers in response to zero- or reduced-tillage systems compared to soil inversion by ploughing is reported, [28–30] note that soil inversion may not decrease earthworm abundance overall but instead modifies the earthworm community structure, with an increase in one functional group simultaneously to the decline of another. A detrimental impact on epigeic and anecic species due to soil inversion is reported by multiple authors (for example [21,31–33]). Epigeic species utilise fresh organic residues on the soil surface. Soil inversion buries these, reducing access to what is their primary food source. Anecic species expend energy constructing permanent burrows within the vertical soil profile. Soil inversion destroys these, meaning that further energy is then expended to reconstruct them, energy that would have otherwise been utilised for reproduction [34]. Due to their large size, anecic species are also vulnerable to direct mechanical damage from the soil inversion process. This is further combined with a loss of soil moisture, increased risk of desiccation, and an increase in exposure to predation [18,35]. Not all earthworm functional groups are disadvantaged however by the soil inversion process. Endogeic species may benefit because their main source of nutrition, humic material, is integrated into the top 15–20 cm of the soil where they are primarily active [21,31–33,36]. Soil organic matter tends to stratify in zero- or reduced-tillage systems i.e., it is higher on the soil surface compared to the sub-surface soil layers [37,38]. This is out of the main activity zone of endogeic species. Ploughing homogenises the organic matter within the soil inversion zone, also the main area of activity of endogeic species. Although endogeic species construct burrows which are subsequently destroyed by the soil inversion process, they are temporary and would need to be reconstructed anyway. There is therefore no additional energy expenditure unlike in the anecic species functional group. Further, the associated decrease in soil bulk density facilitates this creation of temporary tunnels while their smaller body size reduces the risk of mortality caused by mechanical damage [17]. If the previous inversion of the soil was exerting a legacy impact, the number of epigeic and anecic individuals would be expected to be lower in the ZT and ZTMG treatments relative to the PG treatment. Likewise, counts of endogeic species would potentially be higher in the ZT and ZTMG treatment. This was not the case. Counts of endogeic and anecic species were not significantly different between the three treatments, suggesting the legacy impact of tillage, if it were ever present, is absent for these three functional groups. Epigeic counts were significantly higher but only in the ZTMG treatment, not both the ZT and ZTMG treatments relative to the PG treatment. Marinissen and Bosch [39] note that numbers may increase rapidly post-cultivation where soil conditions are favourable. Zero tillage is one potentially favourable management strategy implemented at the site, an increase in crop diversity and the inclusion of a grass-clover ley in the rotation is another.

4.2. Crop Diversification: Grass-Clover Ley

A second key question to address is the impact on the earthworm functional group of the grass-clover ley in the ZT and ZTMG treatments relative to the PG treatment. A combination of zero tillage and undersowing cereal crops with clover have been reported previously to increase earthworm abundance by up to 50% [40]. All three earthworm functional groups are reported to benefit from the inclusion of a ley within an arable rotation compared to a purely annual cropping system [40–43]. Soil cover is maintained all year during the time it is present; there is a greater proliferation of roots in the topsoil relative to annual crop and an increase in SOM upon removal [34]. It may be that this management strategy reduced any negative impact of soil inversion on epigeic and anecic species. It is also noted that the ley differs from permanent grassland in terms of the plant species content and structure. The roots of perennial ryegrass and clover present in the grass-clover ley are relatively shallow, predominantly in the top 15 cm [44], equivalent to the endogeic earthworm activity zone. Deeper-rooted grass species such as common bent (*Agrostis capillaris* Pourr.) and cocksfoot (*Dactylis glomerata* L.), applicable to the activity zone of anecic species, are present in the PG treatment. Plant residues with higher nitrogen (N) content, such as clover, positively impact earthworm abundance since the residues have a low C:N ratio (21–23:1 compared to 30:1 for perennial ryegrass, and 80:1 for wheat straw) which makes them more palatable [45–47]. The C:N ratio of the plant material also determines the composition of humic material in the soil and the value of that material to endogeic species [48]. Low C:N ratio N-fixing leguminous species such as clover [45–47] are present in the grass-clover leys of the ZT and ZTMG treatments but limited in the PG treatment. The anecic *Lumbricus terrestris* (L.) feeds preferentially on the residues of white clover [49]. Piotrowska et al. [50] identified that an increase in plant species diversity reduced earthworm diversity, mainly due to the more species diverse swards containing a lower proportion of legumes. This is not necessarily the case in all grasslands however in this case the PG treatment was limited in clover content. Both zero-tilled plots have management potentially favourable to earthworm populations. A combination of zero tillage and the presence of a grass-clover ley promotes a greater density of low C:N ratio plant root material in the topsoil [45–47]. This may, in part, explain the greater abundance of endogeic species in the ZT and particularly the ZTMG treatments, although not significantly, relative to the PG treatment. These factors in combination appear to be conducive to enhanced earthworm abundance in the top 20 cm of the soil profile at the time of sampling. The ZTMG treatment has an additional management parameter, the mob-grazing of the grass ley.

4.3. Livestock and Mob-Grazing

The ZTMG treatment introduces a further variable, mob-grazing by cattle. As there is no supplementary feed provided when the livestock are grazed there are no additional inputs of N or C into the system. What changes is the form and distribution of the material available to earthworms, namely the quantity of senescing leaves, the proportion of vegetation physically damaged by trampling and the deposition of urine and faeces. The process of grazing tends to decrease the amount of decomposing plant material present as senescing leaves [9] since older leaves are removed by the livestock before senescence occurs. Where permitted, senescence will occur relatively uniformly over the field. The deposition of urine and faeces varies spatially, with an estimated 10–20% of the area receiving coverage [51] although this is impacted by grazing intensity [52]. A risk of mortality exists however, due to trampling [51]. Another important question therefore is, is the form of the material in the ZTMG (grass-clover and manure) more preferential than the ZT (grass-clover) or the PG (native grass species)? Epigeic species were significantly more abundant in the ZTMG treatment relative to the ZT and PG treatments, although lower in abundance compared to endogeic species. The numbers of endogeic species were greater in the ZTMG treatment, although this was not significant. Although the trampling of vegetation and the soil surface may impact epigeic species negatively [51] it did not appear

to be the case for the mob-grazing system of the ZTMG treatment. The 12–24 h grazing period causes physical damage to the vegetation, providing a source of nutrition, but then provides unhindered forage for the epigeic earthworms after removal of the livestock.

Numerous authors report the benefit to earthworms of applying supplementary N in the form of organic manure to arable land, more so than grassland [53–55]. This was not strictly speaking the case in the ZTMG treatment, since in the absence of supplementary feed it was a change in the form of material rather than an additional input of N or C. Grazing deposition contains a greater proportion of readily available C molecules due to the breakdown of plant material during the ruminant digestive process [56–59]. High nutrient material is consumed preferably by earthworms according to [60,61], although this may depend on functional group. Grazing deposition is identified as a resource for earthworms specifically by [51,62,63]. The epigeic *Eisenia fetida* (Savigny.) is reported by [62] to utilise grazing deposition as a preferred source of nutrition and to benefit from higher livestock stocking rates. This would appear to be the case for epigeic species in the ZTMG treatment. The short-term (12–24 h) presence of high cattle stocking rates under mob-grazing did not appear to have a detrimental effect on earthworm abundance due to the physical impact of trampling.

There was no significant impact identified for anecic or endogeic species. The anecic functional group will, in a similar manner to epigeic species, utilise poorly decomposed plant material located on the soil surface [35,48]. Anecic species may also be attracted to grazing deposition but are potentially hindered by their greater activity within the vertical soil plane via permanent burrows, although [64] report they will move on the soil surface to locate dung patches. Endogeic earthworms are geophagous (i.e., feed on soil). They ingest organic matter before mixing it with soil particles and intestinal mucus as it moves through the gut [65]. The undigested material is then egested as casts [66,67]. The form of the organic matter that they consume is reported to be mostly humic, biologically degraded SOM that is physically and chemically stable (i.e., not fresh dung), however [48] argue that this is not necessarily the case. The endogeic *Aporrectodea caliginosa* (Savigny.) also utilises grazing deposition, suggesting a capacity to switch between sources depending on availability. Although not significantly different, endogeic species were more abundant in the ZTMG treatment, a possible reflection of the multiple organic C sources available. The split-plot design in the same field meant that soil type was identical for each treatment and not a further variable.

Earthworms may utilize manure as a key source of nutrition [51,62,63,68,69], the mob-grazing of the grass ley in the ZTMG treatment providing an additional form of organic C [56–59]. Teague et al. [70] outline four elements for successful mob-grazing and, critically, there needs to be enough time for plants to recover from defoliation. The same authors make an important distinction between high grazing pressure and high stocking density. A high stocking density grazes only the tops of the vegetation (no selectivity of the most palatable species) but critically, the animals are removed before grazing of the lower plant parts proceeds (the lower growing points and roots) which may occur at high grazing intensity. The ZTMG grazing system introduces high stocking density for short periods of time (12–24 h), potentially increasing deposition coverage but also permitting vegetation regeneration and leaf senescence when the livestock are removed. The variety of nutrient resource forms (low C:N ratio vegetation, physically damaged plant material, plant material processed by the ruminant digestive process) provides a diversity of food sources, potentially catering for each earthworm functional group. Future work to evaluate the temporal impact of livestock grazing over multiple years and at different times during the season, its duration and the length of the grass-clover ley recovery period would be of further interest.

5. Conclusions

The role of earthworms as ‘ecosystem engineers’ and their link with healthy soils is well documented. Enhancement of abundance and functional group diversity within

arable crop rotations is an important component of sustainable agroecosystem management. Differences in behaviour and morphology between earthworm functional groups requires that sources of nutrition vary in form, have a low C:N ratio and are present in different soil layers to reflect the distinct zones of activity attributed to different earthworm functional groups. The mob-grazing of a grass-clover ley within an arable rotation increases the diversity of available earthworm nutrition sources and appears to be especially beneficial for surface-active epigeic species. This study evaluates a single snapshot in time. A further assessment of how the presence of livestock at different times during the season influences earthworm distribution, coupled with variation in grazing extent and how long the grass-clover ley is given to recover monitored over subsequent years, represents important future work.

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

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