
The effects of soil compaction mitigation on below-ground fauna: how earthworms respond to mechanical loosening and power harrow cultivation.

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The effects of soil compaction mitigation on below-ground fauna: how earthworms respond to mechanical loosening and power harrow cultivation

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**Highlights**

- There was a negative effect of mechanical loosening on anecic earthworms.
- The effects of treatments on earthworm abundance and biomass were broadly similar.
- Mechanical loosening of grassland soil can negatively impact soil macrofauna.

**Abstract**

Soils are one of the most biologically diverse habitats within the terrestrial ecosystem. Although soils are vital to the provision of important ecosystem services, their direct protection and sustainable management are often lacking within conservation policy. Many grassland soils have undergone considerable management intensification and are subject to degradation pressures. Soil compaction is an important form of soil degradation that can reduce soil productivity and crop yields, although the impacts can be reversed through natural processes and mitigated through management interventions. While commonly used, substantial knowledge gaps exist regarding the impact of soil compaction mitigation techniques on key soil macrofauna; many of these organisms are essential to soil function. A complete split-plot design was used to investigate the impacts of mechanical loosening (subsurface soil disturbance using tines or radial blades without significant soil mixing or inversion) and power harrow cultivation (shearing and mixing of soil to prepare a seedbed for the establishment of a deep-rooting forb and legume mix) on the abundance and biomass of earthworms up to two years post-treatment. Mechanical loosening was undertaken at two depths, c. 20 cm and c. 35 cm as two separate treatments. There was a negative effect of mechanical loosening at both depths on the abundance and biomass of anecic earthworms, lasting up to two years post-treatment. There was no significant effect of power harrow cultivation on the abundance or biomass of earthworms. These negative effects are consistent with other studies that have shown mechanical loosening to be a source of earthworm mortality. Although these findings resulted from a single episode of power harrow cultivation and mechanical loosening at a single site, the results...
indicate that the mechanical loosening of grassland soil can have a negative impact on important soil macrofauna and should possibly only be undertaken when the soil is in the most severely degraded conditions. Further work is needed to determine whether the negative impact of mechanical loosening is common to multiple sites and soil types and to link the reduction in earthworm number and biomass to future soil function.

Keywords: soil compaction; ecosystem services; grasslands; intensification; Lumbricus terrestris

1. Introduction

The effects of soil management on soil biodiversity and function are not as well understood in grassland systems as they are in arable systems (Vickery et al., 2001). Grasslands provide multiple benefits to society, including food production, water regulation, carbon storage and the provision of important habitats for a wide range of taxa. These include invertebrates (Hendrickx et al., 2007), in particular lepidopterans (Bourn and Thomas, 2002); mammals (e.g. brown hare Lepus europaeus (Hutchings and Harris, 1996)); and birds (Vickery et al., 2001) (e.g. corncrake Crex crex (Green and Stowe, 1993)). Many of the species supported by grasslands are experiencing serious population declines due to a combination of factors including habitat loss, increases in stocking rates and intensification of management practices (Allan and Bossdorf, 2014). While the above-ground biodiversity of grasslands is of increasing conservation concern, soils are one of the most species-rich habitats within the terrestrial ecosystem (Bardgett, 2005; Heywood, 1995) and are often overlooked within conservation policy (Giller, 1996; Haygarth and Ritz, 2009). Soil biodiversity is likely to play an important role in the provision of important ecosystem services such as regulating water quality, maintaining food security and providing carbon storage (Millennium Ecosystem Assessment, 2005). Facilitative interactions between functionally diverse groups of soil organisms drive ecological processes, such as litter decomposition, nutrient cycling and bioturbation (Bradford et al., 2002; Heemsbergen et al., 2004).
Grasslands account for 40% of global land cover, excluding Greenland and Antarctica (White et al., 2000). UK grasslands contribute 5% of Europe’s permanent grassland (Smit et al., 2008) and within the UK, permanent and temporary grasslands account for approximately 65% of agricultural land (DEFRA, 2014). However, intensification of management practices has led to 17% of vegetated lands experiencing human-induced soil degradation since 1945 (Bilotta et al., 2007; Oldeman, 1994).

Compaction is an important form of physical soil degradation (DEFRA, 2009; European Parliament Council of the European Union, 2013) that threatens soil function and agricultural productivity.

While mitigation methods to alleviate compaction are a field of current interest, significant knowledge gaps exist as to how best to identify high risk areas due to data scarcity and the difficulty in interpreting the often complex interactions that drive soil processes (Troldborg et al., 2013). Soil compaction is a global problem, is widespread in Europe (Jones et al., 2003; Trautner et al., 2003) and is not as visually recognisable as other forms of soil degradation, such as erosion. Several assessment techniques exist to quantify soil condition (Mueller et al., 2009; Peerlkamp, 1967; Shepherd, 2000). Newell-Price et al. (2013) reported on the use of such visual evaluation techniques to assess soil structural condition in 300 grassland fields in England and Wales. They found that c. 10% of grassland soils were in poor condition and c. 60% were in moderate condition.

Soil compaction arises through repeated compressive forces from heavy farm vehicles (Defossez and Richard, 2002; Håkansson et al., 1988) or from livestock trampling that can be exacerbated by high stocking densities or extended grazing periods (Mulholland and Fullen, 1991; Warren et al., 1986). However, the potential risk of compaction can depend as much on the water content and structural strength of the soil as it does on the magnitude of the applied force (Batey, 2009) with compaction a greater risk in soils with higher moisture contents (MAFF, 1970). This change in the spatial arrangement of soil aggregates reduces soil permeability and macroporosity, leading to a reduction in overall soil function. Severe soil compaction can lead to a reduction in soil productivity and ultimately crop yields (Whalley et al., 1995); degraded soils are less able to provide essential ecosystem services (Matson et al., 1997) and maintain soil diversity.
Although there are several soil compaction mitigation techniques that can improve the structure of compacted soils, sustainable soil management should aim to avoid compaction through good practice. Methods such as mechanical loosening (subsurface soil disturbance using tines or radial blades without significant soil mixing or inversion) often cannot fully compensate for the impacts of soil structural degradation and the treated soils can be susceptible to recompaction (Spoor et al., 2003). The three main types of equipment used to mitigate soil compaction in grassland soils vary in terms of their mode of action and the depth of effective operation: aerators can loosen soil to c. 10 cm depth; sward-lifters to c. 35 cm depth; and subsoilers c. 45 cm depth. Aerators or slitters consist of a series of radial blades or spikes, on a horizontal transverse non-powered rotor, that cut through the grass sward into the upper horizon of the topsoil. The nature and angle of the blades (or tines) will affect the degree of disturbance caused by the machine, as does forward speed, soil type and soil moisture level at the time of operation. By contrast, sward lifters and subsoilers work by lifting or fracturing the soil to alleviate the compacted area. Biological approaches to soil compaction mitigation also exist. For example, deep-rooted herb and legume species can have an observable effect on soil compaction by improving soil structure and macroporosity (Głąb, 2008; Latif et al., 1992; Lesturgez et al., 2004).

Any soil disturbance event, such as power harrow cultivation (shearing and mixing of soil to prepare a seedbed) or mechanical loosening, will alter not only the soil structure, but also the physical environment for soil organisms. Earthworms are an important group within soil macrofauna. They create burrows while mixing, ingesting and excreting soil material, thereby modifying the physical structure and availability of soil resources, and fulfilling the role of ‘ecosystem engineers’ (Lavelle, 2012; Lavelle et al., 1997; Pulleman et al., 2012). Earthworms are grouped into three ecological groups or ecotypes that exhibit different behaviours and provide different functions: anecics, endogeics, and epigeics. Anecic species, such as *Lumbricus terrestris*, create large vertical or subvertical permanent burrows from the surface down into the soil, a process that aids litter decomposition and nutrient cycling by pulling leaf-litter down into the soil. These burrows also
increase water infiltration rates and root development by creating macropores that improve soil structure and porosity. Endogeic species feed on mineral soil enriched with organic matter and also improve soil structure by creating a netlike system of smaller subsurface burrows. Epigeic species are found in the hummus layer and feed on plant litter.

This paper investigates the null hypothesis that mechanical loosening, power harrow cultivation, or the addition of deep-rooted forbs, have no effect on the abundance and biomass of earthworms. Although other studies (Emmerling, 2001; Ernst and Emmerling, 2009; Wyss and Glasstetter, 1992) have investigated the impacts of different tillage systems, and land use type and intensity (Boag et al., 1997; Smith et al., 2008) on the taxonomic diversity of earthworms in arable systems; the effect of mechanical loosening on earthworm ecotypes in grassland has not been studied. The experimental results from this study provide an indication of the extent to which mechanical loosening of degraded grassland soils can improve soil function and health.

2. Methods

We tested two methods known to alleviate soil compaction: mechanical loosening, and power harrow (PH) cultivation with the establishment of deep rooting forbs, including legumes. We investigated the effect these treatments had on the abundance and biomass of earthworms up to two years post-treatment. A collective analysis was firstly performed on all earthworm data, before separately analysing how anecic earthworms responded to treatments. Endogeic earthworms were not analysed separately due to the low numbers observed. Only adult earthworms were identified to species level due to uncertainty in the species identification of juveniles.

2.1. Study site and data collection

Field experiments were carried out at Nafferton Farm, northern England (54°98'57 N, 001°90'04 W). The soil was a sandy clay loam and was historically in a grassland-arable rotation; seven years grassland, followed by four years arable production. Arable production consisted of three years in
winter wheat and one year in spring barley (undersown). In the seven years preceding the start of the treatments the study site had been in continuous grassland under an organic dairy production system and was cut for silage two to three times per year. It was grazed by adult dairy cows between September and October each year and 100 ewes were overwintered. Dairy slurry was applied annually and dirty water twice annually amounting to approximately 15 machinery passes per year.

Initial treatments were applied on the 2nd September 2010 and earthworm sampling was undertaken one year after treatment, in October and November 2011 (over 22 days), and in September and October 2012 (over 15 days) two years post-treatment. Sampling was carried out between 0800 and 1400 hrs; data were not collected in adverse weather conditions or when frost or snow covered the ground. The mean monthly soil temperature and precipitation for the duration are presented in Fig. 1. Soil physical measurements (Anon, 1982); maximum penetration resistance (MPR) (0 - 15 cm and 15 - 30 cm depth) and bulk density (BD) (0 - 10 cm depth) were recorded for each plot (n = 36) in 2010 (pre-treatment), 2011, 2012 and 2013.

2.2. Experimental design

The effects of different soil compaction mitigation techniques were tested using a split-plot, randomised block experimental design; there were nine treatment combinations (Table 1) with four replicate plots of each treatment (n = 36 plots in total). Mechanical loosening was the complete block variable (24 x 16 m). Blocks were divided into three subplots each with a different treatment in terms of P/H cultivation (surface cultivation/undisturbed) and the introduction (or not) of deep-rooting herbs and legumes (8 x 16 m). There was 6-12 m between each complete block and 10-12 m headlands.
Table 1. Summary of experimental treatments (see Table 2 for details of forb species). \( n \) shows the number of soil samples (for earthworms) used in the analysis. Sampling was randomised across the four replicates of each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mechanical loosening</th>
<th>Power harrow cultivation / forb species introduction</th>
<th>( n ) 2011</th>
<th>( n ) 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unloosened</td>
<td>Grass sward undisturbed</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Shallow (c.20 cm)</td>
<td>Grass sward undisturbed</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Deep (c.35 cm)</td>
<td>Grass sward undisturbed</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Unloosened</td>
<td>Power harrow, without forb species</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Shallow (c.20 cm)</td>
<td>Power harrow, without forb species</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Deep (c.35 cm)</td>
<td>Power harrow, without forb species</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>Unloosened</td>
<td>Power harrow, with forb species</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Shallow (c.20 cm)</td>
<td>Power harrow, with forb species</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>Deep (c.35 cm)</td>
<td>Power harrow, with forb species</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

Prior to treatments the grass was cut with a Kuhn mower and McHale baler. Mechanical loosening was undertaken at a depth of c. 20 cm and c. 35 cm using a McConnell Shakaerator. Power harrowing was carried out using a standard agricultural PTO-driven power harrow that consisted of a series of bladed rotors that counter-rotated around a vertical axis. The natural vegetation of all plots was dominated by *Lolium perenne* and *Trifolium repens*; *Poa annua*, *Agrostis capillaris* and *Stellaria media* also occurred frequently. The power harrow treatment (treatments 4 - 9) was designed to create at least 50% bare ground within the sward as suitable regeneration niches for the deep-rooting forb and legume mix (Table 2) (RDS, 2004). The seed mix was broadcast by hand and then rolled using a Watson roller. The seed mix included three grass species (Table 2) to ensure that complete vegetation cover established, even if some of the forbs failed to germinate. Between
December and February in the months following mechanical loosening, sheep were introduced to the plots for short single grazing periods (c. 12-24 hrs) to control sward height and avoid shading out of introduced species (Table 3).

**Table 2. Deep-rooting forbs and legume mix used in treatments 7-9. Grass species are marked***

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achillea millefolium</em></td>
<td>Yarrow</td>
</tr>
<tr>
<td><em>Centaurea nigra</em></td>
<td>Common Knapweed</td>
</tr>
<tr>
<td><em>Cichorium intybus</em></td>
<td>Chicory</td>
</tr>
<tr>
<td><em>Hypochaeris radicata</em></td>
<td>Catsear</td>
</tr>
<tr>
<td><em>Lotus corniculatus</em></td>
<td>Birdsfoot Trefoil</td>
</tr>
<tr>
<td><em>Plantago lanceolata</em></td>
<td>Ribwort Plantain</td>
</tr>
<tr>
<td><em>Sanguisorba minor subsp. muricata</em></td>
<td>Forage Burnet</td>
</tr>
<tr>
<td><em>Trifolium pratense var. pratense (wild)</em></td>
<td>Wild Red Clover</td>
</tr>
<tr>
<td><em>Trifolium pratense var. sativum (commercial)</em></td>
<td>Commercial Red Clover</td>
</tr>
<tr>
<td><em>Trifolium repens (small-leaved)</em></td>
<td>Small-leaved White Clover</td>
</tr>
<tr>
<td><em>Trifolium repens (large-leaved)</em></td>
<td>Large-leaved White Clover</td>
</tr>
<tr>
<td><em>Agrostis capillaris</em></td>
<td>Common Bent*</td>
</tr>
<tr>
<td><em>Anthoxanthum odoratum</em></td>
<td>Sweet Vernal-grass*</td>
</tr>
<tr>
<td><em>Cynosurus cristatus</em></td>
<td>Crested Dog’s-tail*</td>
</tr>
</tbody>
</table>

**Table 3 Grazing periods and stocking densities**

<table>
<thead>
<tr>
<th>Livestock</th>
<th>Grazing periods</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewes</td>
<td>13/01/2011 – 25/01/2011</td>
<td>6.2 LU/ha</td>
</tr>
</tbody>
</table>
Treatment replicates were sampled in a random order using 30 x 30 cm metal quadrats. Quadrats were randomly placed five times within each plot and sunk to a depth of approximately 3 cm; a maximum of nine plots were sampled in a day. A non-destructive sampling method was used to avoid impeding additional data collection within plots. Each quadrat was treated with two litres of 0.33% mustard solution (Gunn, 1992); the same batch of Suma Wholefoods organic ground yellow mustard power was used throughout. Before treatment the vegetation within the quadrat was cut to approximately 3 cm to aid the detection of emerging earthworms. Subsequently each quadrat was observed for 15 minutes while the solution percolated into the soil. All adult earthworms that emerged after the solution was applied were identified to species level (Jones and Lowe, 2012; Sims and Gerald, 1999) and weighed on site using a portable balance. After 15 minutes another application of two litres of mustard solution was applied and the process repeated for another 15 minute period. All earthworms were washed and released within the same plot once sampling was complete. Mean abundance and biomass were calculated from the combination of earthworm counts from the five quadrats summed across each plot. Mean daily soil temperature at 15 cm depth was calculated from data recorded at 15 min intervals by the permanent weather station at Nafferton Farm.

2.3. Data analysis

All statistical analyses of earthworm data were performed using R version 3.0.2 (R Core Team, 2013). Soil physical measurements were analysed using two-way ANOVAs between treatments and
between mechanical loosening and year. Predictor variables for the earthworm analyses included mechanical loosening, PH cultivation, year, and soil temperature at 15 cm depth. Soil temperature data were missing between the 17th and 21st of October 2012, replacement values were calculated as a mean of the previous seven days measurements. Mechanical loosening, PH cultivation and year were specified as fixed factors; soil temperature was the only continuous variable, included to account for possible diurnal patterns within earthworm activity.

Due to additional project aims the sampling of replicates was unbalanced. To achieve balanced sampling replicates that had been sampled more than twice in 2011, or more than once in 2012, were randomly subsampled prior to analysis (Table 1). A preliminary analysis was completed for both earthworm abundance and biomass that included all earthworms, i.e. adults and juveniles of all species. Abundance data were analysed using generalized linear mixed models (GLMMs) using the package lme4 1.1-8 (Bates et al., 2015) and a negative binomial error structure (glmer.nb) to account for overdispersion. Biomass data were Box-Cox transformed to fulfil model assumptions prior to analysis using linear mixed models (LMMs) in the package nlme 3.1-120 (Pinheiro et al., 2015). The random effect (1|block/PH cultivation) was included in both models to account for spatial variation within the data and to acknowledge the hierarchal design of the experiment. Main effect models with and without the random effect were compared using AIC (abundance 757.4 GLMM vs 753.41 GLM and biomass 528.3LMM vs 525.74LM). Due to an extremely low level of spatial variation (abundance $\sigma^2 = 1.77^{12}$ and biomass $\sigma^2 = 1.61^8$) and small $n$, the random effect was dropped from the final analyses to avoid models that were over-parameterised. Therefore, generalized linear models (GLMs) were used to model abundance data and were fitted with a negative binomial error structure (glm.nb) using the package MASS to account for overdispersion; linear models (LMs) were used to analyse the biomass data. The significance of main effects and interactions were assessed using likelihood ratio tests to compare nested models.
3. Results

Seven earthworm species were observed within the study site: two anecic species (lob worm *L. terrestris* and black-headed worm *Aporrectodea longa*), four endogeic species (green worm *Allolobophora chlorotica*, blue-grey worm *Octolasion cyaneum*, grey worm *Aporrectodea caliginosa* and rosy-tipped worm *Aporrectodea rosea*) and one epigeic species (redhead worm *Lumbricus rubellus*) (Table 4). Adult anecic earthworms represented 33.6% of the total number of earthworms and 75.0% of the total biomass sampled. Endogeic species represented 24.1% of the total number of earthworms and 4.8% of the total biomass. Juvenile worms accounted for 40.6% of the total number and 19.9% of the total sampled biomass. The remaining earthworms were adult *Lumbricus rubellus*.

3.1. Physical soil structure

There was no significant effect of PH cultivation (*F* = 0.02, d.f. = 2, *p* = 0.980) on MPR (0–15 cm), or evidence of an interaction between treatments (*F* = 0.01, d.f. = 4, *p* = 1.000). There was a significant interaction between mechanical loosening and year (*F* = 6.49, d.f. = 6, *p* < 0.001). Shallow loosened plots had lower MPR (0–15 cm) compared to unloosened plots in 2011 and 2012 (*p* < 0.001 and *p* = 0.030, respectively, TukeyHSD) (Fig. 2A). There was no significant difference between deep loosened plots and unloosened plots in 2011 (*p* = 0.057, TukeyHSD) or 2012 (*p* = 0.992, TukeyHSD), or between shallow and deep loosened plots (2011, *p* = 0.725 and 2012, *p* = 0.441, TukeyHSD). The MPR (0–15 cm) variance was significantly heterogeneous between years and within treatments, however, sampling was balanced. There was no significant effect of PH cultivation (*F* = 0.33, d.f. = 2, *p* = 0.723) or of an interaction between treatments (*F* = 0.12, d.f. = 4, *p* = 0.975) on MPR (15-30 cm), but there was a significant interaction between mechanical loosening and year (*F* = 3.69, d.f. = 6, *p* = 0.002). MPR (15-30 cm) was significantly lower in 2011 in deep loosened plots compared to unloosened plots (*p* < 0.001, TukeyHSD) and compared to shallow plots (*p* = 0.018, TukeyHSD) (Fig. 2B). There was no significant difference between shallow loosened plots and
unloosened plots in 2011 ($p = 0.559$, TukeyHSD). There was significantly lower MPR (15 – 30 cm) in shallow and deep loosened plots compared to unloosened plots in 2012 (both $p < 0.001$, TukeyHSD), but no significant difference between shallow and deep loosened plots ($p = 1.000$, TukeyHSD). There was a significant interaction between mechanical loosening and year ($F = 3.06$, d.f. = 6, $p = 0.007$) on BD (0 – 10 cm); however, there were no significant post-treatment differences between treatments in the same year. There was a significant effect of loosening on BD (0 – 10 cm) ($F = 4.22$, d.f. = 2, $p = 0.017$) (Fig. 2C); BD on shallower loosened plots was significantly lower than on unloosened plots ($p = 0.02$, Tukey HSD). There was no significant difference between loosening treatments or between deeper loosened and unloosened plots. There was no significant effect of PH cultivation on BD (0 – 10 cm) ($F = 1.38$, d.f. = 2, $p = 0.256$) or of an interaction between treatments ($F = 0.17$, d.f. = 4, $p = 0.953$).

3.2. All earthworms

3.2.1. Abundance

There were significant interactions between mechanical loosening and PH cultivation ($X^2 = 12.22$, d.f. = 4, $p = 0.016$), mechanical loosening and year ($X^2 = 10.61$, d.f. = 2, $p = 0.005$), and mechanical loosening, PH cultivation and year ($X^2 = 26.71$, d.f. = 4, $p = 0.008$). There was no significant interaction between PH cultivation and year ($X^2 = 0.31$, d.f. = 2, $p = 0.857$). A model containing an interaction between mechanical loosening, PH cultivation and year was not significantly different from a model containing an interaction between mechanical loosening and year ($X^2 = 16.10$, d.f. = 10, $p = 0.097$) or an interaction between mechanical loosening and PH cultivation ($X^2 = 14.49$, d.f. = 8, $p = 0.070$). The interaction between mechanical loosening and year was chosen over the interaction between mechanical loosening and PH cultivation using AIC (746.80 and 749.19, respectively). Earthworm abundance was not significantly different on shallow loosened compared to unloosened ($p = 0.281$, TukeyHSD), or deep loosened plots in 2011 ($p = 0.827$, TukeyHSD), but they were significantly lower compared to unloosened plots ($p < 0.001$, Tukey HSD), and deep
loosened plots in 2012 ($p = 0.023$, Tukey HSD) (Fig. 3A). Deep loosened plots had significantly lower earthworm abundance compared to unloosened plots in 2011 ($p = 0.012$, Tukey HSD). There was no significant effect of PH cultivation ($p = 0.050$) when included as a main effect.

### 3.2.2. Biomass

There was a significant interaction between mechanical loosening and year ($X^2 = 10.34$, d.f. = 2, $p = 0.006$). There were no other significant interactions; mechanical loosening and PH cultivation ($X^2 = 3.82$, d.f. = 4, $p = 0.431$), PH cultivation and year ($X^2 = 0.13$, d.f. = 2, $p = 0.936$), and mechanical loosening, PH cultivation and year ($X^2 = 19.23$, d.f. = 12, $p = 0.083$). Earthworm biomass was significantly lower in 2012 compared to 2011 in unloosened plots ($p = 0.033$, Tukey HSD) (Fig. 3B) and shallow loosened plots ($p < 0.001$, Tukey HSD). Shallow and deep loosened plots both had significant lower earthworm biomass than unloosened plots in 2011 ($p = 0.005$ and $p < 0.001$ Tukey HSD, respectively). However, only biomass in shallow loosened plots was significantly lower compared to unloosened plots in 2012 ($p = 0.001$ and $p = 0.989$ Tukey HSD, respectively). Shallow and deep loosened plots did not differ from each other in 2011 ($p = 0.998$, Tukey HSD), but did differ significantly in 2012 ($p = 0.012$, Tukey HSD). There was no significant effect of PH cultivation on earthworm biomass when included as a main effect ($X^2 = 4.51$, d.f. = 4, $p = 0.105$).

### 3.3. Anecic earthworm species

#### 3.3.1. Abundance

There was a significant interaction between mechanical loosening and year ($X^2 = 15.32$, d.f. = 2, $p < 0.001$) and between mechanical loosening, PH cultivation and year ($X^2 = 26.23$, d.f. = 12, $p = 0.01$). There was no significant interaction between mechanical loosening and PH cultivation ($X^2 = 4.54$, d.f. = 4, $p = 0.337$) or between PH cultivation and year ($X^2 = 1.41$, d.f. = 2, $p = 0.493$). A model containing an interaction between mechanical loosening, PH cultivation and year was not significantly different from a model containing an interaction between mechanical loosening and
year ($X^2 = 10.91, \text{d.f.} = 10, p = 0.364$). The model containing the interaction between loosening and year was deemed the best fit. There was no significant difference in anecic earthworm abundance between shallow loosened and unloosened plots in 2011 ($p = 0.054$, Tukey HSD) (Fig. 4A), but earthworm abundance was significantly lower in shallow loosened plots compared to unloosened plots in 2012 ($p < 0.001$, Tukey HSD). Earthworm abundance was significantly lower in deep loosened plots in 2011 compared to unloosened plots ($p = 0.003$), but was not significantly different in 2012 ($p = 0.920$, Tukey HSD). There was no significant effect of PH cultivation on anecic earthworm abundance when included as a main effect ($X^2 = 2.69, \text{d.f.} = 2, p = 0.260$).

### 3.3.2. Biomass

There was a significant effect of an interaction between mechanical loosening and year ($X^2 = 11.71, \text{d.f.} = 2, p = 0.003$) on the abundance of anecic biomass. There were no other significant interactions; mechanical loosening and PH cultivation ($X^2 = 2.38, \text{d.f.} = 4, p = 0.665$), PH cultivation and year ($X^2 = 0.17, \text{d.f.} = 2, p = 0.920$), and mechanical loosening, year and PH cultivation ($X^2 = 18.73, \text{d.f.} = 12, p = 0.095$). Anecic earthworm biomass was significantly lower in 2012 compared to 2011 in unloosened plots ($p = 0.022$, Tukey HSD) and shallow loosened plots ($p < 0.001$, Tukey HSD) (Fig 4B). There was significantly lower anecic biomass on shallow and deep loosened plots compared to unloosened plots in 2011 ($p = 0.016$ and $p = 0.003$ Tukey HSD, respectively); in 2012 only shallow plots had significantly lower biomass compared to unloosened plots ($p < 0.001$ and $p = 0.900$ Tukey HSD, respectively). Anecic earthworm biomass was not significantly different between shallow loosened and deep loosened plot in 2011 ($p = 0.995$), but anecic earthworm biomass was significantly lower in shallow loosened plots compared to deep loosened plots in 2012 ($p = 0.005$, Tukey HSD). There was no significant effect of PH cultivation on anecic earthworm biomass when included as a main effect ($X^2 = 4.61, \text{d.f.} = 2, p = 0.100$).
4. Discussion

4.1. Soil physical measurements

A range of physical parameters were impacted by soil mechanical loosening, for example: shallow loosening (c. 20 cm) had a significant effect for at least two years post-treatment on MPR at 0 – 15 cm depths; and there was a significant effect of deep loosening (c. 30 cm) on MPR (15 cm -30 cm) in 2011 and 2012.

The addition of deep-rooting forbs, including legumes, is an effective method for increasing sward diversity (Spehn et al., 2000) and first became available as an Entry Level Stewardship (ELS) agri-environment option in England in early 2013 (EK21- Legume and herb-rich swards). Other studies have found improved soil structure (Latif et al., 1992) and increased macroporosity (Lesturgez et al., 2004) when deep-rooted legumes were included in maize cropping systems. However, in this study they had no significant effect on soil physical measurements or on the abundance and biomass of earthworms. Unfortunately it was not possible to disentangle the effects of power harrowing on vegetation cover from the addition of sown forbs because they were necessarily combined in the same treatment to ensure successful establishment of the sown forbs.

4.2. Earthworms

The negative effect of mechanical loosening on earthworms varied between years when all earthworms were analysed collectively and when anecic earthworms were analysed separately; there was no significant effect of PH cultivation identified by either the collective analysis, or the separate anecic analysis. Patterns across treatments were similar for both abundance and biomass.

4.2.1. Anecic species

Most of the worms sampled belonged to the deeper burrowing anecic earthworms. The effect of mechanical loosening on anecic earthworm abundance and biomass appeared to be greatest on
shallow loosened plots in 2012. The apparent delayed recovery in abundance and biomass
may have been due to the disruptive nature of shallow mechanical loosening in the upper topsoil
layers. As anecic species dig very deep burrows (up to six feet in length) it may be that shallow
loosening treatments impacted them more than deep loosening, as shallow loosening can disrupt
the surface layer and prevent access to the soil surface. Deep loosening, while initially causing
disruption (i.e. in 2011), will most likely retain the upper part of burrows intact, thereby providing
access to the soil surface and allowing earthworm populations to recover more rapidly.
The particularly heavy rainfall in 2012 (Centre for Ecology and Hydrology, 2012) (Fig. 1B)
may have caused the surface soil layer to slump thereby exacerbating the initial effect of shallow mechanical
loosening. Deep loosened plots on the other hand had significantly lower abundance in 2011
compared to unloosened plots, but did not differ significantly in 2012. This suggests a potential
recovery in anecic earthworm abundance two years post-mechanical loosening in deep loosened
plots. The reduction in abundance and biomass may have been due to mortality of large bodied
anecic earthworms linked to loosening treatments and the destruction of permanent anecic
earthworm burrows (Wyss and Glasstetter, 1992).

4.3. Sampling methodology

Due to repeat sampling and additional data collection within plots, destructive sampling methods
were not possible. Mustard extraction (Gunn, 1992), was chosen for its ease of preparation in the
field and non-lethal effects. The same batch of mustard was used throughout to standardise the
chemical composition and extractive properties of the solution between sampling attempts. The
numbers of earthworms observed in this study was lower than would be expected in temperate
grassland (Table 2.3 p45 (Curry, 1994)). Mustard solution may have extracted fewer earthworms
than other forms of chemical extraction (Pelosi et al., 2009; Zaborski, 2003); although the efficiency
of these methods may depend on soil type (Iannone III et al., 2012; Pelosi et al., 2009). There is
however, general agreement that a combination of hand-sorting and chemical expulsion has the
highest efficacy (Pelosi et al., 2009). Mustard extraction is potentially biased towards anecic species (Bartlett et al., 2006; Chan and Munro, 2001) and the number of endogeic earthworms in this study may have been underestimated. The large vertical burrows created by anecic earthworms may provide greater connectivity and water infiltration at the soil surface (Edwards et al., 1992) resulting in greater exposure to the mustard solution. In comparison, horizontally burrowing endogeic earthworms may take longer to be exposed to the mustard solution and may also respond by migrating horizontally through the soil rather than to the surface (Bartlett et al., 2006).

4.4. Management implications

Mechanical loosening is a potentially useful method to alleviate soil compaction (Burgess et al., 2000; Soane et al., 1986) and maintain a range of ecosystem services, such as increased water absorption (Jin et al. 2007). However, our findings indicate that the use of mechanical loosening should be considered with the potential negative impacts on some earthworm species in mind. The experimental results reported in this paper indicate that the negative impact on earthworm populations can last for at least two years.

The aims and objectives of the Environmental Stewardship scheme in England, and its successor Countryside Stewardship (Natural England, 2015), include: to conserve wildlife including farmland birds (biodiversity) and to protect natural resources by improving water quality and reducing soil erosion and surface run-off. To meet the standards of Good Agricultural and Environmental Condition (DEFRA, 2015) soil compaction has to be avoided, while in Countryside Stewardship there is a requirement to ‘remove soil compaction’ when buffer strips are established by reseeding intensive grassland. The establishment of threshold values for the effects of physical and biological soil parameters associated with soil compaction on soil fauna have proved difficult (Beylich et al., 2010). This is due not only to the high variability of soil processes, but also to the variability of experimental approaches making between study comparisons, and therefore generalised recommendations, difficult (Beylich et al., 2010). However, this study suggests that mechanically
loosening a soil that is in moderate condition could have detrimental effects on local earthworm populations and further threaten the provision of important soil processes. When earthworm populations have been reduced due to conventional and minimal tillage, populations were slow to recover and affected areas were not effectively recolonised by earthworms in adjacent undisturbed field margins (Roarty and Schmidt, 2013). Where recolonisation is limited, population recovery will be through reproduction by surviving individuals.

Mild to moderately compacted soil can improve through natural climatic processes and the presence of earthworms can further improve soil structure and alleviate compaction (Capowiez et al., 2012; Joschko et al., 1989). However, soil restoration is a slow process and this ability will differ between species (Bottinelli et al., 2014). While different soils and ecological communities may respond to disturbance events differently (Ernst and Emmerling, 2009) if sustainable soil management is to be further encouraged, unjustified mechanical loosening should be avoided as it may do more harm than good.

4.5. Implications for other species

Although trends vary between regions, depending on whether arable or pastoral systems dominate, improved permanent grassland, temporary grassland and rough grazing have all declined since the 1960s (Chamberlain et al., 2000). Modern agricultural practices (Atkinson et al., 2005; Galbraith, 1988; Shrub, 1990) and agricultural intensification (Chamberlain et al., 2000; Donald et al., 2001) are often cited as major causes of farmland bird declines. Many species have undergone serious declines in the last 20-30 years (Gregory et al., 2004) and changes in grassland management, such as the reduction in ley grass, rough grazing, and increased stocking density are associated with declines of species closely associated with grassland (Chamberlain and Fuller, 2000; O’Brien and Smith, 1992).

Management practices such as conventional and conservation tillage (Capowiez et al., 2012; Chan, 2001; Ernst and Emmerling, 2009; van Capelle et al., 2012), and intensive grazing regimes (Curry et
al., 2008) can often result in changes in the abundance, distribution and community composition of earthworms. As soil macroinvertebrates, including soil-dwelling larvae, constitute an important foraging resource for many vertebrate species (e.g. the European badger *Meles meles* (da Silva et al., 1993; Kruuk and Parish, 1981) and the red fox *Vulpes vulpes* (Macdonald, 1980)), including numerous ground probing birds (e.g. song thrush *Turdus philomelos* (Gruar et al., 2003), northern lapwing *Vanellus vanellus* (Ausden et al., 2003) and European starling *Sturnus vulgaris* (Dunnet, 1955; Rhymer et al., 2012; Whitehead et al., 1995)), these changes could have a further indirect impact on above-ground predators that may experience a change in the availability and accessibility of important below-ground prey.

5. **Conclusion**

This study shows a significant negative effect of mechanical loosening on the abundance and biomass of anecic earthworms. Although limited to one site and mechanical loosening in a single year, these results only support the mechanical loosening of soil in severely degraded conditions due to the potential negative impact on important soil macrofauna that could impede future soil function, as well as indirect effects for earthworm predators.
Acknowledgements

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Figure 1. A) Mean monthly soil temperature at 15 cm depth, B) Mean month rainfall, error bars are ± 1 SE.
Figure 2: Evidence that the treatments used in this experiment did alter soil compaction through changes in maximum penetration resistance: A) Maximum penetration resistance N cm⁻² 0-15 cm depth, B) Maximum penetration resistance N cm⁻² 15-30 cm depth, C) Bulk density g cm⁻³ 10 cm depth. 2010 to 2013 data combined, error bars are ± 1 SE. Light grey = 2010, light blue = 2011, white = 2012 and dark grey = 2013.

Figure 3. The effect of loosening treatments between years on: A) all earthworm abundance, B) all earthworm biomass. Error bars are ± 1 SE. Grey = 2011, white = 2012.
Figure 4. The effect of loosening treatments between years on: A) anecic earthworm abundance, B) anecic earthworm biomass. Error bars are ± 1 SE. Grey = 2011, white = 2012.
Table 4: Mean earthworms per m² sampled in each treatment, ±1 SE.

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>1</th>
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<th>3</th>
<th>4</th>
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<th>7</th>
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<td><em>L. terrestris</em></td>
<td>2011</td>
<td>15.54 ± 2.41</td>
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