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1 **Arable fields as potential reservoirs of biodiversity:**  
2 **earthworm populations increase in new leys**

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5 Miranda T. Prendergast-Miller<sup>1,2\*</sup>, David T. Jones<sup>3</sup>, Despina Berdeni<sup>4,5</sup>, Susannah  
6 Bird<sup>6</sup>, Pippa J. Chapman<sup>7</sup>, Leslie Firbank<sup>8</sup>, Richard Grayson<sup>7</sup>, Thorunn Helgason<sup>6</sup>,  
7 Joseph Holden<sup>7</sup>, Martin Lappage<sup>8</sup>, Jonathan Leake<sup>4</sup>, Mark E. Hodson<sup>1</sup>

8

9 <sup>1</sup>Department of Environment and Geography, University of York, Wentworth Way,  
10 York YO10 5NG, UK

11 <sup>2</sup>Department of Geography and Environmental Sciences, Northumbria University,  
12 Newcastle upon Tyne, NE1 8ST, UK (current address)

13 <sup>3</sup>Life Sciences Department, Natural History Museum, London, SW7 5BD, UK

14 <sup>4</sup>The University of Sheffield, Department of Animal and Plant Sciences, Sheffield,  
15 S10 2TN, UK

16 <sup>5</sup>Crop Physiology, ADAS Gleadthorpe, Meden Vale, Mansfield, Nottinghamshire,  
17 NG20 9PD, UK (current address)

18 <sup>6</sup>Department of Biology, University of York, Wentworth Way, YO10 5DD, UK

19 <sup>7</sup>water@leeds, School of Geography, University of Leeds, Leeds, LS2 9JT, UK

20 <sup>8</sup>water@leeds, School of Biology, University of Leeds, Leeds, LS2 9JT, UK

21

22 \*Corresponding author, Contact email: [miranda.prendergast-](mailto:miranda.prendergast-miller@northumbria.ac.uk)  
23 [miller@northumbria.ac.uk](mailto:miranda.prendergast-miller@northumbria.ac.uk)

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## 27 **Abstract**

28 Managing soil to support biodiversity is important to sustain the ecosystem services  
29 provided by soils upon which society depends. There is increasing evidence that  
30 functional diversity of soil biota is important for ecosystem services, and has been  
31 degraded by intensive agriculture. Importantly, the spatial distribution of reservoirs of  
32 soil biota in and surrounding arable fields is poorly understood. In a field experiment,  
33 grass-clover ley strips were introduced into four arable fields which had been under  
34 continuous intensive/conventional arable rotation for more than 10 years. Earthworm  
35 communities in arable fields and newly established grass-clover leys, as well as field  
36 boundary land uses (hedgerows and grassy field margins), were monitored over 2  
37 years after arable-to-ley conversions. Within 2 years, earthworm abundance in new  
38 leys was  $732 \pm 244$  earthworms  $m^{-2}$ , similar to that in field margin soils ( $619 \pm 355$   
39 earthworms  $m^{-2} yr^{-1}$ ) and four times higher than in adjacent arable soil ( $185 \pm 132$   
40 earthworms  $m^{-2}$ ). Relative to the arable soils, earthworm abundance under the new  
41 leys showed changes in community composition, structure and functional group,  
42 which were particularly associated with an increase in anecic earthworms; thus new  
43 leys became more similar to grassy field margins. Earthworm abundance was similar  
44 in new leys that were either connected to biodiversity reservoirs i.e. field margins and  
45 hedgerows, or not (installed earthworm barriers). This suggests that, for earthworm

46 communities in typical arable fields, biodiversity reservoirs in adjacent field margins  
47 and hedgerows may not be critical for earthworm populations to increase. We  
48 conclude that the increase in earthworm abundance in the new leys observed over 2  
49 years was driven by recruitment from the existing residual population in arable soils.  
50 Therefore, arable soils are also potential reservoirs of biodiversity.

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## 56 **1. Introduction**

57

58 Soil security is an important global issue (McBratney et al., 2014; Amundson et al.,  
59 2015). The need to maintain healthy soils and increase soil organic matter (SOM)  
60 content has been proposed as central to achieving some of the UN's Sustainable  
61 Development Goals (SDGs) (Keesstra et al., 2016; Lal, 2019) such as zero hunger  
62 (SDG2); good health and well-being (SDG 3); climate action (SDG13); and restoring  
63 degraded land (SDG 15), which either directly or indirectly rely on soil as a key  
64 resource. Continuous conventional tillage in arable systems relies on intensive  
65 agricultural management practices which depletes SOM, can destroy soil structure  
66 and can lower soil biodiversity, including key ecosystem engineers such as  
67 earthworms (Beylich et al., 2010; Chan, 2000; Stroud et al., 2016; Briones and  
68 Schmidt, 2017; Pelosi et al., 2014). Intensive soil management practices, such as

69 annual inversion ploughing, lower earthworm abundance, biomass and species  
70 diversity. For example, Holden et al., (2019) reported low earthworm abundance,  
71 biomass and diversity in intensively managed arable fields over a 3-year period  
72 (2015-2017) compared to soils under long-term permanent pasture. The burrowing  
73 and feeding activities of earthworms are related to their functional group (Edwards  
74 and Bohlen, 1996). Anecic earthworms form deep vertical, permanent burrows;  
75 these species are important for pulling plant litter into their burrows. Endogeic  
76 earthworms feed on soil and soil-associated organic matter and form transient  
77 horizontal burrows. Epigeic earthworms live within and feed on the surface litter layer  
78 (Edwards and Bohlen, 1996). The distribution of these functional groups has  
79 important consequences for soil ecosystem functions and soil health and there is  
80 good evidence showing that increased earthworm abundance and diversity benefits  
81 soil ecosystem services (Spurgeon et al., 2013; Blouin et al., 2013). For example,  
82 the permanent burrows formed by anecics like *Lumbricus terrestris* generate  
83 important classes of large pores that control water infiltration rates and facilitate root  
84 growth to access groundwater. In turn, these contribute to reducing overland flow  
85 and soil erosion. Endogeic species promote soil aggregation and soil organic carbon  
86 (SOC) stabilisation and sequestration (Zhang et al., 2013) and also contribute to  
87 increased hydraulic conductivity (Hallam et al., 2021), while epigeics are important  
88 for surface litter processing; all of which increase soil carbon and improve soil fertility  
89 via nitrogen mineralisation (Blouin et al., 2013). Meta-analyses suggest that the  
90 presence of earthworms in agroecosystems can lead to 25% increases in plant  
91 growth (Van Groenigen et al., 2014), however, agricultural intensification, especially  
92 high soil disturbance (Briones and Schmidt, 2017) and low organic matter inputs due  
93 to annual cropping, are detrimental to soil biodiversity and earthworm communities.

94 Continuing with current intensive agricultural management practices will not deliver  
95 soil security. Soil management changes are therefore required which promote soil  
96 biodiversity and sustainable agro-ecosystems, but it remains unclear at what rate, or  
97 even if, earthworm populations can increase after depletion by many decades of  
98 intensive arable management.

99

100 It is generally accepted that pasture soils with permanent plant cover have higher  
101 SOM contents, better soil structure and support a higher biodiversity including  
102 earthworm communities (Spurgeon et al., 2013) compared to soils under continuous  
103 conventional arable cultivation. In arable systems, continuous cultivation destroys  
104 soil aggregates and soil structure and the increased disturbance reduces earthworm  
105 abundance and diversity, especially for the larger anecic species (Gerard and Hay,  
106 1979; Stroud et al., 2016; Chan, 2000; Crittenden et al., 2015). Annual tillage  
107 promotes decomposition of organic matter, decreases food resources and their  
108 availability to earthworms and increases earthworm predation (Edwards and Lofty,  
109 1982; Boström, 1995; Briones and Schmidt, 2017). Previous research has indicated  
110 improvements to soil biology and soil functioning that changes to land management  
111 can bring. For example, the use of rotational grass leys can lead to increases in  
112 SOM and earthworms (Evans and Guild, 1948; Gerard and Hay, 1979). However,  
113 the potential and timescale required for earthworm communities to increase in long-  
114 term intensively managed soils following a change in soil management is poorly  
115 understood. Increasing demand for agricultural productivity has led to a reduction in  
116 the use of leys. Therefore, confirming the timescales involved in the restoration of  
117 earthworm communities and soil function by leys may help decision-making with  
118 respect to the duration of leys needed. This is important especially where leys are

119 being used as part of a transition to less intensive cultivation approaches such as no-  
120 tillage crop production.

121

122 While earthworms are generally considered to be ecosystem engineers (Blouin et al.,  
123 2013), we still do not fully understand their distribution across farming landscapes  
124 and how this varies on different temporal and spatial scales (White et al., 2020).

125 Moreover, while earthworm distributions have generally been compared between  
126 broad land uses, e.g. between pasture, arable and woodland (e.g. Decaëns et al.,  
127 2008; Smith et al., 2008; Spurgeon et al., 2013), earthworm abundance and diversity  
128 vary at more local spatial scales within field boundary soils e.g. between the interior  
129 of fields and their grassy margins. Studies by Crittenden et al., (2015), Roarty and  
130 Schmidt (2013), Nuutinen et al., (2011) and Smith et al., (2008) have looked at  
131 annual changes in earthworms comparing arable fields and different field margins  
132 including newly established margins. Earthworm abundance tends to be higher in  
133 field margin soils and lower in adjacent arable soils. Smith et al., (2008) sampled soil  
134 biodiversity, including *Lumbricidae*, from different habitats, including hedgerows,  
135 margins, and 9 and 27 m into arable fields planted with beans or wheat. Hedgerows  
136 were shown to be distinct in their soil communities and margins were proposed as  
137 acting as corridors to enhance species dispersal within the arable fields. While  
138 arable fields with margins seem to have higher soil biota densities than fields without  
139 margins, there is still debate around the importance of field margins as biodiversity  
140 refugia and whether they are key in facilitating migration, of for example earthworms,  
141 between arable fields and the relatively higher quality soils in field margins. Studies  
142 have shown gradients in earthworm abundance, with abundance in arable fields  
143 declining with increasing distance from the field margin (e.g. Nuutinen et al., 2011;

144 Nieminen et al., 2011), suggesting that migration and refugia in the margins are  
145 important. Dispersal time is important, as migration rates for earthworm species vary  
146 from 1.5 to 14 m yr<sup>-1</sup> (Eijsackers, 2011). In contrast to the refugia-migration  
147 hypothesis, Roarty and Schmidt (2013) and Frazao et al., (2017) propose that  
148 margins do not contribute to earthworm species found in cultivated adjacent fields.  
149 By comparing old and new margins 3 years post-establishment, Roarty and Schmidt  
150 (2013) showed rapid increases in earthworm populations in new margins extending  
151 onto previously cultivated land, and showed no evidence that earthworm populations  
152 were affected by spill-over or migration into the remaining permanent arable part of  
153 the field. However, the study design did not resolve whether the increase in  
154 earthworm populations in the new margins was facilitated by migrations from the  
155 existing permanent margins. Frazao et al., (2017) showed that while the presence of  
156 field margins increased earthworm biodiversity in arable fields, the margins had  
157 limited effect on earthworms within arable fields, as biodiversity did not change.  
158 Therefore, further work is required to test the effect of landscape connectivity in  
159 controlling earthworm abundance in agro-ecosystems, especially under arable-to-leys  
160 conversions.

161

162 Given that soil health and biodiversity are generally lower under continuous arable  
163 cultivation and that viable soil management practices are required to improve soil  
164 function in agro-ecosystems, a field experiment was established with two main  
165 objectives: (1) to determine the rate of change in soil function in new grass-clover  
166 leys; and (2) to determine the importance of field boundary soils (hedgerows and  
167 grassy margins) as biodiversity reservoirs for soil ecosystem engineers like  
168 earthworms to colonise new leys. While several measures of soil biodiversity and soil



169 function were taken in this field experiment (see Holden et al., 2019; Hallam et al.,  
170 2020), this paper focuses on the earthworm communities *in situ*. We monitored  
171 earthworm abundance, biomass, species composition and population structure over  
172 a 3-year period. The experiment used spatial sampling (samples were taken from set  
173 distances from the hedgerow), an earthworm migration barrier at the margin-field  
174 boundary, and temporal sampling (repeated annual and seasonal time points over 3  
175 years) to test the following hypotheses:

176 H1: Agro-ecosystem land uses with a permanent plant cover e.g. hedgerows, grassy  
177 field margins and leys, support higher earthworm abundance, biomass and species  
178 composition than that found in continuous conventional arable cultivation with typical  
179 annual crops (cereals and oilseeds);

180 H2: Earthworm abundance in newly established leys declines with increasing  
181 distance away from the field boundary soils i.e. from hedgerow and grassy margins;

182 H3: Field boundary soils are important reservoirs of earthworm diversity:  
183 belowground connectivity between high quality soils in hedgerow and grassy field  
184 margins and newly-established leys facilitates colonisation via migration;

185

186

## 187 **2. Material and methods**

188

### 189 2.1 Study site

190 The field experiment was established in April 2015 at the University of Leeds Farm,  
191 North Yorkshire, northern England, UK (53°52'06.0"N; 1°19'36.8"W). It is a

192 commercial mixed arable and pasture farm, on loamy, calcareous brown earth soils  
193 50 - 90 cm deep, underlain by dolomitic limestone, in the Aberford series of Calcaric  
194 Endoleptic Cambisols (Holden et al., 2019). Four arable fields were studied (A1-A4,  
195 see Fig. S1), each with mature hedgerow boundaries and grassy field margins. Field  
196 margins were c. 1 m in width and were mown sporadically but were otherwise left  
197 unmanaged. Three of the four fields have been under conventional management  
198 since 1995. One field (A2) was under permanent pasture between 1998 and 2008  
199 when it was returned to the same conventional cropping rotation as the other fields  
200 (see Supporting information Table S1). The main crop was winter wheat. Oilseed  
201 rape, barley, potatoes and vining peas were used as break crops. Arable fields  
202 received 150 kg N ha<sup>-1</sup> and 70 kg K ha<sup>-1</sup> in spring 2016, 23 kg P ha<sup>-1</sup> in autumn 2016,  
203 140 kg N ha<sup>-1</sup>, 86 kg K ha<sup>-1</sup> and 22 kg S ha<sup>-1</sup> in spring 2017, and 8 t ha<sup>-1</sup> of pig  
204 manure in autumn 2017 (Holden et al., 2019). Further site details on climate, farm  
205 management and hedgerow species composition are given in Holden et al., (2019).

206

## 207 2.2 Experimental design

208 Paired grass-clover ley strips (70 m long, 3 m wide) were established in each arable  
209 field (see Supporting Information Figs. S1 and S2) for the field experiment described  
210 below. The strips ran perpendicular to one edge of each arable field and were  
211 approximately 40 m apart. Strips were prepared by subsoiling and were sown in May  
212 2015 with a grass-clover seed mixture (Broadsword Hi-Pro), comprising tetraploid  
213 *Lolium x boucheanum* (12% and 16%), diploid and tetraploid *Lolium perenne* (20%  
214 and 16% respectively), *Festulolium* spp., 16%, *Trifolium repens* 5%, and *Trifolium*  
215 *pratense* 15%, at an application rate of 4.2 g m<sup>-2</sup>. The grass-clover ley strips

216 received no fertiliser or agricultural chemical treatments over the course of the  
217 experiment.

218

219 In order to test the effect of hedgerow-to-field connectivity, one of each pair of ley  
220 strips was disconnected from the hedge-margin field boundary by installing a vertical  
221 steel mesh (104  $\mu\text{m}$  pore size) barrier to 1 m depth to reach the bedrock (see  
222 Supporting Information Figs. S2 and S3). The barrier was inserted at the head of the  
223 Unconnected Arable-Ley strip (UAL), at the field-margin boundary. The first 2 m of  
224 the margin adjacent to the barrier was dug up during barrier insertion and  
225 subsequently kept fallow by regular glyphosate treatment to further discourage  
226 development of large populations of earthworms on plant litter and root carbon  
227 inputs, and movement of earthworms from the remaining margin to the new ley strip.  
228 Both the barrier and the fallow soil extended for 5 m either side of the ley strip giving  
229 a total length of 13 m centred on the ley strip. The height of the barrier was c.15 cm  
230 above the soil surface, supported with a wooden frame to keep the barrier vertical.  
231 The second strip, Connected Arable-Ley strip (CAL), was not modified at the field-  
232 margin boundary and was contiguous with the grassy field margin, giving continuous  
233 vegetation cover from the hedgerow to the end of the ley strip, 70 m into the arable  
234 field. The area of field lying between the two strips was managed in the same way as  
235 the rest of the arable field and served as the arable control (CON). Within the arable  
236 fields and CON area, winter wheat was grown in 2015. In 2016, spring barley was  
237 sown in three fields; one field had winter wheat (A2). In 2017, all fields were sown  
238 with winter barley. The ley strips were mown four times a year: in April and May  
239 2016, clippings were retained within the leys. From June 2016 onwards, grass  
240 clippings were removed from the ley strips to promote clover *versus* grass biomass.

241

### 242 2.3 Earthworm and soil sampling

243 Hedgerow and Margin samples were taken under the hedgerow and from the middle  
244 of the margin at the head of each CON, CAL and UAL strip. Within each of these  
245 strips, further samples were taken at distances 2, 4, 8, 16, 32 and 64 m from the field  
246 margin edge (see Supporting Information Fig. S2). This sampling scheme was  
247 designed to test the effect of distance from the field boundary soils on earthworm  
248 communities and soil properties, providing 'hedgerow to field' transects when all  
249 sample points were taken into account, or 'in-field' transects when the hedgerow and  
250 margin samples were not included.

251

252 CON, CAL and UAL strips were sampled in April 2015 (spring) before the grass-  
253 clover ley strips were established (Baseline study), and then sampled again in April  
254 2016 and April 2017 (Annual study). Additional sampling occurred in one arable field  
255 (A4, Fig. S1) in December 2015 (winter), July 2016 (summer) and October (autumn)  
256 2016, providing a seasonal sampling period from the April 2015 baseline, before the  
257 leys were established, through to April 2017 (Seasonal study).

258

259 For earthworm sampling, a soil block (18 x 18 cm at the surface and 15 cm deep)  
260 was removed at each location and earthworms were collected by hand-sorting.  
261 Dilute allyl isothiocyanate (1.5 L; 0.1 g L<sup>-1</sup>) (Zaborski, 2003; Pelosi et al., 2009) was  
262 poured into each soil pit and left to drain into the soil to facilitate collection of deeper-  
263 dwelling species. Earthworm appearance in the pit was monitored over a 30-min  
264 period. Earthworms were stored in 80% ethanol and adults (with a clitellum) were

265 identified to species using keys in Sims and Gerard (2013). Juvenile earthworms  
266 were identified to functional groups (epigeic, endogeic or anecic) based on  
267 pigmentation and size (Bouché, 1977). Earthworm biomass (the weight of individual  
268 earthworms) was also determined for samples collected from December 2015  
269 onwards. After removal of earthworms, soils were returned to their respective pits  
270 and sample position was recorded to avoid re-sampling the same position on  
271 subsequent surveys.

272

273 Soil moisture and temperature measurements were taken at three positions (within  
274 10 cm) around the excavated soil pit, at 5 and 10 cm depths. Soil moisture was  
275 measured using a ML3 ThetaProbe Soil Moisture Sensor (Delta-T Devices) and soil  
276 temperature using a Checktemp®1 probe (Hanna Instruments). Soil bulk density  
277 samples were taken from the side of the sample pit wall at 5 and 10 cm soil depth  
278 using steel bulk density rings (118 cm<sup>3</sup>); bulk density was calculated on an oven dry  
279 weight basis (dried for 24 hr at 105 °C).

280

### 281 2.3.1 Soil organic carbon and nitrogen analyses

282 SOC and total nitrogen were measured from 100 cm<sup>3</sup> bulk density samples collected  
283 at 2-7 cm depth at each sampling distance across the 4 fields in April 2018 (an  
284 additional sampling event for soil properties only). Samples were oven dried (105°C,  
285 48 hours), weighed and then sieved (1 mm sieve size) to remove roots and large  
286 stones before being re-dried (105°C, 48 hours) and weighed. Dried soil was crushed  
287 to a fine powder using a Fristch Pulverisette agate ball mill. Inorganic C was  
288 removed by reaction with acid as detailed in Holden et al., (2019). Duplicate 25 - 30

289 mg samples of acid-treated soil were analysed using an Elementar vario MICRO  
290 cube for total nitrogen and SOC.

291

### 292 2.3.2 Soil sensors

293 Moisture and temperature sensors (Decagon 5TM) were installed at four distances  
294 along each strip (under hedgerow and 5, 20 and 50 m from margin) and at three  
295 depths for each distance (5, 20 and 50 cm). Data were recorded at 15 min intervals  
296 using Arduino Uno data loggers. Soil-specific calibrations were derived for each field  
297 based on a gravimetric method.

298

### 299 2.4 Statistical analyses

300 Data were transformed where required to reduce the variance. Unless otherwise  
301 stated, analyses were performed using SPSS (IBM v25). All data relating to the  
302 earthworm study described here have been archived in the Natural Environment  
303 Research Centre (NERC) Environmental Information Data Centre (Prendergast-  
304 Miller et al., 2019).

305

#### 306 2.4.1 Effect of distance, field, year and land use on earthworm abundance

307 Earthworm abundance data were first natural log transformed to reduce the  
308 variance. For the 2015 baseline data, the effect of distance across the arable fields  
309 was tested using one-way ANOVAs on hedgerow-to-field (i.e. hedgerow to 64 m) or  
310 in-field distances (i.e. 2 – 64 m). Differences between field and land use were tested

311 using a 2-way ANOVA with field (A1-A4) and land use (arable, hedgerow, margin) as  
312 factors.

313

314 The impact of arable-to-ley conversion was determined using data collected in April  
315 2016 and April 2017. Analyses of variance for repeated measures with sampling  
316 year (2016, 2017) as the within-subject factor were conducted to test the effects of  
317 (1) field (A1-A4), land use (hedgerow, margin, arable, ley) and distance (hedge-to-  
318 field or in-field); and (2) connectivity (CAL vs UAL) and distance (hedge-to-field or in-  
319 field). Post-hoc pair-wise comparisons were performed using Bonferroni tests.

320

321 2.4.2 Effects of field, distance and land use on abundance of earthworm species and  
322 functional groups

323 Only species making up > 1% of total abundance were included in the analyses. The  
324 effects of field, distance, year and land use on abundance of earthworm species,  
325 juvenile functional groups and total functional groups were determined using the  
326 *mva* package in R (Wang et al., 2012), using a negative binomial distribution.

327 Plots of residuals *versus* fitted were visually inspected to confirm model assumptions  
328 and model best fit (Wang et al., 2012). Population structure was calculated as the  
329 juvenile:adult ratio, based on earthworm abundance in each land use for 2015, 2016  
330 and 2017. For each year, a one-way ANOVA on ranks was performed to compare  
331 population structure for each land use.

332

333 2.4.3 Soil properties and relationship with earthworm abundance

334 Soil moisture, temperature and bulk density were analysed by ANOVA with repeated  
335 measures, using year as the within-subjects factor (2016, 2017), and field, distance,  
336 land use and depth as the between-subjects factors. Post-hoc pair-wise comparisons  
337 were performed using Bonferroni tests. Differences in average SOC between land  
338 uses were tested using a Kruskal-Wallis test. Earthworm species abundance data  
339 were square-root transformed and a Bray-Curtis resemblance matrix was created. A  
340 distance-based redundancy analysis (dbRDA) plot was used to visualise the relative  
341 contribution of soil properties to earthworm abundance in each land use using data  
342 collected in April 2017 (April 2018 for SOC and N). Multivariate analyses were  
343 conducted using PRIMER-E (Clarke and Gorley, 2006).

344

#### 345 2.4.4 Seasonal effects on earthworm communities and soil properties

346 One field (A4) was sampled over six time points between April 2015 and April 2017.  
347 As with the annual data, initial analyses confirmed that effects of distance and  
348 connectivity on earthworm abundance were not significant. The seasonal earthworm  
349 abundance and biomass data, as well as soil properties (soil moisture, temperature  
350 and bulk density data) were analysed over 6 seasonal time points: April 2015,  
351 December 2015, April 2016, July 2016, October 2016 and April 2017 using ANOVA  
352 with repeated measures. Month was the within-subject factor, with land use and  
353 depth (for soil properties) as the between-subjects factors. This is a pseudo-  
354 replicated sampling design which may constrain interpretation of the results.  
355 However, because the overall land use trends in field A4 were representative of  
356 changes in the other three fields, and earthworms are known to be sensitive to  
357 seasonality and soil moisture and temperature, we can assume that the temporal



358 changes observed in one field also occurred in the adjacent fields used in this study.  
359 Data generated from the soil sensors installed at A4 field were used to calculate the  
360 number of antecedent dry days (ADD; 0 mm of precipitation), mean soil moisture and  
361 mean soil temperature 7, 14, 21, 30, 50, 80 and 100 days before each seasonal  
362 sampling date.

363

364

### 365 **3. Results**

366

#### 367 **3.1 Earthworm species**

368 The earthworm species and their respective functional groups identified over the  
369 study period are listed in Table 1.

370

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378 **Table 1: Earthworm species and the proportion of individuals (%) recorded in**  
 379 **the annual surveys, 2015-2017. Species/juveniles groups highlighted in bold**  
 380 **accounted for > 1% of the total abundance (total number of individuals) and**  
 381 **were used in subsequent data analyses.**

382

Species name	Functional group	% abundance (Total abundance = 4704 individuals)
<b><i>Aporrectodea longa</i></b> (Ude, 1885)	Anecic	2.0
<b><i>Lumbricus terrestris</i></b> (Linnaeus, 1758)		1.2
<b>Juveniles</b>		10.8
<b><i>Allolobophora chlorotica</i></b> (Savigny, 1826)	Endogeic	14.4
<i>Aporrectodea caliginosa</i> (Savigny, 1826)		0.5
<b><i>Aporrectodea rosea</i></b> (Savigny, 1826)		3.8
<i>Murchieona muldali</i> (Omodeo, 1956)		0.2
<i>Octolasion cyaneum</i> (Savigny, 1826)		0.1
<b>Juveniles</b>		50.1
<i>Dendrodrilus rubidus</i> (Savigny, 1826)	Epigeic	0.04
<b><i>Lumbricus castaneus</i></b> (Savigny, 1826)		4.8
<i>Eisenia fetida</i> (Savigny, 1826)		0.02
<b><i>Satchellius mammalis</i></b> (Savigny, 1826)		1.2
<b>Juveniles</b>		10.7

383

384

## 385 3.2 Baseline study (April 2015 survey)

### 386 3.2.1 Effect of field, land use and distance on earthworm communities in April 2015

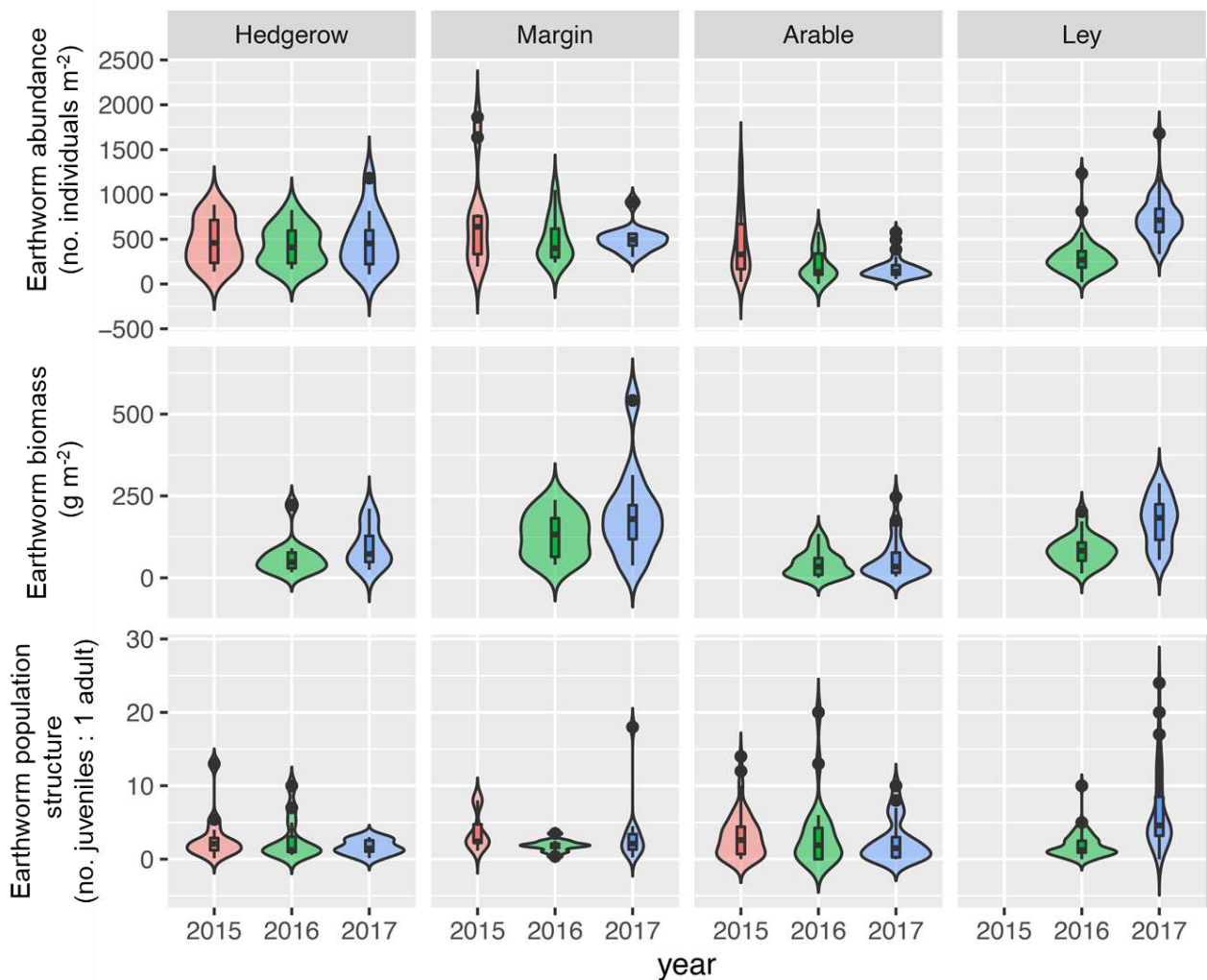
387 The baseline study in 2015 compared earthworm abundance under hedgerow,  
388 margin and continuous arable in four fields. A 2-way ANOVA with field (4 levels) and  
389 land use (3 levels) as factors showed significant effects of field ( $P < 0.001$ ) and land  
390 use ( $P < 0.05$ ), but no significant interaction ( $P > 0.05$ ). For reference, earthworm  
391 abundance in each field CON strip is shown in Fig. S4 (Supplementary information),  
392 indicating higher abundance in field A2 (see management history Table S1). Field  
393 differences are not considered further because the focus of this study is on land use  
394 impacts. In 2015, earthworm abundance was highest in the field margins ( $704 \pm 534$   
395 earthworms  $m^{-2}$ ) compared to the hedgerow ( $473 \pm 257$  earthworms  $m^{-2}$ ) and arable  
396 ( $460 \pm 359$  earthworms  $m^{-2}$ ) soils (Fig. 1A). Earthworm abundance across the  
397 different fields was not affected by in-field sampling distances (i.e. 2 to 64 m) within  
398 the arable strips ( $P > 0.05$ ).

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404 **Fig. 1. Earthworm abundance (top panel), biomass (mid panel) and population**  
 405 **structure (lower panel) in hedgerows, field margins, arable fields and new**  
 406 **arable-to-ley strips. Samples were taken in April 2015 (before new leys were**  
 407 **set up) and again in April 2016 and April 2017. For each year, n = 12 in**  
 408 **hedgerow and margin soils; n = 24 in arable soils (for 2015, n = 72 in arable**  
 409 **soils); n = 48 in ley soils. Biomass was not determined in 2015. The width of**  
 410 **each violin plot indicates the frequency distribution of the data for each land**  
 411 **use and year. Within each violin plot is a box and whisker plot. The central bar**  
 412 **is the median (50<sup>th</sup> percentile), which divides the box into the interquartile**  
 413 **range. The upper vertical line (whisker) extends to the largest value within 1.5**  
 414 **times the interquartile range above the 75<sup>th</sup> percentile, while the lower whisker**

415 **extends to the smallest value within 1.5 times the interquartile range below the**  
416 **25<sup>th</sup> percentile. The dots show outlier values beyond the interquartile range.**

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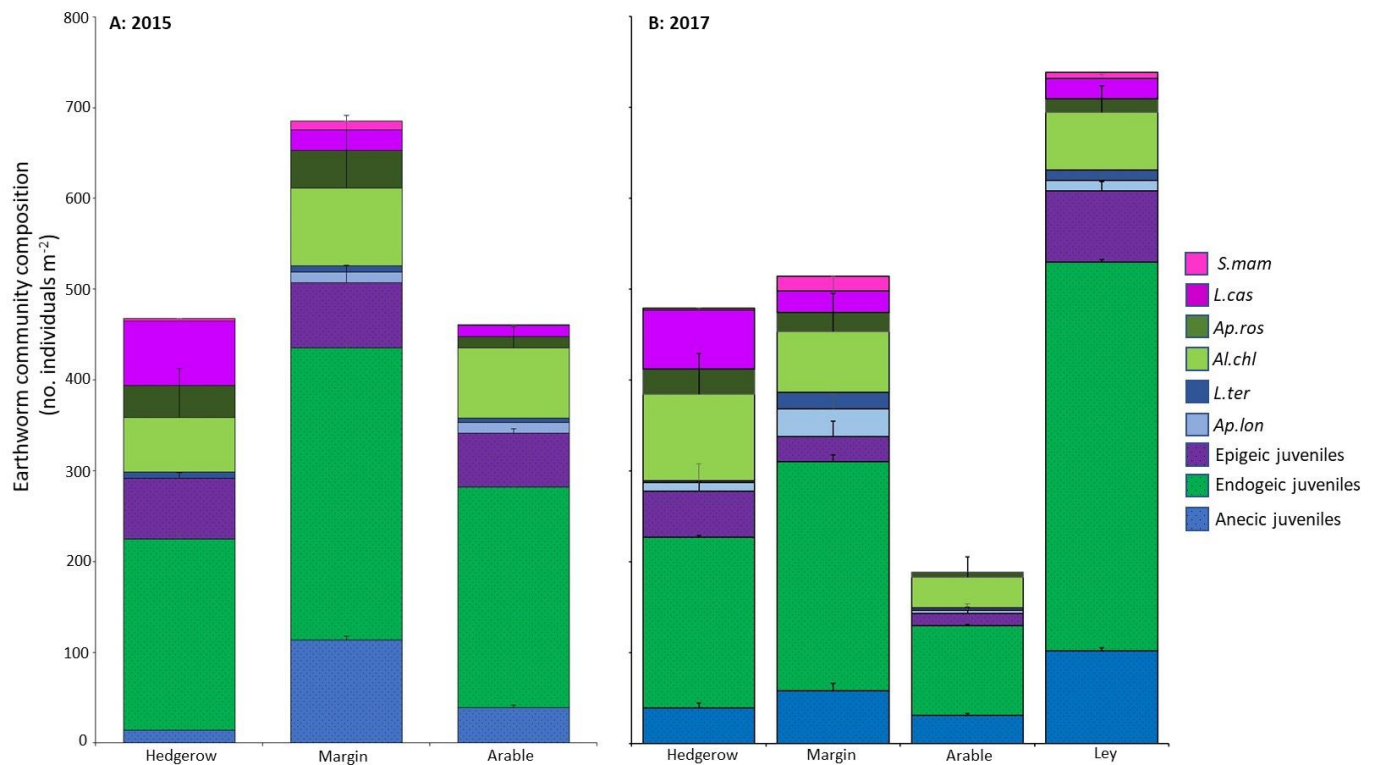
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**Fig. 2. Earthworm community mean abundance and composition by species**

**and functional group in the hedgerow, field margin, arable in April 2015 (A)**

**(baseline data, before ley strips were established) and in new ley soils in April**

**2017 (B). Colours indicate earthworm functional group (purple tones are**

**epigeic species and juveniles; green tones are endogeic species and**

**juveniles; blue tones are anecic species and juveniles). n = 12, 12, 24 and 48 in**

**hedgerow, margin, arable and ley soils respectively. Error bars show +1**

**standard error. Key: *S. mammalis* (*S.mam*; pink); *L. castaneus* (*L.cas*; dark**

**pink); *A. rosea* (*Ap.ros*; dark green); *A. chlorotica* (*Al.chl*; light green); *L.***

***terrestris* (*L.ter*; dark blue); *A. longa* (*Ap.lon*; light blue); Epigeic juveniles**

**(purple); Endogeic juveniles (green); Anecic juveniles (blue).**

### 445 3.2.2 Earthworm community structure and composition in April 2015

446 Baseline earthworm abundance and community composition are shown in Figs. 1  
447 and 2A. Species abundance was affected by land use ( $P < 0.001$ ) and field ( $P <$   
448  $0.001$ ) but not distance ( $P > 0.05$ ). Specifically, the abundance of anecic juveniles ( $P$   
449  $< 0.001$ ; lowest in hedgerow soils), *A. caliginosa* ( $P = 0.058$ ; marginally significant  
450 and most abundant in margin soils) and *L. castaneus* ( $P < 0.01$ ; most abundant  
451 under hedgerows) were affected by land use.

452

453 The three functional groups were represented in each land use (arable, hedgerow  
454 and margin), especially by juvenile earthworms. Margin soils tended to have the  
455 highest abundance of each species, except *L. castaneus* which was highest under  
456 hedgerows (Fig. 2A). Distribution of functional groups was also affected by land use  
457 ( $P < 0.01$ ) and field ( $P < 0.001$ ), but not distance from the margins ( $P > 0.05$ ).

458 Abundance of total anecic ( $P < 0.001$ ; lowest under hedgerows) and epigeic species  
459 ( $P = 0.05$ ) were affected by land use, whereas endogeics ( $P > 0.05$ ) were not.

460

### 461 3.3 Annual study: the arable to ley conversion

#### 462 3.3.1 Does connectivity facilitate earthworm migration into new leys?

463 Connectivity to hedgerows and grassy margins had no effect on earthworm  
464 abundance or biomass in the CAL ( $P > 0.05$ ) or UAL ( $P > 0.05$ ) ley soils. Earthworm  
465 abundance ( $P < 0.001$ ) and biomass ( $P < 0.001$ ) both increased in the CAL and UAL  
466 ley strips between 2016 and 2017. There was no effect of in-field distance on  
467 abundance ( $P > 0.05$ ) or biomass ( $P > 0.05$ ) within the ley strips and no significant

468 interactions between year, strip or distance. Therefore, in all subsequent analyses,  
469 UAL and CAL samples were combined across fields and treated as 'ley' (whereby  
470 ley  $n = 48$ ).

471

### 472 3.3.2. Impact of arable-ley conversion on earthworm abundance and biomass

473 Earthworm abundance increased significantly from a mean arable density of  $472 \pm$   
474  $366$  earthworms  $m^{-2}$  in 2015 (before ley conversion) to  $732 \pm 244$  earthworms  $m^{-2}$  in  
475 2017 in the new ley strips. There was also a significant increase in earthworm ley  
476 abundance between 2016 and 2017 ( $P < 0.001$ ) (Fig. 1A). In contrast to this  
477 increase, between 2015 and 2017, mean earthworm abundance in arable soils  
478 appeared to decline from  $470 \pm 376$  to  $185 \pm 132$  earthworms  $m^{-2}$  but this change  
479 was not significant. Annual densities in field margins ( $619 \pm 355$  earthworms  $m^{-2} yr^{-1}$ )  
480 and hedges ( $495 \pm 277$  earthworms  $m^{-2} yr^{-1}$ ) remained relatively stable over this  
481 period (2015, 2016 and 2017) ( $P > 0.05$ ) and are therefore reported as mean values  
482 over the whole period. Total earthworm biomass (annual data available for 2016 and  
483 2017 only; Fig. 1B) was also affected by land use ( $P < 0.001$ ). In April 2016,  
484 earthworm biomass in margin soils ( $130 \pm 67$  g  $m^{-2}$ ) was significantly greater than  
485 biomass in arable soil ( $43 \pm 39$  g  $m^{-2}$ ) ( $P < 0.001$ ). By April 2017, earthworm biomass  
486 in margin ( $195 \pm 135$  g  $m^{-2}$ ) and ley ( $171 \pm 74$  g  $m^{-2}$ ) soils was similar and both were  
487 significantly higher compared to biomass in arable soil ( $57 \pm 62$  g  $m^{-2}$ ) ( $P < 0.001$ ).

488

489 3.3.3. Effects of land use, year and distance on abundance of earthworm species  
490 and functional groups



491 Species distributions in each land use before and after ley establishment are shown  
492 in Fig. 2. Abundance of individual species was significantly affected by land use ( $P <$   
493  $0.001$ ) and year ( $P < 0.001$ ) but not distance ( $P > 0.05$ ). Species richness in the new  
494 ley soils in 2017 was similar to that found in margin and hedgerow soils, but was  
495 significantly higher compared to arable soil (Fig. S5). While *A. chlorotica* and  
496 endogeic and epigeic juveniles were found in all land uses ( $P > 0.05$ ), land use  
497 affected distribution of species such as *A. longa* and *L. terrestris* (low to no  
498 abundance in hedgerow or arable soils), *A. rosea* (lowest abundance in arable soils)  
499 and *L. castaneus* (low to no abundance in arable soils) (Fig. 2; S6). In particular, *A.*  
500 *chlorotica* and the three juvenile functional groups showed significant annual  
501 increases in abundance. Similar trends were found for the three earthworm  
502 functional groups (adults and juveniles), which were affected by land use ( $P < 0.001$ )  
503 and year ( $P < 0.001$ ), but not distance ( $P > 0.05$ ) (Fig. 2; S6). While anecic  
504 earthworms were found in all land uses, their abundance was lowest in arable and  
505 hedgerow soils.

506

507 In both 2015 and 2017, earthworm abundance was dominated by juvenile  
508 earthworms: juveniles made up at least 60% of the average earthworm abundance in  
509 each land use. In 2015, the proportion of juveniles:adults was generally highest in  
510 the arable soil (~76% juveniles). However, in 2017, the proportion of juveniles was  
511 greatest in the ley soil (~83% juveniles). Abundance of functional groups followed the  
512 order endogeic > epigeic > anecic earthworms. Juvenile earthworms made the  
513 largest contribution to abundance overall and within each functional group. Juvenile  
514 endogeic, anecic and epigeic earthworms made up 50.1%, 10.7% and 10.8% of total  
515 abundance.

516 In terms of biomass (Fig. S7), anecic juveniles and adults dominated earthworm  
517 biomass in margin, ley and arable soils. Endogeic earthworms were dominant in  
518 biomass in hedgerow soils. The biomass of the anecic adult earthworms, especially  
519 *L. terrestris* (which was not the dominant anecic earthworm in terms of abundance)  
520 was highest in the margin soil.

521

### 522 3.4 Population structure

523 In the baseline data (2015), population structure (Fig. 1C) was similar in hedgerow,  
524 margin and arable soils, nor did it change over time in these soils ( $P > 0.05$ ). In the  
525 ley soils, population structure was similar to arable soils in 2016, but changed the  
526 following year ( $P < 0.001$ ). In 2017, population structure in the ley soils had  
527 increased from an arable soil baseline (2015) mean juvenile:adult ratio of 3.1:1 and  
528 3.3:1 in 2016, to a mean ratio of 6.5:1.

529

### 530 3.5 Soil physical properties (annual study)

531 Soil moisture, temperature and bulk density (Fig. S8) varied with sampling year: soils  
532 tended to be drier ( $0.43 \pm 0.003 \text{ g g}^{-1}$  (2016) vs.  $0.38 \pm 0.003 \text{ g g}^{-1}$  (2017);  $P <$   
533  $0.001$ ), warmer ( $9.50 \pm 0.107 \text{ }^\circ\text{C}$  (2016) vs.  $10.55 \pm 0.08 \text{ }^\circ\text{C}$  (2017);  $P < 0.001$ ) and  
534 had a higher bulk density ( $1.02 \pm 0.009 \text{ g cm}^{-3}$  (2016) vs.  $1.12 \pm 0.007 \text{ g cm}^{-3}$   
535 (2017);  $P < 0.001$ ) in 2017 compared to 2016. Overall, soil moisture was lower (~ 6%  
536 drier) and soil temperature higher (~1 °C) in April 2017 compared to April 2016. This  
537 complements the weather station monitoring data, which indicated that April 2017  
538 was drier compared to the 20-year average for the farm (Fig. S9).

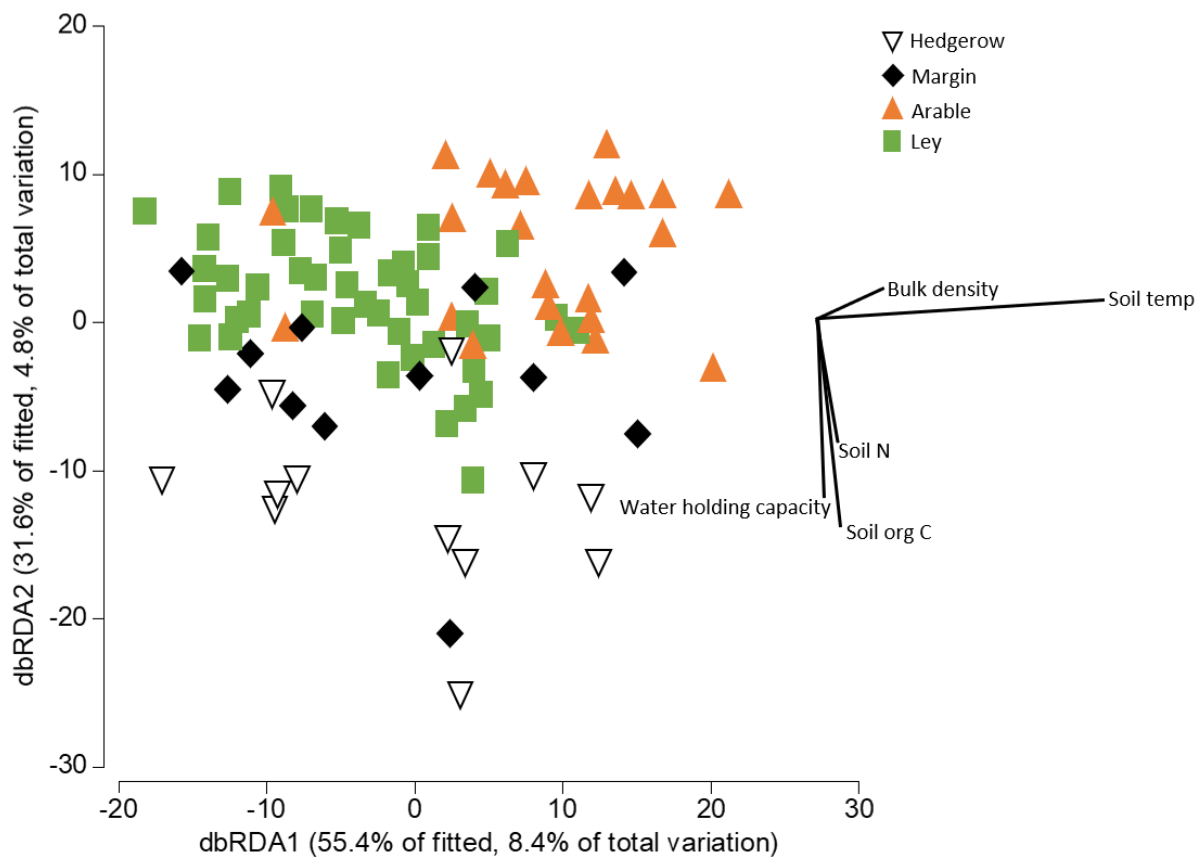
539

540 Soil properties varied with land use and depth, but were not affected by distance  
541 from the field margins. Interactions between year and land use ( $P < 0.001$ ) or year  
542 and depth ( $P < 0.001$ ) in soil moisture reflected the drier and warmer conditions  
543 during sampling in April 2017 compared to April 2016. Overall, mean soil moisture  
544 was significantly higher in margin soils compared to arable, hedgerow and ley soils  
545 ( $P < 0.001$ ). Mean soil temperatures under hedgerows and margin soils were similar,  
546 and the highest mean soil temperatures were recorded in arable soils. Although soil  
547 bulk density tended to be low under hedgerows, there were no significant land use or  
548 depth effects in the soil bulk density data. SOC was highest under hedgerows ( $3.07$   
549  $\pm 0.43\%$ ) and lowest in arable and, to a lesser extent, ley soils ( $1.93 \pm 0.29\%$  and  
550  $2.13 \pm 0.36\%$  respectively) ( $P < 0.001$ ; Fig. S8).

551

552 Earthworm abundance (2017 data) showed weak correlations with soil properties.  
553 For example, abundance and biomass were negatively correlated to soil temperature  
554 ( $r = -0.4, -0.2$ ;  $P < 0.001$ ;  $P = 0.017$  respectively) and bulk density ( $r = -0.05, -0.2$ ;  $P$   
555  $= 0.633, P = 0.043$  respectively) (at 5 cm depth), but showed no correlation with soil  
556 moisture ( $r = 0.2, 0.2$ ;  $P = 0.117, P = 0.117$  respectively) (5 cm depth).

557 The relationship between earthworm species composition and soil properties in each  
558 land use was analysed using dbRDA (Fig. 3), using earthworm, soil temperature and  
559 density data collected in April 2017 and SOC, N and water holding capacity collected  
560 in April 2018. Here, soil temperature is a strong factor differentiating arable soils from  
561 ley, margin and hedgerow soils. Margin and hedgerow soils tend to separate along  
562 increasing SOC contents.



564

565 **Fig. 3. Distance based redundancy analysis (dbRDA) showing the relationship**  
 566 **between earthworm species in hedgerow, margin, arable and ley soils and**  
 567 **associated soil variables**

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570 3.7. Seasonal study

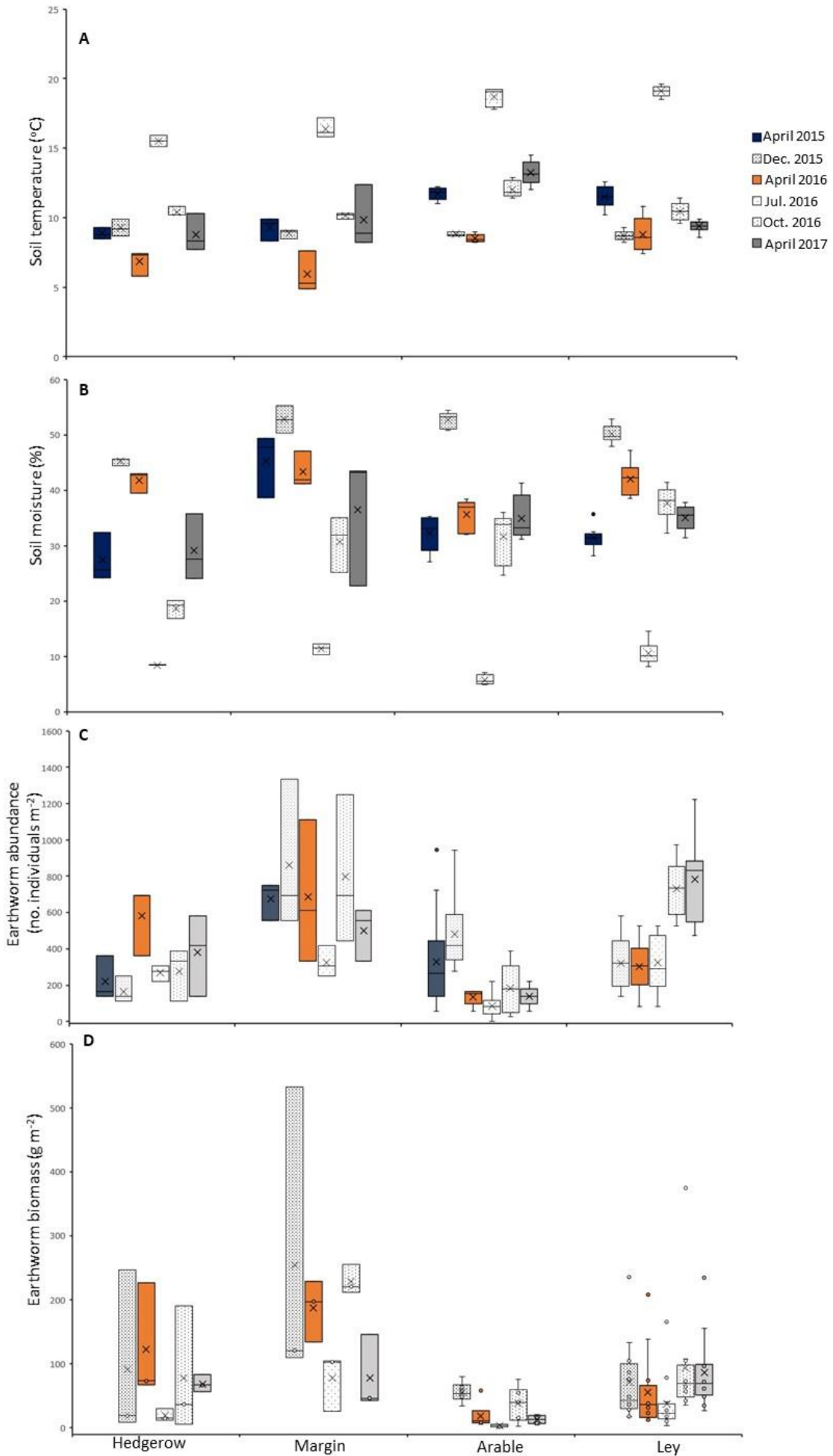
571 3.7.1 Seasonal effects on earthworm abundance, biomass and species abundance  
 572 and functional groups

573 The sampling periods over 2015-2017 had highly variable rainfall patterns, and some  
 574 sampling periods coincided with when rainfall was lower (e.g. April 2015; April, July

575 and October 2017), or higher (e.g. December 2015; April 2016) than the long-term  
576 average (2001-2018) (supplementary information, Fig. S9). In turn, variability in the  
577 weather was also reflected in soil temperature and moisture measured from the  
578 sampled earthworm pits (Fig. 4A&B). There were strong effects of month ( $P < 0.001$ )  
579 and land use ( $P < 0.001$ ) on soil temperature and moisture (at both 5 and 10 cm  
580 depths). Land use also affected temperature: arable and ley soils tended to be  
581 warmer than hedgerow and margin soils. Moisture content indicated relatively drier  
582 soils under hedgerows compared to arable, ley and margin soils.

583

584 As expected, seasonal variability affected earthworm distributions, particularly in the  
585 annual spring surveys where for example, April 2017 which was relatively dry  
586 compared to previous spring samples, had a lower overall total abundance. There  
587 was a significant effect of month ( $P < 0.001$ ) and land use ( $P < 0.001$ ) and a  
588 significant month-land use interaction on earthworm abundance ( $P < 0.001$ ) (Fig.  
589 4C). Earthworm abundance across land uses tended to be higher in wetter and  
590 cooler months, with peak abundance coinciding with greater than average rainfall;  
591 and abundance was lower in drier and warmer months. Similar to abundance data,  
592 earthworm biomass showed similar significant effects of month ( $P < 0.001$ ) and land  
593 use ( $P < 0.001$ ) (Fig. 4D).



595 **Fig. 4. Seasonal variation in soil temperature (A), soil moisture (B), earthworm**  
596 **abundance (C) and biomass (D) in hedgerows, field margins, arable field and**  
597 **new arable-to-ley strips (in field A4). Samples were taken in April 2015 (before**  
598 **new leys were set up) and again in April 2016 and April 2017, with additional**  
599 **seasonal sampling in December 2015, July 2016 and October 2016. For each**  
600 **month, n = 3 in hedgerow and margin soils; n = 6 in arable soils (for April 2015,**  
601 **n = 18 in arable soils); n = 12 in ley soils). Biomass was not determined in April**  
602 **2015. In the boxplots, X marks the mean, with the median line dividing the box**  
603 **into the interquartile range. Outliers are shown beyond the maximum and**  
604 **minimum vertical lines.**

605

606

607

608 Species abundance and functional groups (see Fig. S10) varied with land use ( $P <$   
609  $0.001$ ) and month ( $P < 0.001$ ). Sampling month affected abundance of anecic ( $P <$   
610  $0.01$ ), endogeic ( $P < 0.001$ ) and epigeic ( $P < 0.01$ ) juveniles, as well as *A. chlorotica*  
611 ( $P < 0.001$ ) and *L. castaneus* ( $P < 0.01$ ). While total abundance of each functional  
612 group was affected by land use ( $P < 0.001$ ), total abundance of anecic ( $P < 0.01$ )  
613 and endogeic ( $P < 0.001$ ) groups were also affected by month, but total abundance  
614 of epigeic earthworms was not ( $P > 0.05$ ).

615

616 3.7.2 Effect of seasonal antecedent weather and soil conditions on earthworms

617 Earthworm abundance and biomass did not correlate with antecedent soil moisture  
618 or temperature. However, earthworm biomass did negatively correlate with the  
619 number of dry days in the 14 days before sampling (ADD14;  $r = -0.876$ ;  $P = 0.051$ ).  
620 Antecedent dry day correlations were then calculated for each land use to see if land  
621 use had an effect. Earthworm abundance in arable soil negatively correlated with the  
622 number of dry days in the preceding 30- and 50-day periods before sampling  
623 (ADD30,  $r = -0.884$ ,  $P < 0.05$ ; ADD50,  $r = -0.897$ ,  $P < 0.05$ ). Earthworm biomass in  
624 arable soils also negatively correlated with the number of dry days in the preceding  
625 30-day period (ADD30,  $r = -0.962$ ,  $P < 0.01$ ). Earthworm abundance in margin soils  
626 negatively correlated with each of the short-term antecedent periods, i.e. the number  
627 of dry days 7, 14, 21 or 30 days before sampling ( $r > -0.9$ ,  $P < 0.05$ ), but not the  
628 longer-term periods (50, 80 or 100 days). There were no correlations between ADD  
629 and earthworm abundance in ley soil or under hedgerows.

630

631

#### 632 **4. Discussion**

633

634 This study set out to test three hypotheses relating to earthworm distributions in  
635 agro-ecosystems. We found that although earthworm communities were more  
636 diverse and distinct in hedgerow and margin soils compared to arable soils (H1),  
637 there was no effect of distance from high diversity field boundary soils (H2) or  
638 connectivity to the field margins (H3) on earthworm distributions in newly established  
639 leys. Earthworm distributions varied with annual and seasonal differences in soil  
640 moisture and temperature.



641

642 4.1 Earthworm communities in hedgerow and grassy field margins are distinct  
643 compared to those under continuous conventional arable (Baseline study)

644 In this study, soils under continuous conventional arable had a smaller and less  
645 diverse earthworm community, which was largely dominated by endogeic juvenile  
646 earthworms. In comparison, grassy field margins had the highest and most diverse  
647 earthworm communities. Earthworm abundance under hedgerows was similar to  
648 arable soils, but community composition was dominated by epigeic juveniles under  
649 hedgerows, where there is substantial deciduous litterfall. These findings agree with  
650 other studies showing that land use and cropping history in agricultural landscapes  
651 play a large role in determining earthworm abundance, biomass, species  
652 composition, age structure and functional group distribution (Blouin et al., 2013;  
653 Evans and Guild, 1948).

654

655 Continuous conventional arable cultivation with annual crops is detrimental to  
656 earthworms, due to soil disturbance during tillage, chemical inputs as fertilisers and  
657 pesticides, increased risk of predation after cultivation, and the removal of a  
658 permanent plant cover and crop residues, which reduce earthworm food resources  
659 (Briones and Schmidt, 2017; Stroud et al., 2016; Chan, 2000). In this study, arable  
660 soils had low SOC, high bulk density, and tended to be warmer and drier compared  
661 to margin soils. Additional related studies by Holden et al., (2019) and Hallam et al.,  
662 (2020) at the same field sites confirm the hydrological functioning of these arable  
663 soils have lower infiltration rates and fewer macropores than for the hedgerow and

664 margin soils. There are important links between earthworm communities and soil  
665 water (e.g. Spurgeon et al, 2013; Hallam and Hodson, 2020; Hallam et al., 2021).

666

667 In contrast, environmental stability in grassy field margins supported the highest and  
668 most diverse earthworm community in this study. Earthworm communities in field  
669 margins respond positively to environmental stability, the accrual of SOM and  
670 abundance of food resources for earthworms (Roarty and Schmidt, 2013; Frazão et  
671 al., 2017; Smith et al., 2008; Crittenden et al., 2015).

672

673 Despite the historical dominance of hedgerows and their potential loss in farming  
674 landscapes (Holden et al., 2019), few studies consider earthworm communities  
675 under hedgerows (e.g. Smith et al., 2008). Studies on field boundaries in agricultural  
676 landscapes tend to focus on grassy field margins. In this study, hedgerow soils had  
677 the highest abundance of epigeic earthworms. Soil pH was unlikely to be a strong  
678 factor in earthworm distributions between hedgerow and arable soils, because soil  
679 solution pH values were neutral and were not significantly different (see Holden et  
680 al., 2019). Hedgerow and arable soils had the greatest differences in bulk density  
681 and SOC contents which would affect earthworm abundance and community  
682 composition in these contrasting habitats (Fig. 2). Hedgerow soils also tended to be  
683 drier over the monitoring period of this study, reflecting the potentially large  
684 evapotranspiration of hedges and their interception of rainfall (Holden et al., 2019),  
685 which would tend to lower earthworm abundance compared to margin and ley soils.

686

687 4.2 New grass-clover leys boost earthworm abundance in arable soils (Annual study)

688 In this study, we showed that earthworm communities increased rapidly in new leys  
689 within 2 years of establishment, confirming the benefits of clover-rich ley rotations to  
690 earthworms (Riley et al., 2008; Scullion et al., 2007; Jarvis et al., 2017; van Eekeren  
691 et al., 2008; Schmidt et al., 2001). For example, van Eekeren et al., (2008) showed  
692 that earthworm abundance increased under a grass ley, and abundance was similar  
693 to the long-term grassland within 2 years. However, in their study, the biomass was  
694 still smaller than that observed in permanent grassland. In contrast, in our study, the  
695 abundance and biomass in the leys were similar to that observed in the field  
696 margins. Comparing our data to earthworm abundance in adjacent long-term pasture  
697 fields indicated that earthworm abundance in new leys was also similar to the 3-year  
698 average abundance in pasture soils (see Holden et al., 2019). Our results highlight  
699 that in these soils, 2 years under clover-rich grass ley is sufficient to increase  
700 earthworm populations. Availability of high quality food sources such as clover, as  
701 well as the reduction in ploughing disturbance have been linked to increased  
702 earthworm abundance (Schmidt et al., 2001; Yeates et al., 1998). In particular,  
703 Yeates et al., (1998) demonstrated the value of grass-clover mixtures in supporting  
704 higher earthworm abundance: earthworm biomass increased by only 13 g m<sup>-2</sup> after 5  
705 years in perennial ryegrass compared to 113 g m<sup>-2</sup> under a ryegrass-clover mix.

706

707 The conversion of arable to ley demonstrated the rapid increase in earthworm  
708 abundance following cessation of disruptive ploughing. The positive effect on  
709 earthworms was not only in abundance, but also in species richness, biomass and  
710 population structure. In related studies (Hallam et al., 2020; Hallam and Hodson,  
711 2020; Hallam et al., 2021) we have shown the positive effects earthworm increases  
712 have on soil properties; although it is possible that there might be a time-lag in the

713 field between earthworm population change and changes in hydrological function.  
714 However, in this study, earthworms showed weak correlations to soil physical  
715 properties, and other variables such as soil texture, soil aggregation, plant biomass  
716 and organic matter may be important to consider. Nevertheless, it is plausible that  
717 the lack of soil disturbance and the presence of a permanent plant cover, continuous  
718 inputs of plant litter and plant C contributed to the positive effect on earthworms.  
719 Hallam et al., (2021) found a synergistic relationship between endogeic earthworms  
720 and plants in terms of improved soil hydraulic properties. Meta-analyses of data sets  
721 reporting earthworm abundance after converting arable to grassland showed that  
722 increases in earthworm abundance tended to occur in the short-term (0-3 yrs), with  
723 rapid increases due to the endogeic earthworms which tend to dominate in arable  
724 soils. As soil conditions improved and food resources became more available, so did  
725 the diversity in endogeic, epigeic and anecic earthworms over time; however longer  
726 periods since conversion did not add to the increase (Spurgeon et al., 2013).

727

#### 728 4.3 Arable soils are important biological reservoirs (Annual study)

729 Our experimental design set out to determine the role of migration from biodiversity  
730 refugia into new ley strips. Our data show that the earthworm barrier at the margin-  
731 field boundary had no effect on earthworm distributions within the new leys. Although  
732 abundance was higher in margin soils, there was no gradient in earthworm  
733 distribution with distance from hedgerow and margin. The lack of a gradient in  
734 earthworm density has also been shown by Roarty and Schmidt (2013), Crittenden  
735 et al., (2015) and Smith et al., (2008). Where distance effects have been reported,  
736 these tend to reflect lower abundance at distances close to the margin, where

737 vehicle traffic may have compacted soils, leading to lower earthworm numbers (e.g.  
738 Hof and Bright, 2010). The barriers were only installed at the head of each  
739 unconnected strip, so we cannot completely discount migration of earthworms from  
740 beyond the 13 m-long barrier at the margin-field boundary or from other parts of the  
741 arable field. Evidence from soil surface pitfall traps reveal that several earthworm  
742 species, particularly anecics, but also endogeics and some epigeics crawl over the  
743 soil surface at night (Eijsackers, 2011). Frazao et al., (2017) also noted that *L.*  
744 *terrestris* was likely to colonise plots in which they were not introduced, although  
745 there were 'large' buffer zones between the plots. Therefore, burrowing and soil  
746 surface dispersal from adjacent arable soils could also account for the population  
747 increase, as earthworms in lower quality arable soils are attracted into the new leys  
748 as conditions improved over time. Our study supports previous work by Smith et al.,  
749 (2008), Roarty and Schmidt (2013), Crittenden et al., (2015) and Frazao et al.,  
750 (2017) which demonstrate that although field margins have higher earthworm  
751 abundance, there is no 'spill-over' effect into adjacent arable fields. Roarty and  
752 Schmidt (2013) proposed that the survival of in-field populations supported the  
753 increase in earthworm abundance, rather than immigration from surrounding refugia  
754 (Marinissen and van den Bosch, 1992; Nuutinen et al., 2011). Our data support their  
755 idea of improved survival of in-field earthworm populations. However, we cannot rule  
756 out that the ley populations were also increased by immigration from the surrounding  
757 arable field as soil quality developed in the new leys. Our results demonstrate that  
758 despite low abundance and dominance by juvenile earthworms, intensively managed  
759 arable soils can still be considered as biodiversity reservoirs. Despite very limited  
760 abundance, this arable reservoir held juvenile representatives of the three functional  
761 groups. Our understanding of arable soil earthworm species diversity is incomplete

762 because juveniles were identified to functional group only. However, arable  
763 earthworm diversity would still be relatively lower compared to the margins or new  
764 leys because of soil conditions and limited food resources. The new leys provided  
765 the stability and permanent plant cover which gave the juvenile earthworms the  
766 opportunity to mature and reach adulthood. Furthermore, the change in population  
767 structure suggests a shift from low survival and low abundance in arable soils to  
768 improved conditions in the new ley soils allowing adult earthworms to produce more  
769 offspring. Spurgeon et al., (2013) also suggested that the rapid accrual of  
770 earthworms following arable to grassland conversion was driven by improved  
771 survival of juvenile earthworms. They went on to highlight that endogeic species,  
772 which often dominate agroecosystems, may have an important role in initiating a  
773 positive feedback in soil conditions, thus facilitating the recruitment of epigeic and  
774 anecic species once plant surface litter became more available. Endogeic juveniles  
775 and species were the dominant group in our study. This highlights that arable soils  
776 may be potential reservoirs for all earthworm groups, but this is likely to depend on  
777 soil conditions and the historical legacy of land use management.

778

779 The increase in the number of adult earthworms in the leys could have been due to  
780 migration from the surrounding arable soils, resulting in an apparent decline in arable  
781 soil adult earthworms (Fig. 2). However, population structure remained the same in  
782 arable soils over the study period. Also, April 2017 was relatively drier than previous  
783 years, resulting in relatively lower earthworm abundance overall (the ley soils were  
784 the only land use to show an increase in abundance in 2017). It is likely that cocoon  
785 production and survival increased in the ley soils and also contributed to the increase  
786 in earthworm abundance. In addition, any adult earthworms that dispersed from the

787 adjacent arable soil in the new leys are likely to reproduce and deposit cocoons. This  
788 may help account for the doubling in the juvenile:adult ratio in new leys compared to  
789 no change in arable, hedgerow or margin soils over the monitoring period.

790

#### 791 4.4 Annual and seasonal changes in earthworm abundance

792 Temporal variation in soil moisture and temperature have strong effects on  
793 earthworm populations (Lowe and Butt, 2005; Schmidt et al., 2001; Whalen and  
794 Parmelee, 1999; Evans and Guild, 1947). As expected, earthworm abundance in  
795 our study correlated positively with soil moisture but negatively with temperature, and  
796 numbers declined during summer (July 2016) and the unusually dry spring (April  
797 2017). In addition, our analyses show that earthworm data can be negatively  
798 affected by antecedent dry weather. Therefore, it is important to report weather  
799 conditions for the week before sampling in association with earthworm sampling  
800 data. Our data indicated that there were no correlations between number of  
801 antecedent dry days and earthworm abundance in soils from new leys or under  
802 hedgerows, suggesting that semi-permanent plant cover may help earthworm  
803 populations to be more resilient to fluctuations in soil conditions.

804

805

## 806 **5. Conclusions**

807

808 Results from our field experiments clearly show the positive impact on earthworm  
809 populations of converting arable soils, which are relatively low in earthworms, to

810 grass-clover leys. This conversion increased earthworm abundance, biomass, and  
811 species diversity, while population structure shifted to relatively more adults. After 2  
812 years, earthworm abundance had reached the average levels recorded in long-term  
813 pasture fields at the same site (see Holden et al., 2019), suggesting the maximum  
814 population level had been achieved. This rapid increase has important implications  
815 for soil management as arable farming practices seek to move towards sustainability  
816 and ecological intensification, as leys are also associated with other co-benefits to  
817 soil health, soil function, resilience and plant productivity. The policy mechanisms to  
818 encourage farmers to adopt rotational leys and the barriers to their use now need to  
819 be investigated.

820

821 Our data confirmed that although arable soils are depleted in earthworms, they can  
822 still be important reservoirs of biological diversity. Once converted to grass-clover  
823 ley, the earthworm community inhabiting former arable soils was able to survive  
824 under improved soil conditions, which supported the rapid increase in earthworm  
825 abundance. However, active dispersal from adjacent arable soils to the leys, due to  
826 greater food supply and improved conditions in the new leys, may also be  
827 contributing to the population increase. Our data show that sampling campaigns  
828 coinciding with periods of relatively high or low soil moisture strongly affect  
829 earthworm abundance assessments. This has implications for soil health metrics and  
830 long-term data collection is necessary to avoid misinterpreting changes in soil  
831 management practices.

832

833



834

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849

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