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1 **Effect of earthworms on soil physico-hydraulic and chemical properties,**
2 **herbage production, and wheat growth on arable land converted to ley.**

4 **Running title:** Effect of earthworms on soil properties

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23 **Abstract**

24 Effects of earthworms on soil physico-hydraulic properties, herbage production and wheat
25 growth in long-term arable soils following conversion to ley were investigated. Seven intact
26 soil monoliths were collected from each of four arable fields. One monolith per field served as
27 a control. The other six were defaunated by deep-freezing; three were left defaunated (DeF)
28 and three (DeF+E) were repopulated with earthworms to mimic pasture field density and
29 diversity. The monoliths were planted with a grass-clover ley and inserted into pre-established
30 ley strips in their original fields for 12 months. Hydraulic conductivity measurements at -0.5
31 cm tension ($K_{0.5}$) were taken five times over the year. $K_{0.5}$ significantly increased in summer
32 2017 and spring 2018 and decreased in winter 2017-18. $K_{0.5}$ was significantly greater (47%)
33 for DeF+E than DeF monoliths. By the end of the experiment, pores >1 mm diameter made a
34 significantly greater contribution to water flow in DeF+E (98%) than DeF (95%) monoliths.
35 After only a year of arable to ley conversion, soil bulk density significantly decreased (by 6%),
36 and organic matter (OM) content increased (by 29%) in the DeF treatments relative to the
37 arable soil. Earthworms improved soil quality further. Compared to DeF monoliths, DeF+E
38 monoliths had significantly increased water-holding capacity (by 9%), plant-available water
39 (by 21%), OM content (by 9%), grass-clover shoot dry biomass (by 58%), water-stable
40 aggregates > 250 μm (by 15%) and total N (by 3.5%). In a wheat bioassay following the field
41 experiment, significantly more biomass (20%) was produced on DeF+E than DeF monolith
42 soil, likely due to the changed soil physico-hydraulic properties. Our results show that
43 earthworms play a significant role in improvements to soil quality and functions brought about
44 by arable to ley conversion, and that augmenting depleted earthworm populations can help the
45 restoration of soil qualities adversely impacted by intensive agriculture.

46 **Keywords:** Soil fauna, hydraulic conductivity, soil water release curves, water-holding
47 capacity, plant available water, wheat bioassay.

48

49 **1. Introduction**

50 Soil degradation affects about 33% of land worldwide and is a major threat to future food
51 security, increasing human vulnerability to extreme events resulting from climate change
52 (FAO and ITPS, 2015). Estimates of the costs to the global economy of soil degradation range
53 widely from US\$231 billion per year (Nkonya et al., 2016) to US\$10 trillion per year (The
54 Economics of Land Degradation, 2015), which is equivalent to 160% of the global spend on
55 healthcare (World Health Organisation, 2012). Soil degradation involves both loss of soil
56 functions, such as depleted organic matter content which reduces carbon, water and nutrient
57 storage, and loss of soil volume caused by erosion and compaction. The degradation of soil
58 quality and quantity are interlinked, as reduced water-holding capacity and infiltration rates
59 and poorer crop establishment leave soil more vulnerable to wind and water erosion (Durán
60 Zuazo and Rodríguez Pleguezuelo, 2008; Turner et al., 2018; United Nations Convention to
61 Combat Desertification, 2017). Intensive arable cultivation by growing annual crops on soils
62 that are ploughed and harrowed each year is a major cause of soil degradation, yet as recently
63 as 2016, 60% of arable land in England was cultivated in this way (Townsend et al., 2016).
64 Arable farming accounts for 29% of the land use of England and Wales and is responsible for
65 31% of the total costs associated with soil degradation, in terms of the loss of capacity of soils
66 to deliver ecosystem services (Graves et al., 2015). These costs have been estimated at US\$1.4
67 - 1.9 billion per year without considering the cost of diffuse pollution, soil biota loss and sealing
68 (Graves et al., 2015); the core contributions to these costs are estimated to be loss of soil organic
69 matter (47%), compaction (39%) and erosion (12%).

70 Increasing awareness of the economic and environmental impacts of soil degradation, for
71 example highlighted in the UK by a parliamentary inquiry into soil health (House of Commons,
72 2016), has led to policies around the world to protect soil, for example, the policy goal in the

73 UKs 25 year Environment Plan (House of Commons, 2018) to sustainably manage all of
74 England's soils by 2030. Central to achieving this aspiration is the need to increase soil organic
75 matter content, create a better soil structure, enhance the hydrological function of the soil (e.g.
76 enhanced infiltration and water storage) and to protect the soil surface from erosion (Blanco-
77 Canqui and Lal, 2008). This could be achieved in a number of ways, including through the use
78 of arable-ley rotations and minimum- or no-till methods (van Capelle et al., 2012; van Eekeren
79 et al., 2008). These are less damaging to earthworms (Edwards and Lofty, 1982) and
80 mycorrhizal fungal symbionts of plant roots, that together assist in soil aggregate stabilization
81 and soil carbon sequestration (Asmelash et al., 2016; Wilson et al., 2009; Zhang et al., 2013).
82 While these management approaches favour the development of earthworm populations (Chan,
83 2001; van Capelle et al., 2012) it is unclear as to the extent to which the action of the
84 earthworms, as distinct from other effects of these management methods, such as reduced soil
85 disturbance, greater aggregation of soil by perennial plant roots and mycorrhizal fungal hyphae,
86 and increased organic matter inputs, give rise to observed improvements in soil properties.

87 Earthworms increase soil organic matter content by incorporating organic material into soil
88 (Fahey et al., 2013), enhance soil aggregation in which organic carbon is protected (Sharma et
89 al., 2017), and generate macropores that increase soil water flow (Francis and Fraser, 1998),
90 which in turn protects the soil surface against erosion (Jouquet et al., 2012). Adding
91 earthworms to improve soil properties (Sinha, 2009; Sinha et al., 2010), especially in
92 combination with land-management changes that are more favourable to them such as
93 introduction of leys into arable rotations, has the potential to be economically affordable,
94 environmentally sustainable and socially acceptable. Earthworms can process up to 250 tonnes
95 ha^{-1} of soil each year (Birkas et al., 2010; Zaller et al., 2013) and reproduce rapidly under
96 optimal soil conditions when sufficient food is provided (≈ 27 -82 earthworms per year from a
97 single adult earthworm) (Butt and Lowe, 2011; Johnston et al., 2014; Lowe and Butt, 2005),

98 which could lead to rapid changes in soil properties. The effect of earthworms depends both on
99 which earthworm species are present and the soil conditions (Clause et al., 2014; Hallam, 2018;
100 Hedde et al., 2013). Typically, in field conditions, earthworms are present in mixed
101 communities comprising several species, belonging to the three main ecological groups -
102 epigeic, endogeic and anecic (Kooch and Jalilvand, 2008) - that interact with other soil biota
103 and plant roots. Under laboratory conditions, individual earthworm species interactions with
104 plant roots have resulted in significantly greater improvements in soil physico-hydraulic
105 properties by endogeic compared to anecic earthworm species (Hallam, 2018).

106 This study forms part of the larger NERC Soil Security Programme SoilBioHedge project
107 which tested the hypothesis that grass-clover leys sown into arable fields and connected to
108 hedgerows and unploughed grassy margins enable key ecosystem-engineers (earthworms,
109 mycorrhizal fungi) to recolonize fields, restoring and improving soil quality compared to leys
110 unconnected to field margins. The aim of the experiment detailed here was to isolate the effects
111 of earthworm communities on soil physico-hydraulic properties and plant growth from the
112 effects due to the change in cultivation and vegetation when arable soils are converted to grass-
113 clover leys. To achieve this aim we conducted experiments using intact soil monoliths (Allaire
114 and Bochove, 2006) in arable fields. We set out to test the hypothesis that earthworm
115 populations make a substantial contribution to improvements in soil properties and functions
116 in addition to improvements resulting from converting arable land that has been intensively
117 cultivated for many decades into grass-clover leys. These improvements are expected to
118 include increased soil carbon sequestration, increased aggregate stability, and changes to
119 hydrological functions such as increased infiltration rates and water storage (Blouin et al.,
120 2013).

121

122 Using soil monoliths taken from arable fields, grass-clover leys were established, and
123 earthworm populations manipulated (see Methods for details). We monitored soil hydraulic
124 conductivity at five time points and plant shoot biomass twice over 12 months. At the end of
125 the experiment, we measured soil water release curves, soil water-holding capacity, bulk
126 density, percentage soil mass in water-stable aggregates > 250 μm , organic matter content, total
127 nitrogen content, and earthworm diversity. Soil from each monolith was then used in a 6-week
128 wheat growth bioassay. These studies enabled us to test the effects of earthworms on a set of
129 key measures of soil quality and functions that deliver important ecosystem services such as
130 carbon sequestration, water infiltration and storage, flood risk reduction and crop production.

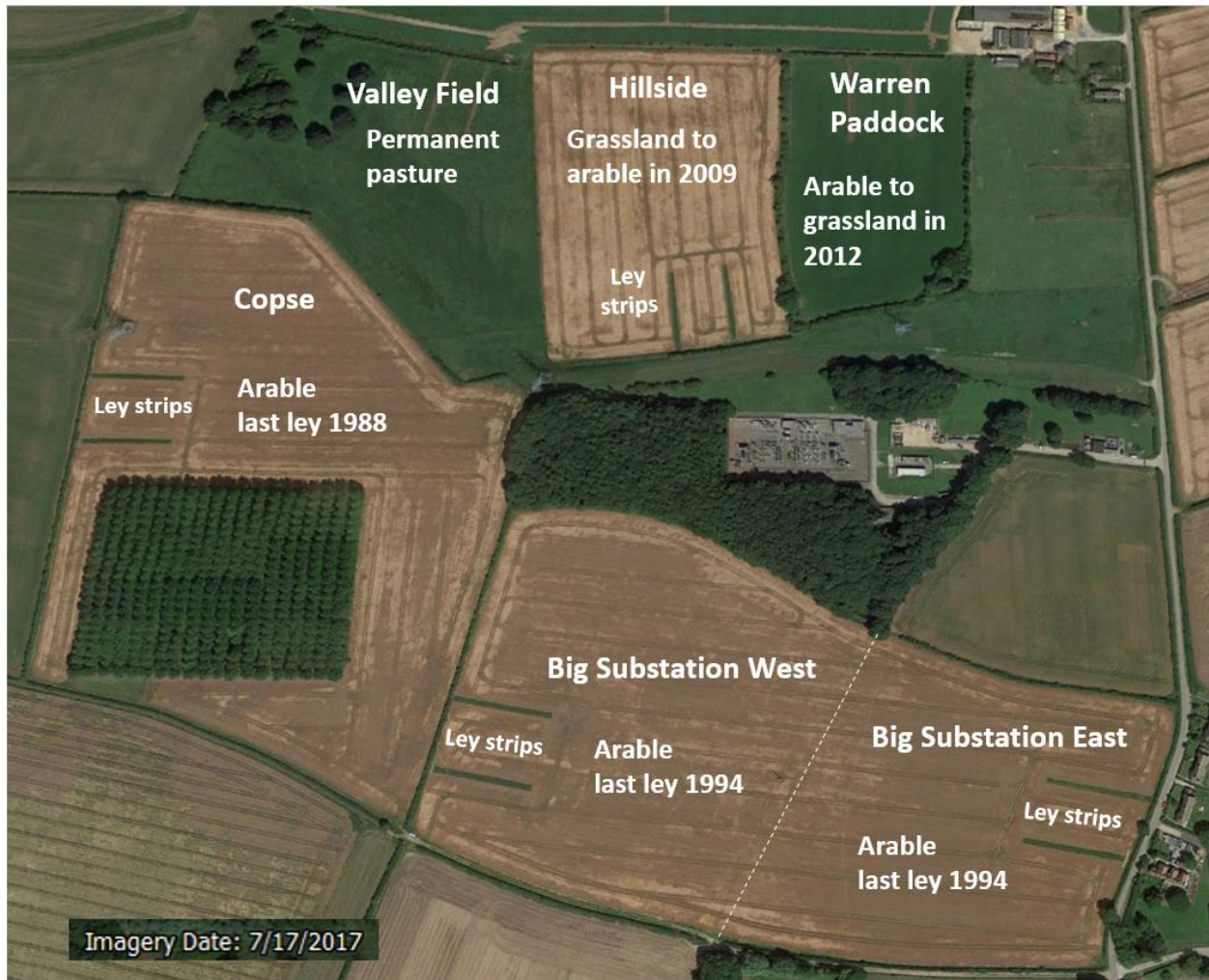
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132 2. Materials and methods

133 2.1. Site and experimental design

134 Seven intact monoliths were extracted from each of four arable fields (approximately 70 m from
135 the field margin) in March 2017 at the University of Leeds Farm (northern England; 53° 52' 25.2"
136 N, 1° 19' 47.0" W; Figure 1). The fields had been cultivated and used to grow annual arable crops
137 every year since they were last converted from ley in 1988 (Copse); 1994 (Big Substation East
138 (BSE) and Big Substation West (BSW)), and 2009 (Hillside (HS)). The monoliths were extracted
139 from the permanent arable area between a pair of ley strips (3 m wide and 70 m long, and 48 m
140 apart), which extended into each of the fields from the hedges, having been sown in May 2015 as
141 part of the NERC Soil Security Programme research project SoilBioHedge (Figure 1). The soil in
142 each field was a Cambisol (WRB, 2006) and basic properties are summarized in Table 1.

143 The seven monoliths from each field were used to produce three treatments: i) unfrozen control,
144 ($n = 1$ per field) ii) frozen (defaunated) monoliths not inoculated with earthworms, abbreviated to
145 DeF ($n = 3$), iii) frozen monoliths inoculated with earthworms, abbreviated to DeF+E ($n = 3$). The
146 monoliths were planted with a grass-clover ley (see below) and were returned to their fields of
147 origin in late March 2017 towards the ends of the 2-year-old ley strips furthest from the field edge.
148 The monolith experiment ran until mid-April 2018.



149

150 Figure 1. The location of the four arable fields, Big Substation East (BSE), Big Substation West (BSW),
151 Copse and Hillside (HS) in which the experiment was carried out and the two pasture fields (Valley field
152 and Warren Paddock) from which earthworms were collected to repopulate the monoliths. The paired
153 green strips within each arable field are the 70 m long ley strips between which the monoliths were
154 sampled from, and near the end of which the monoliths were installed following defaunation by freezing.

155

156 **2.2. *Monolith preparation and grass-clover planting***

157 Seven undisturbed monoliths (22 cm deep, 36 cm long x 27cm wide) were carefully extracted from
158 the arable portion of each field following procedures similar to Allaire and Bochove (2006) and
159 placed into plastic boxes. Each box had drainage holes of 10 mm diameter in the bottom and 8 mm
160 diameter in the sides which were covered in nylon mesh on both the inside and outside (see Figure
161 S1). A mesh size of 0.5 mm was used to try to prevent the entry and exit of earthworms or other
162 soil macrofauna over the duration of the experiment. The control monolith ($n = 1$) from each field
163 was immediately placed in an excavated hole in the ley strip of the field from which the monolith
164 was taken.

165 To maintain soil structural integrity, we needed a non-invasive way of manipulating earthworm
166 populations. Previous studies that have used mustard solution and electro-shocking were found to
167 have an incomplete effect on earthworm extraction (Eisenhauer et al., 2008). Deep freezing (-20
168 °C) has been reported to be totally effective for eliminating earthworms and a range of other soil
169 macro- and meso-fauna such as oribatid mites and collembola though it appears to have little effect
170 on soil micro-fauna such as ciliates, nematodes, rotifers and tardigrades and soil microbiota
171 (Barley, 1961; Bruckner et al., 1995; Kandeler et al., 1994). The remaining 24 monoliths were
172 therefore defaunated by deep-freezing at -20 °C for three weeks. After defaunation, all 28
173 monoliths were planted with the grass-clover ley.

174 Because clover establishment is slow, established plants were collected from the 2-year ley strips
175 in the fields (Figure 1); their roots were thoroughly washed to remove any earthworms and their
176 cocoons. Six white clover plants with extensive lateral root systems, and 3 red clover plants with
177 strong taproots, were carefully transplanted into each monolith. The monoliths were kept indoors

178 for one day and then 2 g of hybrid and Italian ryegrass, using the same mixture of clover-grass
179 seeds “Broadsword Hi Pro” (Oliver Seeds, Lincoln UK) as planted in the leys, were scattered on
180 the surface of each monolith. The monoliths were kept indoors for another 24 hours and then, on
181 the third day, watered to stimulate grass seed germination and moved outdoors. Blocks of soil were
182 excavated from the ley strips of the fields from which the monoliths had been extracted and the
183 monoliths placed in the holes so that they were level with the surrounding soil. Mesh fences of 15
184 cm height and supported by a bamboo frame were placed around the monoliths to prevent
185 earthworms coming in and out over the surface.

186 ***2.3. Earthworm collection and culturing***

187 Three defaunated monoliths per replicate field were repopulated with earthworms. Although
188 earthworm populations will not instantaneously return to pasture levels when arable soils are put
189 into ley, our data from the main SoilBioHedge experiment (unpublished) indicate that earthworm
190 populations within the ley strips reach levels equivalent to the nearby pasture within two years.
191 Therefore, we repopulated the monoliths to give a population diversity and density based on that
192 measured previously by ourselves in nearby pasture fields (Valley Field and Warren Paddock,
193 Figure 1) on the same farm in December 2016 (Table 2). Earthworms were collected from pasture
194 fields by excavating the soil to a depth of 20 cm and hand sorting. The earthworms were classified
195 using the OPAL earthworm identification key (Jones and Lowe, 2009), rinsed with deionized water
196 and placed in containers of soil from each field from which the monoliths had been extracted and
197 maintained at 15 °C in darkness (Butt, 1991) to ensure that individuals were viable prior to the
198 experiment. After 3 days acclimatization, the viable adult earthworms were rinsed again with
199 deionized water, dried with tissue paper, weighed and put in containers ready for inoculation at

200 the surface of the DeF+E monoliths. Earthworms were placed on the surface of the monoliths and
201 watched until they had completely entered the soil to avoid losses to birds or other earthworm
202 predators.

203 To ensure earthworm inoculation success and survival of the more vulnerable species during the
204 experiment we followed the recommendations of (Butt, 2008) in repeating additions after the
205 summer. Our main concern was earthworm survival during high summer temperatures (see Table
206 S3) and low soil moisture conditions, as the depth of the boxes limits the depth to which
207 earthworms can retreat from surface conditions. Earthworms were therefore added to the DeF+E
208 monoliths twice, on 31st of March 2017 at the start of the experiment, and again on the 15th of
209 November 2017, at approximately the same density and species composition (we were unable to
210 collect sufficient *Allolobophora longa* in March 2017 and sufficient *Lumbricus castaneus* and
211 *Aporrectodea rosea* for the November 2017 restock, Table 2, and Table S2 for further details). To
212 reduce the abundance of earthworms, that despite the barriers had managed to recolonize the DeF
213 monoliths, we applied up to 3 L of allyl isothiocyanate at 0.1g L⁻¹ per monolith (Zaborski, 2003)
214 in November 2017, when the soil moisture content was approaching field capacity and earthworms
215 were very active, to expel any earthworms. We found 0 – 8 adults and 1 – 14 juveniles in each
216 monolith, (see Table S4 for details).

217

218 **2.4. Measurements made during the experiment**

219 *2.4.1. Hydraulic conductivity (K)*

220 *K* was measured five times, once per season, over the duration of the experiment (spring 2017, 23-
221 26th May; summer 2017, 21-25th August; autumn 2017, 3rd-10th November; winter 2017-18, 26th
222 January to 2nd February; and spring 2018, 3rd-6th April 2018). The measurements were made using
223 a Decagon Mini Disk Portable Tension Infiltrometer (Decagon Devices Inc, 2016) with an
224 infiltrometer placed on a thin sand layer to ensure good contact between the tension disc and
225 monolith surface (Köhne et al., 2011; Reynolds and Elrick, 1991). Measurements were made at
226 potentials of -6, -3, -1 cm and -0.5 cm until steady-state flow was reached, corresponding to water
227 flow through pores less than 0.5, 1, 3 and 6 mm in diameter respectively. To avoid hysteresis
228 effects, *K* measurements were made in an ascending tension sequence (Baird, 1997). *K* for three
229 dimensional infiltration was computed using the Van-Genuchten Zhang method (Zhang, 1997).
230 The contribution of different pore size classes (< 0.5, 0.5-1, 1-3 and > 3 mm in diameter) to water
231 flow for each set of measurements was calculated after Watson and Luxmoore (1986). In this study
232 the hydraulic conductivity at a tension of -0.5 cm, close to zero, was assumed to be a good
233 approximation for saturated hydraulic conductivity *K_s* (Yolcubal et al., 2004).

234 *2.4.2. Grass-clover shoot biomass*

235 Grass-clover above ground biomass was measured halfway through the experiment (23rd
236 September 2017) and just before the end of the experiment (16th April 2018). At each sampling
237 point all plant shoots were cut at the soil surface. The fresh shoot biomass was weighed and then
238 oven dried at 70 °C to constant weight.

239 *2.5. Measurements made after monolith removal*

240 At the end of the experiment all of the monoliths were removed and weighed. Earthworms were
241 first extracted using up to 3 L of non-toxic allyl isothiocyanate at 0.1 g L⁻¹ per monolith, (Zaborski,
242 2003). Emerging earthworms were collected for approximately 20 minutes after application. Soil
243 core samples were then collected from the monoliths for the measurement of soil water release
244 curves, soil water-holding capacity, bulk density, percentage soil mass in water-stable aggregates,
245 organic matter content and total nitrogen content. These values are all reported on an oven-dried
246 weight basis.

247 After the soil core samples had been removed, any remaining earthworms in the monoliths were
248 recovered by hand-sorting. Stones > 1 cm diameter were removed, and subsamples of this sorted
249 soil were collected for the wheat bioassay. In the laboratory, the recovered earthworms were rinsed
250 with deionized water, dried with tissue paper, identified using the Opal identification key if
251 clitellate (adult) (Jones and Lowe, 2009) and weighed. Juveniles were classed as either *A.*
252 *chlorotica* or *A. caliginosa* based on the Opal identification key (other than the lack of a saddle),
253 anecic (if > 1 g in mass and > 2 cm in length), epigeic (if < 1 g in mass and 1 – 2 cm in length) or
254 “unknown”.

255 *2.5.1. Soil water release curves and water holding capacity (WHC)*

256 Intact soil cores 8 cm diameter x 5 cm high were taken from the surface of the monoliths. The
257 cores were analyzed for water retention at different potentials following the simplified evaporation
258 method (Peters et al., 2015; Schindler et al., 2010) using a HYPROP device (UMS, Munchen,
259 Germany). The measured hydraulic conductivities using the minidisk infiltrometer and the
260 HYPROP measurement campaigns were modeled using the HYPROP-FIT software. The

261 hydraulic function parameters were generated using the bimodal Van Genuchten (1980) model
262 (Durner, 1994). Soil water content at saturation, at field capacity and at wilting point, and plant-
263 available water were calculated from the generated curves.

264 The WHC was determined on 0-5 cm depth x 3.5 cm diameter intact soil cores that were saturated
265 in the laboratory for 48 hours. The cores were then allowed to drain freely, until water was no
266 longer draining out, at which point the cores were weighed and oven dried at 105 °C to a constant
267 weight to establish the water content (ISO 11268-2:1998).

268 *2.5.2. Bulk density (BD) and percentage water stable aggregates (%WSA)*

269 BD was determined in the monoliths at 0-5, 5-10 and 10-15 cm depth using a bulk density corer
270 with rings of 100 cm³ (Eijkelkamp, Agrisearch Equipment). BD measurements were corrected for
271 the mass and volume of stones >2 mm, were averaged across the three depths for each monolith
272 and are expressed on an oven dried weight basis.

273 Four grams of air dried soil that had been sieved through a 2 mm sieve and retained on a 1 mm
274 sieve were placed on 250 µm sieves, pre-moistened and wet-sieved for 3 minutes in deionized
275 water at a rate of 34 times per minute using wet sieving equipment (Eijkelkamp, Agrisearch
276 Equipment). The %WSA was determined as the weight of the stable aggregates remaining on the
277 sieve relative to the total weight of aggregates adjusting for the mass of primary sand particles >
278 250 µm present in the samples (Kodešová et al., 2009; Milleret et al., 2009).

279 *2.5.3. Percentage organic matter (%OM) and total nitrogen (%N) contents*

280 Organic matter was determined by loss on ignition; as the soil contained carbonates an ignition
281 temperature of 350 °C was used to avoid their decomposition (Ayub and Boyd, 1994; CEAE,

282 2003). Total N was measured using a Vario MACRO C/N Analyser (Elementar Analysis System,
283 Germany). The soil samples were first dried at 105 °C, sieved to < 2 mm then homogenized to a
284 fine powder with a laboratory ball mill (Retsch, Germany). The samples were then weighed into a
285 tin-foil cups and sealed for dry combustion.

286 2.5.4. *Wheat bioassay experiment*

287 Moist homogenized soil from each monolith equivalent to an oven dry mass of 200 g was added
288 to plastic pots of approximately 7 cm diameter and 13 cm height and stored at 15 °C for four days
289 until planted with pre-germinated winter wheat seedlings (*Triticum aestivum*, Skyfall variety).
290 Winter wheat seeds were germinated on moist filter paper in Petri dishes kept at room temperature
291 in natural light. Three days after germination, seedlings with approximately 2 cm long radicles
292 were transplanted into the pots and allowed to grow for five days under natural light. The pots
293 were then placed under 50 W LED lights (Massa et al., 2008; Schroer and Hölker, 2016) operating
294 on a 12-hour photoperiod in a controlled temperature room set at 15 °C. Photosynthetically Active
295 Radiation (PAR) measured at the surface of pots was up to 580 $\mu\text{moles m}^{-2} \text{s}^{-1}$. The plants were
296 watered three times a week with distilled water. After 6 weeks, shoots and roots were harvested
297 with roots washed free of soil, weighed and oven dried at 70 °C to a constant weight.

298

299 **2.6. Statistical analysis**

300 Data from monoliths were analyzed using a general linear model analysis of variance (ANOVA).
301 Three-way mixed ANOVA with two main factors (treatment and field) and one repeated factor
302 (seasonal measurements) was used to analyze *K* at different tensions. Ordinary two-way ANOVA
303 was used to analyze data of the other measured parameters at the end of the experiment with
304 treatment and field name as factors. Ideally, we would have had four unfrozen control monoliths
305 per field. However, due to logistic limitations, we only had one unfrozen control monolith per
306 field. Consequently, an ANOVA analysis including control treatments was performed to look at
307 the main effects of treatments (4 control vs 12 DeF vs 12 FeF+E monoliths) and fields (7 monoliths
308 per field) or seasons (all 28 monoliths per season) but not at their interactions, since the design is
309 an unbalanced ANOVA. The unbalanced design resulted in uneven variances for some parameters,
310 we therefore repeated our ANOVA analysis excluding control monoliths; the statistically
311 significant trends were the same. Therefore, here we report the results of the ANOVA analysis
312 including control treatments. However, the ANOVA analysis excluding the controls was used to
313 investigate interactions between the main factors in the DeF+E and DeF monoliths.

314 As part of the regular management of the fields where the monoliths were located, a selective
315 herbicide (ASTROKerb®, MAPP 16184, Dow AgroSciences, Cambridge UK) was applied in late
316 November 2017. The herbicide spray drifted onto the edges of the ley strips in HS field, killing
317 the grass in one replicate of the DeF+E (Replicate 3) and DeF (Replicate 3) treatments; this appears
318 to have had a negative effect on the earthworm populations (see Table S5). For this reason, the
319 infiltration measurements in January and April 2018, in addition to the collected data at the end of
320 the experiment for these two monoliths, were excluded from the statistical analysis. SPSS (IBM

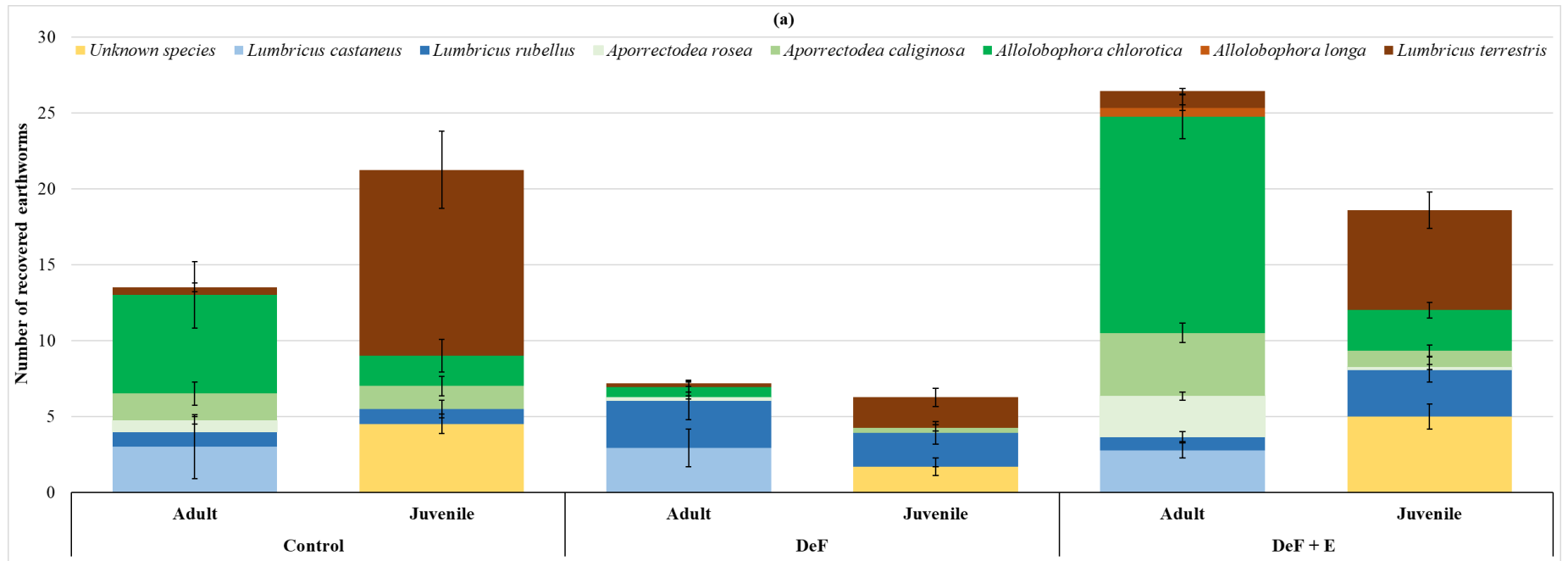
321 Corp. Released 2016, version 24) was used to estimate the statistical significance of mean
322 differences between treatments. *P* values of < 0.05 were used as the threshold for significance.

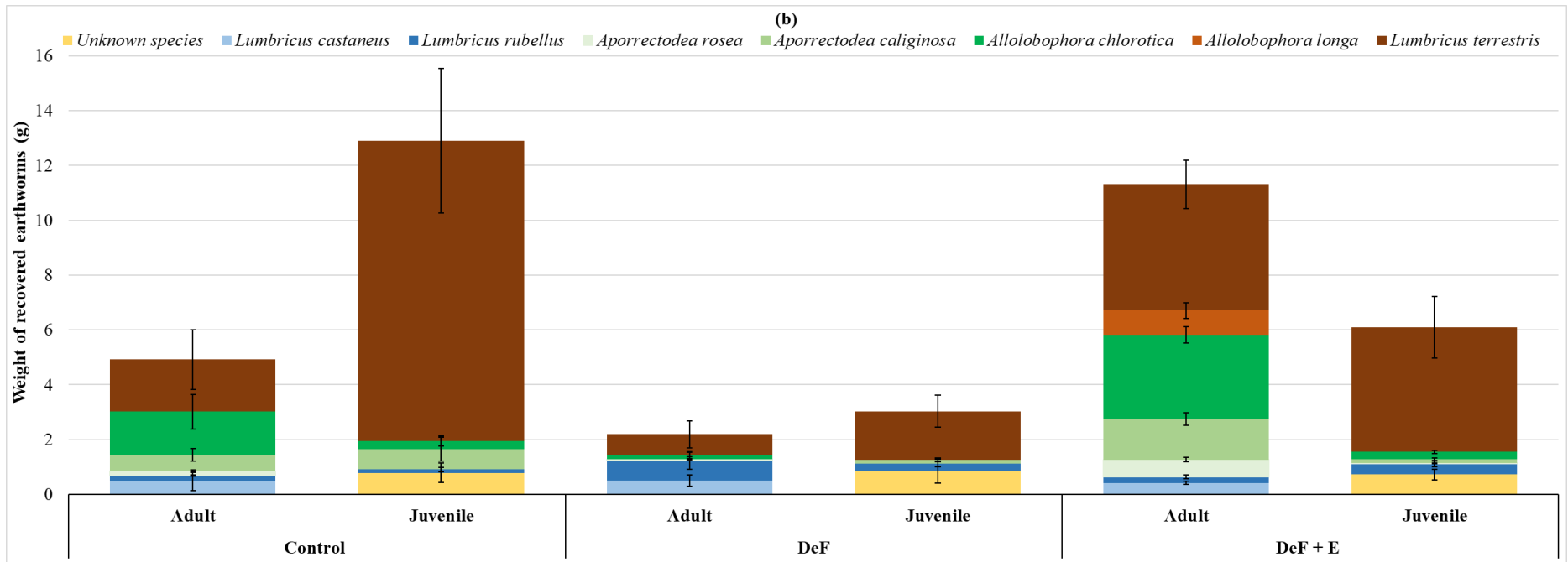
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324 3. Results

325 3.1. Recovered earthworms

326 Figure 2a shows the mean number of each earthworm species recovered from all the treatments
327 and across all the fields in April 2018 and Figure 2b the mean weights of these earthworms.
328 Detailed data for each replicate mesocosm are given in Table S5 and Figure S2. In the DeF+E
329 treatment the number of adults recovered at the end of the experiment (26.42 ± 1.47 ; $n = 11$) was
330 significantly greater than the numbers added either in April 2017 (23 per monolith, $p = 0.025$) or
331 November 2017 (20 per monolith, $p = 0.01$) though the mass of adults was not significantly
332 different. Juveniles were also present in the monoliths at the end of the experiment. Importantly,
333 for testing our hypotheses, at the end of the experiment, the DeF+E monoliths showed significantly
334 higher total earthworm numbers and weights than the DeF treatments ($p < 0.001$) (Figure 2a, b).
335 Total earthworm numbers and weights of the control treatment were significantly higher ($p = 0.013$
336 and $p = 0.001$ respectively) than in the DeF treatment but not significantly different from the
337 DeF+E treatment, although the mass of juveniles in the control treatment appeared to be more than
338 double that in the other two treatments. At the end of the experiment no significant differences
339 were observed between fields for recovered earthworm numbers, but BSE contained a lower total
340 weight of earthworms than BSW field ($p = 0.049$). There were no significant interactions between
341 fields and treatments. The earthworm population in DeF+E treatments was dominated by endogeic
342 individuals ($p < 0.001$ when compared to numbers of individuals of other earthworm types) while
343 epigeics were the dominant earthworm type in the DeF treatments at the end of the experiment (p
344 = 0.02, $p = 0.003$ and $p = 0.008$ when compared to numbers of anecic, endogeic and unknown
345 individuals respectively).





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Figure 2. Mean of the recovered earthworm (a) numbers per monolith and (b) weight (g) per monolith for adults and juveniles across all fields. The figures represent the three treatments; Control = unfrozen monoliths ($n = 4$); DeF = frozen monoliths without earthworm addition ($n = 11$), DeF+E = frozen monoliths with earthworm addition ($n = 11$); error bars = standard error. The chart stack colour and its shades represent the ecological group of earthworm; Brown : anecic, green : endogeic and blue : epigeic species. *L. terrestris* and *L. rebellus* species for juveniles represent anecic and epigiec ecological group respectively, and not species, for the purpose of this graph only. Recovered earthworm numbers and weight for each treatment on a field by field basis is presented in Figure S2.

355 **3.2. Seasonal differences in hydraulic conductivity (K)**

356 Figure 3 presents the seasonal variation in K at -0.5 cm tension ($K_{0.5}$) for all treatments and across
357 all the fields (for K data at different tensions and details of each field see Figure S3). A three-way
358 mixed ANOVA with season, treatment and fields as factors indicated that $K_{0.5}$ increased from
359 spring to summer 2017 ($p < 0.001$), that there were no significant differences between summer and
360 autumn 2017, that there was a significant decrease from autumn 2017 to winter 2017-18 ($p =$
361 0.003), when the values were similar to those in spring 2017, and that subsequently values
362 increased significantly in spring 2018 ($p < 0.001$) to attain values similar to those in summer and
363 autumn 2017. Across treatments $K_{0.5}$ was significantly greater in DeF+E relative to DeF (47%)
364 and control (64%) treatments ($p < 0.001$). There was no significant difference between DeF and
365 control treatments. Only seasons and treatments showed a significant interaction ($p = 0.023$), with
366 $K_{0.5}$ significantly greater in DeF+E compared to DeF treatments only in winter 2017-18 and spring
367 2018 ($p < 0.001$). Across fields $K_{0.5}$ was higher in HS field compared to BSE ($p = 0.006$) and BSW
368 ($p < 0.001$) fields and also higher in Copse compared to BSW ($p = 0.006$).

369 Apart from a significantly lower K at -1 cm tension (K_1) in winter 2017-18 compared to summer
370 2017 ($p = 0.05$), autumn 2017 ($p = 0.022$) and spring 2018 ($p = 0.019$), no significant differences
371 were observed in K_1 between seasons. Across all seasons K_1 was not significantly different
372 between fields ($p = 0.06$) and was greater in DeF+E compared to DeF and control treatments ($p =$
373 0.05). There was no significant difference between DeF and control treatments and no significant
374 interaction effect between main factors.

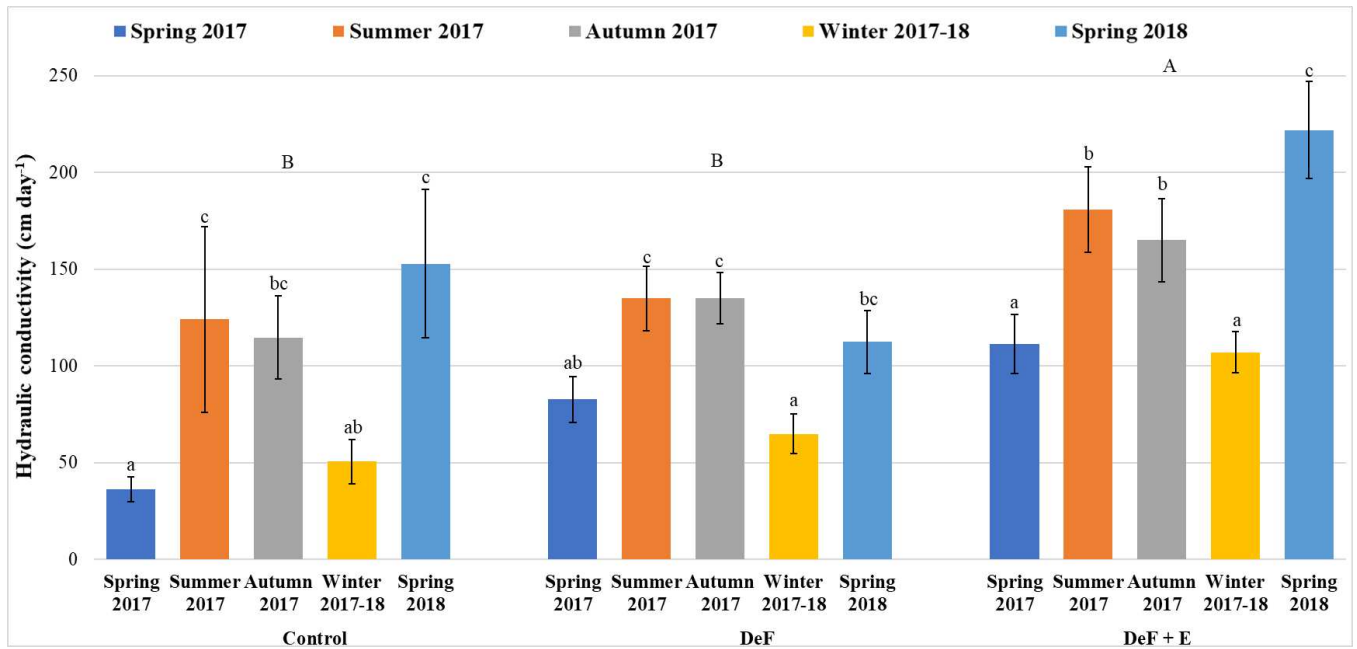
375 At a tension of - 3 cm, K_3 was significantly different between seasons and fields ($p < 0.001$) but
376 not significantly different between treatments. K_3 increased from spring to summer 2017 ($p =$

377 0.001) and from winter to spring 2018 ($p = 0.05$) but decreased from summer to autumn 2017 (p
378 < 0.001) and from autumn 2017 to winter 2017-18 ($p = 0.01$). K_3 was significantly lower in BSE
379 compared to the other fields and higher in HS compared to Copse ($p = 0.002$) and BSE fields ($p <$
380 0.001). There was no significant interaction between fields and treatments. Interactions between
381 seasons and treatments or fields are reported in the Supporting information section.

382 K_6 was not significantly different between treatments. No differences in K_6 were observed between
383 BSE and Copse or between BSW and HS fields through all the seasons. The highest values were
384 reported for BSW and HS fields compared to BSE and Copse fields ($p < 0.001$). All the fields
385 showed a significant decrease in K_6 from summer to autumn 2017 ($p = 0.037$, $p < 0.001$, $p = 0.002$,
386 $p < 0.001$ for BSE, BSW, Copse and HS fields respectively) with no significant differences
387 between the other seasons.

388 The relative flow of water through different pore sizes varied between treatments through the
389 experiment period, but there were no significant differences, so the data are not reported in the
390 main text of this paper (see Figure S4). However, at the end of the experiment (Figure 4) the
391 proportion of water flow through pores wider than 1 mm was significantly greater in the DeF+E
392 treatments (98%) compared to the DeF treatments (95%) ($p = 0.045$). Flow through pores wider
393 than 1 mm in the control treatment was not significantly different from the other two treatments
394 and had a value that lay between them (97%). However, flow through pores 1 – 3 mm was
395 significantly greater in the DeF+E and DeF treatments and through pores > 3 mm was significantly
396 greater in the controls. No significant differences were observed between fields at the end of the
397 experiment for these hydrological properties.

398



399

400 Figure 3. Mean hydraulic conductivity at -0.5 cm tension across seasons and all the fields ($n = 4$) at field

401 temperature. Control = unfrozen monoliths ($n = 4$); DeF = frozen monoliths without earthworm addition

402 ($n = 11$), DeF+E = frozen monoliths with earthworm addition ($n = 11$); error bars = standard error.

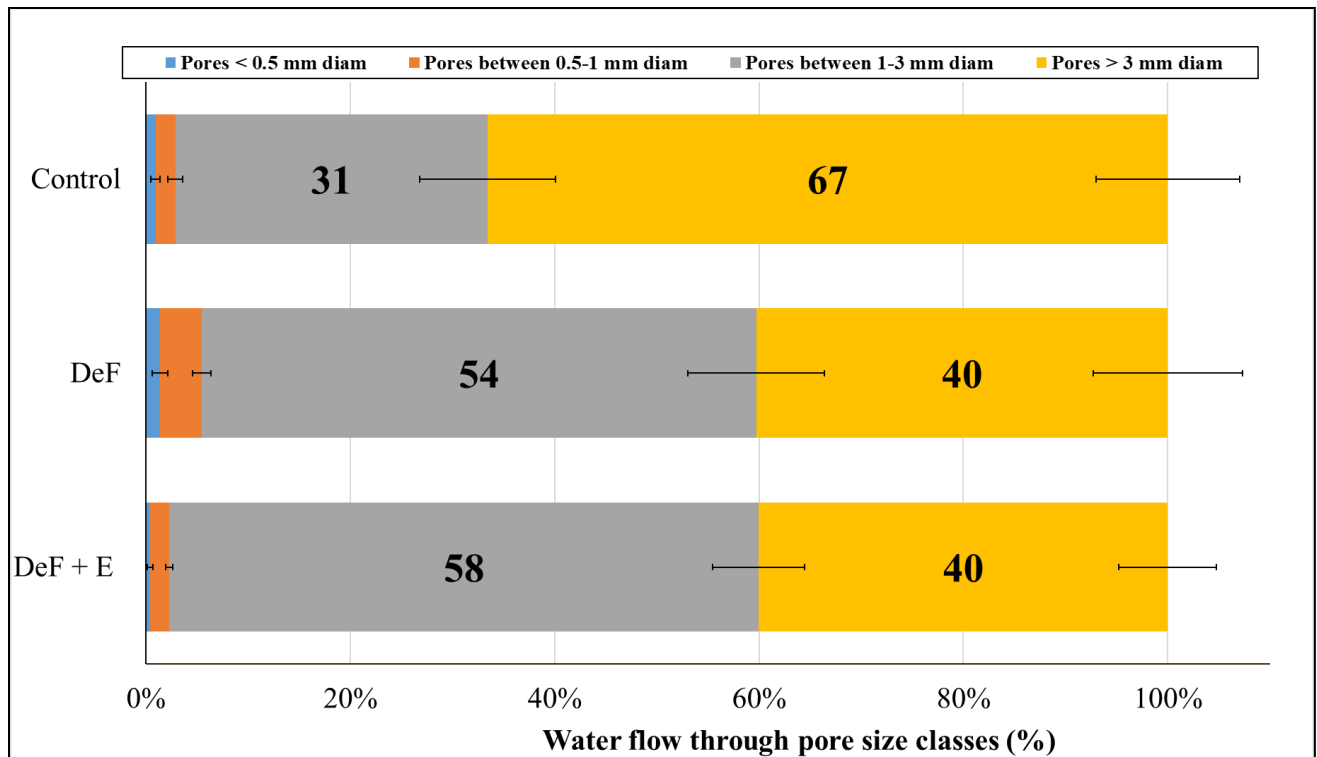
403 Columns with the same letter over them are not significantly different ($p > 0.05$, Bonferroni test); lower-

404 case show differences between seasons within each treatment and upper-case show differences between

405 treatments. Hydraulic conductivity data at different tensions across seasons for each treatment and on a

406 field by field basis are presented in Figure S3.

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409 Figure 4. Mean pore size class contribution to water flow at the end of the experiment across all the fields.

410 Control unfrozen monoliths ($n = 4$); DeF = frozen monoliths without earthworm addition ($n = 11$), DeF+E

411 = frozen monoliths with earthworm addition ($n = 11$), error bars = standard error. Pore size class

412 contribution to water flow across seasons for each treatment on a field by field basis is presented in Figure

413

S4.

414

3.3. Soil water release curves (SWRC) and water-holding capacity (WHC)

415 The SWRC data from the individual cores from each monolith were combined to produce a single

416 SWRC for the DeF and DeF+E treatments from each field and fitted using Hyprop-Fit models.

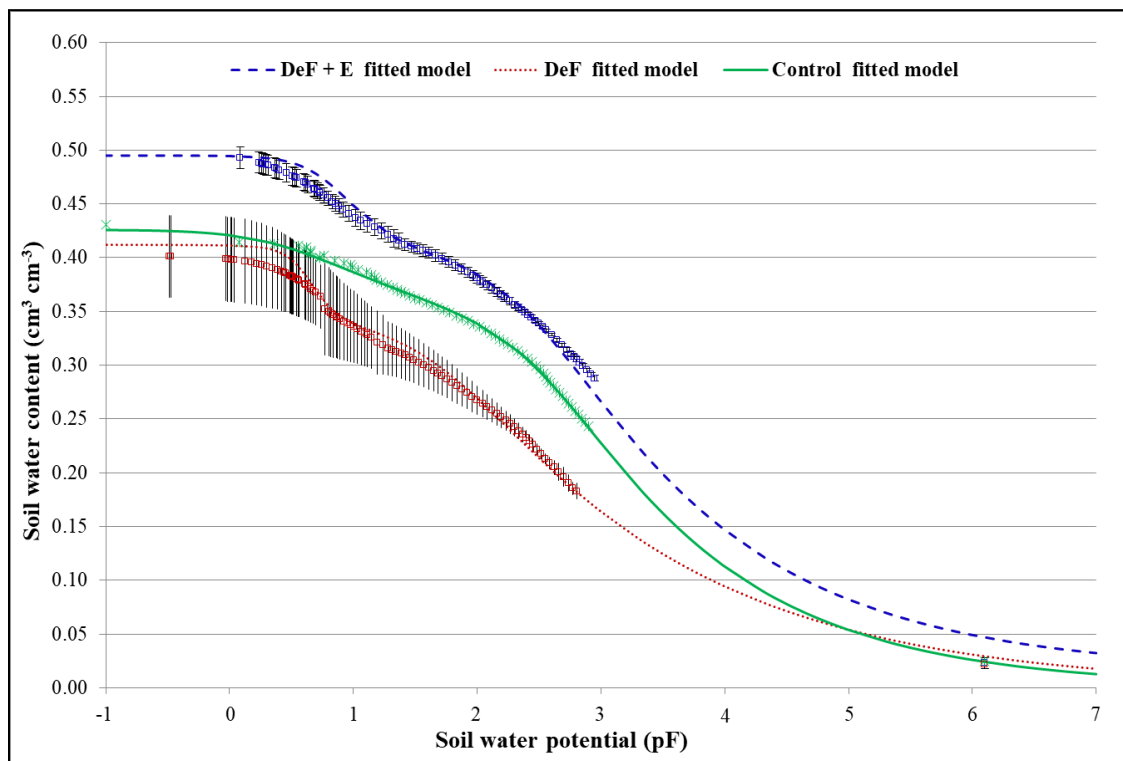
417 SWRC for the controls were from single cores (Figure 5, Figure S5). The generated SWRC were

418 used to derive the soil water content at saturation (WCS) and at field capacity (FC) (at 33KPa;

419 Kirkham (2005)), and also the plant available water (PAW) (Table 3). All these values were

420 significantly greater in the DeF+E treatments relative to DeF (by 11%, 24% and 21% for WCS (p

421 = 0.001), FC ($p < 0.001$) and PAW ($p < 0.001$) respectively) and relative to the unfrozen controls
422 (by 9%, 16% and 19% for WCS ($p = 0.027$), FC ($p = 0.006$) and PAW ($p = 0.011$) respectively).
423 No significant differences were observed between DeF and control treatments. The three
424 parameters showed significant differences between fields ($p = 0.021$, $p = 0.001$, $p = 0.05$ for WCS,
425 FC and PAW respectively). HS field had the highest values, but these were only significantly
426 greater than those for Copse field. There was no significant interaction between treatments and
427 fields.



428
429 Figure 5. Soil water release curves (SWRC) of Copse field fitted to the measured data using the bimodal
430 constrained Van Genuchten (1980) model (Durner, 1994). The curves represent the control, DeF = frozen
431 monoliths without earthworm addition and DeF+E = frozen monoliths with earthworm addition; Three
432 replicates were combined each for the DeF and DeF+E treatments and fitted using Hyprop-Fit models
433 (error bars = Standard deviation). Only one replicate was fitted for the Control. SWRC for BSE, BSW
434 and HS fields are presented in Figure S5.

435 WHC varied significantly between treatments ($p = 0.011$; Table 4). The WHC of the DeF+E
436 monoliths was nearly 9% greater than the DeF monoliths ($p = 0.05$). There was no significant
437 difference between controls and the other treatments. WHC was significantly higher in HS
438 compared to BSE ($p < 0.001$) and BSW ($p = 0.007$) fields and significantly lower in BSE compared
439 to Copse ($p = 0.002$) and HS fields ($p < 0.001$). There was no significant interaction between
440 treatments and fields.

441 **3.4. Soil bulk density (BD)**

442 BD significantly decreased by 6% in the DeF and DeF+E treatments at the end of the experiment
443 compared to the initial soil conditions ($p = 0.01$), suggesting that the growth of the ley for one year
444 increased soil pore space, but there was no effect of adding earthworms. There was no significant
445 difference in BD between the DeF+E and DeF treatments and the control monoliths at the end of
446 the experiment ($p > 0.05$) (Table 4). BD was significantly higher in BSE field compared to the
447 other fields ($p = 0.005$, $p = 0.011$, $p = 0.05$ for BSW, Copse and HS fields respectively). There
448 was no significant interaction between treatments and fields on BD.

449 **3.5. Percentage water stable aggregates (%WSA)**

450 %WSA ($> 250 \mu\text{m}$) in the DeF+E monoliths was significantly greater than that in the DeF
451 monoliths ($70 \pm 3\%$ vs $60 \pm 3\%$, $p = 0.014$). %WSA of the control treatments was between the
452 DeF+E and the DeF treatments with no significant differences (Table 4). %WSA also varied
453 significantly with field ($p = 0.003$); %WSA was highest in the HS field. There was no significant
454 interaction between treatments and fields.

455 **3.6. Percentage organic matter (%OM)**

456 Comparison of the DeF treatments at the end of the experiment (Table 4) with the initial soil
457 conditions (Table 1) indicate that the conversion of arable soil to ley led to a significantly greater
458 %OM in the DeF monoliths in just one year ($3.66 \pm 0.23\%$ vs $4.72 \pm 0.15\%$, $p < 0.001$). In addition,
459 %OM in DeF+E was significantly greater than that in the DeF monoliths ($5.12 \pm 0.19\%$ vs $4.72 \pm$
460 0.15% , $p < 0.001$). The %OM of the control treatments was between the DeF+E and the DeF
461 treatments with no significant differences. The %OM was highest in HS field and lowest in BSW
462 field ($p < 0.0001$). For %OM there was no significant interaction between treatments and fields.

463 **3.7. Total nitrogen content (%N)**

464 The addition of the earthworms to the defaunated monoliths resulted in a significant greater %N
465 compared to the DeF treatment ($0.31 \pm 0.01\%$ vs $0.30 \pm 0.01\%$, $p < 0.027$). %N in the control
466 treatments was between the DeF+E and the DeF treatments with no significant differences (Table
467 4). %N was significantly lower in the HS field compared to the other fields ($p < 0.001$).

468 **3.8. Plant dry biomass**

469 **3.8.1. Grass and clover shoot dry biomass of the monoliths**

470 No significant differences between treatments were observed at the midpoint of the experiment,
471 due to relatively high variance between treatments, but the DeF+E monoliths did produce 34%
472 more shoot biomass than the DeF monoliths. At the end of the experiment, this trend was much
473 stronger with 58% more biomass produced in the DeF+E monoliths compared to the DeF
474 monoliths and had become significant ($p = 0.004$). Plant shoot biomass in the control treatment

475 had an intermediate value and was not significantly different from the DeF+E and DeF treatments.
476 More biomass was collected in September 2017 than in April 2018 (Table 4). Over both periods,
477 the BSE and HS field produced the least dry shoot biomass ($p = 0.001$ and $p = 0.005$ in September
478 2017 and April 2018 respectively). At the end of the experiment only grass was present in HS field
479 monoliths. The low shoot dry biomass in the BSE field and HS field in September 2017 (Table 4)
480 was likely due to voles grazing the grass-clover; plant stems at the soil surface of the monoliths
481 showed evidence of grazing, vole galleries were present around the monoliths and the mesh fences
482 had been pierced at surface level. This impacted the weight of the collected plant material in those
483 fields in spring 2018. Voles have a preference for clover over grass (DeJaco and Batzli (2013),
484 perhaps explaining why only grass was collected in the HS soil at the end of the experiment (see
485 Figure S6 and Figure S7 for details).

486 3.8.2. *Wheat bioassay experiment*

487 Wheat grown in the soil from the DeF+E treatments achieved significantly greater biomass
488 compared to the DeF (20% increase) and control treatments (30% increase) (Table 4, $p = 0.006$
489 for both DeF and control). This was due to an increase in root biomass in DeF+E compared to DeF
490 and control treatments ($p < 0.001$); shoot biomasses were not different ($p > 0.05$). Root and total
491 dry biomass varied significantly between fields ($p < 0.001$) with the highest values recorded for
492 Cope field and the lowest for BSE field. Shoot biomass was not significantly different between
493 fields. There was no significant interaction between treatments and fields.

494

495 **4. Discussion**

496 This study examined the effects of earthworm communities on soil physico-hydraulic and
497 chemical properties and plant growth in arable soil on conversion to grass-clover leys under
498 realistic conditions. Here we focus on differences between treatments. Where relevant, differences
499 between fields are discussed in the Supplementary Information where field specific data are
500 presented.

501 ***4.1. Earthworm populations***

502 The earthworm diversity that we introduced into the DeF+E treatments was maintained for the
503 duration of the experiment. Endogeics dominated the earthworm populations in the DeF+E
504 treatments at the end of the experiment as typically found in pasture fields of the farm (Figure 2;
505 Holden et al.(2019)). Although a greater number of adult earthworm numbers were recovered at
506 the end of the experiment (26.42 per Def+E monolith) than were added to the monoliths in April
507 (23 per monolith) or November (20 per monolith) the numbers were similar, indicating that the
508 second set of additions was necessary. The greater number of adult earthworms can be attributed
509 either to survival of some of the original additions or entry of earthworms into the monoliths over
510 the course of the experiment. The juveniles recovered from the DeF+E treatments represent either
511 entrant earthworms, the hatching of cocoons that survived the defaunation (for example the
512 cocoons of the epigeic *L. rubellus* and *L. castaneus*, two dominant epigeic species found in the
513 monoliths, have been reported to tolerate temperatures as low as -35 °C and -50 °C respectively;
514 (Meshcheryakova and Berman, 2014), or the offspring of some of the added earthworms.

515 Small numbers of earthworms were recovered from the DeF treatments despite the use of mesh on
516 the outside and inside of the plastic containers that contained the monoliths and the use of mesh

517 fences around the monoliths and must represent either hatched cocoons (see above) or entrant
518 earthworms. The earthworms were dominated by epigeics but with some anecic juveniles also
519 present (Tables S4 S5). Epigeics are reported as having high dispersion rates relative to anecic and
520 endogeic earthworms which results in more rapid colonization of new habitats (Bouché, 1977;
521 Chatelain and Mathieu, 2017; Margerie et al., 2001; Migge-Kleian et al., 2006).

522 **4.2. Soil water flow**

523 *4.2.1. Earthworm effects on water flow*

524 The significant increase in $K_{0.5}$ in the DeF+E compared to the DeF and control treatments (Figure
525 3) is consistent with previous studies reporting a positive effect of earthworms on water flow
526 (Blouin et al., 2013; Bouché and AlAddan, 1997; Edwards and Bohlen, 1996; Francis and Fraser,
527 1998; Lamandé et al., 2003). The impact of earthworms was significant in winter 2017-18 and
528 spring 2018 after the second addition of earthworms to the DeF+E monoliths in mid-November
529 2017. The lack of significant differences between treatments in spring 2017, less than 2 months
530 after the first addition of earthworms, is probably due to earthworms having had insufficient time
531 to work the soil. Qualitative observations made whilst measuring K indicate that although
532 earthworm casts were found on the surface of the DeF+E monoliths in summer and autumn 2017
533 these were at a relatively low density compared to spring 2018. Earthworm activity typically
534 reduces in the summer months (Birkas et al., 2010) and the higher than average temperatures
535 during the summer of 2017 may have reduced earthworm populations in the DeF+E monoliths
536 further, which may explain the non-significant differences between the DeF+E and DeF
537 treatments.

538 In spring 2018, DeF+E treatments showed significantly higher $K_{0.5}$ compared to the other seasons.
539 In this period, pores > 1 mm contributed more significantly to water flow in the DeF+E than the
540 DeF treatments (Figure 4). These pores will have been created by earthworms or produced as a
541 result of improved soil structure through aggregation (Table 4). Earthworms facilitate soil
542 aggregation and the incorporation of organic matter within the soil aggregates, which may explain
543 the high %OM content in the DeF+E treatments at the end of the experiment (Fonte et al., 2007).
544 The mean values of $K_{0.5}$ across all seasons were 39 ± 28 , 44 ± 22 and 66 ± 32 mm h⁻¹ for the
545 Control, DeF and DeF+E, treatments respectively. Heavy rainstorms in the UK rarely exceed 200
546 mm day⁻¹, with the greatest rainfall in 2015 being recorded as 341.1 mm day⁻¹ (Friederike et al.,
547 2018), though with the rainfall being concentrated in a shorter time period than 24 hours. The
548 experimental results suggest that the presence of earthworms in the soil will largely reduce
549 infiltration-excess overland flow and flooding which would help to alleviate negative effects of
550 such events. Differences between fields as opposed to treatments are discussed in the text
551 accompanying Figure S3.

552 4.2.2. *Water flow changes between the seasons*

553 K is a dynamic property influenced by, amongst other things, climate, management practices and
554 biological activity (Amer et al., 2014; Elhakeem et al., 2018). As in previous studies (Alletto and
555 Coquet, 2009; Deb and Shukla, 2012; Strudley et al., 2008), K measured at different tensions varied
556 significantly across the seasons. In this study we largely used measures of K_1 , K_3 and K_6 to
557 determine the proportion of water flow through different pore sizes, therefore in this section we
558 focus on $K_{0.5}$ as this is close to hydraulic conductivity at saturation and allows comparison with
559 other studies.

560 Our initial hypotheses were that K would increase with earthworm activity and in line with the
561 seasonal activity of earthworms. By the end of our experiment our data supported our first
562 hypothesis, but it failed to fully support the second part of our hypothesis. $K_{0.5}$ increased
563 significantly in summer 2017 when soils were dry and earthworm activity would be expected to
564 decrease compared to spring 2017 (Spurgeon and Hopkin, 1999) and was unchanged during
565 autumn 2017 when typically earthworms that aestivated over the summer start working the soil
566 again as conditions become more moist and grass and clover litter accumulates on the soil surface
567 (Dar et al., 2006; Michiels et al., 2001). $K_{0.5}$ then decreased considerably in winter 2017-18
568 (January 2018) when soils are wet, facilitating earthworm movement and the hatching activity of
569 some species starts to increase (Potvin and Lilleskov, 2017; Spurgeon and Hopkin, 1999).

570 The high monthly precipitation and temperatures during the summer of 2017 compared to the other
571 seasons (see Table S3) would have induced multiple soil wetting-drying cycles, perhaps resulting
572 in shrink-swell processes increasing aggregation and improving soil structure (Tang et al., 2016).
573 Soil mineralogy data are not available for the soils, so we are not able to say whether the clays
574 present were those which demonstrate shrink-swell behaviours. However, the study site is
575 underlain by limestone and the soils are from the Aberford series of Calcaric Endoleptic Cambisols
576 (Cranfield University, 2019), which are characterized by calcareous clay enrichment, and therefore
577 may be only slightly prone to shrink-swell behavior when compared to non-calcareous equivalents
578 (Avery and Bullock, 1977). Although high rainfall can lead to the disintegration of aggregates and
579 the consequent blocking of pores resulting in reduced K for coarse textured soils with an organic
580 matter content of less than 1% (Hu et al., 2012, 2009), in fine textured soils the formation of small
581 cracks can lead to an increase in K of several order of magnitude (Albrecht and Benson, 2001).
582 These contrasting effects are dependent on soil texture and organic matter content and, in the case

583 of the soils used in this experiment, the formation of small cracks rather than the breakdown of
584 soil aggregates may have dominated and resulted in the increased $K_{0.5}$ in summer 2017. In addition,
585 grass and clover reached maximum growth (see Figure S9) in summer 2017 (with abundant rainfall
586 and suitable temperatures for plant growth in summer) and the cracks and pores associated with
587 well-developed root systems (Angers and Caron, 1998) may have also increased $K_{0.5}$.

588 We expected an increase in earthworm activity and K from summer (21 – 25th August sampling)
589 to autumn 2017 (3 – 10th November sampling) (Hu et al., 2012, 2009) but did not detect a
590 significant change in $K_{0.5}$. There are two possible factors that can explain this:

- 591 1. The numbers of earthworms recovered at the end of the experiment (Figure 2) suggests that
592 earthworms in the DeF+E treatment died over the summer, reducing the populations in the
593 monoliths and therefore earthworm impacts on K . We restocked the monoliths with
594 earthworms on 15th November just after measuring K .
- 595 2. The shoot harvest taken in late September 2017 likely reduced the food supply for any
596 earthworms that had survived over the summer, particularly for vertical burrowing anecics
597 that produce water transmitting vertical pores which may have reduced their activity.
598 Further the harvesting of shoots may have resulted in grass and clover switching from root
599 development that can aid pore formation, to shoot development.

600 By winter 2017-18 (26 January – 2nd February), although K was significantly higher in the DeF+E
601 treatment relative to the DeF treatment indicating a positive impact of earthworms, K had reduced
602 significantly relative to the autumn period. This was counter to our expectation; we expected
603 earthworm activity to have increased due to cocoons continuing to hatch, autumn hatchlings
604 growing in size and the increase in rainfall leading to moister soils. However, relative to autumn

605 2017, the low air temperatures in winter 2017-18 (down to $-5.9\text{ }^{\circ}\text{C}$) may have reduced earthworm
606 activity at the surface. Additionally, the heavier rainfall in the winter period (see Table S3),
607 combined with the reduced plant cover may have led to some surface soil disaggregation and
608 blocking of soil pores. Although the average air temperature during the infiltration measurement
609 campaign was $3\text{ }^{\circ}\text{C}$, on the mornings of the measurements there was often a thin sheet of ice on
610 the soil surface so it seems likely that at least near-surface pores could also have been blocked by
611 ice which would reduce measures of K . In addition the viscosity of water decreases with decreasing
612 temperature (e.g. by a factor of 1.6 between temperatures of $3\text{ }^{\circ}\text{C}$ (Figure 3) and $20\text{ }^{\circ}\text{C}$ (see Figure
613 S3e)) (Aleksandrov and Trakhtengerts, 1974; Haridasan and Jensen, 1972) which would reduce
614 rates of flow and calculated values of K . However, although correcting K values to $20\text{ }^{\circ}\text{C}$ increases
615 the calculated $K_{0.5}$ values for winter 2017-18 (see Figure S3e) they still remain lower than the
616 other seasons with the change in water viscosity only accounting for 6 % of the decrease in K from
617 autumn 2017 to winter 2017-18. Finally, low temperatures and solar radiation in winter reduce
618 water evaporation after frequent rainfall and the increased water content may have led to increasing
619 periods of water saturation and expansion of clays in the soil (Hesseltine, 2016) which can lead to
620 a reduction in pore size and thus a decrease of K (Dexter, 1988; Jabro, 1996; Messing and Jarvis,
621 1990).

622 In some soils K can decrease in spring after winter freeze-thaw cycles due to reconsolidation
623 causing an increase in soil density (Hu et al., 2012, 2009). However, in our experiments $K_{0.5}$
624 increased significantly in spring 2018 (3rd – 6th April) relative to winter 2017-18. Earthworm
625 activity and plant growth during the spring may contribute to an increase in connected soil pores
626 that can conduct more water. At a coarser scale of observation than the hydraulic conductivity
627 measurements we recorded, a decrease in the bulk density and an increase in the %OM content of

628 the soils between the start and end of the experiment, would improve soil structure and also be
629 expected to increase the amount of water movement within the soil (Hillel, 2008).

630 ***4.3. Soil water release curves and water holding capacity***

631 Soil water release curves for the DeF+E treatments shifted to the right relative to the DeF and
632 control treatments resulting in increased predicted water contents at saturation, field capacity and
633 at wilting point for all the fields (Table 3, Figure 5, Figure S5). The DeF+E treatments also had
634 higher water holding capacities (Table 4) and plant available water. This is consistent with an
635 improved soil structure (Huntington, 2006). Earthworms impact soil structure directly by creating
636 pores of different sizes, branching and sinuosity which impact on soil water storage capacity
637 (Bastardie et al., 2005). According to the capillary rise equation, pore radius is proportional to the
638 potential value at which that pore drains (Hillel, 1980). Therefore, at very low potential, water
639 drains through both rapidly and slowly draining pores (Amer, 2012) such as those created by adult
640 earthworms (2 – 9 mm diameter (Pérès et al., 1998)). The wide pores have more impact on soil
641 water content at saturation than at lower water contents. Pores created by juveniles of diameter
642 less than 1 mm would affect capillary water and therefore water content at field capacity, plant
643 available water and water holding capacity (Amer, 2012). Earthworms also impact soil structure
644 by fragmenting organic matter content and mixing it into the soil (Lavelle et al., 1998). This would
645 improve soil aggregation (Table 4) and porosity which in turn increases soil water retention
646 (Smagin and Prusak, 2008; Tisdall and Oades, 1982).

647 Plants roots and associated mycorrhizal fungi also improve soil structure by stabilizing macro-
648 aggregates (Tisdall and Oades, 1982) and creating pores of different sizes. Plant species with dense
649 and fine roots such as grass (Deru et al., 2016) and highly mycorrhizal fibrous lateral root systems

650 such as clover (Wyngaarden et al., 2015) both produce a range of soil pore sizes and increase
651 micropore volume (Bodner et al., 2014; Jarvis et al., 2017). This can increase the water available
652 to plants (Zangiabadi et al., 2017). As is commonly observed (van Groenigen et al., 2014) plant
653 growth was greater in the presence of earthworms (Table 4, Figure S10). This suggests a potential
654 synergistic effect whereby improvements in soil structure may be greater in the presence of
655 earthworms and plants than expected based on improvements in soil structure in the presence of
656 plants or earthworms alone.

657 ***4.4. Plant dry biomass and soil organic matter***

658 Plant shoot dry biomass of grass-clover was greater in the DeF+E compared to the DeF treatments,
659 which is consistent with the majority of studies that report the impact of earthworms on plant
660 growth (Scheu, 2003). In a meta-analysis van Groenigen et al. (2014) reported that the presence of
661 earthworms in agroecosystems increased the aboveground biomass by 23% on average and
662 attributed the majority of this effect to the release of nitrogen from organic matter by earthworms.
663 Consistent with this, in our experiment, the DeF+E treatment of the monoliths increased total soil
664 N content and increased shoot dry biomass by $37 \pm 10\%$ (Table 4). Although no significant
665 increase in shoot biomass was observed in the DeF+E treatments in the bioassay, root biomass did
666 increase significantly, resulting in a significant increase in total dry biomass in the DeF+E
667 treatments. Our data suggest escape or death of at least some of the earthworms added over the
668 duration of the experiment so it is possible that earthworm necromass contributed to this increase
669 in total soil N. However, given a typical earthworm moisture content of 80% (Roots, 1956), and
670 assuming that earthworms have a protein content of 20% comprising 20% N (Currie et al., 2005)
671 even if all the earthworms added to each monolith had died, and all the N present in the earthworms

672 had remained in the soil, the resultant increase in total soil N would be over an order of magnitude
673 less than the increase seen in the DeF+E treatment soils.

674 The significantly higher water holding capacity and available water to plants in the DeF+E
675 treatments (Table 4, Table 3) would also support improved plant growth leading to significant
676 increases in shoot dry biomass in the monoliths and the total dry biomass of the bioassay
677 experiment (Denmead and Shaw, 1962; Veihmeyer and Hendrickson, 1950). These results show
678 the important role of earthworms in supporting food production and security.

679 The increases in the %OM in the DeF and DeF+E treatments relative to the initial arable soil
680 conditions are most likely due to organic exudates from plant roots (Wiesmeier et al., 2019) and
681 increased amounts of plant litter. The precise role that earthworms have on the soil C cycle remain
682 debated (e.g. Lubbers et al. (2013); Zhang et al. (2013)). However, in our experiments, which
683 represent a long-term field trial in the presence of plants, there was an increase in %OM in the
684 DeF+E treatment relative to the DeF treatment. Earthworms play an important role in aggregate
685 formation (e.g. Six et al. (2004)) and %WSA were significantly greater in the DeF+E treatments
686 than in the DeF treatments. Aggregates are thought to protect soil C (e.g. Six et al. (2004)) . Thus,
687 whilst our experiments do not allow us to comment on the contribution of earthworms to
688 greenhouse gas fluxes from soils they do indicate that earthworm activity increases carbon storage
689 in soils.

690

691 **4.5. Research limitations**

692 Despite studies that show that freezing has an impact on soil structure (e.g. Hinman and Bisal
693 (1968); Chamberlain and Gow (1979)) there were no significant differences between the control
694 (unfrozen) and DeF monoliths in terms of hydraulic conductivity, SWRC, WHC, %WSA, BD,
695 %OM, %N and plant biomass at the end of the experiment. Prior to repopulating with earthworms
696 our DeF and DeF+E treatments were treated identically. This gives us confidence that freezing our
697 monoliths to defaunate them did not significantly impact on the physical soil properties that we
698 measured or the conclusions we reached regarding the mechanisms behind the differences in these
699 measurements between the DeF and DeF+E monoliths. However, freezing also removes other soil
700 macro- and meso-invertebrates whilst having little impact on soil micro-invertebrates and the
701 micro-biota (Barley, 1961; Bruckner et al., 1995; Kampichler et al., 1999). We did not compare
702 the invertebrate populations of the monoliths other than the earthworms. Whilst it remains unlikely
703 that these populations would respond differently between the DeF and DeF+E monoliths we can
704 not strictly rule out such differences and consequent impacts on soil properties. Perhaps more
705 significantly, allyl isocyanate has negative effects on at least some types of fungi (e.g. Nazareth et
706 al. (2020); Nazareth et al.(2018)) but was only applied to the DeF+E monoliths. Fungi in particular
707 play an important role in aggregate formation (e.g. Six et al. (2004)). As an assessment of microbial
708 diversity was beyond the scope of this study we can not rule out differences between the DeF and
709 DeF+E monolith soil properties being due, at least in part, to microbial differences rather than the
710 direct actions of earthworms.

711 At the start of our experiment we introduced an earthworm population equivalent to that found in
712 adjacent pasture fields. Therefore, it could be argued that the changes we saw in soil properties

713 over c. 1 year between the DeF and DeF+E treatments would not be observed to occur so rapidly
714 in a natural system as earthworm populations would recover more gradually. However
715 observations in our main experiments indicate that earthworm populations recover very rapidly to
716 pasture levels in our ley strips (within two years, unpublished data) and our experiment does serve
717 to isolate out the important contribution that earthworms, as opposed to changes in vegetation or
718 land management methods, make to soil properties in ley / pasture systems. Further, the data also
719 demonstrate the benefits that could be achieved in a short period of time if arable soils are moved
720 to either pasture or minimum / no till cultivation and are inoculated with earthworms.

721 For logistical reasons our experimental design was unbalanced with only one unfrozen control
722 monolith used for each field. The lack of within-field replication of these controls is not a severely
723 unbalanced design for ANOVA since the experiment is replicated across 4 fields. However, one
724 could be more cautious in interpreting significant differences if p -values are anywhere near the
725 threshold for significance of 0.05. ANOVA analysis was performed to examine the main effects
726 of each factor level (consistent with the experimental design of the fields being the main unit of
727 replication) but not their interactions (which consider observations per field per treatment). We
728 repeated the ANOVA tests excluding the unfrozen control monoliths and the variables with
729 statistical differences between DeF and DeF+E treatments were the same, giving confidence in our
730 statistical analyses that included the controls.

731 Finally, our experiment ran for only one year and we saw improvements in soil properties relative
732 to the arable soil even in our control monoliths. It would be instructive to run earthworm exclusion
733 experiments for longer periods of time to see whether the levels of improvements obtained in the
734 presence of earthworms are greater than those achievable in their absence or whether the
735 achievement of such improvements is simply accelerated.

736 5. Conclusion

737 This experiment examined how the soil properties of long-term arable fields develop when
738 converted into ley and in response to enrichment and depletion of earthworm populations. Within
739 one-year, the conversion led to significant improvements in soil qualities and functions that are
740 widely degraded by intensive cultivation, including reducing compaction (6% decrease in bulk
741 density) and increasing soil organic matter (by 29%). The effects of soil freezing and earthworm
742 enrichment compared to freezing without enrichment, demonstrated significant beneficial effects
743 of earthworms in respect of WHC (9% increase), PAW (21% increase), soil organic matter (9%
744 increase), %WSA > 250 μm (by 15%), and total N (by 3.5%), but no significant effects on bulk
745 density, even though the leys reduced BD. Overall, our study indicates that increases in earthworm
746 populations previously seen in arable land converted to grassland (Roarty and Schmidt, 2013) and
747 in arable rotations that include leys, will make important contributions to the improvements in soil
748 qualities and functions seen in leys. We found organic carbon sequestration, improved soil
749 structure (Jarvis et al., 2017; Johnston et al., 2017) improved herbage (58% increase) and wheat
750 growth (20% increase) all attributable to earthworms.

751 Although earthworms increased K (47% increase in $K_{0.5}$), their impact changed in magnitude
752 through the seasons. This suggests that when modelling the impact of earthworms on water
753 drainage, for example for flood runoff modelling, large estimation errors could occur if the wrong
754 hydraulic conductivity values are used for the wrong season. Seasonal weather conditions
755 influence soil properties and biological activity which in turn impact K , but the presence of
756 earthworms led to an increase in hydraulic conductivity. Given the effect of earthworms, there is
757 a need to better understand whether those effects are only temporary and how they change in the

758 long term. The changed soil proprieties of a converted ley due to the presence of earthworms may
759 be more resilient than the smaller improvements that occur in their absence when exposed to
760 extreme drought or flooding events.

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