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Article:

Kiss, TBW, Chen, X orcid.org/0000-0002-2053-2448, Ponting, J et al. (2 more authors) (2021) Dual stresses of flooding and agricultural land use reduce earthworm populations more than the individual stressors. *Science of The Total Environment*, 754. 142102. p. 142102. ISSN 0048-9697

<https://doi.org/10.1016/j.scitotenv.2020.142102>

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1 **Dual stresses of flooding and agricultural land use reduce**
2 **earthworm populations more than the individual stressors**

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14

15 **Abstract**

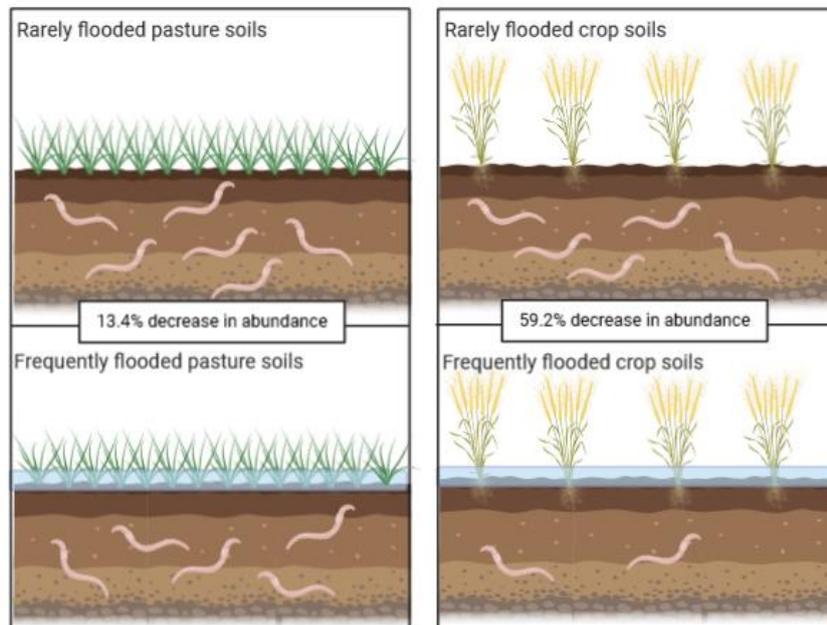
16 Global climate change is leading to a significant increase in flooding events in many countries. Current
17 practices to prevent damage to downstream urban areas include allowing the flooding of upstream
18 agricultural land. Earthworms are ecosystem engineers, but their abundances in arable land are already
19 reduced due to pressure from farming practices. If flooding increases on agricultural land, it is important
20 to understand how earthworms will respond to the dual stresses of flooding and agricultural land use.
21 The earthworm populations under three land uses (pasture, field margin, and crops), across two UK
22 fields, were sampled seasonally over an 18-month period in areas of the fields which flood frequently
23 and areas which flood only rarely. Earthworm abundance in the crop and pasture soils and total
24 earthworm biomass in the crop soils was significantly lower in the frequently flooded areas than in the
25 rarely flooded areas. The relative percentage difference in the populations between the rarely and
26 frequently flooded areas was greater in the crop soils (-59.18% abundance, -63.49% biomass) than the
27 pasture soils (-13.39% abundance, -9.66% biomass). In the margin soils, earthworm abundance was
28 significantly greater in the frequently flooded areas (+140.56%), likely due to higher soil organic matter
29 content and lower bulk density resulting in soil conditions more amenable to earthworms. The findings
30 of this study show that earthworm populations already stressed by the activities associated with arable
31 land use are more susceptible to flooding than populations in pasture fields, suggesting that arable
32 earthworm populations are likely to be increasingly at risk with increased flooding.

33 **Highlights**

- 34 • We surveyed earthworms in frequently and rarely flooded areas of UK fields
- 35 • Flooding increased soil organic matter and reduced soil bulk density
- 36 • Earthworm abundance in regularly flooded soils was lower than in rarely flooded soils
- 37 • Populations decreased due to flooding relatively more in crop than pasture soils
- 38 • Earthworm populations in arable soils are susceptible to future flooding

39

40 Graphical Abstract



41

42 Keywords

43 Flooding, land use, earthworms, climate change, population dynamics

44 1. Introduction

45 The global climate is changing, leading to changes in rainfall frequency and flooding regimes across
46 the world (Kundzewicz et al., 2014; Hirabayashi and Kanae, 2009), including in the temperate regions
47 of Europe (Bronstert, 2003; Blöschl et al., 2017). Models predict an increase in flood discharge rates of
48 10-30% from many rivers globally over the next century (Hirabayashi et al., 2013). In the UK, flooding
49 events associated with increased rainfall have been increasing in both frequency and intensity, with the
50 mean annual floodwater discharge in the UK increasing by approximately 12% between 1960 and 2010
51 (Prudhomme et al., 2003). While these events can cause catastrophic damage to urban conurbations
52 they also affect arable and pasture fields, leading not only to losses of crops and livestock but also to
53 reductions in crop viability and loss of grassland for grazing (ADAS, 2014). With the threat of flooding
54 increasing on agricultural land, due to climatic changes, land use changes, and land management

55 changes, and the flooding of farmland to prevent damage of downstream urban areas (Lane, 2017), the
56 question that arises is; what impact will these flooding events have on soil fauna?

57 Earthworms are important soil fauna. They are a key food source for many animals such as badgers
58 (Skinner and Skinner, 1988), foxes (Macdonald, 1980), birds (Ausden et al., 2001; Wilson et al., 1999)
59 and moles (Funmilayo, 1979). Perhaps more importantly, earthworms are also ‘ecosystem engineers’
60 (Jones et al., 1994); organisms which “directly or indirectly modulate the availability of resources to
61 other species, by causing physical state changes in biotic or abiotic materials” (Lawton, 1994).
62 Earthworms fulfil this role in the soil environment by their behaviours and activities (e.g. movement,
63 consumption, and excretion). Their tunnelling increases soil porosity (Stork and Eggleton, 1992) and
64 soil water infiltration rates (Ernst et al., 2009; Hallam et al., 2020), including in floodplain soils (Schütz
65 et al., 2008). The consumption of soil and organic matter by earthworms contributes to the nutrient
66 turnover of the soil, either through excretion of casts that contain greater macro- and micronutrient
67 availability than the ingested material (Barley and Jennings, 1959; Whalen and Parmelee, 1999; Tomati
68 and Galli, 1995; Sizmur and Hodson, 2009; Sizmur and Richardson, 2020), or through the release of
69 nutrients from earthworm tissues after death (Syers and Springett, 1984). Casting of digested material
70 increases the aggregate stability of the soil (Zhang and Schrader, 1992; Maeder et al., 2002; Hallam and
71 Hodson, 2020) and bioturbates organic matter (Scheu, 1987; Meysman et al., 2006). These activities
72 result in improved plant growth in the presence of earthworms (Tomati et al., 1988; Scheu et al., 1999;
73 van Groenigen et al., 2014; Hallam et al., 2020). For example, earthworms increase crop yield by up to
74 25% when soil nitrogen is limited (van Groenigen et al., 2014).

75 Given that the actions of earthworms in soil give rise to many of the ecosystem services that soils deliver
76 (Blouin et al., 2013), it is important to consider whether changes in flooding regimes with changing
77 climatic conditions and flood management will impact earthworm populations, and the further
78 implications this may have on crop yields or grassland production. Within arable soils, the role of
79 earthworms is particularly important given the boost that earthworms provide for crop growth (van
80 Groenigen et al., 2014; Bertrand et al., 2015). However, in arable soils, earthworm populations are
81 greatly reduced in comparison to pasture soils (Curry et al., 2002; Boag et al., 1997; Holden et al., 2019)

82 due to a number of factors including crush or cutting damage from agricultural machinery (Boström,
83 1995; Tomlin and Miller, 1988), the use of pesticides (Pelosi et al., 2013; Ball et al., 1986) and low
84 organic matter contents resulting in insufficient food to sustain large earthworm populations (Reeleder
85 et al., 2006).

86 It has long been observed that earthworms emerge from the soil after heavy rainfall (Darwin, 1881).
87 The precise reason for this remains unknown, but over repeated flooding events this may lead to
88 reductions in earthworm populations, as earthworms on the soil surface are vulnerable to predation
89 (Tomlin and Miller, 1988). There may also be effects on the earthworm community structure with
90 regular flooding; studies have found that cocoons remain viable following flooding events (Plum and
91 Filser, 2005), but if all adults are removed from the population during a flooding event it will take time
92 for a population to become reproductively viable again. Within the soil itself, inundation may cause
93 physical and chemical changes that create an environment that is either unsuitable for earthworms, such
94 as reduced oxygen concentrations (Ponnamperuma, 1984; Kiss, 2019), or which favours one particular
95 ecotype or behavioural subtype over another. Flooding can lead to increases in the organic matter
96 content of soil through deposition of organic-rich sediment sourced from upstream (Johnston et al.,
97 1984; Venterink et al., 2009) and/or reduced rates of organic matter decay due to reduced oxygen
98 concentrations (Reddy and Patrick Jr, 1975). This increase in organic matter leads to decreases in bulk
99 density (Bronick and Lal, 2005), and increases in soil water holding capacity (Carter, 2002; Rawls et
100 al., 2003), which can lead to higher soil moisture contents. Earthworm population fluctuations in
101 flooded soils, therefore, may depend on a number of factors such as how likely earthworms are to
102 survive flooding events and repopulate the flooded regions; how suitable soil conditions in these flooded
103 areas are for supporting earthworm populations; how viable earthworm cocoons and juveniles remain
104 during and after a flood event; whether earthworm species belonging to different ecotypes respond
105 differently to flooding and rates of earthworm migration after flooding from areas that were not flooded.
106 While some studies have found that earthworm populations in agricultural soils in temperate regions
107 are relatively resilient to one-off, extreme flooding events (Harvey et al., 2019), how populations

108 respond to flooding events of greater frequency and duration, as expected in some global regions with
109 climate change (Hirabayashi et al., 2013), is less well understood.

110 It is clear from the existing literature that both flooding and agricultural soil use effect earthworm
111 populations. However, studies tend to examine these factors in isolation, which is not necessarily
112 representative of how stressors may accumulate or act in the environment. There are very few studies
113 at the time of writing that have examined how combinations of stressors impact earthworm populations
114 in soil, and none of which we are aware that examine the combined stressors of conventional arable
115 farming and flooding. This study aims to understand the effects that flooding has on the soil
116 environment and on earthworm populations under two very different land uses. To achieve this, one
117 pasture field and one arable field (containing soils used for growing crops and soils from the field
118 margin), each with frequently flooded and rarely flooded areas in the same field, were visited on a
119 number of occasions between 2016 and 2018. Soil properties and earthworm populations were
120 measured in the pasture, margin, and crop soils to represent a spectrum of low, medium, and high levels
121 of soil disturbance, in areas known to flood more frequently and areas known to flood rarely. Three
122 broad hypotheses were considered:

123 1. Soil properties differ based both on the frequency of flood events and the land use, with higher soil
124 bulk density, and lower soil moisture, pH, percent carbon and percent nitrogen in the arable soils and
125 the rarely flooded regions than in the pasture soils or the frequently flooded soils.

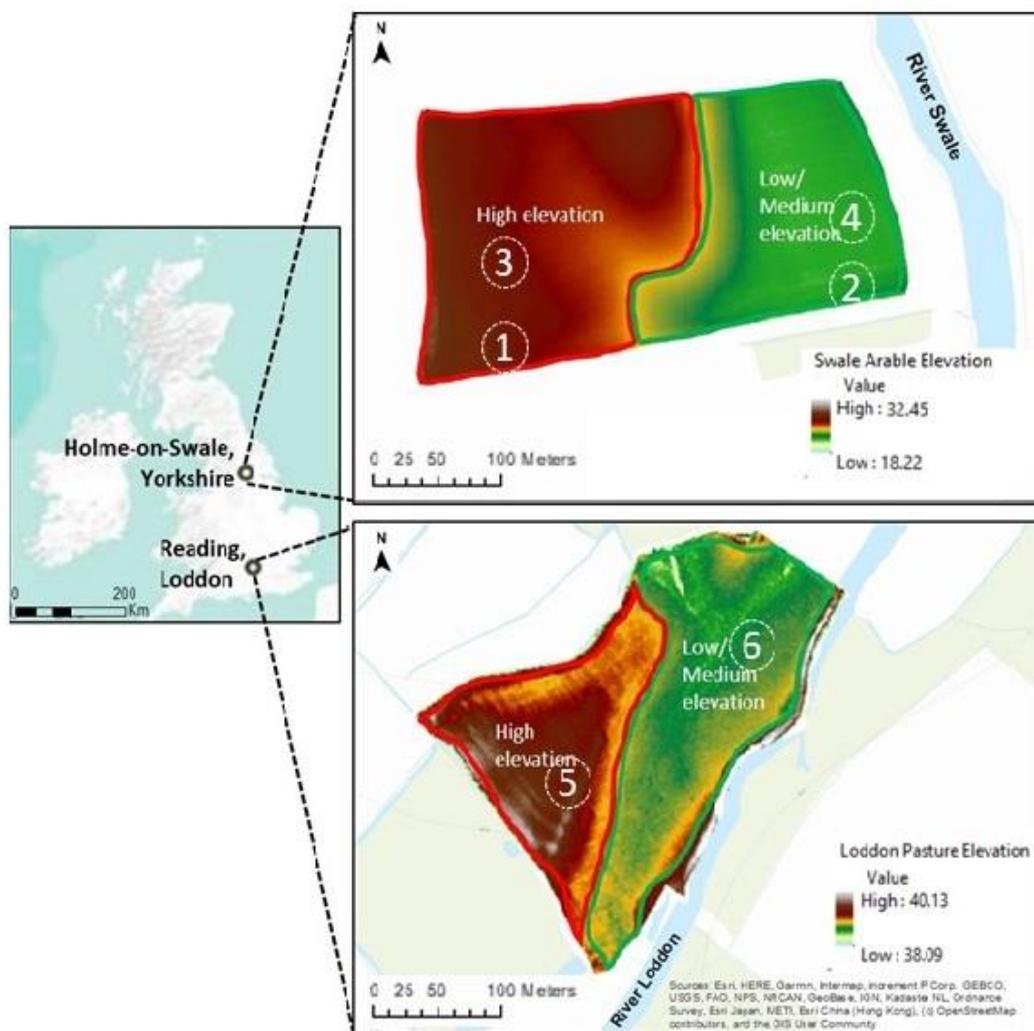
126 2. Earthworm abundance and biomass will be lower in the frequently flooded soils and the crop soils
127 than in the rarely flooded soils or the pasture soils.

128 3. Different earthworm species will respond to the various combinations of flooding and land use
129 differently.

130 **2. Methods**

131 **2.1. Field sites**

132 Two field sites were used for this study. A pasture field located at British National Grid (BNG) reference
133 SU 75153 68746 near Reading, England, and an arable field located at BNG reference SE 36200 81600
134 near Holme-On-Swale in Yorkshire, England. Both of these fields border rivers: the river Loddon
135 borders the pasture field, and the river Swale the arable field (Figure 1) Communication with land
136 managers confirmed that at both sites there are areas of the field subject to frequent flooding and areas
137 of the field which rarely flood, due to both distance from the river and the topography of the field though
138 precise records of the date and duration of individual flood events were not available. As groundwater
139 level data were only available for the frequently flooded pasture soils we were unable to use this data
140 in our analysis of controls on earthworm distributions across the different sampling sites within the
141 same field and between fields. Due to this reason, it is not possible to attribute flooding events
142 specifically to groundwater or riverine flooding.



143

144 **Figure 1 – The location of the Loddon pasture field near Reading, England, and the Swale**
 145 **arable field near Holme-On-Swale, England and LIDAR graphs representing the topography of**
 146 **the fields. Samples for the rarely flooded areas (sites 1, 3 and 5) were taken from areas of high**
 147 **elevation (coloured brown, on the western side of the fields). Samples for the frequently**
 148 **flooding areas (sites 2, 4 and 6) were taken from areas of low and medium elevation (coloured**
 149 **green, on the eastern side of the fields). In the arable field sites 1 and 2 were located in the**
 150 **field margin soil and sites 3 and 4 in the arable soil.**

151 The pasture field was visited every three months over a period of eighteen months, from November
 152 2016 to February 2018. On each visit, six randomly positioned samples were taken from the rarely
 153 flooded area and twelve from the frequently flooded area. A higher number of samples were taken in

154 the frequently flooded area as, according to the land manager, there appeared to be two distinct drainage
155 rates within this area. However, we have combined all the data from the frequently flooded area because
156 our focus is the comparison of frequently and rarely flooded soils. In addition, preliminary data analysis
157 (not reported here) indicated that, when present, any differences in soil properties and earthworm
158 populations in the frequently flooded area between the areas with apparently different drainage rates
159 were minor and rarely significant. Combining the data results in a greater number of frequently flooded
160 than rarely flooded soil samples for the pasture field. The arable field was visited approximately every
161 three months, from April 2017 to January 2018. The decision to only sample for one year was due to
162 the generally low earthworm abundances at this site. On each visit, six randomly positioned samples
163 were taken in each of four locations: a crop soil and a field margin soil, from both the frequently flooded
164 and rarely flooded areas.

165 **2.2. Earthworm and soil sampling**

166 Samples were taken by excavating a pit measuring 20 cm x 20 cm x 20 cm. The soil was extracted using
167 a sharp levering motion with a spade and put into a high sided tray in order to prevent earthworm escape.
168 The extracted soil was hand-sorted for live earthworms. Any earthworms living deeper within the soil
169 were expelled using one litre of 0.13 ml L⁻¹ concentration allyl isothiocyanate in deionised water
170 (Zaborski, 2003; Pelosi et al., 2009), which was poured into the pit and left for 30 minutes to drain into
171 the soil. The combination of hand-sorting soil and use of a chemical expellant is the most effective
172 method of sampling the earthworm community (Pelosi et al., 2009). Emerging earthworms were rinsed
173 with deionised water and stored separately from earthworms collected from the pit. Earthworms were
174 collected live and transported back to the laboratory in moist soil. The soil temperature at 5 cm and 10
175 cm depths for each pit was recorded by inserting a soil temperature probe horizontally into the intact
176 soil adjacent to the pit. A soil sample was collected by hammering a bulk density ring of volume 63.62
177 cm³ (height 4 cm, diameter 5.5 cm) into the side of the freshly dug pit, approximately 10 cm below the
178 soil surface. The sample was brought back to the laboratory for analysis of soil moisture content, bulk
179 density, soil pH, and soil carbon and nitrogen content.

180 In the laboratory, live adult earthworms were identified using the OPAL “Key to Common British
181 Earthworms” (Jones and Lowe, 2016) and weighed. Juvenile and adult earthworms, earthworm
182 fragments or dead earthworms were recorded as such and weighed.

183 **2.3. Soil analysis**

184 Soil samples collected in the bulk density ring were dried at 105°C for 24 hours with pre- and post-
185 drying weights used to calculate gravimetric moisture content and oven-dried soil bulk density. Soil pH
186 was determined by adding 40 ml of deionised water to 10 g of the dried soil sample in 50 ml
187 polypropylene tubes, which were shaken for two hours and left to stand for one hour in order to allow
188 any particulate matter to settle. Soil pH readings were taken using a Thermo Orion 420A plus pH/ISE
189 Meter, calibrated with pH 4, pH 7 and pH 10 buffers. Soil texture was determined by hand texturing
190 (Thien, 1979).

191 Total soil carbon and nitrogen were determined using a Vario Macro C/N analyser. A subsample of the
192 oven-dried soil was finely ground in a ball mill and approximately 100 mg ± 5 mg were analysed to
193 determine soil %C and %N content. The C/N analyser was calibrated using samples of glutamic acid of
194 the same mass as the soil. A certified organic analytical standard of Peaty soil from Elemental
195 Microanalysis Ltd (B2176 – batch 133519) gave recoveries of 97% (std dev = 2.21%, n = 5) and 100%
196 (std dev = 2.94%, n = 5) for certified concentrations of 15.95% C and 1.29% N, respectively.

197 **2.4. Data analysis and statistical methods**

198 Our entire raw data set is provided in the SI. Data were analysed using RStudio (R Core Team, 2019).
199 The soil properties used in further analysis were: soil bulk density (g cm^{-3}), soil moisture content (%),
200 soil pH, soil carbon content (%), and soil nitrogen content (%). For the statistical analysis, soil pH was
201 converted to H^+ activity. Prior to statistical testing, all datasets for soil properties and earthworm
202 populations were tested for normality and heteroscedasticity and, where appropriate, transformed, or
203 non-parametric statistical tests used. The total abundance of earthworms which had been extracted from
204 the pit through both hand sorting and allyl isothiocyanate expulsion was calculated for each pit and
205 expressed on a m^{-2} basis. Partial earthworms were not included in this calculation. Total biomass of

206 earthworms (g m^{-2}) was the sum of the biomass of each individual, including partial earthworm body
207 fragments. The percentage of the total abundance represented by juveniles was calculated, and for
208 analysis arcsine transformed.

209 The data were categorised by both the flooding regime and the land use. Two categories were
210 established for the frequency of flooding: rarely flooded and frequently flooded. Three categories were
211 established for land use: crop and margin soils from the arable field, and pasture soils from the pasture
212 field. To address the hypotheses established for this paper, the data were analysed using linear mixed
213 effect (LME) models, treating the sampling date as a random effect and treating the land use and
214 flooding regime as fixed effects for each soil property or population factor measure. For soil pH, soil
215 percentage carbon, and total earthworm abundance, the linear mixed effect models were overfitted and
216 so generalised linear models were instead used to compare the effects of flooding and land use on these
217 factors. Tukey *post hoc* testing was then performed to determine where differences occurred between
218 flooding and land uses. As samples were collected year-round, with sampling date used as a random
219 factor, the effect of land use and flooding are representative of the populations in general, and therefore
220 not sensitive to the timing of an individual flooding event. Finally, the relative percentage difference in
221 earthworm abundance and earthworm biomass between the rarely and frequently flooded sites were
222 determined for each land use. The means of earthworm abundance and biomass across all pits for each
223 combination of land use and sampling date were used for these calculations with a negative value
224 indicating a decrease from the rarely to the frequently flooded soil. A Kruskal-Wallis test, with *post hoc*
225 testing performed using a Wilcoxon signed ranks test, was used to determine whether these differences
226 were significantly different between land uses and flooding regimes.

227 To determine whether the abundance of different earthworm species varied with flooding and land use,
228 the abundance of each earthworm species was calculated. The only species present at a sufficiently high
229 abundance deemed suitable for statistical analysis were *Aporrectodea caliginosa* ($n = 131$ across the
230 entire data set) and *Allolobophora chlorotica* ($n = 341$ across the entire data set). The abundances of
231 the other species can be found in Table SI-1. The abundances of these species were expressed as
232 individuals m^{-2} and cube root transformed to achieve a normal distribution. The effect of flooding and

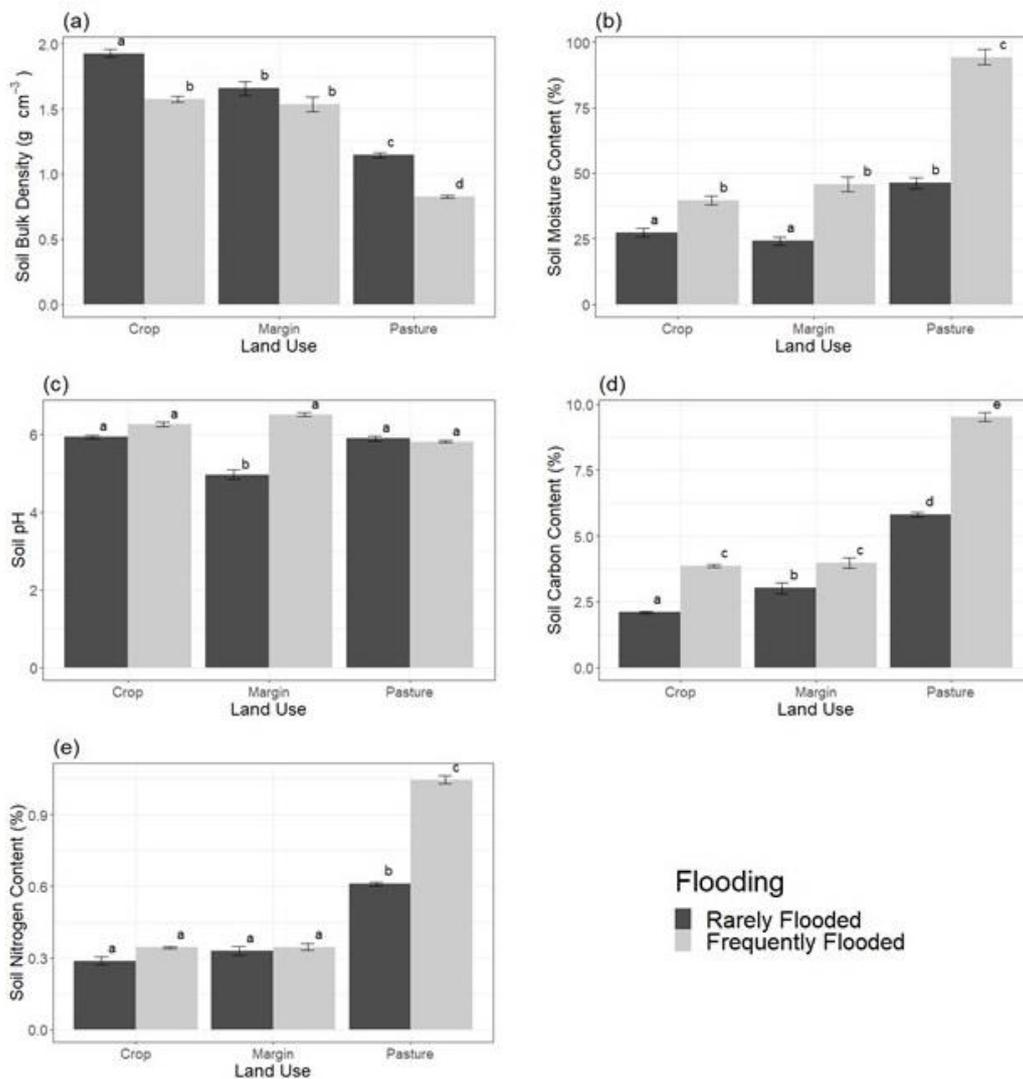
233 land use on the abundances of these two species were determined through the use of LME models,
234 treating the sampling date as a random effect. Tukey *post hoc* testing was then performed to determine
235 where differences occurred between flooding frequency and land use. The process was repeated to
236 determine how the combined biomass of all individuals of the two species varied with flooding
237 frequency and land use; the biomasses of *A. chlorotica* were cube root transformed, but no
238 transformation was required for *A. caliginosa*.

239 **3. Results**

240 **3.1. Soil properties across different land uses and flooding frequencies**

241 The pasture soils were sandy clay loams and the arable soils were silty clay loams. For all soil properties
242 (bulk density, soil moisture, soil pH, and soil percent carbon and percent nitrogen), there was a
243 significant interaction between flooding and land use on the variation observed in the data ($P < 0.001$;
244 Figure 2).

245 The soils from frequently flooded areas had lower bulk densities than the rarely flooded areas. Soil bulk
246 density was significantly lower in the pasture soils than in the crop and margin soils and frequent
247 flooding resulted in the bulk density of crop and margin soils becoming similar. Soil moisture and soil
248 carbon content were both higher in the soils from frequently flooded areas. As with bulk density,
249 frequent flooding resulted in the crop and margin soil moisture and carbon values becoming more
250 similar. Soil nitrogen content was only higher in the frequently flooded pasture soils, with no significant
251 difference in nitrogen content observed between the rarely and frequently flooded areas for either the
252 crop or margin soils. Only margin soil pH showed a significant response to flooding, with the pH in the
253 frequently flooded margin soils significantly greater than the rarely flooded margin soils, to the extent
254 that their pH was similar (not significantly different) to either crop or pasture soils.



255

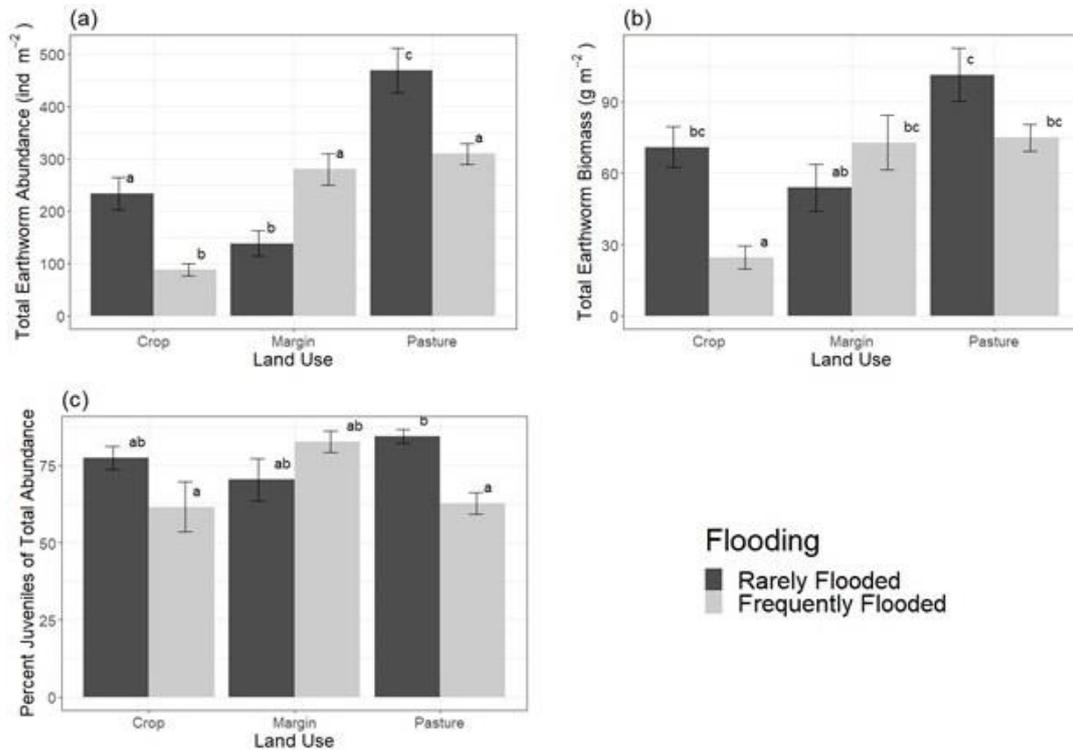
256 **Figure 2 – Mean (a) soil bulk density, (b) soil moisture content, (c) soil pH, (d) soil carbon content**
 257 **and (e) soil nitrogen content in soils under different land uses; crop, margin and pasture, and in**
 258 **areas of the field exposed to different flooding frequencies; rarely and frequently flooded (n =**
 259 **24 for rarely flooded crop, rarely flooded margin, frequently flooded crop, and frequently flooded**
 260 **margin; n = 36 for rarely flooded pasture; n = 72 for frequently flooded pasture). Error bars**
 261 **indicate standard errors of the mean. Bars in the same plot marked with the same letter as each**
 262 **other indicate treatments that are not significantly different from each other ($P < 0.05$).**

263 **3.2. Earthworm populations across different land uses and flooding frequencies**

264 There was a significant interaction between flooding and land use for all earthworm population factors
265 (Figure 3): abundance ($P < 0.001$), total biomass ($P = 0.004$), and the percentage of total earthworm
266 abundance represented by juveniles ($P = 0.002$).

267 Earthworm abundance was significantly lower in the frequently flooded crop and pasture soils relative
268 to the equivalent rarely flooded areas of the same soils. However, the abundance of earthworms in the
269 frequently flooded margin soils were higher than those in the equivalent rarely flooded soils. Total
270 earthworm biomass was significantly lower in the frequently flooded crop soils, but showed no response
271 to flooding frequency in either the margin or pasture soils. The percentage of the total earthworm
272 abundance represented by juvenile individuals was significantly lower in the frequently flooded area of
273 the pasture soils, compared to the rarely flooded area, but there was no significant difference between
274 the rarely and frequently flooded areas of crop or margin soils.

275



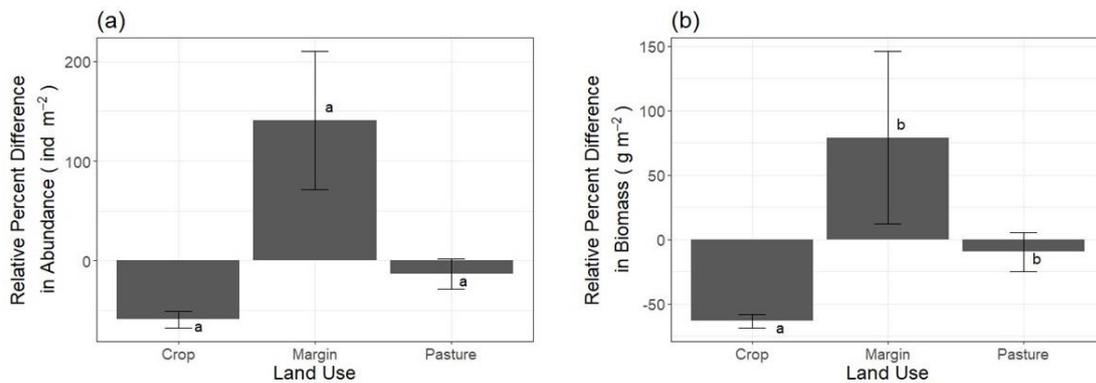
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278 **Figure 3 – Mean (a) total earthworm abundance m⁻², (b) total earthworm biomass (g m⁻²), and (c)**
 279 **percentage of the total abundance of earthworms represented by juvenile individuals in soils**
 280 **under different land uses; crop, margin and pasture, and areas of the field with different flooding**
 281 **frequency; rarely and frequently flooded (n = 24 for rarely flooded crop, rarely flooded margin,**
 282 **frequently flooded crop, and frequently flooded margin; n = 36 for rarely flooded pasture; n = 72**
 283 **for frequently flooded pasture). Error bars indicate standard errors of the mean. Bars in the same**
 284 **plot marked with the same letter as each other indicate treatments that are not significantly**
 285 **different from each other ($P < 0.05$).**

286

287 **3.3. Relative percentage differences in earthworm populations between rarely and**
288 **frequently flooded areas**

289 The relative percentage difference in earthworm abundance and earthworm biomass between rarely and
290 frequently flooded areas differed significantly between land uses ($P = 0.01$ and < 0.05 respectively)
291 (Figure 4). The relative percentage difference in abundance was negative in crop soils (-59.2%) and
292 pasture soils (-13.4%) (i.e. earthworm abundance was lower in the frequently flooded areas than the
293 rarely flooded areas), but was positive in margin soils (+140.6%). Pairwise Wilcoxon *post hoc* testing
294 showed that the differences between these land uses had significance levels of $P = 0.057$ (crop and
295 margin); $P = 0.067$ (crop and pasture) and $P = 0.057$ (margin and pasture). Similarly, the relative
296 percentage difference in total earthworm biomass between rarely and frequently flooded areas was
297 negative in the crop (-63.5%) and pasture soils (-9.7%), and positive in the margin soils. (+78.7%).
298 Pairwise Wilcoxon *post hoc* testing showed that the differences between these land uses had
299 significance levels of $P = 0.043$ (crop and margin); $P = 0.043$ (crop and pasture) and $P = 0.476$ (margin
300 and pasture).



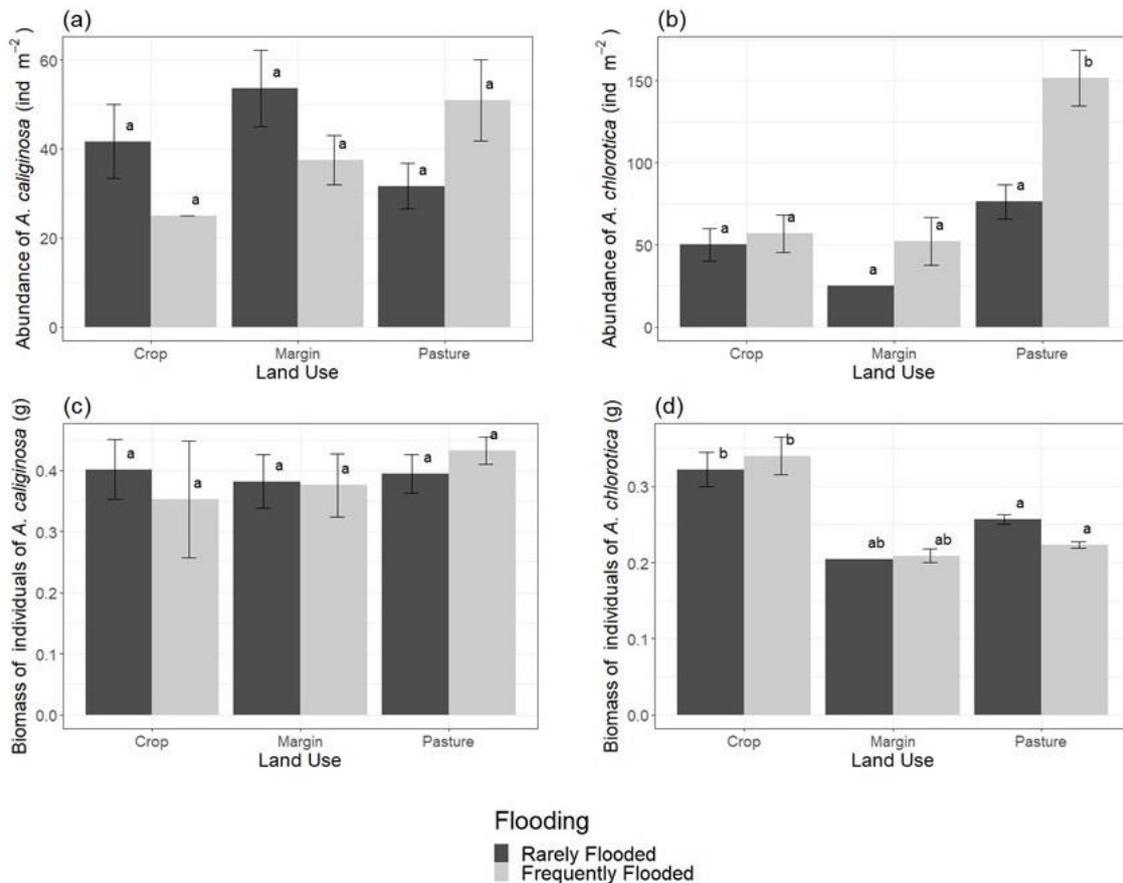
301
302 **Figure 4 – Mean relative percentage difference in (a) earthworm abundance and (b) total**
303 **earthworm biomass (g m⁻²), between rarely flooded and frequently flooded areas of crop, margin**
304 **and pasture soils (n = 4 for crop; n = 4 for margin; n = 6 for pasture). Error bars indicate standard**
305 **errors of the mean. Bars in the same plot marked with the same letter as each other indicate**
306 **treatments that are not significantly different from each other ($P < 0.05$).**

307 **3.4. Influence of land use and flooding on the populations of *A. caliginosa* and *A.***
308 ***chlorotica***

309 Land use had no effect on the abundance of *A. caliginosa*, but significantly affected the abundance of
310 *A. chlorotica* ($P < 0.001$) (Figure 5). *A. chlorotica* was present exclusively as the green morph. Flooding
311 also affected the abundance of *A. chlorotica* ($P < 0.001$), but had no effect on the abundance of *A.*
312 *caliginosa*. *Post hoc* testing showed that the abundance of *A. chlorotica* was significantly higher in
313 frequently flooded pasture soils than in rarely flooded pasture soils, and all crop and margin soils. There
314 was no significant difference in the abundance of *A. chlorotica* between frequently flooded crop and
315 margin soils.

316 There was no significant effect of flooding on the biomass of individuals of either *A. caliginosa* or *A.*
317 *chlorotica*, and no effect of land use on the biomass of individuals of *A. caliginosa*. The combined
318 biomass of *A. chlorotica* individuals was significantly lower in the pasture soils than in the crop soils
319 ($P < 0.05$; Figure 5). The biomass of other species found in the soils at lower abundances can be found
320 in Table SI-2.

321



323

324 **Figure 5 – Mean abundance of *Aporrectodea caliginosa* (a) (n = 24 for rarely flooded crop, rarely**
 325 **flooded margin, frequently flooded crop, and frequently flooded margin; n = 36 for rarely flooded**
 326 **pasture; n = 72 for frequently flooded pasture) and *Allolobophora chlorotica* (b) (n = 24 for rarely**
 327 **flooded crop, rarely flooded margin, frequently flooded crop, and frequently flooded margin; n**
 328 **= 36 for rarely flooded pasture; n = 72 for frequently flooded pasture), and mean biomass (g) of**
 329 **individuals of *A. caliginosa* (c) (n = 10 for rarely flooded crop; n = 2 for frequently flooded crop;**
 330 **n = 15 for rarely flooded margin; n = 9 for frequently flooded margin; n = 34 for rarely flooded**
 331 **pasture; n = 40 for frequently flooded pasture) and *A. chlorotica* (d) (n = 28 for rarely flooded**
 332 **crop; n = 25 for frequently flooded crop; n = 23 for rarely flooded margin; n = 1 for frequently**
 333 **flooded margin; n = 177 for rarely flooded pasture; n = 87 for frequently flooded pasture) across**
 334 **the different land uses of crop, margin and pasture. Error bars indicate standard errors of the**
 335 **mean. Bars in the same plot marked with the same letter as each other indicate treatments that**
 336 **are not significantly different from each other ($P < 0.05$).**

337 **4. Discussion**

338 **4.1. Flooding causes changes in soil properties, reducing differences between crop and** 339 **margin soils.**

340 As with other studies, there were differences in soil properties observed between crop and pasture soils
341 (Figure 2), with higher bulk density, and lower carbon and nitrogen content, in the crop soils than in the
342 pasture soils. Arable fields typically have a lower organic matter content than pasture fields (Bradley,
343 2005), due to a number of factors such as lower levels of plant root exudates and plant residue input,
344 (Haynes and Beare, 1997; Guo and Gifford, 2002; Pausch and Kuzyakov, 2017), and cultivation;
345 cultivation tends to break up aggregates that may protect soil carbon (Beare et al., 1994; Follett, 2001),
346 and which are more protected in the higher root density systems observed in long term pasture compared
347 to the low root density systems found under arable cultivation (Haynes et al., 1991). Arable fields also
348 typically have higher bulk density than grazed pasture sites (Bharati et al., 2002) due to the use of heavy
349 agricultural machinery leading to soil compaction, even in low trafficked fields (Hamza and Anderson,
350 2005). Despite these land use-induced differences between crop and pasture soils, the properties of soils
351 from both land uses responded to flooding in a similar way. In common with other studies, we found
352 that the soils from frequently flooded areas displayed a higher carbon content (Reddy and Patrick Jr,
353 1975; Zehetner et al., 2009; Cierjacks et al., 2010), lower soil bulk density (Bronik and Lal, 2005), and
354 a higher soil moisture content than the soils from rarely flooded areas (Figure 2). The higher soil
355 moisture content can be attributed to the flooding itself but also the increased water holding capacity
356 associated with higher soil organic matter (Carter, 2002; Rawls et al., 2003). Differences in soil nitrogen
357 content between the rarely and frequently flooded areas were only detected in the pasture soils, with
358 significantly higher percent nitrogen observed in the frequently flooded areas. Levels of nitrate are
359 typically high in the River Loddon and in the local groundwater (e.g. Bowes et al., 2018; Environment
360 Agency, 2014; Howden et al., 2011) and it seems likely that this has led to the higher nitrate levels in
361 the frequently flooded areas of the pasture field. Flooding reduced the differences observed between

362 the crop and margin soils (Figure 2), with no significant difference between frequently flooded crop
363 and margin soils observed for any of the soil properties.

364 Soil pH did not respond to flooding in the same way in the arable and pasture fields. All the soils were
365 slightly acidic, but the rarely flooded margin soil was more acidic than the rarely flooded crop soil
366 (Figure 2c). The rarely flooded margin soil contains more organic matter than the crop soil and the
367 greater release of H^+ due to its aerobic decomposition explains the soil's lower pH (Porter et al., 1980).
368 The frequently flooded margin soil had a significantly higher pH than the rarely flooded margin soil, as
369 observed previously (Frohne et al., 2014), likely due to the consumption of H^+ ions during anaerobic
370 decomposition of organic matter whilst the soils are flooded (Xu et al., 2006). Differences in organic
371 matter content of the rarely and frequently flooded areas in the crop and pasture soils are insufficient to
372 cause similar differences in soil pH between the rarely and frequently flooded areas.

373 All of the environmental factors measured in this study influence earthworm populations to a greater or
374 lesser degree. The reduced plant residue input observed in crop soil compared to field margin or pasture
375 soil (Guo and Gifford, 2002) has been shown to reduce earthworm populations, with populations
376 increasing in mulched crop soils compared to un-mulched soils (Pelosi et al., 2009), while the greater
377 below ground root density in pasture soils than in arable soils leads to greater quantities of dead root
378 matter for earthworm consumption (Curry and Schmidt, 2007; Bernier, 1998). The different above-
379 ground plant covers present in pasture and arable soils can also lead to differences in the composition
380 of the rhizosphere, with soil bacterial populations driven in part by different plant root exudates (Dennis
381 et al., 2010; Dey et al., 2012), again influencing soil carbon dynamics (Haichar et al, 2008) and acting
382 as a food source for earthworms (Edwards and Fletcher, 1988). Soil bulk density can influence
383 earthworm burrowing activity, but the responses to compacted soil vary between earthworm ecotypes
384 (Kretzschmar, 1991; Joschko et al., 1989; Langmaack et al., 1999). Soil moisture can influence a range
385 of earthworm behaviours, from escaping behaviour in flooded conditions (Darwin, 1881; Roots, 1956;
386 Zorn et al., 2005) to aestivation in hot and dry conditions (Gerard, 1967). It is evident from the literature
387 that flooding and land use lead to changes in soil properties, and that these, together with a range of
388 other variables such as predator numbers and local weather conditions, in turn influence earthworm

389 populations. By sampling soils from the same field but under different flooding regimes, we have
390 attempted to control for these confounding variables as much as possible in order to understand how
391 the interaction of flooding and land use impacts earthworm populations.

392 **4.2. The dual stresses of flooding and land use reduces earthworm populations more** 393 **than the individual stressors**

394 As expected, the earthworm populations were lower in the crop than in the pasture soils (Curry et al.,
395 2002; Boag et al., 1997; Roarty and Schmidt, 2013). Our observed average abundances of $233.33 \pm$
396 153.84 individuals m^{-2} in the rarely flooded crop soils lie in the 150 – 320 individuals m^{-2} range of
397 abundances in crop soils reported in the literature for temperate climate conventional arable
398 management (Poier and Richter, 1992; Binet and Le Bayon, 1998; Curry et al., 2002; Roarty and
399 Schmidt, 2013; Pelosi et al., 2014), while our observed abundances in the frequently flooded crop soils
400 (87.50 ± 58.98 individuals m^{-2}) fall below this range. However, our observed abundances in both the
401 rarely and frequently flooded field margins were lower than those reported by Roarty and Schmidt (470
402 ± 47 individuals m^{-2} compared to 138.54 ± 120.68 and 280.21 ± 149.63 individuals m^{-2} in the rarely and
403 frequently flooded field margins respectively). We did observe high levels of deviation within the crop
404 and field margin soils, which may be attributed to variability across seasons or reflect patchy
405 distribution of resources in arable soils (Ettema and Wardle, 2002). Within pasture soils, our
406 observations of 468.75 ± 253.92 and 309.38 ± 169.68 individuals m^{-2} in the rarely and frequently
407 flooded soils respectively fall within the literature reported ranges of 218 – 550 individuals m^{-2}
408 (Nuuntinen et al., 1998; Didden, 2001; Piotrowska et al., 2013).

409 In this study, the relative difference in the total earthworm abundance and biomass between the rarely
410 and frequently flooded areas was greater in the crop (-59.18% and -63.49% respectively) than in the
411 pasture (-13.39% and -9.66% respectively) soils (Figure 4). Whilst we do not have quantitative data on
412 the frequency and duration of flooding at the two sites, which could at least in part explain these
413 differences, the populations in the frequently flooded crop soils will have been impacted by two
414 stressors, conventional arable cultivation (leading to compaction, reduced organic matter content, soil

415 disturbance) and flooding, whereas populations in the frequently flooded pasture soils are only impacted
416 by flooding. Barnes and Ellis (1979) also found greater reductions in earthworm populations in sites
417 subject to two rather than one stressor, though in their case they compared sites stressed by both
418 ploughing and straw stubble burning with sites that still experienced straw stubble burning but were
419 direct drilled rather than ploughed. In contrast to the crop and pasture soils, earthworm abundance and
420 biomass in the margin soils showed a relative increase in the frequently flooded area, compared to the
421 rarely flooded area (+140.56% and +78.74% respectively) (Figure 4). This was unexpected, but may be
422 due to the greater organic matter content of the soil caused by flooding and the associated increase in
423 soil moisture leading to soil conditions more suitable for larger earthworm populations than in the rarely
424 flooded margin soils. This hypothesis is supported by the fact that the populations found in the
425 frequently flooded margin soils are not significantly different to those found in the frequently flooded
426 pasture soils.

427 The total earthworm biomass was only significantly different between the rarely and frequently flooded
428 areas in the crop soils. Since total biomass was not lower in the frequently flooded areas in the pasture
429 soils but total abundance was for both crop and margin soils this suggests that flooding led to an increase
430 in the biomass of earthworm individuals in the pasture soil relative to the crop soil. There is evidence
431 that earthworm populations are highly density dependent (Uvarov, 2009), with negative effects of large,
432 multispecies populations on the growth rates of individuals (Eriksen-Hamel and Walen, 2007). In the
433 pasture soils the relative increase in the biomass of earthworm individuals in the frequently flooded area
434 could be due to a reduction in competition between individuals, but may also be due to the reduced
435 juvenile proportion of the population (Figure 3c), with a higher proportion of larger bodied adult
436 individuals present in the population. The lack of a similar response in individual biomass due to
437 reduced numbers in the frequently flooded arable soils may reflect food limitations or reduced
438 competition already being present in the rarely flooded arable areas due to the lower abundances relative
439 to the pasture soil. In the margin soils, the higher earthworm abundance in the frequently flooded area
440 is not accompanied by a higher total earthworm biomass, suggesting a reduction in the biomass of

441 individuals due to competition, particularly between species which overlap niches (Lowe and Butt,
442 1999).

443 The earthworm species present at the highest abundance in both fields were *A. chlorotica* and *A.*
444 *caliginosa*. This is not unexpected; a Natural England survey in 2014 found them to be the most
445 common earthworm species in the UK, together comprising 53% of UK earthworm populations
446 (Natural England, 2014). *A. caliginosa* showed no response to flooding or land use but *A. chlorotica*
447 was most abundant in the frequently flooded pasture soils (Figure 5). As small bodied individuals that
448 belong to the endogeic ecotype, which forage in the upper 20 cm of soil rather than on the soil surface
449 (Bouché, 1977), earthworms such as *A. chlorotica* and *A. caliginosa* are typically less susceptible to the
450 crush damage caused by tillage that leads to the death of larger bodied earthworms (Wyss and
451 Glasstetter, 1992). The lower abundance of *A. chlorotica* in the crop and margin soils than in the pasture
452 soils can therefore be attributed to poor availability of soil organic matter in crop soils (Reeleeder et al.,
453 2006), which is one of the drivers of low earthworm abundance typically observed in arable soils (Curry
454 et al., 2002; Boag et al., 1997; Roarty and Schmidt, 2013). The relatively high abundance of *A.*
455 *chlorotica* in the frequently flooded pasture soils compared to the rarely flooded pasture soils most
456 likely reflects the documented preference of the green morph of *A. chlorotica* for moist soils (Satchell,
457 1967). *A. chlorotica* individuals had a greater biomass in the crop than in the pasture soil (Figure 5).
458 Reduced competition from a less abundant and less diverse population in the crop soil may have allowed
459 individuals of *A. chlorotica* to reach a greater individual biomass. The lack of similar responses for *A.*
460 *caliginosa*, may be due to niche overlap competition with *A. chlorotica* occurring at equal pressure
461 across all soil uses, but this is not certain and would need further investigation. The relative differences
462 in biomass of individuals between the rarely and frequently flooded sites predicted for the different land
463 uses on the basis of the total abundance and biomass data were not observed for either *A. caliginosa* or
464 *A. chlorotica* suggesting that either the differences may have been due to the low abundance species for
465 which statistical testing is not reliable (for example *L. terrestris*, a high biomass earthworm, was not
466 recorded in the frequently flooded arable soil, Table SI-1) or that, for the pasture soil at least, the
467 differences are due to differences in the relative proportion of juveniles.

468 There was a significantly lower proportion of juveniles in the population of the frequently flooded
469 pasture soils than in the rarely flooded pasture soil. No significant difference was observed with
470 flooding in the crop or the margin soils or between land uses. These findings are in contrast to
471 observations made in the literature. Pižl (1992) found that populations in regularly ploughed crop soils
472 had a higher proportion of juvenile earthworms than undisturbed regions, while Plum and Filser (2005)
473 suggested that, following flooding, the proportion of the population represented by juveniles can
474 increase due to the hatching of cocoons and the death of adults caused by the flooding event. In this
475 study, the relatively low percentage of juveniles within the frequently flooded pasture soils may be due
476 to the soil moisture contents. A study by Evans and Guild (1948) found a horseshoe relationship
477 between soil moisture and cocoon production of *A. chlorotica*, with production peaking at between 28%
478 and 42% soil moisture. Average soil moisture content of the frequently flooded pasture soils was 94%
479 ($\pm 25\%$; Figure 2b); it may be the case that the higher soil moisture content of this soil resulted in lower
480 cocoon production overall, leading to a reduced juvenile proportion of the population. The observed
481 results may also be attributed to the effect of reduced competition in the crop soils, with larger bodied
482 individuals in the crop soils better able to maintain cocoon production during the unfavourable
483 conditions caused by flooding, while the higher availability of food sources for earthworms in the
484 margins may lead to higher cocoon production by populations inhabiting margin soils (Evans and Guild,
485 1948).

486 **4.3. Limitations and further study**

487 There are limitations to this study that must be considered. In this study, we did not determine whether
488 groundwater or riverine flooding occurred. This merits further study, as high groundwater levels may
489 not always be evident on the soil surface, and yet still inundate soil where earthworms are active.

490 Larger scale studies across a number of sites with combinations of agricultural land use and flooding
491 over longer periods of time are necessary to provide greater levels of detail about the earthworm
492 populations and biodiversity. Such studies would increase understanding of earthworm population

493 resilience, and information on how the impact of flooding on earthworm populations could ultimately
494 affect the ecosystem services provided by arable and pasture soils.

495 **5. Conclusion**

496 Many of the soil properties measured differed, as expected, between the crop, margin, and pasture soils.
497 The bulk density in the crop and margin soils was higher than the pasture soils, while soil moisture,
498 percent carbon, and percent nitrogen were lower. Similarly, as expected, soil bulk density was lower
499 and soil moisture content, C content and N content higher in frequently flooded areas of the fields due
500 to accumulation and reduced degradation of organic matter, compared to the rarely flooded areas. All
501 the soils were slightly acidic but only the margin soil showed a significantly higher pH in the frequently
502 flooded area, likely linked to the consumption of H⁺ ions during anaerobic respiration. With flooding
503 the significant differences in bulk density, soil moisture, pH, percent carbon, and percent nitrogen
504 between the field margin and the crop soils disappeared. This indicates that increased frequency of
505 flooding overrides some of the effects of land use on soil properties, likely by increasing the organic
506 matter content of the frequently flooded soils.

507 Earthworm populations differed with land use. Total earthworm abundance and biomass was greater in
508 the pasture than in the arable soils. Flooding led to lower earthworm abundance in both pasture and
509 crop soil, and reductions in total earthworm biomass in crop soils. However, the relative difference in
510 population and total biomass with flooding was greater in the crop soils than in the pasture soils. In
511 contrast to the arable and pasture soils, total earthworm abundance was increased in the margin soils
512 with frequent flooding, which may be attributed to the flooding-induced soil environmental properties
513 making the soils more suitable for larger earthworm populations. The percentage of total earthworm
514 abundance represented by juveniles was significantly lower in frequently flooded pasture soils than in
515 rarely flooded pasture soils, but there was no significant response to flooding in crop or margin land
516 uses.

517 The results suggest that earthworm populations are reduced the most when subject to the dual stresses
518 of arable land use and flooding. With changing weather patterns increasing the likelihood of flooding
519 events, including in areas not previously known to flood, earthworm populations in arable soils may be
520 further reduced, leading to a reduction in the ecosystem services they provide and an increase in the
521 time it takes soils to recover following a flooding event.

522 **Acknowledgements**

523 The authors would like to acknowledge the financial support from White Rose University Consortium
524 through the BIOchemical-physical-biological function of Sludge in Agriculture Soils (BioSAS, 2016-
525 2020) PhD studentship network. We acknowledge the help of Jonathan Skidmore, English Nature in
526 identifying the arable field site and Mark Marley for permission to sample there. We thank James
527 Lamburn for granting permission to sample on the pasture field site and his observations on the flooding
528 regime there. Jamal Hallam, Miranda Prendergast-Miller, Henny Omosigho, and Alvaro Leon are
529 thanked for assistance with field work. We thank three anonymous reviewers for their comments which
530 helped improve this paper. The graphical abstract was created using Biorender.com.

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7. Supplementary Information

Table SI-1 – The mean, standard deviation, and number of pits in which individuals were present used to calculate the mean, of the abundance of adults m⁻² of each of the seven earthworm species found in the crop, margin, and pasture sites. Standard deviation N/A indicates earthworm presence in only one pit across all sampling dates.

Species	Crop		Margin		Pasture	
	Rarely flooded	Frequently flooded	Rarely flooded	Frequently flooded	Rarely flooded	Frequently flooded
<i>A. chlorotica</i>	50 (±37.98, 14)	56.82 (± 37.23, 11)	25 (N/A, 1)	52.27 (± 48.03, 11)	76.19 (± 48.40, 21)	151.52 (± 96.61, 33)
<i>A. caliginosa</i>	41.67 (± 20.41, 6)	25 (± 0, 2)	53.57 (± 22.49, 7)	37.5 (± 13.69, 6)	31.67 (± 19.97, 15)	50.93 (± 47.27, 27)
<i>A. rosea</i>	33.33 (± 14.43, 3)	25 (N/A, 1)	25 (± 0, 2)	25 (± 0, 2)	25 (± 0, 9)	34.38 (± 18.60, 8)
<i>L. castaneus</i>			25 (N/A, 1)	43.75 (± 23.94, 4)	50 (N/A, 1)	31.25 (± 12.25, 4)
<i>L. rubellus</i>		25 (N/A, 1)	32.14 (± 12.20, 7)	29.17 (± 10.21, 6)	29.17 (± 10.21, 6)	32.14 (± 11.72, 14)
<i>L. terrestris</i>	25 (0, 2)		25 (N/A, 1)	25 (N/A, 1)	25 (± 0, 3)	37.50 (± 17.68, 2)

Table SI – 2. The mean, standard deviations, and n of the average biomass (g) of individuals of each of the seven earthworm species found in the crop, margin, and pasture sites. Empty cells showed no presence of earthworm individuals. Standard deviation N/A indicates only one earthworm individual.

Species	Crop		Margin		Pasture	
	Rarely flooded	Frequently flooded	Rarely flooded	Frequently flooded	Rarely flooded	Frequently flooded
<i>A. chlorotica</i>	0.32 (± 0.12, 28)	0.34 (± 0.12, 25)	0.20 (N/A, 1)	0.21 (± 0.04, 23)	0.26 (± 0.08, 177)	0.22 (± 0.04, 87)
<i>A. caliginosa</i>	0.32 (± 0.16, 10)	0.35 (± 0.14, 2)	0.38 (± 0.17, 15)	0.36 (± 0.15, 9)	0.39 (± 0.18, 34)	0.43 (± 0.14, 40)
<i>A. rosea</i>	0.24 (± 0.10, 4)	0.21 (N/A, 1)	0.15 (± 0.08, 2)	0.20 (± 0.05, 4)	0.21 (± 0.06, 9)	0.23 (± 0.05, 11)
<i>L. castaneus</i>			0.15 (N/A, 1)	0.11 (± 0.03, 7)	0.14 (± 0.07, 3)	0.15 (± 0.07, 4)
<i>L. rubellus</i>		0.69 (N/A, 1)	0.18 (± 0.03, 9)	0.28 (± 0.19, 7)	0.54 (± 0.25, 16)	0.41 (± 0.17, 9)
<i>L. terrestris</i>	3.15 (± 0.60, 2)		0.32 (N/A, 1)	0.16 (N/A, 1)	2.93 (± 2.09, 3)	1.04 (± 1.16, 3)

Table SI – 3. The mean and standard deviations of Shannon Diversity Index values for the earthworm populations across the different combinations of land use and flooding frequency, and the mean and standard deviations of the percentage of individuals retrieved by allyl isothiocyanate (AITC) poured into the excavated pit.

Flooding frequency	Crop	Margin	Pasture
<i>Total number of individuals recorded</i>			
Rarely flooded	267	157	753
Frequently flooded	106	335	1025
<i>Shannon Diversity index values</i>			
Rarely flooded	1.12 (± 0.46)	0.77 (± 0.60)	1.41 (± 0.32)
Frequently flooded	0.61 (± 0.44)	1.24 (± 0.42)	1.18 (± 0.54)
<i>Percentage of individuals retrieved with allyl isothiocyanate</i>			
Rarely flooded	5.11% (± 2.30)	11.54% (± 14.12)	4.14% (± 1.37)
Frequently flooded	2.54% (± 4.21)	1.41% (± 1.67)	1.79% (± 0.64)