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Impact of different earthworm ecotypes on water stable aggregates and soil water holding capacity

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Abstract
We carried out mesocosm experiments using either the anecic earthworm Lumbricus terrestris or the endogeic earthworm Allolobophora chlorotica and loam, silt loam and sandy loam soils to investigate the differing impact of these earthworm of different ecotypes on aggregate formation (percentage water stable aggregates, %WSA) and soil water holding capacity (WHC), two soil properties that underpin many of the ecosystem services provided by soils. Earthworms significantly increased %WSA (by 16–56% and 19–63% relative to earthworm-free controls for L. terrestris and A. chlorotica, respectively). For L. terrestris, this increase was significantly greater in the upper 6.5 cm of the soil where their casts were more obviously present. Allolobophora chlorotica treatments significantly increased WHC by 7–16%. L. terrestris only caused a significant increase in WHC (of 11%) in the upper 6.5 cm of the sandy loam soil. Linear regression indicated a consistent relationship between increases in %WSA and WHC for both earthworm species. However, for a given %WSA, WHC was higher for A. chlorotica than L. terrestris likely due to the known differences in their burrow structure. Overall, earthworms increased soil %WSA and WHC but the significant species/ecotype differences need to be considered in discussions of the beneficial impacts of earthworms to soil properties.

Keywords Earthworms · Lumbricus terrestris · Allolobophora chlorotica · Water stable aggregates · Water holding capacity

Introduction
The development of sustainable agricultural management systems that deliver a range of ecosystem services would benefit from a full understanding of the impacts of soil fauna on soil properties. Soil macrofauna use the soil as a habitat and a source of food, and consequently, they exert a large influence on the physical properties of soils through the diversity and abundance of the structures they produce (Boivin and Kohler-Milleret 2011). Earthworms change the soil structure by modifying soil aggregation and porosity (Shipitalo and Le Bayon 2004; Blouin et al. 2013; Hallam et al. 2020). Aggregates and the space between them allow the retention and exchange of both air and water (Guber et al. 2004; Saha and Kukal 2015) and thus affect water flow and retention and soil aeration (Bastardie et al. 2003; Capowiez et al. 2014, 2015; Hallam et al. 2020; Lavelle et al. 1992). Additionally, soil aggregates contain the majority of organic carbon in soil and contribute to nutrient release for plant growth (Comforth 1968; Ramachandran Nair et al. 2010). These changes in turn affect the ecosystem services provided by soils such as being a medium for plant growth and providing storage and filtration of water (Edwards 2004; Hallam et al. 2020; Li et al. 2013; Zhang et al. 2016). Soil water holding capacity (WHC) is one measure of water retention and is an important soil parameter for monitoring soil function and processes (Hong et al. 2013; Roussevea et al. 2017). It is a function of a variety of soil properties including texture, organic matter content and soil aggregation (Hudson 1994; Saxton et al. 1986); it is easily measurable; and is a key factor in soil ecology as it influences the distribution and dynamics of animal and plant populations (BIO Intelligence Service and Communities 2014).
Earthworms are major ecosystem engineers in the soil and influence soil structure by creating macropores through their burrowing activities; they play an important role in aggregate formation and stabilization through the ingestion and egestion of soil (Blouin et al. 2013; Bottinelli et al. 2015; Guild 1955; Lee and Foster 1991; Six et al. 2002; Snyder et al. 2009). Earthworms can be grouped into three ecological groups (Bouché 1977): Epigeic earthworms (e.g. Lumbricus rubellus) are litter dwellers that consume plant residue on the soil surface and rarely ingest mineral soil; endogeic earthworms (e.g. Allolobophora chlorotica) are geophagous and inhabit the upper layers of the soil, construct narrow branching sinuous burrows and rarely come out to the soil surface; and anecic earthworms (e.g. Lumbricus terrestris) are deep vertical burrowers that typically inhabit semipermanent deep burrows and feed on organic litter which they collect from the soil surface.

Early studies established the role of earthworms in aggregate formation. Hopp and Hopkins (1946) found that L. terrestris increased the amount of water stable aggregates in sieved soil relative to earthworm-free controls. Swaby (1950) proposed that such increases were related to increased microbial activity in the earthworm gut, in turn related to levels of organic matter in the ingested soil, leading to an increased level of “bacterial gum” binding aggregates together. Guild (1955) suggested that the impact of earthworms on soil aggregation was related to the way different earthworm species process the soil and the proportions of different species and their numbers within the soil. In experiments, he found that, relative to unworked soil that was practically structureless, all earthworm species increased aggregation; epigeic species had the least effect and anecic species the greatest. Since then, investigations have continued to refine our understanding of the interaction of earthworms and other factors such as soil type, land management practices, fungal mycelium and organic matter content as it relates to aggregate production. Studies with epigeic earthworms typically find little effect on soil aggregation compared with other earthworm ecotypes because the earthworms rarely burrow within the soil (Bossuyt et al. 2006; Guild 1955; Shipitalo and Le Bayon 2004). However, contrasting results are found for anecic and endogeic earthworms with most studies recording increases in percentage water stable aggregates (%WSA) (e.g. Buck et al. (2000), Flegel et al. (1998), Hamilton et al. (1988), Ketterings et al. (1997) and Shipitalo and Protz (1989) for the anecic L. terrestris and Blanchart (1992), Bossuyt et al. (2005), Buck et al. (2000) and Haynes and Fraser (1998) for the endogeic A. caliginosa, Millsonia anomala, and Octolasion cyaneum) due to the increased processing of organic matter, increases in microbial activity and increased abundance of fungal hyphae in the presence of earthworms together with age-hardening and age-drying processes of earthworm casts forming new bonds between soil particles. A few studies report either no effect or a decrease in %WSA following earthworm-processing of soil (e.g. the anecic L. terrestris, Aporrectodea longa and Aporrectodea giardi and the endogeic A. caliginosa) (Jégou et al. 2001; Schrader and Zhang 1997; Shuster et al. 2000; Zhang and Schrader 1993). Whilst not discussed in the original studies, possible explanations for this include earthworm ingestion leading to the dispersion of clay particles, there being insufficient organic C to increase the C content of aggregates despite earthworm-processing or simply experiment duration being insufficient for casts to harden and stabilize. Further studies record differences in effect depending on other properties. For example, Blanchart et al. (1997) recorded decreases in %WSA at a soil depth of 0–5 cm, but increases in %WSA at 10–15 cm for the endogeic earthworms M. anomala, Chumiodrilus zielae, and Stuthmannia porifera though legacy effects of old earthworm casts in the soil caused them to question their results.

Whilst there are many studies that find that earthworms increase the amounts of water stable aggregates in soil, there are relatively few studies on the impacts of earthworms on soil WHC (Edwards 2004). The majority of studies consider the impact of soil water content and soil WHC on earthworm distributions rather than the other way around (Palmt et al. 2013; Schneider and Schrader 2012). The few studies that exist are superficially contradictory, but this is most likely because they measure different things. Guild (1955) and Stockdill and Cossens (1969) reported an increase in WHC after A. caliginosa, L. terrestris and A. longa processing of soil but Ernst et al. (2009) observed a decrease in soil water storage in laboratory experiments using L. terrestris or A. caliginosa species due to increased evaporation because of the pores created by the earthworms increasing soil aeration. Guild (1955) did not detect an increase in WHC in the presence of the epigeics L. rubellus and D. subrubicunda, attributing this to them not producing significant amounts of water holding aggregates but both Smagin and Prusak (2008) and Ernst et al. (2009) reported an increase in WHC in the casts of epigeic earthworms from laboratory experiments. In field experiments with kaolinitic soils and tropical earthworms, Blanchart et al. (1999) found that compacting endogeic species (Pontoscolex corethrurus or M. anomala) increased WHC whereas decompacting endogeic species (eudrilid earthworms) decreased WHC. However, Blouin et al. (2007) observed a decrease in WHC in laboratory experiments using a sandy soil in the presence of M. anomala, demonstrating the complex interaction between earthworm type, soil properties and the influence that earthworms have on those properties.

Although many studies have investigated the impacts of earthworms on soil aggregation, and some have investigated the impacts of earthworms on WHC, relatively few investigate the relationship between these two properties (Blanchart et al. 1999; Guild 1955); such studies are vital to fully understand
earthworm drivers of soil properties and the ecosystem services they provide. To address this gap, we investigated the impact of two common globally distributed earthworm species on \%WSA and soil WHC. Our experiments contrast the influence of the anecic earthworm *L. terrestris* and the endogeic earthworm *A. chlorotica* on both aggregate formation and soil water holding capacity. Our use of two different ecotypes in a consistent experimental design allows the evaluation of the potentially differing impacts these ecotypes have on the delivery of ecosystem services. We measured the \%WSA and WHC in the upper and lower portions of the soil in order to investigate the spatial variation of changes in these properties due to earthworm activity. Like many earthworm experiments, in order to detect effects in the course of a relatively short time period experiment (40 days), we used higher earthworm abundances than would be encountered under field conditions and constrained earthworms to a fixed volume of soil. Further, we did not add a food source to the soil to encourage the earthworms to process the soil. The earthworms were added to loam, silt loam and sandy loam soils. We hypothesised that (i) the horizontally burrowing, soil-feeding *A. chlorotica* will have more of an effect on the measured soil properties than surface-feeding, vertical-burrowing *L. terrestris*; (ii) both earthworm species will increase water holding capacity because of changed soil structure, (iii) the least effects will occur in soils with higher organic matter contents due to the significant role that organic matter plays in controlling soil properties regardless of earthworm activity; and (iv) effects will be more marked in soils with finer textures due to the role that clay particles play in aggregation.

**Materials and methods**

**Soils and earthworm selection and mesocosm establishment**

Soil was collected from the top 20 cm from three fields (Big Substation East, Valley and Copse) at the University of Leeds commercial farm (53° 51′ 44″ N, 1° 20′ 35″ W). The soils are Cambisols (WRB 2006) and have different textures. Each soil was air-dried, sieved at 2 mm and thoroughly homogenized to remove any legacy effects of previous earthworm activity. Basic soil properties and land management information are presented in Table 1. pH was determined on 1:2.5 soil:water mixtures (Ministry of Agriculture Fisheries and Food 1986) using an Orion 420Aplus pH meter (Thermo Orion, USA), soil organic matter content by loss on ignition at 350 °C (Ayub and Boyd 1994; CEAE 2003), field dry bulk density using soil density rings of 95 cm³ volume and soil texture using a MasterSizer2000 laser particle size analyser (Malvern Instruments, UK). A standard sand (SiO₂, CAS 14808-60-7) was used as an in house reference material for the particle size analyser which reported the mean grain size distribution at the 10th, 50th and 90th percentile to within 1% percent.

For each soil, 300 g of air-dried soil was wetted with deionized water up to a gravimetric water content of 30% to sustain earthworm activity (Berry and Jordan 2001; Butt and Lowe 2011; Lowe and Butt 2005). The moist soil was put in sealed laboratory bags punctured with pin holes, to prevent earthworms from escaping but to allow the exchange of air. The soil bags were placed in plastic beakers to support the soil and to give irregularly shaped columns of soil of approximately 6 cm diameter and 13 cm height and an approximate density of 0.82–1.00 g cm⁻³. The irregular shape of the soil columns prevents a more accurate calculation. The soil was then stored at 15 °C until the addition of earthworms. *Lumbricus terrestris* can produce burrows of several metres depth (Edwards and Bohlen 1996) which is clearly not possible in our relatively short soil columns. However, *L. terrestris* is also found in shallow soils, and in short soil column experiments such as these are still observed to process the soil to produce vertical burrows in which it rests.

Clitellate, adult earthworms of the vertical burrowing, anecic, *Lumbricus terrestris* and the green morph of the horizontal burrowing, endogeic, *Allolobophora chlorotica* species were used in this experiment. *L. terrestris* were purchased from Blades Biological Ltd. (Edenbridge, UK), and *A. chlorotica* were collected from the same fields as the test soils and identified using the OPAL earthworm identification key (Jones and Lowe 2009). The earthworms were rinsed with deionized water and acclimatized in containers containing the test soils at 15 °C in darkness. After 3 days, the viable adult earthworms were rinsed again with deionized water, dried with tissue paper weighed and added to the mesocosms to give

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### Table 1 Physical and chemical properties of soils selected for the experiment (mean ± standard deviation, *n* = 3 apart from for field dry bulk density measurements where *n* = 2 and both measurements are given)

| Field name       | Land use | pH (mean ± SD) | Organic matter (%) (mean ± SD) | Field dry bulk density (g cm⁻³) | Clay (%) | Silt (%) | Sand (%) | Texture |
|------------------|----------|----------------|---------------------------------|---------------------------------|----------|----------|----------|---------|
| Copse            | Arable   | 7.71 ± 0.10    | 3.41 ± 0.19                     | 1.56, 1.54                      | 8.44 ± 0.44 | 43.65 ± 2.02 | 47.92 ± 2.46 | Loam (L) |
| Big Substation East | Arable   | 7.64 ± 0.11    | 3.60 ± 0.22                     | 1.38, 1.51                      | 4.32 ± 0.13 | 52.12 ± 1.49 | 43.56 ± 1.61 | Silt loam (SiL) |
| Valley           | Pasture  | 7.27 ± 0.05    | 9.59 ± 0.02                     | 1.22, 1.14                      | 3.08 ± 0.54 | 41.57 ± 6.08 | 55.35 ± 6.60 | Sandy loam (SaL) |
either 2 *L. terrestris* (9.04 ± 0.52 g) or 8 *A. chlorotica* (2.16 ± 0.11 g) per mesocosm (*n* = 12 per species). Considered on a mass of earthworm per mass of soil basis, these abundances are far higher than those encountered in the field (Holden et al. 2019). We used these high densities in our experiments to compensate for the relatively short time scale (40 days) of our experiments, in order to be able to detect effects. The mesocosms (4 replicates of control and earthworm-present treatments for each of the three soil textures for each species) were maintained in a controlled environment room (15 ± 1 °C and 60 ± 7% rh) in the dark (Lowe and Butt 2005) for 40 days. They were weighed initially and then every week with any mass loss being corrected by addition of deionized water to maintain a constant soil moisture content. At the end of the experiment, earthworms were removed, rinsed with deionized water, dried with tissue paper and weighed. The soil was divided into “upper soil” (0–6.5 cm) and “lower soil” (6.5–13 cm). Soil from the individual layers was homogenized by gentle mixing. Multiple subsamples of damp material (< 0.5 g) were taken to give sufficient mass for WHC measurement. The rest of the soil was then air-dried, and multiple subsamples were again taken to give sufficient mass for %WSA measurement.

### Soil physical properties measurement

The percentage water stable aggregates (%WSA) and water holding capacity (WHC) of the soils were measured at the start and end of the experiment.

The %WSA was measured using bespoke wet sieving equipment (Wet Sieving Apparatus; Eijkelkamp soil and water Agrisearch Equipment Art no. 08.13) with a 250-μm sieve size on 4 g of air-dried soil that was previously sieved to 1–2 mm. Unstable aggregates were broken up and collected by raising and lowering the soil sample into water at a rate of 34 times per minute for a period of 3 min. Material < 250 μm passed through the sieve and was collected, oven dried at 105 °C and weighed. A dispersing solution of sodium hexametaphosphate was then added, and the process repeated to break up and collect the water stable aggregates, leaving primary particles > 250 μm behind. The %WSA was calculated, after correction for the mass of sand > 250 μm, as the weight of water stable aggregates divided by the total weight of aggregates (Kodešová et al. 2009; Milleret et al. 2009a).

WHC was determined following the method of ISO 11274 (2019). In brief, c. 50 g (oven dried equivalent weight) of damp soil was placed into open tubes of 3.5 cm diameter and 5 cm length with mesh bases. The filled tubes were placed in a container of water and allowed to wet up by capillary action. When the soil surfaces had a glossy appearance, the cores were removed from the water and allowed to drain until they stopped dripping. The soil in the cores was then gently removed and weighed. The water holding capacity of the cores was determined as the weight of water held in the soil cores compared with the 105 °C oven dry weight of the sample.

### Statistical analysis

The change in mass of the earthworms between the start and end of the experiment was analysed separately for *L. terrestris* and *A. chlorotica*, using one-way analysis of variance (ANOVA) with change in mass as a proportion of the original mass as the factor and Bonferroni post hoc pairwise comparisons. As it was impossible to identify individual earthworms, analysis was carried out on total earthworm mass per replicate. Initial values of %WSA and %WHC were compared between soils using one-way ANOVA and Bonferroni post hoc pairwise comparisons. The *L. terrestris* and *A. chlorotica* experiments were run at different times, generating two control groups of data. For ease of interpretation, the post experiment %WSA and %WHC data sets for each species were therefore analysed separately using a three-way analysis of variance (ANOVA) with repeated measures. Factors were soil texture, earthworm absence/presence and upper/lower soil layer with the upper/lower soil layer representing the repeated measure. The relative changes in %WSA and WHC due to the presence of earthworms were analysed using a two-way ANOVA with repeated measures with soil texture and upper/lower soil layer as factors with the upper/lower soil layer representing the repeated measure. All computations were made using SPSS (IBM Corp. Released 2016, version 24) with differences between different combinations of factors in the repeated measures analyses being compared using contrasts (Field 2009). Summary ANOVA tables are presented in the Supplementary Information. We assessed relationships between %WSA and soil WHC for *L. terrestris* and *A. chlorotica* using linear regression. Differences in the slopes and intercepts of the regression lines were tested for their significance between species using GraphPad Prism (GraphPad, Inc. Released on November 2017, version 7.04).

### Results

All the earthworms were recovered at the end of the experiments. The mean mass of *L. terrestris* earthworms decreased significantly over the course of the experiment in all three soils whereas the mass of the *A. chlorotica* only decreased in the loam (L) and silt loam (Sil) soils (*p* < 0.05) (Table 2). Mass loss was less than the critical limit of 30% suggested to ensure the validity of laboratory experimental results (Frend et al. 2010).

Table 3 (*L. terrestris*) and Table 4 (*A. chlorotica*) show the %WSA at the beginning and end of the experiments. Before the experiment began and at the end of the experiment, the sandy loam (SaL) showed a significantly greater %WSA than
the other two soils ($p < 0.001$). The addition of both earthworm species increased significantly the %WSA in the upper and lower soils compared with the control ($p < 0.001$). There was no significant difference between the upper and lower soils for *A. chlorotica* but %WSA was greater in the upper than the lower soils in the *L. terrestris* experiments ($p < 0.05$). For *L. terrestris*, there was a significant interaction between the soil texture and the presence/absence of earthworms with the increase in %WSA in the presence of earthworms being significantly greater in the L than the SiL and SaL soils. For both *L. terrestris* and *A. chlorotica*, there was no significant difference in the relative increase in %WSA between the lower and upper layers but the relative increases were greatest for the L and least for the SaL soils ($p < 0.05$).

WHC of the soils at the start and end of the experiments is shown in Table 3 (*L. terrestris*) and Table 4 (*A. chlorotica*). Before the experiment began and at the end of the experiment, the SaL soil had the highest, and the L soil the lowest, WHC ($p < 0.001$). The presence of *L. terrestris* caused a marginal increase in WHC ($p = 0.06$) with the increase in WHC being almost significantly greater in SaL relative to the SiL soil ($p = 0.06$). In contrast to *L. terrestris*, *A. chlorotica* significantly increased WHC ($p < 0.001$) with this increase being greater in the upper than the lower soil layers ($p < 0.05$). In contrast to %WSA, there were no significant differences in the relative increase of WHC between soils.

For each experiment, there was a statistically significant strong (i.e. $p < 0.0001$, $r > 0.5$, Cohen 1988) positive relationship between the %WSA and soil WHC (Fig. 1). Although the strength of the correlations is greater for the *A. chlorotica* than *L. terrestris* experiments, the slopes of the linear regressions are not significantly different between species. However, the intercepts of the linear regressions are significantly larger ($p < 0.0001$) for *A. chlorotica*.

**Discussion**

**Earthworm biomass**

In our experiments, we deliberately did not feed the earthworms in order to encourage them to intensely process the soil (Abbott and Parker 1981). In almost all the treatments, the earthworms lost weight (Table 2), though weight loss was within the acceptable range for laboratory experiments (Frund et al. 2010). The decrease in mass of the anecic *L. terrestris* is

### Table 2 Mean total *L. terrestris* and *A. chlorotica* mass (g) at the start and end of the experiment and percentage mass loss ($n = 4$ replicates, ± standard deviations)

| Earthworm species | Soil texture | Initial total earthworm mass (g) | Final total earthworm mass (g) | Average percentage mass loss |
|-------------------|-------------|---------------------------------|-------------------------------|-----------------------------|
| *L. terrestris*    | Loam        | 8.88 ± 0.41                     | 6.60 ± 0.39                   | 25.57 ± 4.43%              |
|                   | Silt loam   | 9.18 ± 0.71                     | 7.30 ± 0.81                   | 20.34 ± 7.95%              |
|                   | Sandy loam  | 9.08 ± 0.61                     | 7.76 ± 0.78                   | 14.37 ± 8.25%              |
| *A. chlorotica*    | Loam        | 2.15 ± 0.05                     | 1.56 ± 0.10                   | 27.16 ± 6.42%              |
|                   | Silt loam   | 2.20 ± 0.10                     | 1.74 ± 0.09                   | 21.04 ± 4.01%              |
|                   | Sandy loam  | 2.16 ± 0.15                     | 2.11 ± 0.13                   | 1.60 ± 12.48%              |

*a*The mean difference between the initial and final mass for the given soil texture is significant at the 0.05 level

### Table 3 Mean percentage of water stable aggregates (%WSA) and water holding capacity (WHC) of upper soils and lower soils in the presence (soil + earthworms) and absence (control) of *L. terrestris* within each soil texture. Initial soil is the soil at the start of the experiment. Data from the same row of the same soil texture and soil depth with different superscripts are significantly different ($p < 0.05$). ($n = 4$ replicates, ± standard deviations)

| Soil texture | Measured soil depth | %WSA | WHC (g g⁻¹) |
|--------------|---------------------|------|-------------|
|              | Initial soil        | Control | Soil + earthworms | Initial soil | Control | Soil + earthworms |
| Loam         | Upper soils         | 51.49 ± 5.84 a | 45.91 ± 6.73 a | 71.65 ± 3.47 b | 0.30 ± 0.009 a | 0.30 ± 0.004 a | 0.33 ± 0.056 a |
|              | Lower soils         | 51.49 ± 5.84 a | 42.94 ± 2.80 a | 64.68 ± 6.01 b | 0.30 ± 0.009 a | 0.30 ± 0.014 a | 0.30 ± 0.007 a |
| Silt loam    | Upper soils         | 52.04 ± 3.78 a | 50.31 ± 5.44 a | 68.30 ± 2.34 b | 0.35 ± 0.007 a | 0.33 ± 0.008 a | 0.35 ± 0.017 a |
|              | Lower soils         | 52.04 ± 3.78 ab | 48.07 ± 6.40 a | 61.99 ± 3.49 b | 0.35 ± 0.007 a | 0.34 ± 0.021 a | 0.34 ± 0.013 a |
| Sandy loam   | Upper soils         | 75.96 ± 3.78 b | 63.36 ± 3.45 a | 82.99 ± 1.12 c | 0.39 ± 0.010 b | 0.39 ± 0.010 a | 0.44 ± 0.020 b |
|              | Lower soils         | 75.96 ± 3.78 b | 65.11 ± 4.32 a | 75.27 ± 4.93 b | 0.39 ± 0.010 b | 0.39 ± 0.029 a | 0.40 ± 0.017 b |
typical and reflects their feeding preference of surface grazing on decaying organic matter (Lavelle 1997). Although A. chlorotica is an endogeic earthworm and feeds by consuming soil, the earthworms still lost weight over the duration of the experiment except in the sandy loam (SaL) soil which has the highest organic matter content and therefore presumably more available food (Table 1).

### Impact of earthworms on %WSA

The addition of both earthworm species increased significantly the %WSA of the upper and lower soils compared with the control. Our results agree with the majority of studies on earthworms and aggregation (Bossuyt et al. 2005; Buck et al. 2000; Edwards and Bohlen 1996; Flegel et al. 1998; Hamilton et al. 1988; Ketterings et al. 1997; Swaby 1950) that report an increase of %WSA in the presence of earthworms, although in these studies, it is not always clear what depth of the soil was being inspected. Across the different soil types, L. terrestris increased %WSA to 74 ± 7% in the upper soil but only 67 ± 7% in the lower soil compared with 53 ± 9% in the controls, whereas for A. chlorotica there was no difference with depth with an average value of 74 ± 11%WSA. Despite the restricted size of the mesocosms, this is consistent with the anecic earthworms’ surface casting behaviour that will result in an accumulation of cast-derived aggregates in the surface layer compared with endogeics mixing soil and making extensive burrows filled with casts (Pérès et al. 1998; Whalen et al. 2015).

The SaL soil had the highest organic matter content of the three soils (Table 1) and the highest values of %WSA (Table 2). This is consistent with previous studies that show that increased organic matter content of soils leads to better soil aggregation (Alagöz and Yilmaz 2009; Haynes 2005). Although the L soil has a higher content of clay-sized particles from the same row of the same soil texture and soil depth with different superscripts are significantly different (p < 0.05). (n = 4 replicates, ± standard deviations)

| Soil texture | Measured soil depth | %WSA | WHC (g g⁻¹) |
|--------------|---------------------|------|-------------|
|              | Initial soil        | Control | Soil + earthworms | Initial soil | Control | Soil + earthworms |
| Loam         | Upper soils         | 51.49 ± 5.84ᵃ | 41.30 ± 8.25ᵃ | 67.52 ± 3.90ᵇ | 0.30 ± 0.009ᵃ | 0.31 ± 0.004ᵃ | 0.34 ± 0.020ᵇ |
|              | Lower soils         | 51.49 ± 5.84ᵃ | 43.07 ± 6.57ᵃ | 68.76 ± 2.39ᵇ | 0.30 ± 0.009ᵃ | 0.32 ± 0.011ᵃ | 0.36 ± 0.015ᵇ |
| Silt loam    | Upper soils         | 52.04 ± 3.78ᵃ | 46.65 ± 4.12ᵃ | 64.93 ± 5.64ᵇ | 0.35 ± 0.007ᵇ | 0.36 ± 0.005ᵇ | 0.41 ± 0.015ᵇ |
|              | Lower soils         | 52.04 ± 3.78ᵃ | 47.13 ± 8.04ᵇ | 67.58 ± 8.47ᵇ | 0.35 ± 0.007ᵇ | 0.37 ± 0.010ᵇ | 0.39 ± 0.002ᵇ |
| Sandy loam   | Upper soils         | 75.96 ± 3.78ᵃ | 73.54 ± 3.17ᵃ | 87.70 ± 1.26ᵇ | 0.39 ± 0.010ᵇ | 0.39 ± 0.010ᵇ | 0.45 ± 0.019ᵇ |
|              | Lower soils         | 75.96 ± 3.78ᵃ | 65.25 ± 8.42ᵃ | 87.66 ± 1.62ᵇ | 0.39 ± 0.010ᵇ | 0.40 ± 0.010ᵇ | 0.43 ± 0.014ᵇ |

**Fig. 1** Relationships between soil water stable aggregates and soil water holding capacity for all soil textures and for L. terrestris (blue symbols and correlation trend) and A. chlorotica (orange symbols and correlation trend). Filled and outline symbols indicate the presence and absence of earthworms, respectively. Lines of best fit to the data are y = 0.002648 (0.001882–0.003414) x + 0.187 (0.1388–0.2353) and y = 0.002189 (0.001736–0.002643) x + 0.2381 (0.2085–0.2678) for L. terrestris and A. chlorotica respectively with values in brackets indicating the 95% confidence intervals.
than the other two soils and clay particles also play an important role in soil aggregation, these effects are less significant than those related to organic matter for clay contents of < 10% such as in the soils used in these experiments (Allison 1973). Although the SaL soil had the highest values of %WSA, the largest relative increase in %WSA was seen for the L soil, and the smallest increase in the SaL soil. This suggests that the contribution of earthworms to aggregation is more important in either or both lower %OM or higher %clay particle soils (Fig. 2). This could be because the contribution of earthworm mucus to organic binding agents (Knowles et al. 2016; Schomburg et al. 2018) or the stimulation of fungal activity via the increased supply of soluble organic compounds (Montecchio et al. 2015; Parle 1963; Rashid et al. 2016) is more significant in low–organic matter soils. Alternatively, or additionally, the earthworm activity may have a bigger impact on aggregate formation where there are more clay-sized particles available for aggregation. Interestingly, increased burrowing activity and cast production by endogeic earthworms as they process the soil for food has been observed in soils of lower organic C content which would lead to an increased aggregate production (Bottinelli et al. 2017).

Although our results agree with the majority in the literature, several studies report what appear to be contrasting results, though differences in experimental design and measurement make comparisons and explanations of differences challenging. For example, Swaby (1950) found a bigger earthworm impact on %WSA in organic-rich, pasture soil but considered earthworm casts of unknown age and produced by a different species, Allobophora nocturna, rather than bulk soil. Milleret et al. (2009a) found a reduction in the %WSA in the presence of A. chlorotica but conducted their experiment at a higher than optimum temperature for the earthworms (Butt 1991) which could have reduced the intensity of soil processing relative to our experiment and also have impacted on microbial activities involved in the formation and destruction of aggregates (e.g. Rashid et al. 2016; Shao et al. 2019). In addition, Milleret et al. (2009a) sterilized their soil with gamma irradiation, resulting in the removal of microbiota which may play an important synergistic role with earthworms in soil stabilization and aggregation (Forster 1990). In another experiment with similar conditions, Milleret et al. (2009b) found that the presence of plants partly compensate the decrease in soil aggregation that A. chlorotica caused and hypothesised that this was due to the aggregating effects of microorganisms that fed on the C-rich exudates released by plant roots. Zhang and Schrader (1993) and Schrader and Zhang (1997) reported a lower or the same %WSA in earthworm casts obtained from the soil surface compared with the sieved soil that was initially used to make the substrate for their experiment. Our experimental measures are different from this as we compared the %WSA in bulk soil that was either worked or not worked by earthworms. The experimental designs are very different making comparison difficult. It is possible that the pressure exerted by earthworms on the surrounding soil as they move contributes to the stability of the aggregates in the bulk soil regardless of the %WSA of the casts and that the experimental design of Zhang and Schrader (1993) and Schrader and Zhang (1997) prevented this effect from being measured. In some studies (e.g. Blanchart et al. 1997) earthworms are added to intact soils which will have been in steady state with the previously existing earthworm population. As aggregate formation is a dynamic process with a constant production and destruction of aggregates, earthworms may be seen to reduce %WSA if the %WSA in a soil is moving to a new steady state in response to the new earthworm community composition and abundance. Our experiments deliberately destroyed existing soil structure prior to the start of the experiment, which likely led to rapid soil aggregation. In a final example of apparently contrasting results, Shuster et al. (2000) observed a decrease in the mean size of WSA (of 5–8-, 2–5-, 1–2-, 0.5–1-, 0.25–0.5-, < 0.25-mm size classes) in soils following the addition of a mixture of immature and mature L. terrestris. However, we measured

Fig. 2 A conceptual model of the relationship between water stable aggregate formation and soil organic matter content in the presence and absence of earthworms. The dotted line is the difference between the earthworms absent and earthworms present lines and represents the enhanced formation of aggregates due to the presence of earthworms
%WSA > 250 μm by mass and not whether the size of these water stable aggregates was decreased. Although the mean aggregate size may decrease, the total mass of aggregates greater than > 250 μm, such as measured in our experiment, could still increase. These contrasted results are therefore most likely a function of experimental design and the actual measurements made.

**Impact of earthworms on WHC**

Various studies indicate that a higher %WSA results in a higher WHC (Basche et al. 2016; Blanchart et al. 1999; Jackson 2014; Franzluebbers 2002; Suzuki et al. 2007; Zibilske and Bradford 2007). Similarly, in our work, linear regression of our %WSA and WHC data (Fig. 1) showed a significant increase in the WHC as the %WSA increases. The comparable slopes from the regressions of the A. chlorotica and L. terrestris data reflect a consistent relationship between increases in %WSA and WHC regardless of earthworm species.

In contrast to the regression slopes, the significantly different intercepts of the regression lines for the A. chlorotica and L. terrestris data indicate a greater underlying impact on WHC by A. chlorotica than by L. terrestris separate from any %WSA effect. This could be due to the different burrowing behaviour of the earthworm species. A. chlorotica produces many tortuous small diameter burrows through the soil (Pérès et al. 1998) which help to hold more water whereas L. terrestris produces a smaller number of continuous and wider diameter pores (Capowiez et al. 2015). A simple approach for verifying this would be to perform infiltration measurements at different tensions and produce water release curves for different soils that both species have processed.

**Conclusion**

In these experiments, we have demonstrated that the increase in %WSA due to earthworm-processing of soil results in increases in the important hydrological property WHC. Further, although the slope for the relationship between %WSA and WHC is the same between the two different ecotypes of earthworms, the intercept is not; there was an underlying difference in the impact of the earthworms on WHC most likely due to the differing burrowing behaviours. In this experiment, we have used a high abundance of earthworms in a constrained volume of soil and not provided the earthworms with an additional food source to encourage soil processing in order to compensate for the 40-day period of the experiment compared with the far longer time that earthworms process soils in the field. Nonetheless, given the importance of %WSA and WHC in ecosystem services such as water storage by soils and water availability to plants to support crop growth, our results are consistent with earthworms making an important contribution to these services. Despite the artificial nature of our experiments that constrain the extent to which the different earthworm species can behave, our experiments demonstrate that different ecotypes have different effects, due to their differing lifestyles and nature of the burrows, and that effects can differ with depth. This highlights that caution should be exercised when generalizing about the impacts that earthworms have on soil properties and processes. We also showed that the effects are a function of soil organic matter which also plays an important role in aggregation and water retention and which can have a more dominant effect than the earthworms.

To better understand the impact of earthworms on soil hydrology, further experiments are required that use a more realistic earthworm abundance and diversity. In addition, plant roots are known to have significant impacts on soil hydrology (Beven and Germann 1982; Whalley and Dexter 1994), and therefore, experiments that consider the relative impacts of plant roots and earthworms on soil hydrology are warranted.

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