Soil hydraulic function:
Earthworm-plant root interactions

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Abstract

Relatively little is known about life in the soil, particularly the interactions between its components (such as plant roots, macrofauna and micro-organisms). Many of these interactions drive soil processes that give rise to ecosystem services supporting human wellbeing. Most of the valuable ecosystem services are driven by soil organisms, and earthworms are the key representatives. Earthworm activity underpins a number of soil properties that directly influence soil hydrological functioning and food production. However, the influence of different earthworm ecotypes and plant roots they interact with is largely unknown. This research project examines these effects through laboratory and field experiments.

The data show that the interaction between earthworms and plant roots can significantly increase soil physico-hydraulic properties. However, laboratory experiments indicated that there are species / ecotype effects. The vertical burrowing earthworm *Lumbricus terrestris* increased soil water flow, but in conditions where their burrows are not connected to drainage systems, plant roots had a greater effect. The lateral burrowing earthworm *Allolobophora chlorotica* had a greater impact on soil hydraulic properties than *L. terrestris*. The presence of *A. chlorotica* in soils resulted in the greatest and most rapid increase in soil water flow through macropores > 3 mm diameter as a result of their interaction with plant roots. In the field experiment, the conversion of arable soil to ley caused a significant improvement to soil properties; the presence of earthworms in the ley was responsible for a significant proportion of these improvements. However, the magnitude of earthworm impacts is significantly controlled by seasonal climate variations. Furthermore, the presence of earthworms significantly increased plant biomass.

These results show that the interaction between earthworms and plant roots can lead to increased soil drainage and also soil water retention which could help mitigate the impacts of increasing extreme weather events such as floods and droughts, thereby helping to maintain ecosystem services that are derived from soils.

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Declaration

I declare that the work presented in this thesis is original and is my own, although I did it under the supervision of Professor Mark. E. Hodson. This work has not previously been submitted for any other degree or award at this, or any other, University. All sources are acknowledged as References.

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The data in chapters 3, 4, and 5 were presented at : the European Geosciences Union General Assembly in Vienna , Austria, March 8 - 13th, 2018 (Talk and Poster); the 21st World Congress of Soil Science in Brazil, Rio de Janeiro, August 12 - 17th, 2018 (Poster); and the British Society of Soil Science Early Careers Researcher Conference in UK, Lancaster University ,April 19 - 20th, 2017 (Talk).

The experimental chapters (3, 4, 5 and 6) are presented in this thesis as submitted papers for publication. Full references and the contributed co-authors are indicated in Table 0 - 1 below. I designed the experiments, carried out the practical work, analysed the data and produced the first draft of each paper. Co-authors commented on the initial and subsequent drafts.

Table 0 - 1. References and the co-authors of the submitted papers.

Chapter	Paper title	Journal	Status	Submission date	Co-authors
3	Impact of different earthworm ecotypes on water stable aggregates and soil water holding capacity	The European Journal of Soil Science	Under Review	17/11/2018	Mark. E. HODSON[1].
4	The interacting effects of the anecic earthworm Lumbricus terrestris, winter wheat roots and soil texture on hydraulic function	Hydrological Processes	Under Review	09/08/2018	Mark. E. HODSON[1] David A. ROBINSON[2] Joseph HOLDEN[3]
5	Interaction effects of winter wheat roots and the endogeic earthworms Allolobophora chlorotica on soil physical properties including hydraulic conductivity	Soil Biology and Biochemistry	Under Review	18/11/2018	Mark. E. HODSON[1] Joseph HOLDEN[3] David A. ROBINSON[2]
6	Effect of earthworms on physico-hydraulic properties of arable to ley converted soil	Soil Biology and Biochemistry	Ready for submission		Mark E. HODSON[1], Joseph HOLDEN[3], David A. ROBINSON[2], Jonathan R. LEAKE[4], Miranda T. PRENDERGAST-MILLER[1], Despina BERDENI[4], Martin G. LAPPLLAGE[3], Emily J. GUEST[4], Richard GRAYSON[3], Anthony TURNER[4]

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Chapter 1

General introduction

1.1. Introduction

For a long time, soils were considered to be an inert substrate, however, now it is widely acknowledged that soils are a dynamic system comprising much biodiversity (Plavinet and Coquet, 2013). Earthworms are an essential component of the biological activity in soils (Edwards, 2004). These organisms contribute to plant productivity, plant and soil health and many other ecosystem services.

Because many ecosystem services are performed by organisms (Jax, 2005), Blouin et al. (2013a) reviewed the link between earthworms as part of biodiversity and various ecosystem services, and summarized different soil functions and ecosystem services that earthworms contribute to. The bibliometric analysis conducted by Blouin et al. (2013b) illustrates the growing interest in the use of earthworms in the management of ecosystem services. Using earthworm research citation data from the Science Citation Index Expanded during the period from 2000 to 2015, Xiang et al. (2015) found that the number of records relating to earthworm research (85% of which were peer-reviewed journal articles) increased from 100 to 300 yearly outputs during the 16-year study. Jeanneaux et al. (2012) observed that the rise in the number of scientific publications on ecosystem services related themes occurred following the publication of the Millennium Ecosystem Assessment (MEA) in 2005 (Millennium Ecosystem Assessment, 2005). Indeed, the adoption of the operational use of ecosystem approaches occurred in 2004 as part of the MEA, a UN programme that aimed to better identify and evaluate the importance of ecosystems to human well-being (Millennium Ecosystem Assessment, 2005, Tancoigne et al., 2014). Ecosystem services were defined as the benefits that people obtain from ecosystems, an idea first proposed by Daily et al. (1997).

The role of biodiversity and ecological entities in providing benefits from ecosystem services was signaled by Kremen and Ostfeld (2005). Blouin et al. (2013a) give examples of earthworm as drivers of soil functions such as soil formation and soil structure development, and ecosystem services including water regulation, nutrient cycling, climate regulation, pollution remediation, primary production and cultural services. Amongst these many vital services, water regulation, and soil water in particular, are fundamental in the soil-plant-water system and are of prime importance to modern agriculture.

The ability of earthworms to improve soil properties and the subsequent effects on soil hydrology were first recognized by Gilbert White in 1777 (Shipitalo and Le Bayon, 2004). In the presence of earthworms, soil physical (Darwin, 1881, Zhang and Schrader, 1993, Edwards, 2004, Bohlen et al., 2004, Drouin et al., 2016) and hydraulic (Smettem, 1992, Bohlen et al., 2004, Chan, 2004, Ouellet et al., 2008, Yunusa et al., 2009) properties are dynamic and affect soil water regulation (Blouin et al., 2013a) through transfer and storage processes (Pitkanen and Nuutinen, 1998, Blouin et al., 2007, Capowiez et al., 2014, Bertrand et al., 2015). The Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC, 2014) projected changes in the climate system as "it is very likely that heat waves will occur more often and last longer, and that extreme precipitation events will become more intense and frequent in many regions". Therefore, modification of soil water flow and storage by earthworms would help to alleviate negative effects of such events that limit exposure of many human systems and ecosystem services. Additionally, improved water storage and flux in the soils promote plant growth by increasing water available to plants and diffusion of dissolved nutrient ions within the soil to the root surface, leading to higher crop yields (Chapman et al., 2012). Further, earthworms support plant growth by increasing nutrient mineralization from residue and soil organic matter humification and by stimulation of soil microflora (Cunha et al., 2016, Scheu, 2003). Meta-analysis of 57 published papers before 2013 showed an increase in biomass of plant shoot by 23% and roots by 20% in the presence of earthworms (van Groenigen et al., 2014). The growth of plant roots will create further biopores and modify soil physical properties (Whalley and Dexter, 1994) which in turn influence soil water flow and storage (Figure 1 - 1).

The impact of earthworms on soil hydraulic properties differ according to their species or functional groups (Coleman and Wall, 2015). Anecic earthworms are usually associated with an increase in water infiltration due to their vertical burrowing which creates wide and continuous macropores that function as bypass through saturated soils (Coleman et al., 2004). Endogeic species are generally linked with alteration of soil water retention and storage presumably due to their intense activity and highly branched and tortuous burrows of small diameter (Capowiez et al., 2015), albeit contradicting results have been reported in the few conducted studies (Blanchart et al., 1999, Blouin et al., 2007, Ernst et al., 2009, Stockdill and Cossens, 1969). The litter dwellers, epigeic earthworms, rarely burrow within the soil mineral (Shipitalo and Le Bayon, 2004) but are reported to increase soil water retention (Ernst et al., 2009, Smagin and Prusak, 2008). Regarding the effect of earthworms on plant growth, each of the three ecological groups of earthworm have shown positive effects (van Groenigen et al., 2014). However, 50% to 70% of the conducted studies investigated endogeic species, in particular *Aporrectodea caliginosa* (Scheu, 2003, van Groenigen et al., 2014). This shows the importance of the endogeic ecotype in stimulating shoot and root growth, presumably because of their intense bioturbation compared to the other ecotypes (Capowiez et al., 2015). A better understanding of the interaction between earthworms that occupy different ecological niches and plant roots and their effect on soil physico-hydraulic properties is required. This will allow us to collect the maximum benefit of earthworms to alleviate negative effects of extreme events caused by climate change and sustain food production and other depending ecosystem services.

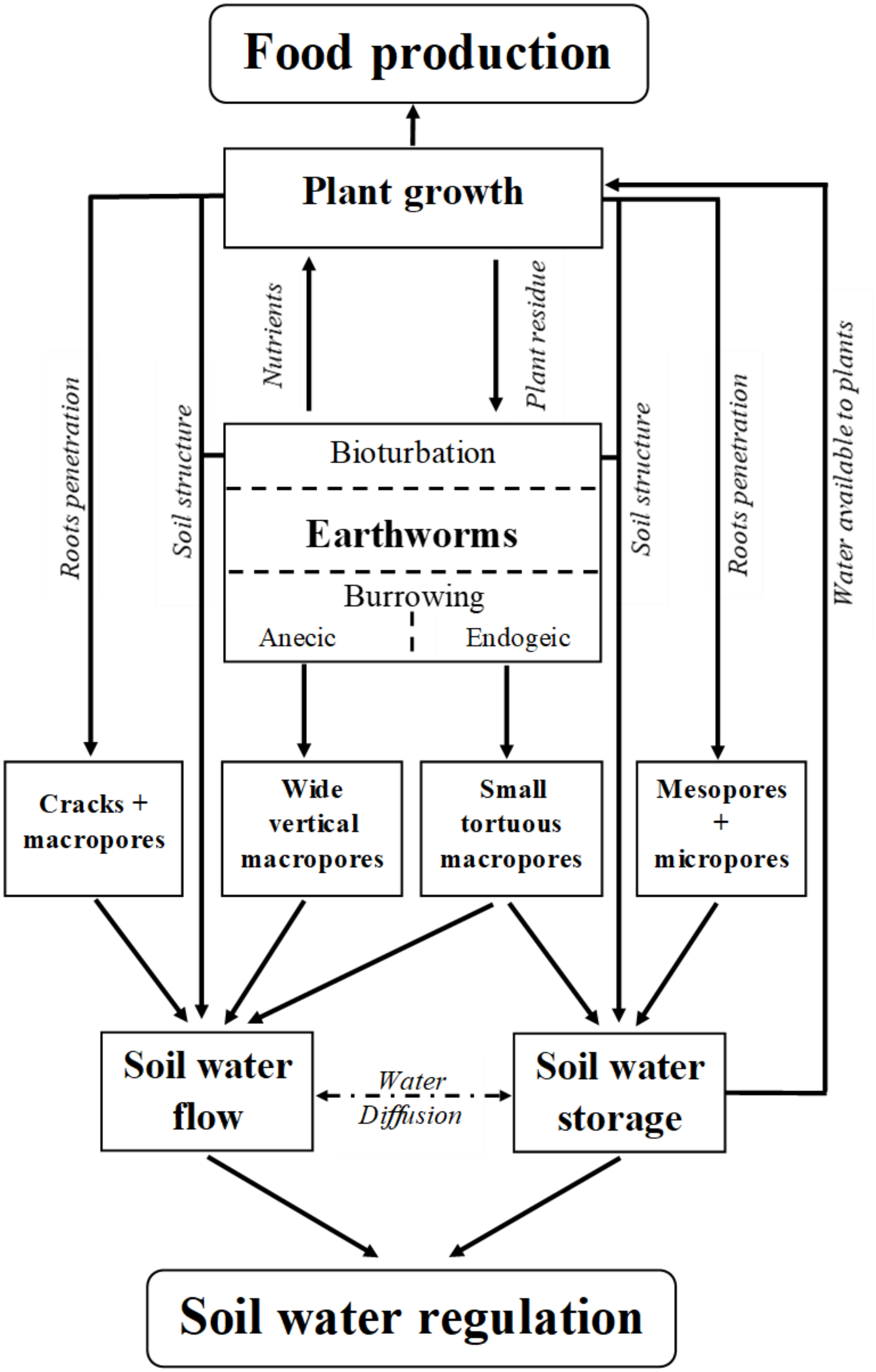

Figure 1 - 1. Schematic diagram of potential effects of the interaction between earthworms and plants on soil water regulation and food production services.

The overall aim of this thesis was to investigate the combined effects of earthworms and plant roots on the way that water interacts with soil. This was achieved through a series of laboratory experiments and a field experiment involving different earthworm ecotypes common in the UK. The experiments were carried out with and without plants and in the presence and absence of earthworms in soils of differing texture (Loam, silt loam and sandy loam soils; two of the fields from which these soils were collected were used in the field experiment). The main outputs of this work were:

- The measurement of soil hydraulic conductivity at different water tensions and the partitioning between macropore flow and micropore flow;
- The generation of soil water release curves showing how much water is held at different water potentials, together with soil water-holding capacity;
- The measurement of the percentage of water stable aggregates as an important component of soil structure which is crucial for water flow and retention in soil;
- The response of plant growth to the presence/absence of earthworms.

1.2. Outlines of the thesis

This PhD comprises seven chapters including three laboratory experiments (Chapters 3, 4 and 5) and a field experiment (Chapter 6). The contents of each chapter are described briefly below:

- Chapter 1 presents a general introduction to the importance of earthworms and their role in providing ecosystem services that benefit people. It introduces the ability of different earthworm ecotypes to influence soil water regulation through modification of soil physico-hydraulic properties and how this is vital to buffer extreme events caused by climate change. It also introduces the role of plant roots in biopores formation and the subsequent benefits in term of soil water regulation. Finally, it summarizes the aim and different outlines of the thesis.

- Chapter 2 reviews our understanding of how earthworms affect soil water regulation. It briefly presents factors, processes and mechanism that influence soil water infiltration and water storage. It provides information on the current state of knowledge on the influence of earthworms on soil water regulation and identifies relevant knowledge gaps.

- Chapter 3 investigates the effects of two earthworm ecotypes, the endogeic *Allolobophora chlorotica* and anecic *Lumbricus terrestris*, on two important soil properties, the percentage water-stable aggregates and water-holding capacity. The measurements were done on the upper and lower layers of soil. The experiment was conducted to clarify contradicting literature findings and establish principles prior to determination of earthworm and plant root interaction effects on soil water storage and transport.

- Chapters 4 and 5 examine the effects of the vertical burrowing earthworm *L. terrestris* and lateral burrowing earthworm *A. chlorotica* in the presence or absence of winter wheat roots on the way that water interacts with soil of differing texture. The measurements were made under undrained soil conditions where earthworm burrows terminate within the soil matrix. In Chapter 5 changes over time of soil hydraulic conductivity and the partitioning between macropore flow and micropore flow in the soil were considered.

- Chapter 6 investigates how earthworm interaction with grass-clover roots changes properties of arable soil when converted into ley. The field experiment described in this chapter was conducted on 28 monoliths of intact soil collected from arable fields of soil textures similar to those used in the laboratory experiments. Changes in soil water flow measured over different seasons of the year and other soil physico-hydraulic properties measured at the end of the experiment were discussed.

- Chapter 7 concludes the key findings of the research, provides research limitations and research needs for future work both in the context of specific findings of the thesis and in a more general context.

The detailed aims of each experiment are given within the relevant chapters.

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Chapter 2

Earthworms and soil water regulation: A review

2.1. Introduction

Previous reviews (Blouin et al., 2013, Bertrand et al., 2015) have highlighted the main ecosystem services provided indirectly by earthworms via their activity in the soil. In this paper we focus on one of the many important ecosystem services provided by earthworms, soil water regulation. This review will consider soil water regulation as the set of actions and interactions controlling soil water flow and availability during a given period for ecological, agricultural and environmental purposes. Addition of earthworms to soil has a significant influence on soil water regulation by changing hydraulic conductivity and infiltration (Bouche and AlAddan, 1997, Capowiez et al., 2009, Capowiez et al., 2014b, Ehlers, 1975) water retention (Ernst et al., 2009, Milleret et al., 2009b, Bertrand et al., 2015) risk of flooding (Edwards and Lofty, 1972) and erosion during runoff (Roth and Joschko, 1991, Blanchart et al., 2004, Jouquet et al., 2012b). To understand the influence of earthworm behaviour and burrows on soil water flow some authors have tried to model the system using soil physico-hydraulic properties (Bastardie et al., 2002, Ouellet et al., 2008, Schneider and Schroder, 2012, Capowiez et al., 2015). However, despite this modelling work, this aspect of earthworm ecology is under represented in the literature compared to other aspects such as earthworm population dynamics and the morphological characteristics of earthworm burrows.

Thus, the aim of this review is to synthesise our understanding of how earthworms affect soil water regulation, particularly soil water infiltration and movement and soil water retention and storage dynamics. Many factors interact in a complex fashion to impact soil water regulation (Figure 2 - 1) such as earthworm species and biomass (Alegre et al., 1996, Blouin et al., 2007), bulk density (Blanchart et al., 1997), burrowing and casting activities (Bastardie et al., 2003, Le Couteulx et al., 2015), soil texture, soil structure and initial water content (Pérès et al., 1998, Fischer et al., 2014). In this review we will briefly review each of these factors in turn along with key findings. We will then report key methodology information and results of a number of the reviewed literature in tables (Table A1, A2) and then identify relevant knowledge gaps, the filling of which would lead to an improved understanding of earthworm-water interaction. Table A1 and A2 summarises experiments detail which are discussed below.

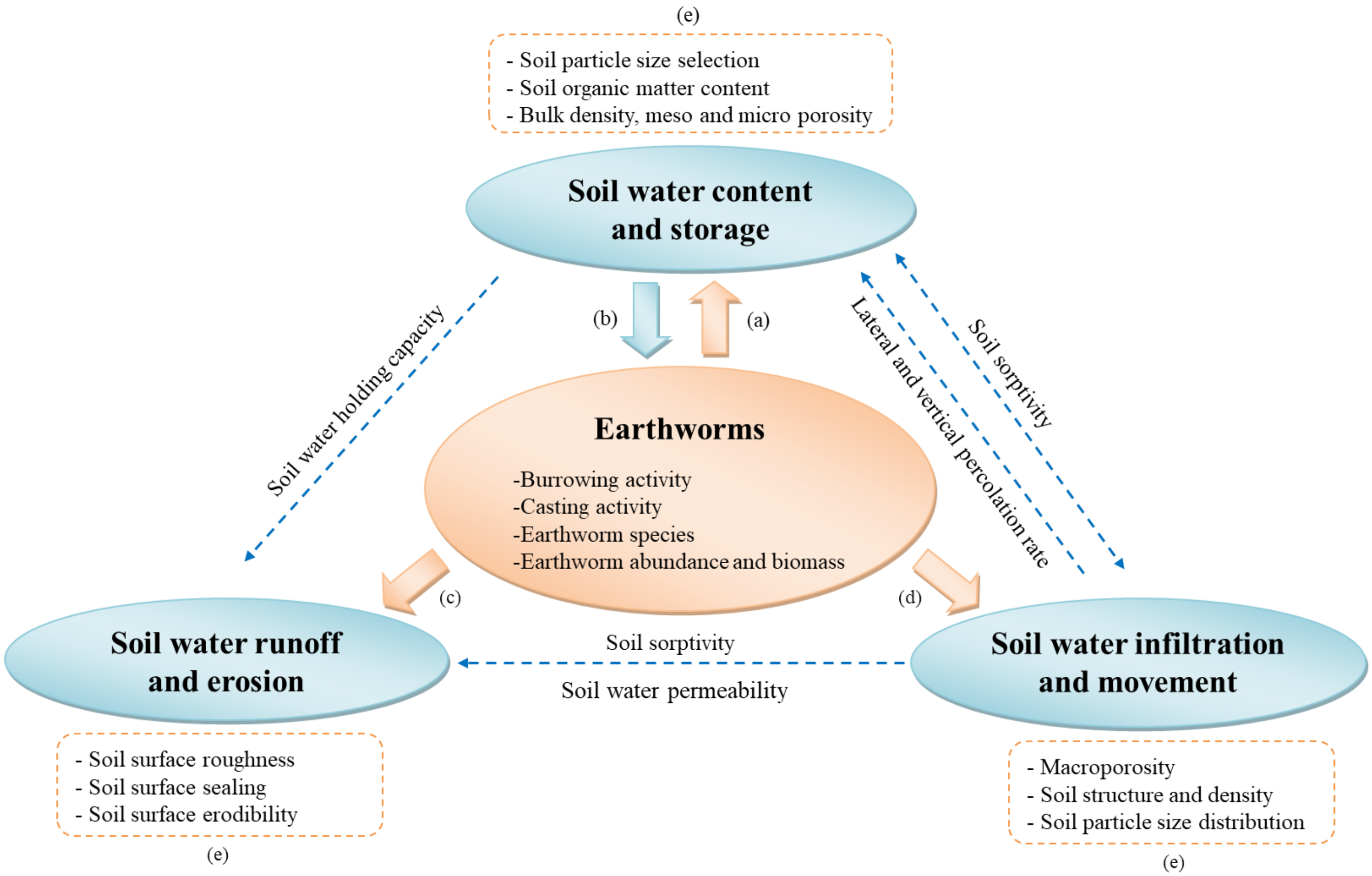

Figure 2 - 1. Potential influences of earthworms on soil water regulation (solid arrows) and the interactions between soil hydrological components (dotted blue arrows). (a): Earthworms affect soil water content and storage, (b): Soil water content affect life cycles of earthworms, (c): Earthworms affect soil water runoff and erosion, (d): Earthworms affect soil water infiltration and percolation, (e): the major variables affected by earthworms.

2.2. Earthworms and soil water infiltration and movement

Water infiltration influences hydrological flows by the transfer of water through the topsoil. Using dye and other tracers McCoy et al. (1994), Chan (2004), Shipitalo and Le Bayon (2004), Shipitalo et al. (2004), Holden and Gell (2009) and Schwartz et al. (1999) have shown that in soils with high populations of earthworms, burrows made by some earthworm species from all three main ecotypes can effectively conduct water and affect infiltration rate of the bulk soil despite the volumes of the burrows not exceeding a few percent (0.2 %) of total soil porosity. Earthworms affect soil water infiltration depending on several factors which are related to each other (Figure 2 - 1).

2.2.1. Earthworm ecotypes

Earthworms can be classified into three ecological groups, epigeic (litter dwelling), endogeic (shallow burrowing) and anecic (deep burrowing) (Bouché, 1977), and these have differing effects on infiltration (Figure 2 - 2). The majority of studies on the impacts of earthworms on infiltration rates have been conducted using anecic species and particularly *Lumbricus terrestris* (Shipitalo and Le Bayon, 2004, Spurgeon et al., 2013). *L. terrestris* are known to increase water infiltration rates and flow in soils (Willoughby and Kladivko, 2002, Fischer et al., 2014) with a number of studies identifying rapid water flow through their burrows because of their large diameter, up to 12 mm, and deep penetration, up to 240 cm, (Shipitalo and Butt, 1999). Bouche and AlAddan (1997) showed that in various soils the infiltration rate with 100 g m^{-2} of anecic species earthworm was close to 282 mm h^{-1} compared to a mean rate of 150 mm h^{-1} per 100 g m^{-2} of fresh earthworm biomass without anecic species. Chan (2004) estimated that the infiltration rate of water through a single burrow in 1 m^2 of soil was 1.9 times that of the remaining bulk soil (6.7 mm h^{-1} against 3.6 mm h^{-1}). Using medical X-ray tomography for the 3D characterization of earthworm burrow systems in natural soil dominated by the anecic species *Nicodrilus giardi*, Bastardie et al. (2005a) found that burrow systems provided a soil surface-accessible burrow volume ranging from 1400 to 10463 cm^3 and wall area ranging from 1069 to 7237 cm^{-2} for 1 m^3 of soil. The accessible burrow volume

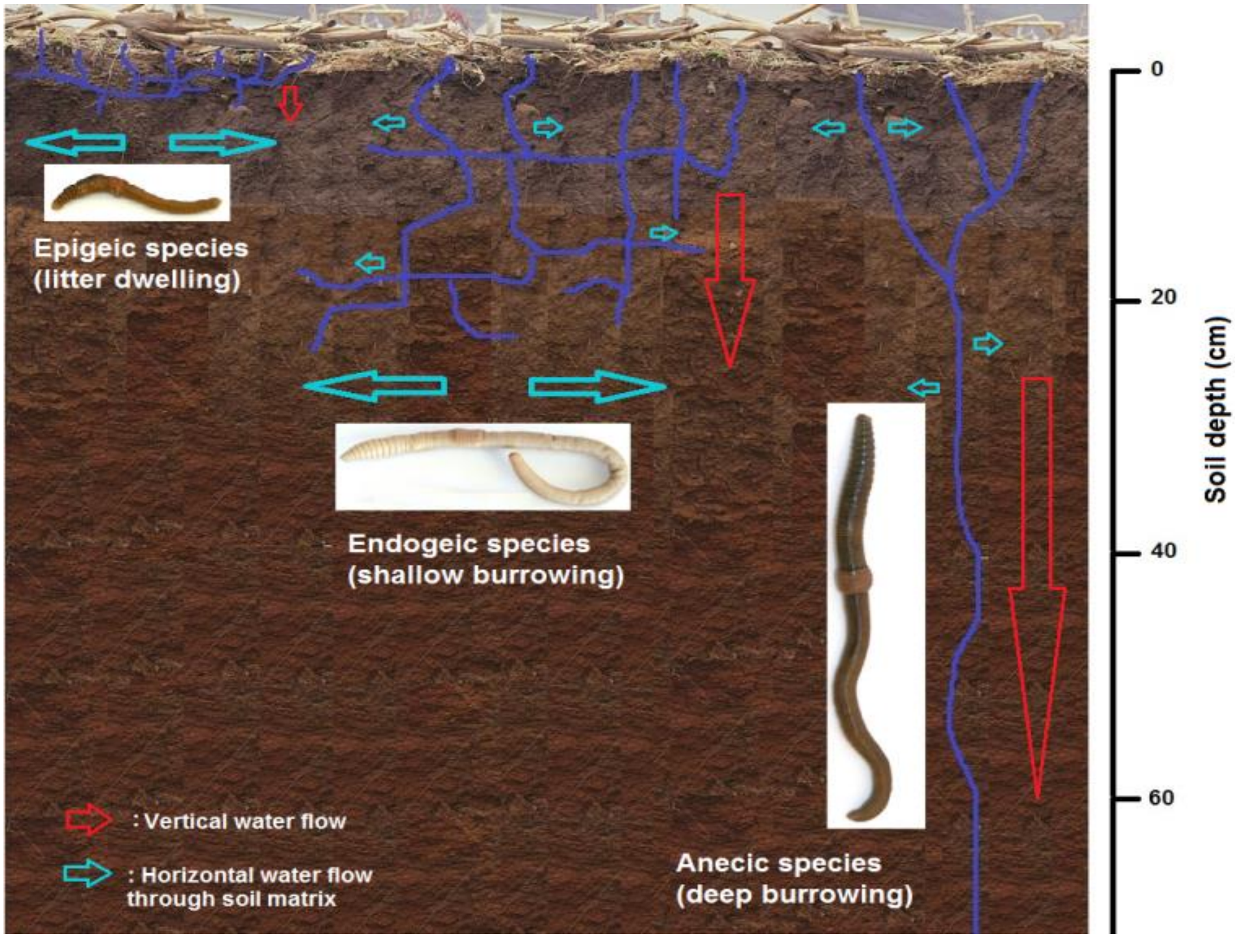

Figure 2 - 2. Potential effects of the three ecological groups of earthworms, epigeic, endogeic and anecic, described by Bouché (1977), on soil water flow. The blue lines are example of burrows filled with water. Arrow sizes are proportional to the impact of the earthworm species on water flow

and area would allow a good vertical water flow, through the burrows, and lateral water flow, within the soil matrix through burrow walls. However, Bastardie et al. (2005b) reported lower lateral water flow through *L. terrestris* burrows compared to that through soil fractures presumably due to high compaction of the burrow walls. In comparison to endogeic species, Capowiez et al. (2015), reported that the burrow systems of anecic species have fewer branched burrows (12.2 to 20.2 vs 28.2 to 37.2 branches m^{-1}) and were far more efficient regarding water infiltration rate (11. 03 to 12.42 vs 2.32 to 5.15 L min^{-1}) due to open burrows linking the top and bottom of the soil cores. The concerns with some of the field experiments (Shipitalo and Butt, 1999, Edwards et al., 1989, Shipitalo et al., 2004, Ehlers, 1975) and most laboratory experiments (Ela et al., 1992, Capowiez et al., 2015, Bastardie et al., 2005b) are that water movement through the burrows would be more than would naturally occur at realistic conditions since the burrows do not terminate within the soil matrix (Smettem, 1992). When earthworm burrows terminate within the soil matrix the flow does not depend strictly on burrow diameter (Sutera and Skalak, 1993, Singh et al., 2013) but also on burrow length and on soil matrix flow once they are water-filled (Smettem, 1986) (Figure 2 - 3). In addition, after initial infiltration the pressure of encapsulated air in the burrow may reduce further water flow (Constantz et al., 1988). Dead-end burrows also improve macropore-soil matrix interaction by increasing lateral water flow as the burrows are filling up (Cey and Rudolph, 2009).

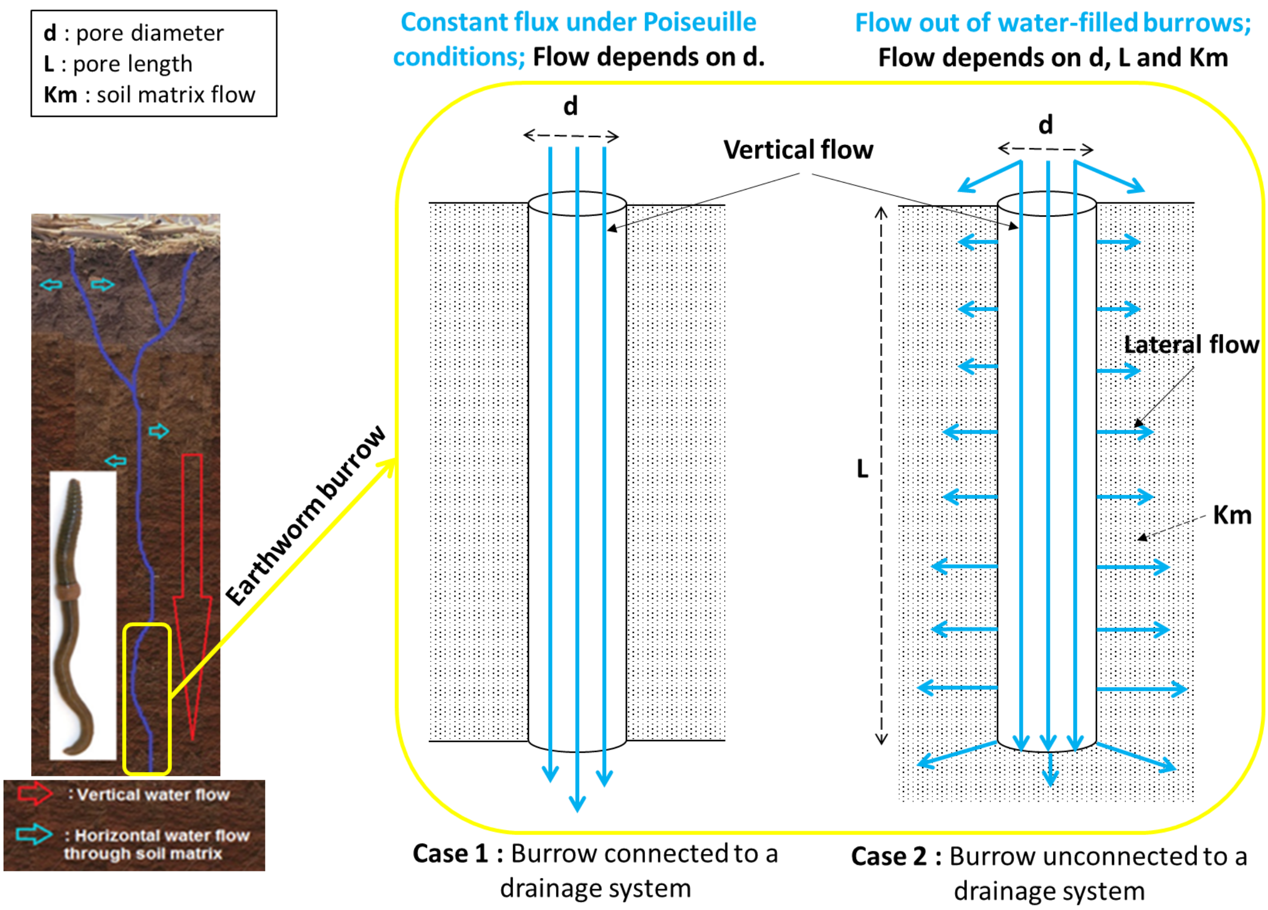

Figure 2 - 3. Empirical relationships between earthworm burrow characteristics and soil water flow. The schematic tubes are a simplification of the morphology of an earthworm burrow. Case 1: earthworm burrow is well drained (connect to field drains, or an underlying more permeable layer) and water flow depends on the burrow radius (Sutera and Skalak, 1993); Case 2: earthworm burrows terminate within the soil matrix and water flow depends on burrow radius, burrow length and soil matrix flow (Smettem, 1992).

Unlike anecic earthworms, significant effects on soil water infiltration have not been widely reported for endogeic earthworms (Ela et al., 1992, Spurgeon et al., 2013). This may be because their burrowing activity is restricted to the top soil horizons and their lateral burrowing preference (Bouche, 1972), sinuous and smaller burrow diameter (ranging between 2 mm and 5 mm in diameter compared to anecic earthworms that are up to 9 mm (Pérès et al., 1998)) and blocking of burrows below the soil surface by casting. Regarding the latter, Whalen et al. (2015) confirmed that macropore continuity can be reduced by endogeic compared to anecic earthworms when refilling no longer in-use burrows by ingested soil redeposited in burrows. Burrow refilling by endogeic earthworms was estimated to be up to 50 % compared to only 20 % for anecic earthworms assuming that all burrows should be connected (Capowiez et al., 2014a). Le Couteulx et al. (2015) reported a greater percentage of burrowed area was refilled with casts in the presence of endogeic earthworms when organic matter was mixed into soil rather than being added to the surface. The authors showed that burrow refilling by endogeic earthworms does not depend on earthworm species and would result in low water movement due to burrow discontinuity. Refilling, caused by endogeic species, can also reduce burrows life span due to the disintegration of no longer in use burrows (separated by casts) compared to anecic earthworms that repair and consolidate the burrows by reusing them (Capowiez et al., 2014a).

In contrast to the above, some studies report an increase in soil water flow in the presence of endogeic earthworms. In column experiments, for example, saturated hydraulic conductivity as well as percolation rates were increased in the presence of the endogeic species *Allolobophora caliginosa* and *Allolobophora rosea* compared to the control (Joschko et al., 1992, Roth and Joschko, 1991). Using the endogeic species *A. caliginosa*, Ernst et al. (2009), reported a larger water infiltration and faster water discharge through the soil column compared to anecic *L. terrestris,* probably due to the greater burrowing activity and connectivity between macropores of endogeic species. Capowiez et al. (2015) observed a positive linear relationship between burrow length and the water infiltration rate ($R^2 = 0.49$, $p < 0.01$) for endogeic species (*Aporrectodea rosea*, *Allolobophora chlorotica*, *Aporrectodea caliginosa*) but when *Aporrectodea icterica* was excluded from the linear regression, R^2 increase to 0.95 ($p < 0.001$). This increased correlation was explained by the low efficiency of water infiltration through *A. icterica* burrows due to lateral compaction or cast compressing along the burrow walls.

Studies that investigated the effect of epigeic species on water infiltration are contradictory. Although they have little effect on soil macroporosity and produce only shallow burrows in the litter layer or in the first 5 cm of soil compared to anecic species (Bens et al., 2007, Bouche, 1972, Fragoso and Lavelle, 1992) the meta-data analysis by Spurgeon et al. (2013) showed that epigeic earthworms increased soil water infiltration significantly. This was attributed to their ability to: i) prevent soil surface crusting due to their surface activity, ii) form stabilized soil aggregates that helped in soil water regulation and iii) form temporary deeper burrows when the earthworms are exposed to extreme climate conditions which helped to conduct water through the soil. Compared to other earthworm ecotypes, Schutz et al. (2008) reported a significant higher correlation between infiltration rate and epigeic earthworm density than anecic earthworms, but the results varied significantly for endogeic earthworms depending on their species. This was explained by the epigeic species *Lumbricus rubellus* preventing the blockage of burrows by mixing the soil litter with the top soil or simply because anecic earthworm density was too low (25 to 65 ind m^{-2}) to have a significant effect on infiltration rates. Ernst et al. (2009) reported lower water infiltration rates and percolation in columns inoculated by the epigeic *L. rubellus* than in those containing the endogeic earthworms *A. caliginosa*. They assumed that the higher burrowing activity of *A. caliginosa* and the high connections between their burrows relative to epigeic earthworms had a big impact on soil water infiltration. In another study Francis and Fraser (1998) showed no significant differences between water flow in columns inoculated by *L. rubellus* and in earthworm-free controls, which was attributed to the lack of burrows in the subsoil that could conduct water.

2.2.2. Soil structure

By ingesting litter and soil, earthworms contribute to the increase of organic matter mineralization and to the development of soil aggregates and structure which have a significant impact on soil aeration and water infiltration (Jouquet et al., 2012a). Earthworms affect soil structure by incorporating surface organic residues in the soil profile within cast aggregates or as coatings on their burrows (Bottinelli et al., 2015). These biogenic aggregates (macroaggregates embedded one in another and produced by earthworms via casting) create a temporary structure composed of particles gathered by weak bounds through organic matter and clays (Puga-Freitas and Blouin, 2015). Larink et al. (2001) found that aggregates produced by *L. terrestris* and *A. caliginosa* showed ca. 10 % lower relative water stability and 10–20 % higher porosity than soil aggregates. The loose structure was attributed to the low quality of the organic matter eaten by the earthworms. Clause et al. (2014) reported that the high porosity and the particular microstructure of cast aggregates may enable better water infiltration compared to soil aggregates. The porosity of casts can, initially, be greater than in other soil aggregates. However, they are less stable and, after breakdown and collapse during rainfall and infiltration, compaction can result in the soil porosity reducing by up to 50 % (Bottinelli et al., 2010).

The activity of earthworms has the potential to compact the soil which is observed through an increase in density and a decrease in porosity of soil; this change in turn results in a decrease in water infiltration rates and sorption (Alegre et al., 1996). The compacting effect of earthworms may be off-set by the decompacting effect of burrows and pores between casts that enhance water infiltration (Jouquet et al., 2012a). Macropore size and number also affects infiltration rate (Smettem and Collis-George, 1985). The diameter of macropores created by earthworms ranges from 2 to 11 mm and a single macropore of 3 mm diameter per 30 cm diameter of soil area could contribute more to the steady infiltration rate through a soil than the cross-sectional area associated with the soil matrix (Ehlers, 1975, Smettem and Collis-George, 1985). This is why anecic species such as *L. terrestris* and adult endogeic species such as *A. caliginosa* (more than 2 mm in diameter) could contribute to a greater water infiltration rate, particularly the wider burrows of anecic species when water is supplied in large quantities (Fischer et al., 2014). Soil macropores, as preferential pathways for water flow, depend also on the soil type and on the interaction between earthworm and soil type. Lower macroporosity was observed in sandy soils compared to soil of finer texture because of the low level, or absence, of earthworm activity and their lower organic matter content; this lower macroporosity may result in slower rates of water infiltration (Luo et al., 2010, Fischer et al., 2014, Bens et al., 2007).

2.2.3. Land management practices

Further understanding of the functional links between earthworms and soil structure and their effect on infiltration requires soil management practices, such as existing crops and tillage management, to be considered. Previous research showed that no-tillage farming management resulted in increases in soil water infiltration and percolation rates of up to 8 times compared to conventional tillage (Ehlers, 1975, Edwards et al., 1990, Wuest, 2001, Chan, 2004, TerAvest et al., 2015). The increased soil water flow was attributed to an up to 9 fold increase in earthworm abundance and activity which resulted in improved soil physical properties (Edwards et al., 1990, Chan, 2004) and an increased number of earthworm burrows wider than 1 mm at the soil surface (Ehlers, 1975, Wuest, 2001). The increased earthworm abundance and activity in the no-till farming was due to an increase in soil residue cover (TerAvest et al., 2015), favourable soil conditions and reduced earthworm mortality due to the cessation of discing the upper soil layers when ploughing (Chan, 2004, Spurgeon et al., 2013).

The decrease of earthworm abundance and biomass in conventional tillage usually comes with an alteration of the species composition (Chan, 2001). Capowiez et al. (2009) and Nuutinen (1992) reported a significant influence of tillage system on ecological groups of earthworms and the abundance and continuity of soil macroporosity. They found less continuous pores, less abundant anecic species, *L. terrestris* and *Aporrectodea giardia*, and more abundant endogeic species, *A. caliginosa*, in conventional tillage compared to reduced tillage. Similarly Spurgeon et al. (2013) reported a tendency of the dominating endogeic earthworm species in arable soil to be the first to increase in numbers in response to grassland conversion. The higher population of endogeic earthworms compared to other ecotypes was attributed to their easy access to decomposed organic matter when plant residue is buried during ploughing (Bertrand et al., 2015). However, Capowiez et al. (2009) reported no significant effect of tillage management on water infiltration (mean values of 81.8 and 96.0 mm h^{-1} in conventional and reduced tillage, respectively; $p = 0.33$)

even with the increase in macroporosity in reduce tillage; this high macroporosity was reported to be offset by a significant increase in soil bulk density which resulted in no effect on soil water infiltration rate. However, they reported a significant effect of the cropping system on water infiltration as a result of different compaction intensities depending on the crops rotation (119 mm h^{-1} in less compacted plots vs 79 mm h^{-1} in most compacted plots). Luo et al. (2010) on the other hand, found higher macroporosity and macropore length density in pasture compared to row crop land use because of greater earthworm activities and higher organic matter content in pasture land use which would probably increase soil water flow.

To understand the interactions between earthworm and soil water flow many interconnected factors should be taken into consideration. Regarding earthworms' species, most studies generally report an increased soil water infiltration and percolation in the presence of anecic species. The influence of endogeic species is highly debated and epigeics were considered in few studies with contrasting effects. Much current research is focussed on modelling using new technologies (e.g. X-ray tomography) to visualize the hidden parameters of the bulk soil that affect water flows.

2.2.4. Seasonality

Various studies have reported variations in infiltration rates through the seasons of the year (Elhakeem et al., 2018, Moujahed and Gifford, 1984, Angulo-Jaramillo et al., 1997). In some cases infiltration rates increased significantly during the summer compared to other seasons (Cerdà, 1999, Bertoni et al., 1958, Sharma et al., 2017, Cerdà, 1996) whereas in other studies infiltration rates decreased during the summer (Johnson and Beschta, 1981, Schumm and Lusby, 1963). These contrasting results are dependent on interactions between changes in soil properties, such as soil texture (swelling effect), dry bulk density and moisture (Fan et al., 2013, Hesseltine, 2016), and external factors, such as land management practices, climate and biotic factors (Starr, 1990, Willoughby et al., 1997, Azooz and Arshad, 1996). Biotic factors, such as plants and earthworms, change through the different seasons of the year. Plants affect soil water flow through the growing season by creating new biopores via developing / decaying roots, but their impact depends on the plant functional group with legumes increasing and grasses decreasing infiltration rate (Meek et al., 1992, Fischer et al., 2014). The proliferation and decay of the tap roots of legumes results in stable macropores and an increase in earthworm biomass because of an increase in organic matter content which increases water infiltration rate, whereas the fibrous roots of grass cause clogging of pore space and decrease water infiltration rate. Earthworm activity is seasonal with increasing activity in spring and autumn (Callaham and Hendrix, 1997, Gates, 1961). Their activity is a function of food availability and abiotic factors (Johnston et al., 2014) but also of root growth that depends on plant functional groups (Eisenhauer et al., 2009). From the few studies reporting seasonal effects of earthworm on soil water flow, Willoughby et al. (1997) showed that the presence of *L. terrestris* increases water infiltration rates gradually throughout the growing season in a no-tillage compared to tillage system. Hu et al. (2012) reported seasonal changes in soil water flow and attributed this to a number of factors including earthworm activity which contributed to the increase of saturated hydraulic conductivity in autumn compared to summer. Further experiments are, however, required to better understand the role of earthworms in changing soil water flow through time (under controlled conditions), or time of year (under field conditions).

2.2.5. Identified knowledge gaps

Studies looking at the relationship between earthworms and soil water infiltration have considered both comparison between species and the effect of interactions between earthworm functional groups (Table A1). In the reviewed studies, different earthworm population densities, initial soil bulk densities and methods of measuring infiltration were used. The studies have been conducted under many different climatic conditions across the globe and on soil textural classes ranging between sandy loam to clay loam. In manipulation experiments, anecic (*Lumbricus terrestris*), endogeic (*A. caliginosa* and *A. chlorotica*) and epigeic (*L. rubellus*) species are most frequently used. Epigeic species were considered in far fewer studies than anecic and endogeic species and have been found to have contrasting effects on soil water flow. Anecic species cause an increase in water infiltration rate in laboratory experiments and under conservation field management practices in field experiments. In these studies, the assessment of infiltration rate was coupled to physical descriptions and quantification of burrow morphology and characteristics of the population, such as earthworm abundance. Studies using endogeic species focus on the effect of casting and compacting activities of earthworms on soil water infiltration through modification of soil porosity, bulk density and macropore continuity. These studies were performed mostly in the laboratory, without any combined cropping system; increased, decreased or no significant effects of earthworms on water infiltration were reported. Studies investigating the combined effects of anecic and endogeic species focus more on the morphological characterization of the burrows and the effect of different cropping system and land management on water infiltration. Under field conditions, earthworm burrows usually terminate in a dead-end, but most laboratory studies have investigated the effect of earthworms on soil water flow under the situation where earthworm burrows are well connected to a drainage system at their end-point which would bias the estimated effects of earthworms during an experimental manipulation.

The relationship between earthworm activity and soil water infiltration has been extensively studied in the literature using a variety of laboratory and field experiments, however, we are still unable to say:

- How interactions of earthworms with crop types impact water flow or more specifically how different root length / densities may enhance the activity of earthworms in increasing water flow;

- How earthworms influence water flow through different soil pore classes (macropores to micropores) and particularly whether these effects are different in the presence of plant roots which earthworms interact with;

- What are the potential behavioural effects of different earthworm species commonly present across the world, such as *L. terrestris*, *A. caliginosa* and *A. chlorotica*, on soil water flow in the presence/absence of plant roots;

- What is the way in which temporal changes in soil structure (e.g. due to crop development, seasonal weather changes and dry and wet cycles) are influenced by different earthworm species or as community and how this in turn can affect the stability of macropores and the potential consequences on water flow;

It would be useful to consider these questions in situations where earthworm burrows terminate within the soil matrix to mimic the conditions found in the majority of fields. This will allow the prediction of soil water infiltration and the sustainability of qualitative and quantitative beneficial effects of earthworms on soil water flows.

2.3. Soil water storage and earthworms

While soil water infiltration implies the downward entry of water into soil (Richards, 1952), stored soil water is considered in this review as that part of the infiltrated water that is held by the soil matrix and that therefore does not flow downwards towards the water table. Soils that can retain more water support more plant growth and are less subject to leaching losses which is highly desirable for rainfed agricultural systems (Kramer and Boyer, 1995, Wang et al., 2013). Soil water storage is known as being spatially variable and affected by many factors such as soil properties, vegetation, topography, and meteorological conditions (Duan et al., 2016).

2.3.1. Soil proprieties influencing soil water storage

Soil water content capacity depends on soil properties such as texture, porosity, bulk density and soil organic carbon content (Wang et al., 2013). Additional factors such as soil structure, cation exchange capacity, carbonate content and plastic limit are also associated with soil water retention but not recognized as the most important factors (Yang et al., 2014). The relationship between predicted soil water content (θ_p m^3/m^3) and the major factors impacting it has been described by Rawls et al. (1982) using a multiple linear regression equation of the form:

$\theta_p = a + b\ (\%\ sand) + c\ (\%\ silt) + d\ (\%\ clay) + e\ (\%\ organic\ matter) + f\ (bulk\ density,\ Mg/m^3)$

Where a, b, c, d, e, and f are regression coefficients.

Soil water content varies widely for different soil texture and particularly depending on clay content (Saxton et al., 1986, Yang et al., 2014). Indeed, the water content held at matric potentials of -10, -33 and -1500 kPa was reported to have an exponential increasing trend with clay content (Minasny et al., 1999). However, the effect of clay is stronger at lower matric potentials, particularly at -1500 kPa, due to the increasing adsorptive effects of the negatively charged clay particles on water retention compared to capillary forces (Khlosi et al., 2013, McBride and Mackintosh, 1984). Sand content has more impact on water content at saturation and silt fraction also has a significant effect on the amount of water available to plants (McBride and Mackintosh, 1984, Petersen et al., 1968, Yang et al., 2014). Soil bulk density is considered as a measure of soil structure because it incorporates soil particle compaction, porosity and water content (Meskini-Vishkaee et al., 2014). The effect of bulk density on the available water is hard to predict because pore geometry, distribution and connectivity can differ for the same soil at a given bulk density (Ohu et al., 1987, Alaoui et al., 2011). However, some authors reported a decrease in water retention at low matric suctions, in the range of 0-10 kPa, and an increase of water retained at 1500 kPa as result of increased bulk density (Saxton et al., 1986, Ohu et al., 1987, Hill and Sumner, 1967, Walczak et al., 2004). Hudson (1994) reported an increase in water holding with increased organic matter due to its influence on soil aggregation and the related pore size distribution. The increased water holding capacity resulted in a significant positive correlation between organic matter content and plant available water for sand ($r^2 = 0.79$, $p < 0.0001$), silt loam ($r^2 = 0.58$, $p < 0.0001$) and silty clay loam ($r^2 = 0.76$, $p < 0.0001$) texture groups. Land management through conservative agriculture systems showed an improved soil moisture content and availability as a consequence of enhanced aggregate stability and total porosity (Souza et al., 2016).

2.3.2. Effect of earthworms on soil water retention

Earthworms as biological actors impact soil proprieties that influence soil water storage (see Table A2). Earthworms support plant growth by increasing soil water retention and nutrient release as a result of improved soil aggregation and porosity through mineral and organic matter mixing ((Darwin (1881) and White (1789) cited by Shipitalo and Le Bayon (2004)). Improvement of hydro-physical proprieties of soil such as porosity and capillary water capacity requires time, for example in the presence of earthworm with an initial quantity of 0.1% of the soil mass the increase of soil water retention may take longer than the growing season (Smagin and Prusak, 2008). Comparison between sites on various soils with and without the endogeic earthworm *A. caliginosa*, showed a 17 % increase in soil moisture holding capacity, 27 % more available water and a near doubling of infiltration rate 10 years after the introduction of the earthworms (Stockdill and Cossens, 1969). The authors reported more water moisture held in high organic matter topsoil as result of an improved soil structure in the presence of earthworms. Earthworm absence was associated with high soil compaction which severely restricted moisture penetration. Furthermore, McDaniel et al. (2015), showed that *A. caliginosa* can change the soil water retention curves with soil depth with an improved soil residual water content from 33 to 41 % in the top 30 cm of the soil. Without giving any quantified measurements, they suggested that the greater concentration of fine soil particles in casts coated with hydrophilic surface layer may explain the increased residual water content in the top 30 cm of soil, where earthworms are most active. In the presence of the epigeic earthworm species *L. rubellus*, Ernst et al. (2009) reported an increase in soil water storage at 10 cm depth as a result of low rates of litter loss relative to *L. terrestris* treatment which reduced water evaporation at the soil surface. *L. terrestris* species buried surface litter leading to an increase in soil surface drying and aeration of their large burrows, resulting in lower water storage.

2.3.3. Effect of earthworm burrows and soil structure

Due to their burrowing activity, earthworms can improve water holding in the top soil layers by modifying soil porosity to a broader range of pore sizes that can retain more water (Boyle et al., 1997, Palm et al., 2013, McDaniel et al., 2015). Also, cracks, smaller than surface holes, and burrows generated by earthworm activity could increase infiltration and help in water retention as the water in water-filled macropores diffuses into the soil matrix through macropore walls (Edwards, 2004, Lee and Foster, 1991, Bastardie et al., 2003). Even though the volume of earthworm burrows accounts for only a few percent of total soil porosity (Schwartz et al., 1999, Kördel et al., 2008), Bastardie et al. (2005a) showed that the total surface of the burrow walls varied between 7721 to 12764 cm^2 m^{-3} with burrows that connected to the surface providing 1069 to 7237 cm^2 m^{-3}. The burrow walls provide an important surface for water diffusion into the soil matrix. In addition, the same accessible burrows offer a volume ranging from 1400 to 10463 cm^3 m^{-3} corresponding to 1–10 mm of a water storage capacity. This amount of water held by the soil matrix through the penetration of water into the earthworm burrow walls, in addition to water stored in water-filled burrows would help to increase soil infiltration and enhance water available for root absorption (Bastardie et al., 2005b). Weiler and Naef (2003) reported that the macropores built by earthworms significantly affect water infiltration rate, but the flow into the surrounding soil matrix and its storage is mainly influenced by the soil properties and initial soil water content. Smettem (1992) and Bastardie et al. (2003) indicated that water diffusion from the burrow to the soil matrix is highly dependent on the burrow wall permeability. Indeed, Bastardie et al. (2005b) showed that the speed and volume of water that infiltrated through burrow walls of *L. terrestris* earthworms is less than that infiltrated through soil fractures due to low soil porosity as a result of the high compaction of burrow walls. In their experiment the density values of artificial burrows (made by a metal rod) were 1.33 and 1.36 g cm^{-3} for the 0-3 mm wall layer and the surrounding soil respectively, whereas the values for burrows made by *L. terrestris* were 1.395 and 1.38 g cm^{-3} for the 0-3 mm wall layer and surrounding soil matrix respectively. Similarly, Rogasik et al. (2014) reported a 30 % increase (from 1.34 g cm^{-3} to at least 1.75 g cm^{-3}) in the bulk density of the burrow walls of *L. terrestris* compared to the soil matrix; from the burrow walls to their outer boundary the bulk density decreased. Since soil bulk density is a proxy for porosity and pore connectivity, high bulk density will therefore greatly reduce the transfer of water and solutes between burrows and the soil matrix. Anecic earthworms such as *L. terrestris* use their burrows for long periods of time and compress egested material onto the burrow walls, whereas epigeic and endogeic earthworms do not reuse their burrows and their casting influence on burrow wall permeability is assumed to be insignificant (Melnichuk, 2016). However, the presence of the compacting endogeic earthworm *Pontoscolex corefhrurus* in three organic residue treatments resulted in a decrease in soil porosity (from 58 to 53 %) and sorptivity (from 0.45 to 0.15 cm $s^{-1/2}$) due to an increased soil bulk density (Alegre et al., 1996). Similarly, Blouin et al. (2007) reported a decrease of soil water retention capacity by more than 6 % in the presence of the endogeic earthworm *Reginaldia omodeoi* as a result of their compacting behaviour which led to a decrease in plant growth.

2.3.4. Effect of earthworm casts

Soil water storage may be enhanced by the good water holding capacity of earthworm casts and the interstitial pores between casts (Bouche and AlAddan, 1997, Blanchart et al., 1999). The hydroscopic swelling of plant remains in the casts and the improved capillarity of their highly porous aggregate structure was reported to cause up to a 20 % increase in soil water holding capacity compared to the surrounding soil (Smagin and Prusak, 2008). The fine fraction of the soil is known to play an important role in soil water and nutrient retention (Yang et al., 2014, Saxton et al., 1986) and was found to be significantly higher in casts of endogeic earthworms compared to the surrounding soil (Asawalam and Johnson, 2007, Duboisset, 1995). During ingestion, the clay and silt fractions in earthworm guts are coated by an extra hydrophilic surface layer (McDaniel et al., 2015, Smagin and Prusak, 2008). The high organic matter content mixed with the high proportion of fine particles in most earthworm casts would increase their ability to retain more water than the bulk soil (Lavelle, 1988). However, Lipiec et al. (2015) reported greater water repellency of old earthworm casts compared to the bulk soil. This was assigned to the high hydrophobic layer of organic carbon coating the surface area of the casts during ingestion (1.99 vs 1.30 % organic carbon in casts vs in natural soil aggregates respectively). These contrasting effects could be dependent on organic matter quality and aging effect of casts on water retention.

2.3.5. Identified knowledge gaps

Compared to soil water infiltration, the impact of earthworms on soil water retention and storage is less well studied. Studies exist that consider all three ecotypes of earthworm, but the majority of studies focus on endogeic species, particularly on *A. caliginosa* (Table A2). Studies either measure the water content of soils, the ability of earthworm casts to retain water under different soil water pressures or water repellency of earthworm casts. *L. terrestris* and *L. rubellus* are the main anecic and epigeic species studied. Studies that considered anecic earthworms focus on compaction due to burrowing activity and rates of water infiltration through burrow walls. Studies using epigeic earthworms stress their ability to maintain soil cover with low rates of litter loss which results in low water evaporation and the capacity of their casts to retain water. Studies in which earthworm numbers were manipulated reported a positive effect of the presence of earthworms on soil water storage and related this to burrow characteristics, cast age and levels of organic matter present. Most studies that consider the impact of earthworms on water storage are laboratory-based and used individual soils ranging in texture from sandy and silty loam to clays, though the majority of studies used sandy and silty loams. Studies using suites of soils did not report textural information. Experiments conducted under a particular cropping system, or where soil water retention curves were drawn, are also infrequent.

The following are the identified knowledge gaps arising from the reviewed literature that should be pursued:

- There is currently little consideration in the literature as to how soil texture impacts on the effect that earthworms have on soil water retention when earthworm ecotype, earthworm density, soil density and soil organic matter content vary.

- Studies involving common earthworm species in Europe and globally are required, particularly *Lumbricus terrestris* (anecic) *Aporrectodea caliginosa* / *A. chlorotica* (endogeic) and *Lumbricus rubellus* (epigeic) to investigate their impact on soil water retention in order to better understand the role earthworms play in soil water regulation. The impact of ecotype/species could be evaluated both by comparison between and within ecotypes;

- Since different earthworms occupy different positions in the soil, water release curves for soils at different soil depth should be produced. Also, earthworm population density could be studied by comparing the influence of abundant, reduced and ambient densities in different soil types;

- Most experiments reported in the literature were performed in vegetation-free soil but most soils are vegetated. Carrying out experiments in the presence of different crop types would highlight the relative effects of crops and earthworms on water retention and any interactions that exist;

- Organic matter (OM) pools in soils are modified by earthworms and this in turn may impact water retention. The composition of organic matter may affect the hydrophilic nature of casts and the location of OM in the soil may be relevant. Experiments where different organic matter compositions are used should emphasis the hydrophilic nature of casts (e.g. by measuring casts water repellency index or water drop penetration time (Cosentino et al., 2010)) of different ecotypes of earthworm and its effect on soil water retention. Characterization of geomorphology and topography of the hydrophilic/hydrophobic surface layer coating soil particles of casts, using electronic microscopy for example, will help understand the processes;

- Most studies were undertaken in the laboratory; field experiments where treatments are exposed to natural condition would be more effective in transferring knowledge into practice.;

- Earthworms are reported to improve crop growth through different processes (e.g. nitrogen mineralization, root aeration), it is likely that this is due in part to changes in water storage as a result of earthworm presence. This can be tested through experiments where soil water storage is measured in the presence of earthworm.

Once the effects are established, crop growth could then be examined at different levels of soil water storage. Further experiments examining the processes by which earthworms/plants impact on soil water storage are to be considered.

2.4. Identified experiments for the thesis

On the basis of the knowledge gaps identified above, the following experiments were designed:

- An initial laboratory experiment (Chapter 3) was carried out to determine the effects of two different earthworm ecotypes - the anecic *Lumbricus terrestris* and endogeic *Allolobophora chlorotica* - on the percentage water-stable aggregates and water-holding capacity of three different soil textures. The upper and lower soil layers were investigated;

- On the basis of the initial experiment results, laboratory experiments with the vertical burrowing earthworm *L. terrestris* (Chapter 4) and lateral burrowing earthworm *A. chlorotica* (Chapter 5) were carried out in order to determine their effects on soil physico-hydraulic properties and whether these effects are different in the presence of plant roots and in soils of three different textures. An experimental design was used to investigate the effects under conditions where earthworm burrows are not well drained;

- A field experiment (Chapter 6) was carried out to test the effects of earthworms on soil physico-hydraulic properties in ambient environmental conditions. Physico-hydraulic properties were measured in different fields of three differently textured soils. Changes in soil water flow were measured over different seasons of the year.

The detailed aims and measured parameters of each experiment are given within the relevant chapters.

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Chapter 3

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

3.1. Declaration

This chapter is presented as a submitted paper for publication without changes to the original submitted main text. The paper was written in the style of European Journal of Soil Science to which it was submitted. For consistency and ease of reading, headings have been numbered, figures and tables inserted and renumbered and citations changed to follow the standard for this thesis.

Running title: *Anecic and endogeic earthworm effect on %WSA & WHC*

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

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Highlights

- Effects of endogeic and anecic earthworm ecotypes on % WSA and WHC in 3 soils are studied
- Understanding the impacts of different earthworm ecotypes may help decisions about soil management
- *A. chlorotica* increased %WSA & WHC; *L. terrestris* increased %WSA but more so in the upper soil
- Earthworms from different ecological niches had different effects which were influenced by soil %OM

3.2. Summary

It is widely accepted that earthworm activity underpins a number of soil properties that give rise to valuable ecosystem services. However, earthworms occupy a range of ecological niches and different ecotypes may have different impacts on soil properties. It is important to understand these differences as different soil management techniques may favour different earthworm ecotypes. We carried out mesocosm experiments using either the anecic earthworm *Lumbricus terrestris* or the endogeic earthworm *Allolobophora chlorotica* and a loam, silt loam and sandy loam to investigate the differing impact of these ecotypes on 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 (16-56 % and 19-63 % 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. *A. chlorotica* treatments significantly increased WHC by 7-16 %. Despite causing an increase in %WSA in all three soils, *L. terrestris* only caused a significant increase in WHC in the upper 6.5 cm of the sandy loam soil. Nevertheless, a significant relationship between increases in %WSA and WHC was found for both earthworm species. Overall, the 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.

3.3. Introduction

In order to develop sustainable agricultural management systems that deliver a range of ecosystem services a full understanding of the impacts of soil fauna on soil properties is required. 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). They change the structure of soil, water flow and retention, soil aeration, and resistance to erosion (Lavelle et al., 1992). 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, Li et al., 2013, Zhang et al., 2016). Soil macrofauna change soil structure by modifying soil aggregation and porosity (Shipitalo and Le Bayon, 2004). The space between aggregates and its distribution allows air and water retention and exchange (Guber et al., 2004, Saha and Kukal, 2015). Soil aggregates contain the majority of organic carbon of the soil and contribute to water retention and nutrient release for plant growth (Ramachandran Nair et al., 2010, Cornforth, 1968). 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, Rousseva et al., 2017). It is a function of a variety of soil properties including texture, organic matter content and soil aggregates (Saxton et al., 1986, Hudson, 1994), is easily measurable and is a key factor in soil ecology as it influence the distribution and dynamics of animal and plant populations (BIO Intelligence Service, 2014).

Earthworms are major ecosystem engineers in the soil and influence soil structure by creating macropores through their burrowing activities and play an important role in aggregate formation and stabilisation through the ingestion and egestion of soil (Lee and Foster, 1991, Six et al., 2002, Snyder et al., 2009). Earthworms can be grouped into three ecological niches (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*) inhabit the upper levels of the soil, construct narrow branching sinuous burrows and rarely come out to the soil surface and; anecic earthworms (e.g. *Lumbricus terrestris*) which are deep burrowers that typically inhabit semi-permanent deep burrows and feed on organic litter which they collect from the soil surface.

Epigeic earthworms have little direct effect on soil aggregation because they rarely burrow within the soil (Shipitalo and Le Bayon, 2004). However, the precise role that anecic and endogeic earthworms play in modifying key soil properties is still unclear. Some studies indicate that earthworms significantly increase soil water stable aggregates (%WSA) relative to aggregates of the same soil without earthworms. For example Ketterings et al. (1997) and Hamilton et al. (1988) measured an increased %WSA in soil dominated by *L. terrestris*. Bossuyt et al. (2005) and Blanchart (1992) reported an increase in %WSA in repacked soil in the presence of the endogeic earthworms *Allolobophora caliginosa* and *Millsonia anomala* respectively. In contrast, Blanchart et al. (1997) showed that the addition of the endogeic species *M. anomala*, *Chuniodrilus zielae* and *Stuhlmannia porifera* to intact soil decreased %WSA compared to treatments without earthworms addition at 0-5 cm soil depth, but increased %WSA at a depth of 10-15 cm, although the legacy effects of old earthworm casts in the intact soil caused them to question their results. No differences between species were detected. Zhang and Schrader (1993) reported that the anecic *L. terrestris* and endogeic *Allolobophora caliginosa* species either had no effect on the %WSA compared to natural arable soil aggregates (Zhang and Schrader, 1993) or decreased the %WSA (Schrader and Zhang, 1997) depending on soil type. In contrast *Aporrectodea longa* decreased the %WSA (Zhang and Schrader, 1993). However, all three species always increased the %WSA compared to mechanically produced artificial aggregates.

Whilst in general, studies support an increase in %WSA in the presence of earthworms (Swaby, 1950, Edwards, 2004, Edwards and Bohlen, 1996) there are relatively few studies on the impacts of earthworms on soil WHC. The majority of studies consider the impact of soil water content and soil WHC on earthworm distributions rather than the influence of earthworms on these properties (Schneider and Schroder, 2012, Palm et al., 2013). The few studies that exist are superficially contradictory, but this is most likely because they measure different things. Smagin and Prusak (2008) and Ernst et al. (2009) reported an increase in WHC in the casts of epigeic earthworms from laboratory experiments and

Stockdill and Cossens (1969) reported an increase in WHC when *A. caliginosa* was introduced to a pasture field, due to an improved soil structure. However, 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 created biopores increasing soil aeration. In field experiments with kaolinitic soils and tropical earthworms, Blanchart et al. (1999) found that compacting endogeic species (*Pontoscolex corethrurus* or *Millsonia 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 *Millsonia anomala*, demonstrating the complex interaction between earthworm type, soil properties and the influence that earthworms have on those properties.

Here we report an experiment to investigate the impact of two earthworm species that are common in the UK (Natural England Commissioned Report NECR145, 2014) on %WSA and soil WHC. Our experiments use the anecic earthworm *Lumbricus terrestris* and the endogeic earthworm *Allolobophora chlorotica*. These earthworms were added to loam, silt loam and sandy loam soils. 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. We hypothesised that i) *L. terrestris* and *A. chlorotica* will have different effects on the measured soil properties due to their differing ecologies, ii) they will increase water-holding capacity in all soils because of improved soil structure and 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.

3.4. Materials and methods

3.4.1. Soils and earthworm selection and mesocosms 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 (WBR, 2006) and have different textures. Each soil was air dried, sieved at 2 mm, and thoroughly homogenised to remove any legacy effects of previous earthworm activity. Basic soil properties and land management information are presented in Table 3 - 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 cm^3 volume and soil texture using a MasterSizer2000 laser particle size analyser (Malvern Instruments, UK). A standard sand (SiO_2, 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.

Table 3 - 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	Organic matter /%	Field dry bulk density /g cm^{-3}	Clay /%	Silt /%	Sand /%	Texture
					< 2 µm	2-50 µm	50-2000 µm	
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)

For each soil, 300 g of air dried soil were wetted with deionized water up to a gravimetric water content of 30 % to sustain earthworm activity (Lowe and Butt, 2005, Butt and Lowe, 2011, Berry and Jordan, 2001). 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 columns of soil of approximately 7 cm diameter and 13 cm height. The soil was then stored at 15 °C until the addition of earthworms.

Clitellate, adult earthworms of the vertical burrowing, anecic *Lumbricus terrestris* and 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 either 2 *L. terrestris* (9.04 ± 0.52 g) or 8 *A. chlorotica* (2.16 ± 0.11 g) per mesocosm (*n* = 12 per species). These earthworm loadings are equivalent to densities of 540 ind m^{-2} for *L. terrestris* and 2100 ind m^{-2} soil for *A. chlorotic*a, far greater than those typically found in pasture fields (100 ind m^{-2} for *L. terrestris*; Ernst et al. (2009), Palm et al. (2013), Rogasik et al. (2014), 200 - to 800 ind m^{-2} for individual adults of endogeic species such as *A. chlorotic*a; Capowiez et al. (2015), Ernst et al. (2009), McDaniel et al. (2015)) and were used to clearly establish possible earthworm effects over the timescale of the experiment. 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) for 40 days. They were weighed initially and then every week with any mass loss being corrected by addition of deionised 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.

3.4.2. Soil physical properties measurement

The percentage water stable aggregates (%WSA) and water holding capacity (WHC) of the soils were measured at the start of the experiment. At the end of the experiment the soils were divided into “upper soil” (0 - 6.5 cm) and “lower soil” (6.5 - 13 cm) and %WSA and WHC measured for soil from each depth.

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 and 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 three minutes. 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 (Milleret et al., 2009a, Kodešová et al., 2009). WHC was determined following the method of ISO 11268-2:1998. In brief a soil sample was saturated, allowed to drain until it stopped dripping and then the moisture content was determined by drying the soil at 105 °C. The WHC was determined as the mass of water held in the soil against gravity.

3.4.3. Statistical analysis

For each treatment %WSA and WHC were analysed using two-way analysis of variance (ANOVA) with soil texture and earthworm presence/absence as the factors. Tukey's honestly significant difference (HSD) procedure was used for pairwise comparisons. All computations were made using SPSS (IBM Corp. Released 2016, version 24). *P* values of < 0.05 were used as the threshold for significance. 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).

3.5. Results

3.5.1. Earthworms biomass

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 ($F_{1,18} = 48.8$, $p \leq 0.001$) whereas the mass of the *A. chlorotica* only decreased in the L and SiL soils ($F_{1,18} = 53.5$, $p \leq 0.01$) (Table 3 - 2).

Table 3 - 2. Mean total *L. terrestris* and *A. chlorotica* mass (g) at the start and end of the experiment (*n* = 4 replicates, ± standard deviations)

Earthworm species	***Soil texture***	***Initial total earthworm mass /g***	***Final total earthworm mass /g***
L. terrestris	Loam*	8.88 ± 0.41	6.60 ± 0.39
	Silt loam*	9.18 ± 0.71	7.30 ± 0.81
	Sandy loam*	9.08 ± 0.61	7.76 ± 0.78
A. chlorotica	Loam*	2.15 ± 0.05	1.56 ± 0.10
	Silt loam*	2.20 ± 0.10	1.74 ± 0.09
	Sandy loam	2.16 ± 0.15	2.11 ± 0.13

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

3.5.2. Water stable aggregates (%WSA)

Figure 3 - 1 (*L. terrestris*) and 3 - 2 (*A. chlorotica*) show the %WSA at the beginning and end of the experiments. At the start of the experiment the SaL showed a significantly greater %WSA than the other two soils ($F_{2,9} = 28.1$, $p < 0.001$). The addition of both earthworm species increased significantly the %WSA in the upper and lower soils compared to the control and the initial soil ($p < 0.01$). The %WSA of the initial soil was between that of the earthworm present and the control treatments for the upper and lower

soils and for both earthworm species ($p < 0.05$). At the end of the experiment and for both sampling depths, SaL soil had the highest value of the %WSA and L and SiL soils were not significantly different from each other ($p < 0.001$).

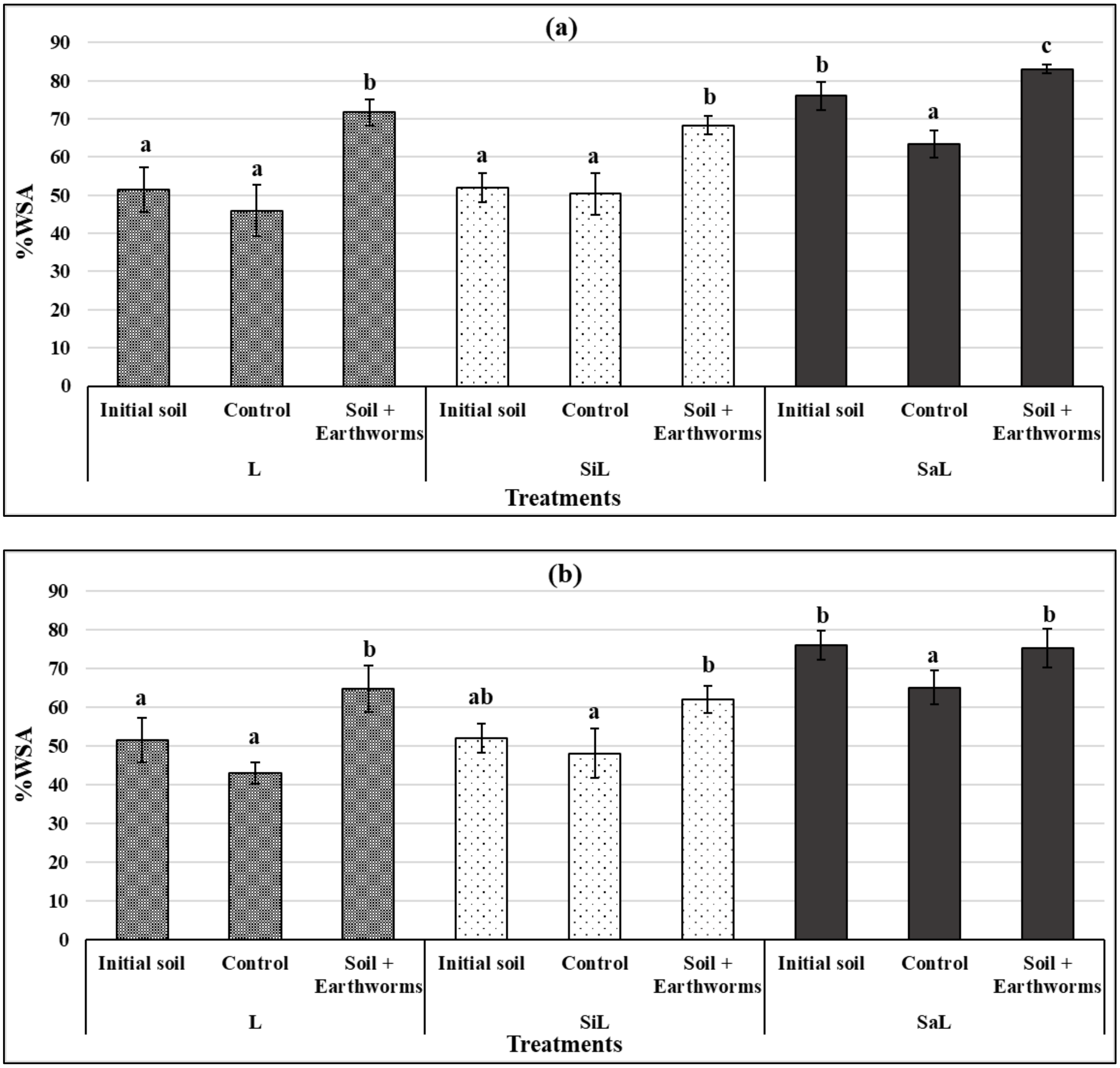


Figure 3 - 1. Mean percentage of water stable aggregates of upper soils (a) and lower soils (b) in the presence (Soil + Earthworms) and absence (Control) of *L. terrestris* within each soil texture. Initial soil is the soil at the start of the experiment. Letters refer to soil textures; L: Loam, SiL: Silt loam and SaL: Sandy loam. Columns with the same letter over them are not significantly different within each soil texture. (*n* = 4, bars = standard deviations).

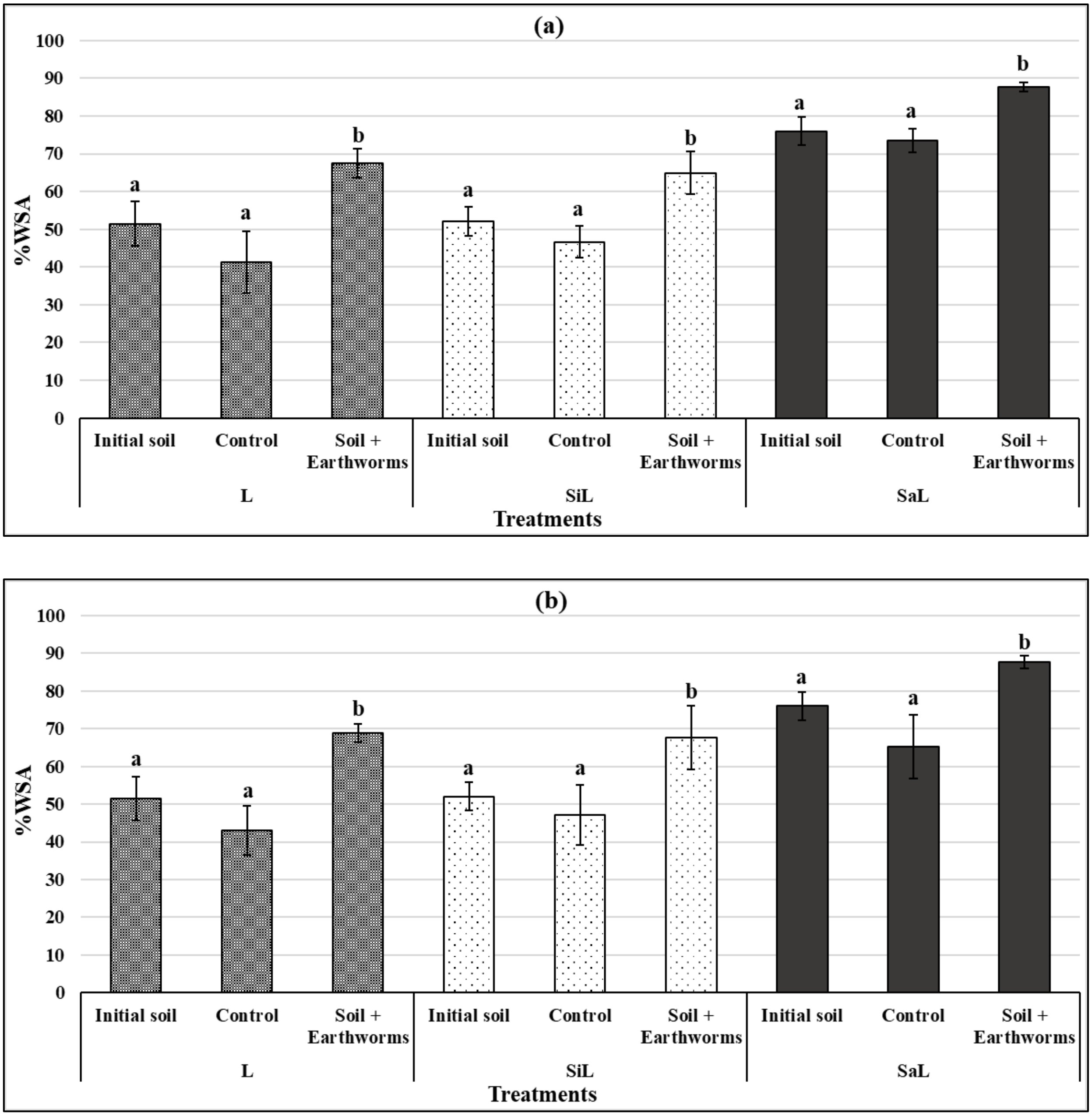


Figure 3 - 2. Mean percentage of water stable aggregates of upper soils (a) and lower soils (b) in the presence (Soil + Earthworms) and absence (Control) of *A. chlorotica* within each soil texture. Initial soil is the soil at the start of the experiment. Letters refer to soil textures; L: Loam, SiL: Silt loam and SaL: Sandy loam. Columns with the same letter over them are not significantly different within each soil texture. (*n* = 4, bars = standard deviations).

3.5.3. Soil water holding capacity (WHC)

WHC of the soils at the start and end of the experiments are shown in Figure 3 - 3 (*L. terrestris*) and Figure 3 - 4 (*A. chlorotica*). Initially the SaL soil had the highest, and the L soils the lowest, WHC respectively ($F_{2,9} = 96.3$, $p < 0.001$). There was a significant difference in WHC in the upper soils between the *L. terrestris* present and absent treatments ($F_{1,18} = 8.2$, $p < 0.01$) but the pairwise comparison indicated that the difference was only significant for the SaL soil ($p < 0.001$) (Figure 3 - 3a). In the *L. terrestris* experiment lower soils (Figure 3 - 3b), no significant effect was associated with the presence / absence of earthworms. There were no significant changes in WHC over the course of the experiment except for the earthworm-present SaL upper soil. In contrast to *L. terrestris, A. chlorotica* caused an increase in WHC in both the upper and lower soils for each soil texture relative to the initial soil and the earthworm-absent treatment ($p < 0.001$) (Figure 3 - 4).

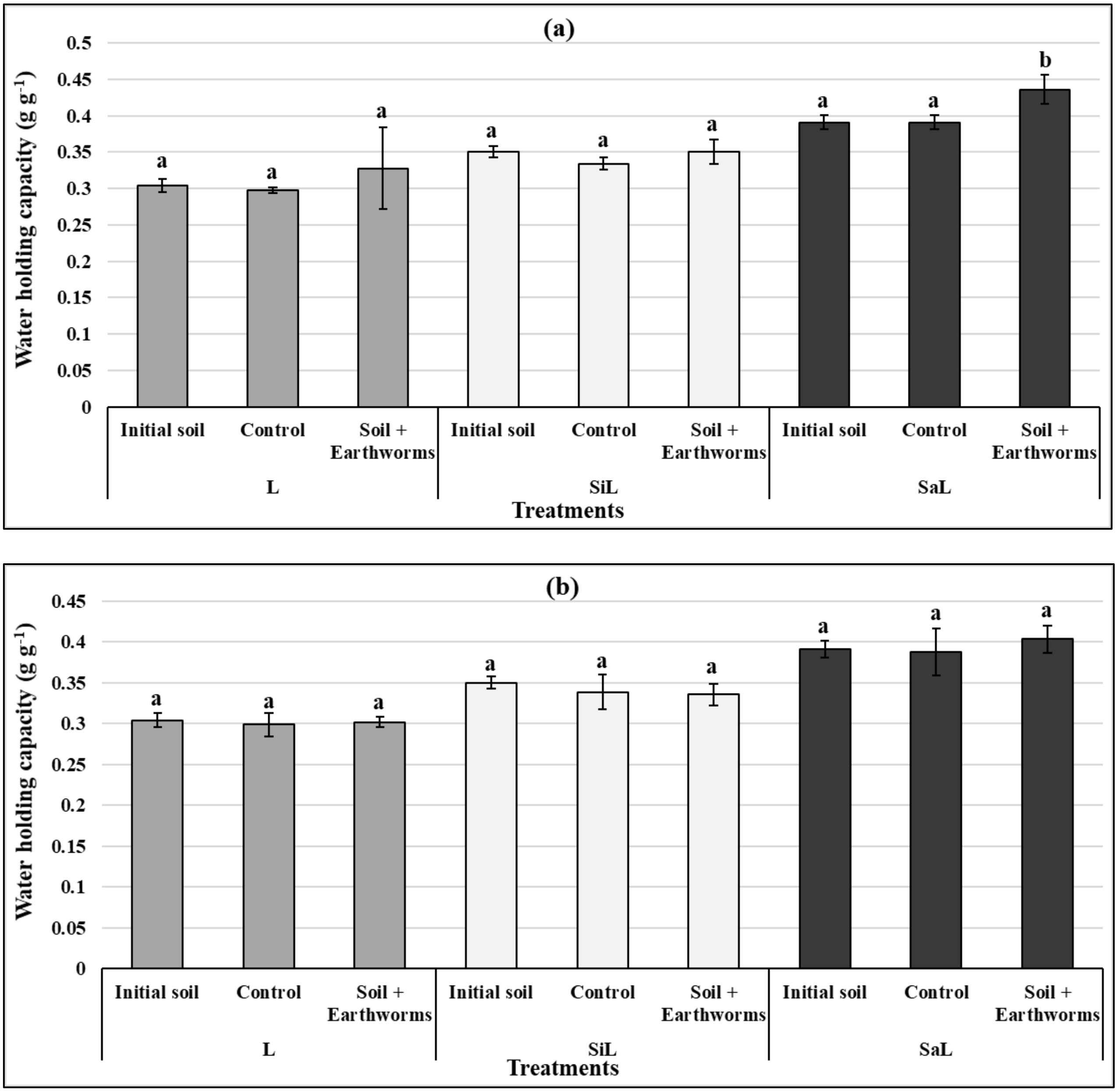


Figure 3 - 3. Mean water holding capacity of upper soils (a) and lower soils (b) in the presence (Soil + Earthworms) and absence (Control) of *L. terrestris* within each soil texture. Initial soil is the soil at the start of the experiment. Letters refer to soil textures; L: Loam, SiL: Silt loam and SaL: Sandy loam. Columns with the same letter over them are not significantly different within each soil texture. (*n* = 4, bars = standard deviations).

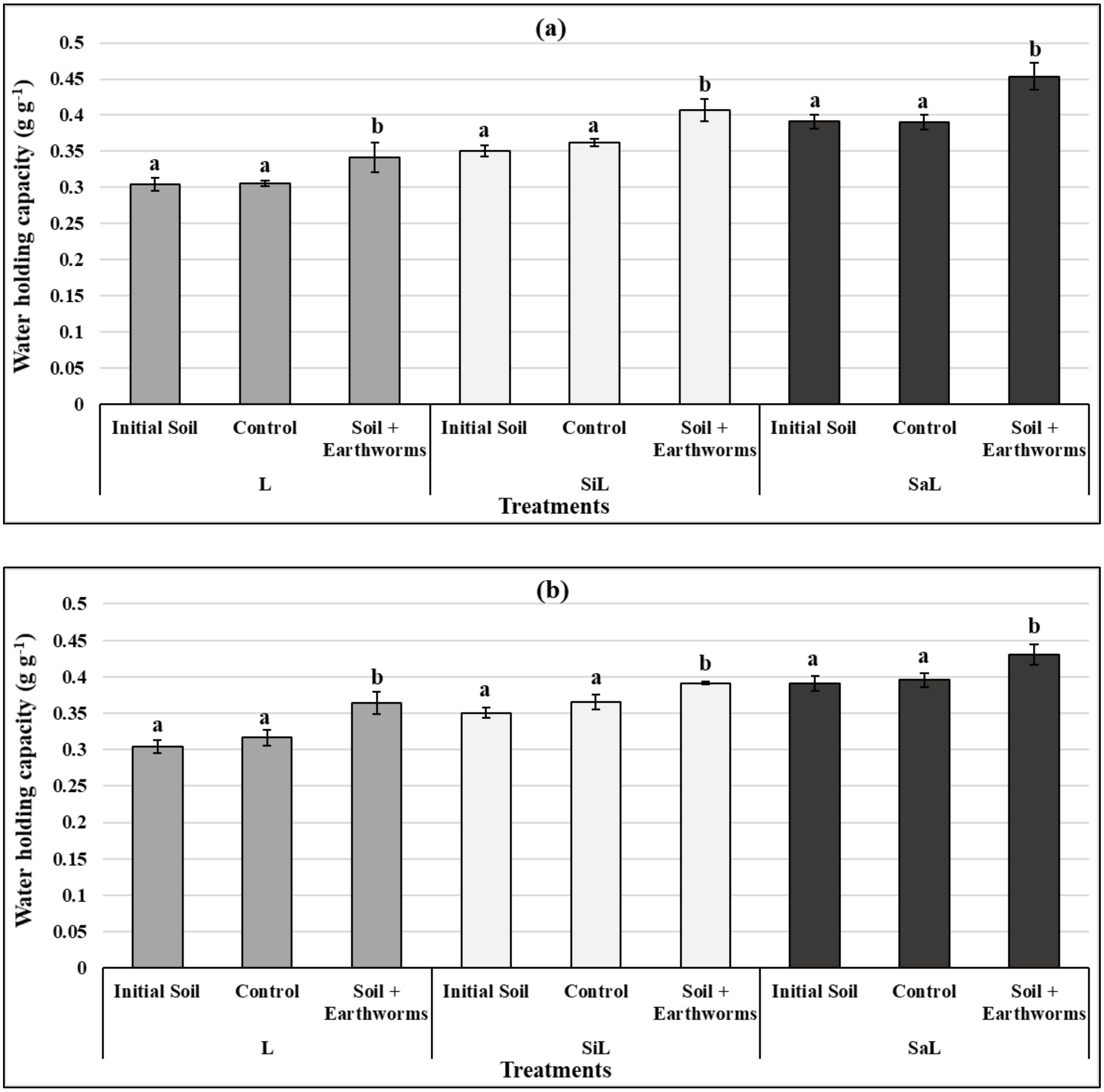
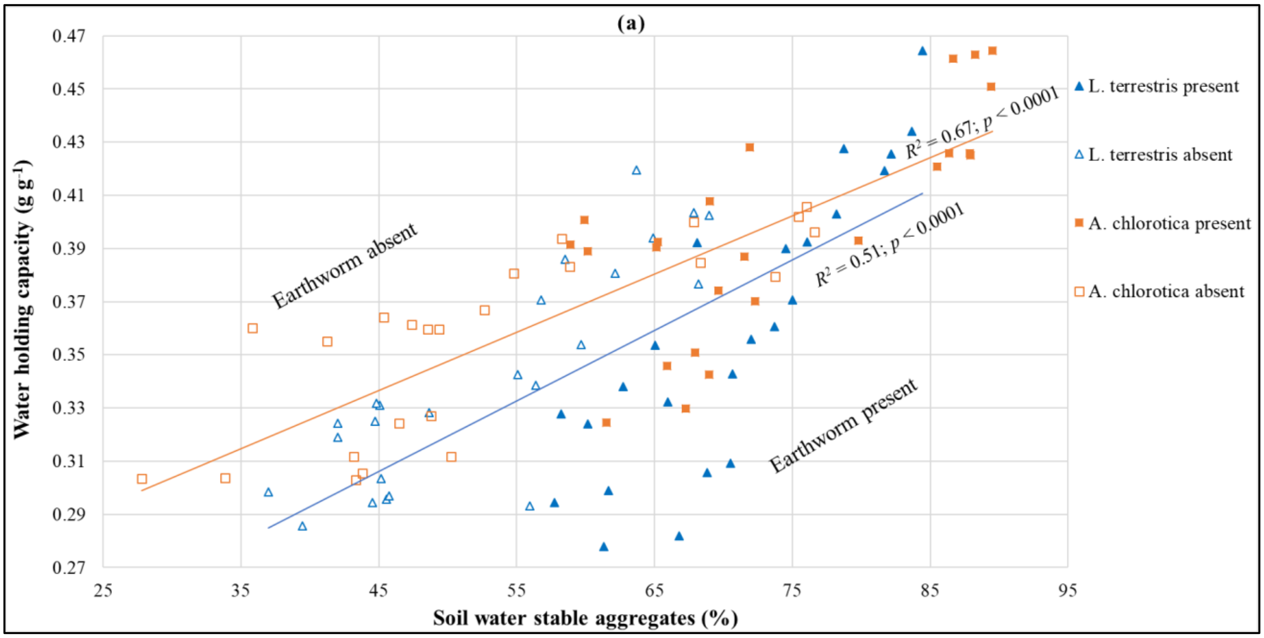
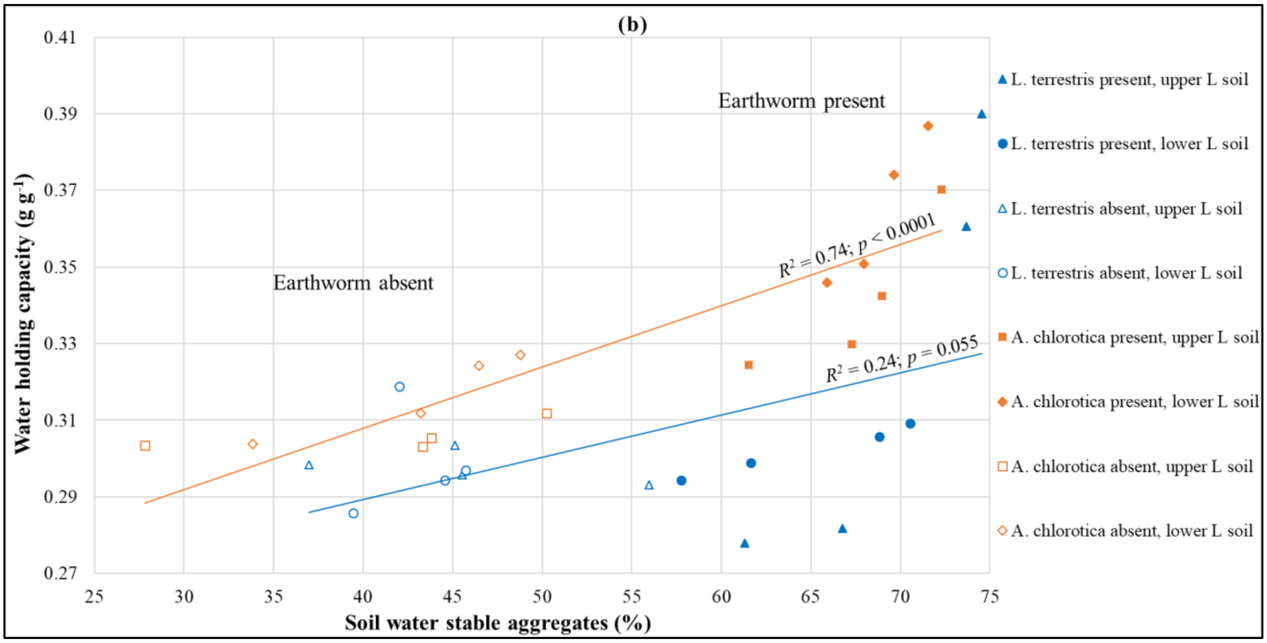


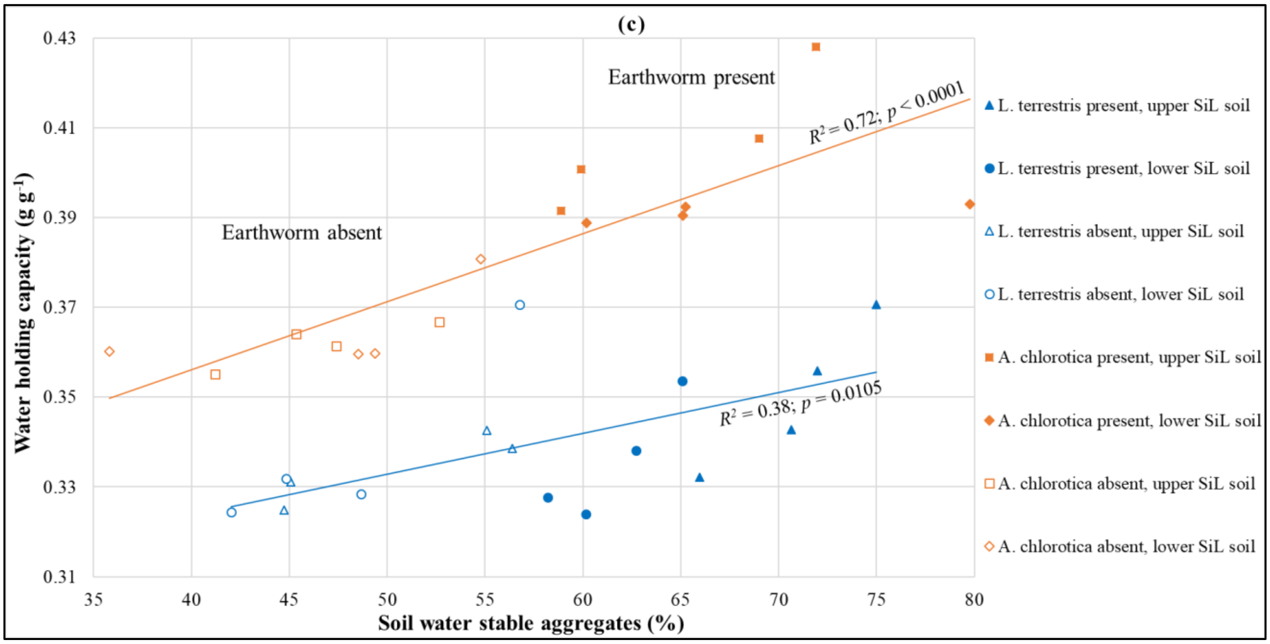
Figure 3 - 4. Mean water holding capacity of upper soils (a) and lower soils (b) in the presence (Soil + Earthworms) and absence (Control) of *A. chlorotica* within each soil texture. Initial soil is the soil at the start of the experiment. Letters refer to soil textures; L: Loam, SiL: Silt loam and SaL: Sandy loam. Columns with the same letter over them are not significantly different within each soil texture. (n = 4, bars = standard deviations).

3.5.4. *Links between earthworms, %WSA and WHC*

For each experiment and for each soil, except for the *L. terrestris* and L soil there is a statistically significant strong (i.e. $p < 0.001$, $r > 0.5$, (Cohen, 1988)) positive relationship between the %WSA and soil WHC (Figure 3 - 5). 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 for each soil texture. However, the intercepts of the linear regressions are significantly higher ($p < 0.001$) for *A. chlorotica* and for the L and SiL soils and also when the data for all three soil types is plotted together.




(b)
Water holding capacity (g g⁻¹)
0.41
0.39
0.37
0.35
0.33
0.31
0.29
0.27
25
30
35
40
45
50
55
60
65
70
75
Soil water stable aggregates (%)
Earthworm present
Earthworm absent
R² = 0.74; p < 0.0001
R² = 0.24; p = 0.055
▲ L. terrestris present, upper L soil
● L. terrestris present, lower L soil
△ L. terrestris absent, upper L soil
○ L. terrestris absent, lower L soil
■ A. chlorotica present, upper L soil
◆ A. chlorotica present, lower L soil
□ A. chlorotica absent, upper L soil
◇ A. chlorotica absent, lower L soil


(c)
Water holding capacity (g g⁻¹)
0.43
0.41
0.39
0.37
0.35
0.33
0.31
35
40
45
50
55
60
65
70
75
80
Soil water stable aggregates (%)
Earthworm present
Earthworm absent
R² = 0.72; p < 0.0001
R² = 0.38; p = 0.0105
▲ L. terrestris present, upper SiL soil
● L. terrestris present, lower SiL soil
△ L. terrestris absent, upper SiL soil
○ L. terrestris absent, lower SiL soil
■ A. chlorotica present, upper SiL soil
◆ A. chlorotica present, lower SiL soil
□ A. chlorotica absent, upper SiL soil
◇ A. chlorotica absent, lower SiL soil

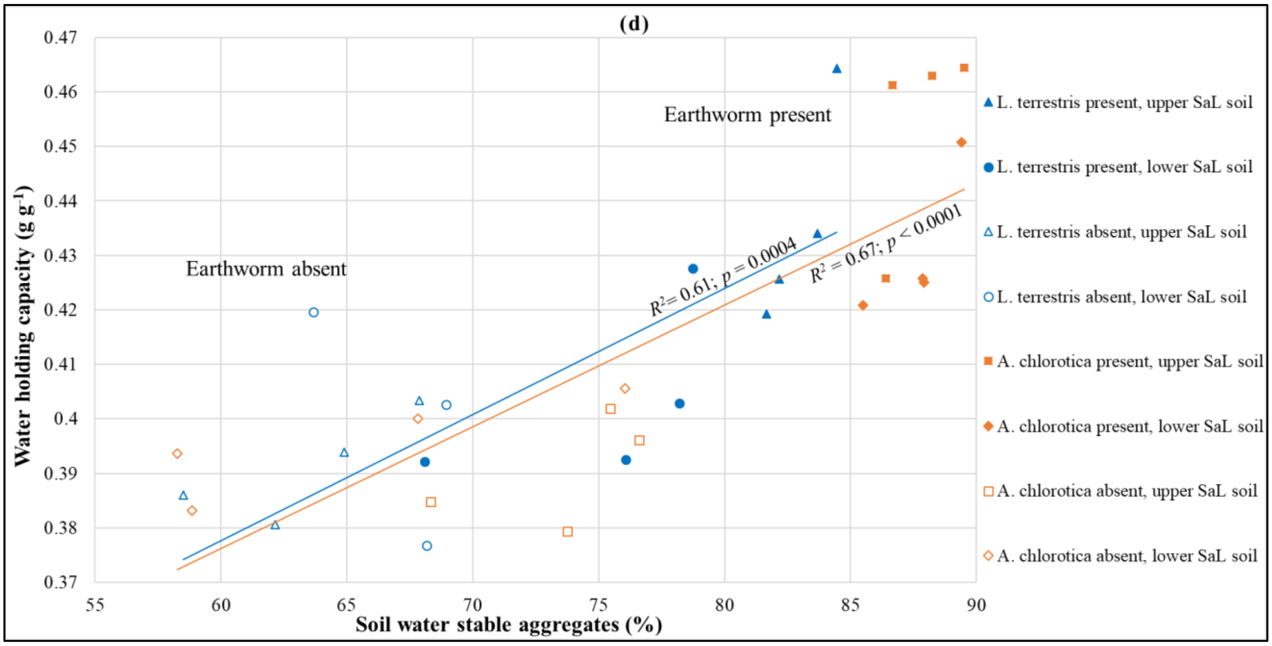


Figure 3 - 5. Relationships between soil water stable aggregates and soil water holding capacity for (a) all soil textures (b) Loam soil, (c) Silt loam soil and (d) Sandy loam soil and for *L. terrestris* (blue symbols and correlation trend) and *A. chlorotica* (orange symbols and correlation trend). Filled and outline symbols indicate the presence and absence of earthworms respectively.

3.6. Discussion

3.6.1. Earthworms 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 still lost weight (Table 3 - 2). The decrease in mass of the anecic *L. terrestris* is 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 SaL soil which has the greatest organic matter content, and therefore presumably more available food (Table 3 - 1).

3.6.2. Impact of earthworms on %WSA

The addition of both earthworm species significantly increased the %WSA of the upper and lower soil compared to the control. Our results agree with reported increase of %WSA in the presence of earthworms (Swaby, 1950, Edwards and Bohlen, 1996, Ketterings et al., 1997), although in these studies it is not always clear what depth of the soil was being inspected. *L. terrestris* increased %WSA by 10 ± 0.32 % in upper soils relative to lower soils, whereas for *A. chlorotica* there was no difference in the increase in the upper and lower soils (between -3.92 and 0.04 %). This is consistent with the anecic earthworms' surface casting behaviour that will result in an accumulation of casting-produced aggregates in the surface layer compared to endogeics mixing soil and making extensive burrows filled with casts (Pérès et al., 1998, Whalen et al., 2015).

For both earthworm species, the SaL soil showed the greatest values of %WSA and the lowest increase in the presence of earthworms (Figure 3 - 1, 3 – 2). Increased organic matter content of soils also leads to better soil aggregation (Haynes, 2005, Alagöz and Yilmaz, 2009). Although clay particles also play an important role in soil aggregation through intimately binding to soil organic matter, at clay contents of < 10 % such as in the soils used in these experiments the effects are far less significant than those related to organic matter content (Allison, 1973, McBride and Mackintosh, 1984). SaL is a pasture soil rich in organic matter whilst SiL and L are arable, have relatively low organic matter contents and, as a result, had lower initial %WSA (du Preez et al., 2011, Haynes et al., 2003). Our results suggest that the contribution of earthworms to aggregation is more important in lower organic matter soils. Perhaps in these soils the contribution of earthworm mucus to organic binding agents is more important for aggregation (Knowles et al., 2016, Schomburg et al., 2018) or the stimulation of fungal activity via the increased supply of soluble organic compounds (Parle, 1963, Rashid et al., 2016, Montecchio et al., 2015), is more significant due to the background of lower organic matter which might otherwise supply these effects (Figure 3 - 6).

Similarly, significant differences between *L. terrestris* and *A. chlorotica* effects on %WSA are only evident in the SaL soil which has the highest organic matter content. It seems likely that the high organic matter content of this soil is responsible for the majority of the %WSA and that the limited processing of soil by the anecic *L. terrestris* is insufficient to further increase %WSA whereas the more intense soil processing by the endogeic *A. chlorotica* is.

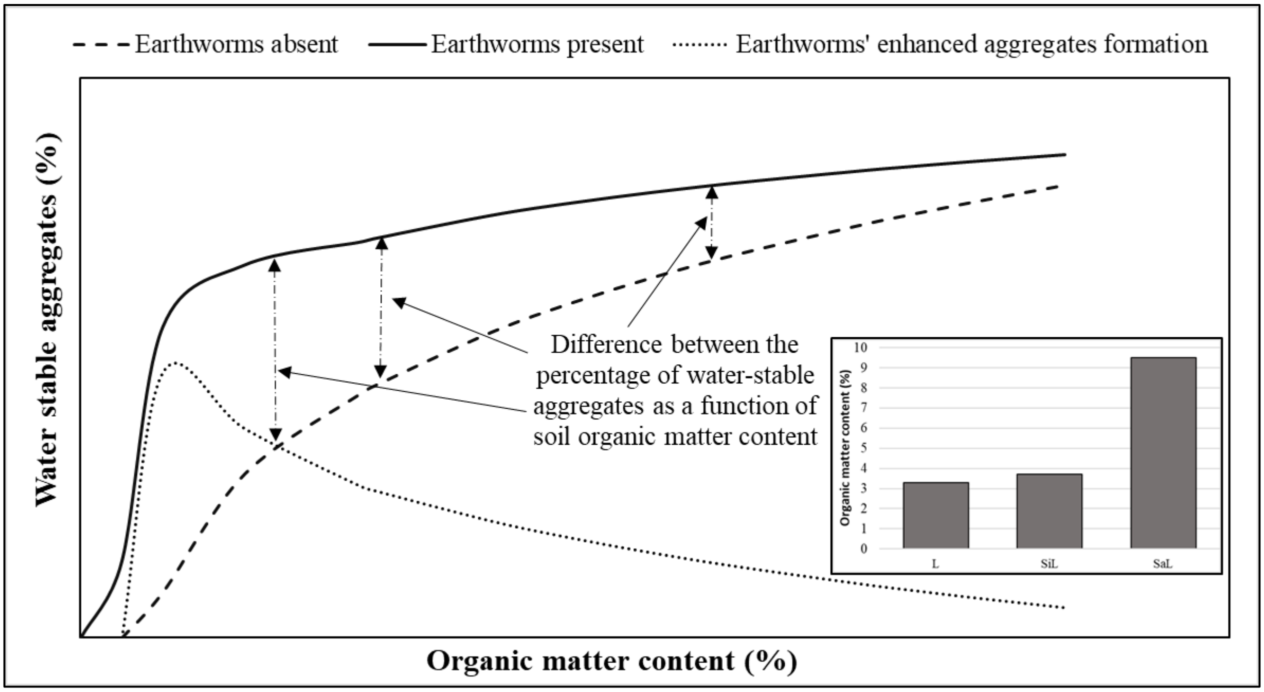


Figure 3 - 6. A conceptual model of the relationship between water stable aggregate formation and soil organic matter content in the presence and absence of earthworms. The dotted line is the difference between the earthworms absent and earthworms present lines and represents the enhanced formation of aggregates due to the presence of earthworms. Insert shows percentage organic matter content of each soil texture used in the current experiments, as presented in Table 3 - 1; L: Loam, SiL: Silt loam and SaL: Sandy loam.

Although our results are in agreement with the positive effect of earthworms on %WSA, they contrast the findings of Milleret et al. (2009a) who found that *A. chlorotica* decreased the % WSA. The low *A. chlorotica* density used in their experiment and a laboratory temperature higher than the optimum temperature for earthworm activity (Butt, 1991), could have reduced the intensity of soil processing relative to our experiment despite the experiment lasting for 35 weeks. 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 stabilisation and aggregation (Forster, 1990). In another experiment with similar conditions Milleret et al. (2009b) found that the presence of plants partly reduced the decrease in soil aggregation that *A. chlorotica* caused. The slightly enhanced soil aggregation was attributed to micro-organisms that feed on carbon from exudates released by plant roots. In a separate experiment using *L. terrestris* Shuster et al. (2000) observed no increase in %WSA in the presence of earthworms. However, these authors conducted field enclosures experiment with an initial immature and adult *L. terrestris* earthworm at a density of 100 ind m^{-2}, far lower than the density of 540 ind m^{-2} that we used.

3.6.3. Impact of earthworms on WHC

Various studies indicate that greater %WSA results in a greater WHC (Franzluebbers, 2002, Zibilske and Bradford, 2007, Suzuki et al., 2007, Blanchart et al., 1999, Jackson, 2014, Basche et al., 2016). Similarly, our linear regression analysis (Figure 3 - 5) 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. Despite the positive relationship seen in the linear regression, our analysis of variance indicates that there was not always a significant increase in WHC due to earthworm processing the soil. Whilst, *A. chlorotica* significantly increased the WHC relative to the controls in all soil textures and for both the upper and lower soils, it is only for the SaL upper soil that a significant increase was seen for *L. terrestris*. For the L and SiL upper soils, the presence of *L. terrestris* slightly increased the WHC but not significantly, presumably because the increase in %WSA of those soils was not high enough.

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. Comparable slopes and intercepts in the SaL soil (Figure

3 - 5c) are consistent with the high organic matter content dominating the %WSA and WHC, rather than earthworm activity.

3.7. Conclusion

In these experiments by using carefully controlled conditions we have demonstrated that earthworms have a significant impact on an important hydrological property (WHC), presumably via the formation of %WSA but also by other species-specific mechanism, most likely their burrowing behaviour. This shows that earthworms can contribute to important ecosystem services such as increased water storage by soils and increased availability of water to plants to support crop growth. We have shown that different earthworm ecotypes have different effects, due to their differing lifestyles and nature of the burrows, and effects can differ with position in the soil mesocosm; further the effects are also a function of soil organic matter which also plays an important role in aggregation and water retention. This helps to explain some of the contradictory work in the literature regarding the impact of earthworms on these properties. It also highlights the point that caution should be exercised when generalising about the impacts that earthworms have on soil properties and processes. To better understand the impact of earthworms on soil hydrology further experiments are required that use a more realistic earthworm density. In addition, plant roots are known to have significant impacts on soil hydrology (Whalley and Dexter, 1994, Beven and Germann, 1982) and therefore experiments that consider the relative impacts of plant roots and earthworms on soil hydrology are warranted.

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Chapter 4

The interacting effects of the anecic earthworm *Lumbricus terrestris*, winter wheat roots and soil texture on hydraulic function

4.1. Declaration

This chapter is presented as submitted paper for publication without changes to the original submitted main text. The paper was written in the style of Hydrological Processes to which it was submitted. For consistency and ease of reading, headings have been numbered, figures and tables inserted and renumbered and citations changed to follow the standard for this thesis.

The interacting effects of the anecic earthworm *Lumbricus terrestris*, winter wheat roots and soil texture on hydraulic function

Running title: *L. terrestris* & root interaction effects on water flow

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4.2. Abstract

Earthworms, and the burrows they make, play an important role in regulating water flow across the landscape. This study examines the combined effects of the vertical burrowing anecic earthworm *Lumbricus terrestris* and crop roots, on soil hydraulic properties. Replicate (n = 4) soil columns, 30 cm high and 11 cm diameter with the presence and absence of both individual winter wheat plants and *L. terrestris* earthworms were established for loam (L), silt loam (SiL) and sandy loam (SaL) soils. Soil water flow measurements were carried out under undrained soil conditions where earthworm burrows have a finite length so that water flow through the soil matrix was considered. The hydraulic conductivity at different tensions was mainly controlled by plant roots ($F_{9,139} = 21.3$, $p \leq 0.001$) compared to earthworms. Although the effect was only significant in the SaL soil ($p \leq 0.05$), field saturated hydraulic conductivity increased in the presence of *L. terrestris*, with the Plant + Earthworm treatments having the greatest value (up to 270 cm day^{-1}). The combined effect of plants and earthworms reduced the amount of water present in L soil as the soil dried in the range 10 to 1500 kPa. The presence of earthworms had no significant effect on either water holding capacity or the percentage of water stable aggregates, relative to the treatments without earthworms. The results indicate that vertical burrowing earthworms can have a significant impact on the hydraulic properties of soils, but when these burrows do not connect to field drains, or an underlying more permeable layer, the impact of plant roots is more significant.

Keywords: *Lumbricus terrestris*, hydraulic conductivity, combined effect, earthworm and plant root interaction, soil physical properties, macropores.

4.3. Introduction

Earthworms have long been recognized for their contribution to ecosystem functions through their action on soil processes (Blouin et al., 2013, Edwards, 2004, Darwin, 1881). Among the ecosystem services, water regulation (Millennium Ecosystem Assessment, 2005) is influenced by earthworms through impacts on soil water transfer and storage processes (Capowiez et al., 2014, Ernst et al., 2009, Pitkanen and Nuutinen, 1998, McDaniel et al., 2015). Different ecological groups of earthworms have different burrowing and bioturbation strategies (Bouché, 1977) each of which may influence soil water movement and storage (Chan, 2004, Joschko et al., 1992, Le Couteulx et al., 2015, Bastardie et al., 2005b).

The anecic species, *Lumbricus terrestris*, produce deep and large burrows, lacking branching, (Shipitalo and Butt, 1999, Capowiez et al., 2015) that have been shown to contribute to water movement through well drained soils (Fischer et al., 2014, Chan, 2004, Bouche and AlAddan, 1997). *L. terrestris* has been associated with a substantial increase of both flow rates and volume of soil water flow in field and laboratory experiments (Shipitalo et al., 1994, Capowiez et al., 2015, Edwards et al., 1989, Shipitalo et al., 2004). However, in those experiments *L. terrestris* burrows were either connected to the bottom of the soil core or to an installed drain within the field. This situation generates free drainage conditions and may lead to an over-estimation of the water flow through the burrows for some environments. In practice, earthworm burrows often have a finite length terminating within the soil matrix at their lower end and so water flows through the soil matrix over the wall of the water-filled burrows (Smettem, 1992). The walls of the accessible bioturbated burrows provide an additional water contact area where water could flow through into the soil matrix (Bastardie et al., 2005a). This may increase the plant available soil water and therefore affect plant growth (Benckiser, 1997, Blouin et al., 2007).

L. terrestris is one of the most thoroughly studied and globally widespread species of earthworm (Gailing et al., 2012) and is the second most frequently reported anecic species in England (Natural England Commissioned Report NECR145, 2014, Kutschera and Elliott, 2010). *L. terrestris* was used in our study due to its abundance and potential for impact on soil water flow due to its burrow morphology. In addition to burrow morphology effects, the increased flow of water through *L. terrestris*-worked soil may be due to other factors. Earthworms often influence the development of soil aggregates and structure (Jouquet et al., 2012, Bottinelli et al., 2015, Puga-Freitas and Blouin, 2015), soil porosity and compaction (Clause et al., 2014, Larink et al., 2001, Bottinelli et al., 2015, Jouquet et al., 2012), macropore size and number of openings (Smettem and Collis-George, 1985, Ehlers, 1975), soil texture (Wang et al., 2013, Yang et al., 2014, Fischer et al., 2014), soil organic matter (Hudson, 1994, Lipiec et al., 2015, Stockdill and Cossens, 1969, Luo et al., 2010) and the interaction with plant root biopores (Ruiz et al., 2015, Weiler and Naef, 2003) may all be important to water transfer.

Plant roots also create biopores that improve water flow within the soil and promote the development of stable aggregates and soil structure (Gyssels et al., 2005, Ghestem et al., 2011). However, the formation of biopores by plant roots causes less bioturbation on average than the formation of earthworm burrows (1 kg m^{-2} yr^{-1} compared to 100 kg m^{-2} yr^{-1}) (Ruiz et al., 2015) and therefore might impact less on soil hydrology compared to earthworm burrows. The biotic interactions between earthworms and plant root bioturbation are very complex and may influence soil hydrology. Just as earthworm burrow morphology could be modified by plant roots, so root distributions are influenced by earthworm bioturbation (Springett and Gray, 1997). In the presence of earthworms, plant roots can easily penetrate the soil by exploiting earthworm burrows which in turn can lead to increases in root biomass, indirectly affecting soil aggregation (Lipiec et al., 2016, Ruiz et al., 2015, Colombi et al., 2017). Equally, the presence of plant roots in soils can modify the number and distribution of burrows produced by newly added earthworms (Springett and Gray, 1997). More burrows are created in topsoils when roots are abundant compared to lower in the profile where there are fewer roots (Springett and Gray, 1997). Both earthworm bioturbation and plant rooting restore soil physical properties, by increasing soil porosity, water stable aggregates and decreasing soil erodibility (Blanchart et al., 2004) and these properties should also influence soil water flow. However, despite some knowledge of the effect of plant roots and earthworm bioturbation activity on water flow and soil physical properties, little is known about the impact of their interactions. Detailed information about the interaction of earthworm ecotypes and plant rooting strategies is necessary if we are to understand processes driving soil functions. Such understanding could lead to the development of earthworm-friendly management practices to help deliver ecosystem services and improved soil hydrological functioning, helping to mitigate and buffer extreme events caused by climate change.

To investigate the complex relationship between plant roots, earthworm bioturbation and the formation of soil aggregates with regards to their impact on soil hydrology, we carried out a laboratory study with the anecic earthworm *L. terrestris*, winter wheat (Skyfall variety) and three soils with different textures. The study was performed in conditions where earthworm burrows have a finite length and are disconnected from the drainage system. Unsaturated and field saturated hydraulic conductivity, soil water release curves, aggregate stability and soil water holding capacity were measured together with plant biomass in the presence and absence of *L. terrestris*. We hypothesize that: i) the presence of plants will increase soil aggregate stability, hydraulic conductivity and water holding capacity within soil; ii) by modifying the distribution of hydrologically functioning pore size classes within the soil matrix, *L. terrestris* will increase the soil hydraulic conductivity and water retention; iii) because earthworms are more active in loam soils (Lowe and Butt, 2005, Jongmans et al., 2003) their modifications of soil physical and hydraulic proprieties will be more significant in L soil than SiL and SaL soils; and iv) *L. terrestris* increases plant biomass production through an increase in water available for root absorption.

4.4. Materials and methods

4.4.1. Experimental design

4.4.1.1. Soil selection and preparation

Topsoil was sampled to a depth of 20 cm in late May 2016 from three Cambisols (WBR, 2006) of differing textures at the University of Leeds commercial farm (53° 51’ 44” N 1° 20’ 35’’W), air-dried, sieved to < 2 mm and then analyzed for pH, organic matter content, dry bulk density and soil particle size (Table 4 - 1) . Soils were repacked into 30 cm high x 11 cm diameter PVC columns to a dry bulk density of 1.3 g cm^{-3} and wetted gravimetrically with deionized water up to 25 – 30 % to sustain earthworm activity (Lowe and Butt, 2005, Butt and Lowe, 2011, Berry and Jordan, 2001). Four replicate columns with the presence and absence of both individual winter wheat plants and *L. terrestris* earthworms as treatments were established for each soil texture. The height of the columns was chosen to allow *L. terrestris* earthworms to produce vertical burrows that terminated within the soil matrix above a buffering soil layer at the foot of the column (Bouché, 1977, Shipitalo and Butt, 1999).

A uniform soil density was achieved in the columns by filling them gradually layer by layer. Sequential 5 cm layers of soil were compacted to the target bulk density and then moistened with deionized water to the correct moisture content to minimize variations with soil depth. Vertical paper barriers of 10 - 15 cm height were placed at the top of all the columns (including earthworm-absent controls) to prevent earthworms from escaping. All columns had a 3 cm thick buffer soil layer at their base. The top of this layer was covered with fine nylon mesh with a mesh size of c. 0.5 mm, i.e. significantly smaller than the earthworms’ diameter to prevent escape and to prevent burrows connecting to the base of the columns. The base of each column was covered with wire mesh with a diameter of 1 mm. The soil columns were weighed, the tops covered with plastic film to prevent water loss and stored at 15 °C until the start of the experiment (up to a maximum of 4 days later).

Table 4 - 1. Physical and chemical properties of soils selected for the experiment (*n* = 3 apart from for field dry bulk density measurements where *n* = 2).

Land use	**pH**[1]	**Organic matter**[2] **(%)**	**Field dry bulk density**[3] **(g cm^{-3})**	**Clay (%)**[4]	**Silt (%)**[4]	**Sand (%)**[4]	**Textural class**
				< 2 µm	**2-50 µm**	**50-2000 µm**	
Arable	7.73	3.63	1.56	8.93	45.93	45.14	Loam (L)
	7.79	3.31	1.54	8.08	42.09	49.82	
	7.62	3.30		8.29	42.93	48.78	
Arable	7.48	3.86	1.38	4.28	51.24	44.47	Silt loam (SiL)
	7.71	3.46	1.51	4.46	53.83	41.70	
	7.73	3.50		4.21	51.28	44.50	
Pasture	7.26	9.61	1.22	3.54	45.11	51.33	Sandy loam (SaL)
	7.33	9.57	1.14	2.48	34.55	62.96	
	7.23	9.58		3.21	41.56	51.76	

[1] Determined using a ratio of 1 : 2.5 (soil : water) (Ministry of Agriculture Fisheries and Food, 1986), [2] by loss on ignition at 350 °C to avoid decomposition of carbonates during ignition (Ayub and Boyd, 1994, CEAE, 2003), [3] using soil density rings of 95 cm^3 volume and [4] using a MasterSizer2000 laser particle size analyzer (Malvern Instruments, UK).

4.4.1.2. Crop growth conditions

Before adding the *L. terrestris*, the columns were planted with pre-germinated winter wheat seedlings (*Triticum aestivum*, Skyfall variety). Winter wheat seeds were germinated on Petri dishes kept at room temperature in natural light for three days. Seedlings with 2 cm radicles were transplanted into the soil columns and allowed to grow for five days under natural light. *L. terrestris* were then added to the earthworm-present treatments and the columns were placed under 50 W LED lights (Massa et al., 2008, Schroer and Hölker, 2016) in a controlled temperature room set at 15 ± 1°C and 60 ± 7 % rh. Photosynthetically Active Radiation (PAR) (Evans and Poorter, 2001, Harding et al., 1990, Bugbee and Salisbury, 1988) was measured at the surface of the soil for each column to ensure a homogeneous distribution of radiation and appropriate light levels for plant growth by adjusting the distance between the LED lights and the plants every week. The position of the columns within each block was changed randomly every week in order to provide the plants with equivalent lighting. A lighting regime of 16-hour days and 8-hour nights was used, and the growing seedlings were watered every week over the 16 weeks of the experiment period with deionized water. Plant water needs were estimated from free water surface evaporation (Pan evaporation method) (Wang et al., 2017, Allen, 1998).

4.4.1.3. Earthworm collection and culturing

Anecic *L. terrestris* were purchased (Biological Blades Ltd, Edenbridge, UK) and maintained in the laboratory prior to the experiment. Clitellate individuals were selected for the experiments. *L. terrestris* to be used in the experiment were moved from the culture into containers containing the same type of soils used in the soil columns and kept at 15 °C in darkness (Butt, 1991), for three days to acclimatize them to the soil and ensure that individuals were active and viable. An individual *L. terrestris* was then added to each soil column giving a density of 100 ind.m^{-2} (Ernst et al., 2009, Palm et al., 2013, Rogasik et al., 2014). Oven dried milled horse manure rewetted with deionized water was used to feed the earthworms. A surface application of 20 g adult^{-1} month^{-1} of horse manure was used (Butt, 2011, Butt and Lowe, 2011, Lowe and Butt, 2003, Svendsen et al., 2002, Lowe and Butt, 2005). At the end of the experiment the following measurements were made in the laboratory at ≈ 20 °C.

4.4.2. Measurements

4.4.2.1. Plant biomass, earthworm biomass and cast production

At the end of the experiment winter wheat shoots were collected, cutting the stems at the soil surface, and the fresh biomass weighed. The shoots were then oven dried at 70 °C to constant weight. *L. terrestris* biomass was recorded at the start and end of the experiment. *L. terrestris* casts (> 2 mm) were collected from the soil surface every week, weighed, oven dried at 105 ºC for 48 h and reweighed; the rate of cast production over the duration of the experiment expressed as mg cast per mass of earthworm per day was calculated.

4.4.2.2. Unsaturated and saturated hydraulic conductivity

There is a range of pore size classifications and an arbitrary value is usually chosen as the boundary between classes (Luxmoore, 1981). We defined micropores as those with a diameter of < 0.5 mm corresponding to a water tension (*h*) of -6 cm and mesopores as those with diameters in the range 0.5 - 1 mm corresponding to water tensions of -6 cm to -3 cm. For macropores, a pore radius corresponding to a capillary potential of zero is not defined when using the capillary rise equation. Therefore, following previous work (Germann and Beven, 1981, Holden, 2008, Holden et al., 2014) we chose a pore diameter of 1 mm, corresponding to a water tension of -3 cm, as the minimum dimension of macropores. Smaller and larger-macropores are pores in the range 1 - 3 mm and ≥ 3 mm respectively. Macropore diameters of anecic species in general are wider than 2 mm (Pérès et al., 1998). Here, pores of 3 mm diameter corresponding to a water tension of -1 cm are assumed to be the minimum size of earthworm produced macropores. A Decagon Mini Disk Portable Tension Infiltrometer (Decagon Devices Inc, 2016) was used to measure the infiltration properties of the soils at potentials of -6, -3, and -1 cm until steady-state flow was reached. A fine moist sand layer was applied to the column surface to improve contact between the tension disk and soil surface (Köhne et al., 2011, Reynolds and Elrick, 1991). Measurements were made starting at the lower tension to avoid hysteresis effects (Baird, 1997). The Van-Genuchten Zhang transient method, proposed by Zhang (1997), was used to estimate the unsaturated hydraulic conductivity (K) for three dimensional infiltration. The field saturated hydraulic conductivity (K_{fs}) was calculated using the Reynolds and Elrick (1991) equation assuming that water flows within all pores of the soil matrix under saturated conditions. The contribution of different pore size classes to water flow and the proportion of the soil volume in which the flow occurred were calculated following the procedure presented by Watson and Luxmoore (1986).

4.4.2.3. Soil water release curves

Water release curves were measured using a HYPROP device (UMS, Munchen, Germany). Soil cores of 250 cm^3 and 5 cm height were taken from the surface of the columns and analyzed following the simplified evaporation method (Schindler et al., 2010, Peters et al., 2015). HYPROP measurement campaigns were modeled using the HYPROP-FIT software to generate the hydraulic function parameters using the Van Genuchten-Mualem model (Van Genuchten, 1980). Because soil water release curve determination takes a considerable time to complete, the measurements were limited to the different treatments applied to the loam soil because earthworms were seen to be more active in this soil texture (Jongmans et al., 2003, Lowe and Butt, 2005). As the pore holding water radius is proportional to the potential value at which that pore drains, the volume of pores can be determined from the slope of water release curves $d\theta/d\psi$. Of the 12 cores from the L soil columns (three cores for each treatment) that were used in the soil water release determinations two cores were excluded, one from the Soil + Wheat treatment and the other from the Soil+ Wheat + Earthworm treatment, because of a power cut to the Hyprop equipment during the measurements which made the data unusable.

4.4.2.4. Water holding capacity and water stable aggregates

The water holding capacity (WHC) was measured in accordance with ISO 11268-2:1998. Intact soil cores 3.5 cm diameter x 5 cm high were taken from the soil surface of the columns. The cores were saturated, allowed to drain for 48 hours and then their water content was determined by drying the soils to constant weight at 105 °C. The percentage wet aggregate stability (%WSA) was measured using wet sieving equipment (Eijkelkamp, Agrisearch Equipment). 250 µm sieves were filled with 4g of the 1-2 mm air dried aggregates, pre-moistened by capillarity for 10 minutes and wet sieved for 3 minutes. The %WSA was calculated, after correction for the mass of sand >250 µm, as the weight of aggregates remaining on the sieve relative to the total initial weight of aggregates (Milleret et al., 2009a, Kodešová et al., 2009).

4.4.3. Statistical analysis

Data were analyzed using general linear model analysis of variance (ANOVA). SPSS (IBM Corp. Released 2016, version 24) software was used to estimate the statistical significance of mean differences between treatments. P values of < 0.05 were used as the threshold for significance. To deal with variability of low replicate numbers, in this paper we presented the median, minimum and maximum values of the data. Mean values ± standard deviation are given when describing data sets of $n > 8$.

4.5. Results

4.5.1. Water flow

Hydraulic conductivity varied significantly with the applied water tension (h), soil texture and treatments, $p \leq 0.001$. Figure 4 - 1 shows the hydraulic conductivity measured at the different water tensions for the different treatments for each soil texture. K_{fs} was significantly higher than K ($p \leq 0.001$) which means that macropores must be important hydrological agents in these soils (Messing et al., 2005, Lin et al., 1999). The hydraulic conductivity measured at -1 cm tension was significantly higher than the conductivities measured at the lower tensions ($0.001 \leq p \leq 0.05$).

The winter wheat treatments for all textures had significantly higher hydraulic conductivities at the different tensions compared to bare soil columns ($p \leq 0.001$), except for the SiL soil at a water tension of -3 and -6 cm ($p > 0.05$). Winter wheat roots appear to be the main factor controlling water flow in these soils. The addition of a single *L. terrestris* earthworm to the planted and bare columns led to a significant increase of K_{fs} in the SaL soil ($0.001 \leq p \leq 0.05$) showing the effect of burrowing activity on matrix flow in this soil texture. With the presence of *L. terrestris* in planted and bare columns, a non-significant increase in hydraulic conductivities was observed for the L and the SiL textures at different water tensions, showing that the burrowing activity did not have a major impact on matrix flow. The SaL soil conducted more water at -1, -3 and -6 cm water tensions than the other soil textures ($p \leq 0.001$). However, K_{fs} was not significantly different between soil textures.

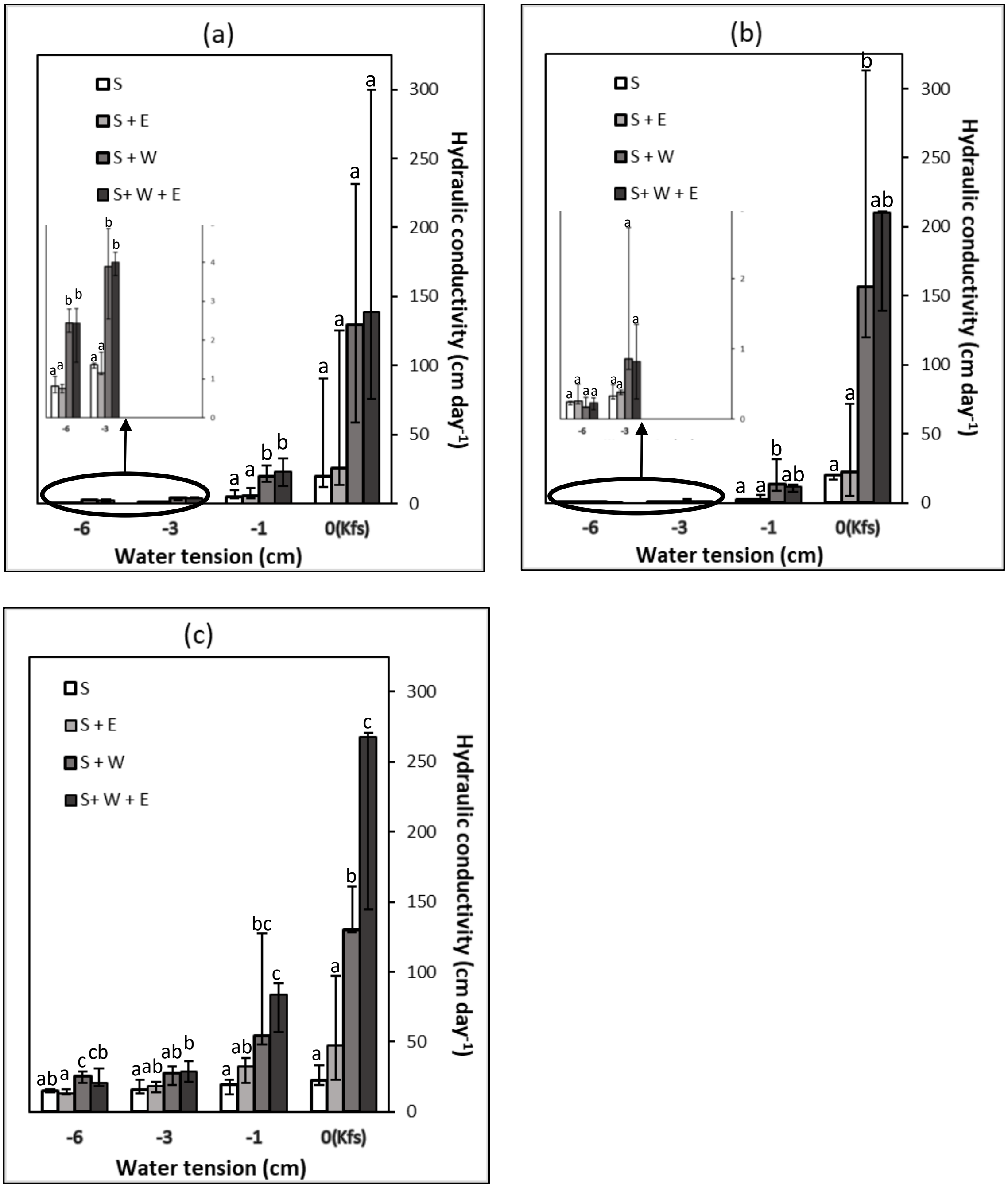

Figure 4 - 1. Median hydraulic conductivity for the treatments and water tension for each soil texture (a, b, c). Letters refer to treatments; S: Soil, S+E: Soil + Earthworms, S + W: Soil + Wheat, S+W+E: Soil +Wheat + Earthworm; and soil textures; (a): The loam soil), (b): The silt loam soil) and (c): The sandy loam soil). Insert shows zooms of hydraulic conductivities at -3 and -6 cm tension. Columns with the same letter over them are not significantly different within each of the applied water tension. (*n* = 4, error bars = max and min values).

The contribution of different pore size classes to water flow was significantly different between soil textures ($p \leq 0.001$) except between the SiL soil and the L soil within micropores. The proportion of water flow within micropores in the SaL soil ranged from 10 % up to 57 % and was greater than the proportion of water flow in the L and SiL soils (< 4 %) through micropores (Figure 4 - 2). In contrast, larger-macropores contributed more significantly to flow through the SiL soils (87 to 94 %) and the L soils (77 to 84 %) compared to the SaL soil (30 to 66%). The proportion of flow within mesopores and smaller-macropores was approximately the same for each soil texture.

The proportion of flow within pore classes was significantly different between treatments only for the SaL soil. Plant roots had a significant effect on the proportion of flow within micropores ($p \leq 0.001$) and larger-macropores ($p \leq 0.05$). In the planted columns, there was on average 30 % less flow through the micropores and 30 % more flow through the larger-macropores compared to the bare soils. A significant effect of *L. terrestris* on pore size class contribution to water flow was found for bare soils for the micropores and mesopores.

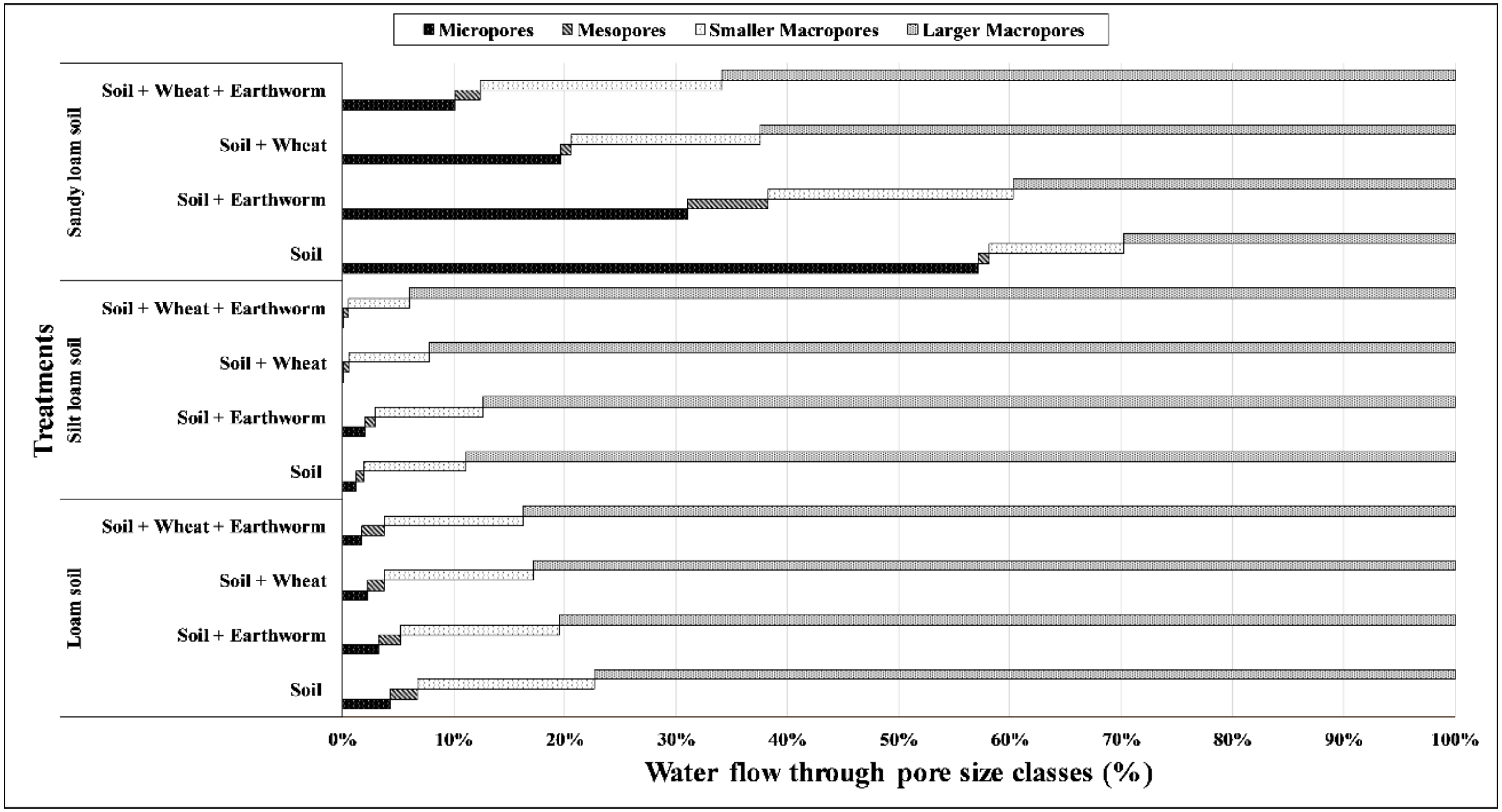

Figure 4 - 2. Pore size class contribution to water flow for all treatments under the loam, the silt loam and the sandy loam soil calculated after the method of Watson and Luxmoore (1986).

Across all the treatments and soils, macropores provide an overall average contribution of 86 ± 6 % of water flow at saturation. This water flow was transmitted through only 0.0037% of the soil volume. The percentage of water flow through macropores larger than 1 mm was significantly less ($p \leq 0.001$) in the SaL soil compared to the L and SiL soils (averages of 65 ± 6 % vs 95 ± 3 % and 98 ± 1 % respectively). In the SaL soil, the flow through macropores increased when planted with winter wheat (46 ± 20 % vs 84 ± 4 % which occurred in 0.001 % vs 0.006% of the soil volume in bare and planted soils respectively). The addition of *L. terrestris* was associated with a greater proportion of macropore flow through both planted and bare soils but the effect was not significant. The combined *L. terrestris* and winter wheat treatments resulted in 94 ± 6 % of water flow through macropores which occurred in only 0.007% of the soil volume. The proportion of flow through macropores in the L and the SiL soils was not significantly different and there were no significant differences for these soils between earthworm or wheat treatments.

4.5.2. Soil water release curves

The Van Genuchten (1980) curve was fitted to the combined water release data of replicates of each treatment (Figure 4 - 3). Water retention at saturation showed no significant differences between treatments ($F_{3,8} = 0.9$, $p > 0.05$, Table 4 - 2). In all treatments, the soil retained the majority of water across the saturation matric potential range.

Table 4 - 2. Analysis of variance of water content and plant available water at different soil water potential pF (pF = Log_{10} (static pressure at cm water)).

	Source of variation	***d.f.***	***SS***	***MS***	***F-ratio***	***P-value***
Water content at pF 1.8	treatments	3	22.99	7.66	0.78	0.535
Water content at pF 2.5		3	57. 90	19.30	2.75	0.112
Water content at pF 4.2		3	46.08	15.36	10.08	0.004
Plant available water from pF 1.8 to 4.2		3	43.50	14.50	1.83	0.219
Plant available water from pF 2.5 to 4.2		3	6.94	2.31	0.39	0.763

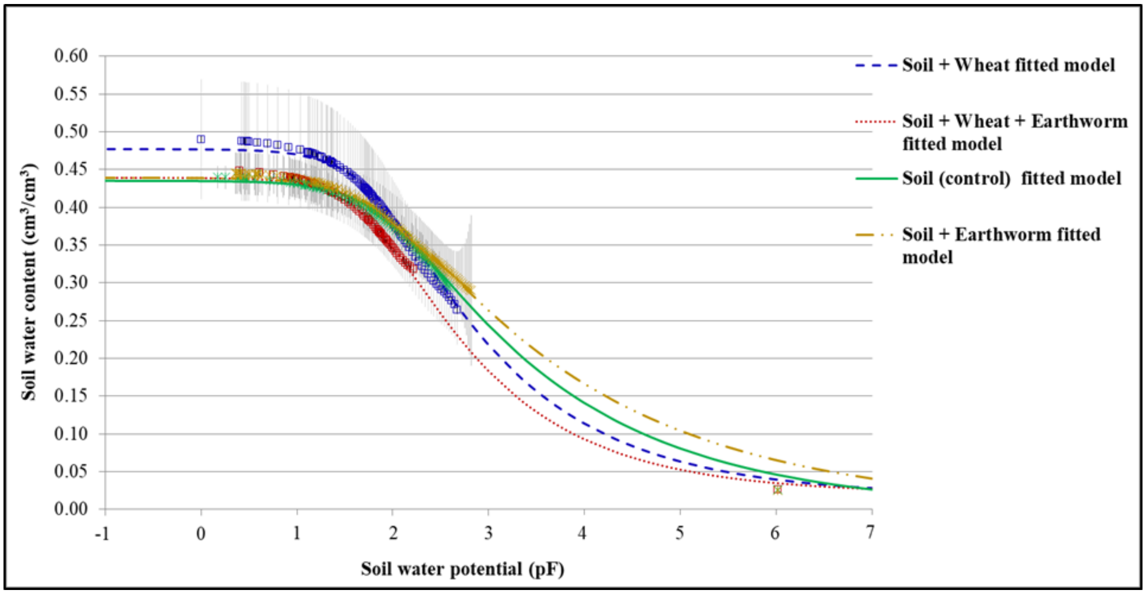

Figure 4 - 3. Water release curves of the four treatments, Soil + Wheat, Soil + Wheat + Earthworm, Soil (control) and Soil + Earthworm, for the loam soil. Standard deviation bars are shown.

The columns with soil and wheat were slightly more porous, but not significantly. Given we are interested in the soil structure it is useful to replot (Figure 4 - 3) as an effective pore size (Figure 4 - 4). Water in the largest pores drains most easily and Figure 4 - 4 shows that such pores are more abundant in columns with plants, as opposed to those with no plants. Figure 4 - 4 clearly shows that it is primarily the plant roots that alter the soil structure at the core scale and not *L. terrestris* earthworm species. This makes sense as the earthworms produce vertical burrows and do not contribute greatly to the bioturbation of the soil matrix as a whole over the time period of the experiment (16 weeks).

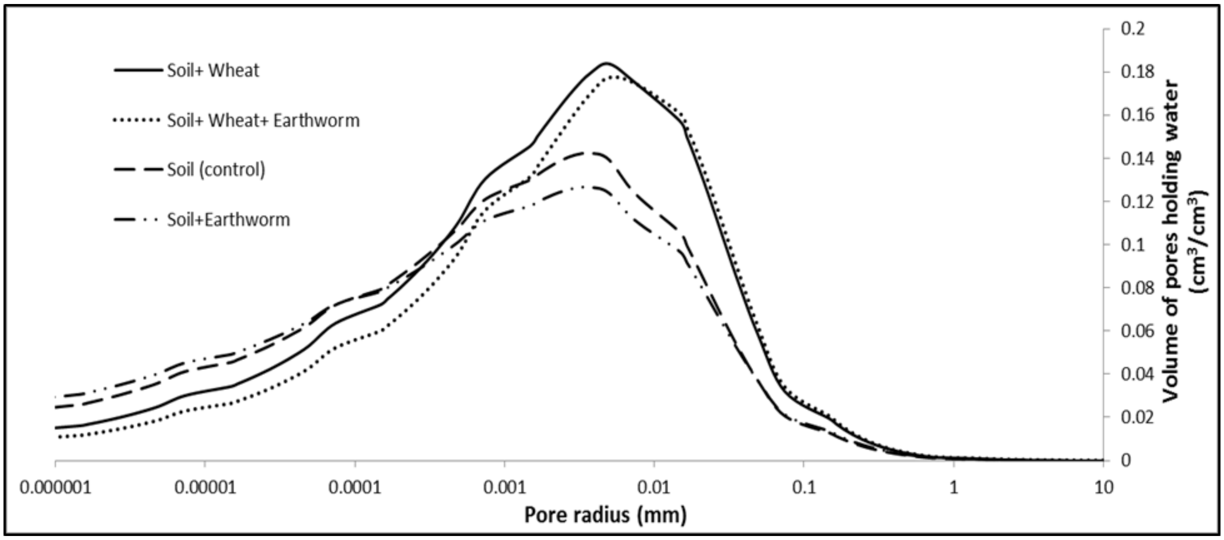

Figure 4 - 4. Volume of pores holding water within the effective pore size class. Radius of pores bearing water is proportional to the potential values from saturation (≈ 10 mm radius) to pF = 7 (10^{-6} mm radius).

4.5.3. Water holding capacity (WHC)

Among the 48 samples used for the measurement of WHC, three samples from the L soil (Soil + Wheat, Soil + Wheat + Earthworm and Soil + Earthworm) were excluded from the statistical analysis because of soil loss from the bottom of the cores when transporting them to the oven for drying after the initial weighing. Soil WHC was greatest in the SaL soil (Figure 4 - 5) and varied significantly between soil textures. The soil WHC (cm^3 cm^-

^{3}) values of the L soil vary in the same range as that measured at saturation on the HYPROP device. For each soil type there were no significant difference in WHC between treatments, except for the SiL soil where Soil + Wheat treatment is significantly lower than earthworms-present treatments.

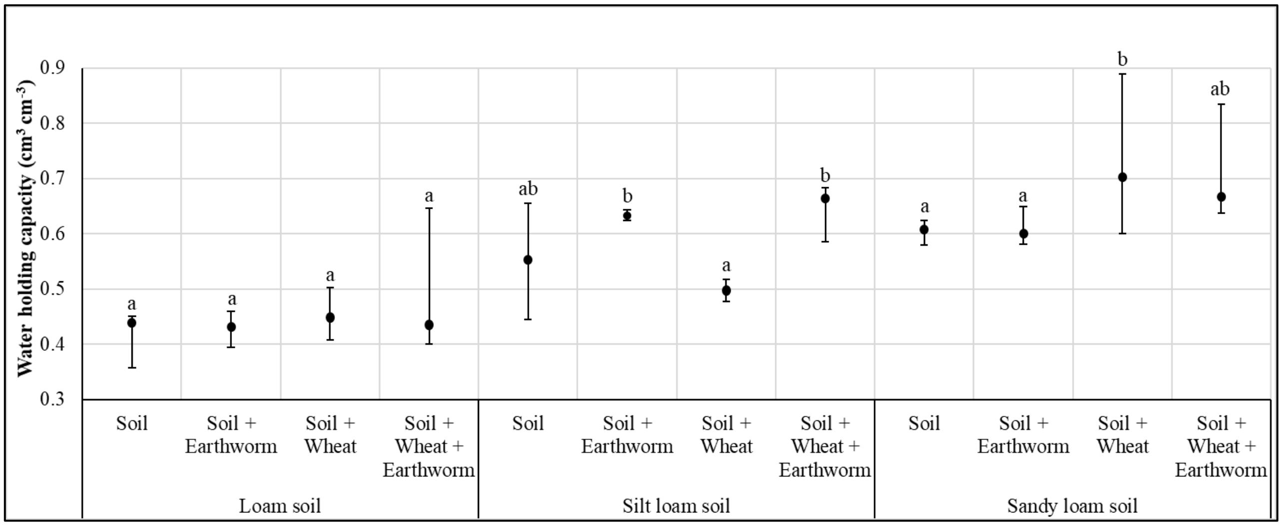

Figure 4 - 5. Median water holding capacity for the different treatments. Columns with the same letter over them are not significantly different within each soil texture. (*n* = 4, error bars = max and min values).

4.5.4. The percentage water stable aggregates (%WSA)

The %WSA in the L and SaL soils are not significantly different from each other but are both significantly greater than the %WSA in the SiL soil (p <0.001). For the bare soils, aggregate stability was much lower for the SiL soil, which had only 51 % aggregates left after wet sieving, compared to the other two soils which had 67 % and 77 % aggregates left. The %WSA were significantly influenced by plant roots (p <0.05). The highest %WSA were observed for the planted L (82 %) and SaL soils (79 %), compared to the SiL soil (65 %). Although the soil in the planted columns contained a higher %WSA compared to bare columns these differences were only significant for the SiL soil (Figure 4 - 6). Aggregate stability in the *L. terrestris* treatments was not significantly different from earthworm-absent treatments.

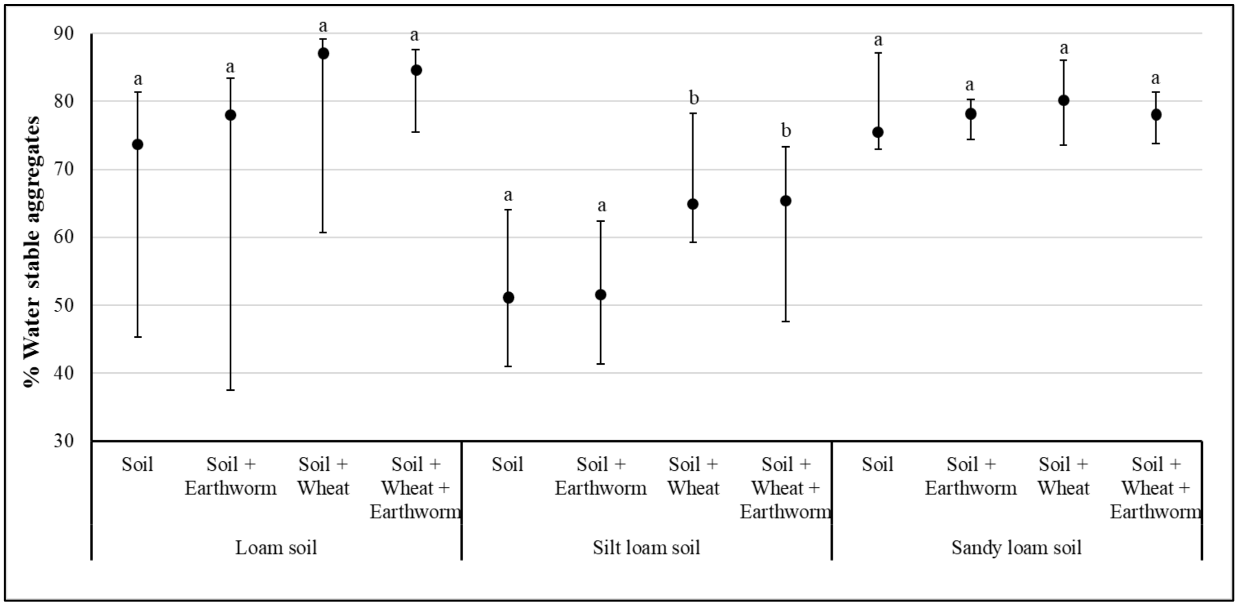

Figure 4 - 6. Median percentage water stable aggregates for the different treatments for each soil. Columns with the same letter over them are not significantly different within each soil texture. (*n* = 4, error bars = max and min values).

4.5.5. Plant biomass

Dry matter biomass weight of winter wheat shoots in the treatments was significantly different between soil textures ($F_{2,18}$ = 63.5, p <0.001) and was lowest in the SiL soil (Figure 4 - 7). The presence of *L. terrestris* was associated with a significantly greater plant biomass in the SiL soil compared to the earthworm-absent treatment ($F_{1,6}$ = 11.1, p = 0.016). The dry matter biomass difference between treatments in the L and SaL soils was not significant.

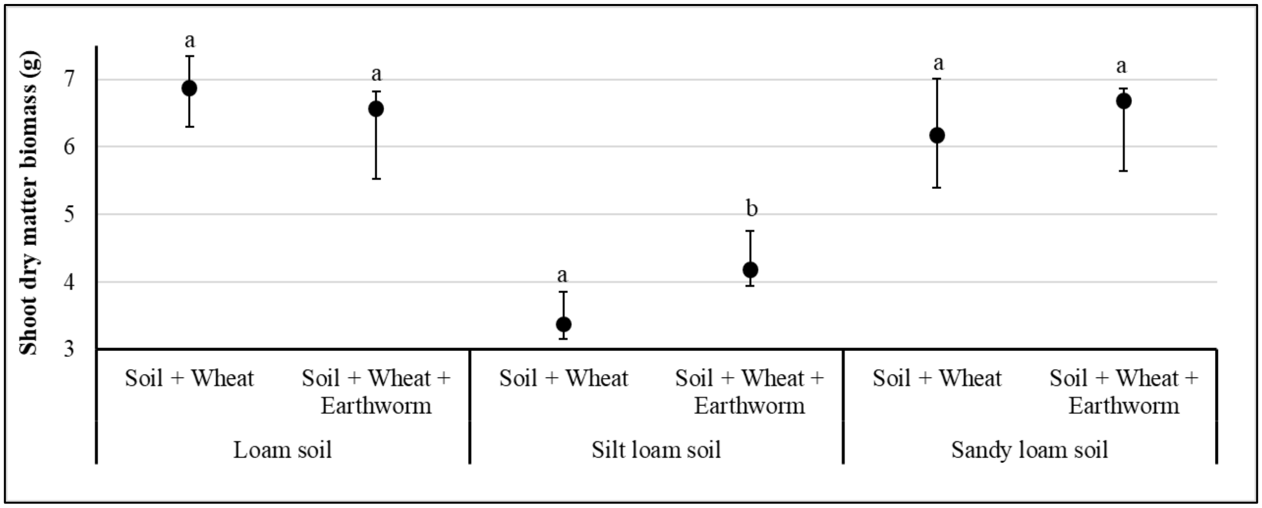

Figure 4 - 7. Median winter wheat shoot dry matter biomass for the treatments with and without *L. terrestris* for each soil. Columns with the same letter over them are not significantly different within each soil texture. (n = 4, error bars = max and min values).

4.5.6. Earthworm biomass and cast production

An earthworm was missing in one replicate of the Soil + Wheat + Earthworm treatments of both SiL and SaL soils. Another earthworm was found decayed in the Soil + Earthworm treatment of the L soil. The *L. terrestris* weight gain was highest, but not significant, in the Soil + Wheat + Earthworm treatments of the SaL soil compared to the other treatments (Table 4 - 3). Cast production was significantly greater ($F_{2,17} = 13.9$, $p < 0.05$) in the SaL soil compared to the L and the SiL soils (Table 4 - 3). The highest value ($p < 0.05$) was recorded in the Soil + Wheat + Earthworm treatments (up to 73.61 g). Based on the initial *L. terrestris* weight, the largest cast production rate was up to 244.32 mg g^{-1} earthworm day^{-1}. This value is an underestimate as only the casts collected at the column surface were weighed and thus burrow wall casts were not included.

Table 4 - 3. *L. terrestris* weight at the start and end of the experiment, total cast production and cast production rate by treatment. S+W+E: Soil + Wheat + Earthworm, S+E: Soil +Earthworm. (*n* = 4).

Soil texture	***Treatments***	***Initial earthworm weight (g)***			***Final earthworm weight (g)***			***Cast dry weight (g)***			***Cast production rate* (mg g^{-1} day^{-1})**		
		Median	***Min***	***Max***	***Median***	***Min***	***Max***	***Median***	***Min***	***Max***	***Median***	***Min***	***Max***
L	S+W+E	3.86	2.60	5.58	3.81	1.64	5.66	29.42	24.40	32.28	71.09	39.04	104.43
	S+E	3.52	2.42	4.54	4.29	3.15	4.59	41.21	24.81	48.89	96.20	91.54	116.62
SiL	S+W+E	3.69	2.90	5.01	4.34	3.32	4.97	42.47	39.28	50.30	120.94	75.69	141.67
	S+E	4.05	3.04	5.90	4.54	3.51	5.79	37.57	28.37	52.13	89.84	54.15	107.14
SaL	S+W+E	3.79	2.69	4.98	4.77	4.67	6.20	63.43	54.04	73.61	151.98	96.89	244.32
	S+E	3.66	2.50	5.70	3.72	2.71	4.94	44.76	38.90	52.63	113.56	76.60	138.93

4.6. Discussion

4.6.1. Unsaturated hydraulic conductivity and field saturated hydraulic conductivity

4.6.1.1. Pore size classes and water flow

Macropores ≥ 1 mm and ≥ 3 mm diameter contributed to 86 % and 74 % of the water flow respectively (Figure 4 - 2). The minor effect of micro- and meso-pores on water flow (11% and 1.8% respectively) is consistent with previous studies in peats (Holden, 2008), forest mineral soils (Watson and Luxmoore, 1986) and pasture mineral soil (Alaoui and Helbling, 2006). Because of the lower hydraulic conductivities in our study compared to those above, our calculations suggest that this flow was through a very small % of soil volume (< 0.01 % compared to 0.2 – 5%). In broad terms these lower hydraulic conductivities reflect differences in experimental systems between our study and others including our use of repacked soils compared to in situ soils and the presence of only a single wheat plant and earthworm in our experiment compared to a combination of fully vegetated soils and invertebrate burrows.

4.6.1.2. Soil texture and organic matter effects

Particle size has often been related to pore size and occurrence of matrix flow and macropore flow (Rawls et al., 1982, Saxton et al., 1986). SaL soil contained relatively fewer effective larger-macropores compared to the L and the SiL soils. In contrast to our findings, coarse grained soils, similar to SaL, are usually more water conducting at high water tension and under field saturated conditions than finer texture soils due to their larger matrix pore sizes (Hopmans et al., 1993, Bresler et al., 1984). It is possible that the relatively high organic matter content in the SaL soil (Table 4 - 1) resulted in high soil aggregate stability (overall %WSA of ≈ 78%, Figure 4 - 6) (Kapur et al., 2007) which resulted in a shift from larger-macropores to narrower pore sizes, apparently to micropores in our case (Figure 4 - 2). Dal Ferro et al. (2012), Tang et al. (2016) and Zaher et al. (2005) studied the contribution of organic matter to the development of mesopores and micropores; the organic matter decreased the number of macropores, increased soil hydrophobicity and decreased soil hydraulic conductivity.

4.6.1.3. Crop root effects

Clear differences were observed in hydraulic conductivities in columns planted with winter wheat compared to bare soils, for all soils except the SiL soil at -3 and -6 cm water tension (Figure 4 - 1). As water tension increased, the relative differences between hydraulic conductivity in planted and unplanted columns increased. Under field saturated conditions, mean hydraulic conductivity was 178 ± 74 cm day^{-1} in planted columns and 35 ± 30 cm day^{-1} in bare soils (Figure 4 - 1). When relating these values to the rainfall records in the region (between 2007 and 2016) with a highest total rainfall recorded of 6.24 cm in a day and a highest rainfall intensity of 17.73 cm h^{-1} (University of York Weather Station, 2017), we do not expect infiltration-excess overland flow to be a frequent occurrence in the region for the planted soils. However, it should be noted that our columns had repacked soils and so the comparison to field rainfall conditions may not be reliable. The high hydraulic conductivities in the planted columns relative to bare soils may be due to winter wheat roots that can extend relatively deep into the columns promoting preferential pathways for water movement (Holden and Gell, 2009). Growing roots physically modify soil properties by creating biopores and contributing to continuous pore systems present in the soil and therefore increasing water transport through soil (Shinohara et al., 2016). Figure 4 - 2 shows the significant effect of the creation of biopores by roots on the SaL soil, where water flow through micropores is, on average, 30 % less in planted columns compared to bare soils.

In addition, winter wheat roots are able to stabilize soil aggregates and structure (Katuwal et al., 2013, Amellal et al., 1998, Rillig et al., 2002). High aggregate stability may improve water movement through soil (Shrestha and Lal, 2008, Kapur et al., 2007). The high %WSA observed in our planted columns compared to bare soil in the L and the SiL soils (Figure 4 - 6) may have an effect on increasing hydraulic conductivities in planted columns. For the SaL soil there was a high %WSA but a lack of earthworm and wheat treatment effects which may be related to the high organic matter content in this soil masking any treatment effects (see above). In the SiL soil, %WSA was lowest compared to the other two soils which may explain the absence of significant differences between hydraulic conductivities at -3 and -6 cm water tension in planted columns. Considering the strong positive correlation that exists between root and shoot biomass (Kimiti, 2011), the lowest %WSA in the SiL soil may be due to the low plant root biomass (Jastrow et al., 1998, Pérès et al., 2013) in this soil (Figure 4 - 7).

4.6.1.4. Earthworm effects

The presence of *L. terrestris* had no significant impact on hydraulic conductivity in the L and SiL soils. However, a significant effect was observed for the SaL soil under field saturated conditions. Figure 4 - 1c shows that the creation of additional pores wider than 3 mm by earthworm burrowing resulted in more water flow in columns with *L. terrestris* than those without *L. terrestris*. The combined effect of *L. terrestris* and plants resulted in the SaL soil having the highest K_{fs} for all the soils and treatments. The reason why *L. terrestris* activity showed a significant effect on K_{fs} in the SaL soil rather than the other soil textures could be due to the soil texture preferences of this earthworm species (Nuutinen, 1992). Contrary to our first hypothesis, in our experiment, *L. terrestris* appears to have been more active in the SaL than in the L and SiL soils; *L. terrestris* produced more casts in the SaL soil than in the SiL or the L soil and, within this soil, produced more casts in the presence of plants (Table 4 - 3). Also, the weight gain of *L. terrestris* was largest in the SaL soil at the end of the experiment (Table 4 - 3), consistent with increased feeding activity and the cast production. Although not always the case (Nuutinen, 1992), often *L. terrestris* occurs more in coarser soil than finer soil textures (Guild, 1948). The large particles of coarser soils, such as SaL, are loosely held together (Lockhart and Wiseman, 1983) which may make it easier for *L. terrestris* to burrow through this soil. Soil texture alone may not explain the effect on hydraulic conductivity in the SaL soil, the organic matter content may also have contributed. The initial high organic matter content in this soil (Table 4 - 1) will have helped maintain the soil moisture at levels suitable for earthworms and provided a food source (Edwards, 2000).

Previous studies have found that *L. terrestris* increased water flow in soils under field conditions (Edwards et al., 1988, Willoughby and Kladivko, 2002, Fischer et al., 2014, Shipitalo and Butt, 1999). In our study under controlled conditions, this effect was only observed at K_{fs} in the SaL soils. No significant effect of *L. terrestris* activity on K (water tensions of -6, -3 and -1 cm) was detected in any of the soils. The reason for such a lack of effect might be that the density of the anecic *L. terrestris* was too low to work the soils and to have a significant effect on infiltration rates given the timeframe over which the columns were studied. Studies where similar *L. terrestris* density was manipulated and which showed an impact on water infiltration rates, were carried out in either field plots in the presence of other earthworm species or in undisturbed soil cores already worked by earthworms for a long period (Schutz et al., 2008, Bastardie et al., 2005a, Palm et al., 2013). In all these cases it is likely that burrows connected to the bottom of the cores or a freely draining substrate. Under laboratory conditions using a *L. terrestris* density relatively similar to that of our experiment and with a short incubation period in repacked cores, Capowiez et al. (2015) and Ernst et al. (2009) found a significant increase in infiltration rate and low bioturbation intensity in *L. terrestris*-present treatments compared to other species. However, earthworm macropores were present throughout the entire core, connecting the surface of the soil to the base of the core unlike our experiment where the bottom extremity was ended by a buffer soil layer and the water flow was therefore only matrix flow. This shows that earthworms by themselves have a limited impact on water regulation unless they connect to conductive pathways or field drains so that water can flow away.

4.6.2. Soil water release curves

The results suggest that the soil water release curves for the L soils are influenced by winter wheat roots in the same way as the other measured parameters. At saturation, Soil + Wheat had a higher water content and drained up to the matric potential of pF= 2.5 much more readily than other treatments. The winter wheat increased the %WSA (Figure 4 - 6), which improves soil structure (Veiga et al., 2009), and will have caused more of the soil water to be held in larger pores (Figure 4 - 4). This would shift the saturation segment of the water release curve upward as shown in (Figure 4 - 3) (Yuge et al., 2012).

In the Soil + Wheat + Earthworm treatment of the L soil, the soils had a similar curve response at saturation as bare soils, but lost water more easily at soil water potentials up to, and above, the field capacity point of pF = 2.5. It is likely that the Soil + Wheat + Earthworm treatment had a similar volume of large water-holding pores, > 0.01 mm diameter, as the Soil + Wheat treatment (Figure 4 - 4). These wide pores, together with the small volume of narrow water-holding pores (Figure 4 - 4), have caused water content to be lower in planted soils with *L. terrestris* than without *L. terrestris* under saturated conditions. From a matric potential of pF = 2.5 up to the wilting point, the few wide pores would have drained easily and plants would have to work progressively harder to pull water from the soil. Plant roots are known to stabilize macro-aggregates and help create large pores in the soil (Veiga et al., 2009, Angers and Caron, 1998). However, the soil around earthworm burrows is typically denser than in the bulk soil (Rogasik et al., 2014) and burrow walls are lined with smeared soil (Cook and Linden, 1996) which may increase the difficulty of root-soil contact and penetration (Jin et al., 2013) leading to a smaller soil volume that roots can access to extract water from in the presence of earthworms. Observations of our soils, on removal from the columns, support the hypothesis that earthworm burrows can be hard for roots to penetrate (Figure 4 - 8). In contrast, unplanted soils with fewer biopores (Scholl et al., 2014), held more residual water above the matric potential of pF = 2.5 due to the high number of smaller pores that hold water by adsorption.

4.6.3. Water holding capacity and plant biomass

Usually, soils with coarser particles, such as the SaL soil, hold less water (Saxton et al., 1986). However, SaL soil had a relatively high organic matter content (Table 4 - 1) which likely contributed to larger WHC (Williams et al., 2016). Considering a range of soil textures, Hudson (1994) showed that available water capacity could be doubled by increasing organic matter content from 0.5 % to 3 %.

Within planted columns, the significant increase in WHC in the presence of *L. terrestris* in the SiL soil was accompanied by a significant increase in plant biomass (Figure 4 - 7). Many mechanisms stimulate plant growth in the presence of earthworms (Brown et al., 1999). Our study shows that one contributing factor could be an increase in water holding capacity. This would lead to an increase in the availability of water in SiL soil ensuring that plant growth was not water limited. In addition, retention of water could reduce the leaching of nutrients from the soil aiding to the process of plant growth.

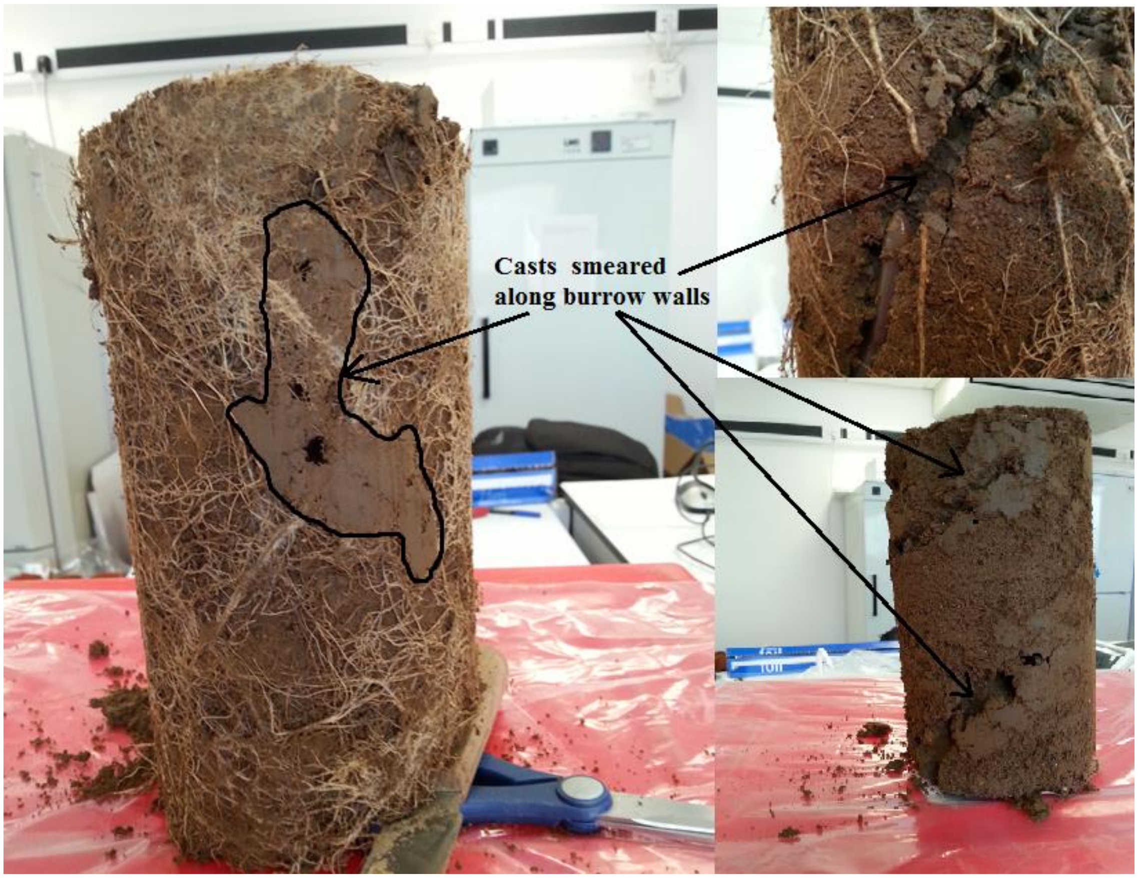

Figure 4 - 8. Smeared casts along the walls of *L. terrestris* burrows. Note the reduced root penetration into the burrow wall material.

4.7. Conclusion

In this study we examined the relative effects of the vertical burrowing earthworm *L. terrestris* and wheat roots, together with their interactions on the partitioning between macropore flow and micropore flow in three soils of differing textures. The experiment was designed so that the earthworm burrows terminated within the soil matrix at the bottom of soil columns. Overall, the results showed that plant-roots were a more important control of soil water flow at different tensions compared to *L. terrestris*. *L. terrestris* significantly increased K_{fs} in the SaL but not the L or SiL soils. In the SaL soil the combined effect of the earthworm and wheat showed the greatest value K_{fs} (up to 270 cm day^{-1}). However, K_{fs} was not significantly different between soil textures. The earthworm density used in this experiment was similar to that typically found in the field. Therefore, our results suggest that vertical burrowing species of earthworm such as *L. terrestris* can have an impact on soil water flow, but their impact is small when burrows are not connected to field drains or a freely draining substrate. Nevertheless, the influence of earthworms and plant root interactions on the partitioning between macropore and micropore flow could be a critical area for further research in other soil types and with other earthworm functional groups with different burrowing strategies.

4.8. References

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Chapter 5

Interaction effects of winter wheat roots and the endogeic earthworm *Allolobophora chlorotica* on soil physical properties including hydraulic conductivity

5.1. Declaration

This chapter is presented as submitted paper for publication without changes to the original submitted main text. The paper was written in the style of Soil Biology & Biochemistry to which it was submitted. For consistency and ease of reading, headings have been numbered, figures and tables inserted and renumbered and citations changed to follow the standard for this thesis.

Interaction effects of winter wheat roots and the endogeic earthworm *Allolobophora chlorotica* on soil physical properties including hydraulic conductivity

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Highlights

- *A. chlorotica* changed soil physico-hydraulic properties;
- Plant root – earthworm interactions caused a bigger change;
- Plant roots and earthworms increased permeability up to 39 times that of the control;
- A higher proportion of flow was through macropores in the presence of earthworms;
- *A. chlorotica* increased soil water holding capacity and plant growth.

5.2. Abstract

Different earthworms have different burrow-styles and these may contribute differently to soil physico-hydraulic processes. Typically, earthworm-hydrology studies focus on vertical burrowing earthworms with little study of the normally more abundant varieties such as the endogeic earthworm *Allolobophora chlorotica* that produces more lateral burrows. Therefore, the interaction between winter wheat roots and *A. chlorotica* was studied in an experiment lasting 16 weeks using soil columns of three different soil textures. Importantly, in our design, the earthworm burrows produced by *A. chlorotica* were prevented from connecting to a drainage system by a buffer layer, separated from the earthworm-bearing soil by a fine nylon mesh. This design results in burrows which have a "deadend", thereby resulting in water flow through the soil matrix rather than specifically through free-draining earthworm burrows and is more likely to mimic conditions found in the majority of field situations. Field saturated hydraulic conductivity (K_{fs}) increased over time in both the plant- and earthworm-present treatments, with a more rapid increase occurring when both plants and earthworms were present. By the end of the experiment, K_{fs} had increased in the plant and earthworm-present experiments by factors of 12, 34 and 39 respectively in loam, silt loam and sandy loam soil textures relative to controls. The presence of the earthworms resulted in an 88.5% increase in the contribution to water flow of pores >3mm diameter. In the majority of treatments, the soil water holding capacity and percentage water stable aggregates in the plant- and the earthworm-present treatments increased significantly compared to controls with the plant and earthworm present treatments showing the greatest increase. Plant growth was greater in the presence rather than absence of earthworms. Our study shows that it is not just vertical burrowing anecic earthworms that can have a significant effect on soil hydraulic properties but that *A. chlorotica*, the most common UK endogeic earthworm, has a role to play in mitigating the impacts of flooding and drought.

Keywords: *Allolobophora chlorotica*, endogeic earthworm, hydraulic conductivity, soil water release curves, soil physical properties, interaction, plant roots.

5.3. Introduction

It is well known that earthworms directly or indirectly affect soil physical and hydrological processes by regulating the surrounding physical environment through their burrowing behavior (Blouin et al., 2013, Jones et al., 1994, Bardgett et al., 2001). Burrows created by earthworms can conduct water and affect other hydrological proprieties of the bulk soil with specific impacts depending on the ecological group (Shipitalo and Le Bayon, 2004). The different ecological groups of earthworms (Bouché, 1977) create burrows of different diameters, from 2 to 11 mm, different lengths, up to 2 m, at different densities and directions and at a rate ranging from 0.1 to 0.2 m day^{-1} (Ehlers, 1975, Ruiz et al., 2015, Shipitalo and Butt, 1999, Bouché, 1977). Burrow diameter is a function of earthworm size and not only of ecological categories. The hydrological effectiveness of these burrows depends not only on parameters cited above but also on the degree of their continuity, connectivity to the soil surface and other macropores and, on the interaction between the water filled burrows with the soil matrix through the burrow walls as well as on the antecedent soil moisture conditions (Beven and Germann, 1982, Smettem, 1992, Weiler, 2005). In field conditions it is only the burrows of some deep burrowing anecic earthworm species that are commonly connected to the drainage systems of poorly drained soils such as tile drains (Shipitalo et al., 2004). In these situations, when it rains and the burrows fill with water a constant flux of water can flow through the burrows at a rate related to their radius as described by Poiseuilles equation. However, non-vertical burrows often terminate within the soil matrix and once they are filled with water, constant flow is not maintained; the water flows through the soil matrix through the burrow wall with the flux of water depending on factors such as the permeability of the soil matrix, burrow length and radius and the wetness of the surrounding soil matrix. The majority of laboratory studies that consider earthworms and hydrology focus on vertical burrows connected to a well-draining base.

Endogeic earthworm species produce lateral burrows within the upper soil layer which are more likely to terminate within the soil matrix compared to the deep burrowing, anecic, earthworms. Although studies on the impact of earthworms on soil-water relations tend to focus on the impact of anecic species on soil water flow (Willoughby and Kladivko, 2002, Shipitalo and Edwards, 1996, Shuster et al., 2002), endogeic species impact on a number of soil properties that influence soil-water relations (e.g. Blouin et al. (2013)). Hallam and Hodson (submitted), showed that the endogeic species *Allolobophora chlorotica* has the potential to increase the percentage water stable aggregates (%WSA) and water holding capacity (WHC) whereas the anecic *Lumbricus terrestris* increased only %WSA. Of the few other existing studies that consider the impact of earthworms on soil water retention and storage, most report an increase in water retention in the presence of endogeic (mainly *A. caliginosa*) (Stockdill and Cossens, 1969, McDaniel et al., 2015, Blanchart et al., 1999) and epigeic (Ernst et al., 2009, Smagin and Prusak, 2008) species or when endogeics were associated with other ecological groups (Boyle et al., 1997). The effect of endogeics on soil water retention could be due to their burrowing behaviour with greater bioturbation of the soil and aggregate formation resulting in a tortuous and complex burrow system that helps to hold water and ensure more flow into the surrounding soil matrix (Weiler and Naef, 2003, Pérès et al., 1998). However, more research is needed to understand their effect on soil hydraulic proprieties particularly when earthworms are associated with plants. In the field, earthworms generally live in vegetated areas and endogeics live in the upper mineral soil where they interact with plant roots (Wu et al., 2017, Springett and Gray, 1997). Therefore, the impact of earthworms on soil hydrology will usually be within the context of the presence of plant roots. Plant roots penetrate the soil more slowly than earthworms, at a rate up to 0.025 m day^{-1} (Ruiz et al., 2015); though like earthworms, as they penetrate the soil they create biopores and modify soil physical properties including hydrological ones (Whalley and Dexter, 1994). Therefore, understanding the interactions between endogeic earthworm and plant roots and their influence on soil physical properties and the surrounding soil matrix flow is crucial to gain an overview of soil processes.

Hallam et al. (submitted), observed a significant impact of plant roots on soil water flow compared to *L. terrestris* when earthworm burrows terminated within the soil matrix; the current work aims to extend that study by investigating the interactive effects of the endogeic earthworm *A. chlorotica* and winter wheat (Skyfall variety) on soil hydrology and soil aggregation. *A. chlorotica* is of special interest as it is the most frequently occurring earthworm species in England, representing 34% of identified specimens (Natural England Commissioned Report NECR145, 2014). *A. chlorotica* is found throughout Europe and is present as an invasive species in North and South America, North Africa and New Zealand; Earthworms that occupy the same ecological niche are present across the world (Dupont et al., 2011). Therefore, we carried out a column experiment in which we studied changes across time of soil hydraulic conductivity and the contribution of different pore sizes to the flow of water through the soil. Treatments with and without winter wheat and in the presence and absence of earthworms were established for three differently textured soils. In addition to soil hydraulic conductivity, we measured soil water release curves, soil water holding capacity, aggregate stability, and plant biomass after a period of 16 weeks of earthworm activity. To establish a metric that could be used for management purposes we define a "Permeability Index" that quantifies earthworm-related changes to field saturated hydraulic conductivity. The main hypothesis that we test is that the combined effect of *A chlorotica* and winter wheat roots will increase soil hydraulic conductivity and soil water retention more than any other treatment for the three tested soil textures. Subsequent hypotheses are that: i) compared to other treatments, the increase over time of field saturated hydraulic conductivity (K_{fs}) will be faster and the K_{fs} will be highest in treatments where both *A chlorotica* and winter wheat are present, ii) as well as increasing soil water retention, the action of the *A. chlorotica* will stabilize soil aggregates which will alter water flow by increasing soil water availability, thereby resulting in increased plant shoot biomass, iii) the trends will be similar across soil textures but the most significant increases in water flow and retention will be in soils with a coarser texture and higher organic matter content.

5.4. Materials and methods

5.4.1. Experiment design

5.4.1.1. Soil columns

PVC columns (16 cm diameter x 30 cm high) were repacked with Cambisols (WBR, 2006) of different textures sampled from a depth of 0-20 cm from the University of Leeds commercial farm (53° 51’ 44” N 1° 20’ 35’’W). The physical and chemical properties of the soils are presented in Table 5 - 1. The soil textures were air-dried and sieved to < 2mm. The columns were filled gradually with c. 1163 g of soil at a time to give a dry bulk density of 1.3 g cm^{-3}. Between soil additions the columns were raised and dropped in order to ensure spatial homogeneity in the pore structure of the soil. This was to allow plant roots to follow the path of least resistance and better exploit of the soil profile, rather than deflecting towards the column wall due to compacted soil layers (Valentine et al., 2012, Burr-Hersey et al., 2017). The soil textures were then wetted with deionized water to approximately 30% gravimetric water content to sustain earthworm activity (Lowe and Butt, 2005, Butt and Lowe, 2011). A layer of melted petroleum jelly was smeared over the inner surface of the columns before adding the soil. The petroleum jelly helped to maintain a good contact between the soil and the column wall in order to avoid preferential flow of water down the sides of the columns (Dawes and Goonetilleke, 2006). The upper and lower surface of the basal 3 cm of soil in the columns was covered with c. 0.5 mm diameter nylon mesh to prevent earthworm escape from the column and connection of their burrows to the base of the columns. Fifteen cm high paper barriers held in place with rubber bands to prevent earthworm escape were placed at the top of the columns. The columns were weighed, covered with plastic film to prevent water loss and stored at 15 °C for four days until planting of winter wheat and addition of earthworms.

Table 5 - 1. Physical and chemical properties of the soils used in the experiment (n = 3 apart from for field dry bulk density measurements where n = 2).

Land use	**pH[1]**	**Organic matter[2] (%)**	**Field dry bulk density[3] ($g\ cm^{-3}$)**	**Clay (%)[4]**	**Silt (%)[4]**	**Sand (%)[4]**	**Textural class**
				< 2 µm	**2-50 µm**	**50-2000 µm**	
Arable	7.73	3.63	1.56	8.93	45.93	45.14	Loam (L)
	7.79	3.31	1.54	8.08	42.09	49.82	
	7.62	3.30		8.29	42.93	48.78	
Arable	7.48	3.86	1.38	4.28	51.24	44.47	Silt loam (SiL)
	7.71	3.46	1.51	4.46	53.83	41.70	
	7.73	3.50		4.21	51.28	44.50	
Pasture	7.26	9.61	1.22	3.54	45.11	51.33	Sandy loam (SaL)
	7.33	9.57	1.14	2.48	34.55	62.96	
	7.23	9.58		3.21	41.56	51.76	

[1] Measured at soil : water ratio of 1:2.5 (Ministry of Agriculture Fisheries and Food, 1986), [2] by loss on ignition at 350 °C (Ayub and Boyd, 1994, CEAE, 2003), [3] using soil density rings of 95 cm^3 and [4] using a MasterSizer2000 laser particle size analyzer (Malvern Instruments, UK).

5.4.1.2. Crop growth and earthworm addition

Winter wheat seeds (*Triticum aestivum*, Skyfall variety) were germinated on Petri dishes. Individual seedlings were transplanted to each column when radicles were ≈ 2 cm length. Plants were grown under natural light for five days and then *A. chlorotica* were added. The columns were placed at 15 ± 1°C and 60 ± 7 % rh in a controlled temperature chamber with a photoperiod of 16 h under 50 W LED lights (Massa et al., 2008, Schroer and Hölker, 2016). To ensure optimum photosynthesis and better distribution of radiation the position of the lights was regularly adjusted during the growing period by measuring the Photosynthetically Active Radiation (PAR) (Evans and Poorter, 2001, Harding et al., 1990, Bugbee and Salisbury, 1988) at the top of each column. The plants were watered every week with deionized water. The *A. chlorotica* were collected from the same site as the test soil textures by hand sorting of soil. In the laboratory active and viable adult earthworms were selected, washed with deionized water and acclimatized to laboratory conditions in containers of the same soil textures used for the experiment. After 3 days at 15 °C in darkness (Butt, 1991), active earthworms were selected again, rinsed, dried with tissue paper and weighed. Eight earthworms of approximately the same total mass (2.32 ± 0.18, $n = 24$) were added to each earthworm-present column. Eight earthworms per column gave a density equivalent to 400 ind m^{-2} equal to the highest density of all adult endogeic species recorded at the University of Leeds farm in December 2016 but greater than the highest density of adult *A. chlorotica* recorded (300 ind m^{-2}). The higher of the two densities was chosen to ensure the burrowing and aggregation of the test soil textures during the 16-week experiments. Earthworm burrowing along column walls as the path of least resistance is a typical bias in mesocosm experiments (Ernst et al., 2009). To discourage this behavior a thin layer of petroleum jelly was applied to the inner wall of the columns and a very shallow vertical hole, slightly less wide than the *A. chlorotica* diameter, was made on the soil surface of each column together with a small amount of water being added to the center of the columns to facilitate earthworms burrowing. Milled and rewetted oven dry horse manure was used to feed the *A. chlorotica* with a surface application of 10 g $adult^{-1}$ $month^{-1}$ (Butt and Lowe, 2011). The columns were established in four replicates for each soil texture in an orthogonal design with the presence and absence of *A. chlorotica* or individual winter wheat plants as the treatments.

5.4.2. Measurements

5.4.2.1. Unsaturated and saturated hydraulic conductivity

Water infiltration rates were measured using a Decagon Mini Disk Portable Tension Infiltrometer (Decagon Devices Inc, 2016) until steady-state flow was reached. Measurements were made over a range of supply tensions of – 6 cm, -3 cm and -1 cm equivalent, according to capillary theory, to water flow through pore diameters of < 0.5 mm, < 1 mm and < 3 mm respectively. The disc infiltrometer was placed on a layer of fine moist sand applied to the soil surface to improve the hydraulic contact between the disc and the soil (Köhne et al., 2011, Reynolds and Elrick, 1991) and measurement was started at the lower tension to avoid hysteresis effects (Baird, 1997). Unsaturated hydraulic conductivity (*K*) at different tensions was derived from cumulative infiltration measurements following the Van-Genuchten Zhang transient method as proposed by Zhang (1997). The field saturated hydraulic conductivity (K_{fs}) was calculated using the Reynolds and Elrick (1991) method that requires cumulative water flow measurements under two or more applied tensions.

Hydraulic conductivities were measured after 3, 9 and 16 weeks of the experiment. The measurements during the experiment (weeks 3 and 9) were made in the controlled temperature room at 15 °C whilst those at the end of the experiment (week 16) were made in the laboratory at 20 °C. All the estimated hydraulic conductivities from the controlled temperature room were adjusted to a standard temperature of 20 °C to account for viscosity differences (Levy et al., 1989, Aleksandrov and Trakhtengerts, 1974).

In this study we define a "Permeability Index (*PI*)" to signify whether the K_{fs} increased and, if so, by how much:

$$PI = \frac{K_{fs(treatment)}}{K_{fs(control)}} \tag{1}$$

where $K_{fs(treatment)}$ is the K_{fs} for a tested treatment and $K_{fs(control)}$ is the K_{fs} for the control treatment.

5.4.2.2. Partitioning flow between different pore classes

The proportion of water flow through different pore size classes and the percent of soil volume accounting for that proportion were calculated based on Watson and Luxmoore (1986). Soil pore classes are not uniformly defined in the literature (Luxmoore, 1981), but here we define macropores as pores > 1 mm (Germann and Beven, 1981, Holden, 2008, Holden et al., 2014, Luxmoore, 1981). Pores of 3 mm diameter were assumed to be the minimum size of *A. chlorotica* burrows (Pérès et al., 1998), therefore we defined smaller and larger macropores as pores of 1 – 3 mm and > 3 mm diameter respectively. Micropores and mesopores were defined as pores < 0.5 mm and in the range 0.5 - 1 mm diameter respectively.

5.4.2.3. Soil water release curves (SWRC)

SWRC determinations are extremely time consuming, therefore measurements were restricted to the loam soil, since earthworms are typically more active in this soil texture (Jongmans et al., 2003, Lowe and Butt, 2005). One soil core of 250 cm^3 (5 cm height x 8 cm diameter) was collected from the surface of each column and analyzed up to pF = 3 using a HYPROP device (UMS, Munchen, Germany) based on the simplified evaporation method (Schindler et al., 2010, Peters et al., 2015). For the very dry end of the SWRC, we measured the relative humidity of a soil sample at equilibrium with potassium carbonate. Using HYPROP-FIT software, the HYPROP measurement campaigns were modeled and adjusted using the measured K_{fs} and K at different tensions. The SWRC were then fitted to our data and hydraulic function parameters were generated using the Van Genuchten (1980) model.

5.4.2.4. Water holding capacity (WHC) and percentage water stable aggregates (%WSA)

Soil cores of 5 cm high x 8 cm diameter were collected from the surface of the columns and measured for WHC following the ISO 11268-2:1998 method. The samples were saturated for 24 hours, drained for 48 hours and then the water content was measured by drying the samples at 105°C overnight. Four grams of 1-2 mm air-dried soils were added into 250 µm sieves to measure the %WSA using bespoke wet sieving equipment (Eijkelkamp, Agrisearch Equipment). The aggregates were pre-moistened for 10 minutes and wet sieved at a rate of 34 times per minute for 3 minutes. The remaining stable aggregates in the sieves were then broken up using sodium hexametaphosphate in order to correct the %WSA for the mass of sand >250 µm. The %WSA was then calculated as the weight of water stable aggregates divided by the sum of unstable and water stable aggregates weight (Milleret et al., 2009a, Kodešová et al., 2009).

5.4.2.5. Winter wheat and earthworm biomass

Winter wheat shoots were collected at the end of the experiment then were oven dried at 70 °C to constant weight. *A. chlorotica* were recovered, rinsed, dried with tissue paper and weighed.

5.4.3. Statistical analysis

The change in hydraulic conductivity with time was tested using two-way mixed ANOVA. Two-way mixed ANOVA was applied to each soil texture in turn with time and treatment as factors. Repeated factor (measurement at different time points) effects were tested for their sphericity and the Bonferroni method was chosen for pairwise comparisons. Tukey's honestly significant difference (HSD) procedure was used for pairwise comparisons between factors. At the end of the experiment ordinary two-way ANOVA was used to analyze the interaction effect between soil textures and treatments for hydraulic conductivities and the other measured parameters. SPSS (IBM Corp. Released 2016, version 24) software was used to estimate the statistical significance of mean differences between treatments. P values of < 0.05 were used as the threshold for significance. In this paper median, minimum and maximum values are presented for directly measured parameters as we make the assumption that the number of replicates ($n = 4$) are insufficient to describe the variation of the data about a mean. Because the PI values are ratios we present mean values calculated as the ratio of the mean K_{fs} of the treatment against the mean K_{fs} of the control, with standard deviations.

5.5. Results

5.5.1. *Soil water flow*

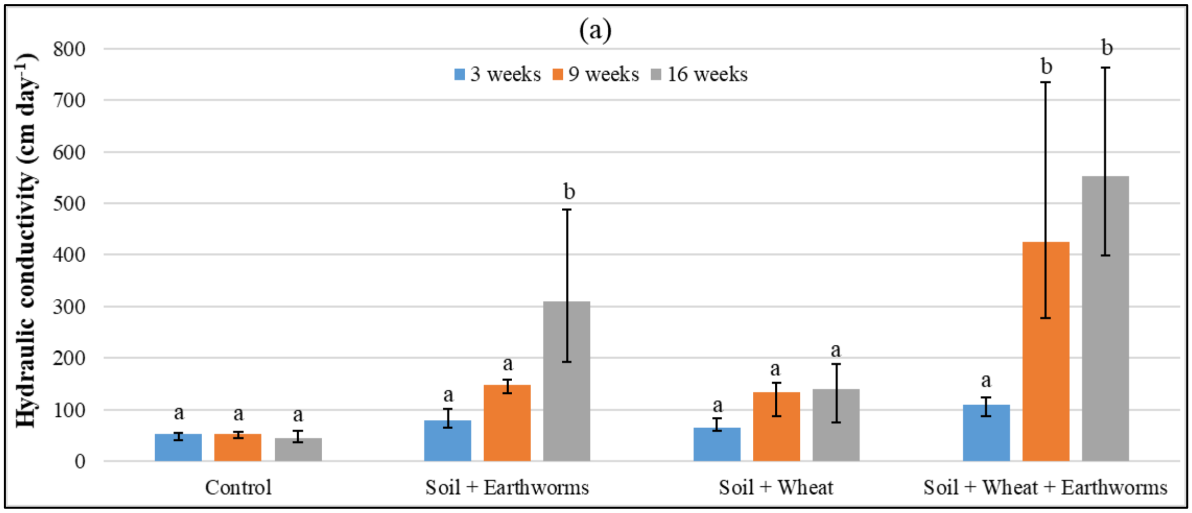
5.5.1.1. *Changes over time of K_{fs}*

K_{fs} varied with soil texture, treatment and time (Figure 5 - 1). For each soil texture there was a significant increase in K_{fs} with time ($p < 0.05$). This increase was significant between each measurement point over the duration of the experiment.

K_{fs} between treatments was also significantly different ($p < 0.001$). There was a significant interaction between treatment and time ($p < 0.001$) indicating that changes in K_{fs} over time varied between the four treatments. The K_{fs} of the bare soils were similar over time across the soil textures (Figure 5 - 1). No significant difference was observed between treatments at week 3 for the SiL and SaL soil but for the L soil the K_{fs} for the earthworm-present treatments was significantly higher than the control ($p < 0.05$). After 16 weeks testing all the treatments showed significantly higher K_{fs} than the controls ($p < 0.05$); the K_{fs} for the earthworm-present treatments had the greatest increase ($p < 0.01$), with the Soil + Wheat + Earthworms treatments showing the greatest value (Table 5 - 2). In all soil textures the K_{fs} in the earthworm-present treatments were significantly larger than Soil + Wheat treatments after 16 weeks testing ($p < 0.01$). Between the three soil textures, SaL soils had the highest and SiL the lowest value of K_{fs} ($p < 0.01$).

Table 5 - 2. Mean of field saturated hydraulic conductivity (cm day^{-1}) of control treatments at the end of the experiment and the Permeability Index (*PI*) (Eq (1)) for the different treatments for the three soil textures (mean ± standard deviation, *n* = 4).

Soil textures	**Treatments**			
	Control (bare soil)	**Soil + Wheat**	**Soil + Wheat + Earthworms**	**Soil + Earthworms**
	Mean of field saturated hydraulic conductivity (cm day^{-1})	Permeability Index (*PI*)		
L	46.23 ± 8.87	2.95 ± 1.17	12.25 ± 4.07	7.00 ± 3.05
SiL	11.92 ± 3.68	4.00 ± 0.30	34.40 ± 2.36	13.50 ± 1,54
SaL	43.89 ± 6.09	5.70 ± 1.20	38.90 ± 7.8	30.60 ± 7.79



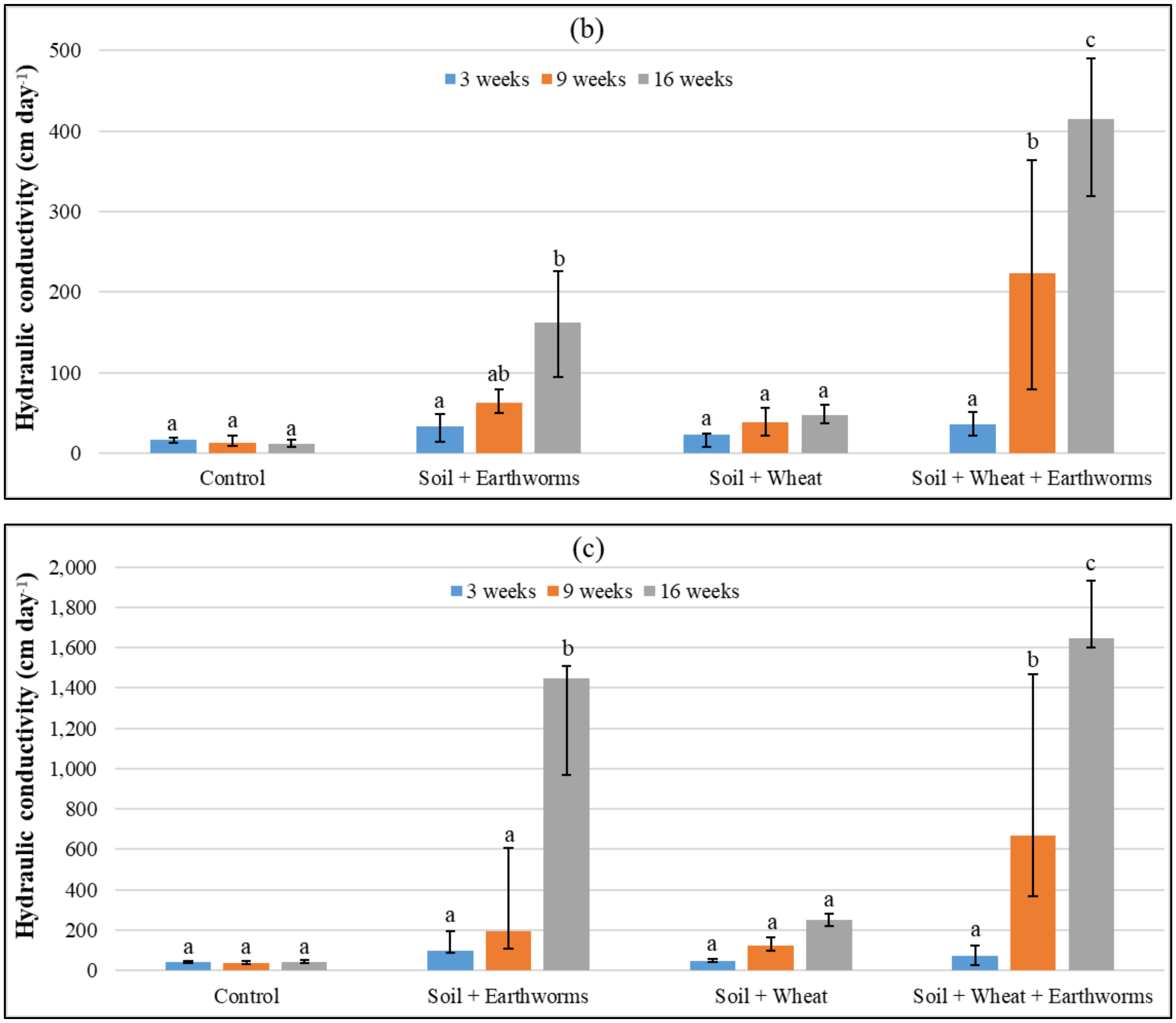


Figure 5 - 1. Median hydraulic conductivity over time at field saturated conditions in (a) the L soil, (b) the SiL soil and (c) the SaL soil. Columns with the same letter over them are not significantly different through time within each treatment (*n*= 4, error bars= max and min values).

5.5.1.2. Changes across time of K

For each soil texture the *K* at -1 cm water tension (K_{-1}) increased significantly across time ($p < 0.05$) and varied significantly between treatments ($p < 0.001$) (Figure 5 - 2). There was also a significant interaction between treatments and time for each soil ($p < 0.001$). K_{-1} showed no significant change with time in the control treatments and, after 16 weeks was greatest in the Soil + Wheat + Earthworms treatment ($p < 0.01$). At 16 weeks, K_{-1} was also significantly greater in the Soil + Earthworms and Soil + Wheat treatments than in the controls, except for the Soil + Wheat treatment in the L soil.

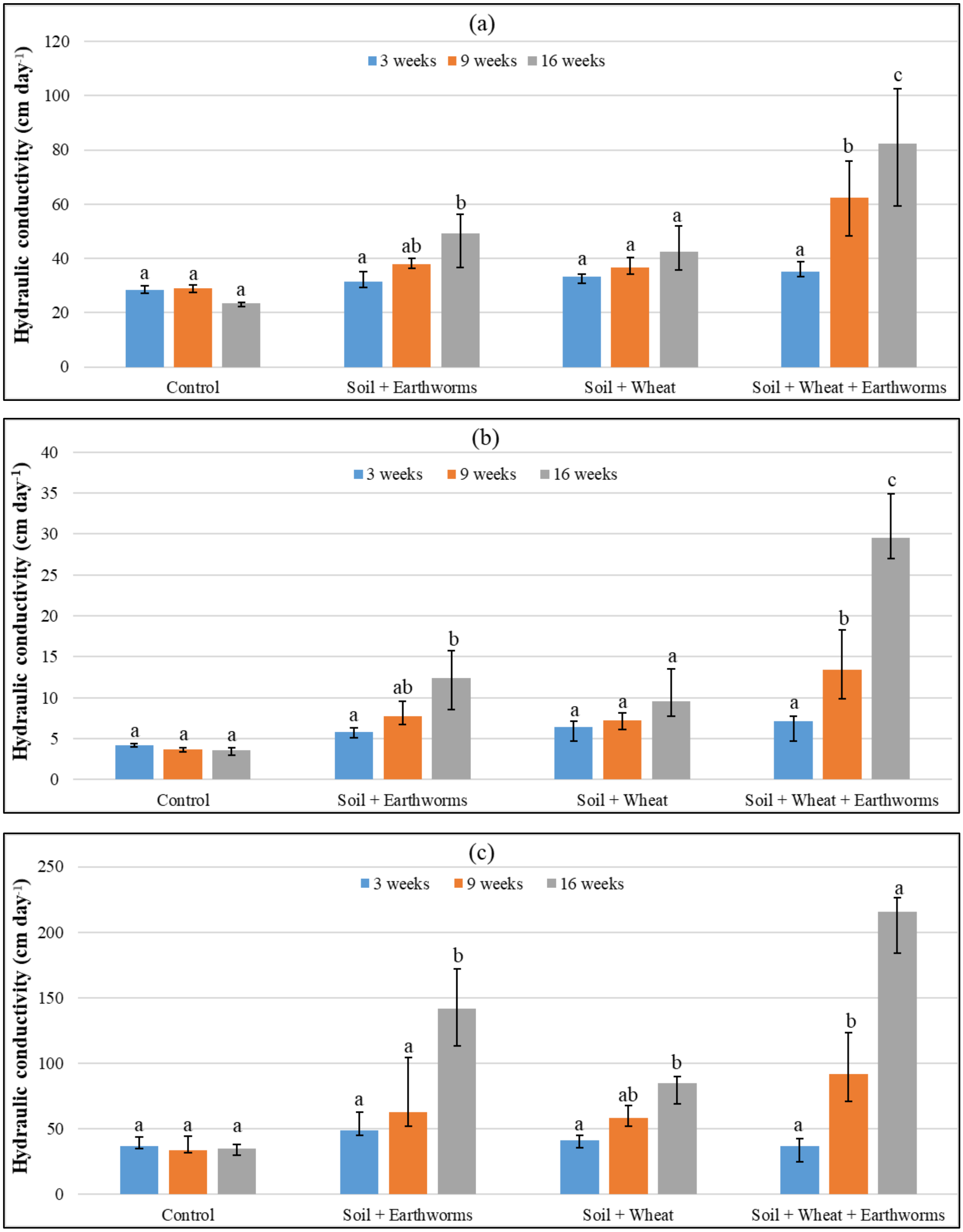
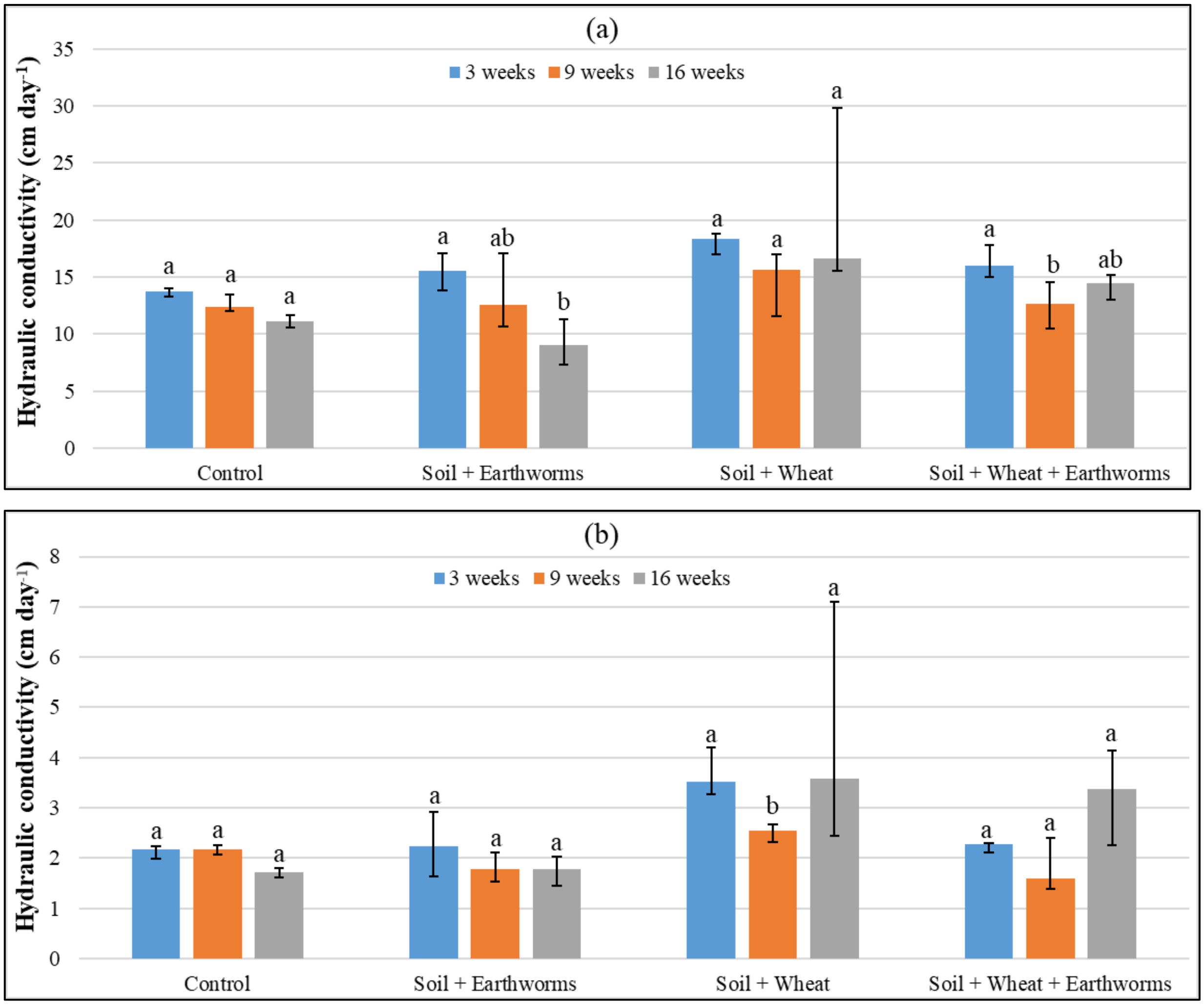


Figure 5 - 2. Median hydraulic conductivity over time at a water tension of – 1 cm in (a) the L soil, (b) the SiL soil and (c) the SaL soil. Columns with the same letter over them are not significantly different through time within each treatment (*n* = 4, error bars = max and min values).

The K at -3 cm water tension (K_{-3}) only changed significantly over time for the L soil texture ($F_{2,24} = 5.2$, $p = 0.013$). In contrast, there were significant differences between treatments for each soil texture ($p < 0.01$) and, apart from the SiL soil, a significant interaction between time and treatment ($p < 0.05$). There were no significant changes in K_{-3} with time in the control treatments. At week 16 K_{-3} of the Soil + Wheat treatment was significantly higher compared to the control for the L and SaL soils. However, by week 16 K_{-3} decreased relative to the controls for the Soil + Earthworms treatment for the L and SaL soils (Figure 5 - 3).



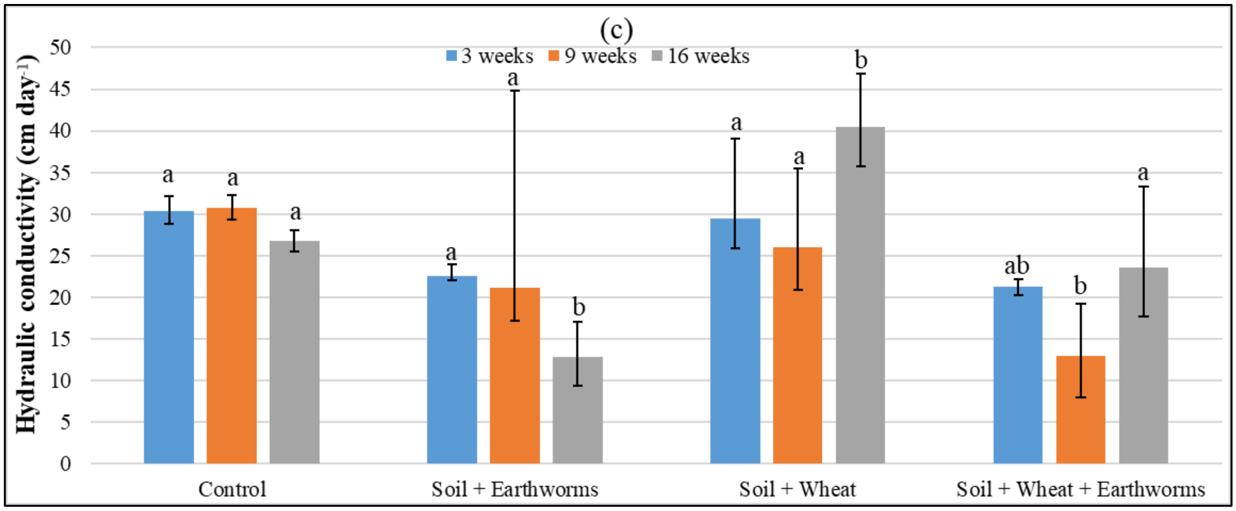


Figure 5 - 3. Median hydraulic conductivity over time at a water tension of – 3 cm in (a) the L soil, (b) the SiL soil and (c) the SaL soil. Columns with the same letter over them are not significantly different through time within each treatment (*n* = 4, error bars = max and min values).

For each soil the *K* at -6 cm tension of (K_{-6}) showed significant differences between treatments and time ($p < 0.05$) (Figure 5 - 4). In all soil textures, K_{-6} did not change over time in the control treatments and decreased significantly over time under the other treatments, except for the Soil + Wheat + Earthworms treatment in the L soil and the Soil + Wheat in the SaL soil where the flow remained the same. At the end of the experiment, for all the soil textures, the earthworm-present treatments had the lowest K_{-6} except for in the L soil where the flow at -6 cm tension of the Soil + Wheat + Earthworms was the same as in the control.

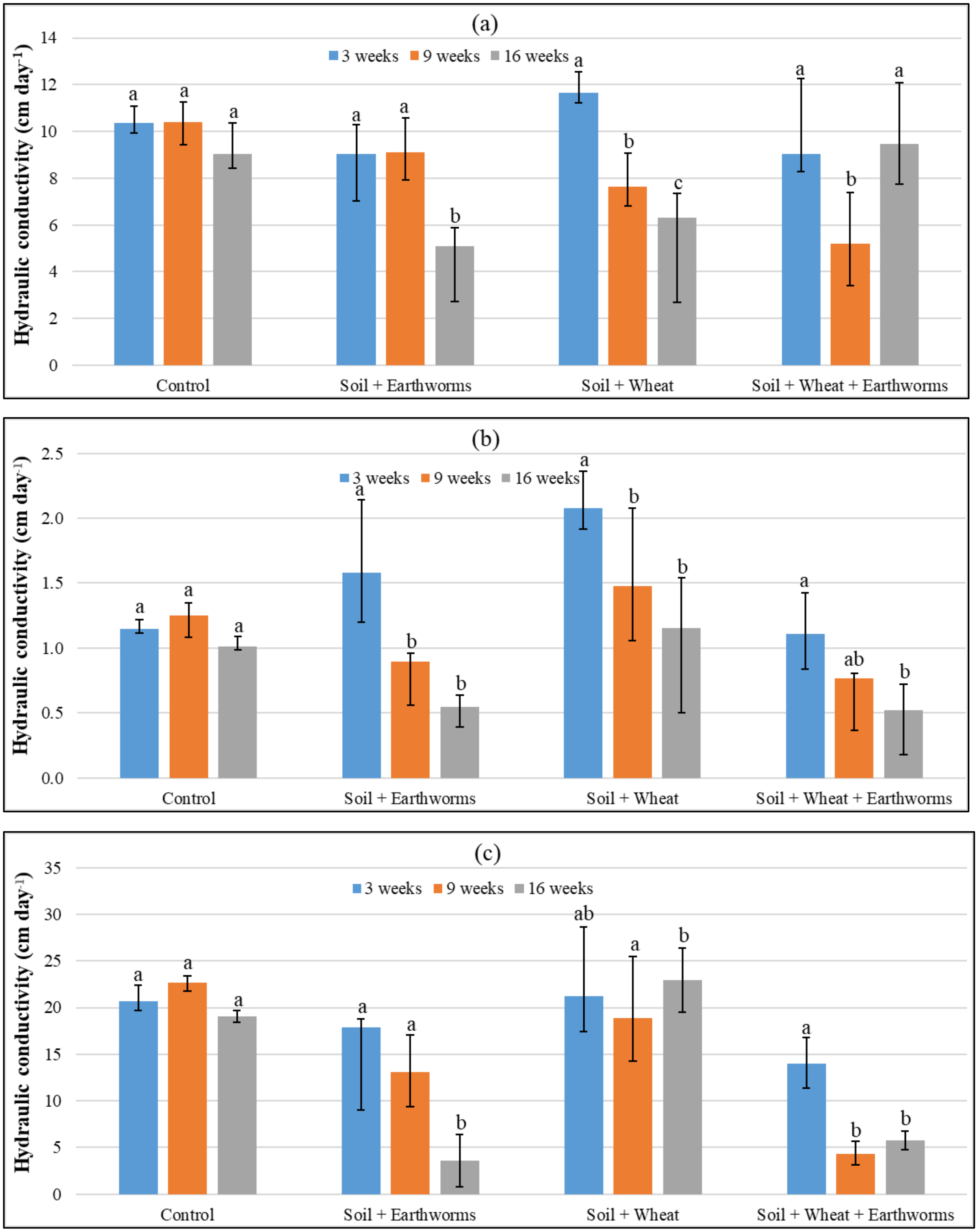


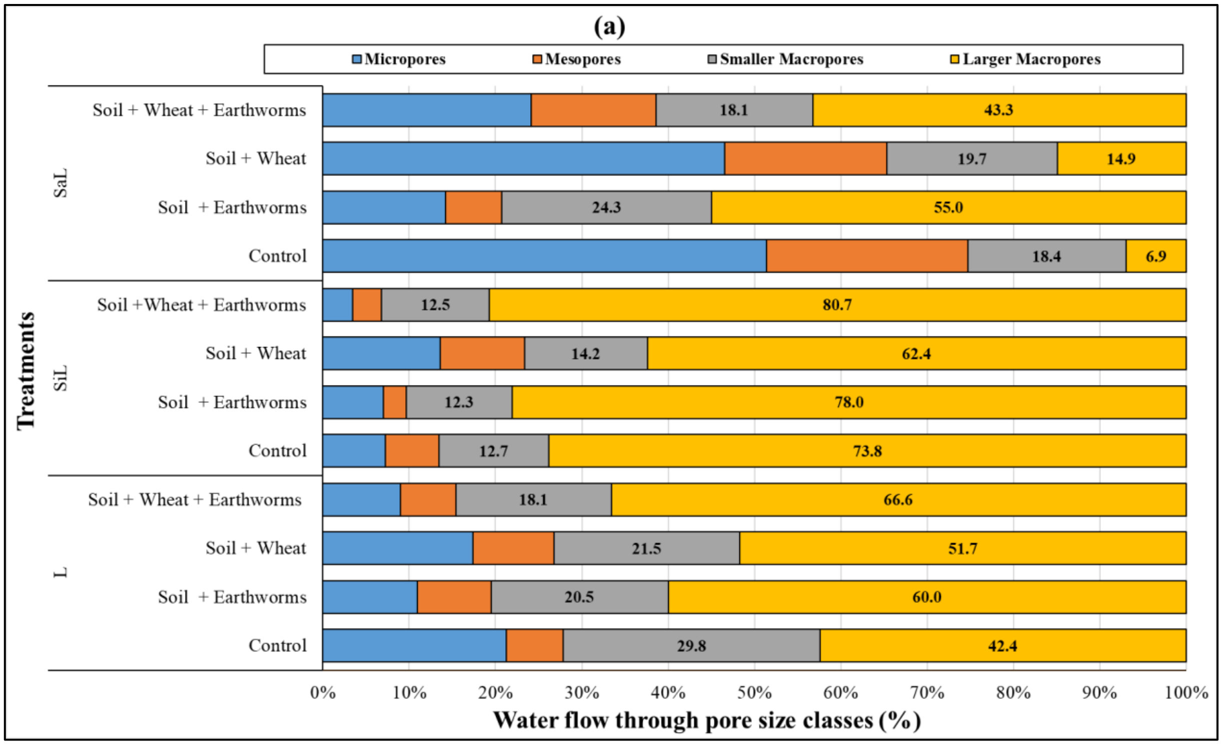
Figure 5 - 4. Median hydraulic conductivity over time at a water tension of – 6 cm in (a) the L soil, (b) the SiL soil and (c) the SaL soil. Columns with the same letter over them are not significantly different through time within each treatment (*n* = 4, error bars = max and min values).

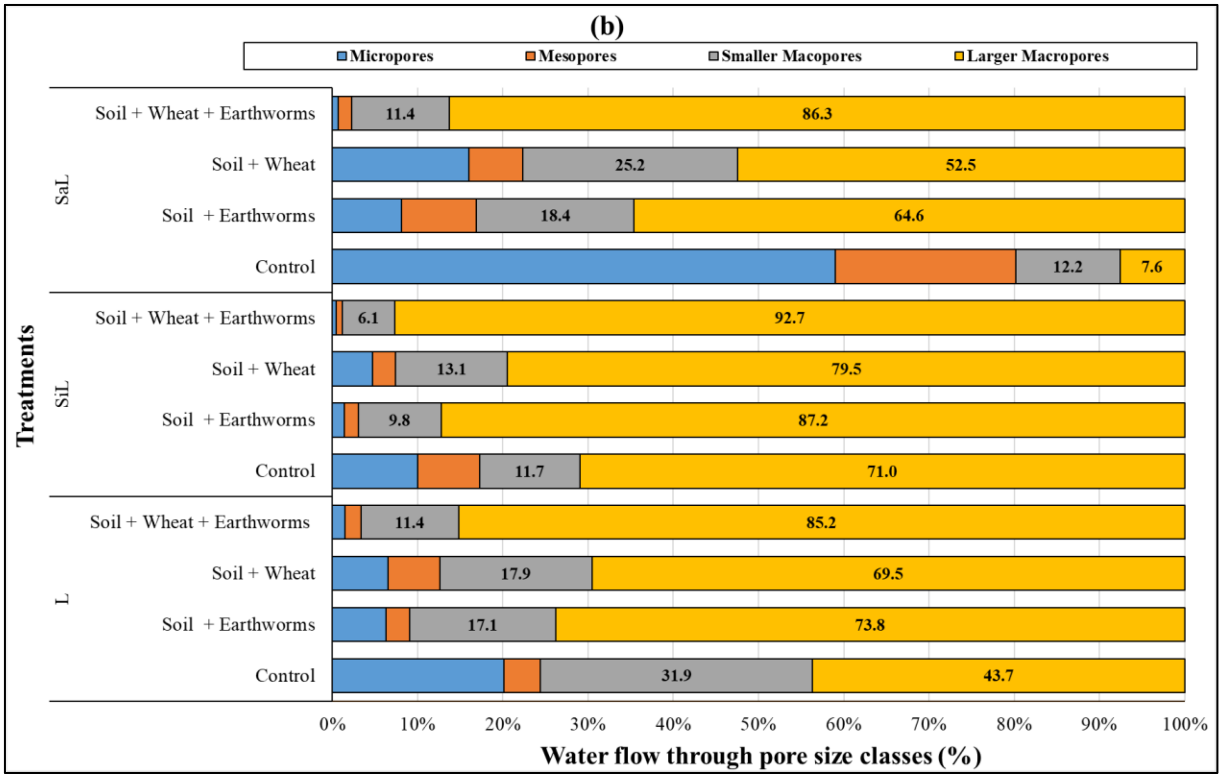
5.5.1.3. Pore size class contribution to water flow

The percentage of flow occurring in larger macropores increased with time in all soil textures and treatments ($p < 0.05$) Figure 5 - 5. However, pairwise comparisons showed that the increase in flow through larger macropores was only significant in the Soil + Earthworms and Soil + Wheat + Earthworms treatments for the L and SaL soils at the end of the experiment. In the control treatment, there were no significant changes across time in the proportion of water flow through the different pore size classes.

The proportion of water flow was also significantly different between treatments ($p < 0.01$) within each pore size class for each soil texture except for smaller macropores in the SaL soils. The proportion of water flow in larger macropores increased gradually over time in the Soil + Earthworms and Soil + Wheat treatments for each soil texture. In the Soil + Wheat + Earthworms treatment, the % flow through the larger macropores reached a maximum after 9 weeks and did not increase further. By the end of the experiment, a higher proportion of flow occurred through the larger macropores in the earthworms-present treatments than in the earthworm-absent treatments (88.5 ± 3 % vs 58 ± 21 %, ($p < 0.05$)). This high proportion of water flow in larger macropores, likely formed by *A. chlorotica*, was transmitted through only 0.0028% of the soil volume as calculated using the method of Watson and Luxmoore (1986). The proportion of flow through each of the different pore ranges was similar for the Soil + Earthworms and Soil + Wheat + Earthworms treatments.

Ordinary two-way ANOVA showed that the greatest proportion of water flow through the larger macropores was seen in the SiL soils. Conversely, the proportion of water flow through pores < 3 mm in diameter was greater in the SaL and L soils.


(a)
Micropores
Mesopores
Smaller Macropores
Larger Macropores
Treatments
SaL
Soil + Wheat + Earthworms
18.1
43.3
Soil + Wheat
19.7
14.9
Soil + Earthworms
24.3
55.0
Control
18.4
6.9
SiL
Soil +Wheat + Earthworms
12.5
80.7
Soil + Wheat
14.2
62.4
Soil + Earthworms
12.3
78.0
Control
12.7
73.8
L
Soil + Wheat + Earthworms
18.1
66.6
Soil + Wheat
21.5
51.7
Soil + Earthworms
20.5
60.0
Control
29.8
42.4
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Water flow through pore size classes (%)


(b)
Micropores
Mesopores
Smaller Macopores
Larger Macropores
Treatments
SaL
Soil + Wheat + Earthworms
11.4
86.3
Soil + Wheat
25.2
52.5
Soil + Earthworms
18.4
64.6
Control
12.2
7.6
SiL
Soil + Wheat + Earthworms
6.1
92.7
Soil + Wheat
13.1
79.5
Soil + Earthworms
9.8
87.2
Control
11.7
71.0
L
Soil + Wheat + Earthworms
11.4
85.2
Soil + Wheat
17.9
69.5
Soil + Earthworms
17.1
73.8
Control
31.9
43.7
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Water flow through pore size classes (%)

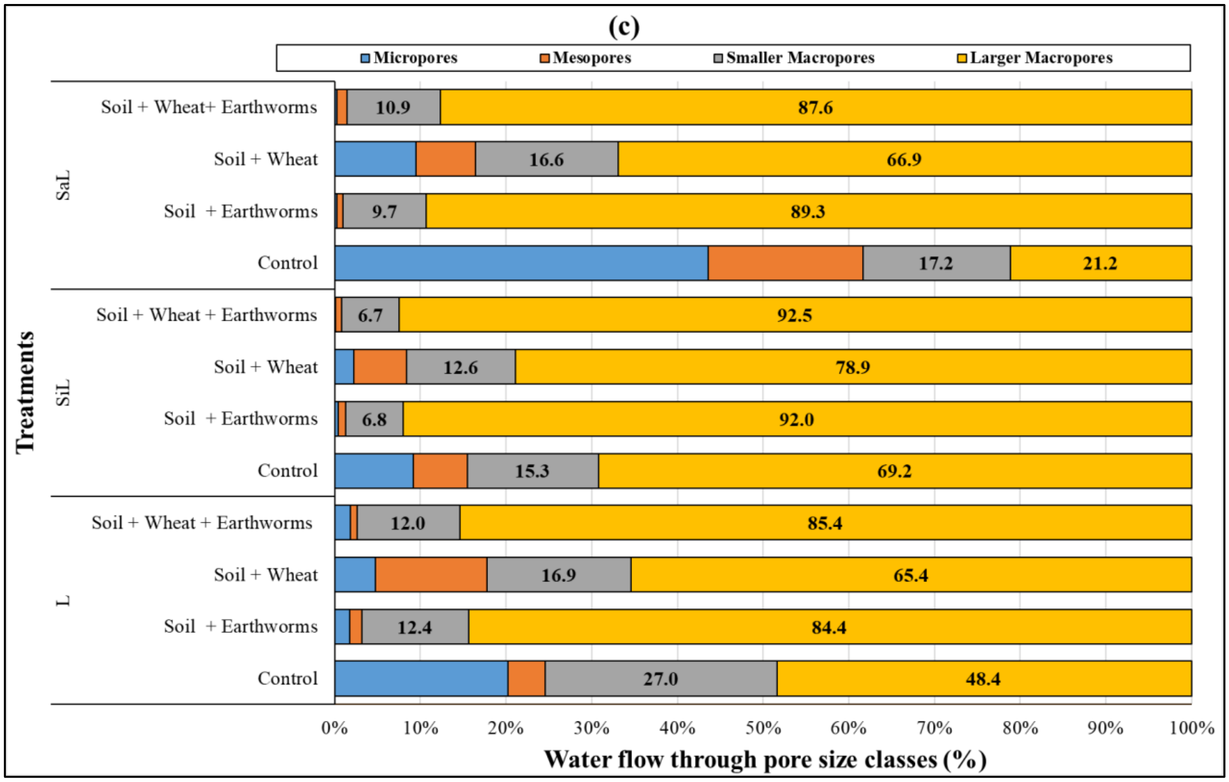


Figure 5 - 5. Pore size class contribution to water flow across time (a, b, c). The figures represent the four treatments, Soil (control), Soil + Earthworms, Soil + Wheat, Soil + Wheat + Earthworms for the L, the SiL and the SaL soils. Letters refer to different time points when measures were made; (a) 3 weeks, (b) 9 weeks and (c) 16 weeks after the start of the experiment.

5.5.2. *Soil water release curves*

The SWRC (Figure 5 - 6) indicate that the volumetric water contents averaged across different treatments were significantly different at different applied water potentials pF. Pairwise comparison shows that at saturation the water content was significantly greater in all the wheat and *A. chlorotica* treatments compared to the control (bare soil treatment). The greatest water content was in the Soil + Earthworms treatment ($p < 0.001$). Water content was measured at potentials of 1.8 and 2.5 corresponding to soils at field capacity for shallow (< 1 m) and deep (> 1 m) water tables (Pertassek et al., 2015, Kirkham, 2005). Under these conditions only the Soil + Earthworms treatment showed a significantly higher water content than the control ($p < 0.01$). The Soil + Wheat + Earthworms treatment also showed a higher water content at field capacity than the control, but the difference was only significant at pF 1.8 ($p = 0.05$ for pF 1.8 and $p = 0.07$ at pF 2.5).

The water content at the wilting point of the soils (pF 4.2), was also significantly higher in the earthworm-present treatments than earthworm-absent treatments, more so for the Soil + Earthworms than the Soil + Wheat + Earthworms treatment. However, despite apparent differences in the plant available water (i.e. the difference between the field capacity and wilting point) for both the shallow and deep-water table conditions between the Soil + Earthworms treatment and the control, these differences were not significant. Apart from at saturation, the Wheat + Soil treatment was not significantly different from the control treatment.

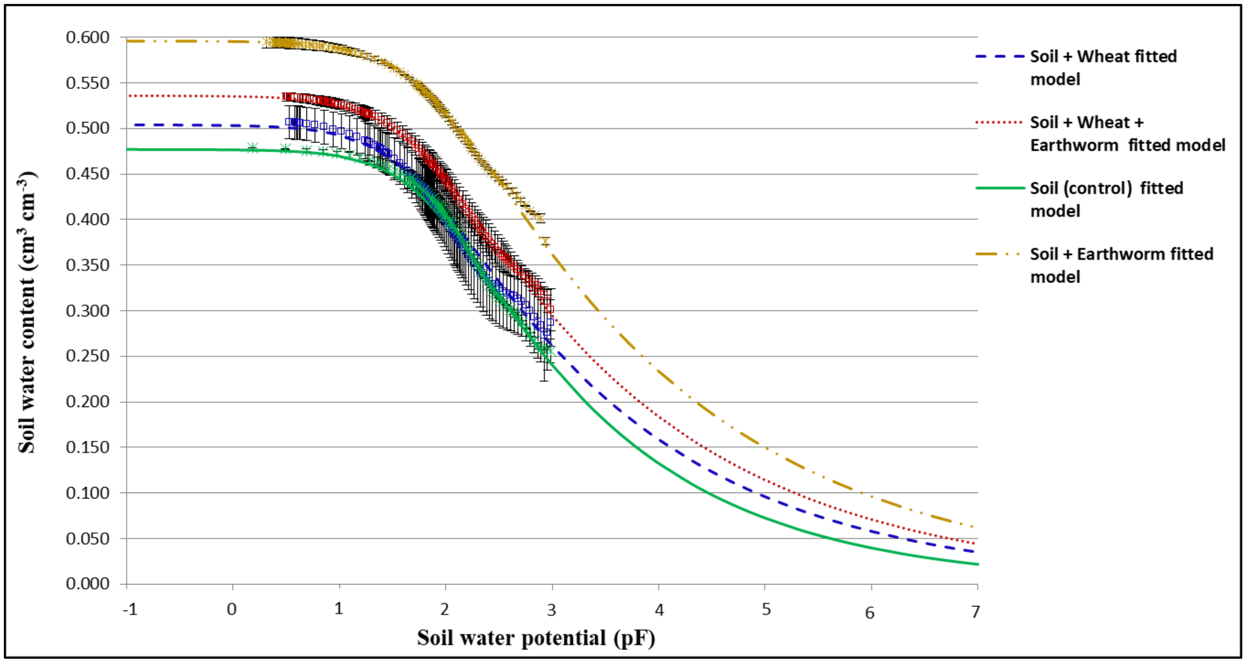


Figure 5 - 6. Water release curves fitted to the measured data using the Van Genuchten (1980) model. The curves represent the four treatments, Soil (control), Soil + Earthworm, Soil + Wheat, Soil + Wheat + Earthworms, for the L soil. Three replicates were combined for each treatment using Hyprop-Fit models. Standard deviation bars are shown.

5.5.3. *Water holding capacity*

The WHC was significantly different between treatments and soil textures ($p < 0.001$) but the treatment effects were similar across the soil textures. Pairwise comparisons indicate that the WHC across treatments was greatest in the SaL and lowest in the L soils ($p < 0.001$). Within each soil texture WHC was significantly higher in planted columns compared to controls with the highest values in the Soil + Wheat + Earthworms treatment (Figure 5 - 7). In all soil textures, the WHC measured in the Soil + Earthworms treatment was not significantly different from that of the Wheat + Soil treatment.

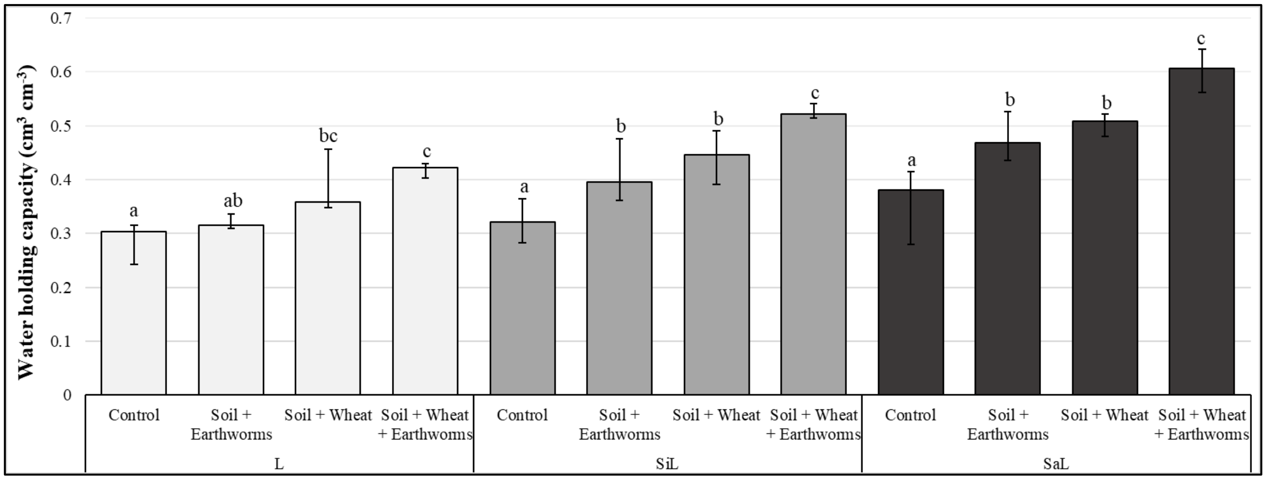


Figure 5 - 7. Median water holding capacity by tested treatments and for the L, the SiL and the SaL soils. Columns with the same letter over them are not significantly different within each soil texture. ($n = 4$, error bars = max and min values).

5.5.4. *Percentage water stable aggregates*

The %WSA varied significantly between soil textures and treatments ($p < 0.001$) (Figure 5 - 8). The SaL soil samples had significantly greater %WSA than the other two soil textures ($p < 0.001$). Between treatments, soil from the planted columns had a greater %WSA than unplanted treatments. The %WSA was significantly highest in the Soil + Wheat + Earthworms treatments. Within unplanted columns of the SiL soil, *A. chlorotica* addition resulted in higher %WSA compared to the control. In L and SaL soils, the %WSA was not significantly different between control and the Soil + Earthworms treatment.

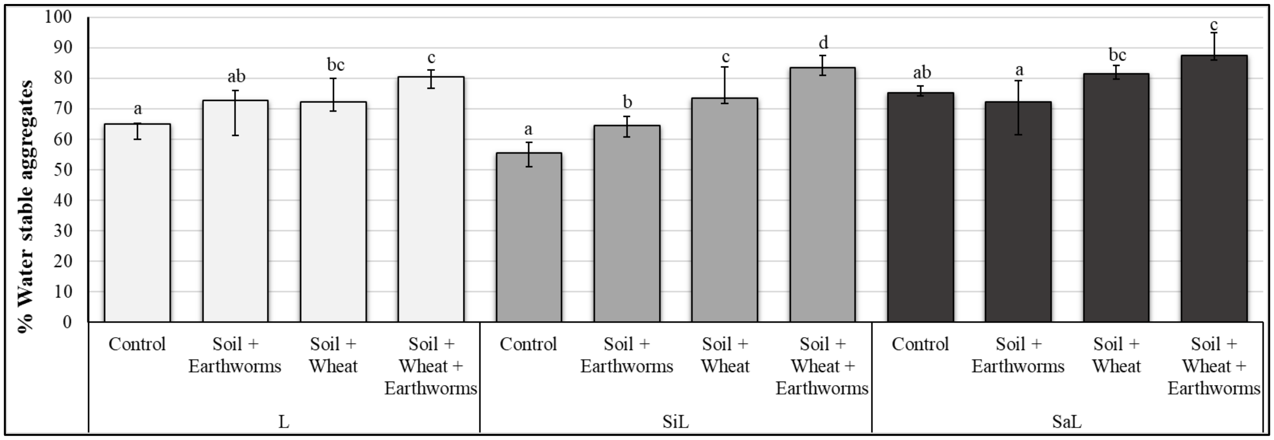


Figure 5 - 8. Median percentage water stable aggregates by tested treatments and for the L, the SiL and the SaL soils. Columns with the same letter over them are not significantly different within each soil texture. (*n* = 4, error bars = max and min values).

5.5.5. Plant biomass

Plant dry matter biomass was greater in the earthworm-present than earthworm-absent treatments ($p < 0.001$, Figure 5 - 9). The dry biomass was lowest in the SiL soils ($p < 0.001$) but was not significantly different between the L and SaL soils. There was no significant interaction between treatments and soil textures.

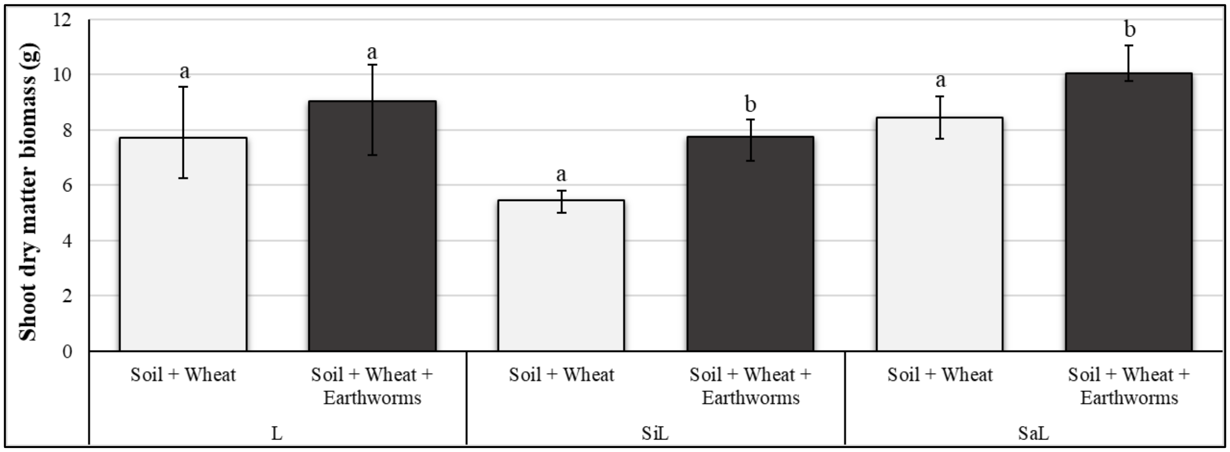


Figure 5 - 9. Median winter wheat shoot dry matter biomass for the treatments with and without *A. chlorotica* and for and for the L, the SiL and the SaL soils. Columns with the same letter over them are not significantly different within each soil texture. (*n* = 4, error bars = max and min values).

5.5.6. Earthworms biomass

All *A. chlorotica* from the columns were recovered at the end of the experiment except for one replicate of the Soil + Earthworms treatment of both the L and SiL soils. One *A. chlorotica* was missing in the L soil and two in the SiL soil. Within other replicates of the same treatment and soil textures, additional juvenile earthworms were found. One juvenile was found in the L soil and four in the SiL soil. Table 5 - 3 shows that the earthworm biomass decreased between the start and the end of the experiment in L and SiL soil. In the SaL soil, the *A. chlorotica* weight increased with time for both the tested treatments. The weight change was, however, not significant.

Table 5 - 3. *A. chlorotica* weight in g at the start and end of the experiment by tested soil textures and treatments. S+W+E: Soil with winter wheat and *A. chlorotica*, S+E: Soil with *A. chlorotica* (*n* = 4).

Soil texture	***Treatments***	***Initial earthworm weight (g)***			***Final earthworm weight (g)***		
		Median	***Min***	***Max***	***Median***	***Min***	***Max***
L	S+W+E	2.42	2.12	2.86	2.24	2.18	2.73
	S+E	2.21	2.11	2.48	2.08	1.81	2.18
SiL	S+W+E	2.35	2.15	2.60	2.39	2.25	2.55
	S+E	2.30	2.11	2.39	1.98	1.85	2.43
SaL	S+W+E	2.33	2.14	2.56	2.75	2.49	2.88
	S+E	2.25	2.16	2.35	2.51	2.31	2.68

5.6. Discussion

5.6.1. Hydraulic conductivity under unsaturated and field saturated conditions

5.6.1.1. Field saturated hydraulic conductivity

The results of this study suggest that the roots of winter wheat and *A. chlorotica* earthworms play a key role in water flow. This impact was common to all the different tested soil textures and was the greatest when wheat and *A. chlorotica* were combined. The K_{fs} data show that this impact was significant within 3 weeks of the start of the experiment in the L soil (Figure 5 - 1). After 9 weeks, the K_{fs} in the Soil + Wheat + Earthworms treatments of all soil textures was much higher than that of all the other treatments. By the end of the experiment the K_{fs} in the Soil + Wheat + Earthworms treatment was greater by a factor of 12, 34 and 39 than the control treatments for L, SiL and SaL soils respectively (Table 5 - 2). In Soil + Earthworms treatments, the K_{fs} was 7, 14 and 31-fold more than the control for L, SiL and SaL soils respectively. Similar trends are reported in the literature both for earthworms in general (Edwards and Bohlen, 1996, Lee, 1985, Hopp and Slater, 1948, Becher and Kainz, 1983) and specifically for endogeic earthworms (Zachmann et al., 1987, Ernst et al., 2009, Ela et al., 1992, Joschko et al., 1992). However, in our study we observed a much higher level of increase in the interaction earthworm-plant root treatments than reported in those earlier studies.

Typically flow rates are low for unstructured soils (Kodešová et al., 2009). Therefore, the high values of the *PI* of the earthworms-present treatments (Table 5 - 2) may in part be due to the comparison between worked soil treatments and a control with an unstructured soil sieved to < 2 mm at the beginning of the experiment. This would be consistent with the extreme findings of Hoogerkamp et al. (1983) who recorded an increase of a factor of 118 to 136 in water infiltration in the presence of earthworms in Dutch polders when using unstructured soil reclaimed from the sea. While some of the hydraulic conductivity values we have found may appear very high (e.g. > 1000 cm day^{-1}), this is still orders of magnitude slower than typical rates of overland flow (Bouma, 1982). If, rather than considering the control soil as the reference value for hydraulic conductivity, we use the Soil + Wheat treatments at 16 weeks as a reference, then the presence of *A. chlorotica* causes an increase of a factor of 4, 9 and 7 in the K_{fs} for L, SiL and SaL respectively, which is more in line with what was reported by previous studies (Blackburn, 1975, Edwards and Bohlen, 1996).

The *PI* indices in Table 5 - 2 could be calculated differently depending on the purposes of land management. As presented in Table 5 - 2, the *PI* are relevant for a management scenario where information is needed regarding the impact of introducing earthworms on water flow through an unstructured bare soil (e.g. sediment deposit, accumulated aeolian or runoff deposit, mine dumps). On the other hand, *PI* calculated with either the wheat-present/earthworm absent or the wheat absent/earthworm-present treatments as the reference values could be used for scenarios where either plant cover or earthworm activity is already established in the absence of earthworms or plants respectively. In the context of land management to reduce the risks of flooding and erosion due to overland flow, our study shows that earthworms and plant roots could be an important tool to increase flow through soils.

5.6.1.2. Unsaturated hydraulic conductivity

Water flow through the soil varied between treatments and soil textures depending on the applied water tension. Flow through < 3 mm diameter pores followed the same trends as that at K_{fs}. By the end of the experiment after 16 weeks, a significant amount of water flows through these pores for each treatment compared to the control for all of the soil textures (Figure 5 - 2). This amount of water was highest in the Soil + Wheat + Earthworms treatments (81.6, 30.2 and 210.3 cm day^{-1} in L, SiL and SaL soils respectively). Although there was higher flow through macropores > 3 mm diameter (Figure 5 - 5) there was still more flow through the < 3 mm pores in the presence of *A. chlorotica* and winter wheat than in their absence. This could be due to the effect of *A. chlorotica* and plant roots on soil structure which influences pore size distributions, connectivity and tortuosity (Carrow and Duncan, 2011). The %WSA was higher in the wheat/earthworm treatments than in the controls (Figure 5 - 8). The changes in the soil structure due to the increase in %WSA results in more tortuous and connected pores which in turn affect the amount of water movement (McDaniel et al., 2015, Veiga et al., 2009).

Water flow through pores < 1 mm and < 0.5 mm diameter was variable depending on the treatment and the soil texture. This indicates that *A. chlorotica* and winter wheat have only a small impact on water movement within those pores and potentially by creating larger burrows (earthworms) and biopores (plant roots) they may also destroy smaller pores.

5.6.1.3. Pore size classes and water flow

Although the K_{fs} at the end of the experiment was greater for the Soil + Wheat + Earthworms treatments than the Soil + Earthworms treatments (Figure 5 - 1), the percentage contribution of pore size classes to water flow was similar between both treatments (Figure 5 - 5). However, the dominance of flow through the larger macropores was reached earlier in the experiment in the Soil + Wheat + Earthworm treatments. Thus, although the proportion of the different pore sizes that contribute to water flow was influenced by the plants (e.g. see the Soil + Wheat treatment, Figure 5 - 5), the main control on water flow through the different pore sizes, at least coarse pores, was the presence of *A. chlorotica.* Both earthworms and plant roots affect soil structure and macroporosity and shape soil hydraulic properties (Fischer et al., 2014). The presence of plant roots in addition to earthworms mainly impacted the amount of water flow by creating more biopores and forming more stable aggregates. The %WSA relative to the controls was increased by the presence of plants and increased further still by the presence of *A. chlorotica* (Figure 5 - 8). SaL soil had both the highest %WSA and a higher water flow compared to L and SiL soils. In addition to the high %WSA, the coarse texture of SaL soils could explain the higher water flow compared to the other soil textures.

After 16 weeks plant growth and *A. chlorotica* burrowing, the Soil + Wheat treatments showed a slightly higher percentage contribution to water flow through smaller macropores, mesopores and micropores compared to earthworms-present treatments suggesting that the earthworms create larger pores but also perhaps destroy smaller pores as soil particles are pushed together. In all treatments, pores < 1 mm diameter contributed less to water flow by the end of the experiment compared to wider pores except for in the controls (Figure 5 - 5). The percentage contribution in these pores was lowest in earthworm-present treatments probably because the wider pores have been created and control the flow water as explained earlier.

Macropores (> 1mm) contributed to 0.015 % of the total soil volume and dominated water flow (87 % of total flow) across all treatments. This result is consistent with findings of previous studies in agricultural (Azevedo et al., 1998) and forest soil (Watson and Luxmoore, 1986). However, when considering only the earthworm-present treatments, only 0.0028 % of the total soil volume was responsible for 88.5 % of water flow through larger macropores, presumably created by *A. chlorotica*. This result reflects the important role of earthworm burrows in controlling water flow.

5.6.2. Soil water release curves

A. chlorotica had a significant effect on the SWRC of the L soil. At saturation, earthworm-present treatments showed a high volumetric water content compared to earthworm-absent treatments (Figure 5 - 6). The saturated and unsaturated hydraulic conductivity data suggest that *A. chlorotica* created wide pores, > 3 mm while burrowing. Endogeic earthworms produce burrows that are more sinuous than those produced by other species (Ernst et al., 2009). Sinuous burrows will create more pore volume for water storage than straight burrows that traverse the same soil volume. Furthermore, water will drain more slowly through sinuous rather than straight burrows because of their geometry (Thomas, 2015, Lal, 2006). These effects will be more significant the more openings there are at the soil surface. In addition, *A. chlorotica* casting behavior increased soil surface roughness (Figure 5 - 10). Micro-depressions of rough soil temporarily store water and may focus it, once they are full, to the opened macropores (Bramorski et al., 2012, Cook and Linden, 1996). Thus, *A. chlorotica* activity may have created “micro-dams” that helped to direct flow down nearby burrows.

Generally endogeic earthworms build complex burrow systems with only a few connections to the surface (Lee and Foster, 1991), though burrowing activity and the number of openings to the surface depend more on the earthworm species than the ecological group (Francis and Fraser, 1998, Bastardie et al., 2003). For example Bastardie et al. (2003) found that the endogeic species *Octalasion lacteum* create burrow systems with more surface openings than the anecic species *Lumbricus terrestris* but fewer openings less than the anecic *Nicodrilus giardia*, whereas Francis and Fraser (1998) reported more soil surface opening in the presence of the endogeic *Aporrectodea caliginosa* compared to *Octalasion cyaneum* (Bastardie et al., 2003). In the current study *A. chlorotica* made several openings to the soil surface (Figure 5 - 10) compared to the anecic *L. terrestris* used under the same conditions in our previous study, Hallam et al. (submitted), and these would have directed water flow into the sinuous burrow network that helps store water. Additionally, the soil surface roughness was more pronounced in Soil + Earthworms treatments compared to Soil + Wheat +Earthworm treatments. *A. chlorotica* was more prone to cast at the surface, making it rougher, when plants were absent. This is most likely related to the availability of food; in this experiment food in the form of manure was added to the soil surface but, in the Soil + Wheat + Earthworm treatments, *A. chlorotica* may have been able to feed in the root zone where conditions are more favorable (See *A. chlorotica* biomass gain in Table 5 - 3). The rougher soil surface, that creates depressions which store water, may in part explain why Soil + Earthworms treatments showed higher water content at saturation condition compared to Soil + Wheat + Earthworms treatment. Casts may also help retain water through the creation of porous aggregates or by the high swelling hygroscopic manure remains in the egested casts (Smagin and Prusak, 2008). However, the data showed higher K_{fs} in Soil + Wheat + Earthworms treatments than in Soil + Earthworms treatment (Figure 5 - 1) which was attributed to flow through > 3 mm pores. Perhaps plant roots impact water flow in the burrows through the improvement of soil structure by increasing the %WSA (Figure 5 - 8) (Katuwal et al., 2013, Filipović et al., 2014) and through root structure that interconnects the soil channels and interact to create a network that act as preferential flow (Ghestem et al., 2011). At field capacity, the soil in the earthworm-present treatments showed higher water content than that in the earthworm-absent treatments although the estimated plant-available water was not significantly different between all treatments.

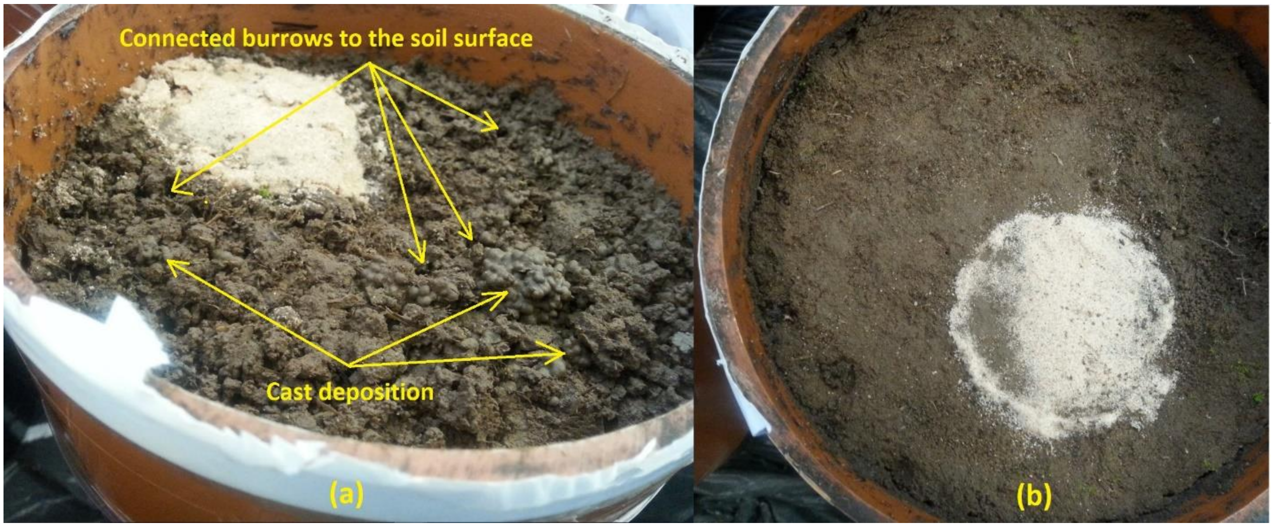


Figure 5 - 10. Soil surface roughness at the end of the experiment of (a): earthworm-present treatment and (b) control treatment.

5.6.3. Water holding capacity

WHC was controlled by both plant roots and *A. chlorotica* burrowing (Figure 5 - 7). For all the soil textures, planted columns showed higher WHC compared to the bare soils. Plant roots modify the soil WHC by direct effects such as creating biopores while growing and soil aggregation by indirect effects such as the excretion of exudates which modify soil physical and hydraulic properties (Doussan et al., 2015). The wheat-present treatments had a higher %WSA than the bare soils (Figure 5 - 8). High levels of stable aggregates improve soil structure including pore size distributions which result in a higher capacity to hold water (Veiga et al., 2009). As in our previous study Hallam and Hodson (submitted) the endogeic earthworm *A. chlorotica* increased the %WSA and consequently the WHC. Earthworms increase aggregates by casting activity which produces interstitial spaces between casts and porosity that helps to hold water (Larink et al., 2001, Kuan Teng et al., 2012, Bouche and AlAddan, 1997). Treatments where both winter wheat and *A chlorotica* were present showed the highest WHC reflecting the impact of both the wheat and earthworms on the soil structure.

The SaL soil had a higher WHC than the other two soil textures across all the treatments. This may be due to higher organic matter content in this soil (Table 5 - 1). Organic matter can increase the soil water content because of the presence of micropores or a variation in pore size distribution through soil aggregation (Bouyoucos, 1939, Reicosky, 2005, Syers and Springett, 1983, Dal Ferro et al., 2012, Tang et al., 2016). The increased processing of high organic matter soils by *A. chlorotica* via ingestion and egestion will lead to the development of hydrophilic coatings on inorganic soil components which may improve further soil water retention in the presence of earthworms (Smagin and Prusak, 2008, Lavelle, 1988).

5.6.4. Wheat shoot biomass

As has been observed in many studies (Stockdill and Cossens, 1969, Laossi et al., 2010, Bertrand et al., 2015), the presence of *A. chlorotica* in the planted treatments increased plant shoot biomass significantly across all soil textures (Figure 5 - 9). This may be due in part to the increase in WHC in those treatments compared to earthworm-absent treatments (Figure 5 - 7) although increases in WHC do not necessarily lead to an increase in plant-available water. Increases in plant biomass in the presence of earthworms has been attributed to increasing N mineralization, K availability in soils and stimulating soil microbial activity (Andriuzzi et al., 2015, Boyle et al., 1997, van Groenigen et al., 2014). Increased aeration in the root zone may also have had an effect (Grable, 1966, Barnes and Ellis, 1979).

In the presence of *A. chlorotica*, the SaL soil produced a higher wheat shoot dry biomass than the other soil textures. This may be due to the increased availability of nutrients due to processing of high organic matter of the SaL soil (Table 5 - 1) by *A. chlorotica* which accelerates organic matter breakdown (Lavelle et al., 1998).

5.7. Conclusion

This study confirmed our main hypothesis that the combination of sub-vertical burrowing *A. chlorotica* earthworms and winter wheat roots would increase soil hydraulic conductivity and the retention of water in soil compared to the presence of either *A. chlorotica* or winter wheat roots alone. The high Permeability Index in the earthworm-present treatments compared to other treatments suggests that endogeic earthworm species can contribute greatly to soil water flow regulation across the landscape. Adopting a minimum till management approach to replace conventional tillage of the upper soil horizons, where endogeic earthworms usually burrow, leads to an increase in earthworm numbers (Spurgeon et al., 2013); our study shows that there would be benefits in terms of water retention and plant growth. We have shown that the effects on soil hydraulic conductivity would be rapid when *A. chlorotica* interact with winter wheat roots. In addition to creating burrows (earthworms) and biopores (plant roots) > 3mm that played a big role in water flow with an average overall contribution of 88.5 % to flow through the soil, *A. chlorotica* and plant roots also induced variation of unsaturated hydraulic conductivity at low water tensions as a result of changing the proportion of micropores (< 0.5 mm). The association of *A. chlorotica* and winter wheat also increased the soil WHC and %WSA which impacts on soil water storage and in part explains the increased plant growth, two vital services that soils provide.

The soil hydraulic conductivity measurements were carried out under conditions where burrows have a finite length and do not connect to a drainage system. We suggest that these conditions are most likely to mimic those found in the majority of field situations, as it is unlikely that the majority of earthworm burrows connect to field drains. Thus, during and after a rain event, burrows and biopores will fill with water which will then flow through the walls and the base of the burrows (Smettem, 1992). During the winter-spring period, most UK soils are wet enough that capillary forces are negligible and only pressure head forces control water flow through burrows. This allows the use of the Permeability Index to estimate infiltration rates under different situations where earthworms and plant roots are present or absent. This may be a useful tool to guide managers to promote soil biota to mitigate surface water erosion or perhaps even downstream flood risk, although further testing of upscaling effects will be required.

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Chapter 6

Effects of earthworms on soil physico-hydraulic properties and wheat growth on arable land converted to ley

6.1. Declaration

This chapter is presented as paper ready for submission. The paper was written in the style of Soil Biology & Biochemistry to which it will be submitted. For consistency and ease of reading, headings have been numbered, figures and tables inserted and renumbered, figures and tables of the Supporting Information section of the original paper renumbered and put in Appendix D and citations changed to follow the standard for this thesis.

Effects of earthworms on soil physico-hydraulic properties and wheat growth on arable land converted to ley

Running title: Seasonal effect of earthworms on soil properties

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6.2. Abstract

The effect of earthworms on the physico-hydraulic properties of soil and on wheat growth in long-term arable soil converted to ley was investigated. Intact monoliths of soil were collected from four arable fields. Six monoliths from each field were defaunated by deep-freezing; earthworms were added to three of the defaunated monoliths (DeF+E) to mimic pasture field density and diversity whilst three were left defaunated (DeF). One monolith from each field was left unfrozen as a control. The monoliths were returned to the field and planted with a ryegrass-clover ley for 12 months. Hydraulic conductivity measurements at -0.5 cm tension ($K_{0.5}$) were taken five times over the year. $K_{0.5}$ significantly increased in Summer and Spring and decreased in Winter. $K_{0.5}$ was significantly greater for the DeF+E monoliths than the DeF monoliths. By the end of the experiment pores > 1 mm in diameter made a significantly greater contribution to water flow in the DeF+E than the DeF monoliths. Arable conversion to ley significantly decreased soil bulk density and increased organic matter content (%OM). Water-holding capacity, plant-available water, %OM content, water-stable aggregates and grass-clover shoot dry biomass were significantly higher in the DeF+E than the DeF monoliths. In a wheat bioassay on the soil following the year-long experiment, significantly more total dry biomass was produced in the DeF+E monolith soil, probably due to the developed soil physico-hydraulic properties. Our results show that earthworms play a significant role in the improvement of soil quality brought about by arable to ley conversion. Boosting earthworm populations is a worthwhile practice to ensure successful and sustainable land reclamation and soil quality improvement.

Keywords: Earthworms, hydraulic conductivity, soil water release curves, water-holding capacity, plant available water, water-stable aggregates, wheat bioassay.

6.3. Introduction

Soil degradation affects about 38% of worldwide agricultural land and is a major threat to future food security, increasing the vulnerability of the increasing global population to impacts of climate change such as flood and famine (United Nations Convention to Combat Desertification, 2017). Estimates of the costs to the global economy of soil degradation range widely from US$231 billion per year (Nkonya et al., 2016) to US$10 trillion per year (The Economics of Land Degradation, 2015), which is equivalent to 160% of the global spend on healthcare (World Health Organisation, 2012). Soil degradation involves both loss of soil functions, such as depleted organic matter content which reduces carbon, water and nutrient storage, and loss of soil volume caused by erosion and compaction. The degradation of soil quality and quantity are interlinked, as reduced water-holding capacity and infiltration rates and poorer crop establishment leave soil more vulnerable to wind and water erosion (Turner et al., 2018, Durán Zuazo and Rodríguez Pleguezuelo, 2008, United Nations Convention to Combat Desertification, 2017).

Intensive arable cultivation growing annual crops on soils that are ploughed and harrowed each year is a major cause of soil degradation causing loss of organic matter, disaggregation and compaction, yet as recently as 2016, 60% of arable land in England has been cultivated in this way (Townsend et al., 2016). Arable farming accounts for 29% of the land use of England and Wales and is responsible for 31% of the total costs associated with soil degradation, in terms of the loss of capacity of soils to deliver ecosystem services (Graves et al., 2015). These costs have been estimated at between £0.9 - 1.2 billion per year without considering the cost of diffuse contamination of soils, soil biota loss and sealing (Graves et al., 2015). The core components the authors identified as contributing to these costs are loss of soil organic matter (47%), compaction (39%) and erosion (12%).

Increasing awareness of the economic and environmental impacts of soil degradation, highlighted by the UK parliamentary inquiry into soil health (House of Commons, 2016), have led to the policy goal in the 25 year Environment Plan (House of Commons, 2018) to sustainably manage all of England’s soils by 2030. Central to achieving this aspiration is the need to increase soil organic matter content, create a better soil structure, enhance the hydrological function of the soil (e.g. enhanced infiltration and water storage) and to protect the soil surface from erosion (Blanco-Canqui and Lal, 2008). This can be achieved in a number of ways, for example through the use of arable-ley rotations and minimum- or no-till methods (van Eekeren et al., 2008, van Capelle et al., 2012) that are less damaging to earthworms (Edwards and Lofty, 1982) and mycorrhizal fungal symbionts of plant roots, that assist in soil aggregate stabilization and soil carbon sequestration (Wilson et al., 2009, Zhang et al., 2013, Asmelash et al., 2016). Whilst these management approaches favour the development of earthworm populations (Chan, 2001, van Capelle et al., 2012) it is not always clear whether it is the action of the earthworms, or other direct aspects of these management methods that give rise to observed improvements in soil properties.

Earthworms are known to increase soil organic matter content by incorporating organic material into soil (Fahey et al., 2013), enhance soil aggregation (Sharma et al., 2017) and generate macropores that increase soil water flow (Francis and Fraser, 1998) which in turn protects the soil surface against erosion (Jouquet et al., 2012). We therefore hypothesized that direct processing of soil by earthworms is responsible for improvements in soil properties and functions including soil carbon sequestration, aggregate stability, and hydrological functions such as infiltration rates (Blouin et al., 2013).

Adding earthworms to improve soil properties has the potential to be economically affordable, environmentally sustainable and socially acceptable (Sinha et al., 2010, Sinha, 2009). Earthworms can process tens of tons of soil each year (Birkas et al., 2010, Zaller et al., 2013) and reproduce rapidly under optimal soil conditions when sufficient food is provided (≈ 27-82 worms per year from a single adult earthworm) (Johnston et al., 2014, Lowe and Butt, 2005, Butt and Lowe, 2011) which could lead to rapid changes in soil properties. The effect of earthworms depends on both which earthworm species are present and on the soil conditions (Clause et al., 2014, Hedde et al., 2013, Hallam and Hodson, submitted). Typically, in field conditions earthworms are present in mixed communities comprising several species, belonging to the three main ecological groups, i.e. epigeic, endogeic and anecic (Kooch and Jalilvand, 2008) that interact with other soil biota and plant roots. Under laboratory conditions, individual earthworm species interactions with plant roots have resulted in significantly greater increases in soil physico-hydraulic properties by endogeic compared to anecic earthworm species (Hallam et al., submitted-a, Hallam et al., submitted-b). The aim of this study was to determine the effects of earthworm communities on soil physico-hydraulic properties and plant growth under realistic conditions by conducting experiments in soil monoliths in arable fields using field relevant earthworm populations.

A field experiment was set up to investigate the influence of earthworms on soil recovery when arable soil is converted to ley. To control earthworm populations we collected intact monoliths from fields (Allaire and Bochove, 2006) and defaunated them by freezing at -20 °C (Bruckner et al., 1995). Other non-destructive earthworm-extraction methods used in previous studies, such as mustard solution and electro-shocking have an incomplete effect on earthworm extraction (Eisenhauer et al., 2008) whereas freezing is totally effective for eliminating them (Bruckner et al., 1995, Barley, 1961). Selected monoliths were reinoculated with earthworm populations typical of local pasture fields and all monoliths were planted with ryegrass and red and white clover to create an arable to ley conversion. The monoliths were monitored for a year with soil hydraulic conductivity measurements being made five times and plant shoot biomass twice. At the end of the experiment, we measured soil water release curves, soil water-holding capacity, bulk density, percentage water-stable aggregates in the 1 - 2 mm size class, organic matter content, total nitrogen content, and earthworm diversity. Soil from each monolith was then used in a 6 week wheat growth bioassay.

6.4. Materials and methods

6.4.1. Site and experimental design

Monoliths were extracted from four arable fields (approximately 70 m from the field margin) in March 2017 at the University of Leeds Farm, Field Research Unit (Bramham, England; 53° 52' 25.2 N 1° 19' 47.0" W; Figure 6 - 1). The fields had been cultivated and used to grow annual arable crops every year since last converted from ley in 1988 (Copse); 1994 (Big Substation East (BSE) and Big Substation West (BSW)), and 2009 (Hillside (HS)). Paired ley strips 3 m wide and 70 m long, extending into the fields from the hedges had been previously established in each of these fields as part of the NERC Soil Security consortium research project SoilBioHedge and the monoliths were extracted from the area of the field between these strips (Figure 6 - 1). The soil in each field was a Cambisol (WBR, 2006) and basic properties are summarized in Table 6 - 1.

Figure 6 - 1. The location of the four arable fields, Big Substation East (BSE), Big Substation West (BSW), Copse and Hillside (HS) in which the experiment was carried out. The paired green strips within each field are the ley strips between which the monoliths were sampled from and near the end of which the monoliths were installed following defaunation by freezing.

Table 6 - 1. Physical and chemical properties of the experimental arable fields (min and max values are shown, *n* = 3; full data are presented in Table D1).

Field	**pH[1]**	**Organic matter[2] (%)**	**Bulk density[3] ($g\ cm^{-3}$)**	**Clay (%)[4]**	**Silt (%)[4]**	**Sand (%)[4]**	**Textural class**
				< 2 µm	**2-50 µm**	**50-2000 µm**	
BSE	7.58	3.14	1.34	4.21	51.24	41.70	Silt loam
	7.75	3.26	1.39	4.46	53.83	44.50	
BSW	7.79	3.13	1.25	4.69	53.03	40.83	Silt loam
	8.00	3.18	1.47	4.82	54.36	42.28	
Copse	7.50	3.51	1.31	8.08	42.09	45.14	Loam
	7.70	3.69	1.53	8.93	45.93	49.83	
HS	6.45	5.03	1.22	3.91	45.75	42.10	Sandy loam
	5.56	5.39	1.28	4.51	53.39	50.34	

[1] Determined using a ratio of 1 : 2.5 (soil : water) (Ministry of Agriculture Fisheries and Food, 1986), [2] by loss on ignition at 350 °C to avoid decomposition of carbonates during ignition (Ayub and Boyd, 1994, CEAE, 2003), [3] using soil density rings of 95 cm^3 volume and [4] using a MasterSizer2000 laser particle size analyzer (Malvern Instruments, UK).

Seven intact soil monoliths were extracted from each field and used to produce three treatments: i) frozen monoliths inoculated with earthworms (DeF+E) (*n* = 3), ii) frozen monoliths not inoculated with earthworms (DeF) (*n* = 3), iii) unfrozen monolith not inoculated with earthworms (control, *n* = 1). The monoliths were planted with a grass-clover (*Lolium-Trifolium repens* and *T. pratense*) mix and were returned to their fields of origin towards the ends of the 2-year-old ley strips furthest from the field edge. The monolith experiment ran until mid-April 2018.

6.4.2. Monolith preparation and grass-clover planting

Seven undisturbed monoliths (22 cm deep, 36 cm long x 27cm wide) were carefully extracted from each field following procedures similar to Allaire and Bochove (2006) and placed into plastic boxes. Each box had drainage holes of 10 mm diameter in the bottom and 8 mm diameter in the sides which were covered in nylon mesh on both the inside and outside (see Figure D1). A mesh size of 0.5 mm was used to try to prevent the entry and exit of earthworms or other soil macrofauna over the duration of the experiment. The control monoliths (n = 1) from each field was immediately placed in an excavated hole in the ley strip of the field from which the monolith was taken. The remaining 24 monoliths were defaunated by deep-freezing at -20 °C for three weeks. After defaunation, all 28 monoliths were planted with the grass-clover ley.

Because clover establishment is slow, established clover plants were collected from the 2-year ley strips in the fields (Figure 6 - 1). The clover roots were thoroughly washed to remove any earthworms and their cocoons. Six white clover plants with extensive lateral root system, and 3 red clover plants with strong taproots, were transplanted into each monolith. The monoliths were kept indoors for one day and then 2 g of hybrid and Italian ryegrass (sorted from an original mixture of clover-grass seeds “Broadsword Hi Pro”, Oliver Seeds, Lincoln UK) were scattered on the surface of each monolith. The monoliths were kept indoors for another 24 hours and then, on the third day, watered to stimulate grass seed germination and moved outdoors. Blocks of soils were excavated in the ley strips of the fields from which the monoliths had been extracted and the monoliths placed in the holes so that they were level with the surrounding soil. Mesh fences of 15 cm height and supported by a bamboo frame were placed around the monoliths to prevent earthworms coming in and out over the surface.

6.4.3. Earthworm collection and culturing

Three defaunated monoliths per replicate field were repopulated with earthworms to give a population diversity and density based on that recorded in nearby pasture fields (Warren Paddock and Valley Field, Figure 6 - 1) on the same farm in December 2016 (Table 6 - 2). Earthworms were collected from pasture fields by excavating the soil to a depth of 20 cm and hand sorting. The earthworms were classified using the OPAL earthworm identification key (Jones and Lowe, 2009), rinsed with deionized water, placed in containers containing the soils from each field from which the monoliths were extracted and maintained at 15 °C in darkness (Butt, 1991) to ensure that individuals were viable prior to the experiment. After 3 days acclimatization, the viable adult earthworms were rinsed again with deionized water, blotted touch-dry with tissue paper, weighed and put in containers ready for inoculation at the surface of the DeF+E monoliths. Earthworms were placed on the surface of the monoliths and watched until they had completely entered the soil to avoid birds or other earthworm predators.

To ensure earthworm inoculation success and survival of the more vulnerable species during the experiment period we followed the recommendations of Butt (2008) in repeating additions after the summer. Our main concern was earthworm survival during high summer temperatures (see Table D6) and low soil moisture conditions. Earthworms were therefore added to the DeF+E monoliths twice, on 31st of March 2017 at the start of the experiment and again on the 15th of November 2017 after the summer, at approximately the same density and species composition (though we were unable to collect sufficient *Allolobophora longa* in March 2017 and sufficient *Lumbricus castaneus* and *Aporrectodea rosea* for the November 2017 restock, Table 6 - 2, see Table D2 for details). To reduce the abundance of earthworms that managed to recolonize the DeF monoliths, we applied up to 3 L of allyl isothiocyanate at 0.1g L^{-1} per monolith (Zaborski, 2003) in November 2017, when earthworms are very active, to expel any earthworms (we found 0 – 8 adults and 1 – 14 juveniles in each monolith, see Table D3 for details).

Table 6 - 2. Number and total weight of the added adult earthworm species across all the fields (*n* = 4) and replicates (*n* = 3) of the frozen monolith with earthworm addition treatments (DeF+E) in March and November 2017. All replicates received the same number of adult earthworms but slightly different weights; (min and max values are shown, see Table D2 for information on individual monoliths).

Earthworm ecological group	**Earthworm species**	**Earthworm addition on the 31st of March 2017**			**Earthworm addition on the 15th of November 2017**		
		Number Added to each monolith	**Total weight (g)**		**Number Added to each monolith**	**Total weight (g)**	
			Min	Max		Min	Max
Anecic	*Lumbricus terrestris*	2	3.83	6.44	2	6.65	9.96
	Allolobophora longa	0	0.00	0.00	1	1.20	3.64
Endogeic	*Aporrectodea rosea*	3	0.50	0.74	2	0.28	0.54
	Allolobophora chlorotica	12	2.78	3.05	12	2.42	3.29
	Aporrectodea caliginosa	3	1.25	1.64	3	0.53	1.45
Epigeic	*Lumbricus castaneus*	3	0.43	0.68	0	0.00	0.00

6.4.4. *Measurements made during the experiment*

6.4.4.1. *Hydraulic conductivity (K)*

K was measured five times, once a season, over the duration of the experiment (Spring 2017, 23-26th May; Summer 2017, 21-25th August; Autumn 2017, 03-10th November; Winter 2018, 26th January to 2nd February; and Spring 2018, 03-06th April 2018). The measurements were made using a Decagon Mini Disk Portable Tension Infiltrometer (Decagon Devices Inc, 2016) with an infiltrometer placed on a thin sand layer to ensure good contact between the tension disc and monolith surface (Köhne et al., 2011, Reynolds and Elrick, 1991). Measurements were made at potentials of -6, -3, -1 cm and -0.5 cm until steady-state flow was reached, corresponding to water flow through pores less than 0.5, 1, 3 and 6 mm in diameter respectively. To avoid hysteresis effects, *K* measurements were made in an ascending tension sequence (Baird, 1997). *K* for three dimensional infiltration was computed using the Van-Genuchten Zhang method (Zhang, 1997). The contribution of different pore size classes (< 0.5, 0.5-1, 1-3 and > 3 mm in diameter) to water flow for each set of measurements was calculated after Watson and Luxmoore (1986). In this study the hydraulic conductivity at a tension of -0.5 cm, close to zero, was assumed to be a good approximation for saturated hydraulic conductivity K_s (Yolcubal et al., 2004).

6.4.4.2. Grass-clover shoot biomass

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

6.4.5. Measurements made after monolith removal

At the end of the experiment all the monoliths were removed and weighed. Earthworms were first extracted using up to 3 L of allyl isothiocyanate at 0.1g L^{-1} per monolith, (Zaborski, 2003). Emerging earthworms were collected for approximately 20 minutes after application. Soil core samples were then collected from the monoliths for the measurement of soil water release curves, soil water-holding capacity, bulk density, percentage water-stable aggregates, organic matter content and total nitrogen content.

After the samples of undisturbed soil had been removed, any remaining earthworms in the monoliths were recovered by hand-sorting. Stones > 1 cm diameter were removed, and subsamples of this sorted soil were collected for a plant bioassay. In the laboratory, the recovered earthworms were rinsed with deionized water, blotted-dry with tissue paper, identified using the Opal identification key and weighed. Because it is hard to differentiate between the juveniles of different earthworm species, juveniles were classified at ecological group level; species that could not be identified to group were classified as unknown.

6.4.5.1. Soil water release curves and water holding capacity (WHC)

Intact soil cores 8 cm diameter x 5 cm high were taken from the surface of the monoliths. The cores were analyzed for water retention at different potentials following the simplified evaporation method (Schindler et al., 2010, Peters et al., 2015) using a HYPROP device (UMS, Munchen, Germany). The measured hydraulic conductivities using the minidisk infiltrometer and the HYPROP measurement campaigns were modeled using the HYPROP-FIT software. The hydraulic function parameters were generated using the bimodal Van Genuchten (1980) model (Durner, 1994). Soil water content at saturation, at field capacity and at wilting point, and plant-available water were calculated from the generated curves.

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

6.4.5.2. Soil bulk density (BD) and percentage water stable aggregates (%WSA)

BD was determined in the monoliths at 0-5, 5-10 and 10-15 cm depth using a bulk density corer with rings of 100 cm^3 (Eijkelkamp, Agrisearch Equipment). Fine earth BD measurements were corrected for the mass and volume of stones >2 mm and were averaged across the three depths for each monolith.

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

6.4.5.3. Percentage organic matter (%OM) and total nitrogen (%N) contents

Organic matter was determined by loss on ignition; as the soil contained carbonates an ignition temperature of 350 °C was used to avoid their decomposition (Ayub and Boyd, 1994, CEAE, 2003). Total N was measured using a vario MACRO C/N Analyser (Elementar Analysis System, Germany). The soil samples were first dried at 105 ºC, sieved to < 2 mm then homogenized to a fine powder with a laboratory ball mill (Retsch, Germany). The samples were then weighed into a tin-foil cups and sealed for dry combustion.

6.4.5.4. Wheat bioassay experiment

Moist homogenized soil from each monolith equivalent to a dry mass of 200 g was added to plastic pots of approximately 7 cm diameter and 13 cm height and stored at 15 °C for four days until planted with pre-germinated Winter wheat seedlings (*Triticum aestivum*, Skyfall variety). Winter wheat seeds were germinated on Petri dishes kept at room temperature in natural light. Three days after germination, seedlings with approximately 2 cm radicles were transplanted into the soil beakers and allowed to grow for five days under natural light. The beakers were then placed under 50 W LED lights (Massa et al., 2008, Schroer and Hölker, 2016) operating 12-hour photoperiod in a controlled temperature room set at 15 °C. The plants were watered three times a week with distilled water. After 6 weeks, shoots and roots were harvested with roots washed free of soil, weighed, oven dried at 70 °C to a constant weight and weighed again.

6.4.6. Statistical analysis

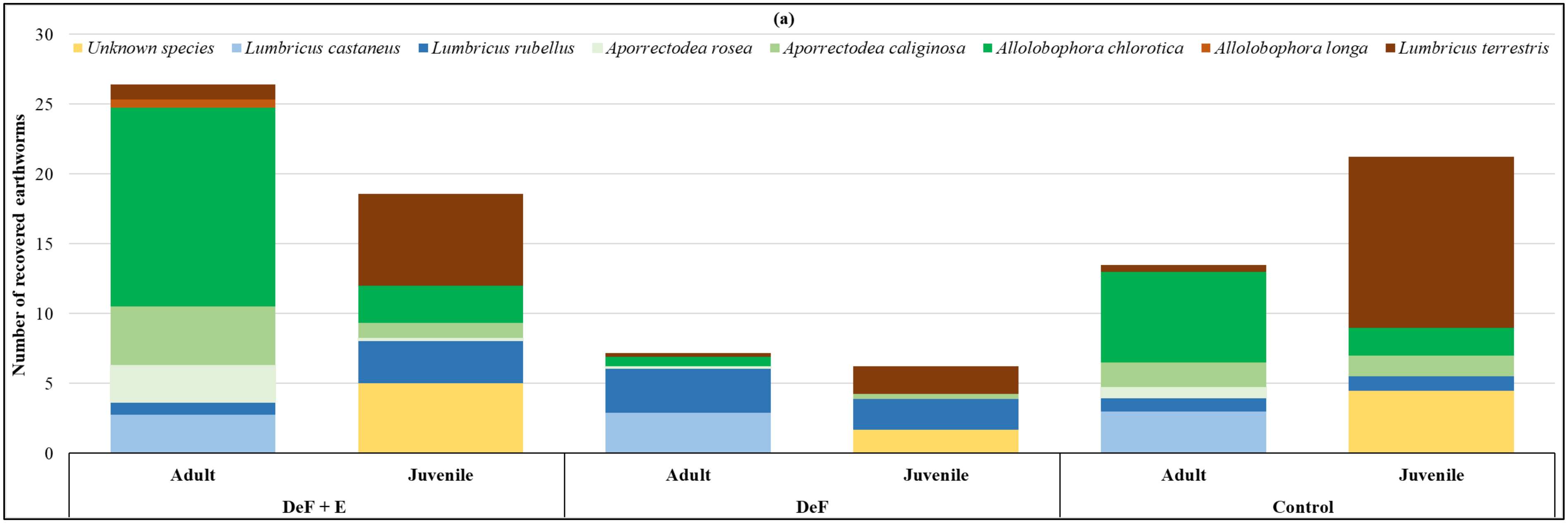
The treatment monolith data were analyzed using a general linear model analysis of variance (ANOVA). Three-way Mixed ANOVA with two between factors (treatment and field name) and one repeated factor (seasonal measurements) was used to analyze K at different tensions. Ordinary two-way ANOVA was used to analyze data of the other measured parameters at the end of the experiment with treatment and field name as factors. Due to logistic limitations we only had one unfrozen control monolith per field. Consequently, ANOVA analysis including control treatments was performed to look at the main effects of treatments and fields or seasons but not at their interactions since the design is an unbalanced ANOVA. The unbalanced design resulted in uneven variances for some parameters, we therefore repeated our ANOVA analysis excluding control monoliths; the statistically significant trends were the same. Therefore, here we report the results of the ANOVA analysis including control treatments. However, the ANOVA analysis excluding the controls was used to investigate interactions between the main factors in the DeF+E and DeF monoliths.

As part of the regular management of the fields where the monoliths were located, a selective herbicide (ASTROKerb®, MAPP 16184, Dow AgroSciences, Cambridge UK) was applied in late November 2017. The herbicide spray drifted onto the edges of the ley strips in HS field killing the grass in one replicate each of the DeF+E (Replicate 3) and DeF (Replicate 3) treatments; this appears to have had a negative effect on the earthworm populations (see Table D4). For this reason, the infiltration measurements in January and April 2018 in addition to the collected data at the end of the experiment for the two monoliths were excluded from the statistical analysis. SPSS (IBM Corp. Released 2016, version 24) was used to estimate the statistical significance of mean differences between treatments. P values of < 0.05 were used as the threshold for significance.

6.5. Results

6.5.1. Recovered earthworms

Figure 6 - 2a shows the mean number of each earthworm species recovered from all the treatments and across all the fields and Figure 6 - 2b the weights. Detailed data for each replicate are given in Table D4 and Figure D3. The number of adults ($F_{1,14} = 55.6$, $p < 0.001$) and the mass of adult ($F_{1,14} = 72.9$, $p < 0.001$) and all (i.e. adult and juvenile) ($F_{1,14} = 13.2$, $p < 0.05$) earthworms recovered from the DeF+E treatments at the end of the experiment were significantly less than the totals added over the course of the experiment (April and November 2017). However, total earthworm numbers recovered (including juveniles) were not significantly different to numbers added. Importantly, for testing our hypotheses, at the end of the experiment, the DeF+E monoliths showed significantly greater total earthworm numbers and weights than the DeF treatments ($p < 0.001$). Total earthworm numbers and weights of the control treatment were significantly higher ($p < 0.05$) than DeF treatment but not significantly different from the DeF+E treatment. At the end of the experiment no significant differences were observed between fields for recovered earthworm numbers, but BSE contained a lower total weight of earthworms than BSW field ($p = 0.049$). There were no significant interactions between fields and treatments. Endogeic species dominated the earthworm population in DeF+E treatments ($p < 0.001$) while epigeics were the dominant species in the DeF ($p < 0.05$) treatments at the end of the experiment.


(a)
Unknown species
Lumbricus castaneus
Lumbricus rubellus
Aporrectodea rosea
Aporrectodea caliginosa
Allolobophora chlorotica
Allolobophora longa
Lumbricus terrestris
Number of recovered earthworms
0
5
10
15
20
25
30
Adult
Juvenile
DeF + E
Adult
Juvenile
DeF
Adult
Juvenile
Control

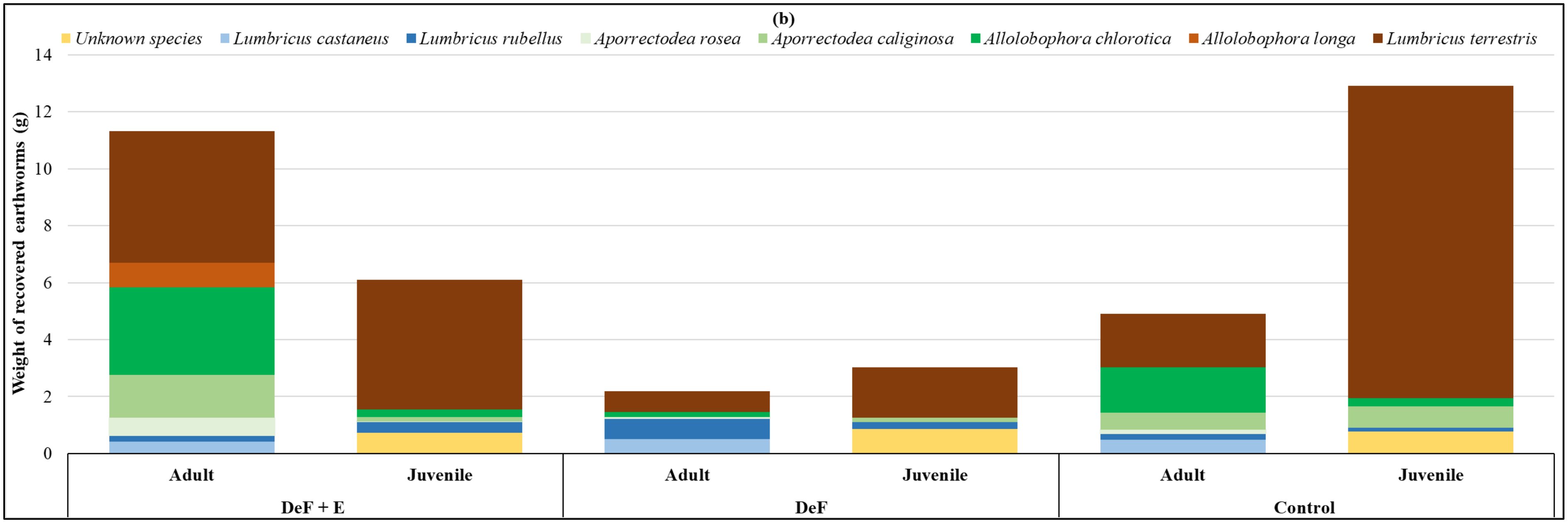


Figure 6 - 2. Mean of the recovered earthworm (a) numbers per monolith and (b) weight (g) per monolith for adults and juveniles across all fields. The figures represent the three treatments; DeF+E = frozen monoliths with earthworm addition (*n* = 12), DeF = frozen monoliths without earthworm addition (*n* = 12) and Control (*n* = 4). The chart stack colour and its shades represent the ecological group of earthworm; Brown : anecic, green : endogeic and blue : epigeic species. *L. terretris* and *L. rebellus* species for juveniles represent anecic and epigiec ecological group respectively, and not species, for the purpose of this graph only. Recovered earthworm numbers and weight for each treatment on a field by field basis is presented in Figure D3.

6.5.2. Seasonal differences in hydraulic conductivity (K)

Figure 6 - 3 presents the seasonal variation in K at -0.5 cm tension ($K_{0.5}$) for all treatments and across all the fields (for K data at different tensions and details of each field see Figure D4). Three-way mixed ANOVA indicated that $K_{0.5}$ varied significantly with season ($F_{4,56} = 17.9$, $p < 0.001$), treatment and field type ($p < 0.001$). $K_{0.5}$ increased from Spring to Summer 2017 ($p < 0.001$), there were no significant differences between Summer and Autumn 2017, a significant decrease from Autumn 2017 to Winter 2018 ($p = 0.003$) and a significant increase from Winter to Spring 2018 ($p < 0.001$). Across treatments $K_{0.5}$ was significantly greater in DeF+E compared to DeF and control treatments ($p < 0.001$). There was no significant difference between DeF and control treatments. Only seasons and treatments showed a significant interaction ($F_{4,56} = 3.1$, $p = 0.023$), with $K_{0.5}$ significantly greater in DeF+E compared to DeF treatments only in Winter and Spring 2018 ($p < 0.001$). Across fields $K_{0.5}$ was higher in HS field compared to BSE and BSW fields ($p < 0.01$) and lower in the BSW field compared to Copse and HS fields ($p < 0.01$).

Apart from a significantly lower K at -1 cm tension (K_1) in Winter 2018 compared to Summer 2017, Autumn 2017 and Spring 2018 ($p < 0.05$), no significant differences were observed in K_1 between seasons. Across all seasons K_1 was not significantly different between fields ($F_{3,14} = 3.1$, $p = 0.06$) and was greater in DeF+E compared to DeF and control treatments ($p = 0.05$). There was no significant difference between DeF and control treatments and no significant interaction effect between main factors.

K_3 at a tension of – 3 cm was significantly different between seasons and fields ($p < 0.001$) but not significantly different between treatments. K_3 increased from Spring to Summer 2017 ($p < 0.01$) and from Winter to Spring 2018 ($p = 0.05$) but decreased from Summer to Autumn 2017 and from Autumn 2017 to Winter 2018 ($p < 0.01$). K_3 was significantly lower in BSE compared to the other fields and higher in HS compared to Copse and BSE fields ($p < 0.05$). There was no significant interaction between fields and treatments. Interactions between seasons and treatments or fields are reported in the Supporting information section.

K_6 was not significantly different between treatments. No differences in K_6 were observed between BSE and Copse or between BSW and HS fields through all the seasons. The highest values were reported for BSW and HS fields ($p < 0.001$). All the fields showed a significant decrease in K_6 from Summer to Autumn 2017 ($p < 0.05$) with no significant differences between the other seasons.

The relative flow of water through different pore size ranges varied between treatments through the experiment period, but there were no significant differences or trends, so the data are not reported in the main text of this paper (see Figure D5). However, at the end of the experiment (Figure 6 - 4) water flow through pores wider than 3 mm showed a significantly greater contribution to total flow in the control treatments compared to the DeF+E and DeF treatments; there was no significant difference between DeF+E and DeF treatments. However, the proportion of water flow through pores wider than 1 mm was significantly greater in the DeF+E treatments compared to the DeF treatments ($p = 0.045$). Flow through pores wider than 1 mm in the control treatment was not significantly different from the other two treatments but had a value that lay between them. No significant differences were observed between fields at the end of the experiment.

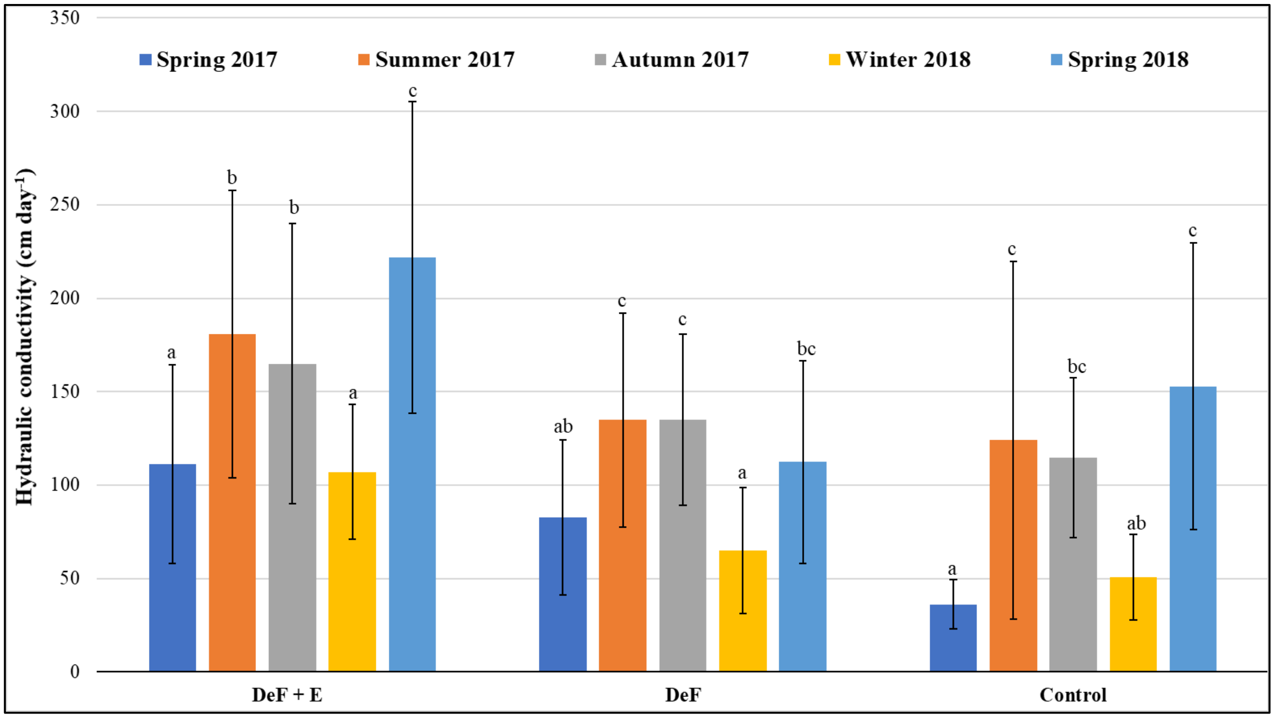


Figure 6 - 3. Mean hydraulic conductivity at -0.5 cm tension across seasons and all the fields (*n* = 4). DeF+E = frozen monoliths with earthworm addition (*n* = 11), DeF = frozen monoliths without earthworm addition (*n* = 11), *n* = 4 for the Control. Columns with the same letter over them are not significantly different through time within each treatment; error bars = standard deviation. Hydraulic conductivity at different tensions across seasons for each treatment and on a field by field basis is presented in Figure D4.

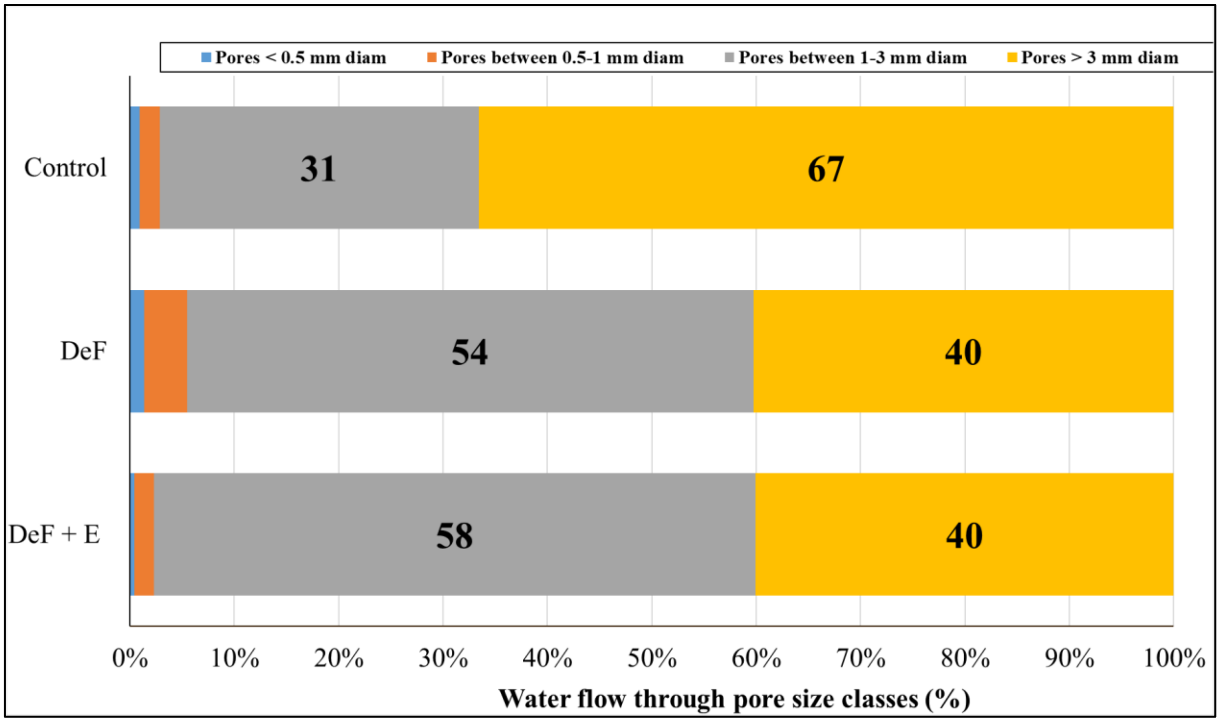


Figure 6 - 4. Mean pore size class contribution to water flow at the end of the experiment across all the fields. DeF+E = frozen monoliths with earthworm addition (n = 11), DeF = frozen monoliths without earthworm addition (n = 11), n = 4 for the Control. Pore size class contribution to water flow across seasons for each treatment on a field by field basis is presented in Figure D5.

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

The SWRC data from the individual cores from each monolith were combined to produce a single SWRC for the DeF+E and the DeF treatments from each field and fitted using Hyprop-Fit models. SWRC for the controls were from single cores (Figure 6 - 5, Figure D6). The generated SWRC were used to derive the soil water content at saturation and at field capacity (at 33KPa (Kirkham, 2005)), and also the plant available water (Table 6 - 3). All these values were significantly higher in the DeF+E compared to DeF and control treatments ($p < 0.05$). No significant differences were observed between DeF and control treatments. All the parameters showed significant differences between fields ($p < 0.05$). HS field had the highest values, but this was only significant compared to Copse field. There was no significant interaction between treatments and fields.

Table 6 - 3. Soil water content (cm^3 cm^{-3}) at different potentials derived from soil water release curves (SWRC) fitted to the measured data using the bimodal constrained Van Genuchten (1980) model (Durner, 1994). DeF+E = frozen monoliths with earthworm addition (*n* = 3), DeF= frozen monoliths without earthworm addition (*n* = 3). Only one treatment was fitted for the Control (*n* = 1). Min and max values are shown; the full data, including values of the combined SWRC of three replicates each for the DeF+E and the DeF treatments, are presented in Table D5.

Field	**Treatment**	**Water content at saturation**	**Water content at field capacity (33 KPa)**	**Water content at wilting point (1500 KPa)**	**Plant available water (33 to 1500KPa)**
BSE	DeF+E	47.5	33.1	11.7	21.4
		50.9	38.2	14.1	25.2
	DeF	45.1	26.4	9.9	16.5
		46.2	33.0	11.6	21.6
	Control	45.7	30.5	12.8	17.7
BSW	DeF+E	46.3	29.7	6.7	21.3
		53.0	33.5	9.5	24.0
	DeF	44.9	23.9	5.5	18.2
		49.9	27.4	7.0	21.8
	Control	50.6	27.0	8.7	18.3
Copse	DeF+E	48.6	33.3	10.8	19.3
		51.9	33.9	14.2	22.5
	DeF	36.0	21.0	6.2	12.6
		43.1	21.9	9.3	15.1
	Control	42.6	29.3	9.8	19.5
HS	DeF+E	46.2	31.6	12.0	19.6
		54.3	42.5	15.7	26.8
	DeF	45.8	30.8	11.6	19.2
		51.5	35.3	14.4	21.3
	Control	45.4	32.2	12.0	20.2

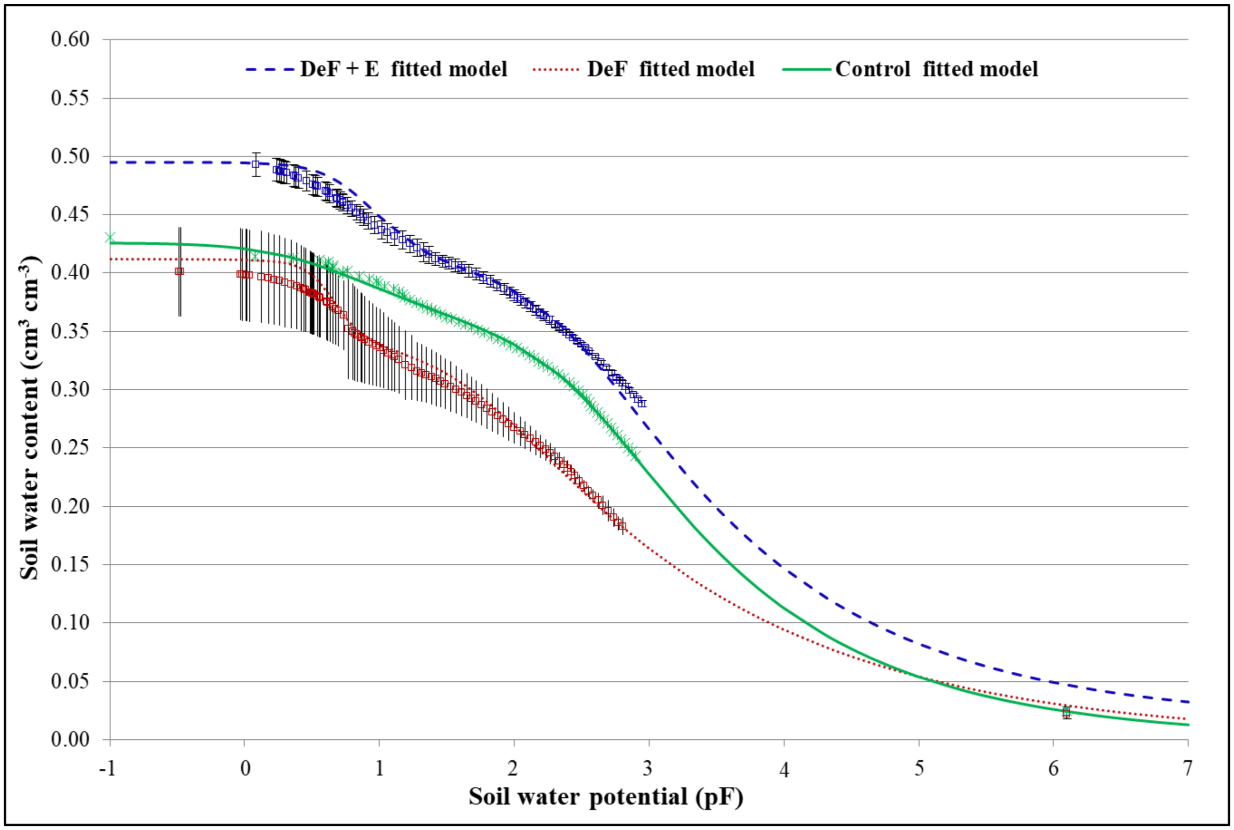


Figure 6 - 5. Soil water release curves (SWRC) of Copse field fitted to the measured data using the bimodal constrained Van Genuchten (1980) model (Durner, 1994). The curves represent the three treatments; DeF+E = frozen monoliths with earthworm addition, DeF = frozen monoliths without earthworm addition and Control. Three replicates were combined each for the DeF+E and the DeF treatments and fitted using Hyprop-Fit models. Only one treatment was fitted for the Control (error bars = Standard deviation). SWRC for BSE, BSW and HS fileds are presented in Figure D6.

WHC varied significantly between treatments ($F_{2,14} = 6.3$, $p = 0.011$; Figure 6 - 6). The WHC of the DeF+E monoliths was greater than the DeF monoliths ($p = 0.05$). There was no significant difference between controls and the other treatments. WHC was significantly higher in HS compared to BSE and BSW fields ($p < 0.01$) and significantly lower in BSE compared to Copse and HS fields ($p < 0.01$). There was no significant interaction between treatments and fields.

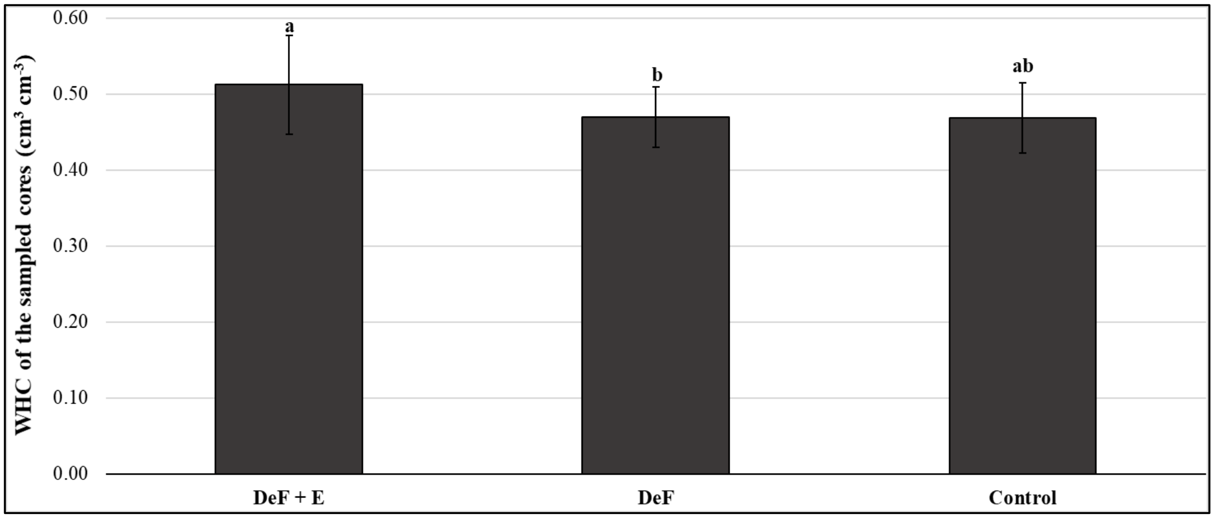


Figure 6 - 6. Mean water holding capacities of the 3.5 cm x 5 cm cores across all fields. DeF+E = frozen monoliths with earthworm addition ($n = 11$), DeF = frozen monoliths without earthworm addition ($n = 11$), $n = 4$ for the Control; error bars = standard deviation. Columns with the same letter over them are not significantly different. WHC for each treatment on a field by field basis is presented in Figure D7.

6.5.4. Soil bulk density (BD)

BD was significantly lower in the DeF treatments at the end of the experiment compared to the initial soil conditions ($F_{1,15} = 8.6$, $p = 0.01$). This suggests that after one year the conversion of arable soil to ley led to a significant decrease in the BD. BD also significantly decreased in the DeF+E treatments relative to starting conditions, however, there was no significant difference in BD between treatments at the end of the experiment (Figure 6 - 7). BD was significantly highest in BSE field compared to other fields ($p < 0.05$). There was no significant interaction between treatments and fields.

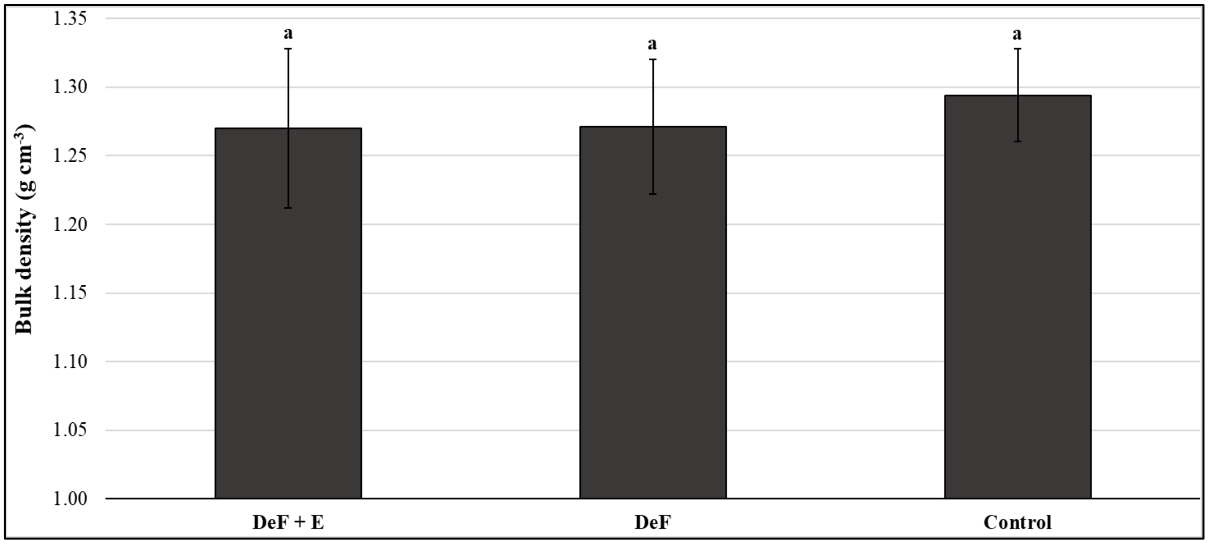


Figure 6 - 7. Mean soil bulk density across all fields. DeF+E = frozen monoliths with earthworm addition ($n = 11$), DeF= frozen monoliths without earthworm addition ($n = 11$), $n = 4$ for Control; error bars = standard deviation. Columns with the same letter over them are not significantly different. Dry bulk density for each treatment on a field by field basis is presented in Figure D8.

6.5.5. Percentage water stable aggregates (%WSA)

The %WSA was greater in the DeF+E monoliths than in the DeF monoliths ($p = 0.014$). %WSA of the control treatments was between the DeF+E and the DeF treatments with no significant differences (Figure 6 - 8). %WSA also varied significantly with field ($F_{3,14} = 7.6$, $p = 0.003$); %WSA was highest in the HS field. There was no significant interaction between treatments and fields.

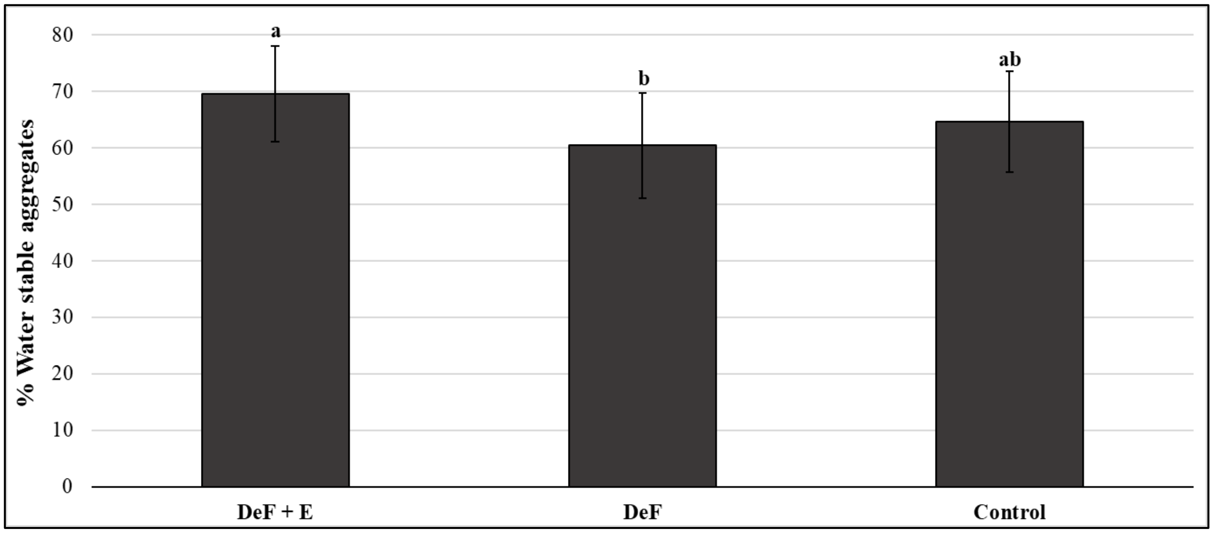


Figure 6 - 8. Mean percentage water stable aggregates (%WSA) across all fields. DeF+E = frozen monoliths with earthworm addition ($n = 11$), DeF= frozen monoliths without earthworm addition ($n = 11$), $n = 4$ for Control; error bars = standard deviation. Columns with the same letter over them are not significantly different. %WSA for each treatment on a field by field basis is presented in Figure D9.

6.5.6. Percentage organic matter (%OM)

Comparison of the DeF treatments at the end of the experiment (Figure 6 - 9) with the initial soil conditions (Table 6 - 1) indicate that the conversion of arable soil to ley led to a significant increase in the %OM content of the monoliths in just one year ($F_{1,14} = 399.2$, $p < 0.001$). In addition, there was a significant increase of the %OM content of the DeF+E treatments compared to DeF treatments ($p < 0.01$). The %OM of the control treatments was between the DeF+E and the DeF treatments with no significant differences. The %OM was highest in HS field and lowest in BSW field ($p < 0.0001$). There was no interaction between treatments and fields.

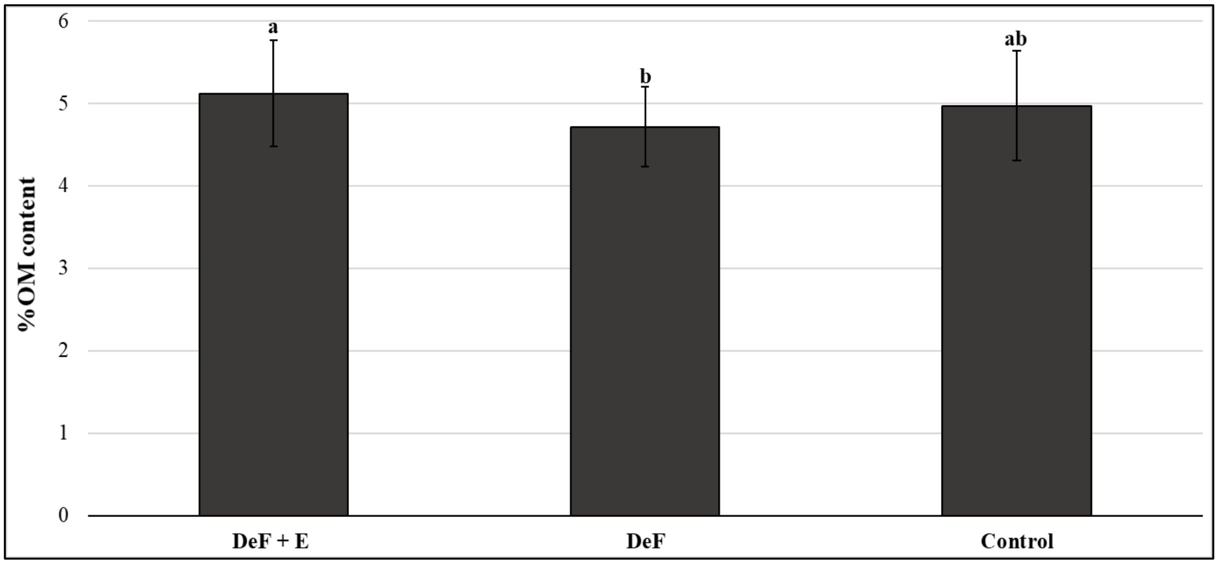


Figure 6 - 9. Mean %OM in the different treatments across all fields. DeF+E = frozen monoliths with earthworm addition ($n = 11$), DeF= frozen monoliths without earthworm addition ($n = 11$), $n = 4$ for Control; error bars = standard deviation. Columns with the same letter over them are not significantly different. %OM for each treatment on a field by field basis is presented in Figure D10.

6.5.7. Total nitrogen content (%N)

The addition of the earthworms to the defaunated monoliths resulted in a significant increase in %N compared to the DeF treatment ($p < 0.05$). %N in the control treatments was between the DeF+E and the DeF treatments with no significant differences (Figure 6 - 10). %N was significantly lower in the HS field compared to the other fields ($p < 0.001$). The interaction between treatments and fields showed significant differences between treatments except in the BSE field.

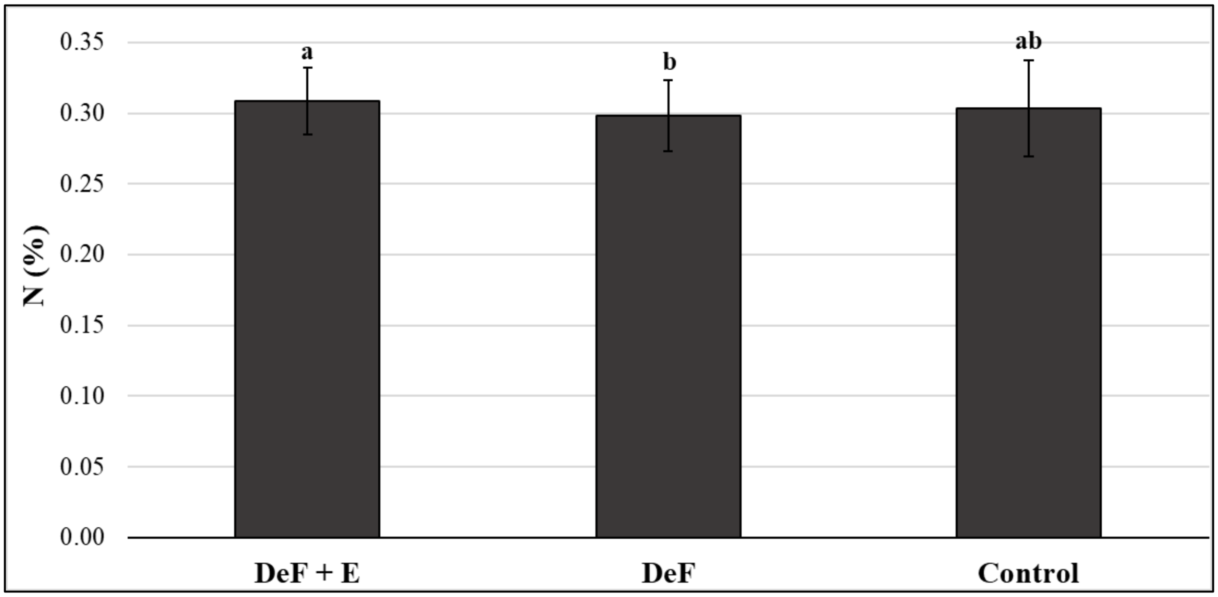
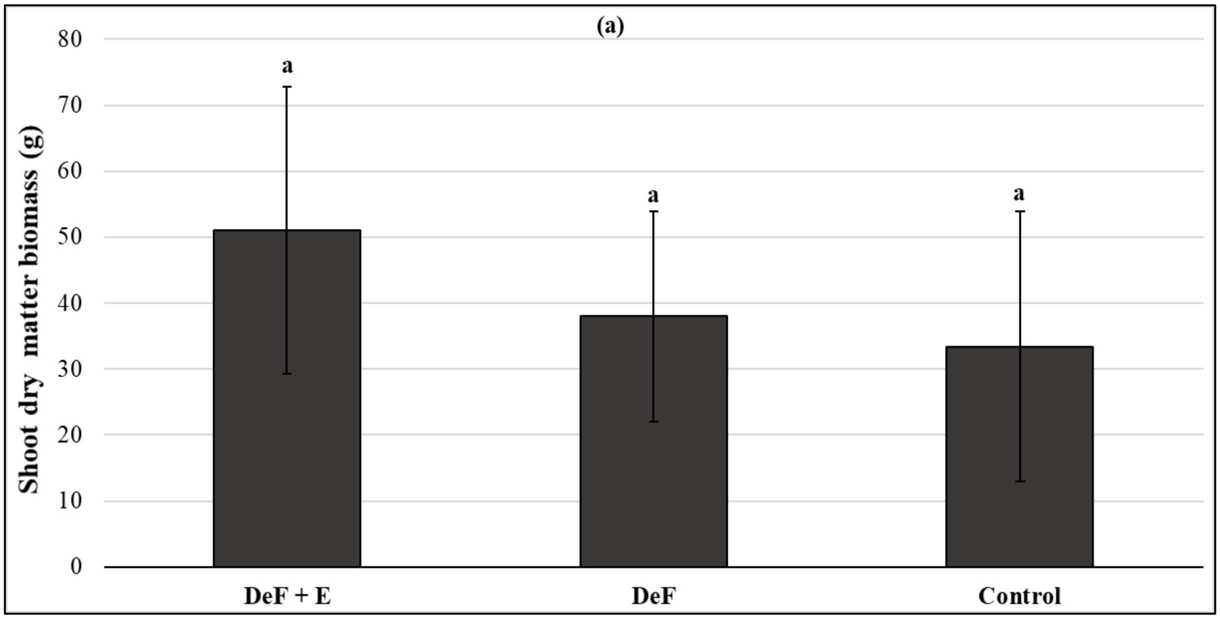


Figure 6 - 10. Mean % N in the different treatments across all fields. DeF+E = frozen monoliths with earthworm addition (*n* = 11), DeF= frozen monoliths without earthworm addition (*n* = 11), *n* = 4 for Control; error bars = standard deviation. Columns with the same letter over them are not significantly different. %N for each treatment on a field by field basis is presented in Figure D11.

6.5.8. *Plant dry biomass*

6.5.8.1. *Grass and clover shoot dry biomass of the monoliths*

No significant differences between treatments were observed at the midpoint of the experiment. At the end of the experiment, more biomass was produced in the DeF+E monoliths compared to the DeF monoliths (p = 0.004). Plant shoot biomass in the control treatment had an intermediate value and was not significantly different from the DeF+E and DeF treatments. More biomass was collected in September 2017 than in April 2018 (Figure 6 - 11). Over both periods, the BSE and HS field produced the least dry shoot biomass (p = 0.001 and p = 0.005 in September 2017 and April 2018 respectively). At the end of the experiment only grass was present in HS field monoliths. The low shoot dry biomass in the BSE field and HS field in September 2017 (Figure 6 - 11a) is thought to have been due to vole activity; plant stems at the soil surface of the monoliths showed evidence of grazing, vole galleries were present around the monoliths and the mesh fences had been pierced at surface level. This impacted the weight of the collected plant material in those fields in Spring 2018. Voles have a preference for clover over grass (DeJaco and Batzli (2013) explaining why only grass was collected in the HS soil at the end of the experiment.



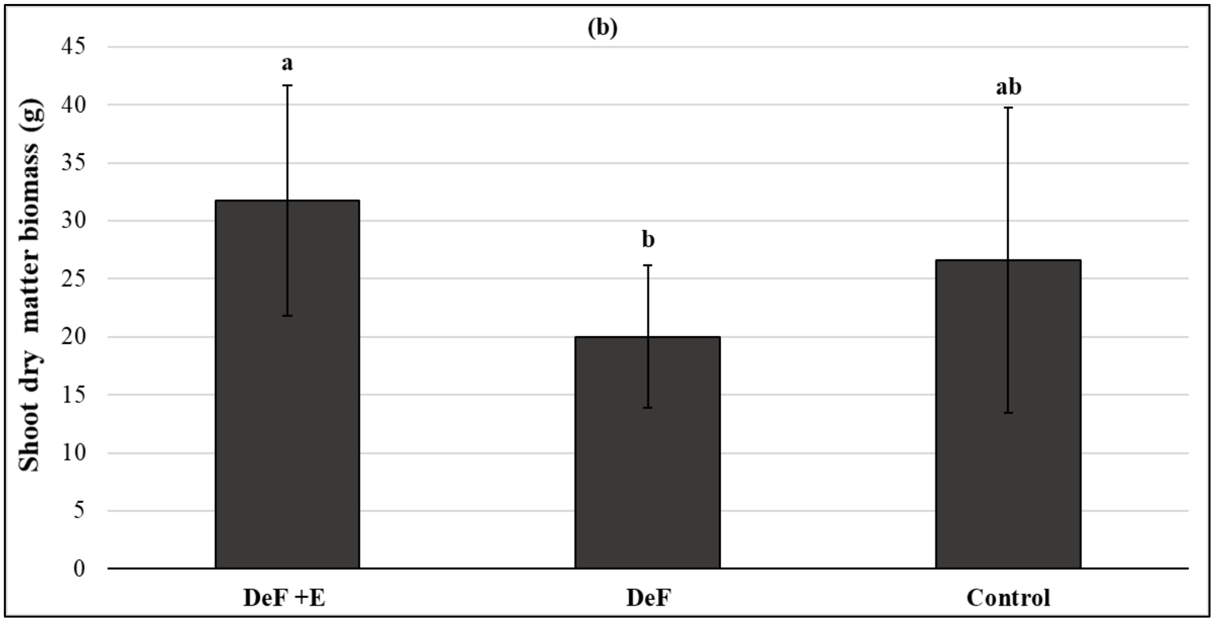


Figure 6 - 11. Mean clover and grass shoot dry matter biomass in (a) September 2017 (at the midpoint of the experiment) and (b) April 2018 (at the end of the experiment) across all field monoliths. DeF+E = frozen monoliths with earthworm addition (*n* = 12 in September 2017 and *n* = 11 in April 2018), DeF= frozen monoliths without earthworm addition (*n* = 12 in September 2017 and *n* = 11 in April 2018), *n* = 4 for Control; error bars = standard deviation. Columns with the same letter over them are not significantly different. Clover and grass shoot dry matter biomass for each treatment on a field by field basis is presented in Figure D12 and separate dry weights for the clover and grass are presented in Figure D13.

6.5.8.2. Wheat bioassay experiment

The wheat in the bioassays had significantly greater total dry biomass when grown in the soil from the DeF+E treatments compared to the DeF and control treatments (Figure 6 - 12, $p < 0.006$). This was due to an increase in root biomass in DeF+E compared to DeF and control treatments ($p < 0.001$); shoot biomasses were not different ($F_{2,14} = 1.6$, $p > 0.05$). Root and total dry biomass varied significantly between fields ($p < 0.001$) with the highest values recorded for Copse field and the lowest for BSE field. Shoot biomass was not significantly different between fields. There was no significant interaction between treatments and fields.

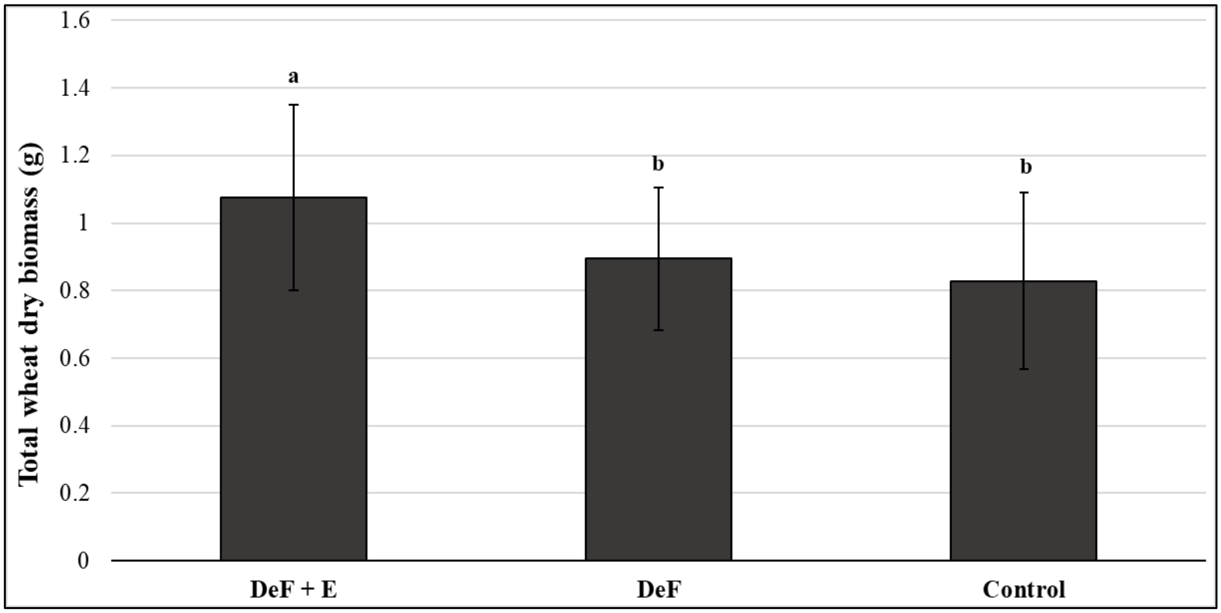


Figure 6 - 12. Mean total dry biomass of wheat grown in the bioassay on soil taken from the BSE, BSW, Copse and HS field monoliths. DeF+E = frozen monoliths with earthworm addition (*n* = 11), DeF= frozen monoliths without earthworm addition (*n* = 11), *n* = 4 for Control; error bars = standard deviation. Columns with the same letter over them are not significantly different. Separate dry weights for the shoots and roots for each treatment on a field by field are presented in Figure D14.

6.6. Discussion

6.6.1. Earthworm populations

The earthworm diversity that we introduced into the DeF+E treatments was maintained for the duration of the experiment. Endogeics dominated the earthworm populations in the DeF+E treatments at the end of the experiment as typically found in pasture fields of the farm (Figure 6 - 2; Holden et al. (submitted). Earthworms did occur in the DeF treatments despite the use of mesh on the outside and inside of the plastic containers that contained the monoliths and the use of mesh fences around the monoliths. These earthworms were dominated by epigeics but with some anecic juveniles also present (Table D3). Epigeics are reported as having high dispersion rates relative to anecic and endogeic earthworms, which results in more rapid colonization of new habitats (Bouché, 1977, Chatelain and Mathieu, 2017, Migge-Kleian et al., 2006, Margerie et al., 2001). Epigeic species produce a higher number of cocoons than other species (Dymond et al., 1997) and the cocoons of the epigeic *L. rubellus* and *L. castaneus* (two dominant epigeic species found in the monoliths) have a temperature tolerance of down to -35 °C and -50 °C respectively (Meshcheryakova and Berman, 2014). It is therefore possible that cocoons produced by these species survived the monolith freezing treatment and hatched when conditions became suitable.

Low earthworm populations in replicate 3 of the DeF+E and DeF treatments (Table D4) may be due to the herbicide spray drift onto the monoliths in late November 2017 (Edwards, 1970, Singh and Singh, 2015, Hackenberger et al., 2018, Gaupp-Berghausen et al., 2015). The selective herbicide used (ASTROKerb®, MAPP 16184, Dow AgroSciences, Cambridge UK) has the active ingredients aminopyralid, which has low ecotoxicological risk to earthworms (European Food Safety Authority, 2013, VKM Report, 2010), and propyzamide, which has significant effects on growth and survival of earthworms (Travlos et al., 2017). According to ISO guideline 11268-3, full recovery of earthworm populations should be observed within one year of application of licensed plant protection products compared to control (de Jong et al., Ockleford et al., 2017). In our study earthworms were collected only 4 months after the herbicide spray so the earthworm populations may still be recovering.

6.6.2. Soil water flow

6.6.2.1. Soil properties and earthworm effect on water flow

$K_{0.5}$ was significantly higher in HS compared to the other fields. HS had the highest %OM content of all the fields at the start and end of the experiment (Table 6 - 1, Figure D10). High %OM content typically improves soil aggregation which in turn increases soil water flow (Boyle et al., 1989). Soil texture is also a factor. The soil in HS field is coarser than that in the other fields (Table 6 - 1) which typically results in higher $K_{0.5}$ (Saxton et al., 1986).

The significant increase in $K_{0.5}$ in the DeF+E compared to the DeF and control treatments (Figure 6 - 3) is consistent with previous studies reporting a positive effect of earthworms on water flow (Blouin et al., 2013, Edwards and Bohlen, 1996, Francis and Fraser, 1998, Bouche and AlAddan, 1997, Lamandé et al., 2003). The impact of earthworms was significant in Winter and Spring 2018 just after the second addition of earthworms to the frozen monoliths in mid-November 2017. The lack of significant differences between treatments in Spring 2017 is probably due to earthworms having had insufficient time to work the soil. Qualitative observations made whilst measuring K indicate that although earthworm casts were found on the surface of the DeF+E monoliths in Summer and Autumn 2017, these were at a relatively low density compared to in Spring. Earthworm activity typically reduces in the Summer months (Birkas et al., 2010) and the higher than average temperatures during the Summer of 2017 may have reduced earthworm populations in the DeF+E monoliths further which may explain the non-significant differences between the DeF+E and DeF treatments.

In Spring 2018, DeF+E treatments showed significantly ($p < 0.01$) higher $K_{0.5}$ compared to the other seasons. In this period pores > 1 mm contributed more significantly to water flow in the DeF+E than the DeF treatments (Figure 6 - 4). These pores will have been created by earthworms or produced as a result of improved soil structure through aggregation (Figure 6 - 8). Earthworms facilitate soil aggregation and the incorporation of organic matter within the soil aggregates which may explain the high %OM content in the DeF+E treatments at the end of the experiment (Fonte et al., 2007).

The mean values of $K_{0.5}$ across all seasons are 66 ± 32, 44 ± 22 and 39 ± 28 mm h^{-1} for the DeF+E, DeF and Control treatments respectively. Heavy rainstorms in the UK rarely exceed 200 mm day^{-1}, with the greatest rainfall in 2015 being recorded as 341.1 mm day^{-1} (Friederike et al., 2018), though with the rainfall being concentrated in a shorter time period than 24 hours. The experimental results suggest that the presence of earthworms in the soil will largely reduce infiltration-excess overland flow and flooding which would help to alleviate negative effects of such events.

6.6.2.2. Water flow changes throughout seasons

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

Our initial hypotheses were that *K* would increase with earthworm activity and in line with the seasonal activity of earthworms. By the end of our experiment our data supported our first hypothesis, but it failed to fully support the second part of our hypothesis. $K_{0.5}$, increased significantly in Summer 2017 when soils are dry and earthworms activity decreases (Spurgeon and Hopkin, 1999), was unchanged during Autumn 2017 when typically earthworms that aestivated over the Summer start working the soil again as conditions become moister and grass and clover litter accumulate on the soil surface (Dar et al., 2006, Michiels et al., 2001), and decreased considerably in Winter (January 2018) when soils are wet facilitating earthworm movement and the hatching activity of some species starts to increase (Potvin and Lilleskov, 2017, Spurgeon and Hopkin, 1999).

The high monthly precipitation and temperatures during the Summer of 2017 compared to the other seasons (see Table D6) would have induced multiple soil wetting-drying cycles resulting in the formation of soil cracks and also increased aggregation leading to improved soil structure (Tang et al., 2016). Although high rainfall can lead to the disintegration of aggregates and the consequent blocking of pores resulting in reduced K for coarse textured soils with an organic matter content of less than 1% (Hu et al., 2009, Hu et al., 2012) in fine textured soils the formation of small cracks can lead to an increase in K of several order of magnitude (Albrecht and Benson, 2001). These contrasting effects are dependent on soil texture and organic matter content and in the case of the soils used in this experiment, the formation of small cracks rather than the breakdown of soil aggregates seems to have dominated and resulted in the increased $K_{0.5}$ in Summer 2017. In addition, grass and clover reached maximum growth (see Figure D2) in Summer 2017 (abundant rainfall and suitable temperatures for plant growth in Summer) and the cracks and pores associated with well-developed root systems (Angers and Caron, 1998) may have also increased $K_{0.5}$.

We did not detect a significant change in $K_{0.5}$ from Summer to Autumn 2017 although we expected an increase in earthworm activity and K (Hu et al., 2009, Hu et al., 2012). It is possible that earthworms died over the Summer reducing the populations in the monoliths and therefore earthworm impacts on K. We restocked the monoliths with earthworms in Autumn 2017 just after measuring K, so that the new earthworms could have a significant impact on K at the next measuring point. Also, plant material was collected in late September 2017 potentially reducing the food supply for any earthworms that had survived over the Summer, particularly for vertical burrowing anecics that produce water transmitting vertical pores which may therefore have reduced their activity. Further, at this time of year the growth of grass and clover would switch from a focus on root development, which can create pores, to shoot production (Willaume and Pages, 2006). This effect would be reinforced by our collection of shoot material in late September 2017 which would also have led to a focus on shoot over root development.

By Winter 2018, K had reduced significantly. The low air temperatures in Winter 2018 (down to -5.9 °C) would have reduced earthworm activity. There was frequent rainfall in the Winter period (Table D6) and although the average air temperature during the infiltration measurement campaign was 3 °C, on the mornings of the measurements there was often a thin sheet of ice on the soil surface so it seems likely that at least near-surface pores could also have been blocked by ice which would reduce measures of K. Conversely, the expansion of the water during freezing could result in soil expansion and a reduction in soil bulk density and consequent increases in K on thawing (Hu et al., 2009, Hu et al., 2012). Thus, K would vary over a finer temporal scale than that measured in our experiments. In addition the viscosity of water decreases with decreasing temperature (e.g. by a factor of 1.6 between temperatures of 3 and 20 °C) (Haridasan and Jensen, 1972, Aleksandrov and Trakhtengerts, 1974) which would reduce rates of flow. Finally, low temperatures and solar radiation in Winter reduce water evaporation after frequent rainfall and the increased water content can lead to increasing periods of water saturation and expansion of clays in the soil (Hesseltine, 2016). Although our soils only contained a small percentage of clay, any swelling could lead to a reduction in pore size and thus a decrease of K (Jabro, 1996, Messing and Jarvis, 1990, Dexter, 1988).

In some soils K can decrease in Spring after Winter freeze-thaw cycles due to the reconsolidation of soils and an increase in soil density (Hu et al., 2009, Hu et al., 2012). However, in our experiments $K_{0.5}$ increased significantly in Spring 2018 (from January to April 2018). Earthworm activity and plant growth increase during the Spring may contribute to an increase in connected soil pores that can conduct more water. At a coarser scale of observation than the hydraulic conductivity measurements we recorded a decrease in the dry bulk density and an increase in the %OM content of the soils between the start and end of the experiment which would improve soil structure and also be expected to increase the amount of water movement within the soil (Hillel, 2008).

6.6.3. Soil water release curves and water holding capacity

Soil water release curves for the DeF+E treatments shifted to the right relative to the DeF and control treatments, resulting in increased predicted water contents at saturation, field capacity and at wilting point for all the fields (Table 6 - 3, Figure D6). The DeF+E treatments also had higher water holding capacities (Figure 6 - 6) and plant available water. This is consistent with an improved soil structure (Huntington, 2006). Earthworms impact soil structure directly by creating biopores of different size, branching and sinuosity which impact on soil water storage capacity (Bastardie et al., 2005). According to the capillary rise equation, pore radius is proportional to the potential value at which that pore drains (Hillel, 1980). Therefore, at very low potential, water drains through both rapidly and slowly draining pores (Amer, 2012) such as those created by adult earthworms (2 – 9 mm diameter (Pérès et al., 1998)). The wide pores have more impact on soil water content at saturation than lower water content. Pores created by juveniles of diameter less than 1 mm would affect capillary water and therefore water content at field capacity, plant available water and water holding capacity (Amer, 2012). Earthworms also impact soil structure by fragmenting organic matter content and mixing it into the soil (Lavelle et al., 1998). This would improve soil aggregation (Figure 6 - 8) and porosity which in turn increases soil water retention (Smagin and Prusak, 2008, Tisdall and Oades, 1982). The highest values of soil water content at different potentials and plant available water in HS field may be due to the high %OM content in this field (Hudson, 1994). These results show the potential for earthworms to increase soil water storage and that the effects could be important in soil with higher %OM content.

Plants roots also improve soil structure by stabilizing macro-aggregates (Tisdall and Oades, 1982) and creating biopores of different sizes. Plant species with dense and fine roots such as grass (Deru et al., 2016) and fibrous lateral root systems as clover (Wyngaarden et al., 2015) both produce a range of soil pore sizes and increase micropore volume (Bodner et al., 2014, Jarvis et al., 2017) which has been shown to increase the water available to plants (Zangiabadi et al., 2017). Plant growth was greater in the presence of earthworms (Figure 6 - 11, Figure D14) and this will also have contributed to the greater predicted water contents at saturation, field capacity and at wilting point in the DeF+E treatments. The results show the positive impact of the combined effect of earthworms and plants on soil water storage. However, the impact could depend on the functional group of plants with different rooting strategies.

6.6.4. Plant dry biomass

Plant shoot dry biomass of grass-clover was greater in the DeF+E compared to the DeF treatments, which is consistent with the majority of studies that report the impact of earthworms on plant growth (Scheu, 2003). In a meta-analysis van Groenigen et al. (2014) reported that the presence of earthworms in agroecosystems increased the aboveground biomass by 23% on average and attributed the majority of this effect to the release of nitrogen from organic matter by earthworms. In our experiment, the DeF+E treatment of the monoliths increased soil N content (Figure 6 - 10) and increased shoot dry biomass by 37 ± 10% (Figure 6 - 11b). Although no significant increase in shoot biomass was observed in the DeF+E treatments in the bioassay, root biomass did increase significantly resulting in a significant increase in total dry biomass in the DeF+E treatments. It seems likely that the 6 week growing period of the bioassay experiment was only enough for wheat root establishment before rapid shoot growth (Weaver et al., 1924).

The significantly higher water holding capacity and available water to plants in the DeF+E treatments (Figure 6 - 6, Table 6 - 3) would contribute to the significant increase in shoot dry biomass in the monoliths and the total dry biomass of the bioassay experiment (Veihmeyer and Hendrickson, 1950, Denmead and Shaw, 1962). The effect of earthworms on plant biomass may also, in part, be due to the plant available nutrients in soil such as nitrogen (Ingestad, 1977, Robinson et al., 1992). Consistent with this the DeF+E monoliths contained a higher %OM and %N than the DeF monoliths (Figure 6 - 9 and 6 - 10). Although the low biomass recovered from the monoliths in BSE and HS fields was due to vole activity, these soils also gave the lowest biomass in the bioassay experiments. This could be due to the low nitrogen content in those fields compared to Copse and BSW fields (Figure D11). The results show the important role of earthworms in supporting food production and security.

6.7. Research limitations

As we have mentioned previously in the statistical analysis section, the experimental design is unbalanced. Only one control was used for each field factor as a result of logistic limitations. One way to avoid this issue would have been to involve only three fields rather than four as in the current experimental design. However, our research was part of the NERC Soil Security consortium research project SoilBioHedge developed originally to draw general conclusions involving the four fields.

It is understood that the tests with unbalanced design would be less powerful than balanced design, particularly when using the non-parametric tests. In this study, we used parametric tests (ANOVAs) and the unreplicated controls within fields compared to other treatments is not considered as severely unbalanced design where factor combinations have no observations at all. However, one should be very cautious if the p-values are anywhere near the threshold for significance of 0.05. Unfrozen control monoliths were not replicated within fields but were replicated across fields. Therefore, ANOVA analysis was performed to look at the main effects of each factor level (consistent with the original concept of the field being the unit of replication within “field” factor level as example) but not at their interactions (which consider observations per field per treatment). To treat the findings with more caution, we repeated the ANOVA tests excluding unfrozen control monoliths and the trends were statistically the same. Thus, within the limitations of the experimental design we can have confidence in the results of the comparisons between DeF+E and the DeF monoliths whilst acknowledging that a better experimental design, had resource permitted, would have been fully balanced with a fully replicated control set as well as the two other treatments.

Consistently, the results were plotted as an average across all fields because the fields were considered the unit of replication and in order to draw conclusions indicating the extent to which the findings were generalizable, rather than presenting the data at the level of individual fields (as differences between fields depend upon the soil-characteristics, experimental errors and design complex, which detract from the main message and aim).

6.8. Conclusion

This experiment examined how the soil properties of long-term arable fields develop when converted into ley in the presence or absence of earthworms. Within one-year, the conversion led to significant decreases in the dry bulk density and increases in the %OM content of the soils. The presence of earthworms resulted in significant greater increases of %OM and also improved other soil physico-hydraulic properties. This resulted in improved plant growth as determined by biomass production both in the field and in bioassays subsequent to the field experiment. Our results suggest that earthworms are a key component that can help achieve the four criteria (increased soil organic matter, better soil structure, enhanced hydrological function and protection from erosion) for successful and sustainable land restoration (Blanco-Canqui and Lal, 2008) and land management practices that encourage earthworm populations in agricultural production systems are to be encouraged. Although earthworms increased *K*, their impact changed in magnitude through the seasons. This suggests, importantly for flood runoff modelling, that large estimation errors could occur if the wrong hydraulic conductivity values are used for the wrong season. Seasonal climate changes influence soil properties and biological activities which in turn impact *K*, but the presence of earthworms fostered positive effects and reduced probable negative effects of climate on soil properties. Given the positive effect of earthworms, there is a need to better understand whether those effects are only temporary and how they change in the long term. The improved soil proprieties of a converted ley due to the presence of earthworms may be more resilient than the smaller improvements that occur in their absence when exposed to extreme drought or flooding events.

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Chapter 7

General discussion, research limitations and future research

7.1. Introduction

The primary aim of this thesis was to examine the influence of different earthworm ecotypes and the plant roots which they interact with on soil properties, that directly influence soil hydrological functioning and food production. It shows the crucial benefits of integrating soil biology, and earthworms in particular, into sustainable land management policies. Laboratory and field experiments were carried out to address this aim. The first laboratory experiment (Chapter 3) was carried out, before taking further steps, to clarify contradicting literature findings on the impact of different earthworm ecotypes on soil properties. In this experiment we tested the impact of two earthworms occupying different ecological niches, the anecic *L. terrestris* and the endogeic *A. chlorotica,* on two important soil properties, %water stable aggregates (%WSA) and soil water holding capacity (WHC), that underpin many of the ecosystem services provided by soils (Chapter 3). We used a higher than realistic density of earthworms to establish possible earthworm effects over the timescale of the experiment. Once principles were verified and we quantified the contribution of earthworms in improving those soil properties, we examined, using a more realistic earthworm density, the effects of the vertical burrowing earthworm *L. terrestris* on hydraulic function of different soil textures and whether these effects are different in the presence of plant roots which earthworms interact with (Chapter 4); then determined whether the interaction effects on soil hydraulic function are different for the lateral burrowing earthworm *A. chlorotica* and how they change with time (Chapter 5). A field experiment was then conducted to determine the effects of earthworms as a community, composed of different ecological groups of earthworms, on soil hydraulic function when exposed to natural climate and how those affects change through seasons (Chapter 6). The thesis also explored the resulting effects on plant growth (Chapters 4, 5, 6).

7.2. Main findings

The research presented in this thesis makes the following contributions: it has (1) examined, for the first-time, how earthworms influence the partitioning between macropore flow and micropore flow, and whether these effects are different in the presence of plant roots which earthworms interact with; (2) assessed the combined effect of plant roots and earthworm burrows on soil hydraulic properties in undrained soil where earthworm burrows terminate within the soil matrix; and (3) explored how earthworms foster positive effects and reduce probable negative effects of seasonal changes of soil hydraulic properties of arable converted to ley soils. The key outputs are (1) the measurement of water flow through different pore sizes of differing soil textures; (2) the generation of soil water release curves showing how much water is retained at different potentials; (3) the measurement of water-holding capacity and percentage water stable aggregates; and (4) the estimation of plant growth.

In the first laboratory experiment (Chapter 3), earthworms were kept at high densities in soil in order to determine the potential for impacts on water holding capacity (WHC) and percentage water stable aggregates (%WSA) at different vertical positions in the soil. Earthworm-processed soil had a higher WHC due to changes in soil structure brought about by earthworm-facilitated aggregation. However, regression analysis of the relationship between WHC and %WSA (Chapter 3, Figure 3 - 5) gave different intercepts for *L. terrestris* and *A. chlorotica* indicating that species specific factors in addition to %WSA had a significant impact on WHC, with the increase in WHC that could not be attributed to %WSA being greater for *A. chlorotica* than *L. terrestris*; *A. chlorotica* bioturbates the soil more significantly and produces smaller, more tortuous burrows that can hold water compared to *L. terrestris*. *L. terrestris* significantly increased the percentage of water stable aggregates in the upper soils of the mesocosm, whereas *A. chlorotica* had the same effect regardless of soil depth. This reflects the effects of the differing lifestyles and nature of the burrows of different earthworm ecotypes on soil properties. The results show the potential of earthworms to influence soil properties that give rise to important ecosystem services, such as an increase in soil water storage and availability of water to plants to support crop production.

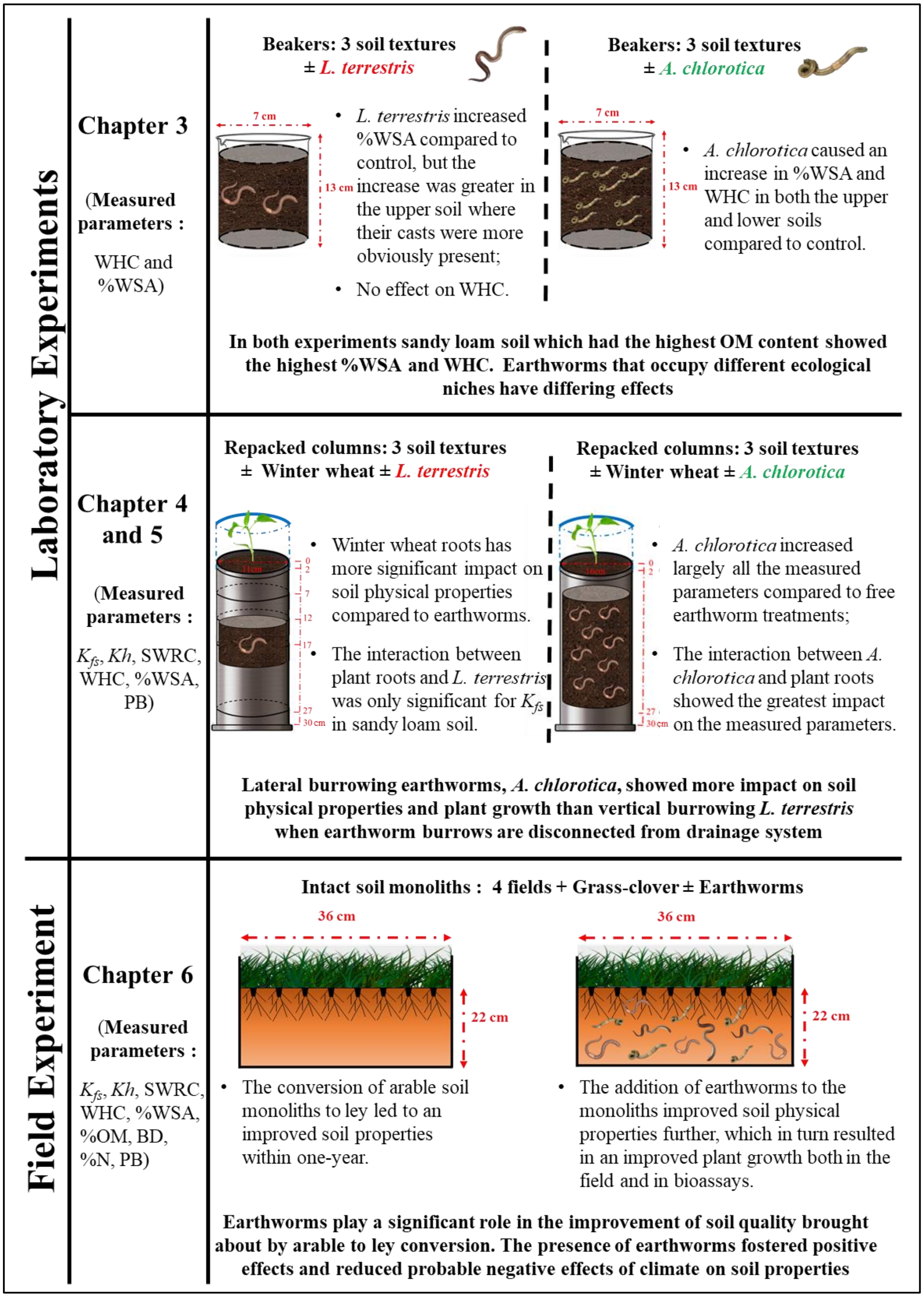
The drain pipe experiments (Chapters 4 and 5) were carried out to better understand the impact of earthworms on soil hydrological processes using a more realistic earthworm field density whilst considering the relative effects of plant roots. The experiments showed that in conditions where burrows do not connect to drainage systems, the vertical burrowing earthworms *L. terrestris* increased water flow at saturation; however, the impact of plants was more significant. Under the same conditions, the lateral burrowing earthworm *A. chlorotica*, showed more significant impact on water flow than both plant roots and the vertical burrowing earthworm *L. terrestris*. The interaction between winter wheat roots and either *L. terrestris* or *A. chlorotica* showed the greatest effect on water flow. In the *A. chlorotica* experiment, the increase in saturated hydraulic conductivity occurred more quickly in the earthworm + winter wheat than the other treatments. In the *L. terrestris* experiment, the contribution of water flow in pores > 3 mm was greatest in the plant-present treatments. In contrast in the *A. chlorotica* experiment water flow in pores > 3mm was greatest in the *A. chlorotica*-present treatments. Similarly, for soil water release curves, the water content in the soil was greatest in the plant-present treatments in the *L. terrestris* experiment. In the *A. chlorotica* experiment water content in the soil was greatest in the *A. chlorotica*-present treatments. *A. chlorotica* burrowing and casting behaviour increased soil surface roughness (micro-dams) and built complex sinuous burrow systems of small diameter that helped to hold more water in soil than *L. terrestris*. These results show the important role of soil biota in modifying soil hydrological processes and suggests that the action of soil biota can mitigate surface water erosion, or perhaps even reduce downstream flood risks. The results show that endogeic earthworm species can have a significant effect on soil hydraulic properties and could have a greater role to play in mitigating the impacts of flooding and drought compared to vertical burrowing earthworms. Also, to reap the maximum benefit of earthworms for successful land management requires the use of the appropriate species.

The use of a mixed population of earthworms under field conditions (Chapter 6) confirmed the significant effect of earthworms on soil water flow as in the laboratory experiment. However, their impact was affected significantly by seasonal changes. Earthworm addition to ley increased hydraulic conductivity in summer and spring and decreased it in winter. Earthworm addition to ley also increased the soil water content at saturation and at field capacity, water holding capacity and available water to plants. This reflects the ability of earthworms to contribute to soil water regulation and support crop growth, two key services provided by soil, through water transfer and storage processes. The decreased soil bulk density and increased organic matter content in the ley monoliths suggest that arable to ley conversion improved soil properties, but the further increase in organic matter content in the presence of earthworms shows the important role of earthworms in fostering/enhancing these effects. The differences in hydraulic conductivity between seasons can affect modelling of hydraulic processes and importantly this suggests the need to use the appropriate hydraulic conductivity for the appropriate season.

The soil from the fields with higher organic matter content had higher water flow and water holding capacity in all the experiments compared to other soils with lower organic matter content. The high soil hydraulic properties appear to be due to the high percentage water stable aggregates in those soils. Overall, the increase in plant dry biomass in all the experiments reflected the increase in soil water holding capacity or the availability of water to plants. Plant growth was the highest in the earthworm addition treatments which suggests improved soil properties in the presence of earthworms.

Overall, earthworms that occupy different ecological niches have differing effects on soil physico-hydraulic properties. However, the interaction between plant roots and earthworm improved soil properties which resulted in rapid water draining through the soils, together with more water being retained by the soil at different volumetric water contents, which in turn result in higher plant growth. The increased soil water flow and storage could help alleviate exposure of many human systems and ecosystem services to negative effects of extreme events due to current climate change.

The main finding of the research presented in this thesis are summarised in the Figure 7 - 1 below.



Chapter 3

(Measured parameters : WHC and %WSA)

Beakers: 3 soil textures ± *L. terrestris*

- *L. terrestris* increased %WSA compared to control, but the increase was greater in the upper soil where their casts were more obviously present;
- No effect on WHC.

Beakers: 3 soil textures ± *A. chlorotica*

- *A. chlorotica* caused an increase in %WSA and WHC in both the upper and lower soils compared to control.

In both experiments sandy loam soil which had the highest OM content showed the highest %WSA and WHC. Earthworms that occupy different ecological niches have differing effects

Chapter 4 and 5

(Measured parameters : K_{fs}, Kh, SWRC, WHC, %WSA, PB)

Repacked columns: 3 soil textures ± Winter wheat ± *L. terrestris*

- Winter wheat roots has more significant impact on soil physical properties compared to earthworms.
- The interaction between plant roots and *L. terrestris* was only significant for K_{fs} in sandy loam soil.

Repacked columns: 3 soil textures ± Winter wheat ± *A. chlorotica*

- *A. chlorotica* increased largely all the measured parameters compared to free earthworm treatments;
- The interaction between *A. chlorotica* and plant roots showed the greatest impact on the measured parameters.

Lateral burrowing earthworms, *A. chlorotica*, showed more impact on soil physical properties and plant growth than vertical burrowing *L. terrestris* when earthworm burrows are disconnected from drainage system

Chapter 6

(Measured parameters : K_{fs}, Kh, SWRC, WHC, %WSA, %OM, BD, %N, PB)

Intact soil monoliths : 4 fields + Grass-clover ± Earthworms

- The conversion of arable soil monoliths to ley led to an improved soil properties within one-year.
- The addition of earthworms to the monoliths improved soil physical properties further, which in turn resulted in an improved plant growth both in the field and in bioassays.

Earthworms play a significant role in the improvement of soil quality brought about by arable to ley conversion. The presence of earthworms fostered positive effects and reduced probable negative effects of climate on soil properties

Figure 7 - 1. Diagram summarising the main results of the research in this thesis (Chapters 3, 4, 5 and 6). Letters refer to the measured parameters; %WSA = % water stable aggregates, WHC = water holding capacity, K_{fs} = field saturated hydraulic conductivity, Kh = unsaturated hydraulic conductivity, SWRC = Soil water release curves, %OM = % organic matter content, BD = bulk density, %N = % total nitrogen, and PB = plant dry biomass. Symbol ± refers to present or absent.

7.3. Research limitations

7.3.1. Experimental design

Hydraulic conductivity measurements were conducted during the *L. terrestris* laboratory experiment (Chapter 4), but because there were no significant differences or trends, data are not reported in this thesis. The reason could be the insufficient contact between the sand layer on the soil surface and the disc infiltrometer. This was due to the narrow diameter of the columns (110 mm outer diameter) used to suit the vertical burrowing behaviour of *L. terrestris* (Bouche, 1972), which turned out to be impractical for the measurement of hydraulic conductivity during the experiment. The barrier surrounding the columns and the wheat tillers pushing the infiltrometer during measurement campaigns resulted in highly biased measurements. This problem was overcome after barriers were removed and plant material had been collected at the end of the experiment. In the *A. chlorotica* experiment (Chapter 5) we did not have the same problem as we used wider columns (160 mm outer diameter) which were chosen to reflect the lateral burrowing behaviour of the earthworms (Bouche, 1972). At the end of the *L. terrestris* experiment (Chapter 4) we observed that plant roots had developed around the walls of some columns, perhaps as a result of repacking the soil by layers and the slight compaction of each layer to a dry bulk density of 1.3 g cm^{-3}. The roots would exploit horizontal planes between layers and grow towards the column wall (Valentine et al., 2012, Burr-Hersey et al., 2017). This is a common problem in most mesocosm experiments using plants, and was overcome in the *A. chlorotica* experiment (Chapter 5) by filling the columns gradually with soil; the columns were then gently raised and dropped downward to reach the targeted bulk density of 1.3 g cm^3. This helped to ensure spatial homogeneity in the pore structure of the soil and allowed plant roots to follow the path of least resistance and better exploit the soil profile. A layer of melted petroleum jelly was smeared over the edges and inner surface of the columns before adding the soil. The petroleum jelly helped to maintain a good contact between the soil and the inner surface of the columns in order to reduce root penetration and avoid side water flow down the columns when irrigating the plants (Dawes and Goonetilleke, 2006). Earthworm burrowing along column walls as the path of least resistance is a typical bias in mesocosm experiments (Ernst et al., 2009). To discourage this behaviour a very shallow vertical hole, slightly less wider than the earthworm diameter, was made on the soil surface for each column together with a small amount of water being added to the centre of the columns to encourage earthworm burrowing.

The field experiment (Chapter 6) had a semi-controlled design; qualitative observations made whilst measuring hydraulic conductivities showed that the fences above the monoliths to prevent earthworms coming in and out created micro-climate that may influence plant growth and earthworm activity compared to the real field conditions. However, this is not expected to have influenced the fact that earthworm addition improved soil properties compared to the earthworm-free treatment. Also, with this design earthworms could not burrow deeper than the depth of the monoliths which may have been a particular problem over the summer as the soil dried out; some species of earthworm (Dar et al., 2006, Potvin and Lilleskov, 2017) respond to such conditions by deep burrowing and limitations on burrow depth may have resulted in earthworm deaths. This limitation was addressed by restocking the monoliths with earthworms in November.

7.3.2. Data collection

There were data collection limitations for the measured hydraulic conductivity and soil water release curves:

For unsaturated hydraulic conductivity *Kh*, we used the equation of Zhang (1997), comprising a hydraulic conductivity term and a soil sorptivity term, as recommended by Decagon Devices Inc (2016) since it was considered simple and reliable. For field saturated hydraulic conductivity K_{fs}, we used the method proposed by Reynolds and Elrick (1991). Both methods require that the applied tension (h_0) for the *Kh* measurements is higher than the soil tension (h_i) before measurements. However, Zhang (1997) reported that only the sorptivity term of his equation was sensitive to high soil moisture and not the *Kh* term, and Reynolds and Elrick (1991) showed that the assumption produced only minor errors particularly for the applied tensions, on the infiltrometer, higher than -15 cm which is within the tension range of the minidisc infiltrometer. In the drain pipe laboratory experiments (Chapters 4 and 5) it is likely that the assumption was fairly met for the measurements at the end of the experiment than during the experiment. In fact, soil was maintained up to a gravimetric water content of 30 % to sustain earthworm activity and *Kh* measurements at the end of the experiment were performed after the columns were given an acceptable time to dry at 20 °C, without affecting earthworms. The measurements were done after the vertical paper barriers were removed and plant material was collected which increases soil surface aeration. However, *Kh* measurements during the *A. chlorotica* experiment (Chapter 5), were made on columns kept typically at a constant water content at 15 °C, would be less reliable for the applied tension of - 6 cm since it may be close to h_i causing the assumption to fail. To take precautions to fairly meet the assumption, we performed *Kh* measurements before plant irrigation and soil moisture adjustment, when the soil surface was drier. In the field experiment (Chapter 6), we chose periods of less rainfall to perform the measurements and rain shelters had been put on the monoliths a few days prior to each measurements campaign.

The Reynolds and Elrick (1991) equation is a modification of Wooding (1968) solution for infiltration from a shallow pond which assumes homogeneity, isotropy and uniform water content of the soil under the disc infiltrometer. This assumption would have been met under laboratory column experiments (Chapter 4 and 5) since the soils were repacked homogeneously into the columns (with the same bulk density without soil layering which also assume isotropic soil under the mini-disc infiltrometer) and watered uniformly. Under field conditions the changes in water content, bulk density and soil texture all occurred near the soil surface making the assumption unrealistic (White et al., 1992, Hussen and Warrick, 1993, Logsdon and Jaynes, 1993). Holden et al. (2001) reported that for most studies using tension infiltrometers this assumption is only met approximatively. The non-homogeneity of the soils under the tension disc infiltrometer in our field experiment could be seen in the soil water release curves (SWRC) in Chapter 6. The measured data points for the SWRC showed a bimodal shape (Chapter 6) compared to the laboratory experiment (Chapter 4 and 5) indicating a heterogeneous pore system distribution that can be due to aggregation, particle size (including stones) or biological effects (Durner, 1994). To avoid Wooding's assumption whilst using Reynolds and Elrick

(1991) method we have used the unsaturated hydraulic conductivity at -0.5 cm calculated using Zhang (1997) method as an approximation for saturated hydraulic conductivity.

For the SWRC, Decagon Devices Inc (2016) recommend complimenting Hyprop measurements with data from a WP4 instrument (Decagon devices, Pullman, Washington, USA) for the dry section of the curves. Since this equipment was not available, we have used the relative humidity, at equilibrium with potassium carbonate, of the soil samples to adjust the dry part of the generated curves. The method was used previously by Fredlund et al. (2012) for the dry end of the soil water release curves.

7.4. Future research

For the better prediction of soil hydraulic processes, simulation models integrating the following properties/factors are needed: 1) the behaviour, population dynamics and distribution (for adults and juveniles) of the three earthworm functional groups; 2) plant root growth and soil penetration; 3) the interacting effect between earthworms and plant roots; and 4) seasonal weather change effects on physico-hydraulic properties. Previous modelling work related to earthworms focuses on the population dynamics of particular species in response to soil hydraulic properties and other abiotic factors (Palm et al., 2013, Schneider and Schroder, 2012). The few studies that model the effect of earthworms on soil hydraulic properties are based on simple models simulating water flow through earthworm-produced macropores that terminate in a drainage system (Bastardie et al., 2002, Jarvis, 2007) and the calibration of those models was mostly done on the basis of laboratory studies (Schneider and Schroder, 2012). I believe that selecting suitable factors (such as earthworm characteristics, behaviour and population dynamics, growth rate of plant roots, interacting effects between earthworms and plant, climate, land management and soil properties), and linking the existing mathematical models considering the different interactions between soil biota calibrated by field experiments will definitely improve the predictive capacity of future models. Based on the main results of this thesis, and by integrating existing models, such as those developed by: 1) Johnston et al. (2014) for predicting earthworms distribution and abundance as response to food resources, soil water and temperature conditions, 2) Ruiz et al. (2015) for predicting earthworms and plant root soil penetration, 3) Bastardie et al. (2002) for simulating the topology of earthworm burrow systems and, 4) Smettem (1992) where water flux through water filled burrow is coupled to soil matrix flow under undrained soil condition, it should be possible to provide useful insights into soil water regulation and earthworm-root interactions.

Studies should exploit new experimental techniques such as three-dimensional computer tomography to study the interaction between different earthworm ecotypes and plant roots. Most studies using X-ray computed tomography have been performed separately for earthworms (Bastardie et al., 2003, Capowiez et al., 2014, Capowiez et al., 2015) and plant roots (Mooney et al., 2012, Zappala et al., 2013); studying their interaction remains to be done. This technique could be used in particular to further study the geometry of soil macropore structure with continuous real-time measurements of water transport and storage through different pore sizes. This is important to understand for example whether the increase of soil water storage is due to pores created via the stimulation of root growth by earthworms or is because of the small tortuous pores created by earthworms through burrowing (such as endogeics, epigeics or juveniles), and/or by soil aggregation.

In addition to soil water flow covered in this thesis, it would be useful to consider the way in which temporal changes in soil structure (e.g. due to crop development, seasonal weather changes and dry-wet cycles) are influenced by different earthworm species or community assemblages and how this in turn can affect the stability of macropores and the potential consequences on soil water flow through different pore sizes.

The field experiment (Chapter 6) showed improved soil properties with earthworm addition after one year of the arable soil being converted to ley. More detailed experiments are needed to test whether the improved soil functions could maintain their resilience over time to severe drought/flooding conditions caused by climate change.

The research in this thesis showed an increased soil water holding capacity and water available to plants in soils in the presence of earthworms. This important result could help to improve crop production in drylands where water supply is irregular and scarce. This could be achieved through a new management practice, which I have named “Bio-irrigation”, where African earthworm species, resistant to high temperatures, could be introduced to farms under irrigation systems. By harnessing the benefits of drip irrigation technology -such as water conservation whilst maintaining optimum soil moisture and production of crops the introduction of earthworms has the potential to improve soil properties, further increasing water conservation and promoting crop production. Also, it would be useful to carry out field experiments to test whether “Bio-irrigation” under reduced tillage regimes conserves water and improves crop yields.

7.5. References

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Appendix A

Appendices of Chapter 2

Tables

Table A 1. Synthesis of the main reviewed literature on earthworm and soil water infiltration. Letters refer to type of experiment; R = Review, L = Laboratory experiment, F = Field experiment; and earthworm groups, AN = Anecic, EN = Endogeic, EP = Epigeic.

Authors	Study objective	Study area	Type of experiment	Earthworm species	Earthworm group	Estimated population	Soil type	Soil density (g cm^{-3})	Cropping system / land management	Burrows description method	Infiltration method	Results
Edwards et al. (1990)	Study the effect of *L. terrestris* burrows on no-till field Hydrology	Ohio, USA	R & F	*Lumbricus. terrestris*	AN	-	Silt loam	-	No-till / corn filed	Dye and chemical tracers	Subtraction of runoff and evapotranspiration from precipitation	Infiltration and ground water recharge could increase by more than 100 mm year^{-1} due to earthworm activity.
Bouche and AlAddan (1997)	Assessment of the effect of earthworms on soil physical functions.	Montpellier , France	F & L	*Scherotheca gigas*	AN	-	17 sites with large variety of soil types	1.1 to 1.3	Dry grassland	Dye	Double ring method (Müntz et al., 1905)	- Infiltration rate in soils with 100 g m^{-2} of earthworms is less than with 100 g m^{-2} of anecic species: (150 vs 282 mm h^{-1}); - Infiltration rate is correlated to earthworm biomass (r = 0.975) and to burrow length, surface and volume (r = 0.99);

												- Infiltration rate is not correlated with burrow diameter, tortuosity, earthworm number and soil profile depth.
Willoughby et al. (1997)	Seasonal variations in infiltration rate under no-till and conventional tillage systems	Indiana, USA	F	*Lumbricus. terrestris*	AN	Qualitative observations of earthworm casts on soil surface as an indication of their presence	Silt loam		No-till and conventional tillage / corn and soybean rotation.	-	Sprinkling infiltrometer (Zegelin and White, 1982)	- Before any tillage, the tilled treatment without casts had the highest infiltration rate than casts presence; - Infiltration rates increased through the growing seasons in no-till with casts presence on soil surface compared to the other treatments.
Chan (2004)	Impact of anecic earthworm burrows on soil hydrology	Central Tablelands, Australia	F	*Spenceriella. hamiltoni*	AN	-	Increase in %clay with depth starting from 25%	-	Permanent pasture	Dye	Twin ring method (Smettem and Clothier, 1989)	- Infiltration rate of 6.7 mm h^{-1} for single burrow vs 3.6 mm h^{-1} for bulk soil (macropores > 0.75 cm diameter excluded for bulk soil); - 53% of the burrow openings were transmitting water
Joschko et al. (1992)	Quantify the effect of earthworm burrows on soil	Germany	L	*Allolobophora caliginosa* *Allolobophora. rosea*	EN	83 g m^{-2} fresh weight 60 g m^{-2} fresh weight	Loamy silt	-	-	Columns with burrows were filled	Constant head permeameter	-High correlation between *Ks* values and total burrow length for *A. caliginosa* ($r_S = 0.943$);

	hydraulic properties									with gypsum		-Increase of percolation rates with burrow length for both species but more for *A. caliginosa*.
Alegre et al. (1996)	Comparing changes in soil physical properties and their effect on water infiltration in the presence / absence of earthworms	Yurimaguas, Peru	F	*Pontoscole. corefhrurus*	EN	36 g m^{-2} fresh weight	Loam	1.12	Plots were cropped to maize-rice-cowpea-rice-rice-rice form March 1990 to January 1993	-	Instantaneous water ponding by driving a 110 mm i.d. and an open-ended steel cylinder into the soil	Earthworm inoculation induced: -An increase of macroaggregates (> 1 cm) from 25.1 to 32.7% in size and a decrease of small aggregates (< 2 cm) from 33.2 to 26.1%; - An increase of bulk density (from 1.12 to 1.23 g cm^{-3}); -A decrease of porosity (from 58 to 53%) and of sorptivity (from 0.45 to 0.15 cm s$^{-1/2}$).
Bottinelli et al. (2010)	Determine the impact of earthworm casting activity on soil porosity	Hanoi, Vietnam	L	*Metaphire posthuma*	EN	14 g m^{-2} fresh weight	Loam	0.8	-	High resolution image analysis	Water infiltration test using 2 syringes placed at 1 cm above the soil surface	After simulated infiltration the cast's porosity decreased by 50 % and was significantly lower than the surrounding soil aggregates.
Jouquet et al. (2012)	Assessment of the effect of earthworm on water infiltration depending on the initial soil bulk density	Hanoi, Vietnam	L	*Metaphire posthuma*	EN	92 ind m^{-2}	Loam	1.1 or 1.4	-	-	Decagon minidisk infiltrometer with a 40 mm diameter base	Earthworms significantly improved hydraulic conductivity at -0.05 kPa for two soil densities: 1.1 and 1.4 gcm^{-3}.

Le Couteulx et al. (2015)	Burrowing activity of endogeic species as affected by organic matter placed at different depths	Brittany, France	L	*Allolobophora chlorotica*	EN	170 ind m^{-2}	Silt loam soil	1.3	Soil from arable field	Drawings of burrows of sliced microcosm using a transparent sheet + digitization	-	- Regardless of earthworm species, there was a high percentage of burrow discontinuity (from 15 to 30 % vs 6-12 %) and fewer burrows (12 to 16 vs 18 to 40 burrows / section) in soil mixed with organic matter vs soil with a surface application of OM; - Burrows of *A. caliginosa* are affected by OM location.
				Aporrectodea caliginosa								
				Allolobophora icterica								
Bastardie et al. (2005a)	3D characterization of earthworm burrow systems of natural soil	Clermont-Ferrand, France	L	*Nicodrilus giardi*	AN	101 ind m^{-2}	Sandy clay loam	1.3	Agricultural research pasture	X-ray tomography	-	- Accessible burrows offer a volume from 1400 to 10463 cm^{3} m^{-3} of soil; - Burrow length density: 687 to 1212 m m^{-3}; - Burrow volume density: less than 2.5% of total soil volume; - 9–43% of the volume was connected to the soil surface.
				Lumbricus. terrestris								
				Dendrobaena mammalis	EN							
				Aporrectodea caliginosa								
Capowiez et al. (2009)	Assessment of the effect of tillage type and cropping system on earthworm communities and on water infiltration	Estrees-Mons, France	F	*Lumbricus. terrestris*	AN	10.1-22.5 ind m^{-2} in conventional and reduced tillage	Silt loam	1.18-1.52	Conventional and reduced tillage coupled to three cropping system	Methylene blue dye tracer	Single-ring infiltration method (Braud et al., 2005)	- No significant effect of tillage management on water infiltration (increased porosity was offset by increased bulk density in reduced tillage compared to conventional tillage); -Significant effect of cropping system on water infiltration
				Aporrectodea giardi		2.2-27 ind m^{-2}						
				Aporrectodea caliginosa	EN	54.2-23.9 ind m^{-2}						

				Aporrectodea rosea		16.6-19.6 ind m^{-2}						(high infiltration in crop system inducing less bulk density).
Fischer et al. (2014)	Assessment of the effect of earthworm, soil texture and plant composition on water infiltration	Thuringia, Germany	F	*Lumbricus. terrestris*	AN	26.88 ind m^{-2}	Sandy loam to silt clay texture	-	Arable land / Grasses, small herbs, tall herbs and legumes	-	Hood infiltrometer (Schwärzel and Punzel, 2007).	- High earthworm populations and well-developed soil structure explain the higher water infiltration in silt clay soil compared to coarse soil; - Burrows of anecic species and of *Aporrectodea caliginosa*, which are larger than 2 mm in diameter, explain the effect of earthworm presence on water flow; -Legumes increased and grasses decreased soil water infiltration.
				Aporrectodea caliginosa	EN							
				Octolasion tyrtaeum								
				Allolobophora chlorotica								
				Aporrectodea rosea								
Capowiez et al. (2014)	Estimation of burrow system area and continuity	Montfavet, France	L	*Aporrectodea caliginosa nocturna*	AN	Earthworm collected at 450 ind m^{-2} from orchard and added to repacked cores at 200 ind m^{-2} in	Clay loam	1.1	Abandoned orchard	Drawings of burrows of sliced cores using a transparent sheet + digitization	-	-Anecic species burrow area was greater than that of endogeic species (c. 40 cm^2 vs c. 15 cm^2); - 40% to 50% of endogeic species burrows and about 20% of anecic species burrows were refilled by casts.
				Aporrectodea caliginosa meridionalis								
				Aporrectodea caliginosa icaliginosa	EN							
				Allolobophora chlorotica								

Capowiez et al. (2015)	3D characterization of reconstituted burrow systems of different earthworm species	Montfavet, France	L	*Aporrectodea nocturna*	AN	100 ind m^{-2}	Clay loam	1.26	Abandoned orchard	X-ray tomography	Single ring method (Braud et al., 2005)	Infiltration rates per core: 11.03 L min^{-1}
				Lumbricus terrestris		100 ind m^{-2}						12.42 L min^{-1}
				Aporrectodea rosea	EN	200 ind m^{-2}						2.32 L min^{-1}
				Allolobophora chlorotica		200 ind m^{-2}						4.41 L min^{-1}
				Aporrectodea caliginosa		200 ind m^{-2}						5.15 L min^{-1}
				Aporrectodea icterica		200 ind m^{-2}						3.76 L min^{-1}
Smettem and Collis-George (1985)	Prediction of steady-state infiltration rates through macropores in a soil under native pasture	Australia	F & L	*Eisenia rose*	EP	-	0-14 cm Sandy loam, 14-100 cm silty loam	1.33 - 1.42 cm^3 cm^{-3}	Pasture	-Resin peels & Methylene blue	-In the laboratory: matrix flow through field-core using permeameter method (Scotter et al., 1982) - In situ: infiltration through soil matrix by double-tube method (Bouwer, 1962) and Infiltration through individual macropores as	-Single macropore of 3 mm diameter contribute greater to steady infiltration rate than the cross-sectional area associated with the soil matrix.
				Allolobophora caliginosa	EN							

											described by Ehlers (1975)	
Schutz et al. (2008)	Study of earthworm population and water infiltration rates in woodland flooding sites	Lange Erlen, Switzerland	F	*Lumbricus terrestris & others*	AN	from 25 to 65 ind m^{-2}	Fluvi-eutric Cambisol -	-	Floodplain (forest and grassland)	-	Bouwer Cylinder Infiltrometer (Bouwer, 1986)	- Low correlation was found between infiltration rate and anecic earthworm density but high correlation for endogeic and epigeic densities.
				Allolobophora chlorotica & others	EN	from 57 to 160 ind m^{-2}						
				Lumbricus rubellus & others	EP	from 11 to 29 g m $^{-2}$ fresh weight						
Ernst et al. (2009)	Quantify the impact of earthworm species on soil water characteristics	Trier, Germany	L	*Lumbricus terrestris*	AN	100 and 113 ind m^{-2}	Sandy loam	1.5	Agricultural use soil	-	Modified infiltrometer according to Hills (1970)	The water infiltration rates were the highest and water discharge was faster in treatments with *A. caliginosa* compared to *L. terrestris* and *L. rubellus* treatments.
				Aporrectodea caliginosa	EN	350 and 370 ind m^{-2}						
				Lumbricus rubellus	EP	270 and 210 ind m^{-2}						
Wuest (2001)	Quantify biopores after long-term no-till compared to recently tilled soil	Pendleton, USA	F	-	-	-	Silt loam	-	No-till / spring and winter wheat rotation	Photographs of horizontal cross-sections of intact cores. + digitization	-	30 to 100% more biopores wider than 1 mm diameter in long-term no-till (17 years) in comparison to recently tilled soil.
TerAvest et al. (2015)	Investigate the effects of three cropping	Nkhotakota and Dowa, Malawi	F	-	-	270 ind m^{-2}	Sandy loam	1.37-1.49	-Three cropping systems:	-	Difference between rainfall applied and runoff	In high potential evapotranspiration zone:

	systems on soil–water relations and crop production								continuous no-till maize, conservative agriculture rotation and conventional tillage rotation		collected using rainfall simulator as described by Thierfelder and Wall (2009)	- No-till and maize residue significantly increased earthworm abundance compared to conservative agriculture rotation and conventional tillage rotation; - Residue retention is positively correlated to infiltration and no till; - Conservative agriculture improved soil water content by 20 mm compared to conventional tillage.
Spurgeon et al. (2013)	Meta-analysis of the relationships between earthworm community change and soil structural properties		R	-	AN EN EP	-	-	-	Conventional / no & reduced tillage / grassland / woodland	-	-	-Positive correlation between earthworm numbers and soil water infiltration; - Epigeic and anecic species are a positively associated with an increased soil water infiltration, whereas endogeic species has no significant effect.

Francis and Fraser (1998)	The effects of earthworm species on soil macroporosity and hydraulic conductivity	New Zealand	F (monoliths)	*Aporrectodea caliginosa* and *Octolasion cyaneum*	EN	300 ind m^{-2}	Silt loam	1.2 - 1.3	Pasture soil	Analysing the binary images (using a standard image analysis system) of macropores filled with sand of a horizontal sectioned monoliths	Disc permeameters (Perroux and White, 1988) were used to measure unsaturated and saturated hydraulic conductivities	- *Lumbricus rubellus* created burrows in top soils; - Endogeic species created continuous burrows in both top and sub soil, but *Aporrectodea caliginosa* burrows were more connected to the soil surface; - *Aporrectodea caliginosa* showed the greatest *Ks* than other earthworm species; - No significant differences in *Ks* were observed between treatments with earthworms and control.
				Lumbricus rubellus	EP	600 ind m^{-2}						

Table A 2. Synthesis of the main reviewed literature on earthworm and soil water storage. Letters refer to type of experiment; R = Review, L = Laboratory experiment, F = Field experiment; earthworm groups, AN = Anecic, EN = Endogeic, EP = Epigeic; and earthworm effect, N = negative, P = positive.

Authors	**Study objective**	**Study area**	**Type of experiment**	**Earthworm species**	**Earthworm group**	**Earthworm population**	**Soil type**	**Soil density (g cm^{-3})**	**Cropping system / land management**	**Soil water retention / moisture / holding capacity method**	**Earthworm effect**	**Results**
Bastardie et al. (2005b)	Assessment of water diffusion through burrow walls	Clermont-Ferrand, France	L	*Lumbricus terrestris*	AN	One worm incubated per 2D terrarium (48 cm high × 33 cm wide)	Sandy clay loam	1.3	-	- Simplified sorptivity equation of Philip (1957) for water diffusion - Micro-tensiometers	N	- The amount of water transits (mean coefficient of sorptivity) through burrows of *L. terrestris* is lower than that transited through soil fractures; - Bulk density of *L. terrestris* burrows are higher than artificial burrows.
Rogasik et al. (2014)	Assessment of the structural changes of drilosphere by compaction	Braunsch-weig, Germany	L	*Lumbricus terrestris*	AN	105 ind m^{-2}	Silt loam	1.34-1.38	-	-	P/N	- Spatial heterogeneous earthworm effect on the bulk density (BD) (increased BD of inner burrows wall, 1.75, and decreased BD from the walls to drilosphere); - Earthworm compacting effect on burrow walls would have a potential impact on lateral water transfer between burrows and the surrounding soil matrix.
Stockdill and	Effects of earthworm activity on	New Zealand	F	*Allolobophora. caliginosa*	EN	-	-	-	Pasture	-	P	- Moisture holding capacity increased by 17%, and 27% more

Cossens (1969)	hydraulic properties of soil under pasture											of available moisture in top soil with earthworm presence; - Increase of root development
Blouin et al. (2007)	Study of drought stress on rice in the presence of compacting earthworm species	Humid savannah, Cote d'Ivoire	L	*Millsonia anomala*	EN	127 g m^{-2} fresh weight	Sandy	0.8	Rice	Daily weight of soil mass after water saturation.	N	- Earthworm reduced soil water retention capacity by more than 6%; - No significant effect of earthworms on plant growth in drought condition; - 40% increase in shoot biomass production with the presence of earthworm in wet condition.
Lipiec et al. (2015)	Determine changes in pore size distribution, stability and water repellency of cast and natural aggregates	Lublin, Poland	L	*Aporrectodea caliginosa*	EN	-	Silt loam	1.33		Water drop penetration time method was used to measure water repellency (Chenu et al., 2000)	N	- One-week old casts collected next to the burrows showed greater repellency than the natural aggregates.
McDaniel et al. (2015)	Investigate the effect of earthworms on soil hydraulic properties	Colorado, USA	L	*Aporrectodea caliginosa*	EN	852 ind m^{-2}	Sandy loam	1.1	-	Bar Pressure Plate Extractor	P	- Water content increased by 33 and 41% in the 0-15 and 15- 30 cm of column sections respectively in the presence of earthworm compared to control;

												- Reduction of pore-size distribution index (greater variety of pore sizes) in the presence of earthworm.
Smagin and Prusak (2008)	Study the effect of earthworm casts on the soil water retention curve	Moscow, Russia	L	*Lumbricus rubellus*	EP	0.1% of soil mass	Loam	-	-	Equilibrium centrifuging method (Smagin et al., 1998)	P	- The soil water retention capacity of casts increased within the entire range of the soil moisture contents compared to the surrounding soils up to 20 wt.% for 0 to –1000 kPa water potentials).
Boyle et al. (1997)	The effects of earthworms on grass growth and soil structure	Clonsant, Ireland	L	*Lumbricus terrestris*	AN	250 ind m^{-2}	-	1.45	Perennial ryegrass in bucket and in glasshouse	Sand box method based on Stakman et al. (1969) procedures	p	- Grass yields were 89% and 19% higher in organically fertilized and inorganically fertilized buckets with earthworm presence respectively than without earthworms; - Soil from the organic fertilizer treatment with earthworm addition held the greatest volume of water than both treatments without earthworms and treatment using inorganic fertilizers. .
				Aporrectodea caliginosa	EN							
				Allolobophora chlorotica	EN							
Bastardie et al. (2005a)	3D characterization of earthworm	Clermont-Ferrand, France	L (natural cores)	*Nicodrilus giardi*	AN	101 ind m^{-2} (in natural burrow cores)	Sandy clay loam	1.3	Agricultural research pasture	Estimation of water storage amount by X-ray tomography	P	- Accessible burrows offer a volume from 1400 to 10463 cm^3 m^{-3} of soil corresponding to 1–10 mm of a water storage capacity;
				Lumbricus. terrestris	AN							

	burrow systems of natural soil			*Dendrobaena mammalis*	EN							- Surface diffusion from 1069 to 7237 cm^2 m^{-3}.
				Aporrectodea caliginosa	EN							
Ernst et al. (2009)	Quantify the impact of earthworm species on soil water characteristics	Trier, Germany	L	*Lumbricus terrestris*	AN	100 and 113 ind m^{-2}	Sandy loam	1.5	-	Tensiometers at 10 and 40 cm + FD-probes at 10 cm	P	- *L. rubellus* tended to enhance the storage of soil moisture at 10 cm depth; - *A. caliginosa* enhanced water infiltration rates compared *to L. terrestris* and *L. rubellus*
				Aporrectodea caliginosa	EN	350 and 370 ind m^{-2}						
				Lumbricus rubellus	EP	270 and 210 ind m^{-2}						
Palm et al. (2013)	Modelling distribution patterns of earthworms depending on soil proprieties, land management and topography	Baden-Württemberg, Germany	Modelling of collected field data	*Lumbricus terrestris*	AN	0 to 92 ind m^{-2}	Different texture containing clay from 9.3–23.6%	-	No till and reduced Ploughing	-	P	Distribution of epigeic earthworms are controlled by topographic features and endogeics species by soil moisture, clay content and organic matter.
				Octolasium cyaneum, Aporrectodea caliginosa and Aporrectodea rosea	EN	0 to168 ind m^{-2}						
				Lumbricus rubellus, Lumbricus castaneus	EP	0 to128 ind m^{-2}						

Appendix B

Appendices of Chapter 4

Figures

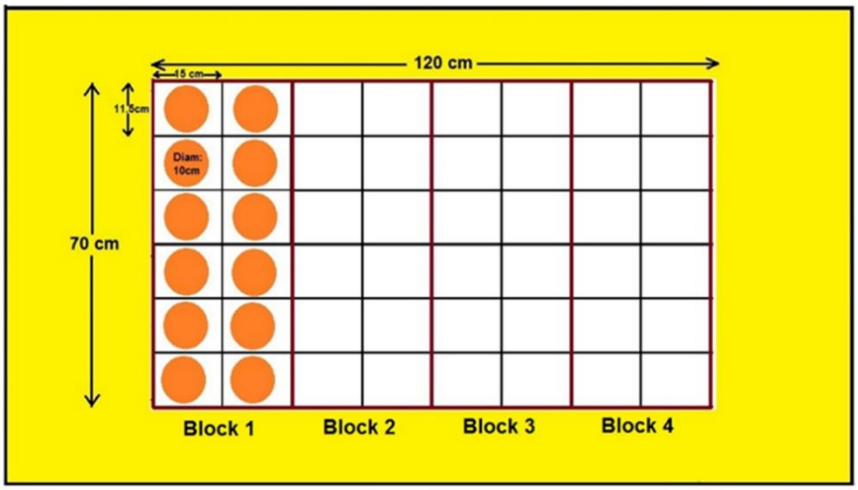


Figure B 1. Diagram of the experimental set up viewed from above. Circles in orange are the randomized position of the columns of one replicate of each treatment.

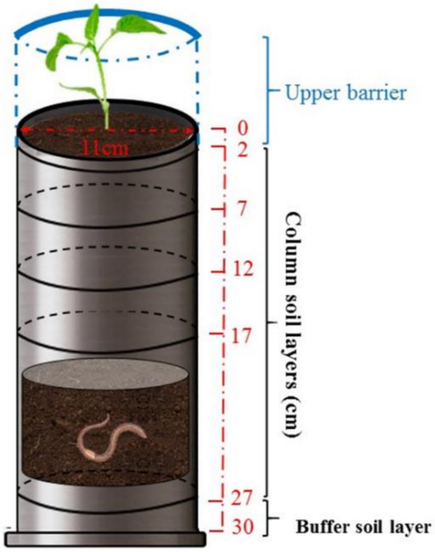


Figure B 2. Repacked soil in PVC column used to test the effect of anecic *L. terrestris* earthworm on soil hydraulic proprieties. Upper barrier is designed to stop earthworm escape; the buffer soil layer is to smoothly isolate earthworms' active area from the direct external effects on the bottom of columns.

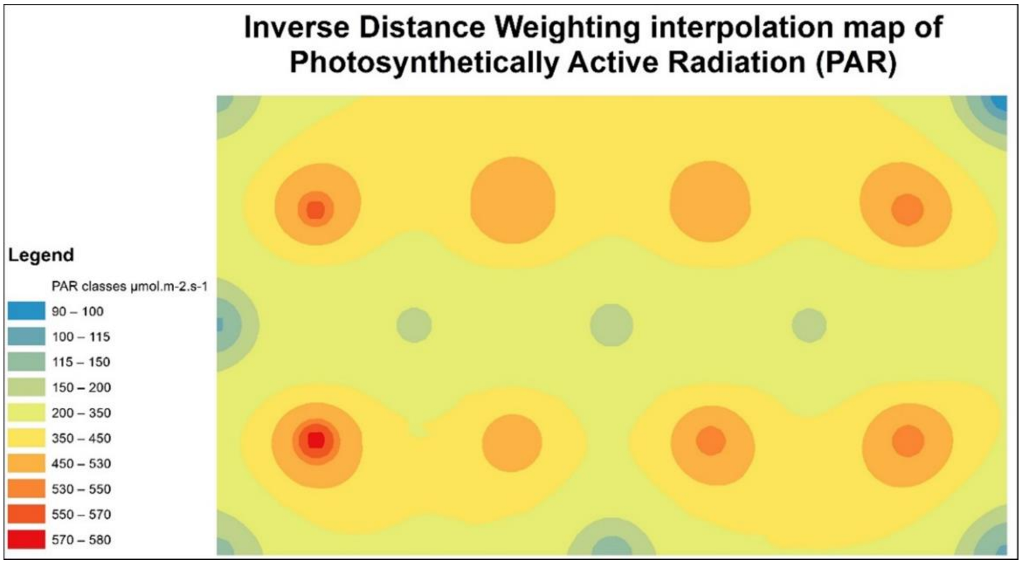


Figure B 3. Inverse Distance Weighting interpolation map of Photosynthetically Active Radiation (PAR) measured above the blocks at the upper surface of soil columns (Crop growth conditions section of the main text).

Figure B 4. PVC column of all treatments placed under LED lights.

Tables

Table B 1. Analysis of variance of hydraulic conductivity by applied water tension, soil texture and treatments.

Source of variation	*d.f.*	*SS*	*MS*	*F-ratio*	*P-value*
Water tension	3	298614.18	99538.06	122.62	<0.001
Texture	2	21123.18	10561.59	13.01	<0.001
Treatments	3	92009.58	30669.86	37.78	<0.001

Table B 2. Pairwise comparisons of water holding capacity means between soil textures by Tukey's honestly significant difference (HSD) procedure.

Soil textures (I)	*Soil textures (J)*	*Mean Difference (I-J)*	*Std. Error*	*Sig.*	*95% Confidence Interval*	
					Lower Bound	*Upper Bound*
L soil	SiL soil	-13.57	2.78	.000	-20.39	-6.74
	SaL soil	-21.22	2.78	.000	-28.04	-14.40
SiL soil	SaL soil	-7.65	2.63	.017	-14.12	-1.19

Measurements made in detail

Hydraulic conductivity measurements were conducted during the experiment, but because we couldn't identify any significant differences or trends, data are not reported in the paper. At the end of the experiment the following measurements were made in the laboratory at ≈ 20 °C.

Plant biomass, earthworms' biomass and cast production

At the end of the experiment winter wheat shoots were collected, cutting the stems at the soil surface; the fresh biomass was weighed to the nearest thousandth of a gram balance. The fresh biomass was then oven dried to constant weight at 70 °C for 48 hours. L. terrestris biomass was recorded at the start and end of the experiment. L. terrestris casts (> 2 mm) on the soil surface were collected, weighed and then oven dried at 105 ºC for 48 h and reweighed. The total cast production for each treatment during the experiment was expressed in g dry weight and the cast production rate was calculated as:

$$\begin{aligned} &Cast\ production\ rate\ (mg\ g^{-1}day^{-1}) \\ &\qquad = \frac{Total\ cast\ production\ in\ g}{(Initial\ earthworm\ weight\ in\ g)\ X(112)} X\ 1000 \end{aligned}$$

Where 112 is the duration of the experiment in days.

Unsaturated hydraulic conductivity

A Decagon Mini Disk Portable Tension Infiltrometer was used to measure the infiltration properties of the soils under the different treatments. Measurements were made at potentials of -6, -3, and -1 cm until steady-state flow was reached. The equivalent pore radii of the applied suctions 0.5, 1 and 3 mm respectively) were predicted from the capillary rise equation which assumes that pore radius is proportional to the potential value at which that pore drains:

$$r = \frac{2.\,\sigma_w.\cos(\alpha)}{\rho_w.\,\mathrm{g.\,h}} \qquad (1)$$

Where r (m) is the pore radius at capillary rise h (m), σ_w (N m^{-1}) is the surface tension of the water, α is the contact angle degree between capillary and water, ρ_w (Mg m^{-3}) is the density of water, and g (N kg^{-1}) is the gravitational acceleration constant. For water-capillary interfaces it was assumed that the contact angle (α) is 0 for a wetted surface so $\cos(\alpha) \approx 1$ (Amer, 2012), $\rho_w = 0.998$ Mg m^{-3}, $\sigma_w = 0.0728$ N m^{-1} (at 20 °C), and g = 9.81 N kg^{-1}. The supplied heads in the Mini Disk Infiltrometer do not let pores with an effective radius greater than the equivalent pore radius of the applied suction to drain water. By replacing the variables in Eq.1 with their values, the applied potentials of -6, -3 and -1 cm allowed water to flow respectively through pores of diameter less than 0.5, 1 and 3 mm.

After harvesting the winter wheat and before starting hydraulic conductivity measurements, a very fine moist sand layer of a similar diameter to that of the infiltrometer (4.5 cm) was applied to the column surface to improve the contact between the tension disk and soil surface. As long as the applied contact layer is very thin (1 to 2 mm), has sufficient porosity and has a greater hydraulic conductivity than that of the soil, it should not be hydrologically limiting for the soil steady-state infiltration rates and for the range of applied tensions (Köhne et al., 2011, Reynolds and Elrick, 1991). In order to avoid any osmotic effect of the applied thin sand layer, the sand was first washed and drained three times with ultra-purified water until reaching an electrical conductivity of $EC_{(1/5)} = 20$ µS/cm. The measurements were taken on one infiltration surface per column. The lowest negative potentials (-6 cm) were measured first to avoid hysteresis phenomena that can arise when applying the following negative potentials (-3, -1 cm) (Baird, 1997). Cumulative infiltration volumes against time were determined for each suction set. The Van-Genuchten Zhang transient method, proposed by Zhang (1997), was used to estimate the unsaturated hydraulic conductivity for three dimensional infiltration. The data were fitted to Eq.2 where I is the cumulative infiltration (cm), t is the time (s), C_1 ($cm.s^{-0.5}$) is the soil sorptivity and C_2 ($cm.s^{-1}$) is related to hydraulic conductivity.

$$I = C_1\sqrt{t} + C_2 t \tag{2}$$

The unsaturated hydraulic conductivity K (ψ) was then calculated from:

$$K(\psi) = \frac{C_2}{A} \tag{3}$$

where C_2 is the slope of a second-order polynomial curve fitted to the cumulative infiltration vs. square root of time data, and A is a value computed from tables relating the van Genuchten parameters of a given soil texture to the applied suction rate and radius of the infiltrometer disk (Decagon Devices Inc, 2016).

Field saturated hydraulic conductivity

The linear regression method of Reynolds and Elrick (1991) was used to estimate the field saturated hydraulic conductivity K_{fs} from steady state tension disk infiltrometer data. Reynolds and Elrick (1991) proposed a logarithmic transformation of the combined Wooding's solution for infiltration from a shallow circular pond (Wooding, 1968) with the unsaturated hydraulic conductivity equation of Gardner (1958). Based on Wooding (1968) assumptions, we assumed that the method is suitable for the instrument and the studied soils. Wooding (1968) solution assumes that the steady state tension disk infiltrometer measurements should be made on homogeneous, isotropic and uniformly unsaturated soils. Previous studies showed that in practice the assumptions are only met approximately (Holden et al., 2001). In our study, the soils were sieved and packed homogeneously in columns and the measurements were made at the end of the experiment when the soils started to dry. To determine the hydraulic conductivity $K(\psi)$ by using the methods of Reynolds and Elrick (1991) there is no need for information on the sorptive behavior of the soil or changes in the volumetric water content at the soil surface, nor the thickness of the contact sand layer. The method requires one infiltrometer radius configuration and only one infiltration spot which eliminates errors resulting from the successive placement of the infiltrometer and the use of different infiltrometer radii.

Soil water release curves

Water release curves were measured using a HYPROP device (UMS, Munchen, Germany) at the Center for Ecology and Hydrology (CEH) of Bangor. Soil cores of 250 cm^3 and 5 cm height were taken from the surface of the columns and were analyzed following the simplified evaporation method (Schindler et al., 2010, Peters et al., 2015). The method derives soil hydraulic functions from soil core weight changes and the subsequent matric potential during drying through evaporation. HYPROP measurement campaigns were modeled using the HYPROP-FIT software (UMS, Munchen, Germany) in which the commonly used traditional constrained unimodal Van Genuchten-Mualem model (Van Genuchten, 1980) was chosen to generate hydraulic function parameters. Because soil water release curve determination takes a considerable time to complete, the measurements were limited to the different treatments applied to the soil columns from the Copse field (loam).

As the pore holding water radius is proportional to the potential value at which that pore drains (Eq. 1), the volume of pores can be determined from the slope of water release curves $d\theta/d\psi$. The larger the specific water retention capacity $d\theta/d\psi$ the wider is the volume of pores holding water within the pore size class. The maximum value of $d\theta/d\psi$ can be used to determine the most frequent pore size class.

Water holding capacity

Water holding capacity measurement is based on the water held against gravity without any external pressure applied after a soil has been saturated. Soil cores (3.5 cm inner diameter by 5 cm long) were collected from the surface of the soil column. Fine mesh was used to prevent soil loss from the bottom of the cores. Cores were then placed in a container of water at a depth equal to half the core height and allowed to wet up by capillary action. After one day, more water was added to return the water level to its original value and the container was covered with perforated foil to prevent evaporation. Once the water level became stable, it was increased up to the rim of the soil cores and left for 48 hours. When the soil surfaces had a glossy appearance, the cores were removed from the water, positioned above beakers 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 by the weight of water held in the soil cores compared to the 105 °C oven dry weight of the sample.

Soil aggregate stability

The wet aggregate stability was measured using bespoke wet sieving equipment (Wet Sieving Apparatus; Eijkelkamp soil and water Agrisearch Equipment Art no. 08.13). Soil samples were taken from each treatment, air dried and sieved to 1 – 2 mm. 250 µm sieves were filled with 4g of the 1-2 mm air dried aggregates and pre-moistened in a wetting chamber for 5 minutes to avoid air entrapment during shaking of the samples. Sieves of moistened aggregates were then placed in the wet sieving apparatus, above a first set of cans filled with distilled water. The aggregate laden sieves were raised and lowered into the underlaying water at a rate of 34 times per minute. After 3 minutes, the first set of cans underneath the sieves contained unstable aggregates and were replaced by a second set of cans filled with a dispersing solution of Calgon (35.7g of sodium hexametaphosphate $(NaPO_3)_6$ and 7.9 g sodium carbonate $NaCO_3$ in 2 liters of distilled water) and a second continuous shaking cycle was carried out until all stable aggregates are destroyed and only the sand and other fragments were left in the sieves. Both sets of cans were oven dried at 105 °C for 24 hours and weighed. The weight of dispersing solute was subtracted from the weight of the content of cans filled with dispersing solution to obtain the corrected stable soil weight.

The wet stable aggregates percentage was calculated as:

$$Stable\ aggregate\ (\%) = \frac{Wds}{(Wds + Wdw - Ws)} \times 100$$

where Wds is the weight of stable aggregates left in the sieves after dispersing with Calgon solution (g), Wdw is the weight of unstable aggregate dispersed in distilled water (g) and Ws is the weight of dispersing solute (g).

Appendix C

Appendices of Chapter 5

Figures

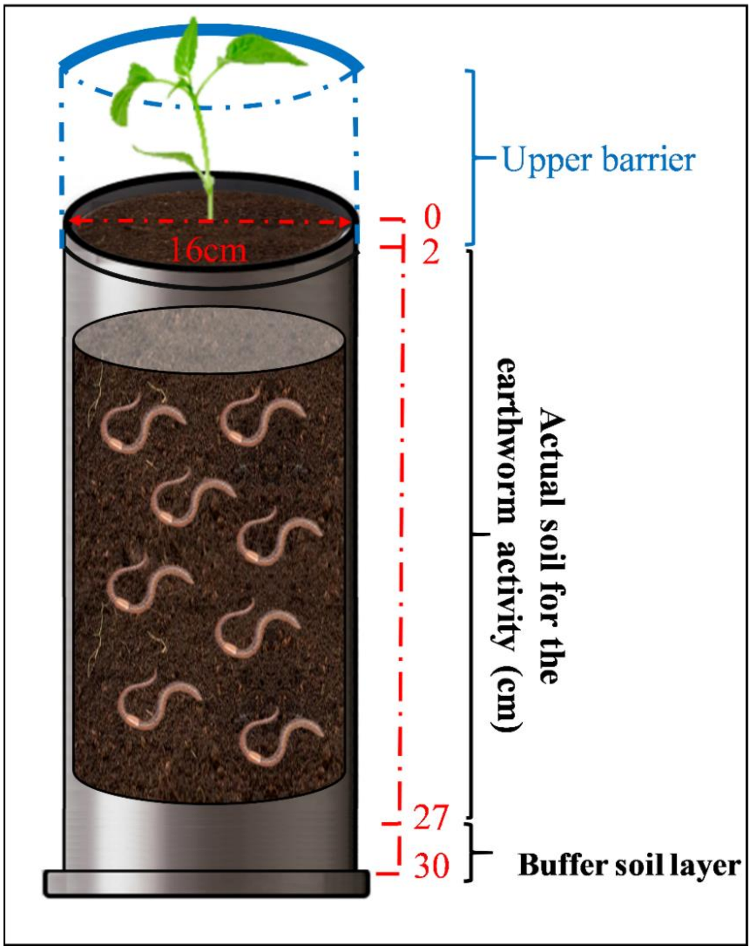


Figure C 1. Repacked soil in PVC column used to test the effect of endogeic Allolobophora chlorotica earthworm on soil hydraulic proprieties. Upper barrier is designed to stop A. chlorotica escape; the buffer soil layer is to isolate A. chlorotica active area from the direct external effects on the bottom of columns.

Tables

Table C 1. Pairwise comparisons (Bonferroni method) of the field saturated hydraulic conductivty means between time points for the three soil textures.

Soil texture	***(I) Time points***	***(J) Time points***	***Mean Difference of K_{fs} (I-J)***	***Std. Error***	***Sig.***	***95% Confidence Interval for difference***	
						Lower Bound	***Upper Bound***
L	3 weeks	9 weeks	-120.39	29.48	.005	-202.33	-38.46
		16 weeks	-191.54	27.96	.000	-269.24	-113.85
	9 weeks	16 weeks	-71.15	19.90	.011	-126.46	-15.84
SiL	3 weeks	9 weeks	-58.38	16.33	.011	-103.76	-12.99
		16 weeks	-131.49	12.35	.000	-165.80	-97.17
	9 weeks	16 weeks	-73.11	20.45	.011	-129.95	-16.26
SaL	3 weeks	9 weeks	-237.61	64.35	.009	-416.48	-58.74
		16 weeks	-765.45	37.16	.000	-868.74	-662.16
	9 weeks	16 weeks	-527.84	82.03	.000	-755.83	-299.85

Table C 2. Analysis of variance of water content and plant available water at different soil water potential pF across all the treatments. pF = Log_{10} (static pressure at cm water).

	Source of variation	***d.f.***	***SS***	***MS***	***F-ratio***	***P-value***
Water content at saturation	treatments	3	195.59	65.20	78.17	< 0.001
Water content at pF 1.8		3	192.66	64.22	24.07	< 0.001
Water content at pF 2.5		3	231.30	77.10	16.07	0.002

Water content at pF 4.2		3	165.96	55.32	10.37	0.006
Plant available water from pF 1.8 to 4.2		3	31.74	10.58	3.43	0.081
Plant available water from pF 2.5 to 4.2		3	27.44	9.15	6.60	0.019

Table C 3. Analysis of variance of water holding capacity by tested treatments and soil textures.

Source of variation	***d.f.***	***SS***	***MS***	***F-ratio***	***P-value***
Soil texture	2	0.144	0.072	52.314	< 0.001
Treatment	3	0.228	0.076	55.102	< 0.001
Soil texture * Treatment	6	0.014	0.002	1.722	0.144
Error	36	0.050	0.001		

Appendix D

Appendices of Chapter 6

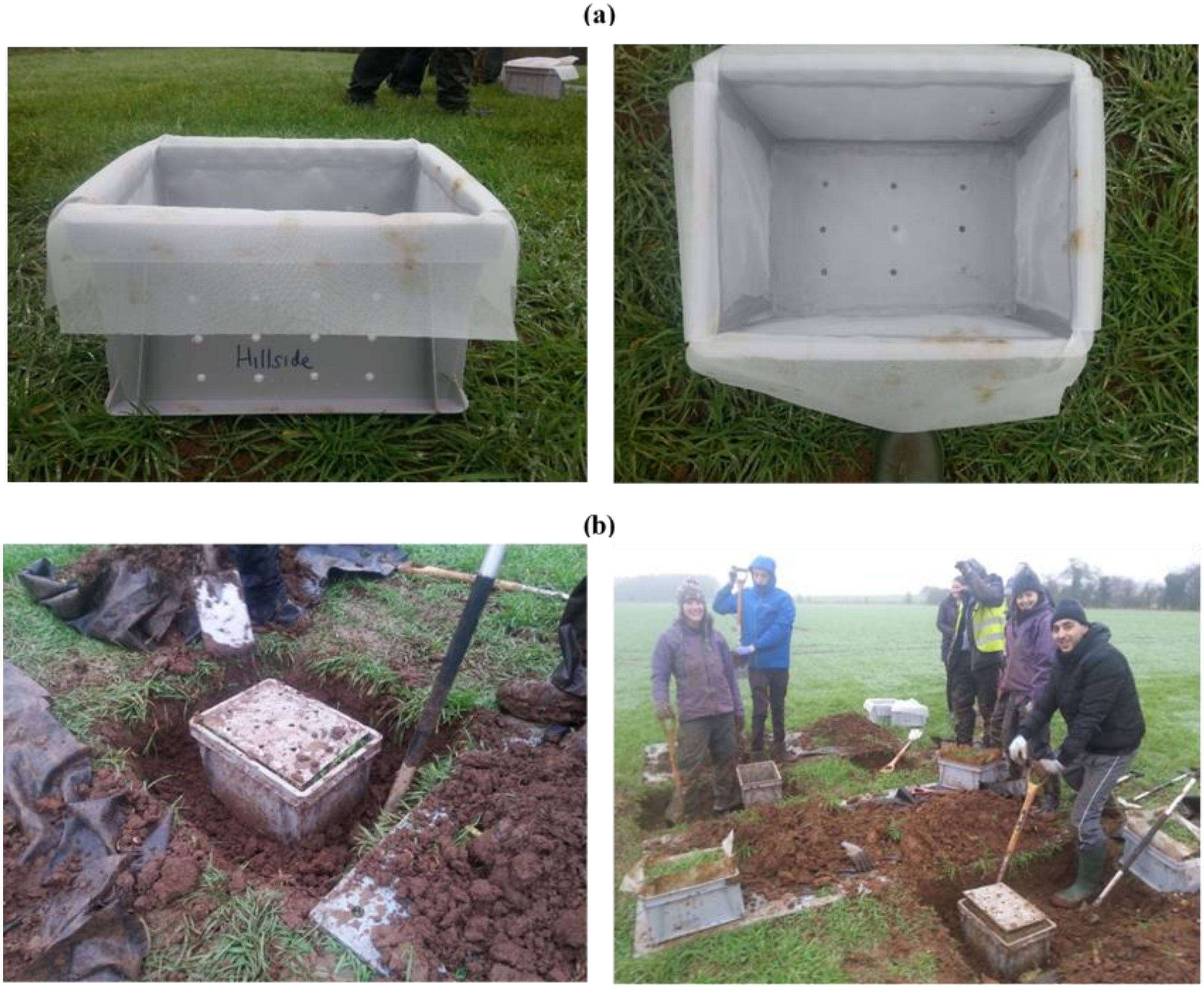


Figure D1. Plastic boxes used to hold the monoliths (a) and monoliths removal and placing into the plastic boxes (b).

Figure D2. Examples of grass-clover growth on the monoliths through different seasons of the experiment period; (a) at the beginning of the experiment in April 2017 just after the first addition of earthworms, (b) May 2017, (c) August 2017, (d) September 2017, (e) January 2018 and (f) April 2018.

Initial soil properties of the experimental fields

Table D1. Physical and chemical properties of the experimental arable fields (*n* = 3).

Field	**pH**[1]	**Organic matter**[2] **(%)**	**Field dry bulk density**[3] **(g cm^{-3})**	**Clay (%)**[4]	**Silt (%)**[4]	**Sand (%)**[4]	**Textural class**
				< 2 µm	**2-50 µm**	**50-2000 µm**	
BSE	7.58	3.26	1.39	4.28	51.24	44.47	Silt loam
	7.74	3.21	1.34	4.46	53.83	41.70	
	7.75	3.14	1.38	4.21	51.28	44.50	
BSW	7.99	3.13	1.31	4.69	53.03	42.28	Silt loam
	8.00	3.18	1.25	4.82	54.35	40.83	
	7.79	3.17	1.47	4.80	54.36	40.84	
Copse	7.50	3.51	1.31	8.93	45.93	45.14	Loam
	7.70	3.65	1.53	8.08	42.09	49.83	
	7.55	3.69	1.48	8.29	42.93	48.78	
HS	5.56	5.28	1.27	3.91	45.75	50.34	Sandy loam
	6.51	5.03	1.28	4.26	50.21	45.53	
	6.45	5.39	1.22	4.51	53.39	42.10	

[1] Determined using a ratio of 1 : 2.5 (soil : water) (Ministry of Agriculture Fisheries and Food, 1986), [2] by loss on ignition at 350 °C to avoid decomposition of carbonates during ignition (Ayub and Boyd, 1994, CEAE, 2003), [3] using soil density rings of 95 cm^3 volume and [4] using a MasterSizer2000 laser particle size analyzer (Malvern Instruments, UK).

Detailed earthworm data

Table D2. Number and weight of the added adult earthworm species to each replicate (*n* = 3) of the frozen monolith with earthworm addition treatments for each field in March and November 2017. Letter R refers to replicates; all replicates received the same number of adult earthworms but slightly different weights.

Field	**Earthworm ecological group**	**Earthworm species**	**Earthworm addition on the 31st of March 2017**				**Earthworm addition on the 15th of November 2017**			
			Number	**Weight (g)**			**Number**	**Weight (g)**		
				R1	**R2**	**R3**		**R1**	**R2**	**R3**
BSE	Anecic	*Lumbricus terrestris*	2	4.65	5.35	6.44	2	9.28	8.74	8.85
		Allolobophora longa	0	0.00	0.00	0.00	1	2.34	1.20	1.49
	Endogeic	*Aporrectodea rosea*	3	0.63	0.60	0.50	2	0.44	0.34	0.31
		Allolobophora chlorotica	12	3.03	2.80	2.89	12	2.78	2.73	2.75
		Aporrectodea caliginosa	3	1.55	1.34	1.35	3	0.78	0.90	0.94
	Epigeic	*Lumbricus castaneus*	3	0.55	0.53	0.45	0	0.00	0.00	0.00
		Total	**23**	**10.41**	**10.63**	**11.63**	**20**	**15.61**	**13.91**	**14.34**
BSW	Anecic	*Lumbricus terrestris*	2	5.63	4.03	4.50	2	6.65	8.34	9.82

		Allolobophora longa	0	0.00	0.00	0.00	1	1.32	1.66	1.38
	Endogeic	*Aporrectodea rosea*	3	0.65	0.68	0.52	2	0.37	0.47	0.28
		Allolobophora chlorotica	12	3.05	2.83	3.01	12	3.29	2.60	2.71
		Aporrectodea caliginosa	3	1.41	1.46	1.39	3	0.93	0.65	0.53
	Epigeic	*Lumbricus castaneus*	3	0.68	0.44	0.45	0	0.00	0.00	0.00
		Total	**23**	**11.41**	**9.44**	**9.86**	**20**	**12.56**	**13.72**	**14.73**
Copse	Anecic	*Lumbricus terrestris*	2	4.88	4.25	4.09	2	8.51	7.27	7.53
		Allolobophora longa	0	0.00	0.00	0.00	1	1.60	1.50	1.49
	Endogeic	*Aporrectodea rosea*	3	0.74	0.57	0.53	2	0.41	0.31	0.29
		Allolobophora chlorotica	12	3.00	2.88	2.93	12	2.93	2.71	2.71
		Aporrectodea caliginosa	3	1.39	1.25	1.39	3	0.62	0.74	0.85
	Epigeic	*Lumbricus castaneus*	3	0.59	0.62	0.43	0	0.00	0.00	0.00
		Total	**23**	**10.60**	**9.56**	**9.36**	**20**	**14.08**	**12.52**	**12.87**
HS	Anecic	*Lumbricus terrestris*	2	4.94	4.38	3.83	2	8.59	9.96	6.71
		Allolobophora longa	0	0.00	0.00	0.00	1	1.53	1.78	3.64
	Endogeic	*Aporrectodea rosea*	3	0.55	0.59	0.52	2	0.54	0.45	0.51

		Allolobophora chlorotica	12	2.88	2.92	2.78	12	2.42	2.69	2.69
		Aporrectodea caliginosa	3	1.64	1.47	1.36	3	0.87	1.45	0.81
	Epigeic	*Lumbricus castaneus*	3	0.64	0.52	0.46	0	0.00	0.00	0.00
		Total	**23**	**10.64**	**9.89**	**8.95**	**20**	**13.96**	**16.34**	**14.37**

Table D3. Number and weight of removed juvenile and adult earthworms from each replicate ($n = 3$) of the frozen monoliths without earthworm addition (DeF treatments). Earthworms were extracted in November 2017 by applying mustard solution to the monolith surface. Juveniles and adults were counted separately but weighed together. Letters between brackets refer to, an = anecic, en = endogeic and ep = epigeic earthworm.

Field	**Monolith replicate**	**Earthworm species**	**Number**		**Total number**	**Total weight (g)**
			Juvenile	**Adult**		
BSE	1	*Lumbricus terrestris* (an)	1	1	4	5.32
		Allolobophora chlorotica (en)	0	1		
		Aporrectodea caliginosa (en)	0	1		
	2	*Lumbricus terrestris* (an)	5	0	9	2.61
		Allolobophora chlorotica (en)	0	1		
		Aporrectodea caliginosa (en)	1	0		
		Lumbricus castaneus (ep)	2	0		
	3	*Lumbricus terrestris* (an)	2	0	3	1.2
		Aporrectodea caliginosa (en)	1	0		
BSW	1	*Lumbricus terrestris* (an)	5	0	10	2.45
		Lumbricus castaneus (ep)	5	0		
	2	*Lumbricus terrestris* (an)	10	0	18	8.56
		Allolobophora chlorotica (en)	0	1		
		Aporrectodea caliginosa (en)	1	1		
		Lumbricus castaneus (ep)	3	2		
	3	*Lumbricus terrestris* (an)	8	0	18	8.53
		Aporrectodea caliginosa (en)	0	2		
		Lumbricus castaneus (ep)	2	6		
Copse	1	*Lumbricus terrestris* (an)	3	0	3	2.17
	2	*Lumbricus castaneus* (ep)	6	2	8	1.03
	3	*Lumbricus terrestris* (an)	2	0	11	1.98
		Lumbricus castaneus (ep)	4	5		

HS	1	*Allolobophora chlorotica* (en)	1	2	3	0.77
	2	*Lumbricus terrestris* (an)	3	0	3	0.98
	3	*Lumbricus terrestris* (an)	4	0	7	3.81
		Aporrectodea rosea (en)	1	0		
		Aporrectodea caliginosa (en)	1	0		
		Lumbricus castaneus (ep)	1	0		

Table D4. Recovered earthworm species in number and weight for adults and juveniles of each treatment replicate for each field at the end of the experiment. Letters refer to, DeF+E = Frozen monoliths with earthworm addition, DeF = Frozen monoliths without earthworm addition, and R = replicates.

Field	**Earthworm ecological groups**	**Earthworm species**		**DeF+E (n = 3)**				**DeF (n = 3)**				**Control (n = 1)**			
				Adult		**Juvenile [a]**		**Adult**		**Juvenile [a]**		**Adults**		**Juvenile [a]**	
				Number	**Weight (g)**	**Number**	**Weight (g)**	**Number**	**Weight (g)**	**Number**	**Weight (g)**	**Number**	**Weight (g)**	**Number**	**Weight (g)**
BSE	**Anecic**	*Lumbricus terrestris*	R1	2	9.3879	9	4.6122	0	0	1	1.1309	0	0	11	7.6592
			R2	1	3.3806			0	0						
			R3	1	3.3553	4	1.1472	0	0	1	0.7528				
		Allolobophora longa	R1	1	2.4773			0	0			0	0		
			R2	0	0	4	1.0356	0	0	0	0				
			R3	1	1.2305			0	0						
	Endogeic	*Aporrectodea rosea*	R1	3	0.6479	0	0	1	0.2274	0	0	1	0.2394	0	0
			R2	2	0.5498	0	0	1	0.1971	0	0				
			R3	2	0.4511	0	0	0	0	0	0				
		Allolobophora chlorotica	R1	13	2.7001	3	0.7271	0	0	0	0	8	1.8487	1	0.1535
			R2	12	2.4079	3	0.1164	3	0.5669	0	0				
			R3	24	4.7297	2	0.2263	0	0	0	0				
		Aporrectodea caliginosa	R1	2	0.7507	2	0.1673	0	0	0	0	1	0.5897	1	0.5006
			R2	4	1.2379	1	0.0869	0	0	1	0.4084				
			R3	5	1.3695	3	0.5431	0	0	0	0				
	Epigeic	*Lumbricus rubellus*	R1	0	0	4	0.372	5	1.2168	2	0.2967	1	0.2315	2	0.294

			R2	0	0			2	0.3274						
			R3	0	0	6	0.6442	1	0.1628	2	0.1365				
		Lumbricus castaneus	R1	3	0.5685			2	0.3168			9	1.4422		
			R2	3	0.4715	3	0.2346	12	2.0991	1	0.0313				
			R3	2	0.4073			0	0						
		Unknown [b]	R1	0	0	3	1.9634	0	0	0	0	0	0	5	0.4083
			R2	0	0	6	0.4156	0	0	6	0.5808				
			R3	0	0	8	0.3754	0	0	0	0				
	Total			**81**	**36.1235**	**61**	**12.6673**	**27**	**5.1143**	**14**	**3.3374**	**20**	**4.3515**	**20**	**9.0156**
BSW	**Anecic**	*Lumbricus terrestris*	R1	1	4.3868	14	10.7982	0	0	1	0.1544	1	3.557	18	18.2749
			R2	0	0			0	0						
			R3	2	8.3376	10	12.1997	0	0	7	4.3081				
		Allolobophora longa	R1	0	0			0	0			0	0		
			R2	0	0	13	7.7835	0	0	4	0.5958				
			R3	1	1.1351			0	0						
	Endogeic	*Aporrectodea rosea*	R1	3	0.5712	0	0	0	0	0	0	0	0	0	0
			R2	2	0.4702	0	0	0	0	0	0				
			R3	4	0.9162	0	0	0	0	0	0				
		Allolobophora chlorotica	R1	15	3.37753	4	0.34	0	0	0	0	12	3.2395	2	0.1562
			R2	11	2.5758	3	0.3868	3	0.9162	0	0				
			R3	15	3.218	0	0	1	0.3234	0	0				
		Aporrectodea caliginosa	R1	9	3.2614	0	0	0	0	0	0	4	1.262	2	0.4781
			R2	1	0.389	0	0	0	0	2	0.5815				
			R3	7	2.6506	2	0.1766	0	0	0	0				

	Epigeic	*Lumbricus rubellus*	R1	2	0.3562	9	0.8697	0	0	0	0	1	0.2069	2	0.19917
			R2	3	0.6842			13	2.8299						
			R3	1	0.4744	7	0.8895	12	3.1377	7	0.8589				
		Lumbricus castaneus	R1	7	0.833			0	0			3	0.4925		
			R2	3	0.446	1	0.1907	7	1.1536	8	1.0653				
			R3	2	0.4297			2	0.4827						
	Unknown [b]		R1	0	0	2	0.2083	0	0	4	0.6455	0	0	3	0.4475
			R2	0	0	3	1.739	0	0	1	0.2931				
			R3	0	0	7	0.6214	0	0	2	4.6186				
	Total			**89**	**34.51293**	**75**	**36.2034**	**38**	**8.8435**	**36**	**13.1212**	**21**	**8.7579**	**27**	**19.55587**
Copse	**Anecic**	*Lumbricus terrestris*	R1	1	5.9073	7	5.8723	0	0	0	0	1	4.0253	14	6.5617
			R2	1	3.1006			1	4.4381						
			R3	1	5.8133	6	2.9997	0	0	3	1.8163				
		Allolobophora longa	R1	2	2.5511			0	0			0	0		
			R2	1	1.4511	4	2.2335	0	0	1	2.2708				
			R3	1	1.7261			0	0						
	Endogeic	*Aporrectodea rosea*	R1	4	0.9991	0	0	0	0	0	0	1	0.1722	0	0
			R2	2	0.5028	0	0	0	0	0	0				
			R3	3	0.5047	0	0	0	0	0	0				
		Allolobophora chlorotica	R1	19	4.548	5	0.4303	0	0	0	0	3	0.7478	0	0
			R2	12	2.6072	4	0.3786	0	0	0	0				
			R3	16	3.3077	3	0.2363	0	0		0				
		Aporrectodea caliginosa	R1	4	1.6445	0	0	0	0	0	0	1	0.2128	0	0
			R2	3	1.0733	0	0	0	0	0	0				

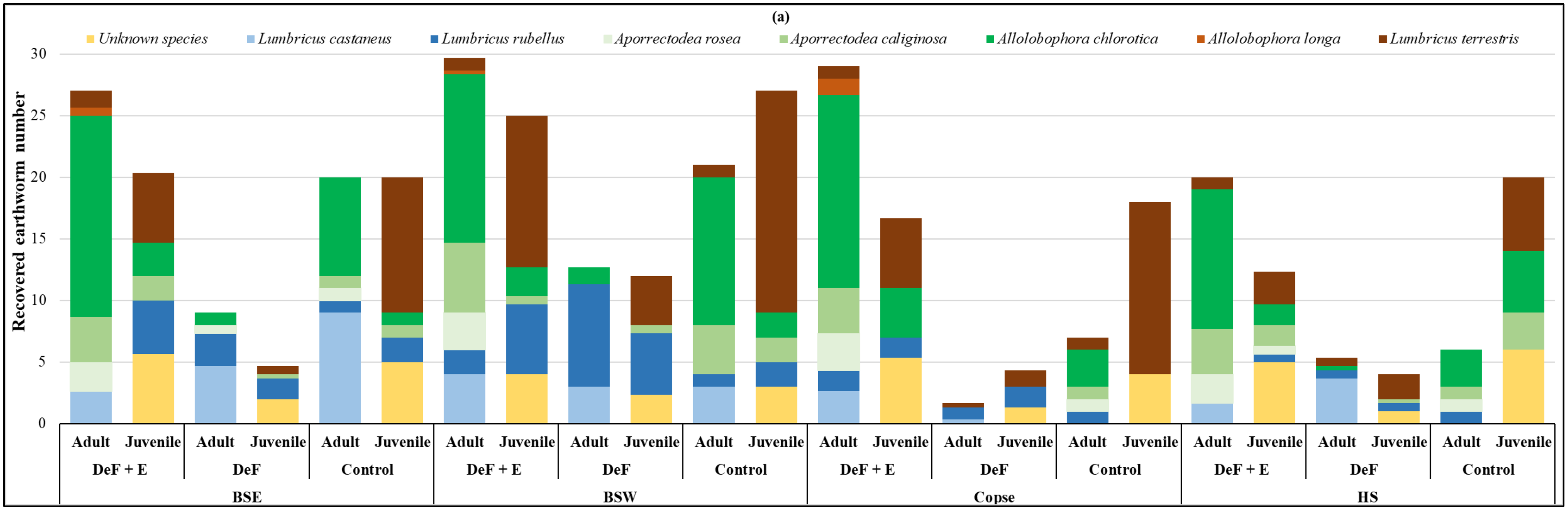
			R3	4	1.3888	0	0	0	0		0				
	Epigeic	*Lumbricus rubellus*	R1	1	0.193	1	0.1039	1	0.3225	3	0.361	1	0.259	0	0
			R2	2	0.449			1	0.1923						
			R3	2	0.4171	1	0.1545	1	0.255	1	0.1239				
		Lumbricus castaneus	R1	3	0.3572			1	0.0963			0	0		
			R2	3	0.4185	3	1.1181	0	0	1	0.0908				
			R3	2	0.3339			0	0						
	Unknown [b]		R1	0	0	5	0.542	0	0	0	0	0	0	4	0.4114
			R2	0	0	4	0.3463	0	0	0	0				
			R3	0	0	7	0.5656	0	0	4	0.5361				
	Total			**87**	**39.2943**	**50**	**14.9811**	**5**	**5.3042**	**13**	**5.1989**	**7**	**5.4171**	**18**	**6.9731**
HS [c]	**Anecic**	*Lumbricus terrestris*	R1	1	3.3782	4	3.047	0	0	3	5.8947	0	0	6	11.3594
			R2	2	8.2334			0	0						
			R3	0	0	1	2.6212	2	4.4105	3	4.3454				
		Allolobophora longa	R1	0	0			0	0			0	0		
			R2	0	0	3	0.3173	0	0	0	0				
			R3	0	0			0	0						
	Endogeic	*Aporrectodea rosea*	R1	4	0.6808	0	0	0	0	0	0	1	0.1672	0	0
			R2	2	1.1216	0	0	0	0						
			R3	1	0.1164	2	0.1216	0	0						
		Allolobophora chlorotica	R1	17	3.5114	5	0.4094	1	0.3105	0	0	3	0.4749	5	0.8492
			R2	14	3.195	0	0	0	0	0	0				
			R3	3	0.7022	0	0	0	0	0	0				
			R1	4	1.9064	2	0.2285	0	0	0	0	1	0.3419	3	2.0082

	Aporrectodea caliginosa	R2	5	1.4755	3	0.4857	0	0	1	0.7041				
		R3	2	0.7294	0	0	0	0	0	0				
Epigeic	*Lumbricus rubellus*	R1	0	0	1	0.0745	1	0.2154	1	0.1055	1	0.1536	0	0
		R2	0	0			0	0						
		R3	0	0	0	0	1	0.2444	1	0.0819				
	Lumbricus castaneus	R1	4	0.6314			10	1.6232			0	0		
		R2	1	0.1373	1	0.0641	0	0	0	0				
		R3	0	0			1	0.2066						
	Unknown [b]	R1	0	0	1	0.0865	0	0	1	3.3939	0	0	6	1.8639
		R2	0	0	11	1.7409	0	0	2	0.149				
		R3	0	0	3	0.1661	0	0	0	0				
	Total		**60**	**25.819**	**37**	**9.3628**	**16**	**7.0106**	**12**	**14.6745**	**6**	**1.1376**	**20**	**16.0807**

[a] Differentiation between two species of the same ecological group is not obvious for juveniles. Therefore, some species were classified at ecological group level for anecic and epigeic earthworms.

[b] To distinguish between very young juvenile species is not obvious and were therefore classified as unknown species.

[c] Replicate 3 of the DeF+E and DeF treatments of the HS field are excluded from the statistical analysis because of the herbicide spray drifted onto the edges of the ley strip affecting the earthworm populations in monoliths.

(a)
Unknown species
Lumbricus castaneus
Lumbricus rubellus
Aporrectodea rosea
Aporrectodea caliginosa
Allolobophora chlorotica
Allolobophora longa
Lumbricus terrestris
Recovered earthworm number
30
25
20
15
10
5
0
Adult
Juvenile
DeF + E
DeF
Control
BSE
BSW
Copse
HS

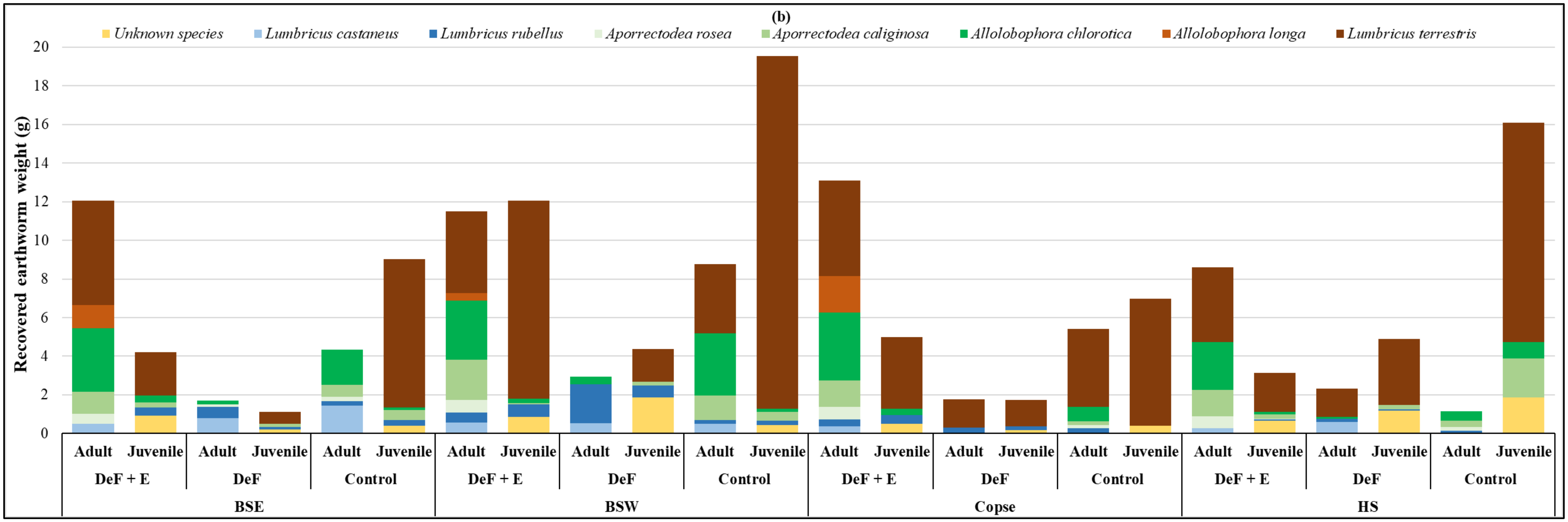
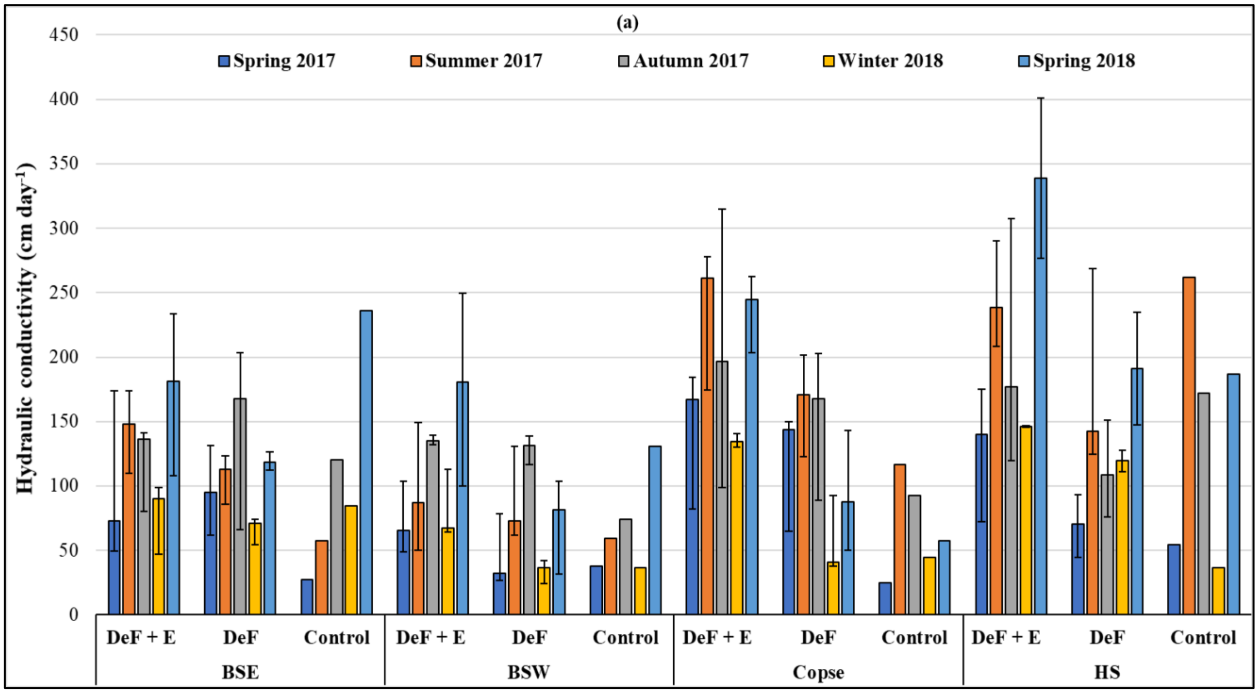
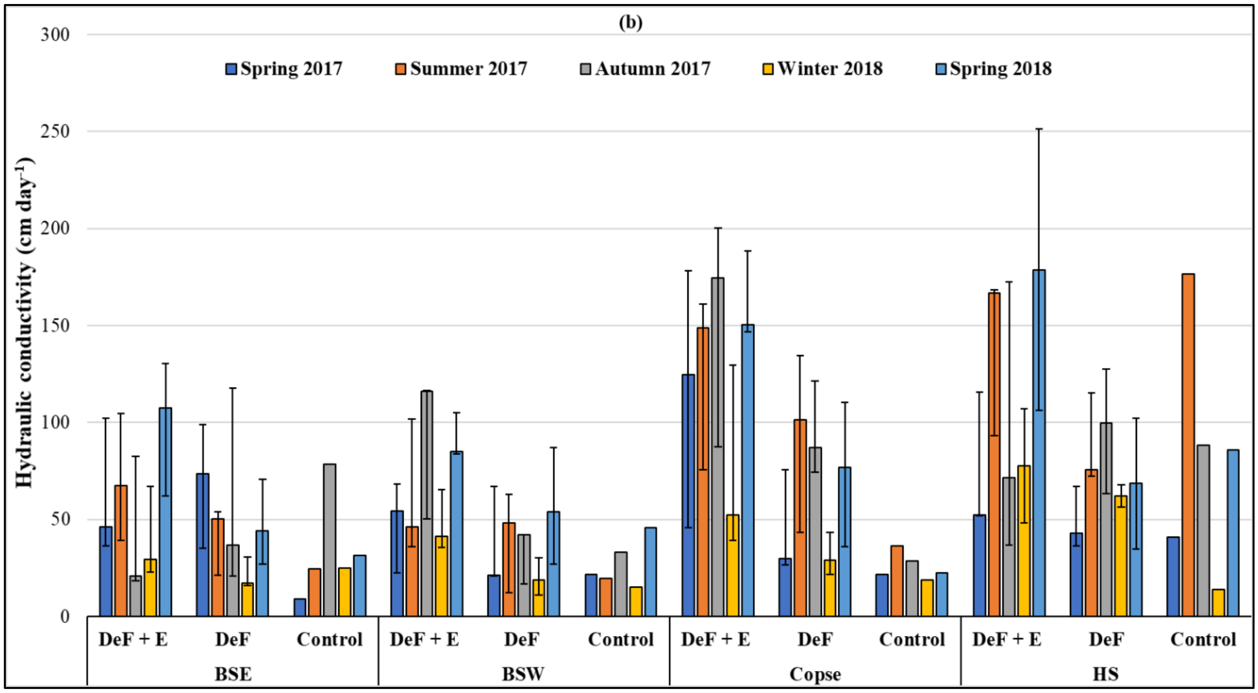
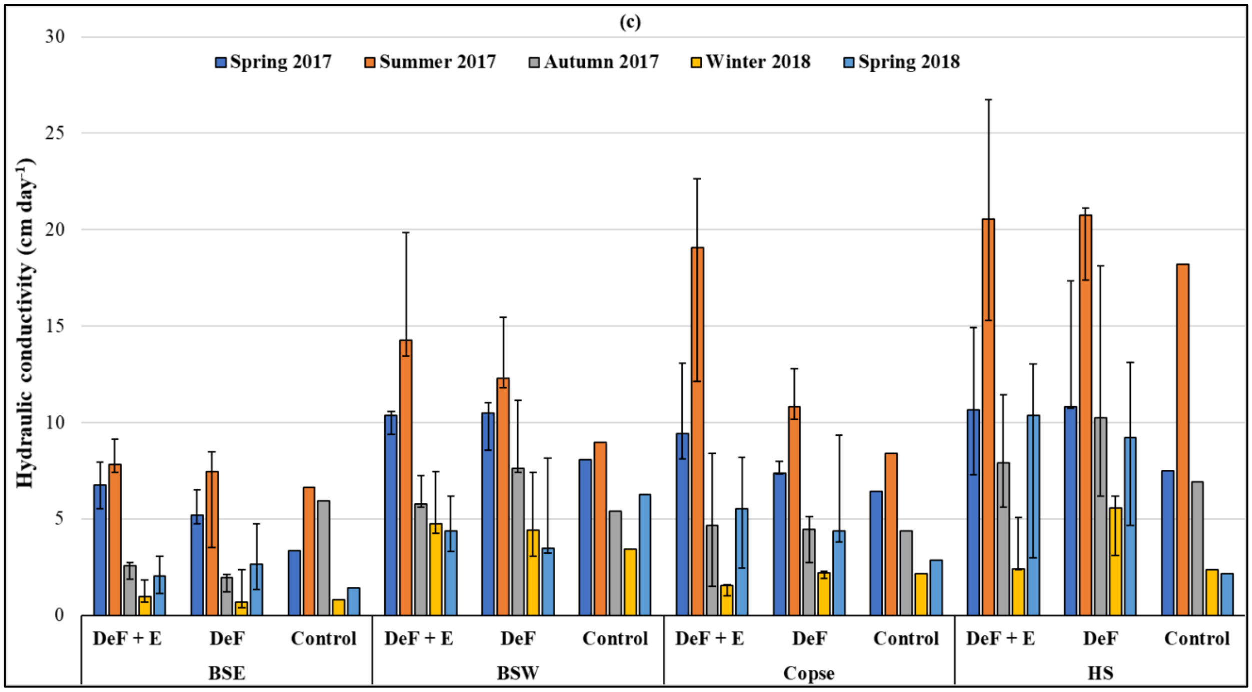


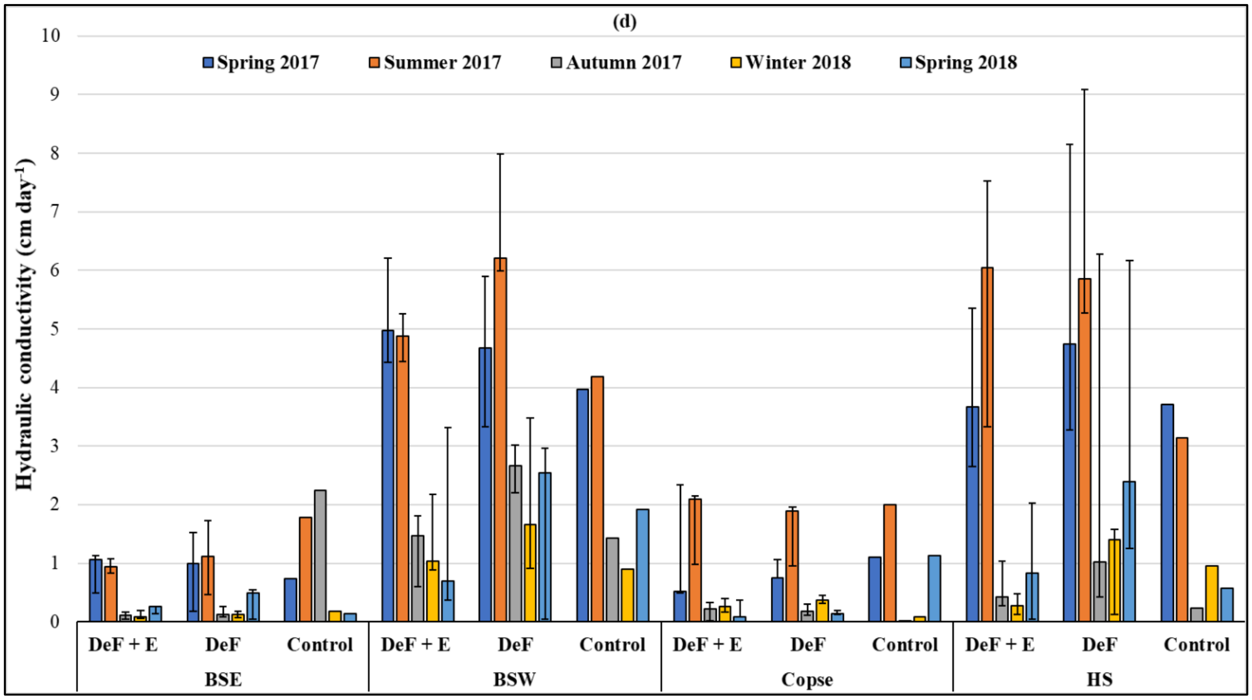
Figure D3. Mean of the recovered earthworm numbers (a) and weight (b) for adults and juveniles for each field. The figures represent the three treatments; DeF+E = frozen monoliths with earthworm addition (*n* = 3), DeF = frozen monoliths without earthworm addition (*n* = 3) and Control (*n* = 1). The chart stack color and its shades represent the ecological group of earthworm; Brown : anecic, green : endogeic and blue : epigeic species. *L. terretris* and *L. rebellus* species for juveniles represent anecic and epegiec ecological group respectively, and not species, for the purpose of this graph only.

Hydraulic conductivity at different tensions and across seasons for each field






(c)
Spring 2017
Summer 2017
Autumn 2017
Winter 2018
Spring 2018
Hydraulic conductivity (cm day^{-1})
30
25
20
15
10
5
0
DeF + E
DeF
Control
BSE
BSW
Copse
HS


(d)
Spring 2017
Summer 2017
Autumn 2017
Winter 2018
Spring 2018
Hydraulic conductivity (cm day^{-1})
10
9
8
7
6
5
4
3
2
1
0
DeF + E
DeF
Control
BSE
BSW
Copse
HS

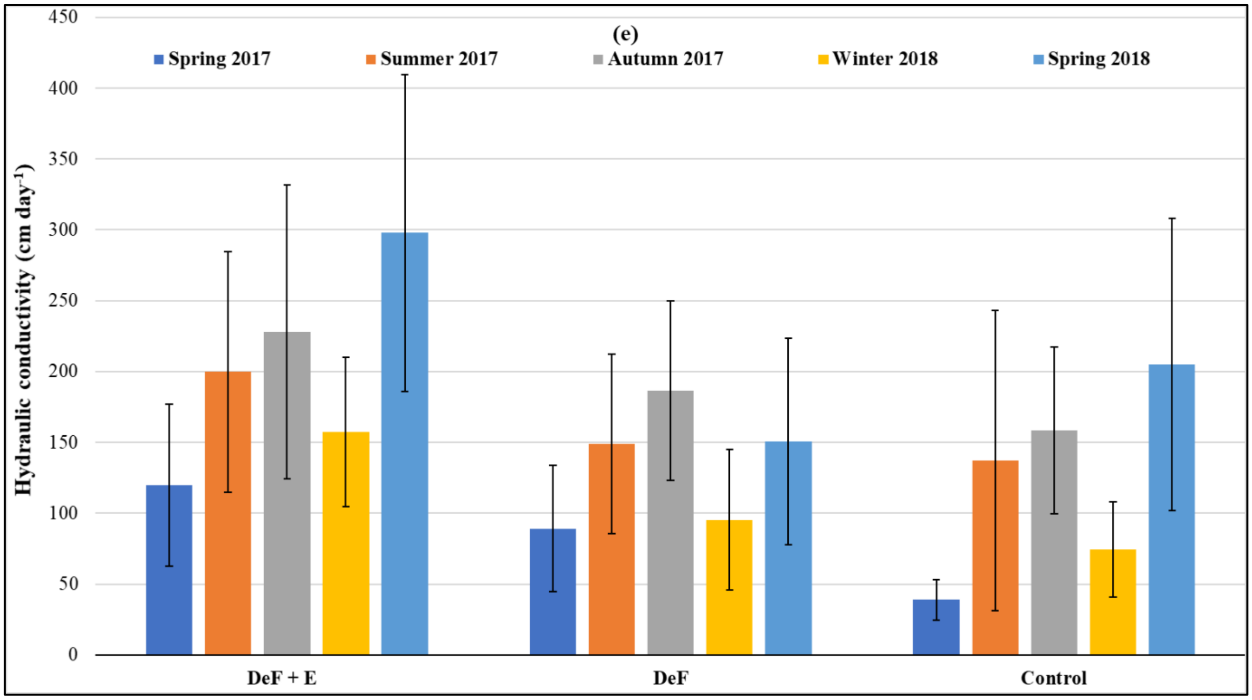
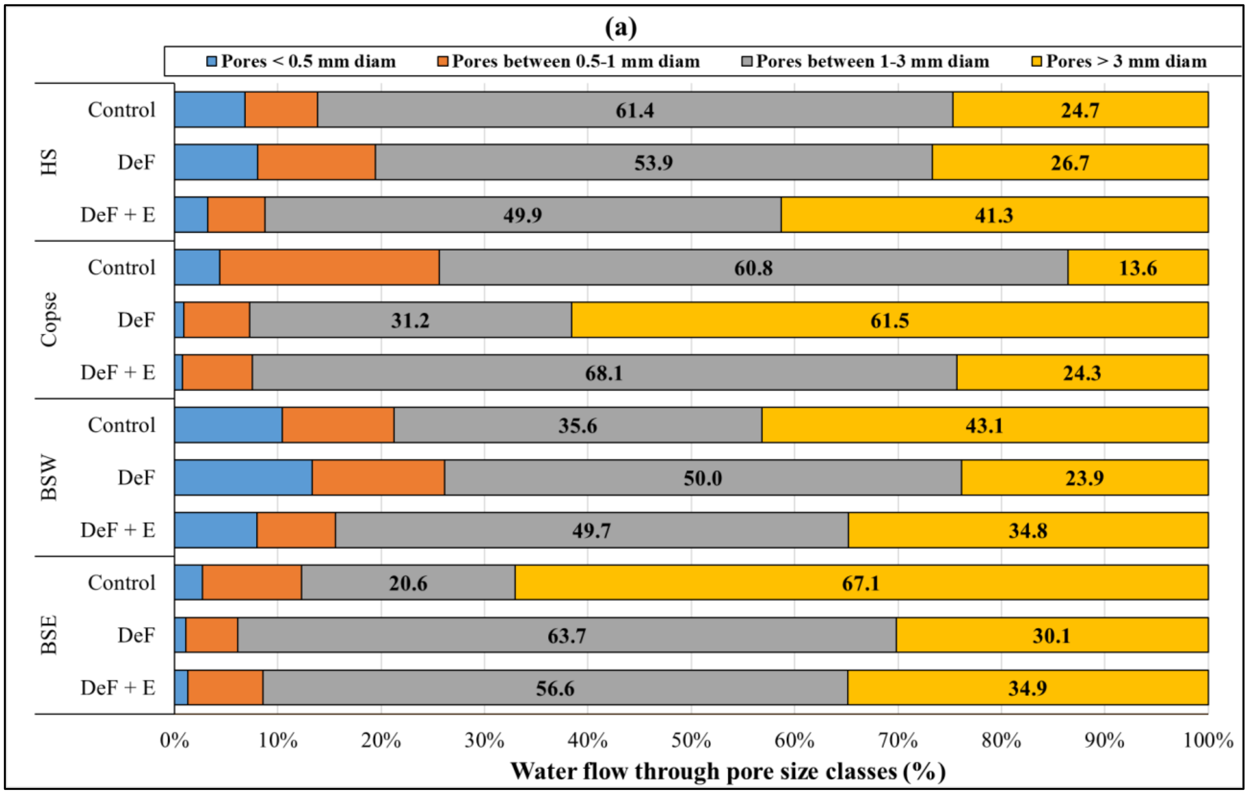


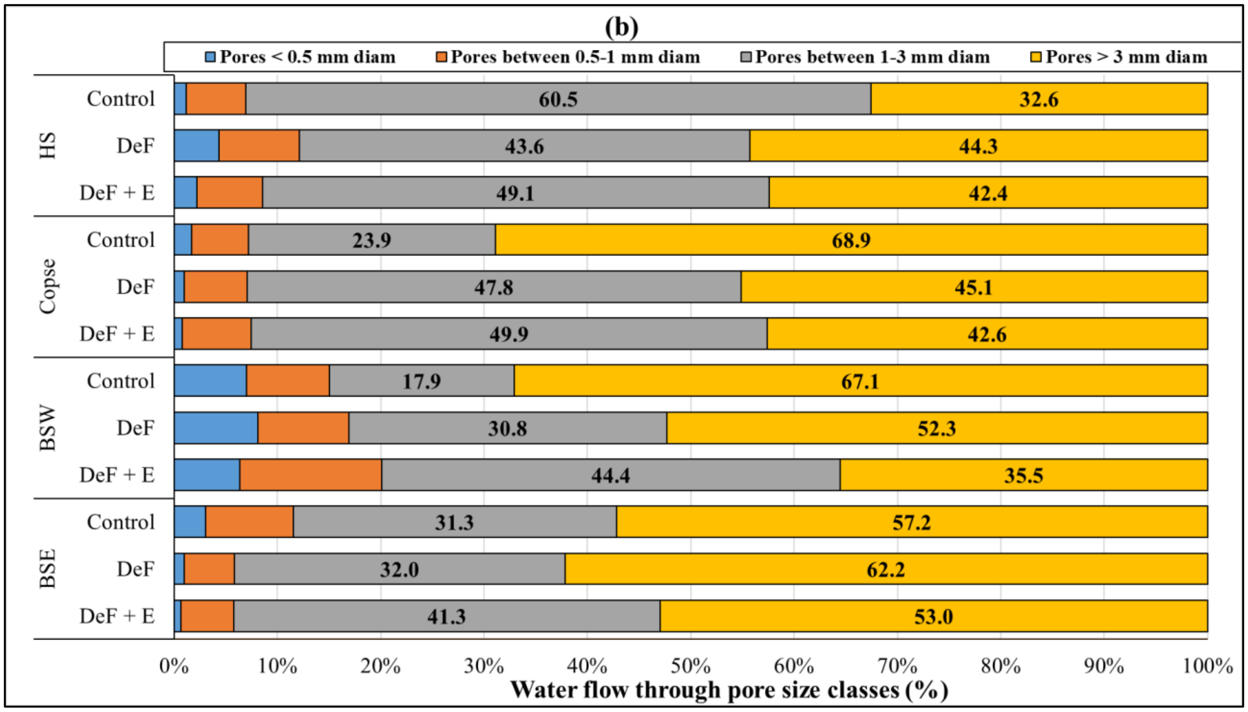
Figure D4. Median hydraulic conductivity at (a) -0.5 cm, (b) -1 cm, (c) -3 cm and (d) -6 cm tensions in the four different fields (BSE, BSW, Copse and HS). (e) shows the mean hydraulic conductivity at -0.5 cm tension adjusted for water viscosity to 20 °C equivalent across seasons and all the fields. DeF+E = frozen monoliths with earthworm addition (n = 3), DeF = frozen monoliths without earthworm addition (n = 3), and Control (n = 1); error bars = max and min values (a, b, c, d) and standard deviation (e).

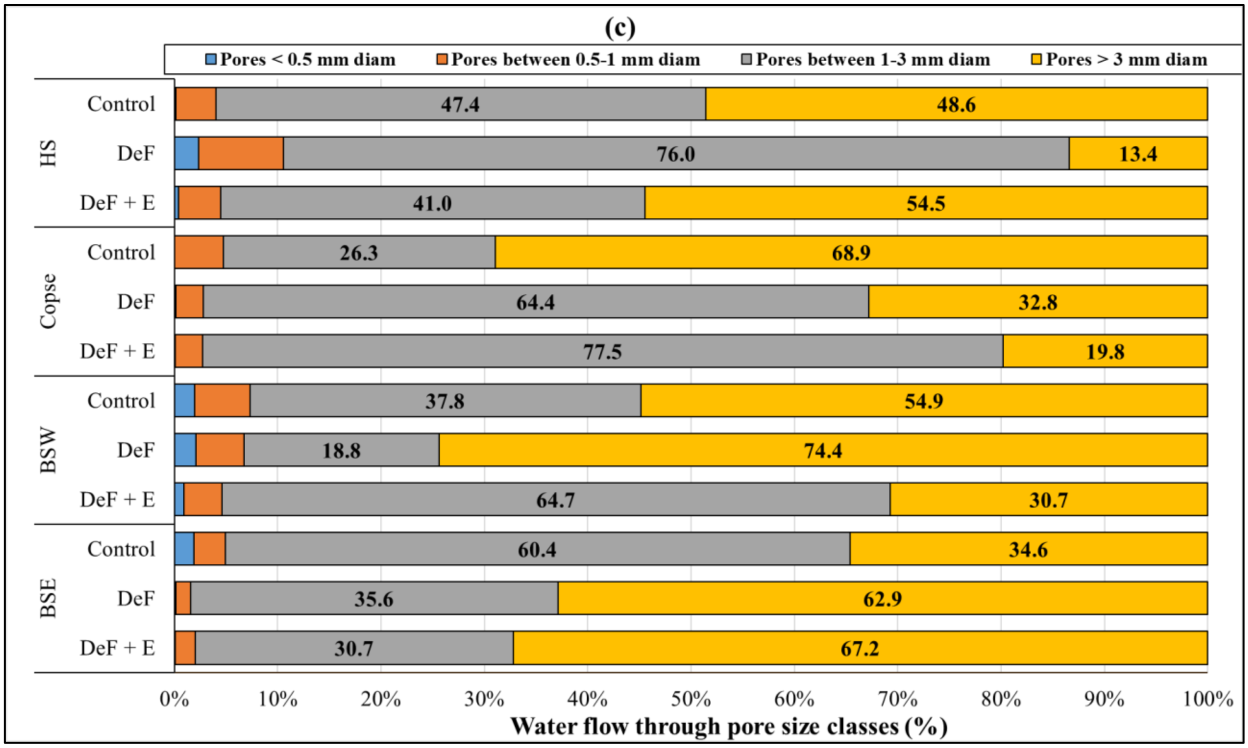
Results of the interaction between factors for K_3

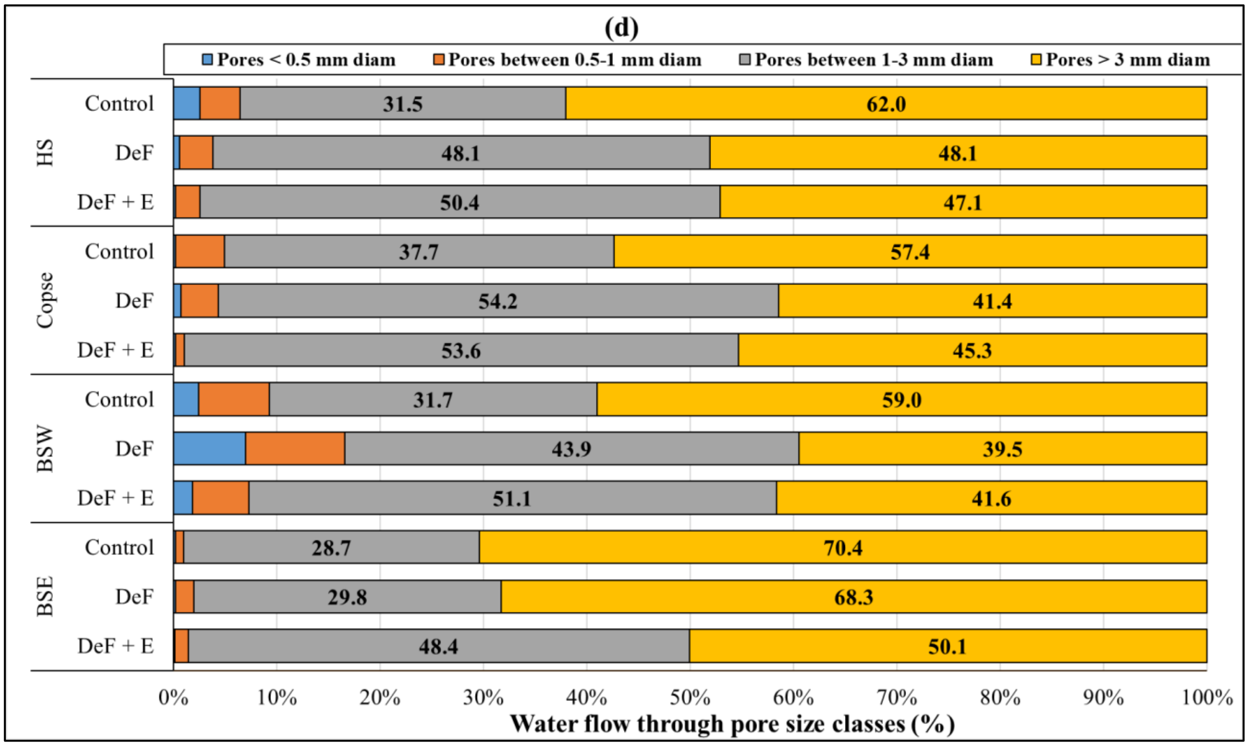
At a tension of – 3 cm K_3 showed a significant interaction between seasons and treatments ($p < 0.05$) or fields ($p < 0.01$) (Figure D4 (c)). K_3 was only greater in the DeF+E monoliths compared to the DeF monoliths in Summer 2017 ($p = 0.043$), no differences were observed between treatments in the other seasons. K_3 increased in both DeF+E and DeF treatments from Spring to Summer 2017 and from Winter to Spring 2018 ($p < 0.01$) but decreased from Summer to Autumn 2017 and from Autumn 2017 to Winter 2018 ($p < 0.01$). K_3 of Copse and HS fields showed a significant increase from Spring to Summer 2017 and from Winter to Spring 2018 ($p < 0.01$) and a decrease from Summer to Autumn 2017 and from Autumn 2017 to Winter 2018 ($p < 0.05$). BSE and BSW fields showed a significant decrease of K_3 from Summer to Autumn 2017 ($p < 0.05$).

Pore classes contribution to water flow across seasons for each field






(c)
Pores < 0.5 mm diam
Pores between 0.5-1 mm diam
Pores between 1-3 mm diam
Pores > 3 mm diam
HS
Control
47.4
48.6
DeF
76.0
13.4
DeF + E
41.0
54.5
Copse
Control
26.3
68.9
DeF
64.4
32.8
DeF + E
77.5
19.8
BSW
Control
37.8
54.9
DeF
18.8
74.4
DeF + E
64.7
30.7
BSE
Control
60.4
34.6
DeF
35.6
62.9
DeF + E
30.7
67.2
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Water flow through pore size classes (%)


(d)
Pores < 0.5 mm diam
Pores between 0.5-1 mm diam
Pores between 1-3 mm diam
Pores > 3 mm diam
HS
Control
31.5
62.0
DeF
48.1
48.1
DeF + E
50.4
47.1
Copse
Control
37.7
57.4
DeF
54.2
41.4
DeF + E
53.6
45.3
BSW
Control
31.7
59.0
DeF
43.9
39.5
DeF + E
51.1
41.6
BSE
Control
28.7
70.4
DeF
29.8
68.3
DeF + E
48.4
50.1
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Water flow through pore size classes (%)

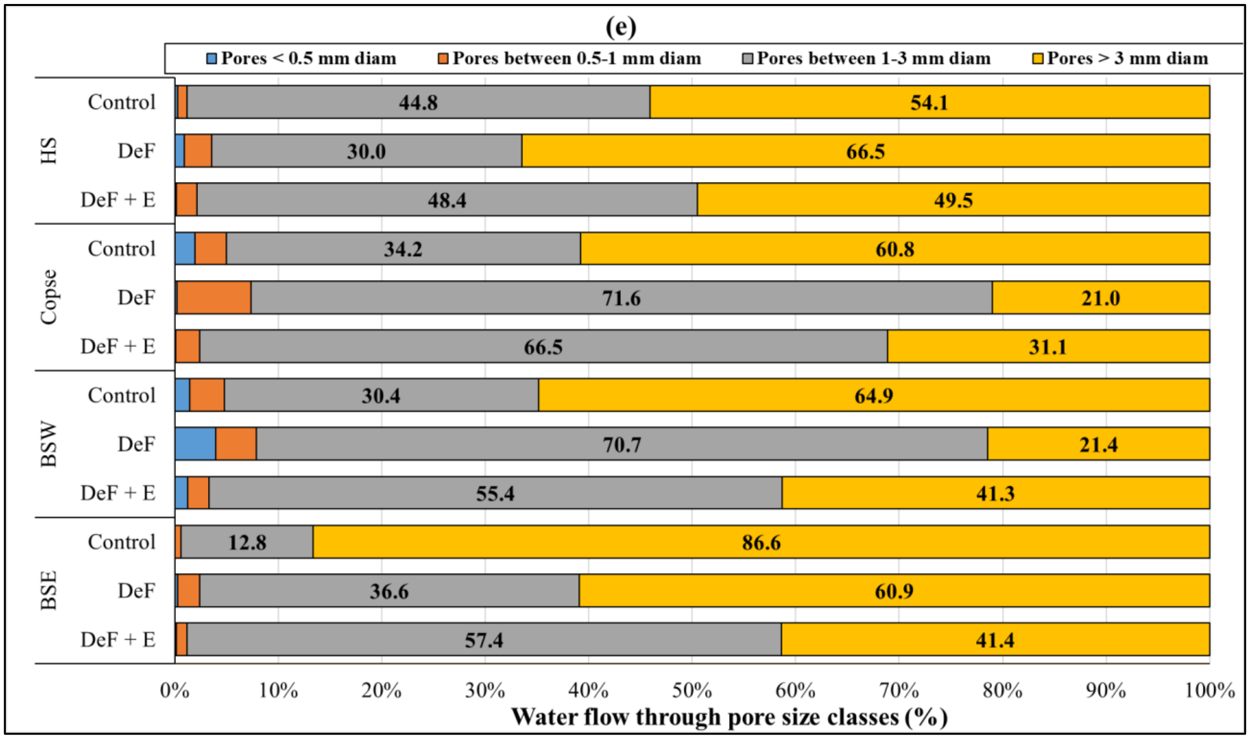
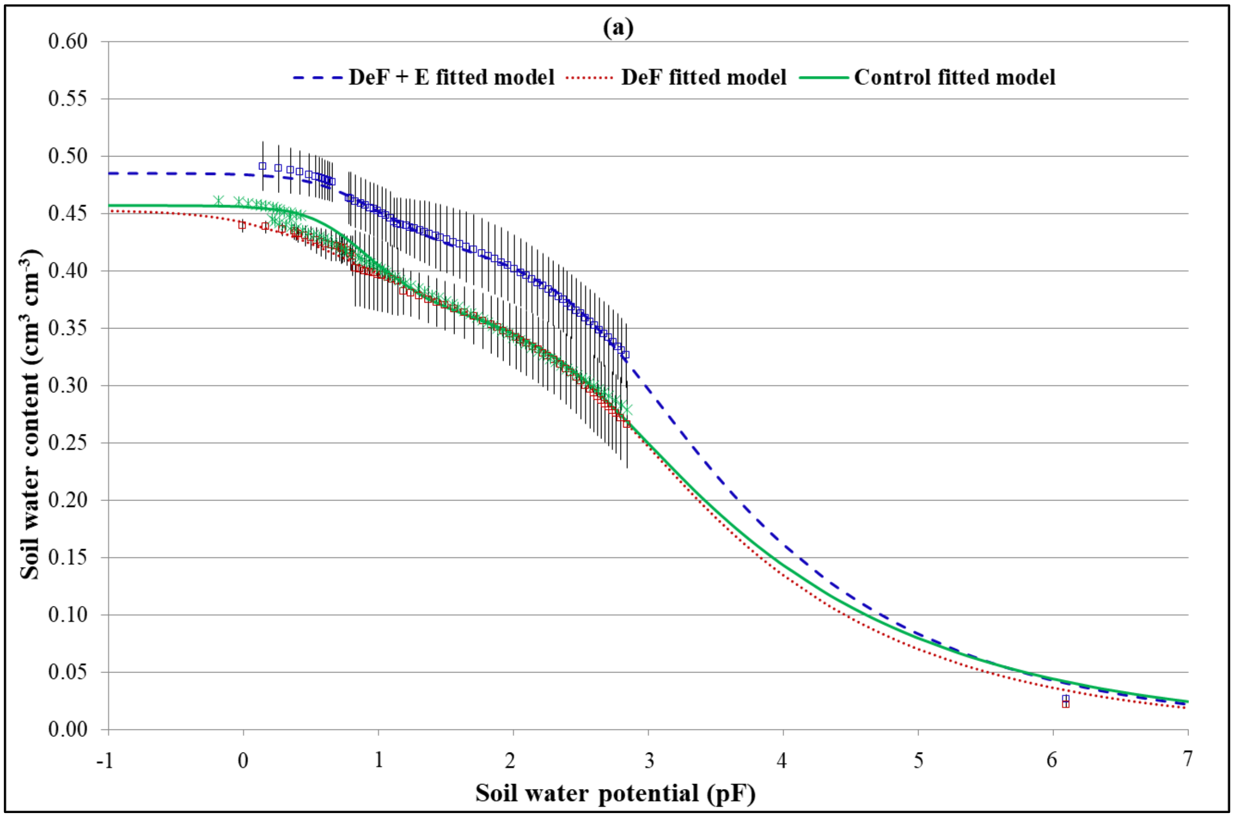
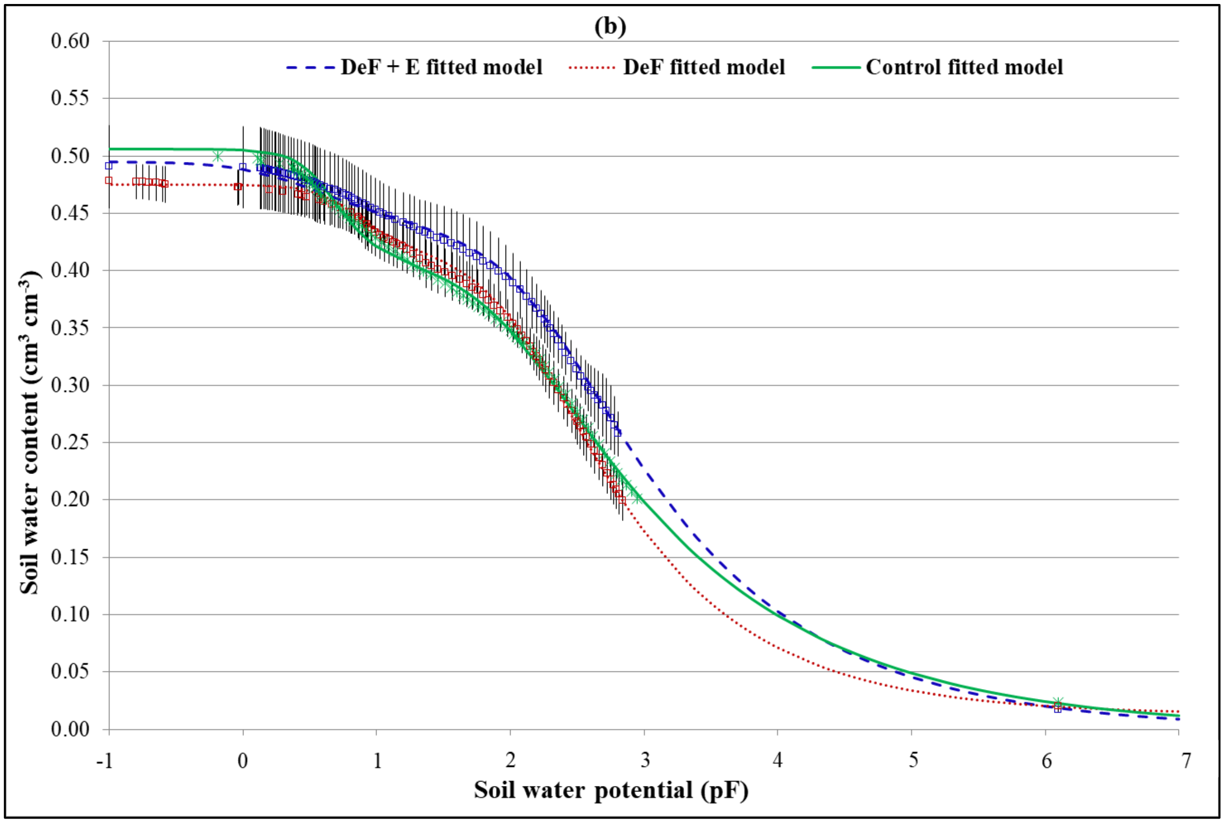
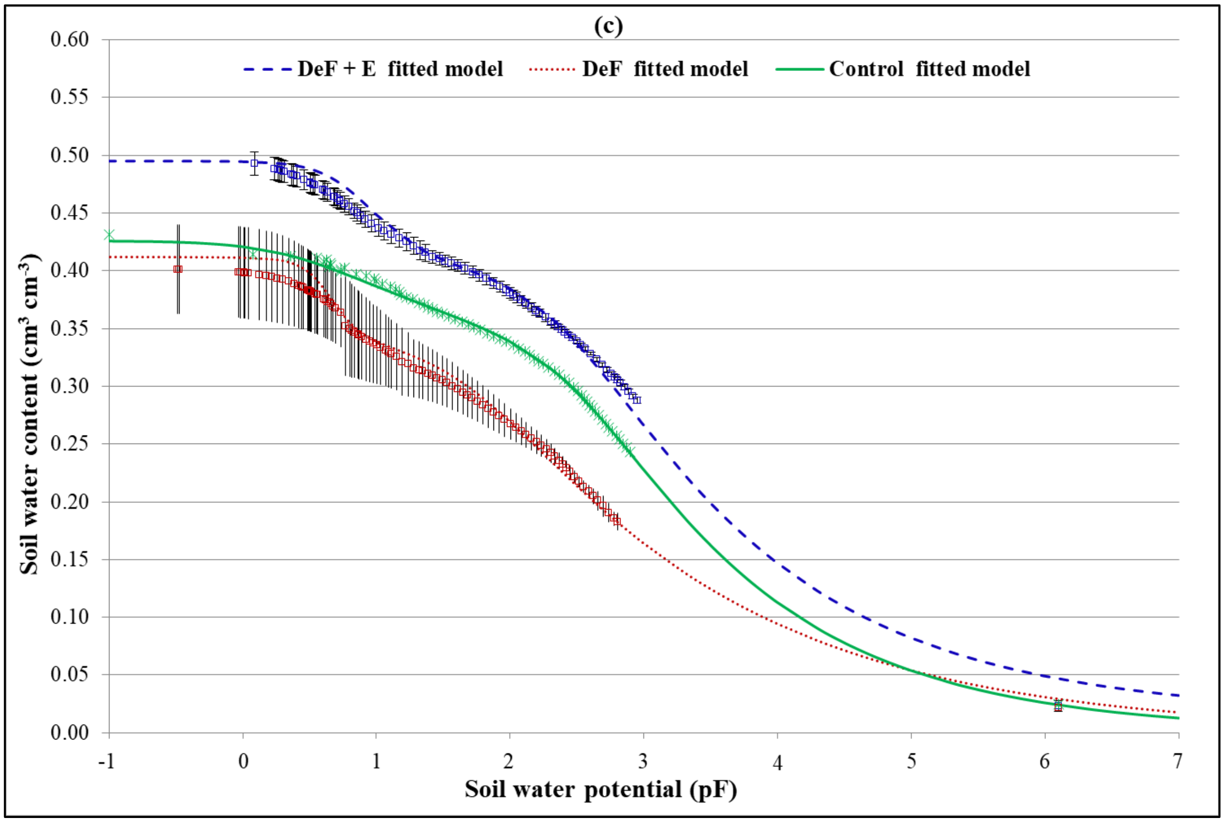


Figure D5. Pore size class contribution to water flow across seasons. The figures represent the three treatments; DeF+E = frozen moniliths with earthworm addition (*n* = 3), DeF = frozen monoliths without earthworm addition (*n* = 3) and Control (*n* = 1) for the BSE, the BSW, the Copse and the HS fields. Letters refer to different time points when the measures were made; (a) May 2017, (b) August 2017, (c) November 2017, (d) January 2018 and (e) April 2018.

Soil water release curves data




(b)
DeF + E fitted model
DeF fitted model
Control fitted model
Soil water content (cm^3 cm^{-3})
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-1
0
1
2
3
4
5
6
7
Soil water potential (pF)


(c)
DeF + E fitted model
DeF fitted model
Control fitted model
Soil water content (cm^3 cm^{-3})
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-1
0
1
2
3
4
5
6
7
Soil water potential (pF)

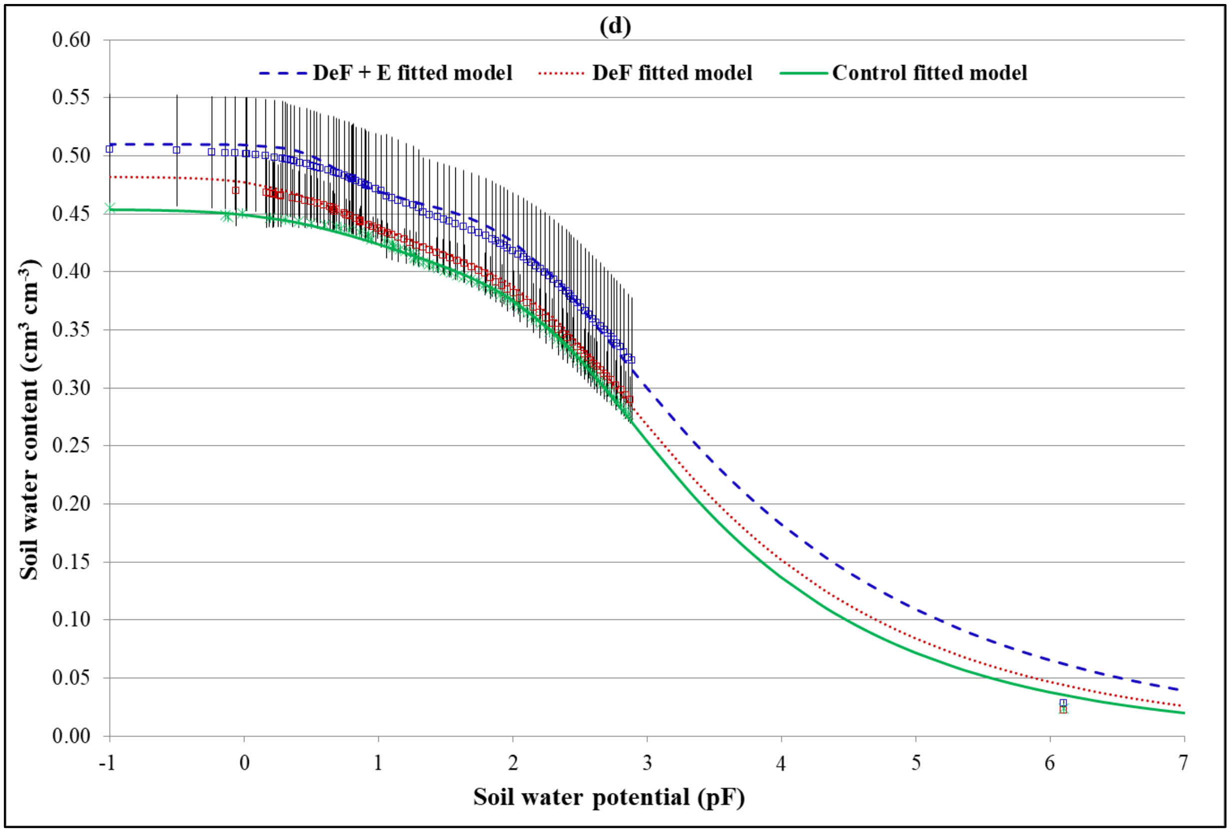


Figure D6. Soil water release curves fitted to the measured data using the bimodal constrained Van Genuchten (1980) model (Durner, 1994). The curves represent the three treatments; DeF+E = frozen monoliths with earthworm addition, DeF = frozen monoliths without earthworm addition and Control. Letters refer to (a) the BSE, (b) the BSW, (c) the Copse and (d) the HS fields. Three replicates were combined each for the DeF+E and the DeF treatments and fitted using Hyprop-Fit models. Only one treatment was fitted for the Control (error bars = Standard deviation).

Table D5. Soil water content at different potentials derived from soil water release curves (SWRC) fitted to the measured data using the bimodal constrained Van Genuchten (1980) model (Durner, 1994). DeF+E = frozen monoliths with earthworm addition (*n* = 3), DeF = frozen monoliths without earthworm addition (*n* = 3) and Combined data = values derived from the combined SWRC of three replicates each for the DeF+E and the DeF treatments and fitted using Hyprop-Fit models. Only one replicate was fitted for the Control treatment.

Field	**Treatment**	**Replicates**	**Water content at saturation**	**Water content at field capacity (33 KPa)**	**Water content at wilting point (1500 KPa)**	**Plant available water (33 to 1500KPa)**
BSE	DeF+E	1	50.9	38.2	13.3	25.2
		2	47.5	33.1	11.7	21.4
		3	49.1	37.1	14.1	23
		Combined data	**48.5**	**36.3**	**14.2**	**22.1**
	DeF	1	46.2	33	11.4	21.6
		2	45.1	26.4	9.9	16.5
		3	45.2	31.4	11.6	19.8
		Combined data	**45.3**	**30.4**	**11.9**	**18.5**
	Control	1	45.7	30.5	12.8	17.7
BSW	DeF+E	1	46.3	33.5	9.5	24
		2	53	30.1	6.7	23.4
		3	51.7	29.7	8.4	21.3

		Combined data	**49.5**	**31.4**	**8.8**	**22.6**
	DeF	1	44.9	27.3	5.5	21.8
		2	49.9	27.4	7	20.4
		3	46.3	23.9	5.7	18.2
		Combined data	**47.5**	**26.1**	**6.1**	**20**
	Control	1	50.6	27	8.7	18.3
Copse	DeF+E	1	49.9	33.3	10.8	22.5
		2	48.6	33.5	14.2	19.3
		3	51.9	33.9	13.5	20.4
		Combined data	**49.5**	**33.5**	**13**	**20.5**
	DeF	1	41.7	21.8	6.7	15.1
		2	43.1	21.9	9.3	12.6
		3	36	21	6.2	14.8
		Combined data	**41.2**	**21.2**	**8.4**	**12.8**
	Control	1	42.6	29.3	9.8	19.5
HS	DeF+E	1	52.4	42.5	15.7	26.8
		2	54.3	37.1	14.5	22.6
		3	46.2	31.6	12	19.6
		Combined data	**51**	**36.8**	**16.5**	**20.3**
	DeF	1	46.6	33.6	12.3	21.3
		2	51.5	30.8	11.6	19.2
		3	45.8	35.3	14.4	20.9
		Combined data	**48.2**	**33.5**	**13.5**	**20**
	Control	1	45.4	32.2	12	20.2

Soil water holding capacity for each field

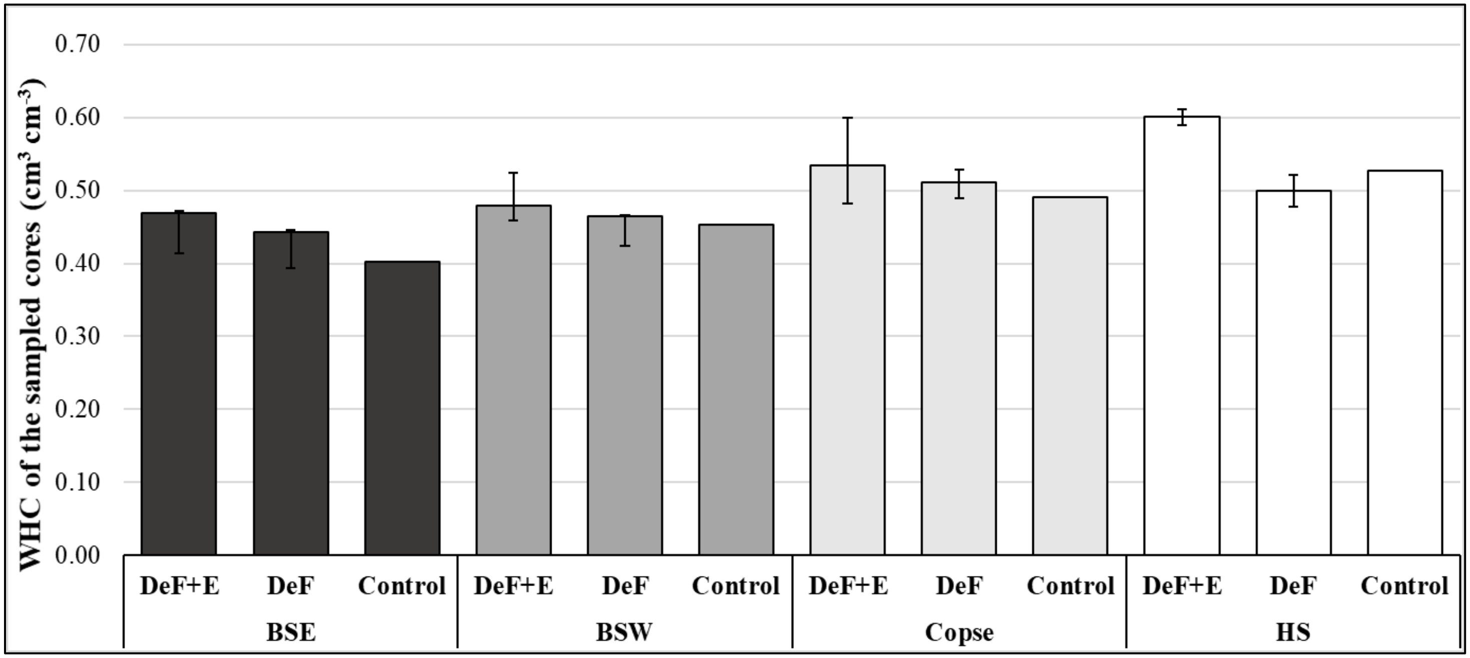


Figure D7. Median water holding capacities of the 3.5 cm x 5 cm cores taken from the different treatments in the BSE, BSW, Copse and HS fields. DeF+E = frozen monoliths with earthworm addition (*n* = 3), DeF = frozen monoliths without earthworm addition (*n* = 3), *n* = 1 for the Control; error bars = max and min values.

Soil dry bulk density for each field

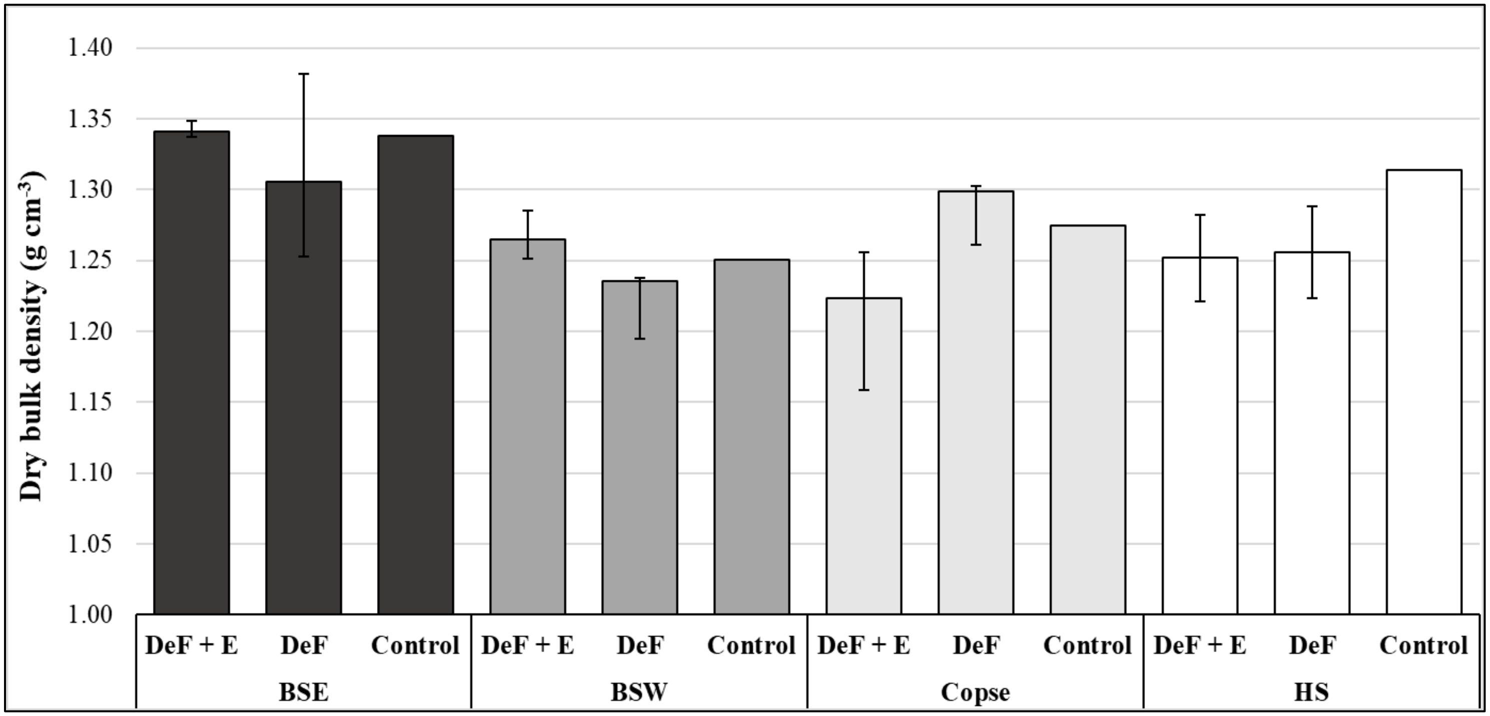


Figure D8. Median of soil dry bulk density in the different treatments in the BSE, BSW, Copse and HS fields. DeF+E = frozen monoliths with earthworm addition (*n* = 3), DeF = frozen monoliths without earthworm addition (*n* = 3), *n* = 1 for Control; error bars = max and min values.

Percentage soil water stable aggregates for each field

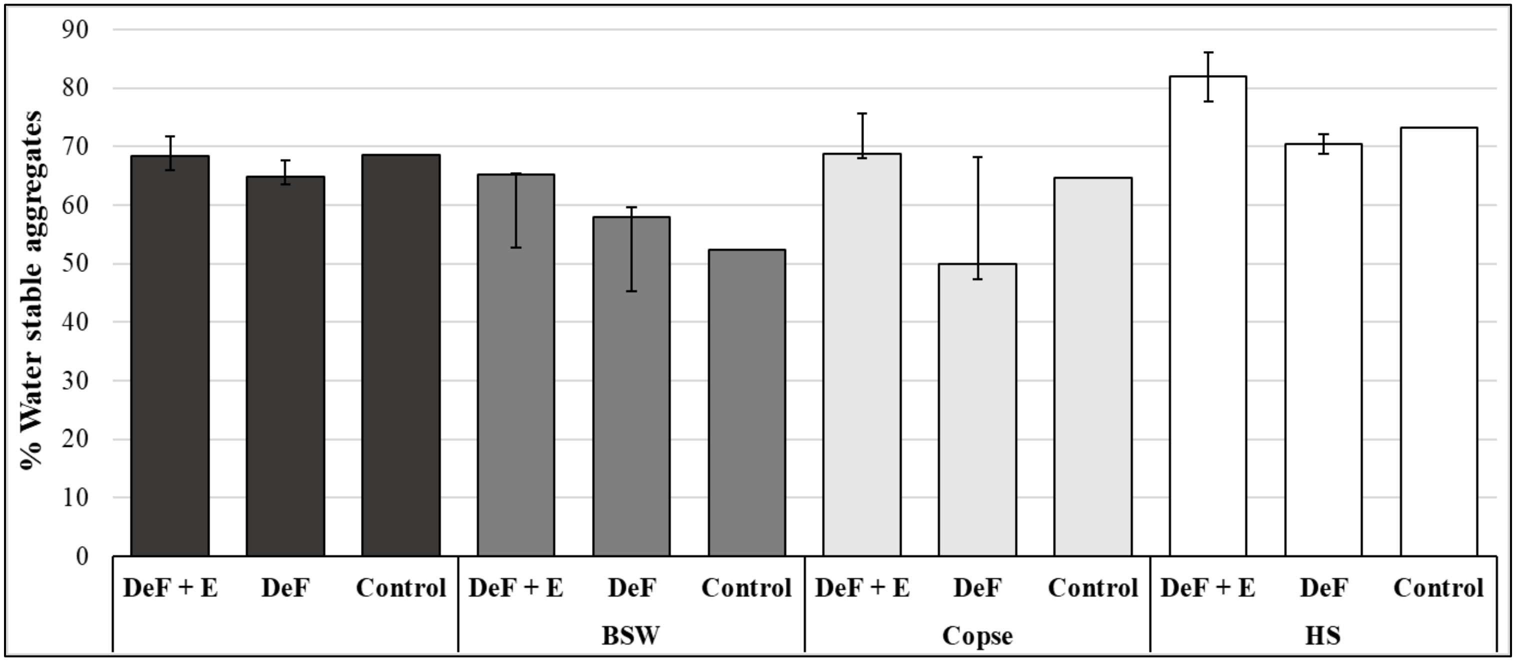


Figure D9. Median of percentage water stable aggregates (%WSA) in the different treatments in the BSE, BSW, Copse and HS fields. DeF+E = frozen monoliths with earthworm addition (*n* = 3), DeF = frozen monoliths without earthworm addition (*n* = 3), *n* = 1 for Control; error bars = max and min values.

Soil organic matter content for each field

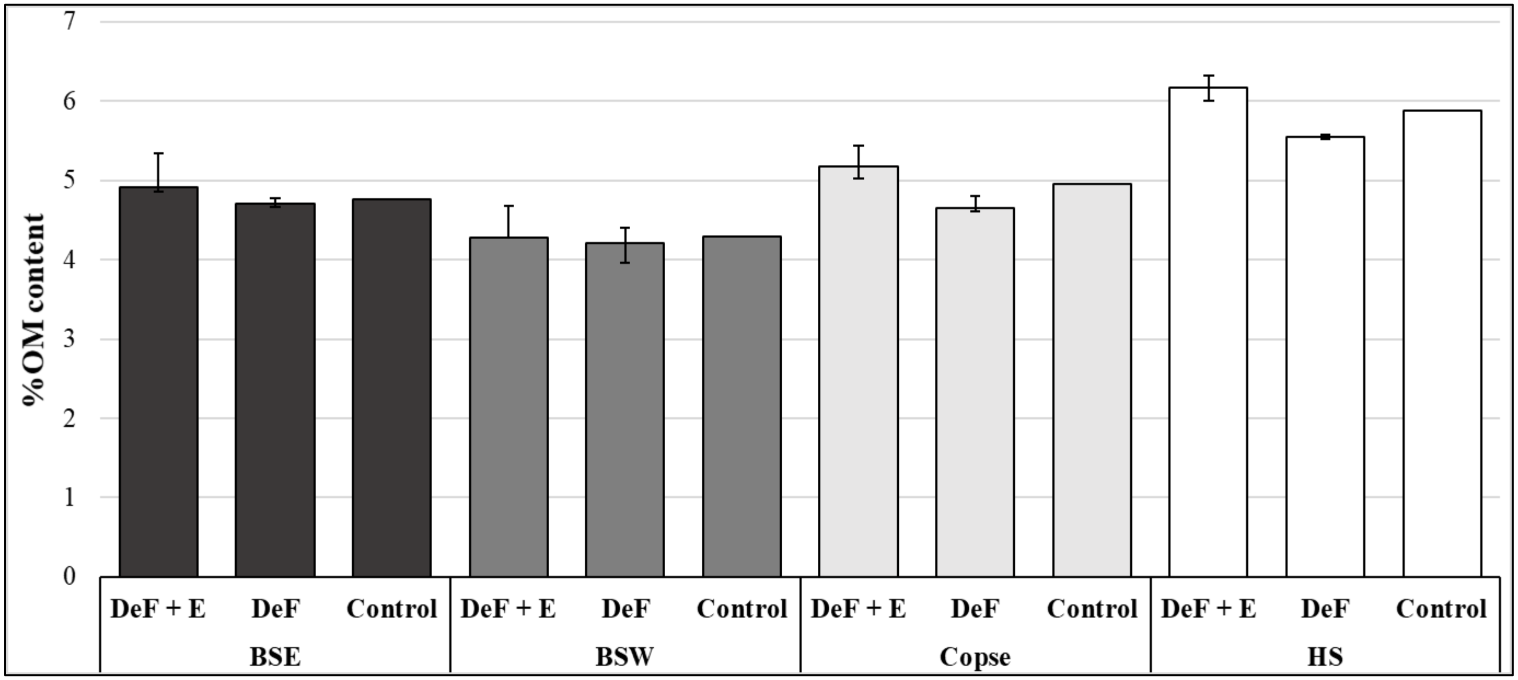


Figure D10. Median %OM in the different treatments in the BSE, BSW, Copse and HS fields. DeF+E = frozen monoliths with earthworm addition (*n* = 3), DeF = frozen monoliths without earthworm addition (*n* = 3), *n* = 1 for Control; error bars = max and min values.

Total nitrogen content for each field

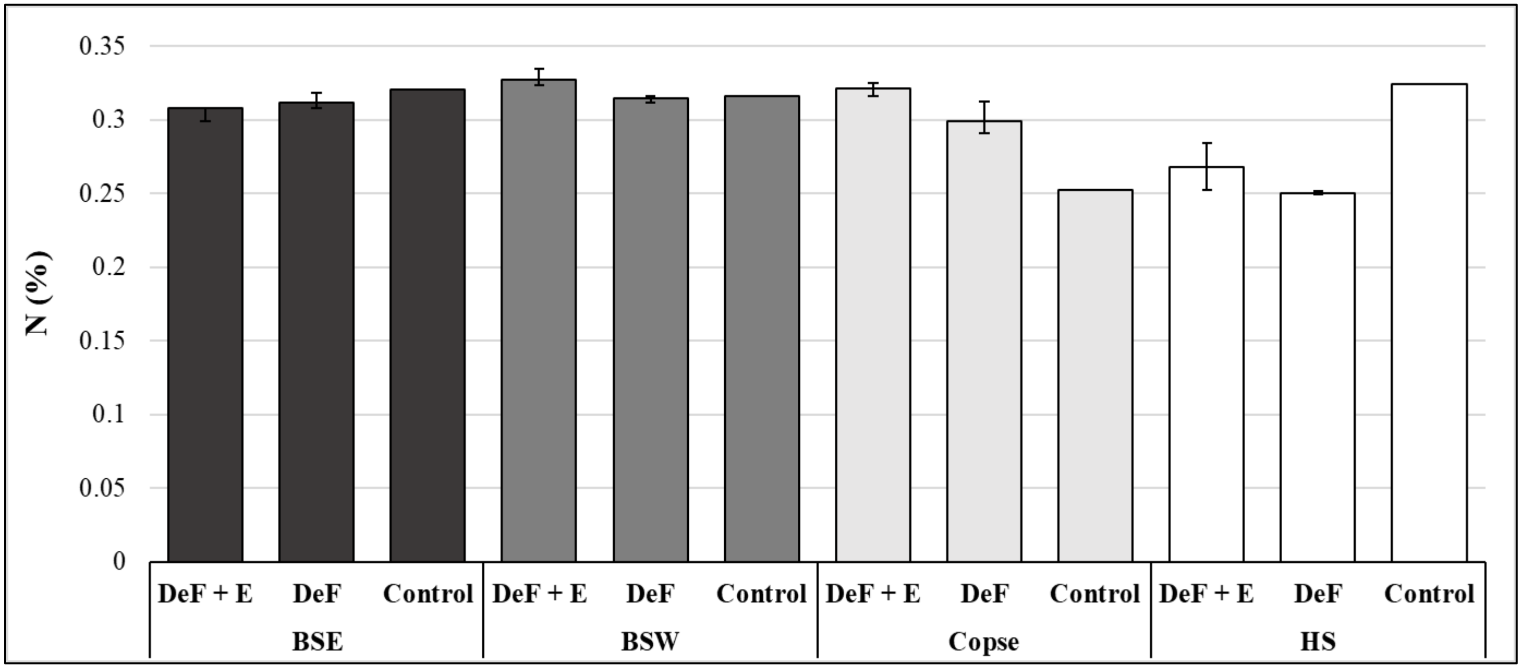


Figure D11. % N in the different treatments in the BSE, BSW, Copse and HS fields. DeF+E = frozen monoliths with earthworm addition (*n* = 3), DeF = frozen monoliths without earthworm addition (*n* = 3), *n* = 1 for Control; error bars = max and min values.

Soil monoliths dry shoot biomass for each field

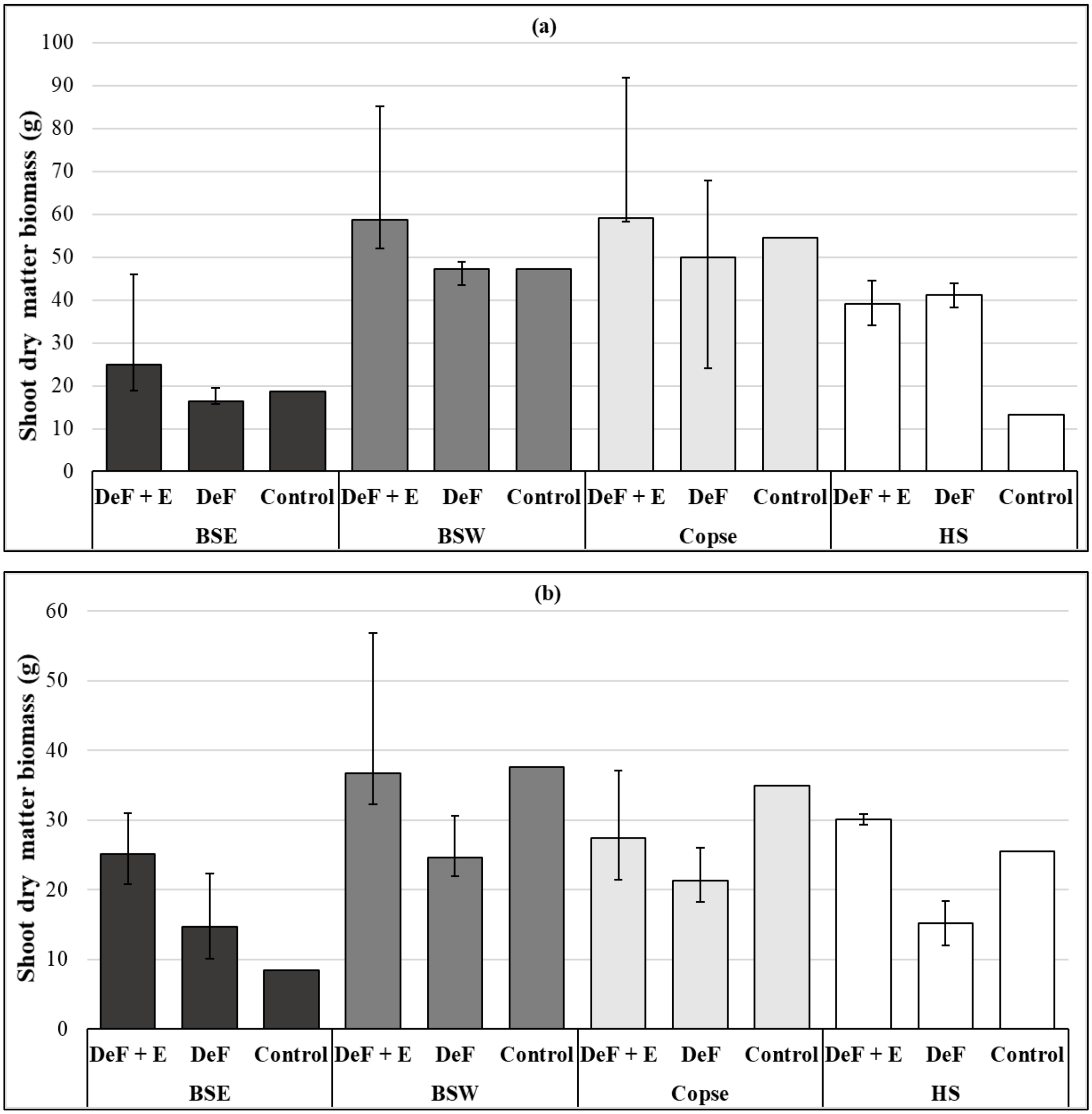


Figure D12. Median clover and grass shoot dry matter biomass in (a) September 2017 (at the midpoint of the experiment) and (b) April 2018 (at the end of the experiment) in the BSE, BSW, Copse and HS field monoliths. DeF+E = frozen monoliths with earthworm addition (*n* = 3), DeF = frozen monoliths without earthworm addition (*n* = 3), *n* = 1 for Control; error bars = max and min values.

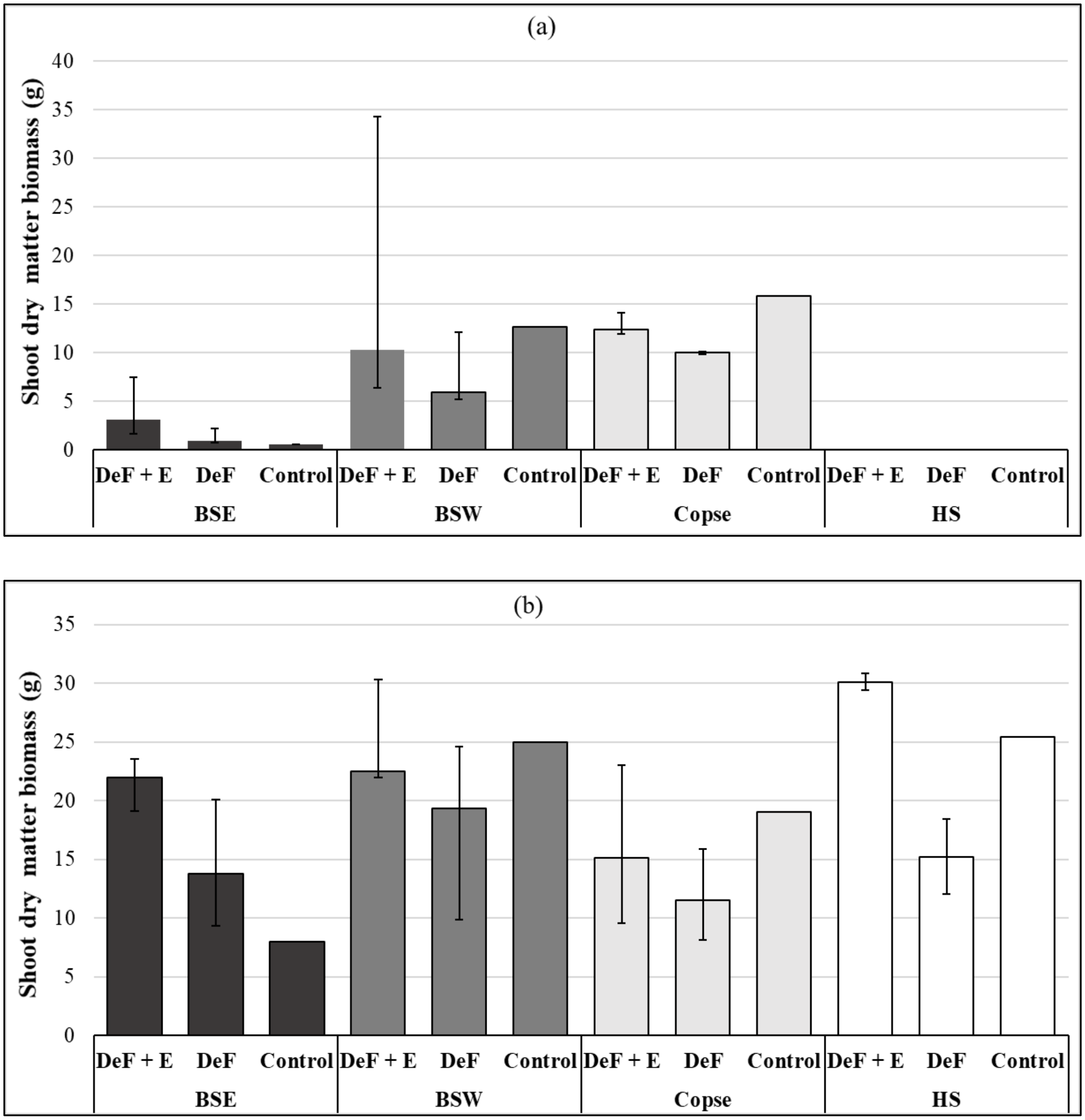
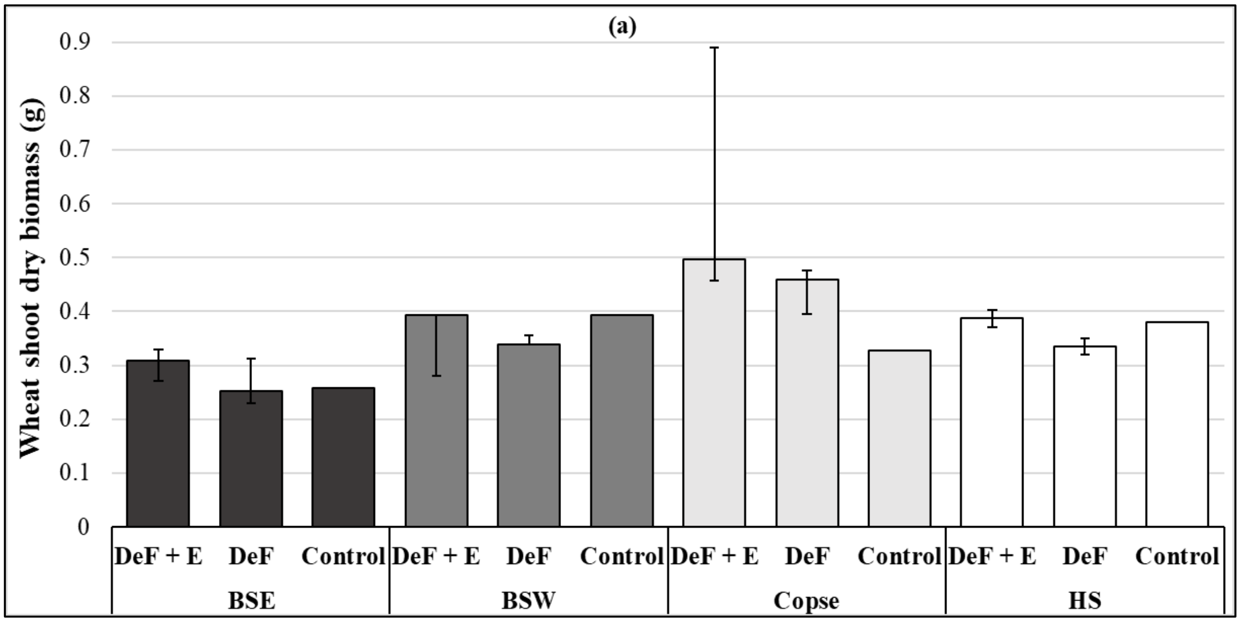
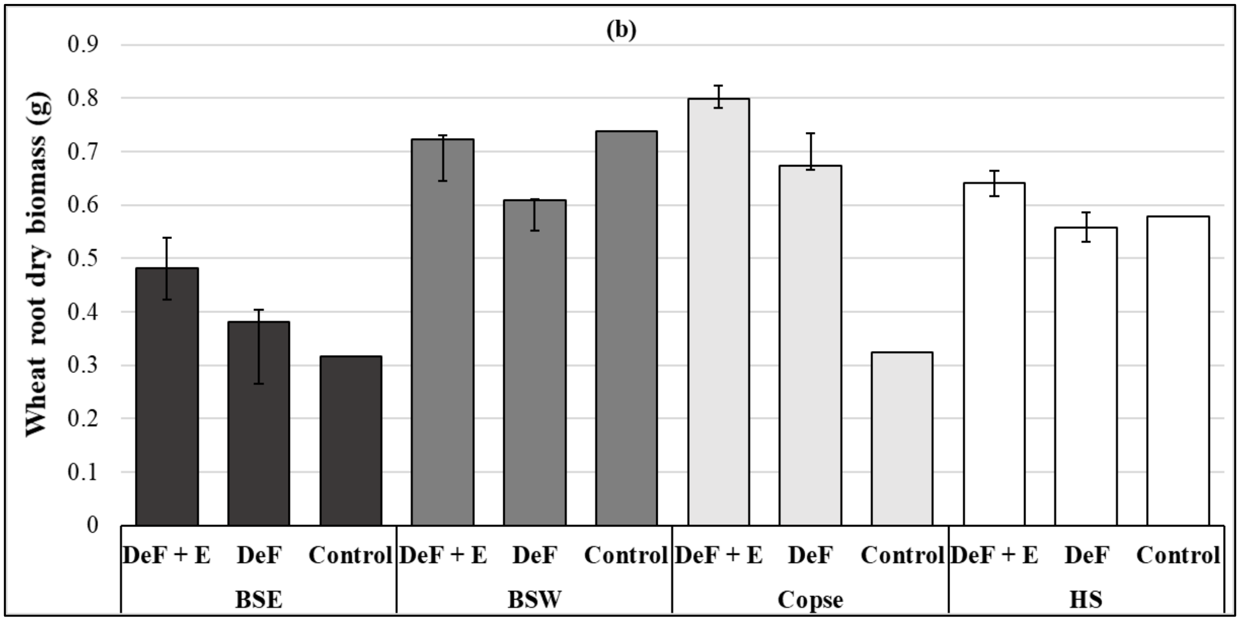


Figure D13. Median (a) clover and (b) grass shoot dry matter biomass in April 2018 (at the end of the experiment) in the BSE, BSW, Copse and HS field monoliths. DeF+E = frozen monoliths with earthworm addition (*n* = 3), DeF = frozen monoliths without earthworm addition (*n* = 3), *n* = 1 for Control; error bars = max and min values

Wheat biomass form the bioassay experiment for each field





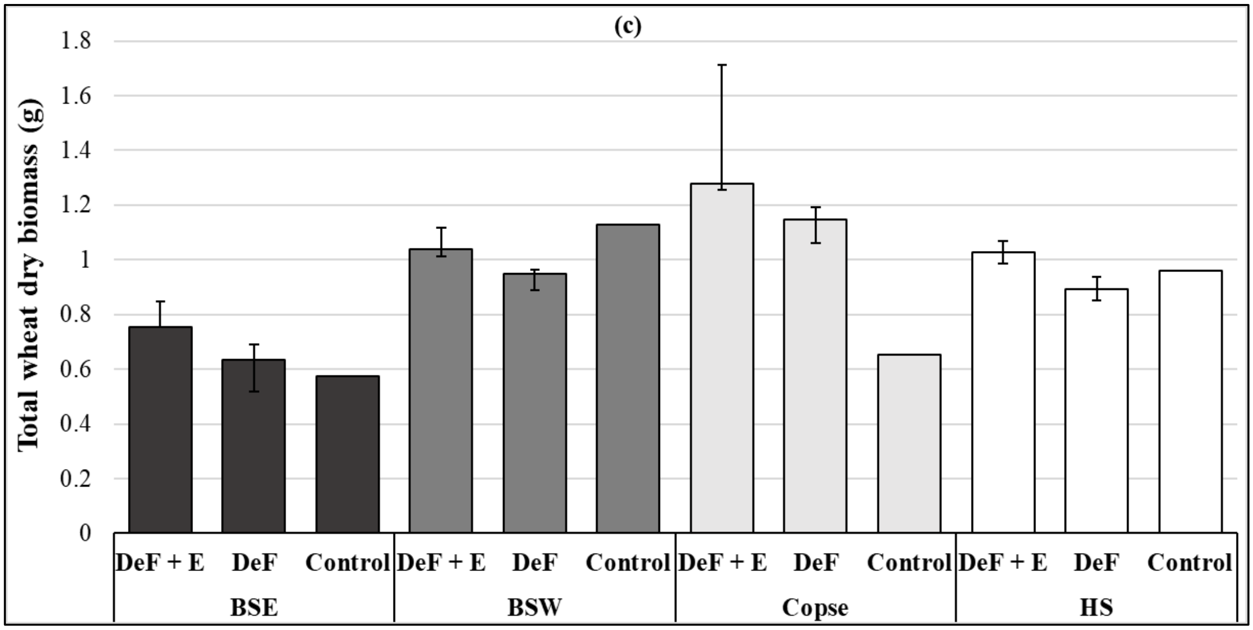


Figure D14. Median (a) shoot, (b) root and (c) total dry biomass of wheat grown in the bioassay on soil taken from the BSE, BSW, Copse and HS field monoliths. DeF+E = frozen monoliths with earthworm addition (*n* = 3), DeF = frozen monoliths without earthworm addition (*n* = 3), *n* = 1 for Control; error bars = max and min values.

Weather data

Table D6. Monthly weather data from the COSMOS station installed near the ley strips at the University of Leeds commercial farm. COSMOS weather station characteristics are available in the web link: https://cosmos.ceh.ac.uk/network-instruments

Months	**Total monthly precipitation (mm)**	**Mean monthly Air Temperature (°C)**	**Minimum monthly Air Temperature (°C)**	**Maximum monthly Air Temperature (°C)**
March 2017	54.3	8.11	-1.20	18.00
April -2017	13.6	8.53	-1.00	20.90
May-2017	42.9	12.26	3.10	24.80
Jun-2017	68.7	15.37	7.10	28.50
July-2017	65.9	15.78	8.60	26.30
August-2017	103.6	15.35	7.90	22.80
September-2017	83.0	13.17	5.50	21.20
October-2017	49.4	12.33	2.40	20.10
November-2017	32.5	6.87	-0.60	15.60
December-2017	38.5	4.93	-3.20	13.30
January-2018	68.0	4.75	-3.30	13.20
February-2018	32.1	2.55	-5.90	9.90
March-2018	96.7	4.26	-5.30	11.50

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