Earthworms, Ecosystem Services, and Environmental Change



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Abstract

Biodiversity underpins human health and wellbeing through the provision of ecosystem services. These services will become increasingly important due to climate change and the food production requirements of feeding an ever-growing human population. Earthworms (Oligochaeta: Lumbricidae) are particularly important ecosystem engineers that contribute to provisioning, regulating, supporting, and cultural ecosystem services through their burrowing and feeding activities. In this DPhil thesis, I focus on the effects of earthworms on plant growth and cattle dung decomposition in manipulative experiments reported across five data chapters. I performed experiments investigating the potential of earthworms to improve plant growth in peat-free horticultural substrates, focussing on the effects of earthworms in different substrates, at different population densities, and during times of drought or waterlogging stress. The effects of earthworms differed across different earthworm abundances and substrates. In one experiment, increasing earthworm abundance improved plant growth but there were diminishing returns at higher earthworm densities for root growth. In another, earthworms provided benefits in one horticultural substrate but not in the other two tested. However, in other experiments, large benefits provided by earthworms in horticultural substrates were conferred during periods of environmental stress, as well as under normal conditions. These results suggest that earthworms could be a valuable addition to horticultural plant growth media, particularly in the UK where the horticultural industry must soon shift to peat-free media in line with an upcoming ban on widely-used peat-containing substrates. I also conducted an experiment investigating the effects of earthworms on plant growth and fruit yield in topsoil during manipulated rainfall but, unlike the compost experiments, and in contrast with the literature, earthworms had no significant effects on plant growth suggesting that earthworm benefits to plant growth may not be universal. The effects of earthworms on

cattle dung decomposition during flooding or warming stress were also investigated across two large-scale factorial experiments. Earthworms had a significant effect on dung decomposition in both experiments with functioning unaffected by environmental stress or the presence of dung insects. Therefore, earthworms could be important in maximising pasture productivity in a changing climate. Overall, the results presented here showcase the great importance and value of earthworms for providing ecosystem services and that these services may be robust to some perturbation from climate change.

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Chapter 1: General introduction

The global human population is growing rapidly (Gu et al., 2021; Ritchie et al., 2023) and is predicted to grow to 9 billion by 2037 and 10 billion by 2050 (Zeifman et al., 2022) while becoming increasingly urban (Ritchie et al., 2024). These global trends are mirrored in the UK, which is experiencing both population growth (Office for National Statistics (ONS), 2023) and urbanisation (Office for National Statistics (ONS), 2021). To feed this growing population and to meet growing *per capita* consumption of meat (Godfray et al., 2018), food production will need to increase sustainably without major increases in the land area dedicated to farming (Godfray, 2014; Godfray et al., 2010).

A great challenge facing food production, both within the UK and globally, is climate change driven by anthropogenic greenhouse gas and aerosol emissions (IPCC, 2023). Within the UK, mean summer temperatures are expected to increase with summer maximum temperatures being particularly affected (Met Office, 2022; Patterson, 2023). Record-breaking temperatures have recently been recorded in the UK: mean air temperature was above 10 °C for the first time in 2022 (Kendon et al., 2023), and an all-time highest temperature of 40.3 °C (since records began in 1884) was recorded on 19 July 2022 (Kendon, 2022). Rainfall in the UK is also predicted to change: winters will have increased rainfall and flooding risk while summers will have decreased rainfall and reduced soil moisture, although heavy rainfall events in summer may also increase (Met Office, 2022).

Biodiversity supports human wellbeing through ecosystem functions and services and may offer a means to improve agricultural yields and resilience in spite of a changing climate (Millennium Ecosystem Assessment, 2005). In many temperate farming systems, including the UK, earthworms (Oligochaeta: Lumbricidae) could provide a range of

ecosystem services to improve productivity of agricultural and horticultural (Blouin et al., 2013) or livestock (Schon and Dominati, 2020) systems.

Earthworms

Earthworm diversity

Globally, there are currently ~5400 valid earthworm species, although total species numbers are predicted to be in excess of 8000 (Misirlioğlu et al., 2023). The UK has ~29 species (Sherlock et al., 2018) and all are in the family Lumbricidae, except for *Microscolex phosphoreus* (Dugès, 1837) (Acanthrodrilidae) and *Sparganophilus tamesis* Benham, 1892 (Sparganophilidae) (Sherlock et al., 2018). The UK earthworm fauna is relatively depauperate; for example, France has in excess of 180 species (Bouché, 1972), and the low number of UK species is likely a result of slow natural colonisation following glacial activity (Sims and Gerard, 1999). However, the UK species list is still increasing: *Kenleenus armadas* was described as new to science in 2012 having possibly been introduced from Europe (Blakemore, 2012) and *Aporrectodea nocturna* Evans, 1946, formerly considered a subspecies of *Ap. caliginosa* (Savigny, 1826) (Sims and Gerard, 1999), is now considered a distinct species (Sherlock et al., 2018). Additionally, several UK species may be cryptic species complexes with common species such as *Allolobophora chlorotica* (Savigny, 1826) potentially consisting of multiple lineages (King et al., 2008). Many earthworm species, including multiple UK species, are considered cosmopolitan and may have been moved large distances by prehistoric (or more recent) human activities (Blakemore, 2009). Multiple ‘exotic’ earthworm species have been recorded from glasshouses in the UK via movement of plants, soils, and growth media (Sherlock and Carpenter, 2009).

Earthworms are found across a range of habitats including pastures (Bacher et al., 2018), arable fields (Stroud, 2019), grasslands (Eisenhauer et al., 2009), forests (Eggleton et al., 2009), and domestic gardens (Natural England, 2014), although earthworm population density, biomass, and community composition differ across habitat types (Coleman et al., 2018; Fründ et al., 2010; Lee, 1985). The UK earthworm fauna is numerically dominated by a few common species and data compiled from 6309 specimens collected across the UK showed that ~84% of specimens consisted of the six most common species: *Allolobophora chlorotica* (34.06%), *Aporrectodea caliginosa* (Savigny, 1826) (19.3%), *Lumbricus castaneus* (Savigny, 1826) (12.14%), *Murchieona muldali* (Omodeo, 1956) (nationally rare but may be locally abundant, 10%), *Aporrectodea rosea* (Savigny, 1826) (8.34%), and *Lumbricus rubellus* Hoffmeister, 1843 (4.31%) (Natural England, 2014).

Earthworm ecotypes and ecology

Earthworms are members of the soil macrofauna (Coleman et al., 2018) with adults in the UK occupying a size range of ~10 – 350 mm in length (Sherlock, 2016; Sherlock et al., 2018). Earthworms feed on organic matter in varying states of decay and typically live in the soil or in the substrate on which they feed (Curry and Schmidt, 2007). Lumbricid earthworms all reproduce via cocoons, hardened balls of mucus produced by an adult earthworm that contain albuminous fluid, ova, and spermatozoa. Earthworm embryos develop inside the cocoons before emerging as hatchlings (Domínguez and Edwards, 2011; Domínguez and Velando, 2013). Most UK earthworm species are cross-fertilising hermaphrodites, although a number of UK species are parthenogenetic (Diaz Cosin et al., 2010) such as *Ap. rosea* (Terhivuo and Saura, 1993) and *Octolasion cyaneum* (Savigny, 1826) (Lowe and Butt, 2008a).

Earthworms can be divided into different ‘ecotypes’ based on their ecology and morphology (Bouché, 1977, 1972). *Anecic* earthworms are large-bodied, form permanent vertical burrows, and have pigmented anterior ends and paler tails. *Endogeic* earthworms have pale, unpigmented bodies and live in the topsoil where they make multiple temporary horizontal burrows and feed on organic material and mineral soil. *Epigeic* earthworms are red-bodied and feed on leaf litter and high organic-content matter at the soil surface (Bouché, 1972). These groups can be further subdivided; for example, endogeics can be divided into polyhumic, mesohumic, and oligohumic endogeics depending on the depth at which they burrow and feed (Brown, 1995; Lavelle, 1988). Additionally, species may partly occupy multiple ecotypes, such as *Lumbricus terrestris* L. which can be considered epi-anecic as it forms permanent vertical burrows but feeds at the soil surface (Hoeffner et al., 2019). Some epigeic species are grouped in an additional ‘compost earthworm’ (or composter) category (Sherlock et al., 2018). Compost earthworms are typically large-bodied, reproduce quickly, and show rapid consumption rates of organic matter (Domínguez and Edwards, 2011). The UK earthworm fauna contains representatives of all ecotypes including compost earthworms.

The initial ecotype definitions focussed on European Lumbricid earthworms and were largely based on morphology (Bouché, 1972). This caused some discrepancies in ecotype definitions across studies (Bottinelli and Capowiez, 2020), leading to efforts to formalise the ecotypes (Bottinelli et al., 2020). More recently, standardised laboratory testing of 50 European species facilitated definition of six functional groups (intense tunnellers (anecic), deep bioturbators (hypo-endogeic), litter dwellers (epigeic), shallow bioturbators (epi-endogeic), burrowers (epi-anecic), and intermediates) based on measurements of burrowing activity, litter consumption, and casting location (Capowiez et al., 2024).

Natural history of the earthworms used in this thesis

Lumbricus terrestris Linnaeus, 1758 (Fig. 1.1) was used in the experiment described in Chapter 4 and is the largest earthworm species in the UK with a body size of 90 – 350 mm x 6 – 10 mm (Sherlock, 2016; Sherlock et al., 2018). *Lumbricus terrestris* is an epi-anecic species that forms permanent vertical burrows that can be in excess of 1 m deep (Shipitalo and Butt, 1999), although burrowing behaviour is highly plastic and *L. terrestris* can burrow and settle in soils as shallow as 15 cm in the field (Butt and Nuutinen, 2024), or 10 cm in laboratory culture (Lowe and Butt, 2005). *Lumbricus terrestris* forms 'middens', a combination of castings, organic matter, and stones (Stroud et al., 2016a) which may act as an 'external rumen' for the earthworm (Brown et al., 2000). Middens can also act as hotspots for soil mesofauna (Hamilton and Sillman, 1989; Schrader and Seibel, 2001; Stroud et al., 2016a), slugs (Nieminen et al., 2015), and other earthworms (Butt and Lowe, 2007). *Lumbricus terrestris* is one of the few commercially-available non-composter earthworm species in the UK (Lowe and Butt, 2007b), although many earthworms are collected and imported from pastures and golf courses in Canada rather than being cultured locally (Tomlin, 1983).



Fig 1.1: Photograph of three adult *Lumbricus terrestris*.

Allolobophora chlorotica (Savigny, 1826) (Fig. 1.2) was used in experiments reported in Chapters 4 and 6 and is an endogeic species with a body size of 30 – 38 mm x 2 – 7 mm (Sherlock, 2016; Sherlock et al., 2018). It is one of the most abundant and widespread species within the UK (Natural England, 2014). The species exists as two visually distinct morphs; a green morph and a pink morph (Lowe and Butt, 2007a). The pink morph may be paraphyletic and the species may represent a cryptic complex (King et al., 2008). The two morphs are able to interbreed but cocoon viability is reduced following inter-morph mating (Lowe and Butt, 2008b), suggesting a degree of reproductive isolation. The two morphs differ in their tolerances and preferences for soil moisture content: the green morph shows faster growth at high soil moisture content but slower growth at reduced soil moisture contents while the pink morph shows similar growth at high and low soil moisture (Lowe and Butt, 2007a). Both morphs frequently co-occur, although the green morph is more common in wet sites and the pink morph is more common in dryer sites (Evans and Guild, 1948; Satchell, 1967a).

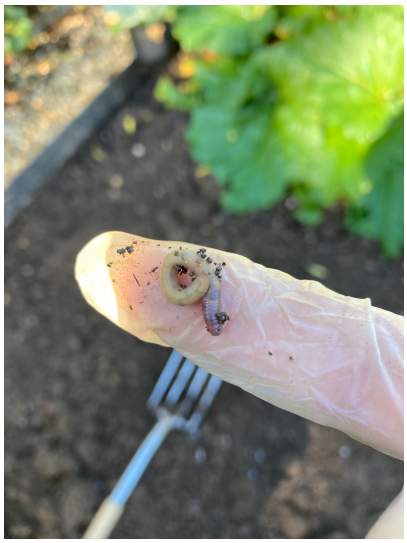


Fig 1.2: Photograph of an adult *Allolobophora chlorotica* (pink morph).

Dendrobaena veneta (Rosa, 1886) (Fig. 1.3) was used in experiments reported in Chapters 2 and 5 and is a compost earthworm with a body size of 50 – 155 mm x 4 – 8 mm (Sherlock, 2016; Sherlock et al., 2018). This species was originally described in Italy but is now distributed widely over Europe due to its popularity in vermicomposting (Domínguez, 2018; Szederjesi et al., 2019). As a compost earthworm (Sherlock, 2016; Sherlock et al., 2018), *D. veneta* is found in areas of high organic matter (Domínguez and Edwards, 2011). *Dendrobaena veneta* and has a larger body size than other commonly-used vermicomposting species (Domínguez and Edwards, 2011) and is commercially available in the UK (Katsiamides and Stürzenbaum, 2021), where it is marketed for fishing bait, pet food, or vermicomposting (personal observation).



Fig 1.3: Photograph of adult *Dendrobaena veneta*.

Ecosystem functions and services provided by earthworms

> *It may be doubted where there are many other animals which have played so important a part in the history of the world, as have these lowly organised creatures.*
>
> - Charles Darwin, 1881

Ecosystem functioning can directly translate to human welfare in the form of ecosystem services consisting of *supporting* (*e.g.,* nutrient cycling and soil formation), *regulating* (*e.g.,* maintenance of climate conditions and water quality), *provisioning* (*e.g.,* food, water, and material production), and *cultural* (*e.g.,* recreational and aesthetic) services (Millennium Ecosystem Assessment, 2005). Through their feeding, burrowing, and casting activities, earthworms contribute to all these ecosystem service categories.

Supporting services

Earthworms are ecosystem engineers with strong impacts on soils (Lavelle et al., 2016). Through burrowing and casting, earthworms influence fundamental soil properties such as

soil texture, porosity, soil nutrients, mineral weathering, and formation of soil structure (Le Bayon et al., 2017). Earthworms also create hotspots of biological activity through casting (Van Groenigen et al., 2019) and through construction of biogenic structures such as burrows and middens with strong effects on other members of the soil fauna (Butt and Lowe, 2007; Stroud et al., 2016a) and strong impacts on soil microbial activity (Medina-Sauza et al., 2019).

Regulating services

Through their burrowing activities, earthworms influence soil moisture content and water drainage after extreme rainfall events (Andriuzzi et al., 2015). Earthworms are also important for the decomposition of animal dung in pastures. Earthworms are highly attracted to dung pats and accumulate under them in large numbers where they modify soil properties through their burrowing (Bacher et al., 2020, 2018; Knight et al., 1992). Earthworms feed on dung directly and strongly contribute to dung decomposition, integration of dung carbon into the soil, and stimulation of soil microbes (Hendriksen, 1991a, 1991b, 1997; Holter, 1979a; Kaartinen et al., 2013; Schon et al., 2015, 2017, 2020; Schon and Dominati, 2020).

Provisioning services

Earthworms have a marked beneficial effect on plant production and growth (Brown et al., 1999; Scheu, 2003; Van Groenigen et al., 2015; Xiao et al., 2018). These effects can lead to large increases in crop yields and earthworms globally may contribute to as much as 6.5% of grain and 2.3% of legume production (Fonte et al., 2023). Earthworms also contribute to plant growth indirectly through the controlled production of vermicompost, organic material such as livestock dung, plant material, or kitchen waste, consumed by

compost earthworms (Domínguez, 2018; Domínguez and Edwards, 2011; Ferraz Ramos et al., 2022; Gómez-Brandón et al., 2010; Suthar, 2009). Vermicompost has a significant positive effect on plant growth when applied to horticultural substrates (Atiyeh et al., 2000a, 2000b) and soils (Arancon et al., 2004; Singh et al., 2008), and the level of benefit can be similar to that provided by earthworms directly (Blouin et al., 2019).

Cultural services

The burrowing and casting activities of earthworms can also contribute to the burial of flint and other stones which may have effects on preservation and burial of archaeological objects and structures (Butt et al., 2016; Darwin, 1881). Additionally, *L. terrestris* was recently voted the 2024 UK Invertebrate of the Year in a public vote held by The Guardian newspaper (Barkham, 2024a, 2024b), suggesting that earthworms have a strong cultural appeal with the British public.

Effects of environmental stress on earthworms

Although earthworms provide a wide range of ecosystem services, their ability to deliver these services is strongly influenced by environmental factors and stress. Environmental stressors can have severe negative effects on the performance (growth, survival, reproduction, and functioning) of earthworms (Singh et al., 2019). Environmental stress may reduce the beneficial effects of earthworms if it causes mortality or reduces earthworm activity levels. Flooding, drought, and warming are the environmental stressors investigated in this thesis (see below). However, other stressors such as pesticide residues (Pelosi et al., 2014) and anthropogenic mechanical disturbance such as tillage (Briones and

Schmidt, 2017; Johnston et al., 2018; Stroud et al., 2016b) can also negatively impact earthworms.

Flooding

Sustained submersion in water can lead to mortality of earthworms; tolerance duration differs across earthworm ecotypes with endogeics showing higher tolerance that other ecotypes (Kiss et al., 2021a). However, survival in aerated water can exceed 100 days for some UK species representing all ecotypes (Roots, 1956). Flooding events can severely reduce earthworm population sizes (Kiss et al., 2021b; Plum and Filser, 2005; Zorn et al., 2005) although these reductions may result entirely from mortality as earthworms utilise a combination of phenological, physiological, and reproductive responses to tolerate flooding, as well as vertical or horizontal migration to escape waterlogged areas (Plum, 2005). Population reductions may indicate a mixture of mortality and escape from flooded areas, with earthworms showing a strong tendency to escape from flooding, even if they are able to survive for extended periods when submerged (Ausden et al., 2001). Extreme rainfall is expected to increase in frequency and severity due to climate change which may lead to increased occurrences of flooding (Seneviratne et al., 2021), Therefore, flooding may negatively affect the functions and services provided by earthworms if earthworm burrowing activities are disrupted or populations are reduced due to mortality or escape.

Drought

Earthworm activities are strongly affected by soil moisture (Evans and Guild, 1948; Lee, 1985; Lowe and Butt, 2007a; Wever et al., 2001), so drought could have severe negative impacts on earthworm performance, functioning, and services. The effects of drought on earthworms differ strongly across ecotypes: while epigeic species are very vulnerable to

drought and may show large population declines in summer months (Eggleton et al., 2009) and during drought events (Plum and Filser, 2005), endogeic species can tolerate prolonged drought by entering a period of dormancy (diapause or aestivation) in which activity ceases and water loss is reduced, allowing prolonged survival during droughts (Lee, 1985). As with flooding, drought may alter or disrupt ecosystem services provided by earthworms by reducing earthworm population sizes due to mortality or reducing levels of earthworm activity through triggering aestivation in endogeic species. Due to climate change, the UK is expected to experience a decrease in summer and autumn precipitation (Cotterill et al., 2023; Met Office, 2022), so there is a need to better understand how drought stress may influence earthworms and their services.

Warming

Substrate temperatures can have a strong effect on a number of earthworm performance measures with different effects across species (Singh et al., 2019; Uvarov et al., 2011). Temperature affects somatic growth and development rates (Daniel et al., 1996; Eriksen-Hamel and Whalen, 2006; Wever et al., 2001), rates of reproduction (Boström and Lofs, 1996), and survival of both adults and cocoons (Johnston and Herrick, 2019; Presley et al., 1996; Richardson et al., 2009). Excessive temperatures can negatively affect earthworm communities, reducing overall abundance and species richness (Briones et al., 2009; Eisenhauer et al., 2014; Johnston, 2019). In cases where increased temperatures are still within earthworm tolerances, or where increased temperatures are closer to temperature optima for earthworm species, warming may result in an increased level of functioning and services provided by earthworms. However, if warming increases above earthworm tolerances, functioning may be reduced due to earthworm mortality. UK summer temperatures are expected increase due to climate change (Met Office, 2022) and record

high summer temperatures were recorded in the UK in 2022 (Kendon et al., 2023) so further work is needed to understand what effects these temperature increases may have on earthworms and their functions and services.

Research gaps addressed in this thesis

Effects of earthworm on plant growth in horticultural substrates and during periods of environmental stress

The positive effects of earthworms on plant growth in soil are well known (Fonte et al., 2023; Van Groenigen et al., 2015). Additionally, vermicompost produced by compost earthworms processing organic matter also has strong benefits for plant growth (Blouin et al., 2019) in both soil (Arancon et al., 2004) and horticultural growth substrates (Arancon et al., 2003). However, the potential benefits of inoculating soil-less horticultural growth substrates (composts) with earthworms have received little attention. These substrates are used by both amateur gardeners and in commercial horticulture. Within the UK, recent policy developments have caused large changes to both the amateur and professional horticulture industries with a proposed ban on the sale of peat-containing composts for amateur growers, to be followed in due course by a total ban (Department for Environment, Food and Rural Affairs, 2022, 2021). Peat-based composts have been enormously popular with both professional and amateur gardeners for many years but industrial extraction of peat causes great damage to peatlands (Alexander et al., 2008). Peatlands are unique habitats that provide a number of different ecosystem services (Bain et al., 2011; Whitfield et al., 2011). Peat-free composts are widely available in the UK and are produced from a range of materials (Bek et al., 2020; Holmes and Bain, 2023, 2021; Leyman and Aplin, 2007). However, despite general public support for the upcoming ban

(Department for Environment, Food and Rural Affairs, 2022), members of the public are concerned about the performance of peat-free composts (Environment and Forestry Directorate, 2023).

Compost earthworms are relatively easy to obtain (either from the field, or commercial suppliers) and 'wormeries' are widely marketed for home culture of earthworms (Butt and Williams, 2010). Therefore, earthworms could be a valuable addition to horticultural substrates if they are able to provide similar yield benefits to those reported from soils (Van Groenigen et al., 2015). However, garden containers are small and require regular irrigation and maintenance (Nagase and Lundholm, 2021) and may be highly exposed to environmental stress. Therefore, it is necessary to understand the effects of earthworms on plant growth in horticultural substrates and how environmental stress may influence earthworm-plant interactions in these substrates. Additionally, the effects of earthworms on plant growth can depend on earthworm population densities (Demetrio et al., 2017; Eisenhauer et al., 2009; Eriksen-Hamel and Whalen, 2007; Van Groenigen et al., 2015), and in some cases high earthworm densities can have negative effects on plant growth (Brown et al., 1999). Therefore, as the number of earthworms added to a garden container can be freely controlled by the grower, there is a need to understand potential density-dependent effects of earthworms on plant growth in these systems. Additionally, although a large volume of work has focussed on the effects of earthworms on overall plant growth or biomass (Van Groenigen et al., 2015), in many cases it is a specific plant trait or part (*e.g.,* edible fruit or ornamental flowers) that is of interest when growing plants for horticultural purposes and earthworms can also influence plant growth form and traits (Poveda et al., 2005; Zaller et al., 2011), which may be of particular interest in horticultural contexts.

As earthworm activities and performance are strongly influenced by substrate moisture content (Evans and Guild, 1948; Lee, 1985; Lowe and Butt, 2007a; Wever et al., 2001), effects of climate change on rainfall, flooding, or drought may have strong influences on earthworm effects on plants. Additionally, earthworms can also influence substrate moisture content through their activities (Hodson et al., 2023; Ma et al., 2021) which may have either positive (Hodson et al., 2023; Johnson et al., 2011) or negative (Blouin et al., 2007) effects on overall plant growth during periods of drought stress. As rainfall is predicted to change due to climate change (Seneviratne et al., 2021), there is an increased need to understand how drought or increased rainfall, including flooding, may alter earthworm effects on plant performance in both soils and horticultural substrates.

Effects of earthworms on cattle dung decomposition during periods of environmental stress

In addition to providing beneficial effects on plant growth, earthworms also act as important decomposers of animal dung (Kaartinen et al., 2013), supplementing the activity of the insect dung fauna (Skidmore, 1991). Earthworms accumulate under dung pats (Bacher et al., 2018; Knight et al., 1992) and strongly contribute to breakdown and burial of dung through burrowing, feeding, and casting (Hendriksen, 1991a, 1991b; Schon et al., 2020, 2015). Dung insects arrive at dung pats earlier than earthworms and it is thought that this preliminary insect activity may 'prime' dung pats for earthworm activity (Holter, 1983). However, experimental work has indicated that earthworms and dung insects may have additive (Holter, 1977; Kaartinen et al., 2013), positive interactive (O'hea et al., 2010a), or negative interactive (O'hea et al., 2010a) effects on dung decomposition. Additionally, earthworms can have negative effects on dung insects by reducing food availability for larvae (Xie et al., 2021), or by complete removal or fragmentation of dung

pats before insect larvae can complete development (Gittings et al., 1994). Environmental stress is also likely to add further complexity to these ecological interactions and their resultant effects on dung decomposition if earthworms and dung insects respond differently to stress. As rapid decomposition of cattle dung is important for maximising pasture productivity and reducing parasite risk in cattle, there is a need to understand how environmental stress, earthworms, and the insect dung fauna may interact to affect this ecosystem service.

Thesis outline

In this thesis I present multiple data chapters (Chapters 2 – 6) reporting individual or multiple manipulative experiments investigating the ecosystem services provided by earthworms during periods of environmental stress. All experiments were conducted using microcosms (Chapters 2, 3, and 5), mesocosms (Chapter 4), or pots (Chapter 6) in order to facilitate application of manipulative treatments. Each data chapter is a self-contained piece of work with an independent introduction, methods, results, and discussion section. The data chapters can be broadly split into two groups focussing on two major ecosystem services provided by earthworms: increasing plant growth (Chapters 2, 3, and 6) and contributing to cattle dung decomposition in pasture agroecosystems (Chapters 4 and 5).

In Chapters 2 and 3, I report on microcosm experiments that aimed to increase understanding of the potential beneficial applications of earthworms for increasing crop growth in horticultural composts in container gardens using the widely-available compost earthworm *D. veneta* as a representative species. In Chapter 2, I report on experiments investigating the effect of these earthworms on crop yields of radish in different horticultural substrates (*compost variety experiment*), and at different earthworm

abundances (*earthworm abundance experiment*). In Chapter 3, I report an expansion of this work that investigated the potential benefits of *D. veneta* in horticultural composts during periods of environmental stress across two experiments (a flood experiment and a drought experiment) using radish as an example crop.

Chapters 4 and 5 report on experiments investigating the effects of earthworms on cattle dung decomposition during periods of environmental stress. In Chapter 4, I report results of a mesocosm experiment investigating the effects of earthworms (*L. terrestris*., *A. chlorotica*, and *D. veneta*) on cattle dung decomposition during periods of flooding and in the presence or absence of the naturally colonising insect dung fauna. In Chapter 5, I report on a microcosm experiment investigating the effects of *D. veneta* and the dung beetle *Acrossus rufipes* (Linnaeus, 1758) on the decomposition of cattle dung under ambient and warmed (+6 °C mean) conditions.

In Chapter 6, I return to investigating the effects of earthworms on plant growth and report results of a pot experiment investigating the effects of *A. chlorotica* on growth and fruit yields of alpine strawberry across watering treatments simulating expected late summer rainfall (control) and a 'dry' (0.5 x control) and 'wet' (1.5 x control) scenario.

Following the data chapters, I present a General Discussion of my work in Chapter 7. As each data chapter reports on a self-contained experiment (or set of experiments), each has its own detailed discussion section. Therefore, for the General Discussion I focus on overall patterns in the horticultural compost experiments (Chapters 2 and 3) and the dung decomposition experiments (Chapters 4 and 5). I also review the pros and cons of the methods that I used in the experiments reported in this thesis, discuss my work in a wider context, and make recommendations for future research areas.

Supplementary information for each chapter is provided after the main text of that chapter. Reference lists for individual chapters have been combined as a single bibliography presented at the end of the thesis. Figures and tables are labelled according to the order in which they appear in each chapter with a chapter-specific prefix (*e.g.* Figure 3 in Chapter 2 is reported as Fig. 2.3). Tables and figures in the supplementary have an additional *S* in the prefix (*e.g.,* Table S3.1). Chapter 4 also contains an additional Appendix where tables and figures have an *A* in the prefix (*e.g.,* Table A4.2) Individual brief acknowledgement sections are provided at the end of each data chapter and an overall Thesis Acknowledgements section is presented after the General Discussion (Chapter 7).

Statement of authorship

All written work presented in this thesis is my own. This thesis was completed with regular discussion with, and feedback from, my primary supervisor Owen T. Lewis (OTL). My secondary supervisor Kevin R. Butt and a collaborator František X. J. Sládeček both provided comments and feedback on drafts of chapters 6 and 5, respectively.

Although this thesis consists wholly of my own work, I have listed multiple authors for each data chapter to reflect the proposed authorship that will be included when the chapters are adapted to a manuscripts and submitted to journals. Chapters 4 and 5 report on collaborative experiments so additional detail is provided below.

Chapter 4

The experiment reported in this chapter was conducted jointly by me and a MBiol student (Katie Jones, KJ) during September – December 2021 investigating effects of earthworms, dung insects, and flooding on dung decomposition. Analysis of selected data from this

experiment also formed part of KJ's MBiol Dissertation (submitted May 2022). Any data used in both KJ's dissertation and this thesis were completely re-analysed by me prior to inclusion.

Experiment contributions

I was responsible for the earthworm aspects of this experiment. I selected earthworm species, acquired them, and maintained them prior to use in the experiment. When setting up the experiment I prepared the earthworms (washing, gut voidance, allocation to experimental mesocosms) and applied them to the mesocosms. During data collection I recorded earthworm mass change and survival.

Additionally, I was responsible for selecting and preparing the site of the experiment including preparing and maintaining rain shelters. I also selected and acquired the soil that was used in the experiment.

KJ was responsible for collecting adult insect samples from emergence traps (including constructing emergence traps), preparing insect samples, and weighing and identifying emergence trap samples. During data collection, KJ was responsible for hand sorting experimental dung pats, preparing insect larvae, and identifying and weighing larvae. KJ allocated experimental dung pats to mesocosms during setup.

Both KJ and I were responsible for collecting and homogenising cattle dung, preparing experimental dung pats, and designing and constructing the experimental mesocosms. We also shared responsibility for applying flooding treatments, collecting surface soil samples, and recording final dung dry masses.

Reporting of experimental results

For their MBiol thesis, KJ analysed and reported on dung decomposition, soil moisture, and dung insect performance data.

For this DPhil chapter, I combined the insect data collected by KJ, the earthworm data I collected, and the dung and soil data collected by both of us to make the final dataset for analyses. I was fully responsible for all data analysis presented in this chapter, including necessary data tidying and curation prior to analysis. I completely re-analysed dung decomposition, soil moisture, and dung insect data in addition to performing analysis on the earthworm survival and mass change data. I also wrote all text in Chapter 4 and produced all figures and tables.

Chapter 5

Experiment contributions

This experiment was designed and setup by both myself and František X. J. Sládeček (FXJS), with input and advice from Owen T. Lewis (OTL).

Both FXJS and I were responsible for acquiring experimental materials, setting up microcosms, and applying treatments (heating cables). Both FXJS and I performed site surveys around the John Krebs Field Station to attempt to find earthworms for the experiment, collected the cattle dung for the experiment, and prepared it for the experiment.

FXJS collected the dung beetles and maintained them prior to their use in the experiment. FXJS also divided the dung beetles into groups that would be added to experimental microcosms.

I selected the earthworm species used, acquired them, and maintained them prior to use in the experiment. I prepared the earthworms for the experiment (removal from culture containers, cleaning, starving, weighing, and allocation to microcosms) and added earthworms to experimental microcosms. I was responsible for day-to-day running and maintenance of the experiment. I was also responsible for designing the microcosms used in this experiment and designing the spatial arrangement of the experiment.

Reporting of results

Results from this experiment have not yet been reported elsewhere. I was fully responsible for all data collection (with consultation with FXJS and OTL), tidying, and curation. I conducted all data analysis presented in this chapter and all written work, figures, and tables also consist wholly of my own work.

Chapter 2: Effects of the compost earthworm *Dendrobaena veneta* on the growth of radish in peat-containing and peat-free composts

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Author contributions: MJT designed and set up the experiment, collected all data, performed all analyses, and wrote the manuscript. OTL provided feedback on experimental design, running, data collection, and analysis and provided multiple rounds of feedback on draft sections and complete drafts.

Status: In preparation for submission to a journal.

Abstract

Peat-containing plant growth media, or composts, are popular with both amateur and professional growers of crops and ornamental plants. However, sale of peat-containing composts is soon to be banned in England and Wales to protect valuable peatland habitats and there are concerns about the performance of peat-free alternative products. Earthworms (Oligochaeta: Lumbricidae) are ecosystem service providers that can increase plant growth in soils. Several 'compost earthworm' species are commercially available for vermicomposting and may be able to provide benefits to plants grown in soil-less peat-free growth media. However, commercial plant growth substrates are produced from a variety of materials which may affect earthworm performance and earthworm effects on plants.

Two glasshouse microcosm experiments were conducted investigating the effects of the compost earthworm *Dendrobaena veneta* on growth of *Raphanus sativus*. The first experiment manipulated earthworm presence or absence across two peat-free and one peat-containing commercially available composts. The second experiment manipulated earthworm abundance (0 – 10 individuals) in a peat-free compost. Overall earthworm survival was extremely high and approached 100%. Radish growth differed strongly across the composts tested and earthworms significantly affected radish growth in the lowest-performing compost only. In this compost, radish yields (fresh mass) were 3.7 – 8.0x lower (shoot mass) and 5.2 – 10.2x lower (root mass) than in the other composts although earthworms increased fresh shoot yields by 44% and fresh root yields by 340%. In the earthworm abundance experiment, there was a positive linear relationship between shoot mass and earthworm abundance, equivalent to ~0.5 g extra fresh shoot mass per additional earthworm. Root mass showed a non-linear relationship with earthworms, with growth maximised at ~7 earthworms where fresh yields were increased by ~11 g (~57% increase). These results suggest that commercially available compost earthworms may provide an effective means to increase crop yields in commercially available plant growth substrates. However, effects are influenced by both substrate type and earthworm abundance.

Keywords: Peat-free horticulture, earthworms, radish, crop yields, container gardens, peatlands

Introduction

Peat has been a major component of horticultural substrates in the UK since the 1960s (Bek et al., 2020) which has caused widespread damage and degradation of peatlands (Alexander et al., 2008; Evans et al., 2017; Minasny et al., 2023; UNEP, 2022). This could have major consequences for greenhouse gas emissions (Leifeld and Menichetti, 2018) and maintenance of regulating, supporting, and cultural ecosystem services provided by peatlands (Bain et al., 2011; Millennium Ecosystem Assessment, 2005; Whitfield et al., 2011). In response, the UK government has proposed a ban on the sale of peat-containing compost for horticultural use as part of the *England Peat Action Plan* (Department for Environment, Food and Rural Affairs, 2021; Department for Environment, Food and Rural Affairs et al., 2022). The proposal has been largely supported by both members of the public and businesses (Department for Environment, Food and Rural Affairs, 2022) and peat-free composts produced from a number of materials including wood, bark, coconut coir, green waste, and wool are now commercially available (Bek et al., 2020; Holmes and Bain, 2023, 2021). However, many amateur and professional growers have concerns regarding the cost, quality, consistency, and performance of peat-free products and the availability and sustainability of peat-free compost ingredients (Bek et al., 2020; Environment and Forestry Directorate, 2023). Soil-less multi-purpose horticultural composts are popular for use in container gardening involving ornamental or edible plants (Durham et al., 2018; Nagase and Lundholm, 2021). Due to the small size, and high maintenance and irrigation requirements of garden containers (Hillier, 1991; Nagase and Lundholm, 2021; Relf, 2020), substrate quality is important for maximising plant growth and yields.

One approach that could be used by growers to improve the performance of peat-free composts, and horticultural substrates more generally, is encouraging or enhancing the activity of earthworms (Oligochaeta: Lumbricidae). Earthworms have positive effects on plant production in soils (Scheu, 2003; Van Groenigen et al., 2015; Xiao et al., 2018) and can influence soil microbiota and soil fertility through their casts (Medina-Sauza et al., 2019; Van Groenigen et al., 2019). Vermicompost produced by controlled decomposition of organic material by 'compost earthworms' (Domínguez, 2018; Ferraz Ramos et al., 2022; Gómez-Brandón et al., 2010; Suthar, 2009) provides benefits in horticultural substrates (Atiyeh et al., 2000a, 2000b). However, the potential benefits of directly adding earthworms to horticultural substrates is less well studied, although inoculation of earthworms into a mixture of peat-based compost, bark, and crushed wood increased yields of tomatoes and lettuce but reduced yields of rye and clover (Dorr et al., 2017). Peat-free composts are produced from a wide range of materials (Bek et al., 2020) and, although there are few experimental studies, different composts can have a large influence on crop performance in container gardens (Holmes and Bain, 2021; Otway, 2022). The effects of earthworms on plant growth can also differ depending on soil properties (Doan et al., 2013; Hallam et al., 2021; Van Groenigen et al., 2015) and the performance of compost earthworms is highly dependent on substrate composition and quality (Borang et al., 2016; Gunadi et al., 2003; Vodounnou et al., 2016). Additionally, the effects of earthworms on plant growth can depend on earthworm population densities (Demetrio et al., 2017; Eisenhauer et al., 2009; Eriksen-Hamel and Whalen, 2007; Van Groenigen et al., 2015). The performance (*i.e.* growth and reproduction) of earthworms is also density dependent (Domínguez and Edwards, 1997) as well as their effects on substrate macroporosity and water content (Ma et al., 2021), litter decomposition (Gaudon et al., 2020), and soil chemical composition (McLean and Parkinson, 1997). Care may be needed

when introducing earthworms to garden containers as increased earthworm abundances can also have non-linear, and sometimes negative, effects on plant growth (Agapit et al., 2018; Brown et al., 1999; Chan et al., 2004; Demetrio et al., 2017). Therefore, while commercially available compost earthworms in the UK (Katsiamides and Stürzenbaum, 2021) may provide a useful means to improve crop yields in horticultural substrates in both amateur and professional contexts, an understanding of earthworm effects on crop growth in different peat-containing and peat-free composts, as well as an understanding of the effects of different earthworm abundance on plant growth in small garden containers is required. Additionally, it is important to understand the performance of earthworms (*i.e.,* survival and body mass maintenance) in horticultural composts and at different population densities to determine if, and how, earthworms can provide services in these substrates. As the composition of garden containers, including the abundance and population densities of added earthworms, can be freely controlled by the grower, earthworm abundances may far exceed those seen in natural ecosystems. Therefore, it is important to investigate how the abundance of earthworms added to garden containers influences performance of both the earthworms and plants to determine optimum earthworm densities to add to garden containers.

Two glasshouse microcosm experiments were conducted to investigate the effects of the compost earthworm *Dendrobaena veneta* (Rosa, 1886) on the growth of radish plants (*Raphanus sativus* L.). In the first experiment, the presence or absence of earthworms was manipulated across two peat-free and one peat-containing commercially available horticultural composts. The second experiment manipulated earthworm abundance in a commercially available peat-free compost. Radishes are grown for consumption of both the hypocotyl (storage root) and leaves (Gamba et al., 2021; Singh, 2021), so fresh and dry shoot mass, root mass, and shoot : root ratio, were measured. As earthworm performance

in horticultural composts will influence the suitability of earthworms for addition to these substrates, earthworm survival and mass change were also recorded to infer effects of compost variety and earthworm abundance on earthworm performance. Cocoon production in the different composts was also recorded in the first experiment. It was predicted that, due to differences in compost ingredients and composition, earthworm performance would differ across substrates. Additionally, it was predicted that earthworm performance would be reduced at higher earthworm densities, due to increase competition for food and space. It was also predicted that radish performance would differ among the composts tested and, while earthworms would have generally positive effects on radish yields, the level of benefit provided by earthworms would differ between the composts due to different levels of earthworm activity in each compost and differences in plant performance across composts. It was also predicted that increasing earthworm abundances would increase radish shoot and root yields, but that the two plant parts might respond differently to earthworm abundance (altering shoot : root ratios) due to potential direct effects of earthworm on the root but only indirect effects on the shoot. Finally, it was also predicted that relationships may be non-linear with diminishing returns or possible negative earthworm effects at high abundances if excessive levels of earthworm activity disrupted plant growth in the small experimental microcosms.

Methods

Two experiments were performed. A compost variety experiment manipulated the presence or absence of earthworms across three commercially available multi-purpose composts and was conducted from 16 February – 28 March 2023. An earthworm abundance experiment manipulated earthworm abundance in a commercially available multi-purpose compost and was conducted from 27 April – 5 June 2023.

Study system

Dendrobaena veneta (Lumbricidae: Rosa 1886) was used as a representative earthworm for both experiments, with both experiments using adult earthworms. *Dendrobaena veneta* is a medium-bodied (~50 – 80 mm x 5 – 7 mm) species with an average adult body mass of 0.92 g (Domínguez and Edwards, 2011). The species is considered a 'compost epigeic' (or 'composter') earthworm (Sherlock et al., 2018), characterised by high tolerance to handling and environmental conditions, fast growth and reproduction, and rapid consumption of organic material and is commonly utilised in vermicomposting (Domínguez, 2018; Domínguez and Edwards, 2011). The morphology of *D. veneta* is very similar to the more commonly used *Eisenia fetida* (Savigny, 1826) (Katsiamides and Stürzenbaum, 2021), although *D. veneta* is slightly larger and has a longer life cycle (Domínguez and Edwards, 2011) so was chosen for these experiments to maximise chance of earthworms surviving the experiment duration. *Dendrobaena veneta* is an iteroparous hermaphroditic species that reproduces via cocoons, balls of hardened mucus containing albuminous fluid, ova, and spermatozoa (received from another individual during mating), in which offspring develop, and from which hatchlings emerge (Domínguez and Edwards, 2011; Domínguez and Velando, 2013). Growth is indeterminate, and can continue after reaching sexual maturity (Domínguez and Guimaraěs, 2024). Earthworms were purchased from Yorkshire Worms Ltd. (Holme-on-Spalding-Moor, Yorkshire, UK) and were kept in a mixture (by volume) of 3 parts moist compost to 1 part dried and rewetted cattle dung to act as food (Lowe and Butt, 2005) in 10 L white polypropylene buckets, sealed with 40 g m^{-2} nylon insect mesh, in the same glasshouse as the experiment (described below) prior to use.

Radish (Brassicaceae, *Raphanus sativus* L. var. “Cherry Belle”, supplier: Mole Seeds (UK) Ltd. Essex, UK) was used as a representative horticultural crop species for both experiments. Radish is a salad crop (Monaghan and Beacham, 2017) that has been grown in the UK since its introduction by the Romans (Field, 2000) and today is a popular crop for amateur growers. Radishes are typically grown for their swollen hypocotyl storage root (Monaghan and Beacham, 2017) but leaves are also edible and commonly consumed (Singh, 2021) and may have higher nutritional value than the roots (Gamba et al., 2021), meaning that root growth, shoot growth, and the shoot : root ratio are of importance for growers. “Cherry Belle” is a red coloured spherical variety (George and Evans, 1981) that was chosen due to its small size, rapid growth, and sensitivity to environmental conditions (Kostka-Rick and Manning, 1993).

Microcosm design

Both experiments used microcosms (Fig. 2.1) constructed from 10 cm diameter, 20 cm height, PVC piping (manufacturer Manrose, Crawley, West Sussex, UK). Adhesive hook tape (2 cm width) was applied around the upper rim of the microcosms to prevent earthworm escape (Lubbers and Van Groenigen, 2013). The open base of each tube was covered with two layers of mesh. The inner layer was thin, 40 g m^{-2}, nylon insect mesh and the outer layer was 1 mm plastic mesh. The mesh layers were secured with a stainless-steel vent clamp to contain the substrate and prevent earthworm escape from the base of microcosms. Microcosms in both experiments first received a layer of washed gravel (10 cm diameter) to facilitate drainage before being filled with commercially available multi-purpose compost (described below). Microcosm lids were produced from transparent plastic 500 ml deli tubs with a single 1 mm hole for ventilation. These lids were

temporarily added to microcosms during seed germination and when earthworms were added.

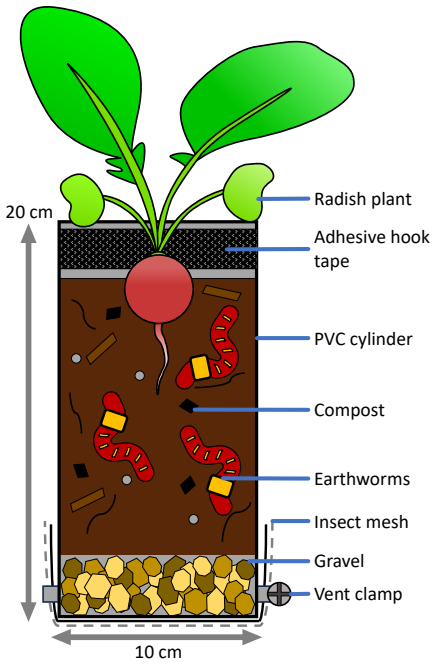


Fig. 2.1: Diagram of earthworm-containing microcosms used in both experiments.

Compost variety experiment protocol

The experiment was conducted in a research glasshouse at the John Krebs Field Station (Department of Biology), University of Oxford near the village of Wytham, Oxfordshire, UK. Air temperatures were recorded with data loggers (SensorBlue Thermometer Hygrometer, Oria, Hongkong Siuon Technology CO Ltd.) for part of the experiment duration. Mean temperatures during this experiment were 13.4 °C (SD = 5.2, range = 2.1 – 32.7 °C, recording period = 21 – 28 February 2023).

On 16 February, microcosms received ~350 g of gravel followed by one of three commercially available multi-purpose composts; Levington, New Horizon, or SylvaGrow (Table 2.1). The Levington compost was a conventional peat-containing compost while the

New Horizon and SylvaGrow composts were representative peat-free composts. New Horizon consists primarily of wood fibre made from wood chips (Holmes and Bain, 2023) while SylvaGrow is made from wood-fibre and fine bark (Holmes and Bain, 2021). Microcosms were filled to ~3 cm from the upper rim (~1.18 L) and the compost surface was gently pressed by hand to ensure good contact between the seeds and the compost. Starting compost mass differed across the compost varieties due to differences in compost density (Table 2.1). Microcosms were watered with 200 ml of tap water after filling.

Table 2.1: Summary of the composts used in this experiment.

Compost	Full product name	Manufacturer	Type	Compost mass	
				Before seeds	After planting
Levington	Levington® Multi purpose compost	Evergreen Garden Care (UK) Ltd, Frimley, Surrey, UK	Peat containing	480 g	510 g
New Horizon	New Horizon® All Plant Compost	Westland Horticulture Ltd, Dungannon, Tyrone, Northern Ireland, UK	Peat-free: wood fibre from wood chips (Holmes and Bain, 2023)	650 g	680 g
SylvaGrow	SylvaGrow® Multi-Purpose	Melcourt Industries Ltd, Tetbury, Gloucestershire, UK	Peat-free: wood-fibre and fine bark (Holmes and Bain, 2021)	500 g	530 g

Radish seeds were planted on 16 February in a hexagonal arrangement (~2 cm spacing) with a seventh seed planted in the centre. For each seed, a ~1 cm deep hole was made with a dibber, a single seed was placed into the hole, and the hole was resealed by hand. Once all seeds were planted, ~30 g of extra compost (Table 2.1), of the same type already added to the microcosm, was added, and gently firmed by hand. After planting, each microcosm was watered with 100 ml of tap water and lids (described above) were added. Germination occurred rapidly (<1 week) and microcosms were thinned to three seedlings per microcosm on 24 February, to two seedlings on 6 March, and to one on 8 March. During

thinning events, the most developed seedlings (based on visual inspection of stem diameter and leaf size) were left unless seedlings had grown 'leggy' and were in danger of collapsing. Seedlings were pulled from the compost by hand for the first two thinning events but cut at the compost surface for the third event due to seedling size.

Earthworms were added to microcosms on 17 February. On 15 February, earthworms were removed from the storage bucket, washed with tap water, and placed into ventilated containers on moist tissue in a 15 °C incubator in darkness for 48 hours for gut voidance (Fründ et al., 2010). On 17 February, earthworms were removed from the incubator and groups of five adults were randomly assigned to each earthworm-containing microcosm, representing a density of 636.6 earthworms m^{-2}. Average total starting earthworm mass was 5.56 g ±SE 0.02 g and there were no significant differences in starting earthworm mass across compost varieties ($F_{2,33} = 0.46$, $p = 0.64$). Microcosm lids were removed from each microcosm and earthworms were placed onto the compost surface and seen to burrow rapidly into the compost. Lids were then reapplied before being removed on 22 February.

The experiment was fully factorial with twelve replicates of each of the earthworm (zero or five *D. veneta*) and compost (New Horizon, SylvaGrow, or Levington) treatment combinations for a total of 72 microcosms. Microcosms were randomly arranged in a 12 x 6 grid adjacent to the south-facing glasshouse window on 16 February and re-arranged to a 16 x 4 grid on 24 February to increase light received by microcosms on rows furthest from the window. Microcosm positions within the grid were re-randomised weekly and microcosms at the edge of the grid were shielded from direct sun exposure with a row of empty PVC tubes placed around the experimental microcosms. Microcosms were watered with 100 ml of tap water three times per week from 17 February, increasing to 125 ml per event from 1 March, with a final event of 200 ml on 22 March. Artificial

supplemental lighting (High Pressure Sodium lights) was used in the glasshouse from 17 February after earthworms were introduced to microcosms with an initial regime of 07:00 – 19:00 (12 h light : 12 h dark) that was increased to 06:00 – 20:00 (14 h light : 10 h dark) on 1 March as the radish plants grew.

Earthworm abundance experiment protocol

On 27 April, microcosms received 300 g of gravel (10 cm diameter) followed by ~650 g of New Horizon compost (Table 2.1). Six radish seeds were planted in a hexagonal arrangement (2 cm spacing) in holes (1 cm depth) made with a dibber. After seeds were placed in holes, ~50 g of additional compost was added to each microcosm and gently pressed by hand to ensure good contact between seeds and compost. Microcosms then received 300 ml of tap water, and a lid (described above) was applied to each. Germination occurred rapidly (<1 week), and microcosms were thinned to two seedlings per microcosm on 9 May, and to one seedling on 11 May by gently pulling seedlings from the compost by hand.

Earthworms were added to microcosms on 4 May after being removed from storage containers for gut voidance as described above on 2 May. On 4 May, earthworms were allocated to groups of two, four, six, eight, or ten individuals with each abundance level replicated eight times. These earthworm abundance levels were selected to provide a wide range of population densities that could be produced by growers adding earthworms to garden containers. Earthworm groups were then randomly allocated to microcosms (Table 2.2). Mean starting earthworm mass was 0.94 g ±SE 0.005 g and did not differ across earthworm abundance levels ($F_{4,35} = 0.01$, $p = 1.000$). Eight additional microcosms did not receive earthworms. Microcosm lids were removed, and earthworms were placed on the

compost surface before lids were replaced. Lids were removed on 5 May after no earthworms were seen on the compost surface of microcosms.

Table 2.2: Summary table showing starting earthworm mass (total mass of earthworms per microcosm and mean mass of earthworms in a microcosm) and population density (n and mass m^{-2}) across earthworm abundance levels.

Earthworm abundance	Total mass (g)		Mean mass (g)		Density m^{-2}	
	Mean	SE	Mean	SE	n	Mass (g)
2	1.87	0.03	0.937	0.017	254.6	238.5
4	3.75	0.05	0.937	0.011	509.3	477.1
6	5.62	0.07	0.936	0.012	763.9	715.2
8	7.48	0.06	0.935	0.008	1018.6	952.7
10	9.38	0.13	0.938	0.013	1273.2	1194.9

Microcosms were arranged in a 6 x 8 grid adjacent to the south-facing window of the same experimental glasshouse described above with positions re-randomised at weekly intervals. Microcosms at the edge of the grid were protected from direct sun exposure with a row of empty PVC tubes. Air temperature was recorded for part of the earthworm abundance experiment with data loggers (SensorBlue Thermometer Hygrometer, Oria, Hongkong Siuon Technology CO Ltd.). Mean temperatures were 16.5 °C (SD = 2.8, range = 10.1 – 21.5 °C, recording period = 15 – 27 May 2023). Supplemental lighting (460 – 730 nm, Attis-7 LED Growth Light, Plessey Semiconductors Ltd., Plymouth, Devon, UK) was provided daily from 07:00 – 19:00 (12 h light : 12 h dark) but was temporarily turned off from 4 – 5 May when earthworms were added to microcosms. Microcosms were watered with 200 ml of tap water on 1 May and 2 May and 100 ml of tap water on 4 May. After this point, microcosms were watered with 200 ml of tap water three times per week (Monday, Wednesday, and Fridays). Due to high temperatures drying the compost, microcosms were watered with 400 ml of tap water on 26 May. A final watering took place on 30 May with 300 ml of tap water.

Data collection

For both experiments, radish plants and earthworms were collected in rounds. Each round consisted of one replicate of each treatment combination in a random order. Radish plants were harvested by gently pulling plants from the compost, washing them with tap water, and wrapping them in moist tissue paper in individual sealed plastic bags to prevent wilting. Plants were separated into leaves, cotyledons, hypocotyls, and taproots and parts were weighed separately before being dried at 70 °C until constant mass when dry masses were recorded. Earthworms were retrieved by emptying the microcosms and hand sorting the compost. Earthworms were cleaned with tap water and transferred to an incubator at 15 °C as described above before end masses were recorded after 48 hours for gut voidance. All masses were recorded to 0.001 g precision using a OHAUS Pioneer PX323 microbalance (OHAUS Europe GmbH, Switzerland).

For the compost variety experiment, all radish plants were harvested and weighed on 23 March, 35 days after seeds were planted, and dry masses were recorded on 30 March while earthworms were retrieved on 28 March and weighed on 30 March (after being transferred to the same incubator for 48 hours for gut voidance, as described above). Additionally, earthworm cocoons were collected from microcosms and counted and weighed on 30 March. For the earthworm abundance experiment, all radish plants were harvested on 2 June, 36 days after seeds were planted, and dry masses were recorded on 8 June. After plants were harvested, microcosms were watered with ~500 ml of tap water and lids were reapplied until earthworms were retrieved on 5 June and weighed on 7 June (after 48 hours for gut voidance in the incubator, as described above).

Data analysis

All data were analysed in R version 4.3.1 (R Core Team, 2023) and all plots were produced with *ggplot2* (Wickham, 2016). Radish response variables for both experiments were fresh and dry shoot mass, root mass, and shoot : root ratio. For both experiments, earthworm survival was extremely high (see *Results*), so earthworm survival was not analysed.

In the compost variety experiment, radish responses were analysed with Analysis of Variance (ANOVA) with earthworms, compost variety, and their interaction included as explanatory factors. All radish response variables were log-transformed to better fit the assumptions of ANOVA. All earthworm response variables were analysed with compost variety as an explanatory factor. Mean earthworm mass change and mean and total cocoon mass were analysed with ANOVA, and cocoon number was analysed with a Poisson Generalised Linear Model (GLM). When variables, or their interactions, had statistically significant effects on response variables, *post hoc* group comparisons were performed with the *emmeans* package (Lenth, 2023) with group contrasts and confidence intervals back transformed to the response scale for interpretation and reporting if log-transformation or GLMs were used.

For the earthworm abundance experiment, all radish response variables and mean and total earthworm mass change were analysed using linear regression, with earthworm abundance as a continuous predictor variable. Dry shoot mass and fresh and dry shoot : root ratios were log-transformed to better fit the assumptions of linear regression. Additionally, one microcosm with 10 earthworms was excluded as an extreme outlier from analysis of fresh and dry shoot : root ratio. The hypocotyl of the radish in this microcosm appeared to be malformed and was highly elongated. Therefore, it was an extreme outlier for both fresh

shoot : root ratio (7.5x standard deviation above grand mean) and dry shoot : root ratio (7.8x standard deviation above grand mean). For each response variable, the relationship with earthworm abundance was first plotted. If it appeared that there may be a non-linear relationship, a linear regression with a quadratic term included was performed. If the relationship appeared linear, a quadratic term was not included in the model. If a quadratic term was statistically significant, the AIC scores of a model with the quadratic term included and one without were compared. The model with the quadratic term included was kept only if it had a lower AIC than the model without the quadratic term included.

Results

Plant performance

Compost variety experiment

In the compost variety experiment, there was a statistically significant interaction between *D. veneta* and compost variety for all radish response variables (Table 2.3). Fresh and dry shoot and root mass were highest in Levington, lowest in New Horizon, and intermediate in SylvaGrow (Fig. 2.2a – d) and *D. veneta* increased fresh shoot mass (Fig. 2.2a, ratio = 1.44, $CI_{95\%}$ = [1.22, 1.71], $p < 0.001$), dry shoot mass (Fig. 2.2b, ratio = 1.31, $CI_{95\%}$ = [1.03, 1.68], $p = 0.021$), fresh root mass (Fig. 2.2c, ratio = 3.40, $CI_{95\%}$ = [2.08, 5.57], $p < 0.001$), and dry root mass (Fig. 2.2d, ratio = 2.60, $CI_{95\%}$ = [1.73, 3.91], $p < 0.001$), but only in the New Horizon compost (Table 2.3, Fig. 2.2a – d). For fresh and dry shoot : root ratio, all significant pairwise differences involved the New Horizon + No Earthworms treatment group (Table S2.1, S2.2). In the New Horizon compost, *D. veneta* reduced both fresh shoot

: root ratio (Fig. 2.2e, ratio = 0.42, $CI_{95\%}$ = [0.26, 0.70], $p < 0.001$) and dry shoot : root ratio (Fig. 2.2f, ratio = 0.50, $CI_{95\%}$ = [0.31, 0.82], $p = 0.001$).

Table 2.3: Summary of ANOVA results showing effects of earthworms, compost variety, and their interaction, on radish plant response variables.

Response variable	Earthworms			Compost			Earthworms x Compost		
	Df	F	P	Df	F	P	Df	F	P
Fresh shoot mass	1,66	9.95	**0.002**	2,66	1322.79	**<0.001**	2,66	14.94	**<0.001**
Dry shoot mass	1,66	0.00	0.989	2,66	418.21	**<0.001**	2,66	7.93	**0.001**
Fresh root mass	1,66	23.00	**<0.001**	2,66	249.77	**<0.001**	2,66	15.48	**<0.001**
Dry root mass	1,66	18.63	**<0.001**	2,66	235.77	**<0.001**	2,66	14.47	**<0.001**
Fresh S:R ratio	1,66	13.43	**<0.001**	2,66	11.51	**<0.001**	2,66	6.59	**0.002**
Dry S:R ratio	1,66	13.18	**0.001**	2,66	8.31	**0.001**	2,66	3.18	**0.048**

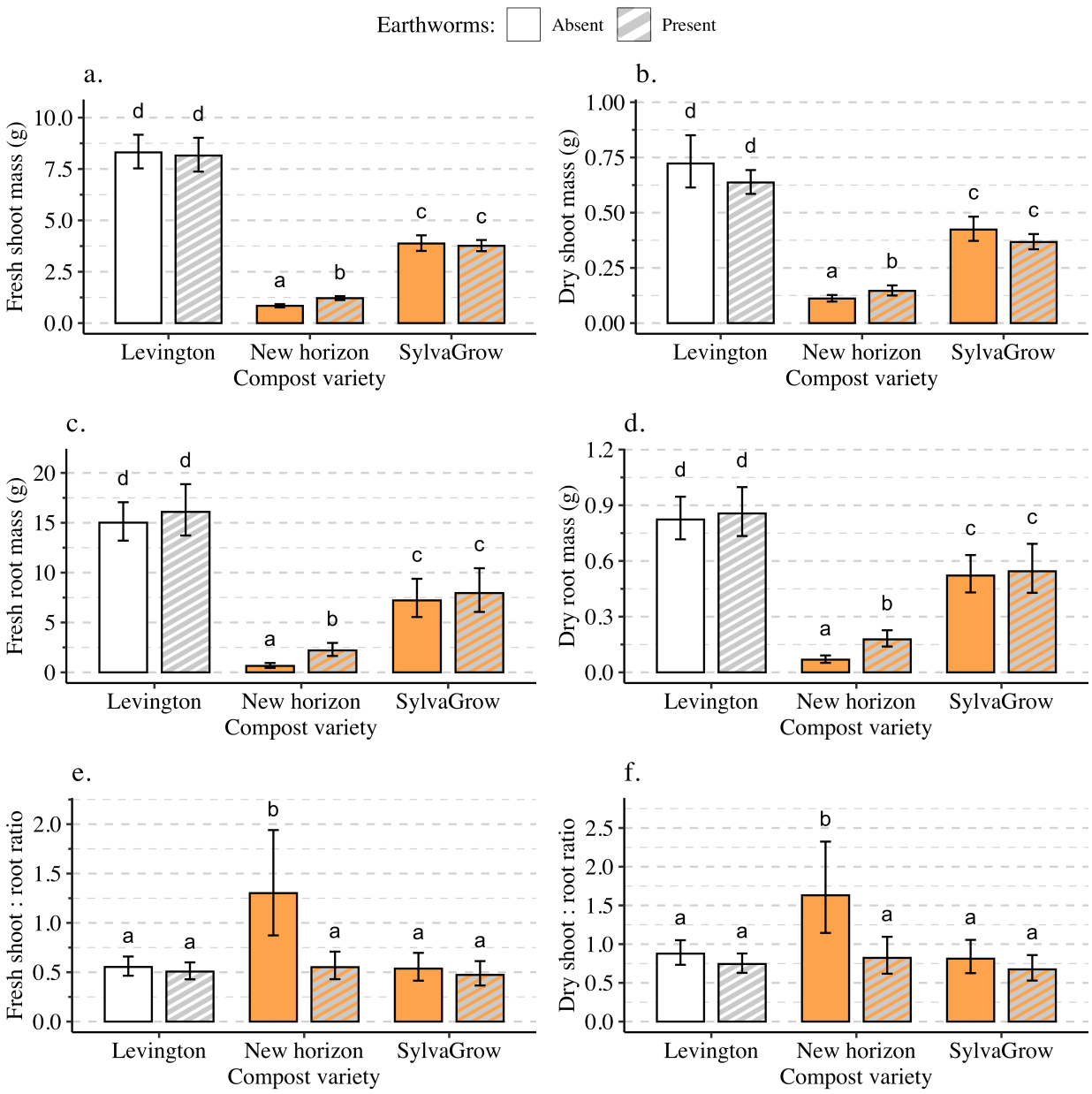


Fig. 2.2: Bar plots showing group means and 95% confidence intervals for *a.* fresh shoot mass, *b.* dry shoot mass, *c.* fresh root mass, *d.* dry root mass, *e.* fresh shoot : root ratio, and *f.* dry shoot : root ratio across earthworm (solid fill = earthworms absent, grey stripe pattern = earthworms present) and compost variety treatment groups. Levington (white bar fill colour) was a conventional peat-containing compost. New horizon and SylvaGrow (orange bar fill colour) were peat-free composts. All means and confidence intervals have been back transformed from the log scale. Letter displays over bars indicate group differences. The means of bars that do not share letters are significantly different from one another ($p < 0.05$, analysis performed on log scale).

Earthworm abundance experiment

Increasing *D. veneta* abundance significantly increased both fresh shoot mass (Fig. 2.3a, Table 2.4, $b = 0.49$, $CI_{95\%} = [0.35, 0.64]$, $p < 0.001$) and log-transformed dry shoot mass (Fig. 2.3b, Table 2.4, $b = 0.05$, $CI_{95\%} = [0.03, 0.08]$, $p < 0.001$). There was a statistically significant quadratic relationship between *D. veneta* abundance and fresh root mass (Fig. 2.3c, Table 2.4) but no effect of *D. veneta* abundance on dry root mass (Fig. 2.3d, Table 2.4). For fresh root mass, there was a positive effect of *D. veneta* abundance ($b = 3.16$, $CI_{95\%} = [1.00, 5.32]$, $p = 0.005$) but a negative effect of *D. veneta* abundance^2 ($b = -0.22$, $CI_{95\%} = [-0.43, -0.14]$, $p = 0.037$), resulting in a hump-shaped relationship with a peak fresh root mass predicted between 6 and 8 earthworms (Fig. 2.3c). *Dendrobaena veneta* abundance had no significant effect on log-transformed fresh shoot : root ratio (Fig. 2.3e, Table 2.4) but significantly increased log-transformed dry shoot : root ratio (Fig. 2.3f, Table 2.4, $b = 0.025$, $CI_{95\%} = [0.002, 0.049]$, $p = 0.035$).

Table 2.4: Regression summaries for the relationship between earthworm abundance and radish response variables.

Response variable	Df	F	P	R^2 adj.
Fresh shoot mass	1,46	45.00	**<0.001**	0.48
Dry shoot mass[a]	1,46	16.17	**<0.001**	0.24
Fresh root mass	2,45	7.25	**0.002**	0.21
Dry root mass	1,46	2.59	0.115	0.03
Fresh S:R ratio[a]	1,45	1.29	0.263	0.01
Dry S:R ratio[a]	1,45	4.72	**0.035**	0.07

[a]Analysis performed on the log scale

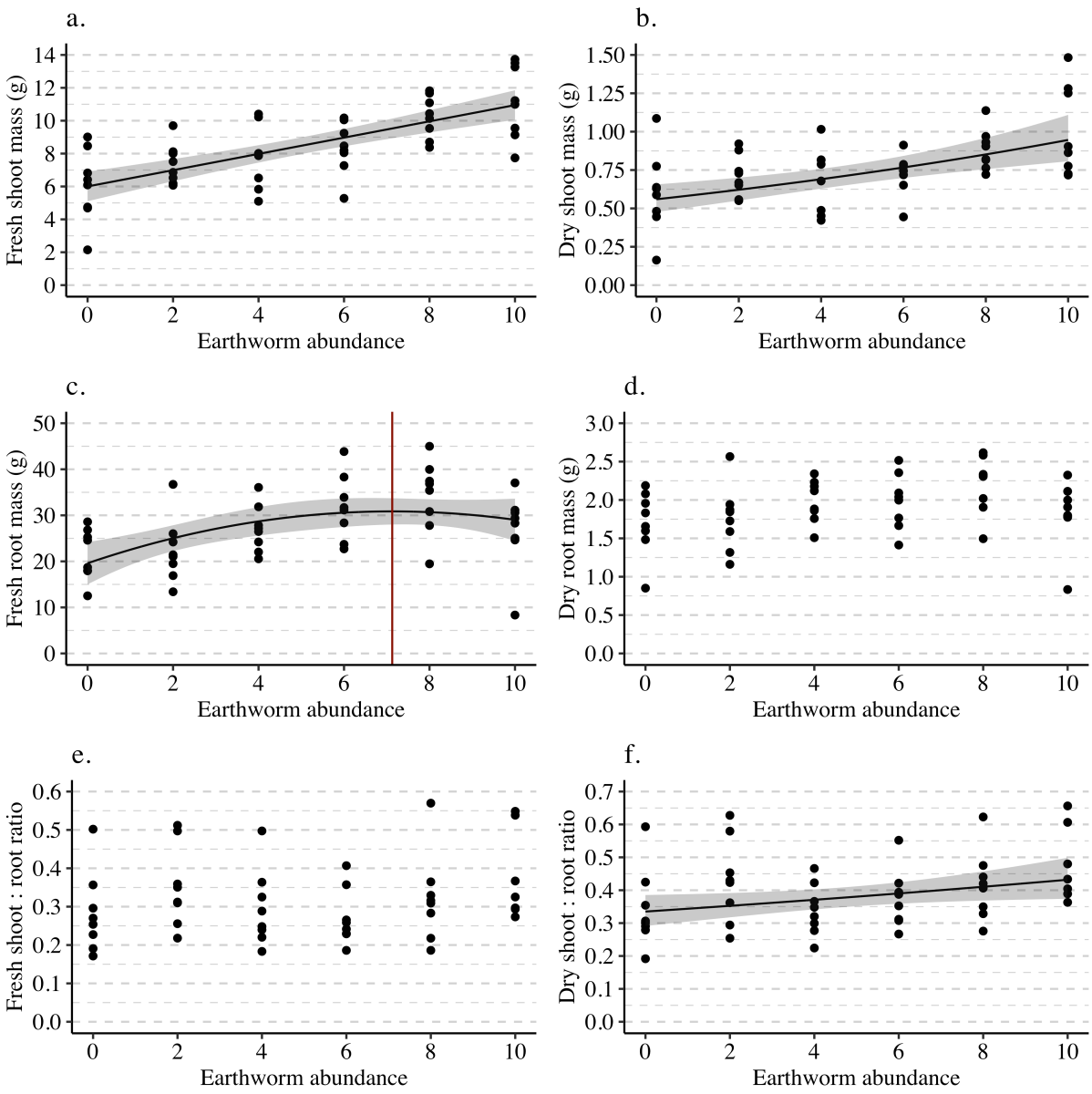


Fig. 2.3: Scatter plots showing the relationship between earthworm abundance and *a.* fresh shoot mass, *b.* dry shoot mass, *c.* fresh root mass, *d.* dry root mass, *e.* fresh shoot : root ratio, and *f.* dry shoot : root ratio. Lines of best fit and 95% confidence interval ribbons are shown only when relationships were statistically significant ($p < 0.05$). The lines of best fit and confidence intervals for dry shoot mass (*b.*) and dry shoot : root ratio (*f.*) have been back-transformed from the log scale. For plots *e.* and *f.*, one outlier microcosm with ten earthworms has been excluded from both the plots and regression analyses. This was because the hypocotyl of the radish appeared to be malformed which greatly increased the fresh and dry shoot : root ratio (see *Methods*). The vertical red line in *c.* shows the predicted earthworm abundance (7.13) resulting in the greatest fresh root mass.

Earthworm performance

Compost variety experiment

Survival of *D. veneta* was extremely high in the compost variety experiment: 174 out of 180 individuals (96.7%) were recovered alive. All individuals that died were in the Levington compost treatment where several earthworms were seen dead on the compost surface in the first few days after being added to microcosms. For mean earthworm mass change, cocoon number, and mean cocoon mass, there were significant differences across compost treatments (Table 2.5). On average, mean earthworm mass decreased in the experiment by 120.12 mg ($CI_{95\%}$ = [95.93, 144.35], $p < 0.001$) with individuals in Levington compost losing significantly less mass than those in New Horizon (Fig. 2.4a, difference = 77.70 mg, $CI_{95\%}$ = [27.82, 127.50], $p = 0.002$) and SylvaGrow (Fig. 2.4a, difference = 126.4 mg, $CI_{95\%}$ = [76.52, 176.20], $p < 0.001$) compost. The average number of cocoons recovered from microcosms was 17.83 ($CI_{95\%}$ = [15.63, 20.04]) with significantly more per microcosm in SylvaGrow than in New Horizon (Fig. 2.4b, ratio = 1.29, $CI_{95\%}$ = [1.04, 1.61], $p = 0.02$) and in Levington (Fig. 2.4b, ratio = 1.56, $CI_{95\%}$ = [1.24, 1.97], $p < 0.001$). Mean cocoon mass across microcosms was 22.29 mg ($CI_{95\%}$ = [21.41, 23.17]) and mean cocoon mass was significantly higher in Levington than in New Horizon (Fig. 2.4c, difference = 3.33 mg, $CI_{95\%}$ = [1.33, 5,34], $p < 0.001$) and in SylvaGrow (Fig. 2.4c, difference = 3.86 mg, $CI_{95\%}$ = [1.85, 5.87], $p < 0.001$). Average total cocoon mass per microcosm was 390.62 mg ($CI_{95\%}$ = [343.94, 437.30]) but there were no significant differences across composts (Table 2.5, Fig. 2.4d).

Table 2.5: Summary of ANOVA results showing differences in earthworm response variables across compost treatments.

Response variable	Test	Compost variety		
		Df	Test statistic	P
Mean earthworm mass change	F	2,33	19.69	**<0.001**
Cocoon number	X^2	2	21.76	**<0.001**
Mean cocoon mass	F	2,33	13.09	**<0.001**
Total cocoon mass	F	2,33	2.62	0.088

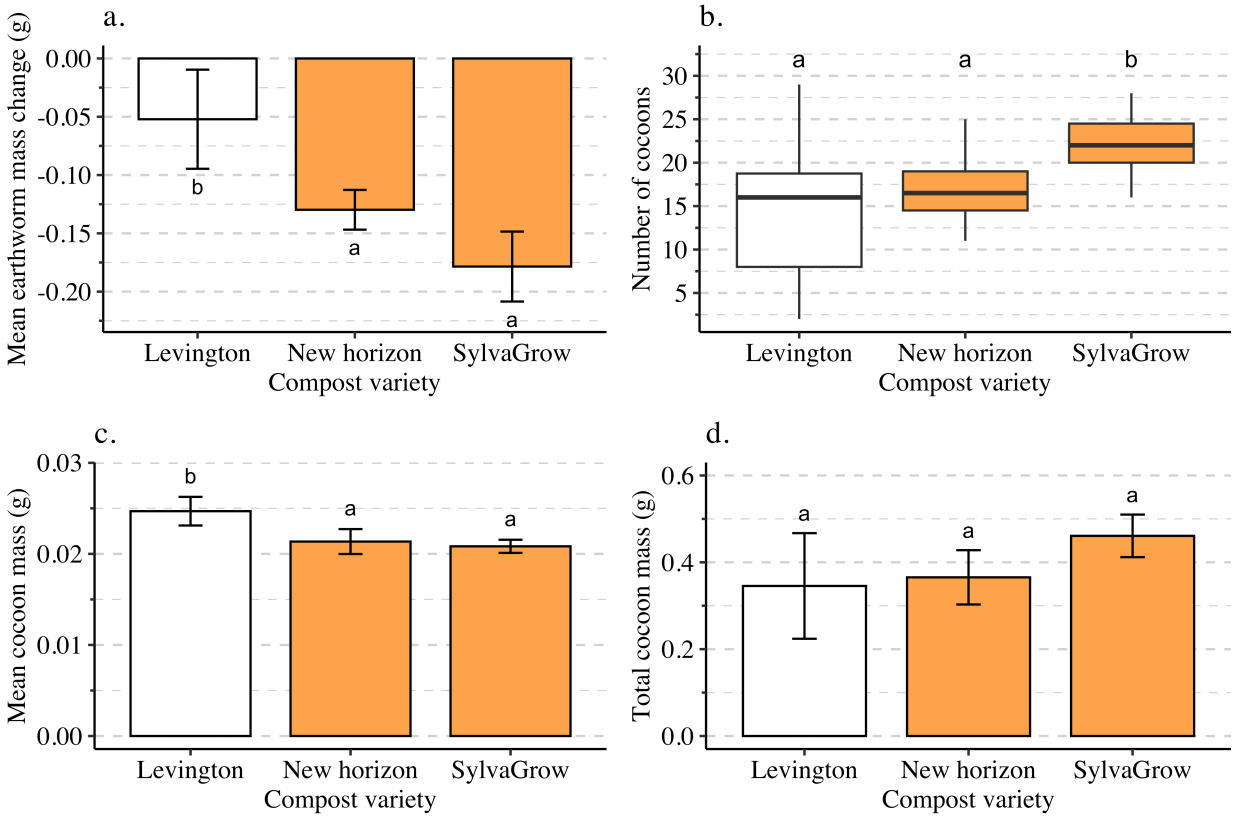


Fig. 2.4: Bar plots (*a.*, *c.*, and *d.*) and boxplot (*b.*) showing *a.* mean earthworm mass change, *b.* cocoon number, *c.* cocoon mean mass, and *d.* total cocoon mass across compost treatments. Levington (white bar or box fill colour) was a conventional peat-containing compost. New horizon and SylvaGrow (orange bar or box fill colour) were peat-free composts. For the bar plots, bar height and error bars show group means and 95% confidence intervals respectively. For the box plot, box outer horizontal lines show group interquartile ranges, central horizontal lines show group medians, and extended vertical lines show full data range for each group. Different letters above / below bars / boxes indicate that group means are significantly different within a graph ($p < 0.05$).

Earthworm abundance experiment

Dendrobaena veneta survival was 100% in the earthworm abundance experiment, so survival was not analysed. There was no statistically significant relationship between earthworm abundance and mean earthworm mass change (Fig. 2.5a, $F_{1,38} = 0.23$, $p = 0.63$) or total earthworm mass change (Fig. 2.5b, $F_{1,38} = 0.29$, $p = 0.59$). There was also no overall trend for either mean earthworm mass ($\mu = 16.85$ mg, $CI_{95\%} = [-2.50, 36.19]$, $p = 0.086$) or total earthworm mass ($\mu = 88.01$ mg, $CI_{95\%} = [-17.74, 193.75]$, $p = 0.10$) to increase or decrease in the experiment.

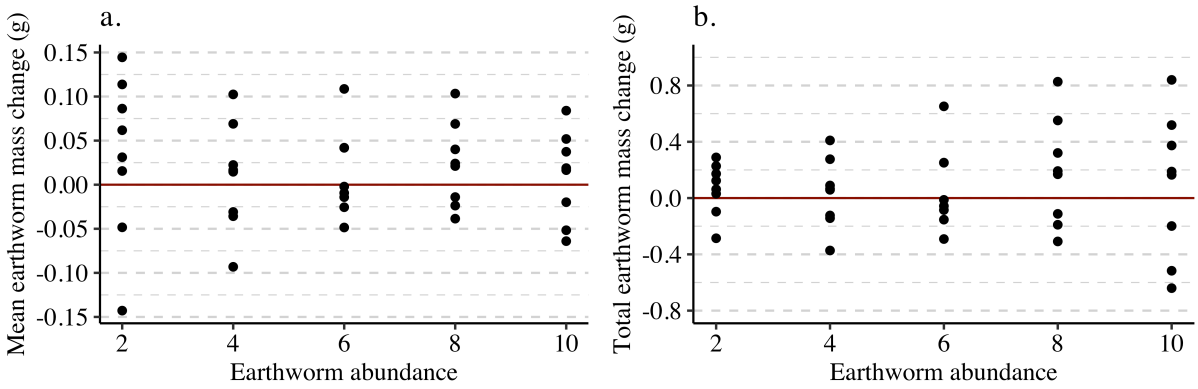


Fig. 2.5: Scatter plots showing *a.* mean earthworm mass change and *b.* total earthworm mass change across earthworm abundance levels.

Discussion

In the compost variety experiment, earthworms caused an increase in radish plant growth but only in one of the three composts tested. In the earthworm abundance experiment, radish plant performance was influenced by the number of *D. veneta* added to containers although shoot and roots showed different responses to increasing earthworm abundance. In both experiments, earthworm survival was very high, suggesting that the horticultural composts used were suitable substrates for *D. veneta*.

Earthworm effects on radish yields differed across composts

Compost variety had a significant impact on radish plant growth (Fig. 2.2): in the peat-containing Levington compost, fresh root and shoot mass were ~13.1x and ~8.1x higher than in the peat-free New Horizon compost, and ~2.1x and ~2.2x higher than in the peat-free SylvaGrow compost, respectively (Fig 2.2a, 2.2c). While, the beneficial effects of earthworms on plant growth are well known (Scheu, 2003; Van Groenigen et al., 2015) and earthworms may contribute up to 6.5% of grain and 2.3% legume global production (Fonte et al., 2023), effects on plants can differ depending on soil properties (Hallam et al., 2021), and in some cases earthworm only provide benefits in substrates with reduced plant performance (Doan et al., 2013). In the compost variety experiment, *D. veneta* only significantly increased radish growth in the New Horizon compost, the worst performing of the three composts used when considering radish growth. *Dendrobaena veneta* increased fresh shoot and root yields by 44% and 340%, respectively, but this was insufficient to raise yields to levels obtained in the other two composts (Fig. 2.2a, 2.2c), despite providing greater increases than the mean 23% shoot mass and 20% root mass increases estimated for earthworms in a meta-analysis of earthworm effects on plant primary production (Van Groenigen et al., 2015). Earthworms also significantly reduced shoot : root ratios in the New Horizon compost to the same levels as the higher performing composts (Fig 2.2e, 2.2f). However, this likely reflects a general improvement in plant performance, instead of a change in resource allocation, as radish shoot : root ratios are decreased by the swelling of the hypocotyl during development (Kostka-Rick and Manning, 1993). With the upcoming ban on the sale of peat-containing composts in England and Wales (Department for Environment, Food and Rural Affairs, 2021; Department for Environment, Food and Rural Affairs et al., 2022), there are concerns among both professional and amateur gardeners regarding the performance of peat-free

composts (Department for Environment, Food and Rural Affairs, 2022; Environment and Forestry Directorate, 2023). The much higher performance of the peat-containing compost in comparison to both peat-free composts in the compost variety experiment suggests that, in some cases, conventional peat-containing composts may offer superior performance.

Multi-purpose horticultural composts typically contain added plant nutrients: for example, the manufacturers of the SylvaGrow compost state that the compost contains "balanced nutrients sufficient for the first 4 – 6 weeks of growth" (Melcourt Industries Limited, 2024) while the manufacturers of Levington compost state it contains "essential plant food for 6 weeks" (Evergreen Garden Care (UK) Ltd., 2024). Both of these composts contain wood fibre as a main ingredient (Holmes and Bain, 2023, 2021) which must be treated with slow-release fertiliser during manufacture to prevent nitrogen immobilisation in the growing medium (Schmilewski, 2008). This added fertiliser, in addition to short plant growth periods in the two experiments (35 and 36 days), may partially explain the limited effects of *D. veneta* on radish plant yields in the compost variety experiment. It may be the case that the rapid growth of radish plants occurred too quickly for benefits of the earthworms to become apparent, and that earthworms may provide benefits in horticultural composts for plants with growth durations longer than six weeks.

Effects of increasing earthworm abundance on radish plant growth

In the earthworm abundance experiment, radish shoot mass significantly increased with increasing *D. veneta* abundance (Fig. 2.3a). Increasing benefits of earthworms with increasing abundances have been reported previously, although often following manipulation of field densities (Brown et al., 1999; Eisenhauer et al., 2009; Eriksen-Hamel and Whalen, 2007). In a pot experiment, increasing abundances of *Amynthas* spp. Kinberg, 1866 (Megascolecidae) increased tomato (*Solanum lycopersicoides* Dunal) plant biomass,

although earthworm survival was only 39% (Demetrio et al., 2017). High earthworm escape or mortality makes interpretation of earthworm effects challenging (Fründ et al., 2010), as survival below 50% may remove benefits provided to plants (Van Groenigen et al., 2015) and decomposition of dead earthworms can increase plant growth, with potentially stronger effects than live earthworms (Kos et al., 2017). Therefore, the 100% survival in the earthworm abundance experiment reported here makes it easier to interpret the relationship between *D. veneta* abundance and plant performance. There are multiple mechanisms that may have contributed to increased plant growth. Earthworm casts cause hotspots of soil fertility through concentration of material through preferential feeding, and transformation of material in the earthworm gut that increases nutrient availability (Van Groenigen et al., 2019), with nitrogen availability likely being particularly important (Van Groenigen et al., 2015). Additionally, carbon and nitrogen may be rapidly transferred from earthworms to plants via secreted cutaneous mucous (Shutenko et al., 2022). Earthworms may also indirectly stimulate plant growth through modification of the soil microbiota (Medina-Sauza et al., 2019) or through release of molecules that interact with plant hormone signalling pathways (Puga-Freitas et al., 2012; Puga-Freitas and Blouin, 2015). By increasing earthworm abundances, these beneficial effects on plant growth may also be increased, leading to a linear improvement in shoot growth.

However, the relationship between *D. veneta* abundance and fresh radish root mass was non-linear, with maximum yield estimated at ~7.13 earthworms (Fig 2.2c, ~908 earthworms m^{-2}), in agreement with non-linear relationships recorded for pasture productivity (Chan et al., 2004), soil microbial activity (Demetrio et al., 2017), plant root morphology (Agapit et al., 2018), and grain and root yields of crops in the tropics (Brown et al., 1999). The non-linear relationship may have been caused by root herbivory or damage counteracting beneficial effects provided by earthworms at high abundances.

Eisenia fetida can reduce radish (cv. "Granat") biomass in a mixture of soil and vermicompost with greater reductions at higher earthworm densities (Kováčik et al., 2021, 2018). While earthworms primarily feed on decaying organic material (Curry and Schmidt, 2007; Lee, 1985), soil rhizotron observations (Gunn and Cherrett, 1993), gut dissections of field collected earthworms (Bouché and Kretzschmar, 1972; Piearce, 1978), phosphorous radio labelling of clover roots (Baylis et al., 1986), and carbon radio labelling of grass roots (Cortez and Bouche, 1992) suggest that earthworms partially feed on roots and may, to some degree, consume live roots. The small volumes of compost (~1.2 L) used in containers may have provided insufficient space or food at high abundances of *D. veneta*, leading to root damage and reductions in yields via grazing or burrowing.

The earthworm abundances of two, four, six, eight, and ten individuals used in the earthworm abundance experiment corresponded to approximate densities of 255, 509, 764, 1019, and 1273 individuals m^{-2} respectively. These values are higher than field densities in most ecosystems (Lee, 1985), although temperate pastures may contain 300 – 1000 individuals m^{-2} (Fründ et al., 2010) and cattle dung pats can attract very high earthworm densities in temperate pastures, equivalent to 4000 individuals m^{-2} and 1182 g m^{-2} (Bacher et al., 2018). A meta-analysis divided earthworm densities from previous studies into four categories (<100, 100 – 199, 200 – 399, ≥400) and showed that the positive effect of earthworms on plant growth was increased at the highest density (≥400 individuals m^{-2}) compared to lower densities (Van Groenigen et al., 2015). Gardens containers typically consist of a vessel, substrate, and plant(s) (Nagase and Lundholm, 2021) and each of these parameters (including earthworm abundances, if added) can be freely controlled by the grower. Therefore, abundances of earthworms are not constrained to levels seen in natural ecosystems (Coleman et al., 2018; Fründ et al., 2010; Lee, 1985). However, the non-linear effect of earthworms on root yields, with negative effects at high abundances, suggests that

care may be needed to ensure that earthworm densities are selected that maximise growth of the plant trait of interest in a garden container system.

Earthworm performance in horticultural substrates

Overall *D. veneta* survival across both experiments was 98.6% with 100% survival in the earthworm abundance experiment and 96.7% (New Horizon and SylvaGrow = 100%, Levington = 90%) survival in the compost variety experiment. Despite using 2 cm-width hook tape, which can completely prevent the escape of epigeic earthworms from open-topped microcosms (Lubbers and Van Groenigen, 2013), individual *D. veneta* were seen crawling on the underside of microcosm lids shortly after being introduced to microcosms so had the potential to escape. However, the high earthworm survival rates, with only a few dead earthworms found at the compost surface in the Levington compost suggest that the composts provided a suitable substrate for the earthworms, even at the high densities in the earthworm abundance experiment. The lack of earthworm escape during the experiments indicate that earthworms could be a suitable additive to horticultural substrates. However, integration of earthworms into composts before filling containers may be needed to reduce chance of escape.

In the compost variety experiment, cocoon production, but also body mass loss, were highest in the SylvaGrow compost (Fig 2.4a, 2.4b). Compost earthworms are affected by substrate composition; *E. fetida* growth rates can differ greatly in different animal manures (Gunadi et al., 2003; Vodounnou et al., 2016) or plant-based substrates (Borang et al., 2016) and earthworm cocoon production rates can differ across substrates (Fayolle et al., 1997; Sirithanakorn et al., 2014; Sonia et al., 2016; Suthar and Ram, 2008). Substrate quality can affect growth and reproduction simultaneously: growth rates and cocoon production were increased for *E. fetida* fed cattle dung compared to dried leaves (Siddique

et al., 2005). Both body size and cocoon production of adult *L. terrestris* increase with higher food quality, suggesting that somatic growth and reproductive output flexibly respond to environmental properties (Butt, 2011). However, reproduction and growth can also show divergent responses to substate properties with growth and cocoon production rates of *Perionyx excavatus* Perrier, 1872 (Megascolecidae) and *Eudrilus eugeniae* (Kinberg, 1866) (Eudrilidae) being maximised in different substrates (Coulibaly and Zoro Bi, 2010; Sadia et al., 2020). Surface dwelling species, which live in areas of greater environmental stress, produce more cocoons than subterranean species (Satchell, 1967a). Therefore, the increased cocoon production and body mass loss in the SylvaGrow compost might indicate reduced suitability for the earthworms.

Dendrobaena veneta may have also allocated resources to reproduction and body mass maintenance differently across the compost varieties. Earthworm populations show reduced growth rates, and potential mass declines, at the onset of reproduction before later declines during senescence (Mathieu, 2018). Individual *Eisenia andrei* (Bouché, 1972) and *E. fetida* show large body mass declines and cessation of weight gain, respectively, following mating and commencement of cocoon production (Cluzeau et al., 1992). The growth rates of *E. eugeniae* also reduce upon reaching sexual maturity, which may be caused by shifting resource allocation from growth to mating and cocoon production (Jesikha and Lekeshmanaswamy, 2013). Conditions that are optimal for earthworm reproduction rate may also reduce body size through reproductive fatigue (Lowe and Butt, 2005). Therefore, the reduced body mass, but increased cocoon production, in the SylvaGrow compost relative to the other two varieties likely reflects altered resource allocation towards reproduction, at the expense of body size, in this substrate. The three composts differed in ingredients: Levington compost is peat-based, New Horizon compost is comprised of wood-fibre made from wood-chips (Holmes and Bain, 2023) while

SylvaGrow compost is made from wood-fibre and fine bark (Holmes and Bain, 2021). Commercially produced composts are made from a variety of materials including peat, coconut coir, wood fibre, green waste, wool, bark, and others (Bek et al., 2020; Holmes and Bain, 2023, 2021; Leyman and Aplin, 2007) and it is likely that compost earthworm performance may differ across different formulations.

Survival of *D. veneta* was 100% in the earthworm abundance experiment and neither mean nor total mass were affected by earthworm abundance (Fig. 2.5). Earthworm populations can be strongly influence by food availability (Lee, 1985; Satchell, 1967a) and the growth rate of *E. fetida* in pig slurry is reduced at increasing population densities (Domínguez and Edwards, 1997). However, growth of hatchling *Lumbricus rubellus* Hoffmeister, 1843, and size at maturity, were reduced in small containers at higher abundances despite being provided with food *ad libidem* (Klok, 2007), suggesting that population density may directly affect growth. Juvenile earthworms have higher energy requirements than adults (Curry and Schmidt, 2007), so, by using large adults, the negative effects of increased population density on earthworm performance may have been reduced in the earthworm abundance experiment, resulting in no negative effects on mass change. Juvenile *Aporrectodea rosea* (Savigny, 1826) show greater burrow construction and casting rates for their size and reduced gut clearance time than adults (Bolton and Phillipson, 1976), so juvenile earthworms may have increased functioning in gardens containers and these benefits may be provided for longer as individuals will be earlier in the life cycle and may therefore live longer (Domínguez and Edwards, 2011). However, juveniles have lower abiotic tolerances than adults so additional care will be needed with their use (Reinecke and Venter, 1987). Additionally, under controlled conditions, compost earthworms can have extremely long life spans in excess of 144 weeks for *E. andrei* (Cluzeau et al., 1992) and *D. veneta* can show continued growth over a 200 day period (Viljoen et al., 1991).

Therefore, utilisation of adult earthworms may maximise benefits while limiting risk of mortality or poor performance due to population density. Additionally, compost earthworms show indeterminate growth and individuals of *E. andrei*, *E. fetida*, and *Dendrobaena hortensis* (Michaelsen, 1890) can reach masses of 9.1 g, 7.0 g, and 7.8 g respectively when fed on ox ruminal content (Domínguez and Guimaraěs, 2024). The earthworm abundance experiment here manipulated abundance, but not individual mass; juvenile earthworms, or giant earthworms, might have different effects in horticultural containers than average-sized adults.

Conclusions

The performance of *D. veneta* was very high in both experiments, suggesting that soil-less multi-purpose composts can act as a suitable substrate for compost earthworms. While earthworms did provide benefits to radish yields, this was only in the lowest performing compost in the compost variety experiment. In addition, while shoot yields increased linearly with increasing earthworm abundance, root yields showed a non-linear relationship with yields maximised at intermediate earthworm densities. Therefore, while earthworms could provide great benefits to crop yields in gardens containers, care must be taken to select optimal earthworm densities and substrates in which earthworms can provide a benefit to plant growth.

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Supplementary information

Table S2.1: Between group contrasts for fresh shoot : root ratio across earthworm (EW) and compost variety treatment groups. Contrasts and confidence intervals have been back transformed from the log scale.

Comparison	Ratio	95% CI		t	P
		Lower	Upper		
Levington + No EW / New Horizon + No EW	0.43	0.26	0.70	⁻5.06	**<0.001**
Levington + No EW / SylvaGrow + No EW	1.03	0.63	1.69	0.18	1.000
Levington + No EW / Levington + EW	1.09	0.67	1.79	0.52	0.995
Levington + No EW / New Horizon + EW	1.00	0.61	1.65	0.02	1.000
Levington + No EW / SylvaGrow + EW	1.17	0.71	1.92	0.93	0.938
New Horizon + No EW / SylvaGrow + No EW	2.42	1.48	3.98	5.24	**<0.001**
New Horizon + No EW / Levington + EW	2.57	1.56	4.22	5.58	**<0.001**
New Horizon + No EW / New Horizon + EW	2.36	1.44	3.88	5.08	**<0.001**
New Horizon + No EW / SylvaGrow + EW	2.75	1.67	4.52	5.99	**<0.001**
SylvaGrow + No EW / Levington + EW	1.06	0.65	1.74	0.34	0.999
SylvaGrow + No EW / New Horizon + EW	0.97	0.59	1.60	⁻0.16	1.000
SylvaGrow + No EW / SylvaGrow + EW	1.13	0.69	1.86	0.75	0.975
Levington + EW / New Horizon + EW	0.92	0.56	1.51	⁻0.50	0.996
Levington + EW / SylvaGrow + EW	1.07	0.65	1.76	0.41	0.999
New Horizon + EW / SylvaGrow + EW	1.17	0.71	1.91	0.91	0.943

Table S2.2: Between group contrasts for dry shoot : root ratio across earthworm (EW) and compost variety treatment groups. Contrasts and confidence intervals have been back transformed from the log scale.

Comparison	Ratio	95% CI		t	P
		Lower	Upper		
Levington + No EW / New Horizon + No EW	0.54	0.33	0.87	⁻3.76	**0.005**
Levington + No EW / SylvaGrow + No EW	1.08	0.67	1.75	0.47	0.997
Levington + No EW / Levington + EW	1.18	0.73	1.91	1.00	0.915
Levington + No EW / New Horizon + EW	1.07	0.66	1.73	0.39	0.999
Levington + No EW / SylvaGrow + EW	1.30	0.80	2.11	1.60	0.601
New Horizon + No EW / SylvaGrow + No EW	2.01	1.24	3.26	4.23	**0.001**
New Horizon + No EW / Levington + EW	2.19	1.35	3.56	4.77	**<0.001**
New Horizon + No EW / New Horizon + EW	1.98	1.22	3.21	4.15	**0.001**
New Horizon + No EW / SylvaGrow + EW	2.42	1.49	3.92	5.36	**<0.001**
SylvaGrow + No EW / Levington + EW	1.09	0.67	1.77	0.53	0.995
SylvaGrow + No EW / New Horizon + EW	0.99	0.61	1.60	⁻0.08	1.000
SylvaGrow + No EW / SylvaGrow + EW	1.20	0.74	1.95	1.13	0.867
Levington + EW / New Horizon + EW	0.90	0.56	1.47	⁻0.61	0.990
Levington + EW / SylvaGrow + EW	1.10	0.68	1.79	0.60	0.991
New Horizon + EW / SylvaGrow + EW	1.22	0.75	1.98	1.21	0.831

Chapter 3: The compost earthworm *Dendrobaena veneta* increases radish yields in peat-free horticultural compost during simulated waterlogging and drought events

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Abstract

Drought and waterlogging stress have major negative impacts on crop yields. These stressors are a particular problem in garden containers due to their small size and high irrigation and maintenance requirements. In the UK, there is an upcoming ban on the sale of peat-containing horticultural composts and there are concerns about the performance of peat-free alternatives in times of environmental stress. Earthworms (Oligochaeta: Lumbricidae) are ecosystem service providers that can provide great benefits to crop yields in soils. The potential benefits of earthworms in horticultural substrates during periods of environmental stress have received less attention. This chapter reports on two factorial glasshouse microcosm experiments (a flood experiment and a drought experiment)

investigating the effects of the compost earthworm *Dendrobaena veneta* on the growth of *Raphanus sativus* across multiple stress levels. Earthworms significantly increased plant growth in both experiments. In the flood experiment, fresh shoot mass was increased by 27% and fresh root mass was increased by 25%. In the drought experiment, fresh shoot mass was increased by 88.4% and fresh root mass by 115.1%. Earthworm survival was high in both experiments at 81.3% in the flood experiment and 99.6% in the drought experiment. Earthworm survival was significantly reduced in the flood experiment in 24-hour flood event treatments, although this may reflect earthworm escape from microcosms, rather than mortality. In both experiments there was no significant interaction between earthworms and the environmental stress treatment. In the flood experiment, radish shoot mass was reduced only in the most severe flooding treatment (4 x 24-hour events), but root mass was reduced by both 24-hour events and by frequent, short, events (4 x 4-hour events). In the drought experiment, plant performance did not differ significantly across individual drought treatments but longer droughts (two-week duration) significantly reduced plant performance compared to controls when treatments were pooled by duration. Commercially available compost earthworms may therefore provide a way for growers to greatly improve crop yields in peat-free composts during both normal conditions and periods of waterlogging stress or drought stress. However, the high mobility of compost earthworms may lead to earthworms escaping from waterlogged containers.

Keywords: Peat-free compost, earthworms, radish, container gardens, drought, flooding, waterlogging

Introduction

Plant growth and crop yields are strongly influenced by water availability and can be negatively impacted by drought (Farooq et al., 2009; Lipiec et al., 2013; Seleiman et al., 2021) or waterlogging (Rao and Li, 2003; Zhou et al., 2020). These stressors can be particularly severe in garden containers; small containers consisting of a container vessel, substrate, and one or more plants (Nagase and Lundholm, 2021) due to their small size and potential challenges with drainage which necessitate regular irrigation and maintenance (Durham et al., 2018; Hillier, 1991; Relf, 2020). Within the UK, growers face additional challenges as conventional peat-containing composts are soon to be banned for amateur use in England and Wales (Department for Environment, Food and Rural Affairs et al., 2022) and there are ongoing consultations about a proposed ban in Scotland (Environment and Forestry Directorate, 2023). There are concerns among professional and amateur growers regarding the cost, quality, and consistency of peat-free products (Environment and Forestry Directorate, 2023) and the availability and sustainability of peat-free compost ingredients, which include wood, bark, coconut coir, green waste, and wool (Bek et al., 2020; Holmes and Bain, 2023, 2021). Peat is typically added to horticultural composts for its water holding properties (Leyman and Aplin, 2007) so drought and water stress are a major concern regarding peat-free alternatives (Environment and Forestry Directorate, 2023).

Earthworms (Oligochaeta: Lumbricidae) are functionally important invertebrates that could provide a means to improve performance of peat-free horticultural substrates during times of environmental stress. Earthworms have generally positive effects on plant production (Scheu, 2003; Van Groenigen et al., 2015), and may contribute up to 6.5% of global grain production (Fonte et al., 2023). Earthworms influence soil microbiota

(Medina-Sauza et al., 2019), plant growth in the presence of herbivores (Xiao et al., 2018), and can produce areas of increased soil fertility through casts (Van Groenigen et al., 2019). A number of earthworm species are commercially available in the UK (Katsiamides and Stürzenbaum, 2021; Lowe and Butt, 2007b). Several of these are 'compost earthworms' (Domínguez and Edwards, 2011) that have applications for processing organic material (Ferraz Ramos et al., 2022; Gómez-Brandón et al., 2010; Suthar, 2009) to produce 'vermicompost' that can improve plant performance in soils and horticultural substrates (Atiyeh et al., 2000a, 2000b). Despite the known beneficial effects of earthworms on plant growth in soils (Van Groenigen et al., 2015) and the benefits to plant growth provided by vermicompost (Blouin et al., 2019), the potential benefits of direct application of 'compost earthworms' into horticultural substrates has been less well studied. However, results of work reported elsewhere in this thesis (Chapter 2) suggest that commercially available compost earthworms could provide benefits to crop growth in some peat-free composts.

In the context of garden containers, there are a number of mechanisms by which earthworms could increase plant performance during normal conditions or periods of environmental stress. Earthworms may be able to alleviate negative effects of waterlogging or flooding through their burrowing activities, which can increase porosity and drainage of soils (Hallam et al., 2021; Ma et al., 2021) and alter soil moisture and water holding capacities (Hallam and Hodson, 2020; Hodson et al., 2023), which can reduce effects of flooding stress on crops (Andriuzzi et al., 2015). Additionally, earthworms can improve plant growth and performance during times of drought stress (Hodson et al., 2023; Johnson et al., 2011), although they can also exacerbate effects of drought on plants in some circumstances (Blouin et al., 2007). However, earthworms themselves are also negatively impacted by environmental stressors (Singh et al., 2019). Flooding and waterlogging have strong negative effects on earthworm populations (Kiss et

al., 2021b; Plum and Filser, 2005; Singh et al., 2019). Epigeic (surface dwelling) species are particularly affected (Kiss et al., 2021a; Klok et al., 2006; Zorn et al., 2005) and demonstrate avoidance behaviours to flooding (Plum, 2005; Zorn et al., 2008) which may reduce beneficial effects on plants due to earthworm mortality or escape from garden containers. Additionally, drought or dry periods can negatively impact earthworm populations (Eggleton et al., 2009; Singh et al., 2019). Compost earthworms are strongly affected due to their high substrate moisture requirements (Domínguez and Edwards, 2011) where insufficient moisture can reduce growth rates (Domínguez and Edwards, 1997; Reinecke and Venter, 1987) or cause mortality (Gunadi et al., 2003; Hallatt et al., 1992). Therefore, while direct application of earthworms to horticultural substrates could benefit plant growth and crop yields (Chapter 2, this thesis), further study of how the benefits provided by earthworms may be altered by environmental stress is required.

Two glasshouse microcosm experiments were conducted to examine the effects of the compost earthworm *Dendrobaena veneta* (Rosa, 1826) on radish (*Raphanus sativus* L.) growth in soil-less peat-free compost during times of environmental stress. In the first experiment, the presence or absence of earthworms was manipulated across four short-term waterlogging treatments that varied in frequency and duration. In the second experiment, the effects of earthworms on plant growth were examined across seven drought treatment levels that differed in timing, duration, and number. It was predicted that, while earthworms would have a positive effect on plant growth, their own performance would be negatively affected by waterlogging or drought stress, which may reduce the benefits for plants provided by earthworms.

Methods

Two factorial experiments were performed in a period from May – August 2023. The first experiment manipulated the presence or absence of earthworms across five flood treatment levels and was conducted from 12 May to 14 June. The second experiment manipulated the presence or absence of earthworms across seven drought treatment levels and was conducted from 12 July to 12 August.

Study system

Dendrobaena veneta (Rosa, 1886) (Lumbricidae), a commercially available 'compost epigeic' earthworm species commonly used in vermicomposting (Domínguez, 2018), was chosen as a study species for both experiments. *Dendrobaena veneta* is similar in morphology to other compost species such as *Eisenia fetida* (Savigny, 1826) (Katsiamides and Stürzenbaum, 2021), although *D. veneta* is larger (Podolak et al., 2020), reaching masses of ~0.92 g compared to ~0.55 g for *E. fetida* (Domínguez and Edwards, 2011). *Dendrobaena veneta* also has a longer life cycle of 100 – 150 days compared to 45 – 51 days for *E. fetida* (Domínguez and Edwards, 2011). Therefore, *D. veneta* was chosen for this experiment to increase the likelihood of individuals surviving the experiment duration. Earthworms were purchased from Yorkshire Worms Ltd. (Holme-on-Spalding-Moor, Yorkshire, UK) and were kept in a mixture (by volume) of three parts moist compost to one part dried and rewetted cattle dung (Lowe and Butt, 2005) in 10 L white polypropylene buckets, sealed with 40 g m^{-2} nylon insect mesh, in a research glasshouse (described in detail below).

Radish (Brassicaceae: *Raphanus sativus* L. cv. "Cherry Belle", supplier: Mole Seeds (UK) Ltd. Essex, UK) was used as a representative horticultural crop species for both

experiments. Radish is a salad crop (Monaghan and Beacham, 2017) that has been grown in the UK since its introduction by the Romans (Field, 2000) and today is a popular crop for amateur gardeners. Radishes are typically grown for consumption of their swollen hypocotyl storage root (Monaghan and Beacham, 2017) but leaves are also edible and commonly consumed (Singh, 2021) and may have higher nutritional value than the roots (Gamba et al., 2021). Therefore, root growth, shoot growth, and the shoot : root ratio are of importance for growers. "Cherry Belle" is a red coloured spherical variety that was chosen due to its small size, rapid growth, and sensitivity to environmental conditions (Kostka-Rick and Manning, 1993).

Experimental setup and protocol

Both experiments were conducted in a research glasshouse at the John Krebs Field Station (Department of Biology), University of Oxford near the village of Wytham, Oxfordshire, UK. Air temperatures were recorded for part of the flood experiment and for the full duration of the drought experiment. For the flood experiment, air temperatures were recorded with data loggers (SensorBlue Thermometer Hygrometer, Oria, Hongkong Siuon Technology CO Ltd.). Mean air temperature was 17.21 °C (SD = 2.96, range = 10.1 – 25.9 °C, recording dates = 15 May – 27 May 2023, and 1 June – 14 June 2023). For the drought experiment, temperature readings were recorded with data loggers (RC-51H, Elitech (UK) Ltd., London, UK) both in air and in the same compost as used for the experiment (described below). Dataloggers placed into compost were pressed into compost in the same microcosms used as experimental units (described below) without plants or earthworms. Mean air temperature was 20.84 °C (SD = 3.15, range = 13.75 – 32.45 °C, recording dates = 11 July – 11 August 2023) while mean temperature within the compost was 20.33 °C (SD = 2.08, range = 14.65 – 25.6 °C, recording dates = 20 July – 11 August 2023).

Both experiments used microcosms constructed from 10 cm diameter, 20 cm height, polyvinyl chloride (PVC) cylinders (Chapter 2 – Fig. 2.1, manufacturer Manrose, Crawley, West Sussex, UK). The open base of each tube was covered with two layers of mesh that were secured with a stainless-steel vent clamp. The inner layer was thin 40 g m^{-2} nylon insect mesh, and the outer layer was 1 mm plastic mesh. Adhesive hook tape (2 cm width) was applied around the upper rim of the microcosms to prevent earthworm escape (Lubbers and Van Groenigen, 2013). Microcosms in both experiments first received a layer of washed gravel (10 cm diameter) to facilitate drainage before being partially filled with New Horizon Peat Free Multi Purpose Compost (Westland Horticulture Ltd, Dungannon, Tyrone, Northern Ireland, UK). This is a popular commercial peat-free compost in the UK (Bek et al., 2020), consisting primarily of wood fibre made from wood chips (Holmes and Bain, 2021), that was chosen as a representative soil-less peat-free compost. Microcosm lids were produced from transparent plastic 500 ml deli tubs with a single 1 mm hole for ventilation. These lids were temporarily added during seed germination and when earthworms were introduced to the microcosms. Lid application duration, gravel mass, and compost mass are detailed in the respective protocol sections for both experiments.

Flood experiment

The substrate was added to microcosms on 12 May with microcosms receiving ~150 g of gravel followed by ~650 g of compost. Six radish seeds were then planted on the same day in ~1 cm deep holes made with a garden dibber in a hexagonal arrangement (~2 cm spacing). An additional ~50 g of compost was added to each microcosm and gently pressed to ensure good contact between the seeds and the substrate. Each microcosm was

watered with 200 ml of tap water and lids were applied. Germination rates were high, and microcosms were thinned by hand to three seedlings on 23 May and to one on 26 May.

Earthworms were added to microcosms on 17 May, five days after seeds were planted. On 15 May, earthworms were removed from storage containers, washed with tap water, and placed into ventilated 3 L polypropylene containers lined with moist tissue paper. Earthworms were transferred into a 15 °C incubator in darkness for 48 hours for gut voidance (Fründ et al., 2010). On 17 May, earthworms were removed from the incubator, washed with tap water, and were haphazardly assigned to 40 groups of six adults which were randomly assigned to microcosms after some exchanges were made between earthworm groups to ensure all groups had similar starting mass. Starting earthworm population densities were 763.9 earthworms m^{-2} and average total earthworm mass was 9.52 g ±SE 0.04 g, with no significant differences in starting earthworm mass across flood treatment groups ($F_{4,35} = 0.35$, $p = 0.841$). As the composition of garden containers, including the number of earthworms, if added, can be freely controlled by growers, it was not necessary to mimic field densities of earthworms for the flood experiment or the drought experiment (described below). The earthworm abundances used in both experiments were therefore selected to provide a high population density of earthworms, which can increase effects on plant growth (Van Groenigen et al., 2015), while ensuring that the experimental logistics were manageable. Earthworms were placed on the compost surface of their microcosm and were seen to quickly begin burrowing into the substrate. Lids were then reapplied and left overnight to prevent earthworm escape. On 18 May, microcosms were inspected, and lids were removed after no earthworms were visible on the compost surface.

The experiment was fully factorial with eight replicates of each of the earthworm (zero or six *Dendrobaena veneta*) and flooding (Table 3.1) treatment combinations for a total of 80 microcosms. Microcosms were arranged in a 16 x 5 grid (positions re-randomised weekly) adjacent to the south-facing window of the experimental glasshouse with microcosms at the edge of the grid shielded from direct sun exposure by empty 3 L plant pots (described below). Supplemental lightning (460 – 730 nm, Attis-7 LED Growth Light, Plessey Semiconductors Ltd., Plymouth, Devon, UK) was provided from 07:00 – 19:00 (12 hours light : 12 hours dark) for the duration of the experiment but was temporarily switched off from 17 – 18 May when earthworms were added to microcosms.

Table 3.1: Summary of flooding treatment levels and dates of flood events.

Treatment	Flooding event		Total duration (hrs)	Flood dates
	Number	Duration (hrs)		
Control	0	0	0	Not flooded
2x4hr	2	4	8	30 May 2023, 5 June 2023
4x4hr	4	4	16	30 May 2023, 1 June 2023, 5 June 2023, 8 June 2023
2x24hr	2	24	48	30 May 2023, 5 June 2023
4x24hr	4	24	96	30 May 2023, 1 June 2023, 5 June 2023, 8 June 2023

The flooding treatment commenced on 30 May, 17 days after seeds were planted, with the treatment consisting of five levels (Table 3.1: *Control* / *2x4hr* / *4x4hr* / *2x24hr* / *4x24hr*) that differed in number of events (two or four) and the length of an individual flooding event (4 or 24 hours). Radishes are sensitive to waterlogging events as short as five hours (Silva et al., 2012) and epigeic earthworms can exhibit mortality after 28 hours of

submersion in non-aerated water (Kiss et al., 2021a). Additionally, pilot work using the same microcosm design described above showed that *D. veneta* would remain under the compost surface during 4-hour waterlogging events but would come to the compost surface and attempt to escape microcosms after 12 – 24 hours. Therefore, 4-hour events were selected as a 'mild' waterlogging stress that would affect the radish plants but have reduced effects on the earthworms, whereas 24-hour events were selected as a 'severe' waterlogging stress that would negatively impact both the earthworms and the radish plants and may alter the effects of the earthworms on the radish plants.

On 30 May, all microcosms were placed into individual 3 L 'long tom' plant pots with drainage holes and a square 14 x 14 cm upper rim and remained in pots until the end of the experiment. For each flooding event, the pots of flooded microcosms were lined with waterproof plastic bags and filled with tap water to flood the microcosms from the bottom up to a water level of ~2 cm above the compost surface (Fig. 3.1). Bags were not added to the pots of microcosms that were not flooded to allow water drainage during regular watering. At the end of flooding periods, each flooded microcosm was transferred into an empty, unlined, plant pot to allow water drainage. Water left in the flood chambers was poured through a 2 mm sieve and any live earthworms that had escaped were returned to their original microcosms. No dead earthworms were found in the flood chambers after any of the floods. Dead earthworms seen on the compost surface were not removed from microcosms. Outside of flooding events, microcosms were watered with 250 ml of tap water twice per week. Tap water from the same source was used for both the flooding events and regular watering.

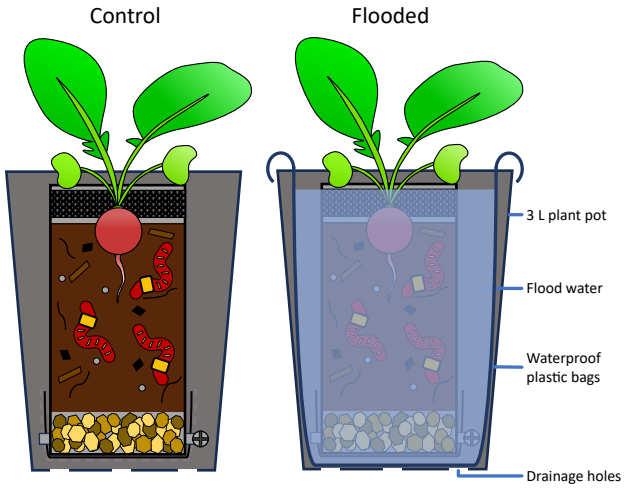


Fig. 3.1: Diagram of flooding method used in this experiment. All microcosms were placed inside 3 L plant pots with drainage holes. For flooded microcosms, a waterproof plastic bag was used to line the inside of the pot to allow flooding of the microcosm. A labelled diagram of experimental microcosms is shown in Fig 2.1 (Chapter 2) of this thesis.

Drought experiment

The compost was added to microcosms on 11 July with microcosms receiving ~350 g of gravel followed by ~600 g of compost. Six radish seeds were planted in each microcosm on 12 July in a hexagonal arrangement (~2 cm spacing) in 1 cm deep holes made with a garden dibber. An additional ~50 g of compost was added to each microcosm and was gently pressed by hand to ensure good contact between the seeds and the substrate. Microcosms were then watered with 300 ml of tap water and lids (described above) were applied before being removed on 17 July. Germination rates were high, and microcosms were thinned by hand to three seedlings on 17 July, two seedlings on 19 July, and to one on 21 July.

Earthworms were added to microcosms on 19 July (seven days after seeds were planted) after being removed from storage containers on 17 July and having followed the same gut voidance process used with the flood experiment (described above). Earthworms were

haphazardly assigned to groups of six individuals which were randomly allocated to earthworm-containing microcosms. Starting earthworm population densities were 763.9 earthworms m^{-2} and average total start mass was 7.62 g ±SE 0.02 g of earthworms with no significant differences in earthworm starting mass across drought treatment ($F_{6,35} = 0.28$, $p = 0.943$). Earthworms were placed on the compost surface and lids (the same as described previously) were reapplied to prevent earthworm escape. On 20 July, microcosms were inspected, and lids were removed as no earthworms were visible.

The experiment was fully factorial with six replications of each of the earthworm (zero or six *D. veneta*) and drought (Table 3.2: *Control* / *1a* / *1b* / *1c* / *2a* / *2b* / *2c*) treatment combinations for a total of 84 microcosms. The microcosms were arranged in a 14 x 6 grid (with positions re-randomised weekly) adjacent to the south-facing window of the experimental glasshouse with microcosms at the edge of the grid shielded from direct sun exposure by empty PVC tubes. Supplemental lighting was provided daily from 06:00 – 20:00 during the experiment (16 hours light : 8 hours dark, using the same lights described above), but lights were turned off from 19 July – 20 July when earthworms were added to microcosms. Microcosms were watered with 300 ml of tap water on 12 July and 200 ml of tap water on 17 July and 19 July. From 21 July onwards, microcosms were watered with 250 ml of tap water three times per week (Mondays, Wednesdays, and Fridays) outside of drought periods (described below).

Drought treatments commenced on 21 July (nine days after seeds were planted) and took place over a three-week period until 12 August. Radish plants are sensitive to the timing of short drought stress events (dos Santos Bregonci et al., 2008) so seven drought treatment levels were applied, consisting of six droughts varying in timing, number, and duration in addition to a control treatment level that was not exposed to drought. Droughts were

performed across the three-week treatment period (Table 3.2) during which regular watering was halted. Treatments *1a*, *1b*, and *1c* were each exposed to a one-week drought in weeks 1, 2, or 3 of the drought period, respectively. Treatments *2a* and *2b* were both exposed to a two-week drought over weeks 1 and 2 or 2 and 3 of the drought period, respectively. Finally, treatment *2c* was exposed to two one-week droughts in weeks 1 and 3 of the drought period.

Table 3.2: Summary of drought treatment levels and dates of drought events.

Treatment	Drought duration	Drought week			Drought dates
		1	2	3	
Control	No drought				No drought
1a	1 week	X			21 July 2023 – 28 July 2023
1b	1 week		X		28 July 2023 – 4 August 2023
1c	1 week			X	4 August 2023 – 11 August 2023
2a	2 weeks	X	X		21 July 2023 – 4 August 2023
2b	2 weeks		X	X	28 July 2023 – 11 August 2023
2c	2x 1 week[a]	X		X	21 July 2023 – 28 July 2023 + 4 August 2023 – 11 August 2023

[a]Excluded from drought duration analyses.

Data collection

For both experiments, radish plants and earthworms were collected in rounds. Each round contained one replicate of each earthworm + drought or flood treatment combination in a random order. Radish plants were harvested by gently pulling plants from the compost, washing them with tap water, and placing them in sealed plastic bags, wrapped in moist tissue paper, to prevent wilting. Plants were separated into leaves, cotyledons, hypocotyls, and taproots and all parts were weighed separately. Plant parts were then dried at 70 °C until constant mass before dry masses were recorded. Shoot mass was calculated by

summing leaf and cotyledon mass and root mass was calculated by summing hypocotyl and taproot mass.

Earthworms were retrieved by emptying the compost out of microcosms and hand sorting. Surviving earthworms were cleaned with tap water and placed into ventilated ~350 ml plastic containers lined with moist tissue paper and transferred to a 15 °C incubator for 48 hours in darkness for gut voidance (Fründ et al., 2010) before end masses were recorded.

For the flooding experiment, all radish plants were harvested on 13 June, 32 days after seeds were planted. Dry plant masses were recorded on 19 June. All earthworms were retrieved on 14 June and end masses were recorded on 16 June. For the drought experiment, all plants were harvested on 11 August, 30 days after seeds were planted and dry masses were recorded on 15 August. Earthworms were retrieved on 12 August and end masses were recorded on 14 August.

Additionally, for the drought experiment, microcosm masses were recorded prior to each watering event. The empty mass of each microcosm and the starting mass of earthworms (for earthworm-containing microcosms) was subtracted from each measure to estimate compost + plant mass at different time points to provide a proxy for compost water content.

Data analysis

All data analysis performed using R version 4.3.1 (R Core Team, 2023) and figures were produced with *ggplot2* (Wickham, 2016). For both experiments, radish response variables consisted of fresh and dry shoot mass (leaf mass + cotyledon mass), root mass (hypocotyl + taproot mass), and shoot : root ratios, and were analysed with Analysis of Variance

(ANOVA). For the flooding experiment, earthworms, the flooding treatment, and their interaction were included as explanatory factors. For the drought experiment, earthworms, the drought treatment, and their interaction were included as explanatory factors. Additionally, analysis was performed for the drought experiment with drought treatments grouped by length: treatments *1a*, *1b*, and *1c* were grouped as '1 week', treatments *2a* and *2b* were grouped as '2 weeks', and treatment *2c* was excluded as it consisted of two separate drought events. Models analysing the effect of drought duration used Type II ANOVA with the *car* package (Fox and Weisberg, 2019) to account for uneven group sizes. Diagnostic plots were inspected for each radish model and log-transformation was performed if necessary to better fit the assumptions of ANOVA. For the flooding experiment, all response variables were log-transformed. For the drought experiment, fresh and dry root mass and shoot : root ratios were log-transformed for analyses with both drought treatments and drought duration.

For the flooding experiment, earthworm survival was analysed with a binomial generalised linear model (GLM) with flooding treatment as an explanatory factor. Survival was 100% for two treatment groups (see *Results*) so a reduced-bias binomial GLM was performed with package *brglm2* (Kosmidis, 2023; Kosmidis et al., 2020; Kosmidis and Firth, 2021) to avoid complete separation. Additionally, earthworm mean and total mass change were analysed with Kruskal-Wallis tests, with flooding treatment as an explanatory factor as these response variables did not meet the assumptions of ANOVA and log-transformation was not possible due to negative values. *Post hoc* Dunn's tests were used when flooding had a statistically significant effect on these variables. Earthworm survival was very high in the drought experiment (see *Results*) so only mean earthworm mass change was analysed with ANOVA with separate analyses for drought treatments and droughts grouped by duration.

In the drought experiment, compost + radish plant mass was analysed with repeated measures ANOVA with the *nlme* package (Pinheiro et al., 2023). Earthworms, drought treatment, day, and all possible two-way and three-way interactions were included as explanatory factors with microcosm ID included as a random factor. Effects of the droughts on mass were analysed by comparing treatment group means to the control treatment (no drought + no earthworms) using the *emmeans* package (Lenth, 2023). Effects of earthworms on mass were compared for each treatment at each time point.

For all parametric models, *post hoc* group comparisons were performed with the *emmeans* package (Lenth, 2023) if explanatory variables, or their interactions, had statistically significant effects on response variables. When response variables were log-transformed, group contrasts and 95% confidence intervals were back-transformed to the response scale for interpretation and reporting.

Results

Flood experiment

Earthworm performance

On average, 4.93 *D. veneta* were recovered from microcosms for an overall survival rate of 82.3%. Two earthworm-containing microcosms (one *4x4hr* and one control) contained seven earthworms at the end of the experiment. As all seven individuals in these microcosms were adults, it was assumed that the additional earthworms had entered from other earthworm-containing microcosms, rather than resulting from reproduction during the experiment. For survival analysis, these microcosms were treated as containing six earthworms (100% survival) and for total mass change analysis the mean end earthworm

mass was multiplied by six to estimate total end mass for six earthworms. Additionally, five earthworms were found in control microcosms (to which no earthworms were added) at the end of the experiment: two *4x4hr* + no earthworms microcosms each contained two earthworms and one *4x24hr* + no earthworms microcosm contained one earthworm. Two *4x24hr* treatment microcosms contained no earthworms so these replicates were excluded from the mean mass change analyses. The flooding treatment significantly affected earthworm survival (Fig. 3.2), total earthworm mass change (Fig. 3.3a), and mean earthworm mass change (Fig. 3.3b, Table 3.3). Earthworm survival was reduced, and total mass declined more, in *2x24hr* and *4x24hr* floods compared to controls, *2x4hr*, and *4x4hr* floods (Fig. 3.2, 3.3a, Table S3.1, S3.2). Additionally, total earthworm mass declined when averaged across treatments ($\mu = -1.90$ g, $CI_{95\%} = [-2.83, -0.97]$, $p < 0.001$). There was no overall trend for mean earthworm mass to increase or decrease across all treatments ($\mu = -27.07$ mg, $CI_{95\%} = [-61.30, 7.15]$, $p = 0.11$). However, individual earthworms lost more mass in the *2x24hr* treatment compared to controls (Fig. 3.3b, Table S3.3).

Table 3.3: Summary of analysis results showing effects of the flooding treatment on earthworm response variables.

Response variable	Test method	Flooding treatment		
		Df	X^2	P
Earthworm survival	Binomial GLM	4	86.05	**<0.001**
Total earthworm mass change	Kruskal-Wallis	4	27.89	**<0.001**
Mean earthworm mass change	Kruskal-Wallis	4	11.15	**0.025**

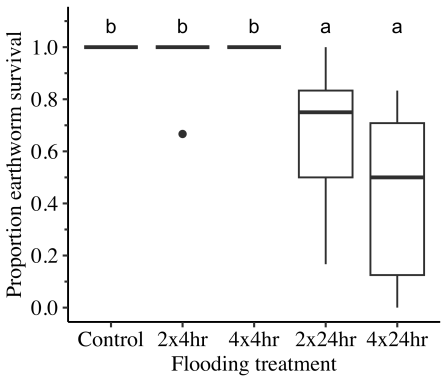


Fig. 3.2: Box and whisker plot showing proportion of earthworms surviving across flood treatments. Groups that do not share a letter have significantly different survival rates ($p < 0.05$). Controls and the *4x4hr* treatment are each represented by a single horizontal line as earthworm survival was 100% for these treatments. For the *2x4hr* treatment, survival was 100% except for one single replicate, indicated by the single outlier data point (>1.5x interquartile range below 25th percentile). For the other two treatments, the central horizontal line in the box shows the group median, the upper and lower limits of the box show the third and first quartile, respectively, and the vertical lines extending from each box show the range of the data for each treatment group. Sample sizes were 8 for each treatment group.

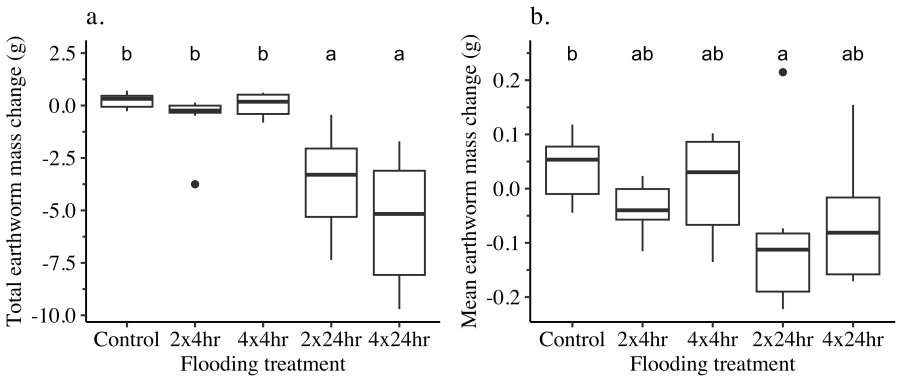


Fig. 3.3: Box and whisker plots showing *a.* total earthworm mass change and *b.* mean earthworm mass change across flooding treatments. Boxes that do not share letters above them are significantly different from one another on an individual graph ($p < 0.05$, *post hoc* Dunn tests). For each treatment group, the central horizontal line in the box shows the group median, the upper and lower limits of the box show the third and first quartile, respectively, and the vertical lines extending from each box show the range of the data for each treatment group. Points below box medians show lower outliers (>1.5x interquartile range below 25th percentile) while points above medians show upper outliers (>1.5x interquartile range above 75th percentile). Sample sizes were 8 for each treatment group, except for the 4x24hr flood treatment in *b.* where two data points have been excluded as they contained zero surviving earthworms

(these data points were also excluded from the Dunn test comparing pairwise group differences).

Radish performance

There was no statistically significant interaction between earthworms and flooding for any of the radish response variables (Table 3.4). *Dendrobaena veneta* significantly increased fresh shoot mass (Fig. 3.4a, ratio =1.47, $CI_{95\%}$ = [1.28, 1.69], $p < 0.001$), dry shoot mass (Fig. 3.4b, ratio = 1.33, $CI_{95\%}$ = [1.14, 1.55], $p < 0.001$), fresh root mass (Fig. 3.4c, ratio = 1.26, $CI_{95\%}$ = [1.1, 1.44], $p < 0.001$), dry root mass (Fig. 3.4d, ratio = 1.13, $CI_{95\%}$ = [1.01, 1.26], $p = 0.04$), fresh shoot : root ratio (Fig. 3.4e, ratio = 1.17, $CI_{95\%}$ = [1.04, 1.31], $p = 0.01$), and dry shoot : root ratio (Fig 3.4f, ratio = 1.18, $CI_{95\%}$ = [1.05, 1.33], $p = 0.008$). The flooding treatment had a significant effect on all radish response variables except dry shoot mass (Table 3.4, Fig. 3.4b). Fresh shoot mass was reduced in the *4x24hr* treatment compared to controls (Fig. 3.4a, ratio = 0.69, $CI_{95\%}$ = [0.51, 0.94], $p = 0.011$). Longer flooding treatments also reduced fresh and dry root mass compared to controls (Fig. 3.4c, 3.4d, Table S3.4, S3.5) and increased fresh and dry shoot : root ratios compared to controls and shorter flood treatments (Fig. 3.4e, 3.4f, Table S3.6, S3.7).

Table 3.4: Summary of ANOVA results showing effects of earthworms, flooding, and their interaction, on radish plant response variables.

Response variable	Earthworms			Flooding			Earthworms x Flooding		
	Df	F	P	Df	F	P	Df	F	P
Fresh shoot mass[a]	1,70	30.62	**<0.001**	4,70	3.37	**0.014**	4,70	0.31	0.869
Dry shoot mass[a]	1,70	13.82	**<0.001**	4,70	0.86	0.491	4,70	0.26	0.903
Fresh root mass[a]	1,70	11.93	**0.001**	4,70	18.74	**<0.001**	4,70	0.82	0.514
Dry root mass[a]	1,70	4.37	**0.040**	4,70	18.26	**<0.001**	4,70	0.96	0.432
Fresh S:R ratio[a]	1,70	7.05	**0.010**	4,70	10.15	**<0.001**	4,70	0.62	0.649
Dry S:R ratio[a]	1,70	7.55	**0.008**	4,70	12.95	**<0.001**	4,70	0.59	0.671

[a]Analysis performed on the log scale

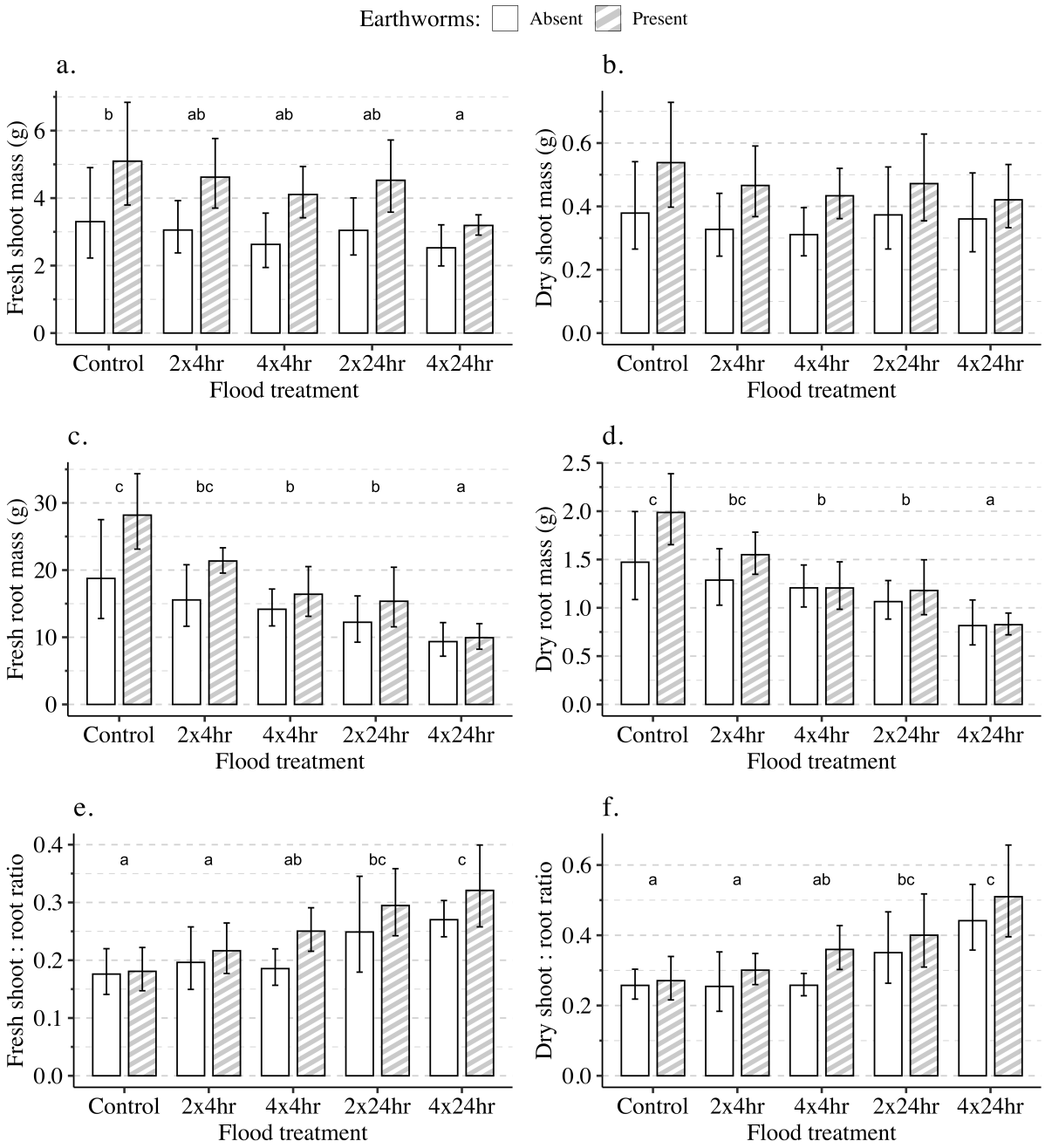


Fig. 3.4: Bar plots showing mean *a.* fresh shoot mass, *b.* dry shoot mass, *c.* fresh root mass, *d.* dry root mass, *e.* fresh shoot : root ratio, and *f.* dry shoot : root ratio across earthworm and flood treatment groups. Error bars show 95% confidence intervals. All means and confidence interval limits have been back-transformed from the log scale. Flooding treatment groups that do not share letters are significantly different from one another on an individual graph ($p < 0.05$, pairwise comparisons performed on log scale). Group sizes for each treatment group are 8 for all graphs.

Earthworm performance

Survival of *D. veneta* was extremely high at the end of the experiment with 251 of the 252 earthworms recovered alive for a survival rate of 99.6%. Therefore, analysis of survival was not performed, and only mean earthworm mass change was analysed. Earthworms lost mass overall in the experiment, with mean earthworm mass decreasing by 38.08 mg ($CI_{95\%}$ = [−55.11, −21.05], $p < 0.001$). However, there were no significant differences in mean mass change across drought treatment groups (Fig. 3.5a, $F_{6,35} = 1.66$, $p = 0.16$). When drought treatments were grouped by length (with treatment *2c* excluded, see *Methods*) the effect of the drought treatment approached statistical significance at the conventional critical value of alpha = 0.05 ($F_{2,33} = 3.25$, $p = 0.052$) with mean mass decreasing significantly more in 1-week drought treatments than in controls (Fig. 3.5b, difference = −61.00 mg, $CI_{95\%}$ = [−119.90, −2.18], $p = 0.041$).

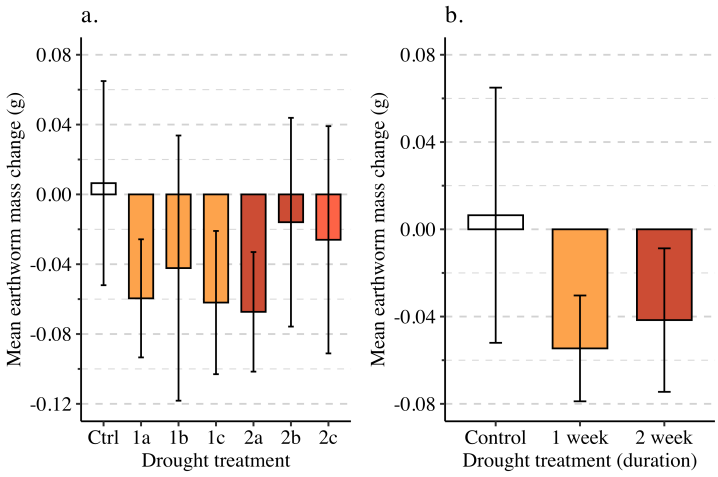


Fig. 3.5: Bar plots showing mean earthworm mass change (g) with 95% confidence intervals plotted against *a.* drought treatment and *b.* drought duration (total weeks). Drought treatment *2c* was excluded when analysing drought time. Bar fill colour indicates length of drought treatment. Group sizes in *b.* differ across groups. Control $n = 6$, 1 week $n = 18$, 2 week $n = 12$.

Radish performance

Drought treatments

There was no significant interaction between *D. veneta* and drought for any of the radish plant response variables (Table 3.5). Earthworms significantly increased fresh shoot mass (Fig. 3.6a, difference = 1.6 g, $CI_{95\%}$ = [1.36, 1.84], $p < 0.001$), dry shoot mass (Fig. 3.6b, difference = 0.12 g, $CI_{95\%}$ = [0.08, 0.16], $p < 0.001$), fresh root mass (Fig. 3.6c, ratio = 2.15, $CI_{95\%}$ = [1.89, 2.45], $p < 0.001$), and dry root mass (Fig. 3.6d, ratio = 1.51, $CI_{95\%}$ = [1.35, 1.69], $p < 0.001$). Drought only had a significant effect on fresh shoot mass (Table 3.5) which was lower in treatment *2b* than in *controls* or in droughts *1a*, *1c*, and *2c* (Fig. 3.6a, $p < 0.05$). There was no significant main effect of either earthworms or drought on fresh or dry shoot : root ratio (Table 3.5, Fig. 3.6e, 3.6f).

Table 3.5: Summary of ANOVA results showing effects of earthworms, the drought treatment, and their interaction, on radish plant response variables.

Response variable	Earthworms			Drought			Earthworms x Drought		
	Df	F	P	Df	F	P	Df	F	P
Fresh shoot mass	1,70	175.26	**<0.001**	6,70	3.73	**0.003**	6,70	1.11	0.368
Dry shoot mass	1,70	34.31	**<0.001**	6,70	1.06	0.396	6,70	1.11	0.364
Fresh root mass[a]	1,70	136.80	**<0.001**	6,70	2.14	0.059	6,70	1.40	0.227
Dry root mass[a]	1,70	54.30	**<0.001**	6,70	1.57	0.170	6,70	1.44	0.213
Fresh S:R ratio[a]	1,70	3.00	0.088	6,70	0.61	0.720	6,70	0.98	0.444
Dry S:R ratio[a]	1,70	1.58	0.213	6,70	1.07	0.386	6,70	0.81	0.563

[a]Analysis performed on the log scale

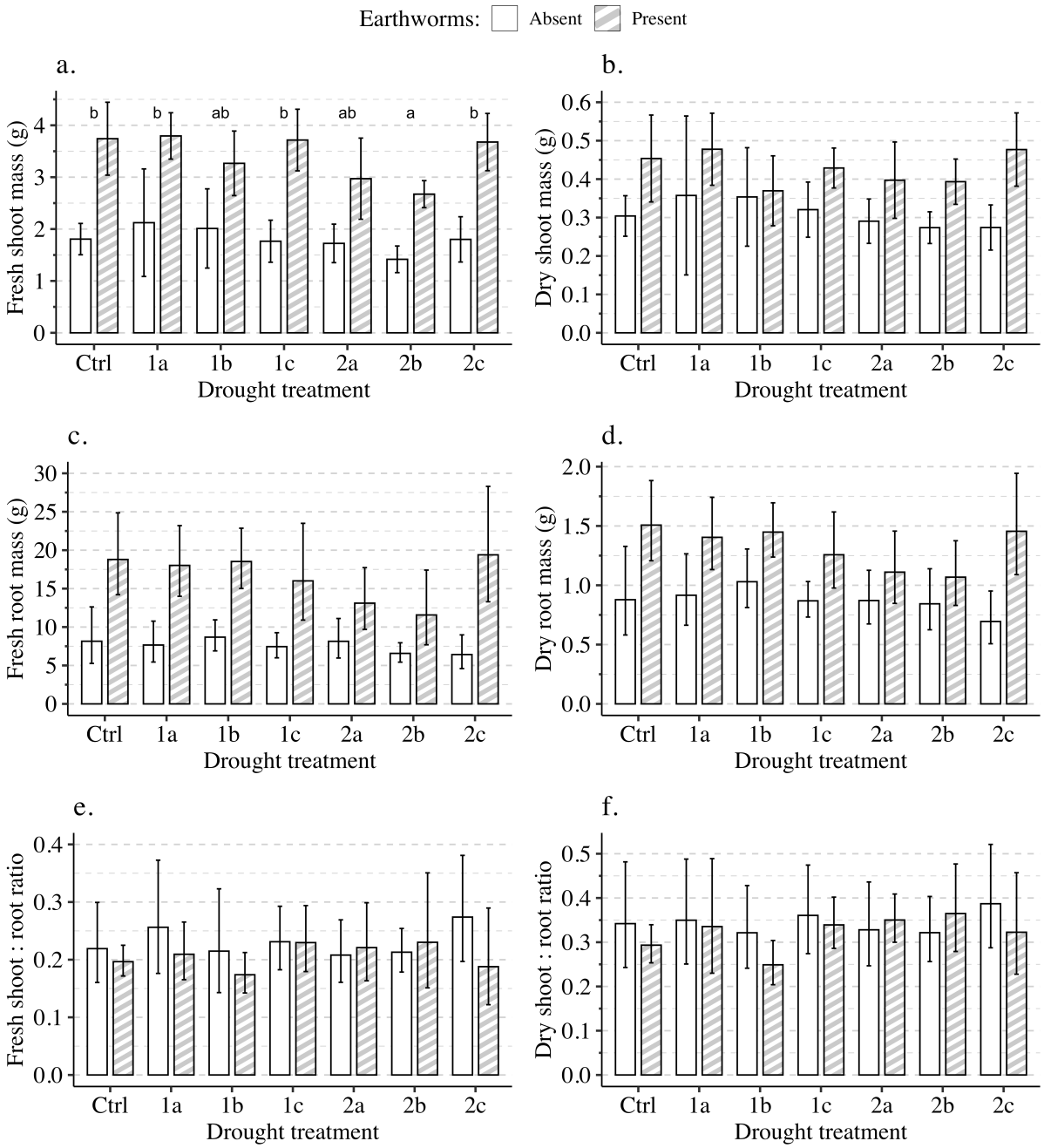


Fig. 3.6: Bar plots showing group means and 95% confidence intervals for *a.* fresh shoot mass, *b.* dry shoot mass, *c.* fresh root mass, *d.* dry root mass, *e.* fresh shoot : root ratio, and *f.* dry shoot : root ratio across earthworm and drought treatment groups. Means and confidence interval limits in *c.*, *d.*, *e.*, and *f.* have been back-transformed from the log scale. Drought treatments in *a.* that do not share letters are significantly different from one another ($p < 0.05$). Group sizes for each treatment group are 6 for all graphs.

Total drought duration

Analysis was also conducted with drought treatments combined by duration, where treatment *2c* was excluded (see *Methods*). There was no significant interaction between *D. veneta* and drought duration for any of the radish response variables (Table 3.6). Earthworms significantly increased fresh shoot mass (Fig. 3.7a, difference = 1.60 g, $CI_{95\%}$ = [1.31, 1.90], $p < 0.001$), dry shoot mass (Fig. 3.7b, difference = 0.11 g, $CI_{95\%}$ = [0.07, 0.16], $p < 0.001$), fresh root mass (Fig. 3.7c, ratio = 2.05, $CI_{95\%}$ = [1.76, 2.38], $p < 0.001$), and dry root mass (Fig. 3.7d, ratio = 1.47, $CI_{95\%}$ = [1.30, 1.67], $p < 0.001$). Drought duration had a significant effect on shoot mass (Fig. 3.7a), fresh root mass (Fig. 3.7c), and dry root mass (Fig. 3.7d, Table 3.6). Fresh shoot mass was reduced significantly in two-week droughts compared to one-week droughts (difference = 0.58 g, $CI_{95\%}$ = [0.23, 0.94], $p = 0.001$) and controls (difference = 0.58 g, $CI_{95\%}$ = [0.10, 1.06], $p = 0.001$). Fresh root mass was also significantly lower in two-week droughts than in both one-week droughts (ratio = 0.81, $CI_{95\%}$ = [0.67, 0.97], $p = 0.017$) and controls (ratio = 0.77, $CI_{95\%}$ = [0.60, 0.98], $p = 0.030$) while dry root mass was only reduced significantly in two-week droughts compared to one-week droughts (ratio = 0.85, $CI_{95\%}$ = [0.73, 1.00], $p = 0.046$). Neither drought duration nor earthworms affected shoot : root ratios significantly (Table 3.6, Fig. 3.7e, 3.7f).

Table 3.6: Summary of ANOVA results (Type II) showing effects of earthworms, drought duration, and their interaction, on radish plant response variables.

Response variable	Earthworms			Drought duration			Earthworms x Drought duration		
	Df	F	P	Df	F	P	Df	F	P
Fresh shoot mass	1,66	135.61	**<0.001**	2,66	8.47	**0.001**	2,66	1.61	0.207
Dry shoot mass	1,66	22.02	**<0.001**	2,66	1.83	0.168	2,66	0.64	0.528
Fresh root mass[a]	1,66	109.05	**<0.001**	2,66	5.12	**0.009**	2,66	1.95	0.151
Dry root mass[a]	1,66	38.67	**<0.001**	2,66	3.50	**0.036**	2,66	1.56	0.218
Fresh S:R ratio[a]	1,66	1.03	0.314	2,66	0.16	0.856	2,66	1.11	0.336
Dry S:R ratio[a]	1,66	0.80	0.376	2,66	0.43	0.650	2,66	1.57	0.215

[a]Analysis performed on the log scale.

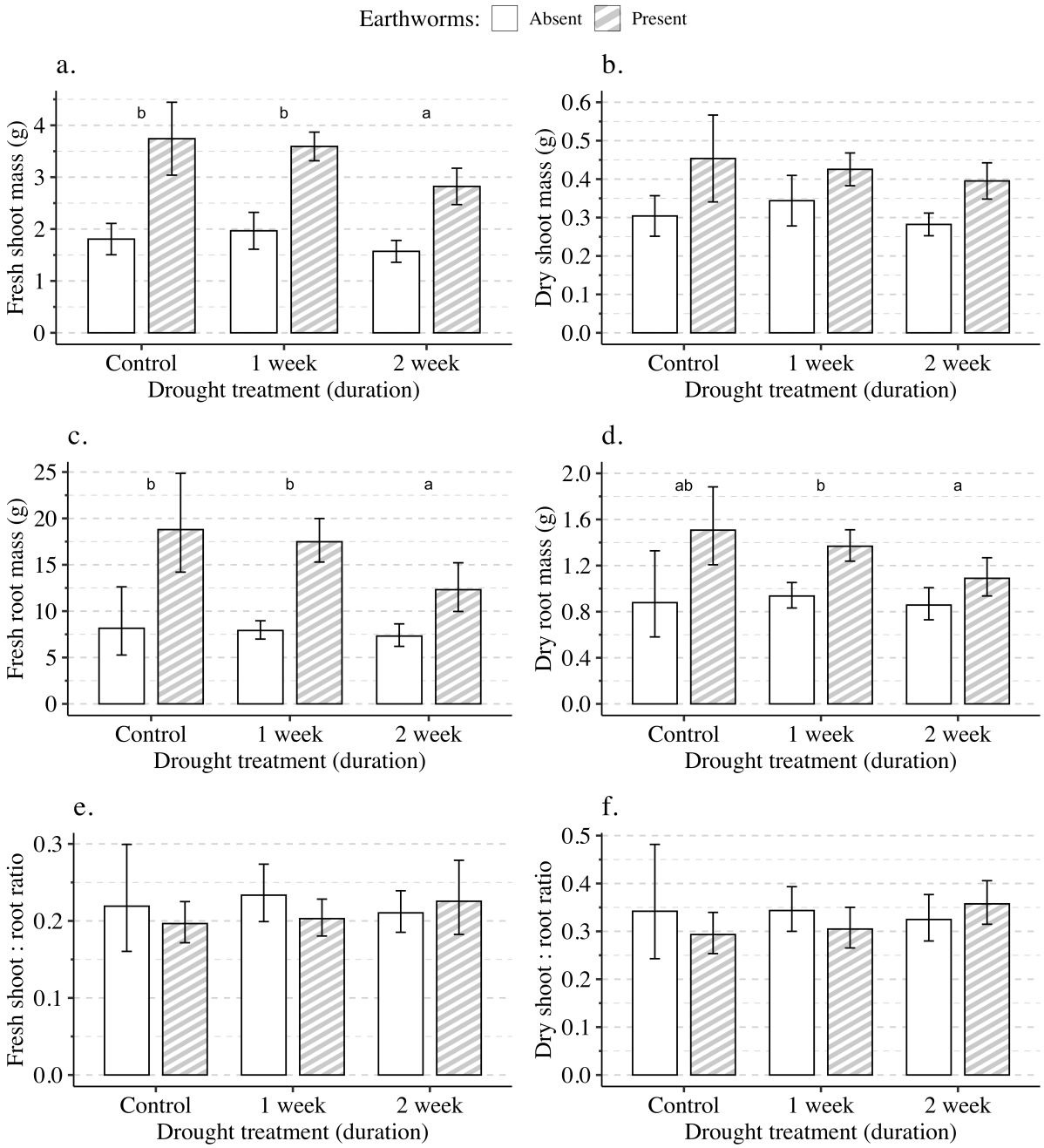


Fig. 3.7: Bar plots showing groups means and 95% confidence intervals for *a.* fresh shoot mass, *b.* dry shoot mass, *c.* fresh root mass, *d.* dry root mass, *e.* fresh shoot : root ratio, and *f.* dry shoot : root over earthworm and drought time treatment groups. Drought time groups were created by pooling data from different drought treatments with the same drought length. Drought treatment *2c* was excluded from these analyses, see *Methods*. Means and confidence interval limits in *c.*, *d.*, *e.*, and *f.* have been back-transformed from the log scale. Group sizes differ across drought treatments for control ($n = 6$), 1 week ($n = 18$), and 2 week ($n = 12$) across both earthworm treatment levels. Drought treatments that do not share letters are significantly different from one another within an individual graph ($p < 0.05$, pairwise comparisons performed on log scale for fresh and dry root mass and response scale for fresh shoot mass).

Compost mass

There was a statistically significant three-way interaction between weighing day, earthworms, and drought treatment on compost + plant mass (Table 3.7). During droughts, compost mass was significantly reduced compared to *controls* before recovering to *control* levels when watering was resumed (Fig. 3.8) but earthworms partially alleviated the effects of drought in treatments *2a* and *1b* (Fig. 3.8, Table S3.8). Earthworms increased compost + plant mass for treatment *2a* (day 20) and *1b* (days 22 and 24) during droughts with compost drying more slowly in the presence of *D. veneta* (Fig. 3.8). Additionally, earthworms increased compost mass for treatment *2a* after watering was resumed (day 27, 29, and 31) indicating that compost rehydrated more rapidly in the presence of earthworms (Fig. 3.8). By the final weighing event, treatment *2a* mass had only recovered to *control* levels in the presence of earthworms and was still significantly reduced compared to *controls* without earthworms.

Table 3.7: Summary of repeated measures ANOVA results (Type II) for effects of weighing day, earthworms, drought treatment, and their interactions on Compost + Plant mass.

Variable	Compost + Plant mass		
	Df	X^2	P
Day number	13	15,981.87	**<0.001**
Earthworms	1	9.93	**0.002**
Drought	6	365.94	**<0.001**
Day number x Earthworms	13	128.94	**<0.001**
Day number x Drought	78	19,618.82	**<0.001**
Earthworms x Drought	6	13.57	**0.035**
Day number x Earthworms x Drought	78	338.23	**<0.001**

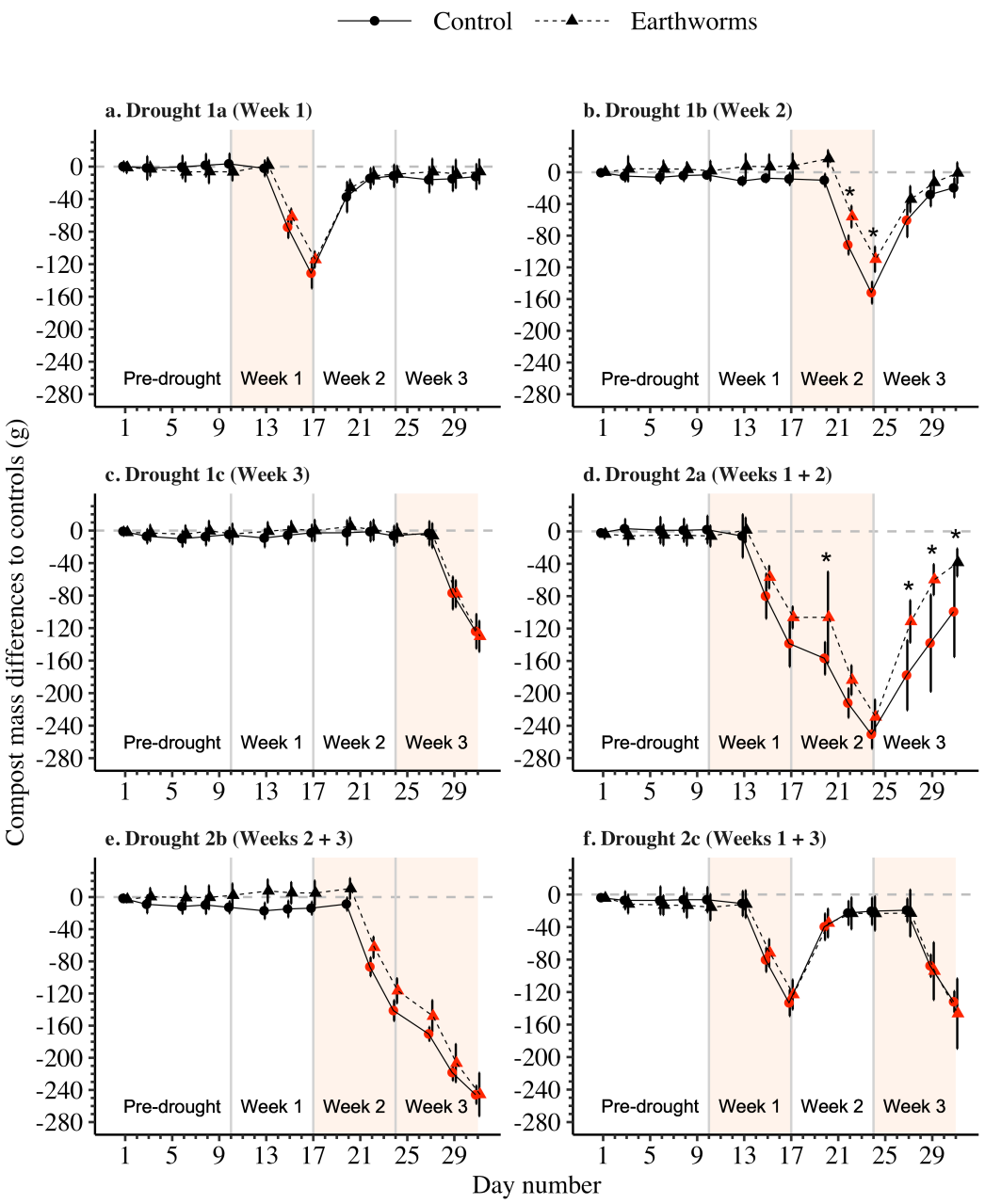


Fig. 3.8: Line plot showing compost + plant mass relative to controls (no drought + no earthworms, indicated by the horizonal dashed grey line) at different time points through the experiment. Text labels and vertical grey lines within plots indicate pre-drought (day 1 – 10) and drought time periods for drought week 1 (day 10 – 17), week 2 (day 17 – 24), and week 3 (day 24 – 31). Orange shaded regions on individual plots indicate when treatments did not receive water. Outside of drought periods, all microcosms were watered after being weighed at each time point (see *Methods*). Points indicate group means for microcosms with (triangles) and without (circles) earthworms and vertical black lines indicate 95% confidence intervals. Red points indicate group means that are significantly lower ($p < 0.05$) than controls at a time point.

Asterisks indicate that compost + plant mass was significantly higher ($p < 0.05$) for microcosms with earthworms than for those without for a drought treatment and time point.

Discussion

In the flood experiment, radish growth was negatively affected by longer flood durations (24 hours) while in the drought experiment, growth was reduced in two-week duration droughts but not one-week duration droughts, suggesting that radish plants may be tolerant to short stress events. Survival of *D. veneta* was high in both experiments, although some mortality or escape occurred in the two 24-hour duration treatments of the flood experiment. Earthworms increased radish plant growth in both experiments and provided benefits to plants independently of the stress treatment in both experiments.

Effects of waterlogging and drought stress on earthworms

In the flooding experiment, earthworm survival was extremely high in the control (100%), *2x4hr* (95.8%), and *4x4hr* (100%) flood treatments, but was significantly reduced in the *2x24hr* and *4x24hr* flooding treatments to rates of 66.7% and 43.8%, respectively (Fig. 3.2). Earthworms use a combination of vertical and horizontal migration, and physiological, reproductive, and phenological responses to tolerate flooding (Plum, 2005). Therefore, while some dead earthworms were seen after flooding events, earthworm escape, rather than mortality, likely also contributed to the reduced survival rates in the 24-hour flood treatments. Many live *D. veneta* were found outside microcosms in the flood chambers after 24-hour flooding events. In non-aerated water, the epigeic earthworm *Lumbricus castaneus* (Savigny, 1826) exhibits 66% mortality after 28 hours, although 100% mortality can take up to 168 hours (Kiss et al., 2021a). Another epigeic species, *Lumbricus rubellus* Hoffmeister, 1843, can survive an average of 78 days (range: 3 – 195

days) in aerated tap water (Roots, 1956). Therefore, in this flood experiment, *D. veneta* may have been able to survive for extended periods of time in the open-topped microcosms during flooding. During 24-hour flooding treatments, individual *D. veneta* were visible floating in water (Fig. S3.1) or attempting to climb over the adhesive hook tape. Although observed individuals would retract into the water after only partially crossing the adhesive hook tape, five earthworms were found in control (no earthworm) microcosms and two earthworm-containing microcosms had seven earthworms at the end of the experiment, suggesting that some individuals were able to escape both the microcosms and the surrounding flood chamber during flood events.

Dendrobaena veneta is similar in both size, morphology, and ecology to *L. rubellus* (Domínguez and Edwards, 2011; Sherlock, 2016; Sherlock et al., 2018), another epigeic species that can be completely prevented from escaping open topped microcosms using the same adhesive hook tape used in the flood experiment (Lubbers and Van Groenigen, 2013). This suggests that this method should have prevented escape of *D. veneta* from flooded microcosms. However, as a 'compost epigeic' earthworm species, *D. veneta* is considered to have high abilities to colonise organic matter (*i.e.* high mobility) and high endurance to handling (*i.e.* tolerance to physical disturbance) (Domínguez and Edwards, 2011), which may have facilitated escape from microcosms to avoid flooding. While some dead individuals were seen in flooded microcosms, a much larger number were found to have escaped and were returned alive to their microcosms. Earthworm mortality may have been caused by injury against the relatively sharp upper rim of the PVC microcosms used, rather than being directly caused by the flooding. Although earthworm survival in the flooding chambers was high, and earthworms could be safely re-introduced to microcosms, this high ability to escape containers, even when preventative measures were applied, may indicate reduced potential applications of 'compost' earthworms in container gardens.

Individual earthworms also lost more mass in *2x24hr* floods compared to controls which suggests that the longer floods reduced performance of the earthworms (Fig. 3.3). Although mass loss between *4x24hr* and controls was not significantly different, this likely occurred as data points with 100% earthworm mortality were excluded for mean mass analysis. As earthworms were often seen floating in flood water at the compost surface during floods, rather than burrowing, these prolonged flooding events may have reduced feeding opportunity for the earthworms, resulting in body mass reduction.

In the drought experiment *D. veneta* survival was also extremely high, and approached 100%, across all treatments with only one individual out of 252 not recovered at the end of the experiment. However, there was a tendency for earthworms to lose body mass. While this did not differ across individual drought treatments, earthworms lost significantly more mass in the two-week droughts than in controls (Fig. 3.5), suggesting that the decreased compost moisture negatively impacted earthworm performance. Optimum substrate moisture for *D. veneta* is 75% (tolerance: 65 – 85%) which is lower than *Eisenia andrei* (Bouché, 1972) and *E. fetida*, other compost earthworm species with 80 – 85% (tolerance: 70 – 90%) optimum moisture levels (Domínguez and Edwards, 2011). Therefore, the high performance of *D. veneta* across the drought treatments in our study might reflect its relatively lower moisture requirements and not be representative of other compost earthworm species. Compost earthworms move vertically in substrates to remain in their optimum moisture levels in the presence of a moisture gradient (Reinecke and Venter, 1987). While the upper levels of compost rapidly appeared to dry during droughts, movement into deeper, moister, areas may have facilitated survival. The drought periods in this experiment were relatively short (maximum of two weeks) but still caused large reductions in compost water content (Fig. 3.8, determined by compost + plant mass as a proxy). Epigeic earthworms are unable to undergo aestivation, a period of inactivity that

reduces water loss (Lee, 1985; Plum and Filser, 2005), so have little tolerance to drought, and populations can strongly decline during dry periods (Eggleton et al., 2009; Monroy et al., 2006). Therefore, although survival and performance were high in the present experiment, droughts of greater severity might cause mortality in compost earthworms in gardens containers and reduce the benefits that they could provide plants.

Beneficial effects of earthworms on radish growth

Dendrobaena veneta had a significant positive effect on radish plant growth in both the flooding experiment (Fig. 3.6), where both shoot mass (fresh = +47%, dry = +33%) and root mass (fresh = +25%, dry = +13%) were increased. Earthworms had a greater positive effect in the drought experiment for both shoot mass (fresh = +115%, dry = +38%) and root mass (fresh = +88%, dry = +51%). Meta-analyses show that earthworms cause an average increase of 23% for shoot biomass and 20% for root biomass, although effects are increased by higher earthworm densities (Van Groenigen et al., 2015), while vermicompost may increase shoot and root biomass by 78% and 57% respectively (Blouin et al., 2019). A large number of studies have focussed on the effects of earthworms on growth of grass species, particularly arable crops (Van Groenigen et al., 2015). The large yield improvements demonstrated in both the flood and drought experiments suggest that earthworms can also provide great benefits to yields of root crops such as radish. However, in contrast to the results reported here, the compost earthworm *E. fetida* can reduce yields of radish (cv. "Granát") grown in a mixture of vermicompost and soil (Kováčik et al., 2021, 2018). Both *R. sativus* cv. "Granát" (Osiva Moravia s.r.o, Czech Republic) and cv. "Cherry Belle" are red globe varieties with small leaves (George and Evans, 1981), and both *E. fetida* and *D. veneta* are 'compost earthworms' with similar morphology and ecology (Domínguez and Edwards, 2011; Katsiamides and Stürzenbaum, 2021). Kováčik

et al. hypothesised that root herbivory reduced radish yields in their study (Kováčik et al., 2021). It may be the case that the horticultural compost used in the present study contained sufficient organic material to act as food for the earthworms so that the radish plants received the benefits of the earthworms without risk of root damage.

The high densities of *D. veneta* used in the two experiments here may be responsible for the large increase in shoot and root growth. The six adult *D. veneta* added to microcosms corresponded to a density of ~764 ind. m^{-2} and ~970 g m^{-2}. These population densities are much higher than those in most habitats, although densities of 300 – 1000 ind. m^{-2} are reported from some temperate pastures (Fründ et al., 2010). However, by using large adult *D. veneta*, the biomass of earthworms per m^2 was much higher than values recorded in natural habitats (Lee, 1985), although very high earthworm densities, equivalent to 4000 individuals m^{-2} and 1182 g m^{-2}, have been recorded accumulating under dung pats in temperate pastures (Bacher et al., 2018). Garden containers are (small) vessels for growing plants: typically consisting of the outer container, substrate, plant, and optional drainage and fertiliser (Nagase and Lundholm, 2021). The contents of a container garden (including earthworm densities, if added) can be freely controlled by the grower, so it is challenging to compare earthworm densities from the field with the high densities of earthworms used in these experiments. However, the effects of earthworms on plant growth can be non-linear, and even negative, at high densities (Agapit et al., 2018; Brown et al., 1999; Chan et al., 2004; Eriksen-Hamel and Whalen, 2007), and shoot and root yields of radish cv. "Cherry Belle" can show different responses to increasing earthworm abundances (Chapter 2, this thesis), so care may be needed when selecting earthworm application densities for container gardens.

Dendrobaena veneta had inconsistent effects on radish plant shoot : root ratios across the two experiments, increasing shoot : root ratios in the flood experiment (Fig. 3.4) but having no effect in the drought experiment (Fig. 3.6, 3.7). Radish cv. "Cherry Belle" grows very quickly and the shoot : root ratio declines during development as the hypocotyl swells, greatly increasing root mass relative to shoot mass (Kostka-Rick and Manning, 1993). The change in relative shoot and root mass can occur rapidly in radish, with cv. "VIP Crimson" fresh root diameter increasing ~90% from 28 to 35 days after sowing (dos Santos Bregonci et al., 2008). Radish shoot : root ratios can also be influenced by soil fertility, with shoot : root ratios of a late sown Japanese variety increasing with increased nitrogen fertilisation in a loam soil (Yousaf et al., 2021). Earthworms can increase nitrogen availability through their casts (Van Groenigen et al., 2019), which may have altered shoot : root ratios in the flood experiment. Therefore, there may have been a number of potential causes for the inconsistent effects of earthworms on shoot : root ratios across the flood and drought experiments. The two experiments were conducted at different times of year (flood experiment = May – June 2023; drought experiment = July – August 2023) with different mean temperatures, day lengths, and supplemental lighting regimes in the experimental glasshouse. Additionally, while both experiments used the same commercially available horticultural compost as a substrate, there may have been batch differences in the composts used for the two experiments that may have led to differences. A large number of crops species are grown in container gardens, with some species grown for their shoots (*e.g.* lettuce, spinach), roots (*e.g.* carrot, beetroot), both (*e.g.* radish), or for their fruits (*e.g.* strawberry). Therefore, an understanding of how earthworms influence plant resource allocation and growth under different conditions would be valuable for understanding the benefits that they can provide in horticultural contexts.

Effects of waterlogging, droughts, and earthworms on radish yields

In both the flood and drought experiment the effects of earthworm treatment did not differ depending on stress treatments (Fig. 3.4, 3.6, 3.7). Radishes appear to be very sensitive to flooding events, and waterlogging events as short as five hours can reduce root yields of radish cv. "VIP Crimson", especially when occurring early in development (Silva et al., 2012). Radish cv. "Crimson Giant" showed reduced root and shoot growth when waterlogged for three weeks, although effects were partially ameliorated by inoculation with the plant growth promoting bacterium *Azospirillum brasilense* (Salazar-Garcia et al., 2022). In the flood experiment here, the more severe treatments (24-hour flood events) reduced radish growth although the *4x4hr* flood treatment also reduced fresh and dry root mass compared to controls (Fig. 3.4). Shoot growth appeared to be less sensitive to flooding, with no effect of the flooding treatment on dry shoot mass and only the most severe flood treatment (*4x24hr*) reducing fresh shoot mass compared to controls. Although radish leaves are edible and have high nutritional value (Gamba et al., 2021; Singh, 2021), it is primarily the swollen hypocotyl that is consumed (Monaghan and Beacham, 2017), so even relatively short waterlogging events could be detrimental for yields in garden containers.

Through their burrowing activities, earthworms can influence soil moisture content (Ma et al., 2021) and water holding capacity (Hallam and Hodson, 2020) and the anecic earthworm *Lumbricus terrestris* L. can reduce surface soil moisture following extreme rainfall events, preventing negative effects on biomass of *Lolium multiflorum* Lam. (Poaceae) (Andriuzzi et al., 2015). The endogeic earthworm *Allolobophora chlorotica* (Savigny, 1826) can also increase hydraulic conductivity of soils and increase flow of water through soil macropores (Hallam et al., 2021). Although the beneficial effects of

earthworms on radish yields in the flood experiment appeared to be reduced for the longer flood treatments (24-hour events), the lack of significant interaction between earthworms and the flooding treatment suggests that the effects of earthworms were largely robust to the flooding stress. The trend towards reduced earthworm benefits in the longer flood treatments may reflect the reduced earthworm survival rates in these flood treatment levels. Earthworm effects on plants can be density-dependent (Van Groenigen et al., 2015); therefore, reduced earthworm densities in the longer flooding treatments may have reduced the overall effects of the earthworms on radish growth in these microcosms. In the flood experiment, live earthworms that had escaped from microcosms during flood events were returned to microcosms after flooding events. However, this might not be possible for container gardens and earthworm benefits may be further reduced as successive waterlogging events gradually deplete earthworm numbers in containers.

Radishes appear sensitive to drought stress and short term droughts can cause >50% root yield reductions in radishes with droughts experienced during early stages of growth having the most negative impact on root yields (dos Santos Bregonci et al., 2008). However, in the drought experiment here, it was drought duration, rather than drought timing, which caused reduction in radish shoot and root yields (Fig. 3.6, 3.7). Additionally, of the two 2-week drought treatments, only the later treatment (*2b* occurring over week 2 and 3 of the drought treatment period) significantly reduced shoot growth compared to controls showing that water stress experienced in later development had a more negative effect on yields (Fig. 3.6). The beneficial effects of earthworms on radish growth were not affected by the drought treatment, suggesting that benefits are robust to minor water stress. However, reported effects of earthworms on plant growth during droughts are not consistent. The endogeic earthworm *Millsonia anomala* Omodeo, 1954 (Megascolecidae) increased shoot biomass of *Oryza sativa* L. only when plants were well-watered, with

benefits largely disappearing when exposed to drought (Blouin et al., 2007). Additionally, *A. chlorotica*, another endogeic earthworm, increased above-ground biomass of *Triticum aestivum* L. during droughts but provided greater benefits to well-watered plants (Hodson et al., 2023), suggesting that earthworm-derived benefits may be reduced in drought. However, *Metaphire guillelmi* (Michaelsen, 1895) (Megascolecidae) can increase drought tolerance in *Solanum lycopersicoides* Dunal (=*Lycopersicon esculentum* Mill.) (Solanaceae) during droughts by up-regulating synthesis of drought tolerance compounds (Li et al., 2022). *Aporrectodea caliginosa* (Savigny, 1826) increased growth of *Hordeum vulgare* L. (Poaceae) during reduced or normal rainfall but provided no benefits to polycultures of *H. vulgare*, *Senecio vulgaris* L. (Asteraceae), and *Capsella bursa-pastoris* (L.) Medik. (Brassicaceae) under ambient or drought conditions (Johnson et al., 2011), suggesting that earthworm effects on plants during drought can be context dependent. Therefore, while the robustness of the benefits provided by earthworms to radish plant growth in this study are encouraging, the wide range of effects demonstrated in soil-based studies suggests that additional work examining different substrates, species, and species mixtures, is necessary to determine if benefits of earthworms in horticultural substrates are widespread.

Effects of earthworms on compost water content during and after droughts

Due to the useful water holding capacities of peat (Leyman and Aplin, 2007), plant growing businesses are concerned about increasing water use requirements from a transition to peat-free composts (Environment and Forestry Directorate, 2023). In the drought experiment, earthworms provided the same benefit to radish growth in treatment *2c* (2x 1-week droughts) as they did in controls while using 1.5 L less water per microcosm (from six 250 ml watering events missed during droughts). Additionally, while

it was not possible to directly measure compost moisture in the drought experiment, using compost + plant mass as a proxy suggested that earthworms reduced compost drying during droughts (Fig. 3.8, treatments *2a* and *1b*) and increased compost rewetting after droughts (treatment *2a*). The mass differences were greater than the difference in fresh plant mass between earthworm treatment levels (*2a* = 6.32 g, *1b* = 11.23 g) suggesting that the differences in compost + plant mass reflected differences in compost water content as well as plant mass. Earthworm activities visibly disrupted and loosened the surface of the compost compared to earthworm-free microcosms and this layer may have reduced water loss by evaporation from deeper compost. Additionally, this loosening of the surface compost may have facilitated rewetting of the compost when droughts were ended. In earthworm-free microcosms, the compost contracted away from microcosm edges during drought while compost in earthworm-containing microcosms did not. When droughts were ended and watering was resumed, the dry, flat layer of at the top of the compost in earthworm-free microcosms caused water to pool and then rapidly drain out of microcosms by flowing down the gap between the dried compost and microcosm interior walls. The loosened compost surface from earthworm activity provided multiple channels into which water could infiltrate during watering and reduced water loss from the base of microcosms after droughts.

In soils, earthworms demonstrate a range of effects on substrate moisture content. *Allolobophora chlorotica* (green morph) can increase water holding capacity of soils (Hallam and Hodson, 2020), although increases may disappear during droughts (Hodson et al., 2023). However, the epigeic earthworm *L. rubellus* can increase water content near the soil surface during simulated drying (Ernst et al., 2009). However, earthworms can also reduce soil water content: burrowing activities of endogeic *Metaphire guillelmi* can reduce soil water content (Ma et al., 2021) and epi-anecic *L. terrestris* can reduce soil moisture

after extreme rainfall (Andriuzzi et al., 2015). Additionally, burrowing activities of *Millsonia anomala* reduced maximum soil water holding capacity and exacerbated effects of drought on *Oryza sativa* (Blouin et al., 2007). The positive effects of earthworms on compost moisture content during and after droughts in the present study may mean that earthworms have valuable applications in reducing irrigation requirements and increasing drought resistance in garden containers. However, horticultural composts are produced from a range of materials including peat, coconut coir, wood fibre, green waste, wool, bark, and more (Bek et al., 2020; Holmes and Bain, 2023, 2021; Leyman and Aplin, 2007). The variety of effects of earthworms on soil moisture content may indicate that earthworm effects in horticultural composts may not be universal and will differ depending on formulation.

Conclusions

In both the flooding and drought experiments, *D. veneta* provided great benefits to radish plant growth in soil-less peat-free horticultural compost during times of environmental stress. The high survival of earthworms when exposed to mild stress, and the lack of interaction between the stressors and the beneficial effects of earthworms, suggests that earthworms could provide great benefits in container gardens and improve crop yields under both normal and stressed conditions. However, care may be needed to prevent the escape of compost earthworms from horticultural containers, especially during times of waterlogging stress, to maintain the benefits provided by earthworms.

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Supplementary information

Table S3.1: Pairwise group comparisons of earthworm survival across flood treatment levels. Odds ratios and confidence intervals have been back-transformed from the Logit scale.

Contrast	Odds ratio	95% CI		P
		Lower	Upper	
Control / 2x4hr	5.22	0.07	387.03	0.834
Control / 4x4hr	1.00	0.00	254.79	1.000
Control / 2x24hr	49.25	0.90	2702.97	0.061
Control / 4x24hr	124.07	2.28	6755.08	**0.009**
2x4hr / 4x4hr	0.19	0.00	14.23	0.834
2x4hr / 2x24hr	9.44	1.31	67.97	**0.016**
2x4hr / 4x24hr	23.79	3.36	168.45	**<0.001**
4x4hr / 2x24hr	49.25	0.90	2702.97	0.061
4x4hr / 4x24hr	124.07	2.28	6755.08	**0.009**
2x24hr / 4x24hr	2.52	0.80	7.96	0.183

Table S3.2: Dunn test comparisons of total earthworm mass change across flood treatment levels.

Contrast	Z	P
2x24hr – 2x4hr	⁻2.20	**0.046**
2x24hr – 4x24hr	0.53	0.659
2x4hr – 4x24hr	2.74	**0.012**
2x24hr – 4x4hr	⁻3.16	**0.004**
2x4hr – 4x4hr	⁻0.96	0.420
4x24hr – 4x4hr	⁻3.70	**0.001**
2x24hr – Control	⁻3.61	**0.001**
2x4hr – Control	⁻1.41	0.226
4x24hr – Control	⁻4.15	**<0.001**
4x4hr – Control	⁻0.45	0.653

Table S3.3: Dunn test comparisons of mean earthworm mass change across flood treatment levels.

Contrast	Z	P
2x24hr – 2x4hr	⁻1.46	0.287
2x24hr – 4x24hr	⁻0.79	0.536
2x4hr – 4x24hr	0.56	0.574
2x24hr – 4x4hr	⁻2.36	0.091
2x4hr – 4x4hr	⁻0.90	0.526
4x24hr – 4x4hr	⁻1.40	0.271
2x24hr – Control	⁻3.01	**0.026**
2x4hr – Control	⁻1.55	0.302
4x24hr – Control	⁻2.00	0.152
4x4hr – Control	⁻0.65	0.571

Table S3.4: Pairwise group comparisons of fresh root mass across flood treatment levels (averaged across earthworm treatment levels). Contrasts and confidence intervals have been back-transformed from the log scale.

Contrast	Ratio	95% CI		P
		Lower	Upper	
Control / 2x4hr	1.26	0.94	1.70	0.195
Control / 4x4hr	1.51	1.12	2.03	**0.002**
Control / 2x24hr	1.68	1.25	2.26	**<0.001**
Control / 4x24hr	2.38	1.77	3.21	**<0.001**
2x4hr / 4x4hr	1.20	0.89	1.61	0.451
2x4hr / 2x24hr	1.33	0.99	1.79	0.066
2x4hr / 4x24hr	1.89	1.40	2.54	**<0.001**
4x4hr / 2x24hr	1.11	0.83	1.50	0.854
4x4hr / 4x24hr	1.58	1.17	2.13	**<0.001**
2x24hr / 4x24hr	1.42	1.06	1.91	**0.012**

Table S3.5: Pairwise group comparisons of dry root mass across flood treatment levels (averaged across earthworm treatment levels). Contrasts and confidence intervals have been back-transformed from the log scale.

Contrast	Ratio	95% CI		P
		Lower	Upper	
Control / 2x4hr	1.21	0.94	1.56	0.226
Control / 4x4hr	1.42	1.10	1.83	**0.002**
Control / 2x24hr	1.53	1.18	1.97	**<0.001**
Control / 4x24hr	2.08	1.62	2.69	**<0.001**
2x4hr / 4x4hr	1.17	0.91	1.51	0.416
2x4hr / 2x24hr	1.26	0.98	1.62	0.090
2x4hr / 4x24hr	1.72	1.33	2.22	**<0.001**
4x4hr / 2x24hr	1.08	0.84	1.39	0.926
4x4hr / 4x24hr	1.47	1.14	1.89	**0.001**
2x24hr / 4x24hr	1.36	1.06	1.76	**0.009**

Table S3.6: Pairwise group comparisons of fresh shoot : root ratio across flood treatment levels (averaged across earthworm treatment levels). Contrasts and confidence intervals have been back-transformed from the log scale.

Contrast	Ratio	95% CI		P
		Lower	Upper	
Control / 2x4hr	0.87	0.67	1.12	0.512
Control / 4x4hr	0.83	0.64	1.07	0.242
Control / 2x24hr	0.66	0.51	0.85	**<0.001**
Control / 4x24hr	0.61	0.47	0.78	**<0.001**
2x4hr / 4x4hr	0.96	0.74	1.23	0.988
2x4hr / 2x24hr	0.76	0.59	0.98	**0.030**
2x4hr / 4x24hr	0.70	0.54	0.90	**0.002**
4x4hr / 2x24hr	0.80	0.62	1.03	0.101
4x4hr / 4x24hr	0.73	0.57	0.94	**0.009**
2x24hr / 4x24hr	0.92	0.71	1.19	0.890

Table S3.7: Pairwise group comparisons of dry shoot : root ratio across flood treatment levels (averaged across earthworm treatment levels). Contrasts and confidence intervals have been back-transformed from the log scale.

Contrast	Ratio	95% CI		P
		Lower	Upper	
Control / 2x4hr	0.95	0.73	1.25	0.988
Control / 4x4hr	0.87	0.66	1.13	0.570
Control / 2x24hr	0.70	0.54	0.92	**0.004**
Control / 4x24hr	0.56	0.43	0.73	**<0.001**
2x4hr / 4x4hr	0.91	0.70	1.19	0.852
2x4hr / 2x24hr	0.74	0.57	0.96	**0.018**
2x4hr / 4x24hr	0.58	0.45	0.76	**<0.001**
4x4hr / 2x24hr	0.81	0.62	1.06	0.200
4x4hr / 4x24hr	0.64	0.49	0.84	**<0.001**
2x24hr / 4x24hr	0.79	0.60	1.03	0.107

Table S3.8: Summary table showing statistically significant differences between microcosms with and without earthworms for each drought treatment on individual weighing days. The *Mass difference* column shows the reduction in compost + plant mass of microcosms without earthworms compared to those with earthworms.

Drought	Day	Mass difference (g)	95% CI		P
			Lower	Upper	
2a	20	⁻50.57	⁻83.65	⁻17.50	**<0.001**
1b	22	⁻35.71	⁻68.78	⁻2.63	**0.019**
1b	24	⁻42.21	⁻75.28	⁻9.13	**0.001**
2a	27	⁻66.41	⁻99.48	⁻33.33	**<0.001**
2a	29	⁻78.41	⁻111.48	⁻45.33	**<0.001**
2a	31	⁻60.74	⁻93.82	⁻27.66	**<0.001**

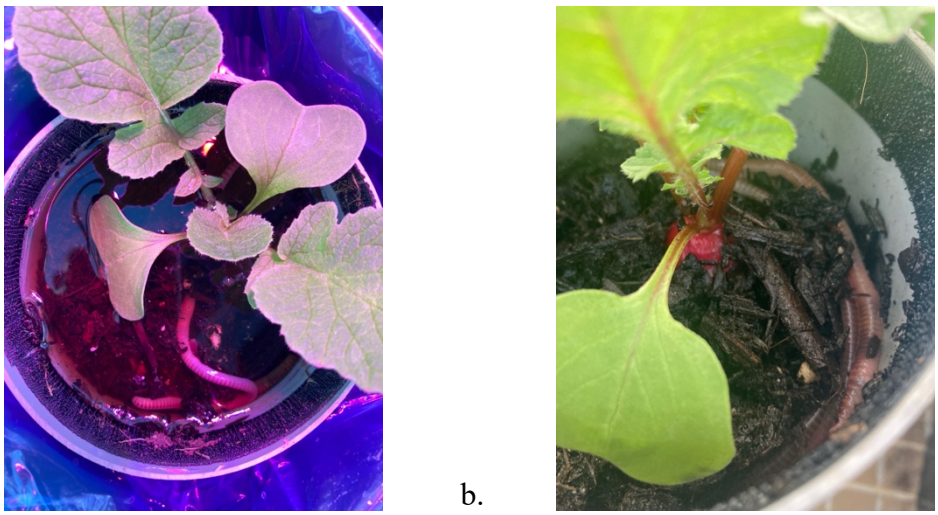


Fig. S3.1: Photos showing *a. D. veneta* floating in flood water during a flooding event and *b. D. veneta* at compost surface after flooding event was ended and water was drained.

Chapter 4: Effects of earthworms and regular flooding events on cattle dung decomposition and performance of the insect dung fauna.

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Abstract

Cattle dung decomposition is an important process for maximising pasture productivity and reducing parasitism risk in cattle. Cattle dung decomposition is performed by the insect dung fauna (Coleoptera and Diptera) as well as by earthworms (Oligochaeta: Lumbricidae) in temperate pasture ecosystems. Earthworms have strong effects on cattle dung decompositions but the delivery of their functions and services may be strongly affected by environmental stressors such as flooding. Additionally, by consuming cattle dung, earthworms may have negative impacts on the performance of the insect dung fauna. In a fully factorial mesocosm experiment conducted in autumn, the effects of earthworms

(three species that represented the three main ecotypes) and weekly flooding (unflooded controls, an intermediate flooding treatment, and an intense flooding treatment) on the decomposition of cattle dung and the performance of the insect dung fauna were investigated. The performance of earthworms (mass change and survival) was also measured. Earthworms had a significant positive effect on dung pat decomposition, and their functioning was not affected significantly by the flooding treatment. Earthworms had significant negative effects on dung insect performance, reducing adult and larva abundances in the intermediate flooding treatment and reducing larva abundance in the intense flooding treatment, although more larvae were found in control mesocosms with earthworms present. These results suggest that earthworms can be important decomposers of cattle dung in wet autumnal conditions in the UK, but their activity may have negative effects on dung insect performance.

Keywords: Dung insects, earthworms, cattle dung, decomposition, flooding, pasture ecosystems.

Introduction

The UK contains ~9.6 million cattle, with a breeding herd of ~3.24 million individuals (Department for Environment, Food and Rural Affairs, 2023). Grazing cattle can produce enough dung to cover ~0.68 m^2 of pasture per cow per day (MacLusky, 1960). Plant growth immediately under pats is suppressed (Williams and Haynes, 1995) and cattle avoid the smell of dung (Dohi et al., 1991), resulting in them avoiding feeding on grass too close to dung pats, likely as a means of reducing parasite load (Seó et al., 2015). It has been previously estimated that dung pats produced by cattle in the UK, and the avoided area around pats, could remove as much as 4.8% of useable pasture per year (Beynon et al., 2012b). Rapid degradation and decomposition of cattle dung is thus desirable for maintaining pasture productivity and reducing parasite load in cattle. Within the UK, this service is provided by both the insect dung fauna (Skidmore, 1991) and earthworms (Gittings and Giller, 1999).

The insect dung fauna is primarily comprised of Coleoptera in the families Scarabaeidae (Aphodiinae and Scarabaeinae) and Geotrupidae and Diptera in the families Muscidae, Anthomyiidae, and Acalyptratae of a wide range of species (Skidmore, 1991; Sladecek et al., 2021). While dung flies contribute to dung decomposition, dung beetles are more effective decomposers (Kavanaugh and Manning, 2020; Wu and Sun, 2010). Alongside dung decomposition (Holter, 2016; Lee and Wall, 2006; Manning et al., 2016), dung beetles provide a range of ecosystem functions and services including reduction of cattle parasite abundance (Sands and Wall, 2017), and improvement of soil drainage (Keller et al., 2022), among other benefits (Nichols et al., 2008). Dung beetles utilise dung to provision their offspring (Holter, 2016) and they can be divided into guilds based on their dung usage behaviours (Hanski and Cambefort, 1991). Diverse assemblages of dung

insects are necessary to maximise ecosystem functioning during typical conditions and during periods of environmental stress (Beynon et al., 2012a; Manning et al., 2016, 2017b; Slade et al., 2007; Slade and Roslin, 2016).

Earthworms (Oligochaeta: Lumbricidae) are also important decomposers of cattle dung in pasture ecosystems (Gittings et al., 1994; Holter, 1977, 1979a, 1983; Kaartinen et al., 2013; Schon and Dominati, 2020). Earthworms are attracted to dung and accumulate under dung pats (Bacher et al., 2018; Barth et al., 1994; Holter, 1983; Hughes et al., 1994; Knight et al., 1992) where they contribute to dung pat degradation (Kaartinen et al., 2013). Earthworms feed on dung and incorporate dung into the soil through casting and burrowing (Hendriksen, 1997; Schon et al., 2020, 2017, 2015), and also modify soil properties beneath dung pats (Bacher et al., 2020). Earthworms of all ecotypes (Bouché, 1977, 1972) are attracted to cattle dung (Bacher et al., 2018) and cattle dung is the preferred food source for controlled earthworm cultivation (Lowe and Butt, 2005). While dung insects colonise dung pats soon after defecation (Sladecek et al., 2021), earthworms typically arrive later and increase in abundance over multiple weeks (Bacher et al., 2020, 2018; Holter, 1983; Knight et al., 1992). It may be the case that the arrival and activities of earthworms at cattle dung pats is influenced by the insect dung fauna 'priming' cattle dung for earthworm activity (Holter, 1983, 1977). However, the interactions between earthworms and the dung fauna are unclear, with the combined effects of earthworms and dung insects having positive (Holter, 1977; Kaartinen et al., 2013; O'hea et al., 2010a) or negative (O'hea et al., 2010a) impacts on dung decomposition. In addition, earthworms can reduce dung beetle performance by consuming larval food supplies (Gittings and Giller, 1999; Xie et al., 2021). Therefore, there is a need to increase understanding of how the insect dung fauna and earthworms interact, especially during times of environmental stress such as flooding events.

Flooding may have strong impacts on the performance of both the earthworm and dung insect faunas. Flooding has negative effects on earthworm populations (Kiss et al., 2021b; Plum and Filser, 2005; Singh et al., 2019; Zorn et al., 2008) although earthworms can also influence the effects of flooding by affecting soil water content and water drainage (Hallam et al., 2021; Hallam and Hodson, 2020; Hodson et al., 2023; Ma et al., 2021). Earthworm responses to flooding differ across ecotypes (Kiss et al., 2021a). Epigeic (surface dwelling) earthworm species cannot tolerate long periods of submersion (Kiss et al., 2021a) and populations may only persist as cocoons (Klok et al., 2006; Zorn et al., 2005), balls of hardened mucus in which earthworm embryos develop (Domínguez and Velando, 2013). However, endogeic (soil dwelling with temporary horizontal burrows) species can withstand much longer periods submerged than species of other ecotypes (Kiss et al., 2021a). Anecic earthworm species form large vertical burrows which can have strong effects on water drainage and plant performance during extreme rainfall (Andriuzzi et al., 2015) and may reduce flooding risks. The effects of earthworms on dung decomposition depend on community abundance and functional richness (Schon et al., 2017); therefore, flooding could have negative effects on the ecosystem services provided by earthworms if earthworm communities are negatively impacted.

The tunnels produced by dung beetles into soil can influence water infiltration so may alleviate the impacts of intense rainfall or flooding (Keller et al., 2022). Moist conditions can also increase performance and reproduction of endocoprid (living and breeding within dung) species (Vessby, 2001). However, many dung beetle larvae develop under the soil surface (Hanski and Cambefort, 1991) and adults will avoid egg laying in excessively moist soil as excessive soil moisture can lead to larval mortality (Osberg et al., 1994; Sowig, 1995). Dung pats themselves can be highly anoxic (Holter, 1991) and adult dung beetles can maintain aerobic respiration in extremely low atmospheric oxygen

concentrations (Holter and Spangenberg, 1997); however, mortality will occur when adult beetles are submerged in water for extended periods of time (Whipple et al., 2013). Regular flooding can impact dung beetle community composition at the landscape level (Correa et al., 2022; Nunes et al., 2014; Tissiani et al., 2015), suggesting that species differ in their tolerance to flood stress. Dung-breeding flies also show negative responses to flooding (Lühken et al., 2014) so flooding stress could have negative impacts on the ecosystem services provided by the insect dung fauna.

Due to the strong effects of flooding on soil moisture and on the activities and survival of both dung insects and earthworms, the effects of flooding on the interactions between these groups, and the resulting effects on dung decomposition, are unclear. In this mesocosm experiment, the presence or absence of earthworms representing the three main ecotypes (Bouché, 1977, 1972) and a weekly flooding treatment (unflooded controls, an intermediate flooding treatment, and an intense flooding treatment) were manipulated. Dung decomposition (determined by end wet and dry dung mass), surface soil moisture, earthworm performance (survival and mass change) and dung insect performance (adult and larval abundance and biomass) were recorded. It was predicted that the presence of earthworms would reduce dung mass at the end of the experiment, but that earthworms may have a negative impact on dung insect performance. It was also predicted that earthworm performance would be negatively affected by flooding, resulting in reduced dung decomposition through reduced earthworm activity.

Methods

Overview of study design and mesocosm construction

A fully factorial mesocosm experiment was conducted from October – December 2021 with three levels of a flooding treatment (*Control*, *Flood 1*, and *Flood 2*, described below), two levels of an earthworm treatment (earthworms absent or present, described below) and two levels of a dung fauna treatment (natural colonisation prevented or enabled. Note: mesocosms with dung fauna colonisation prevented were excluded from analyses, see below. Mesocosms with dung fauna colonisation prevented are included in analyses presented in the *Appendix*). Each of the 12 treatment combinations were replicated six times for a total of 72 mesocosms. This experiment was conducted in an outside grassy area at the John Krebs Field Station (Department of Biology), University of Oxford, near the village of Wytham, Oxfordshire, UK. The field station is situated next to an organic cattle farm and is surrounded by grazed pasture. Mesocosms were randomly arranged in a 12 x 6 grid under two rain shelters constructed from 4 x 5 m 200 g m^{-2} tarpaulin (Table S4.1). Due to the size and weight of the mesocosms, position re-randomisation was not possible while the experiment was running.

Experimental mesocosms (Fig. 4.1) were constructed from white 21 L polypropylene buckets (height 331 mm, upper diameter 326 mm; manufacturer Involvement Packaging Ltd., Cheshire, UK) fitted with sealable lids. Mesocosms were filled to a depth of ~26 cm with a sandy-loam soil (17% clay, 22% silt, 61% sand, C:N ratio = 11.4; supplier North Oxfordshire Topsoil Ltd., Banbury, Oxfordshire, UK) that was passed through a 10 mm sieve and homogenised by hand to remove soil macro-invertebrates. Each mesocosm was raised and dropped five times from a height of ~5 cm to provide consistent compaction across buckets before being watered with 2 L of tap water with a watering can with a rose

from a height of ~30 cm. Mesocosms were fitted with emergence traps constructed from clear 500 ml plastic bottles containing ~100 ml of antifreeze (primary ingredient: ethylene glycol). Emergence traps were connected to mesocosms via a clear plastic tube (2 cm outer diameter, 8 cm length) and secured in place with hot-melt adhesive and duct tape, to a 2 cm diameter hole drilled into each mesocosm near the upper rim during construction. A strip of adhesive hook tape (2 cm width, 10 cm length) was placed under the hole to discourage earthworms from exiting the mesocosms and entering the emergence traps (Lubbers and Van Groenigen, 2013). Bucket holes allowing entry to emergence traps were initially sealed with duct tape before sampling commenced (described below). A ~25 cm disk was cut from the centre of each bucket lid and the remaining ring-shaped lid was used to secure a layer of 40 g m^{-2} nylon insect mesh over the upper opening of the bucket. This facilitated air exchange and watering without removing lids while preventing dung insect and earthworm escape. Two ~1 cm diameter drainage holes were drilled into the base of each mesocosm on 9 October prior to the flooding treatment (see below) to allow mesocosms to slowly drain after flooding treatments.

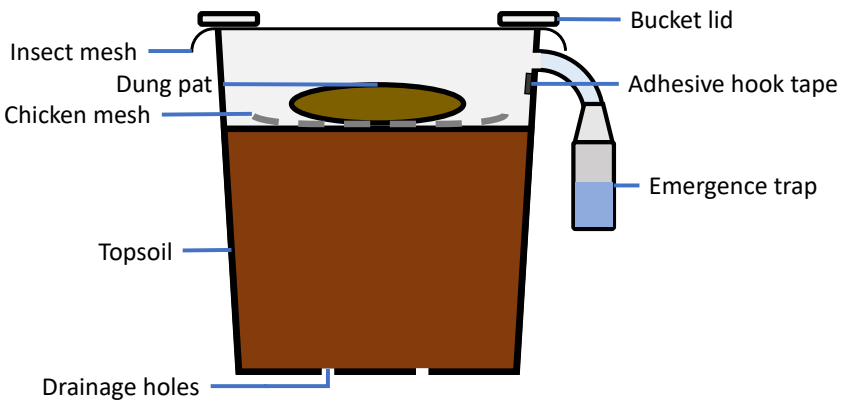


Fig. 4.1: Diagram of mesocosms used in this experiment.

Colonisation of the artificial dung pats

Fresh cattle dung (<24 hours old) was collected from an organic farm adjacent to the John Krebs Field Station (described above) on 9 September. The herd consisted of mixed-sex Angus-Saler-Stabiliser cattle that had not recently been treated with anthelminthic medication at the time of dung collection. The grazing pasture of the farm was divided into sections and the herd was moved to an ungrazed section every 24 hours, ensuring that dung collected from the grazing area of the previous day was <24 hours old. Dung was stored in 20 L polypropylene buckets with air-tight lids after collection to prevent ingress of dung insects.

Artificial dung pats were prepared for natural colonisation by the insect dung fauna (Skidmore, 1991). On 10 September, the cattle dung was homogenised by hand in a large plastic container. Ninety-nine artificial dung pats (~15 cm diameter, ~550 g fresh mass) were formed and arranged in a 9 x 11 grid in an open grassy area at the John Krebs Field Station, adjacent to the cattle shed and near pastures of the neighbouring farm. Half of the pats were left exposed to insect colonisation while half were placed into 1 mm mesh litter bags to prevent ovipositing of dung insects. This was performed to exclude dung insects from the pats to create an insect-free control. However, this method was only partially successful (see below and *Supplementary information – Dung fauna exclusion method*). All pats were placed onto 6 cm diameter hexagonal steel mesh to facilitate moving them. Artificial pats were left for one week (10 – 17 September) before being transferred to mesocosms. On 17 September, 72 artificial dung pats (36 exposed and 36 covered) were randomly selected and allocated to mesocosms. Mesocosm lids were removed and pats, with wire mesh, were placed onto the soil surface and gently pressed to ensure contact with the soil before lids were replaced.

Experimental manipulations

Three earthworm species, *Dendrobaena veneta* (Rosa, 1886), *Allolobophora chlorotica* (Savigny, 1826), and *Lumbricus terrestris* L., were selected to represent the three main earthworm ecotypes (Bottinelli et al., 2020; Bouché, 1977, 1972), with all species native to the UK (Sherlock et al., 2018). *Dendrobaena veneta* is a red-coloured epigeic earthworm, commonly used for vermicomposting (Domínguez, 2018), with an adult body size of 5 – 7 x 50 – 80 mm and mass of ~0.92 g (Domínguez and Edwards, 2011). *Allolobophora chlorotica* is an endogeic species with an adult body size of 2 – 7 x 30 – 38 mm (Sherlock et al., 2018) that forms temporary horizontal burrows under the soil surface (Lowe and Butt, 2007a) and can be found as pink or green morphs that differ in preference for soil moisture (Lowe and Butt, 2007a). Following previous work, a mixture of pink and green morph individuals of similar body size and mass were used in this experiment (Ashwood et al., 2017). *Lumbricus terrestris* is an anecic species with an adult body size of 6 – 10 x 90 – 350 mm (Sherlock et al., 2018) that forms permanent vertical burrows and feeds on organic material at the soil surface (Hoeffner et al., 2019). The species forms 'middens' at the entrance to their burrows (Stroud et al., 2016a) which earthworms and other soil invertebrates can accumulate under (Butt and Lowe, 2007). Earthworm-containing mesocosms received six *D. veneta*, six *A. chlorotica*, and two *L. terrestris* for a total of 14 earthworms and a density of ~168 individuals m^{-2}. While this is lower than densities reported from UK dairy pastures (Hodson et al., 2021) and is lower than densities that can occur under dung pats (Bacher et al., 2020, 2018), this density was selected due to the relatively small pat sizes used in the experiment and the lack of other food sources for the earthworms in the mesocosms. *Dendrobaena veneta* and *L. terrestris* were purchased from Yorkshire Worms Ltd. (Holme-on-Spalding-Moor, Yorkshire, UK) while *A. chlorotica* were hand collected from soil in a private garden in Wiltshire, UK. All earthworms were

kept in ventilated sealed buckets containing the same soil used in the experiment prior to being introduced to mesocosms.

Earthworms were added to mesocosms on 18 September. Earthworms were removed from storage on 16 September and were washed with tap water and moved into ventilated containers on moist tissue paper and stored in a laboratory (~19 °C) for 48 hours for gut voidance (Fründ et al., 2010). On 18 September, earthworms were randomly allocated to 108 ventilated tubs lined with moist tissue paper (one tub per species per mesocosm). Earthworms were weighed individually, and some individuals were exchanged between containers to ensure similar starting masses. One container of each species was then randomly allocated to each earthworm-containing mesocosm. Earthworm-containing mesocosms received 1.18 g ±SE 0.03 g of *A. chlorotica*, 6.31 g ±SE 0.15 g of *D. veneta*, and 11.13 g ±SE 0.17 g of *L. terrestris* with no statistically significant differences in starting masses across dung fauna and flooding treatment groups (Table S4.2). Earthworms allocated to each mesocosm were combined into one tub, the mesocosm lid was removed, and earthworms were placed onto the soil surface and observed to burrow quickly into the soil. The mesocosm lids were then replaced to minimise chance of dung insects leaving mesocosms or entering from outside.

Mesocosms were exposed to three levels of a watering treatment: *Control*, *Flood 1*, and *Flood 2*. *Control* mesocosms were not watered for the duration of the experiment but soil remained moist due to shading from the rain shelters and bucket rims (end *Control* surface soil gravimetric water content = 39.0% ±SE 0.9, see below and *Results*). *Flood 2* mesocosms were watered weekly to produce a standing water level of ~1 cm which slowly drained via the mesocosm drainage holes. *Flood 1* mesocosms were watered with half of the mean amount of water added to *Flood 2* mesocosms at each weekly watering event.

This corresponded to ~2 L (equivalent to ~24 mm rainfall) of water for *Flood 2* mesocosms and ~1 L (equivalent to ~12 mm rainfall) of water for *Flood 1* mesocosms per watering event. The flooding treatment was conducted from 14 October – 9 December with a total of nine watering events.

Data collection

Dung insect sampling commenced on 6 October. Emergence traps were attached to mesocosms (Fig. 4.1; described above) and duct tape covering the exit hole from each mesocosm was removed. Opaque lids were placed over mesocosms on 4 November until the end of the experiment to encourage emerging invertebrates to enter the emergence traps. The contents of emergence traps were collected every two weeks. Contents were passed through a 1 mm sieve, and insects were stored in 98% ethanol at −20 °C. Destructive mesocosm sampling began on 13 December and was completed across three days. Dung pats were removed from mesocosms and hand sorted for adult insects and larvae which, as with those collected from emergence traps, were stored in 98% ethanol at −20 °C. Juvenile and adult insects of the dung fauna were then counted, identified to family level (Skidmore, 1991), and dried for 24 hours at 45 °C before being weighed.

A surface soil sample (~300 ml) was collected from each mesocosm and dried at 70 °C until constant mass to estimate surface soil gravimetric water content. Dung pats, after being sorted for insects, had their wet mass recorded and were dried at 70 °C until reaching constant mass, which was recorded. Soil was then removed from mesocosms, and hand sorted to recover earthworms. Surviving earthworms were washed with tap water, transferred to ventilated containers lined with moist tissue paper and stored in the laboratory (~19 °C) for 48 hours for gut voidance (Fründ et al., 2010), before being

weighed to determine mass changes. Any missing earthworms were assumed to have died during the experiment.

Exclusion of dung insect-excluded mesocosms

During data collection, it was identified that the dung fauna exclusion method was only partially successful (*Supplementary Information – Dung fauna exclusion method* Table S4.2). Therefore, for analyses, only mesocosms with dung fauna colonisation facilitated were included. Analysis of the full dataset, including mesocosms with dung fauna excluded, is presented in the *Appendix* of this Chapter after the *Supplementary Information.*

Data analysis

All data were analysed using R version 4.3.1 (R Core Team, 2023). Due to partial failure of the rain shelter, treatments in six mesocosms were compromised by rainwater. Three of these mesocosms contained pats with facilitated dung insect colonisation. These three mesocosms were excluded from all analyses (Table S4.1, S4.3; additionally, all six compromised mesocosms were excluded from the full analyses presented in the *Appendix*). Two compromised mesocosms were from the *Control* + earthworms absent + dung fauna present treatment group, two were from the *Flood 1* + earthworms absent + dung fauna absent treatment group, and one mesocosm was excluded from both the *Flood 1* + earthworms present + dung fauna present and *Flood 2* + earthworms present + dung fauna absent treatment groups. Type II Analysis of Variance (ANOVA) was performed for all response variables using the *car* package (Fox and Weisberg, 2019) to account for differences in sample size across groups. For dung and soil response variables, the flooding and earthworm treatments, and their interaction, were included in models as

explanatory variables. Earthworm response variables were analysed separately for each species with the flooding treatment as an explanatory variable.

End dung wet mass, end dung dry mass, surface soil moisture content, mean and total earthworm mass change, and dung insect biomass were all analysed with ANOVA, with dung insect biomass log-transformed to better fit the assumptions of ANOVA. Counts of insect adults, total insect larvae, and Diptera larvae were analysed with Poisson Generalised Linear Models (GLMs). Earthworm survival was analysed with Binomial GLMs. The probability of a mesocosm containing Coleoptera larvae was analysed with a Binomial GLM. All dung insect response variables were analysed with earthworms, the flooding treatment, and their interaction included as explanatory variables. Additionally, the effects of surviving earthworm number on end dung dry mass were analysed. Analysis of Covariance (ANCOVA) was used to analyse effects of total surviving earthworm number and surviving *A. chlorotica* number. All mesocosms contained either one or two *L. terrestris* and five or six *D. veneta* so the individual effects of survival of these species were analysed with ANOVA with each earthworm abundance treated as a factor. Diagnostic plots of all models were inspected, and end dung dry mass was log-transformed for all analyses of effects of surviving earthworm number of end dung dry mass. When factor variables or interactions had statistically significant effects on response variables, *post hoc* group comparisons were performed with the *emmeans* package (Lenth, 2023). Group differences were back-transformed to the response scale for interpretation and reporting for GLMs. All plots and figures were produced in R with the *ggplot2* package (Wickham, 2016).

Results

Dung decomposition and soil moisture

For both end dung dry mass (Fig. 4.2a) and wet mass (Fig. 4.2b), there was a statistically significant interaction between earthworms and the flooding treatment (Table 4.1, S4.4, S4.5). Earthworms reduced end dung dry mass in both *Flood 2* (difference = −41.33 g, $CI_{95\%}$ = [−59.72, −22.95], $p < 0.001$) and *Flood 1* (difference = −23.27 g, $CI_{95\%}$ = [−42.55, −3.99], $p = 0.011$) but had no significant effect in *Controls* (difference = −18.92 g, $CI_{95\%}$ = [−39.47, 1.64], $p = 0.085$). For dung wet mass, earthworms reduced mass for all flooding treatment levels but had the greatest effect in *Flood 2* (difference = −257.50 g, $CI_{95\%}$ = [−351.50, −199.50], $p < 0.001$), followed by *Flood 1* (difference = −210.30 g, $CI_{95\%}$ = [−271.17, −149.50], $p < 0.001$), and the lowest effect in *Controls* (difference = −100.6 g, $CI_{95\%}$ = [−165.3, −35.7], $p = 0.001$). For surface soil moisture content, there was no statistically significant interaction between earthworms and the flooding treatment (Table 4.1, Fig. 4.2c). However, soil moisture was significantly increased by earthworms (difference = 4.97, $CI_{95\%}$ = [2.90, 7.04], $p < 0.001$). For the flooding treatment, soil moisture was significantly higher than in *Controls* for both *Flood 2* (difference = 6.43, $CI_{95\%}$ = [3.35, 9.50], $p < 0.001$) and *Flood 1* (difference = 5.74, $CI_{95\%}$ = [2.60, 8.89], p , 0.001) but there was no significant difference between *Flood 2* and *Flood 1* (difference = 0.68, $CI_{95\%}$ = [−2.23, 3.65], $p = 0.838$).

Table 4.1: Summary of ANOVA (Type II) results showing effects of earthworms, flooding, and their interactions on end dry dung mass, end wet dung mass, and soil moisture content.

Variable	Dung dry mass			Dung wet mass			Soil moisture		
	Df	F	P	Df	F	P	Df	F	P
Earthworms	1	61.76	**<0.001**	1	288.41	**<0.001**	1	26.26	**<0.001**
Flooding	2	2.15	0.136	2	0.30	0.741	2	17.37	**<0.001**
Earthworms x Flooding	2	3.65	**0.039**	2	15.70	**<0.001**	2	2.16	0.135
Residuals	27			27			27		

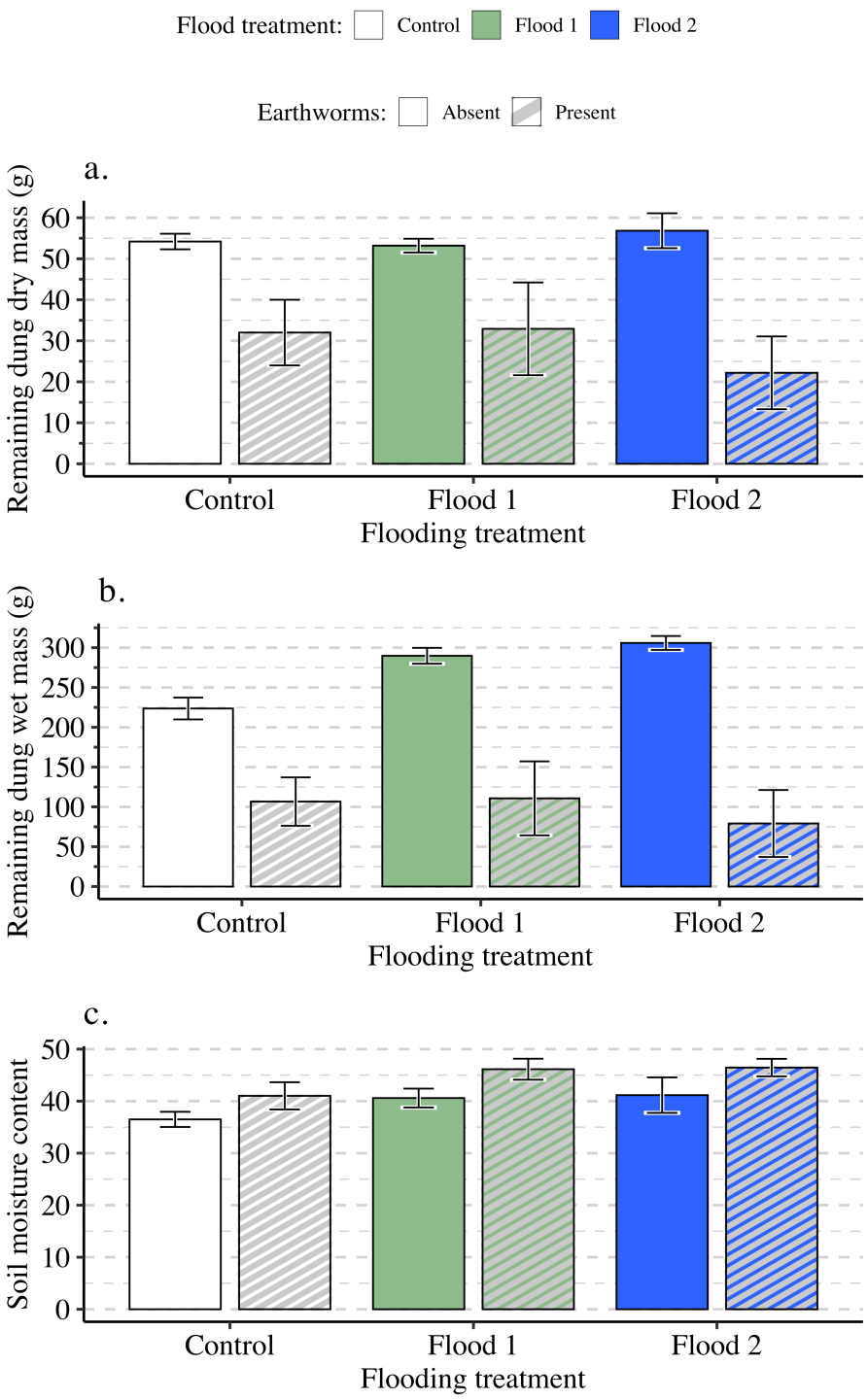


Fig. 4.2: Bar plots showing *a.* end dry dung mass, *b.* end wet dung mass, and *c.* surface soil moisture across flooding and earthworm treatment groups. Bar heights show group means and error bars show 95% confidence intervals.

Earthworm performance

Survival and mass change

Seventeen earthworm-containing mesocosms were kept for analysis (see *Methods,* Table S4.3). Of the 238 earthworms added to these mesocosms, 208 were recovered alive representing an overall survival rate of 87.4%.

Lumbricus terrestris survival (overall = 76.5%) was not significantly affected by the flooding treatment (Table 4.2, Fig 4.3a). *Lumbricus terrestris* total mass change did not change significantly across the whole experiment ($\mu = -1.78$ g, $CI_{95\%} = [-3.65, 0.10]$, $p = 0.062$) but was significantly affected by the flooding treatment (Table 4.2, Fig 4.3b). However, pairwise comparisons between flooding treatments levels did not show significant differences in total mass change (Table S4.6). Overall mean mass change also did not change during the experiment ($\mu = 0.48$ g, $CI_{95\%} = [-0.11, 1.07]$, $p = 0.102$) and was not significantly affected by the flooding treatment (Table 4.2, Fig. 4.3c).

Allolobophora chlorotica survival (overall = 84.3%) was not significantly affected by the flooding treatment (Table 4.2, Fig. 4.3d). Both total ($\mu = 0.24$ g, $CI_{95\%} = [0.12, 0.36]$, $p < 0.001$) and mean ($\mu = 0.09$ g, $CI_{95\%} = [0.07, 0.10]$, $p < 0.001$) *A. chlorotica* mass both significantly increased in the experiment, but neither were significantly affected by the flooding treatment (Table 4.2, Fig 4.3e, 4.3f).

Dendrobaena veneta survival (total = 94.1%) was not significantly affected by the flooding treatment (Table 4.2, Fig. 4.3g). Total *D. veneta* mass significantly decreased in the experiment ($\mu = -0.44$ g, $CI_{95\%} = [-0.86, -0.01]$, $p = 0.045$) but there was no significant trend for mean mass to either increase or decrease ($\mu = -0.01$ g, $CI_{95\%} = [-0.09,$

0.06], $p = 0.751$). There was no significant effect of the flooding treatment on either total or mean mass change for *D. veneta* (Table 4.2, Fig. 4.3h, 4.3i).

Table 4.2: Summary of ANOVA (Type II) results for effects of flooding on earthworm response variables. Analyses were performed separately for each species.

Species	Variable	Survival			Total mass change			Mean mass change		
		Df	X^2	P	Df	F	P	Df	F	P
Lumbricus terrestris	Flooding	2	3.48	0.175	2	3.97	**0.043**	2	2.69	0.103
	Residuals	14			14			14		
Allolobophora chlorotica	Flooding	2	0.14	0.933	2	0.94	0.413	2	1.74	0.211
	Residuals	14			14			14		
Dendrobaena veneta	Flooding	2	0.77	0.681	2	1.78	0.205	2	0.59	0.565
	Residuals	14			14			14		

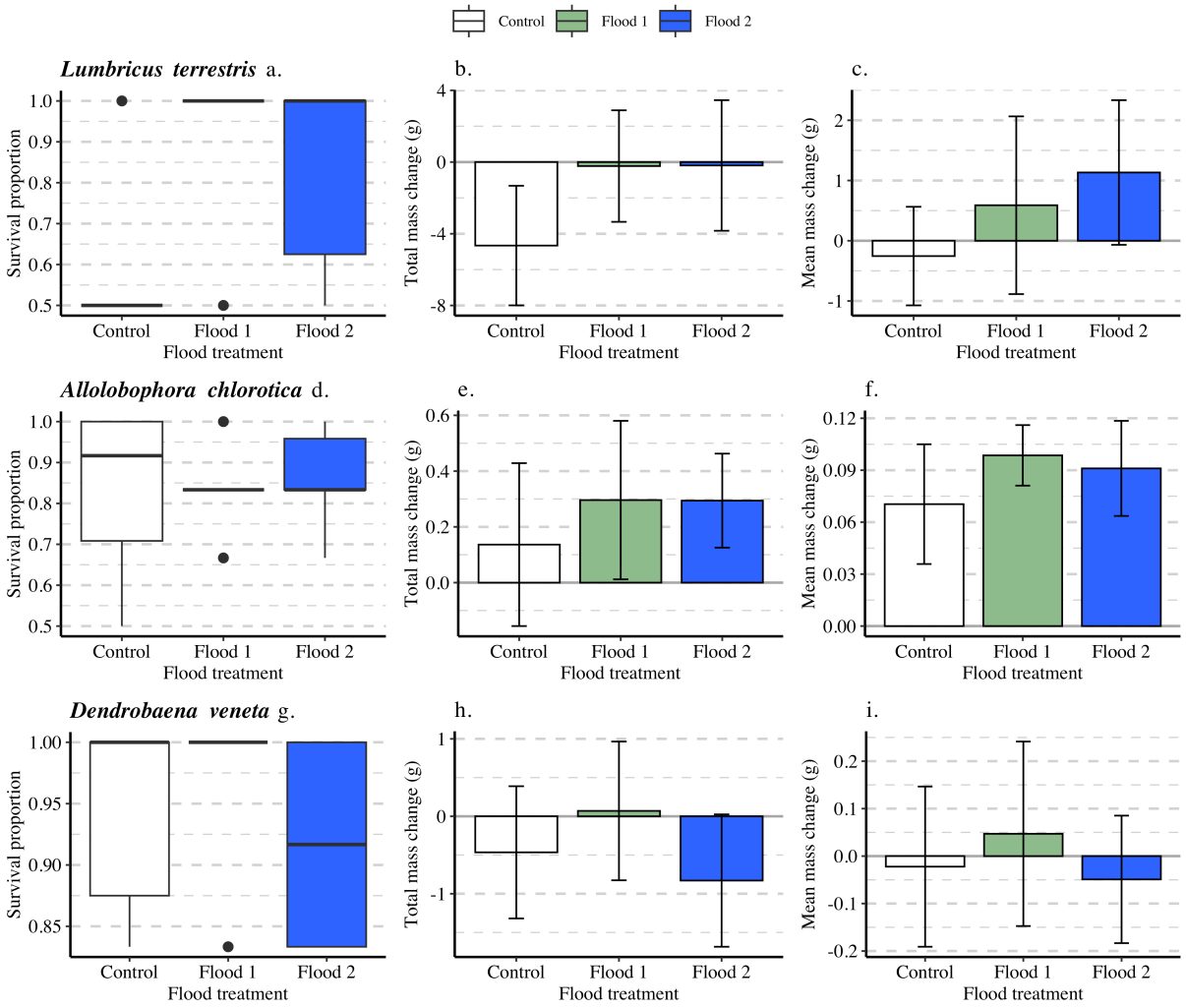


Fig. 4.3: Box plots showing survival and bar plots showing mean and total mass change of *Lumbricus terrestris* (*a.* - *c.*), *Allolobophora chlorotica* (*d.* - *f.*), and *Dendrobaena veneta* (*g.* - *i.*) across flooding treatment groups. For bar plots, bar heights show group means and error bars show 95% confidence intervals. For box plots, box outer horizontal lines show group interquartile ranges, central horizontal lines show group medians, and extended vertical lines show full data range for each group. Box plot points below medians show lower outlier data points (>1.5x interquartile range below 25th percentile) while points above medians upper outlier data points (>1.5x interquartile range above 75th percentile).

Effects of earthworm survival on dung decomposition

There was a statistically significant effect of *L. terrestris* survival on log-transformed end dung dry mass (Table 4.3), with mass significantly reduced when two *L. terrestris* were recovered alive compared to when one *L. terrestris* was recovered (ratio = 0.536, $CI_{95\%}$ =

[0.314, 0.915], $p = 0.025$). However, there was no significant effect of overall earthworm survival, surviving *A. chlorotica* number, or surviving *D. veneta* number (Table 4.3, Fig 4.4a, 4.4c, 4.4d).

Table 4.3: Summary of ANOVA (Type II) and ANCOVA (Type II) results showing effects of surviving earthworm number on end dung pat dry mass. Separate models with survival of all earthworms and each individual species as explanatory variables were constructed.

Species	Analysis	Variable	Df	F	P
All	ANCOVA	Surviving earthworms	1	1.02	0.328
		Residuals	15		
Lumbricus terrestris	ANOVA	Surviving earthworms	1	6.17	**0.025**
		Residuals	15		
Allolobophora chlorotica	ANCOVA	Surviving earthworms	1	0.03	0.861
		Residuals	15		
Dendrobaena veneta	ANOVA	Surviving earthworms	1	0.05	0.821
		Residuals	15		

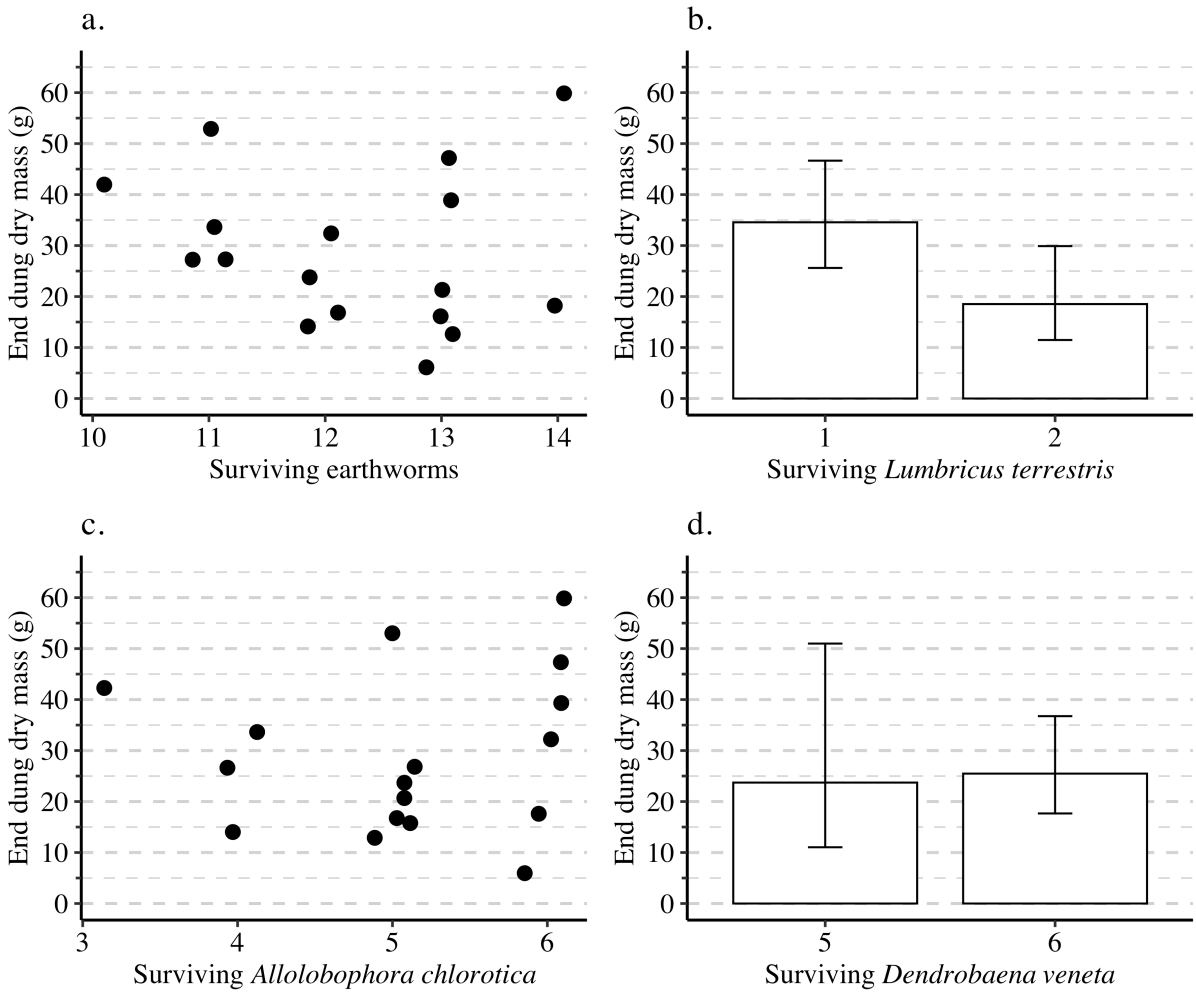


Fig. 4.4: Scatter plots and bar plots showing correlation between end dry dung mass and *a.* total earthworm survival, *b. Lumbricus terrestris* survival, *c. Allolobophora chlorotica* survival, and *d. Dendrobaena veneta* survival. Bar plots are used for *b.* and *d.* as all mesocosms contained either one or two earthworms for *L. terrestris* or five or six earthworms for *D. veneta*. Group sizes in *b.* and *d.* differ. For *b*, eight mesocosms contains one surviving *L. terrestris* while nine contained two surviving *L. terrestris*. In *d.*, six mesocosms contained five surviving *D. veneta* while 11 mesocosms contained six surviving *D. veneta*. A jitter has been applied on the x axis for all points in *a.* and *c.* to reduce overplotting. Lines of best fit are not displayed in *a.* and *c.* as there was no statistically significant correlation between variables in these plots.

Dung fauna performance

For larva counts, there was a statistically significant interaction between the effects of earthworms and the flooding treatment (Table 4.4, S4.7; Fig. 4.5a, S4.3). Larva counts were significantly lower in the presence of earthworms in the *Flood 1* treatment (ratio =

0.34, $CI_{95\%}$ = [0.24, 0.49], $p < 0.001$) and in the *Flood 2* treatment (ratio = 0.16, $CI_{95\%}$ = [0.10, 0.24], $p < 0.001$) but were marginally higher in the *Control* watering treatment (ratio = 1.26, $CI_{95\%}$ = [1.00, 1.59], $p = 0.057$). For total insect counts, there was also a statistically significant interaction between earthworms and flooding treatment (Table 4.4, S4.8; Fig. 4.5b, S4.3) where earthworms reduced insect counts only in the *Flood 1* treatment (ratio = 0.51, $CI_{95\%}$ = [0.35, 0.73], $p < 0.001$). For total insect biomass, there was no statistically significant interaction between earthworms and the flooding treatment or main effects of either treatment (Table 4.4; Fig. 4.5c). As with total larva counts, for Diptera larva counts there was a significant interaction between the earthworm and flooding treatments (Table 4.4, S4.9; Fig. 4.5d, S4.3), where Diptera larva counts were significantly lower in the presence of earthworms in the *Flood 1* treatment (ratio = 0.37, $CI_{95\%}$ = [0.26, 0.53], $p < 0.001$) and in the *Flood 2* treatment (ratio = 0.16, $CI_{95\%}$ = [0.11, 0.24], $p < 0.001$) but were higher in the *Control* watering treatment (ratio = 1.34, $CI_{95\%}$ = [1.04, 1.73], $p = 0.015$). The probability of finding live Coleoptera larvae was significantly reduced in the presence of earthworms (Table 4.4; odds ratio = 0.11, $CI_{95\%}$ = [0.02, 0.62], $p = 0.01$) with larvae being recovered from 81% and 32% of no-earthworm and earthworm-containing mesocosms respectively. There was no significant effect of flooding on the probability of mesocosms containing Coleoptera larvae, and no significant interaction between earthworms and flooding (Table 4.4).

Table 4.4: Summary of ANOVA (Type II) results showing effects of flooding, earthworms, and their interactions on dung fauna response variables.

Response variable	Test	Flooding			Earthworms			Flooding x Earthworms		
		Df	Test statistic	P	Df	Test statistic	P	Df	Test statistic	P
Larvae count	X^2	2	109.67	**<0.001**	1	113.00	**<0.001**	2	218.74	**<0.001**
Insect count	X^2	2	10.94	**0.004**	1	19.71	**<0.001**	2	13.34	**0.001**
Adult insect biomass	F	2,27	0.79	0.465	1,27	0.00	0.950	2,27	0.14	0.874
Diptera larva count	X^2	2	110.55	**<0.001**	1	105.22	**<0.001**	2	210.11	**<0.001**
Coleoptera larva prob.	X^2	2	2.09	0.352	1	8.58	**0.003**	2	2.07	0.355

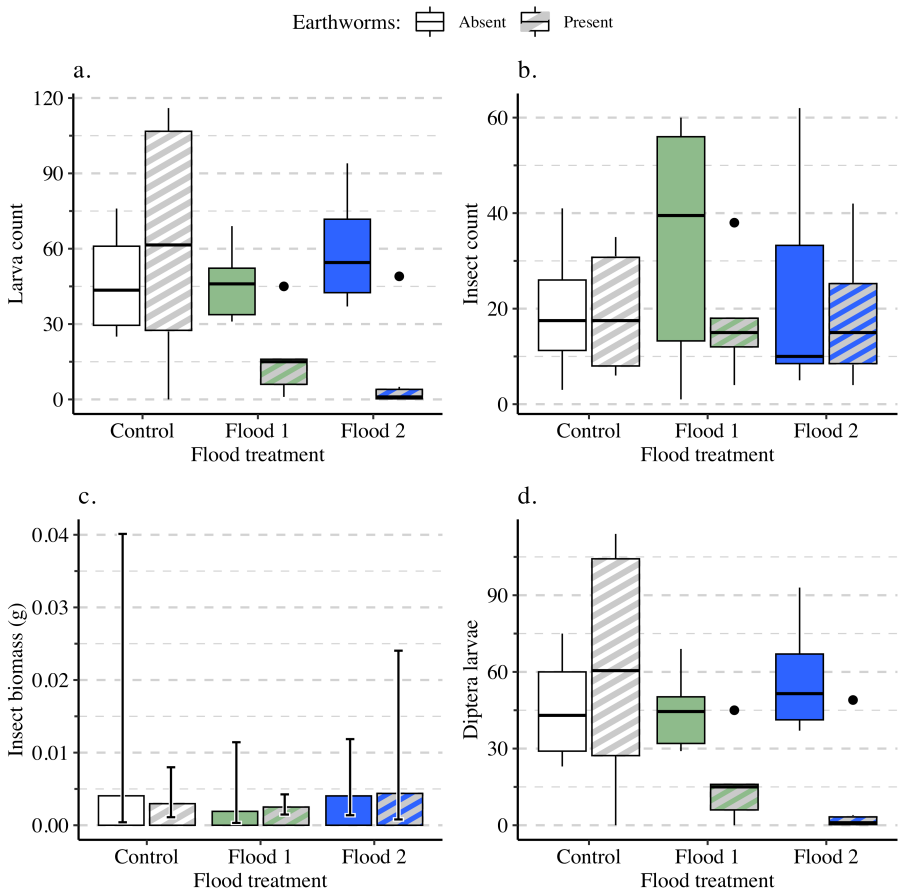


Fig. 4.5. Box plots and bar plot showing *a.* Total larva abundance, *b.* Total insect abundance, *c.* Total insect biomass, and *d.* Diptera larva abundance across flooding and earthworm treatment groups. For *a.*, *b.*, and *d.*, box outer horizontal lines show group interquartile ranges, central horizontal lines show group medians, and extended vertical lines show full data range for each group. Box plot points show outlier data points (>1.5x interquartile range above 75th percentile). Bar heights in *c.* show group means and error bars show 95% confidence intervals with both back-transformed from the log scale.

Discussion

In this study, earthworms contributed significantly to dung decomposition and the effects of earthworms on dung decomposition were robust to the flooding treatment (Fig. 4.2). However, dung insect performance was negatively affected by earthworms in the two flooded treatment levels (Fig. 4.5). This suggests that, although earthworms could provide

benefits for dung decomposition during cool and wet periods, they could also have potential negative impacts on the performance of the wider dung community.

Robustness of earthworms and their services during regular flooding

Earthworms had a significant effect on dung decomposition in this study (Fig. 4.2), consistent with studies documenting their importance for cattle dung decomposition (Holter, 1977; Kaartinen et al., 2013; Schon et al., 2017, 2015). Earthworm community biomass and functional richness impacts dung decomposition (Schon et al., 2017, 2015). Although earthworm community composition was not manipulated in this study, there was a significant effect of surviving *L. terrestris* number on dung decomposition (Fig 4.4). Although this correlation does not necessarily indicate causation, large amounts of cattle dung lining the burrows of *L. terrestris* were observed when mesocosms were deconstructed, suggesting that *L. terrestris* was responsible for removing large amounts of surface dung in this experiment. *Lumbricus terrestris* is an epi-anecic species that feeds at the soil surface from its permanent burrow (Hoeffner et al., 2019) but appears not to move towards dung pats (Bacher et al., 2018). However, in pastures, *L. terrestris* may contribute to cattle dung decomposition if dung is deposited near burrow entrances. Additionally, *L. terrestris* is the largest earthworm species in the UK (Sherlock et al., 2018) and collects large amounts of organic and plant material to form middens at burrow entrances (Stroud et al., 2016a) which act as hotspots for both earthworms and other soil invertebrates (Butt and Lowe, 2007; Hamilton and Sillman, 1989; Stroud et al., 2016a). Therefore, cattle dung incorporated into *L. terrestris* burrows and middens may have beneficial impacts on other soil invertebrates that accumulate in and around middens and burrows.

In this study, earthworm-mediated dung decomposition was highly robust to the flooding treatment, with flooding having no effect on final dung dry mass, either alone or in

interaction with earthworms (Fig 4.2). None of the earthworm species tested were significantly affected by the flooding treatment (Fig. 4.3a – i). Earthworms tolerate flooding through a combination of vertical and horizontal migration, as well as physiological, reproductive, and phenological responses (Plum, 2005); however, due to the sealed nature of the mesocosms in this experiment, horizontal migration as an escape strategy was prevented which may have been expected to lead to mortality of earthworms. Survival of different ecotypes in non-aerated water varies where 100% mortality of *L. terrestris* can occur after 36 hours while *Lumbricus castaneus* (Savigny, 1826), an epigeic species, may show 66% mortality after 28 hours and 100% mortality after 168 hours (Kiss et al., 2021a). *Lumbricus terrestris* can survive in aerated water for several weeks (Roots, 1956), which may explain why it was unaffected by the flooding treatment in this experiment. Sampling of floodplain soils in the Netherlands showed that epigeic *Lumbricus rubellus* Hoffmeister, 1843 abundance and biomass were severely reduced by flooding events of several weeks in duration, while endogeic *A. chlorotica* and *Aporrectodea caliginosa* (Savigny, 1826) were not affected (Zorn et al., 2005). Additionally, *L. rubellus* populations in a floodplain meadow in Northern Germany declined sharply following flooding (Plum and Filser, 2005). However, earthworms can show a strong tendency to escape from flooding, even if they have the capacity of surviving for extended periods when submerged (Ausden et al., 2001), and *L. rubellus* in microcosm flooding experiments has shown a strong tendency to escape enclosures during flooding (Zorn et al., 2008), reflecting the horizontal migration flood avoidance behaviour of epigeic species (Plum, 2005). In the experiment reported in this Chapter, the drainage of the mesocosms after simulated flooding events may have meant that submersion times were insufficient to cause earthworm mortality.

Interactions between the insect dung fauna and earthworms

Interactions between earthworms and the insect dung fauna can have both positive and negative effects on dung decomposition. In a microcosm setting, *Eisenia fetida* (Savigny, 1826) showed positive interactions with the dung fly *Scathophaga stercoraria* (Linnaeus, 1758) (Scathophagidae) but negative interactions with the dung beetles *Agrilinus ater* and *Acrossus rufipes* for dung decomposition (O'hea et al., 2010a). In other cases, the performance of a combination of earthworms and dung beetles may be no better than beetles (Xie et al., 2021) or earthworms (Hirschberger and Bauer, 1994) alone. However, dung beetles and earthworms can also display a positive additive effect on dung decomposition (Holter, 1977; Kaartinen et al., 2013). Dung beetles may be more important for dung decomposition initially, when pat moisture content is high, while earthworms contribute more as the dung dries (Bang et al., 2009). However, in the present study, the incomplete exclusion of dung insects meant that it wasn't possible to analyse interactions between earthworms and the dung fauna on dung decomposition.

The presence of earthworms had a negative effect on dung insect abundances in the two flooded treatments in this study (Fig. 4.5). Insect larva counts were reduced in the presence of earthworms for the two flooding treatments while adult insect counts were reduced in the *Flood 1* treatment. Soil feeding endo-anecic earthworms such as *Aporrectodea nocturna* Evans, 1946 can reduce larva size of tunneller dung beetles such as *Onthophagus yubarinus* Matsumura, 1937 by feeding on buried dung balls (Xie et al., 2021). However, the epigeic earthworm *E. fetida* has been shown to have no effect on brood ball production of the tunneller species *Copris tripartitus* Waterhouse, 1875, while the dung beetles had no effect on earthworm mass change (Bang et al., 2009). Both earthworms (Bouché, 1977, 1972) and dung beetles (Doube et al., 1988) can be divided into functional groups

depending on their ecology and morphology. The functional diversity of both earthworms (Schon et al., 2017, 2015) and dung beetles (Slade et al., 2007) influence dung decomposition due to complementarity in dung utilisation behaviour. Therefore, interactions between dung beetles and earthworms are likely to be highly dependent on the functional composition and traits of the two groups, which may make drawing general conclusions difficult. However, high levels of earthworm activity can result in complete removal of pats from pastures before larvae of Aphodiine beetles can complete development (Finn and Gittings, 2003; Gittings and Giller, 1999), which could result in mortality of these larvae. As earthworms can reach very high densities under dung pats (Bacher et al., 2018), this large-scale dung removal may be common. Additionally, the activity of earthworms may increase exposure of dung insects to environmental stressors. Flooding stress can negatively affect performance of dung-breeding *Culicoides* flies (Diptera, Ceratopogonidae) (Lühken et al., 2014), but in the present study, negative effects of flooding on insect adult and larval abundances were only detected in the presence of earthworms. By disrupting the dung pats and consuming and burying dung, earthworms may have increased larval exposure to the flooding stress in this experiment, increasing mortality and reducing recorded abundances.

Diptera larval abundances increased in the presence of earthworms in *Control* mesocosms (Fig. 4.5), further suggesting that insect and earthworm interactions are context dependent. Gittings and Giller (1999) noted that complete pat removal by earthworms largely occurred during cool, wet, weather which was similar to the conditions present during the experiment reported in this Chapter and may further explain the negative effects of earthworms on insects in the flooded treatments of this study. In this experiment, earthworm performance was also affected by the dung fauna treatment, where the endogeic *A. chlorotica* showed lower growth rates in the presence of the dung fauna, while *L.*

terrestris, a surface-feeding epi-anecic species (Hoeffner et al., 2019), and *D. veneta* showed increased growth. The potential of the 'priming effect' (Holter, 1983), where dung insects increase accessibility of dung pats for earthworms, may depend on earthworm species traits with surface feeding earthworms being positively affected by dung insect activity.

Limited activity of the insect dung fauna

Dung insect colonisation of experimental pats was fairly limited in this experiment, particularly for Coleoptera. The timing of the experiment (September – December) may account for the limited dung insect colonisation in this experiment. Dung beetle assemblages show high degrees of seasonality and spatial segregation, likely as a result of competition (Hanski, 1991). In the UK, dung beetle and dung fly abundances peak in early spring and summer, respectively, although several dung beetle species show peaks between late summer and autumn (Sladecek et al., 2017). Previous sampling conducted in pastures around the study location found that while some species such as *Agrilinus ater* (Degeer, 1774) showed a peak of abundance in June and July, other species such as *Acrossus rufipes* (Linnaeus, 1758) and *Bodilopsis rufus* (Moll, 1782) peaked in late August or September and were still active as late as October (Hanski, 1980). Therefore, while the pat colonisation window was late in the season (10 – 17 September), several common dung beetle species should still have been active and able to colonise the pats.

Few artificial dung pats appeared to have been colonised by dung beetles in this experiment: 35 Coleoptera larvae were recovered from 19 mesocosms, with an additional 13 larvae recovered from six non-exposed mesocosms (see *Supplementary Information*). Although larva counts may reflect larval mortality during development, they do suggest limited dung beetle colonisation of experimental pats. Dung beetles are more effective

dung decomposers than dung flies (Kavanaugh and Manning, 2020; Wu and Sun, 2010) so the low colonisation rates of dung beetles in this study may also be responsible for the limited effects of dung insects on dung decomposition in this experiment (in full analyses presented in the *Appendix*). The low levels of dung beetle colonisation also make it difficult to determine the effects of flooding on their performance. Dung pats are highly anoxic environments (Holter, 1991) and adult dung beetles are able to continue aerobic respiration at very low oxygen concentrations (Holter and Spangenberg, 1997). Although prolonged submersion is fatal to dung beetles (Whipple et al., 2013), regular wetting of pats can increase egg and larva production of *Teuchestes fossor* (Linnaeus, 1758) in pasture (Vessby, 2001). Therefore, the lack of an effect of the flooding treatment in the study reported in this Chapter does not necessarily mean that larvae were not negatively affected.

Conclusions

The study reported here has shown that earthworms can act as major decomposers of cattle dung in the UK and the services provided by these earthworms appear to be robust to flooding. However, fewer insect larvae were found in flooded pats with earthworms than in those without, suggesting that, during times of flooding stress, the activity of earthworms could severely disrupt development of dung insects. While this may have positive effects by reducing pest fly populations, effects could also be negative if dung beetle performance is negatively affected.

Acknowledgements

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Supplementary information

Table S4.1: Spatial arrangement of mesocosms in the experiment showing dung fauna, earthworm, and watering treatments. Mesocosms in red were excluded due to rain shelter failure. Mesocosms were arranged across two rain shelters (A and B). For the analysis, only mesocosms with dung fauna present (F+) were included. Analyses included mesocosms with dung fauna absent (F-) are presented in the *Appendix*.

		Column					
Row	A	1	2	3	4	5	6
	1	1 E+/F-	2 E+/F-	3 E+/F+	4 E+/F+	5 E-/F-	6 E+/F-
	2	7 E-/F+	8 E-/F+	9 E-/F-	10 E+/F-	11 E+/F+	12 E-/F+
	3	13 E+/F-	14 E-/F-	15 E-/F+	16 E+/F+	17 E-/F+	18 E+/F-
	4	19 E-/F+	20 E+/F+	21 E+/F-	22 E+/F+	23 E+/F-	24 E+/F+
	5	25 E-/F-	26 E-/F-	27 E-/F-	28 E-/F-	29 E-/F+	30 E+/F-
	6	31 E-/F-	32 E-/F+	33 E+/F+	34 E+/F-	35 E-/F+	36 E-/F+
	B	1	2	3	4	5	6
Row	7	37 E+/F-	38 E+/F-	39 E-/F+	40 E+/F-	41 E+/F-	42 E-/F-
	8	43 E-/F-	44 E+/F+	45 E+/F+	46 E+/F+	47 E+/F+	48 E-/F+
	9	49 E+/F+	50 E-/F-	51 E-/F-	52 E+/F-	53 E+/F+	54 E-/F-
	10	55 E-/F+	56 E+/F+	57 E-/F-	58 E+/F-	59 E+/F+	60 E+/F-
	11	61 E-/F-	62 E-/F+	63 E+/F-	64 E+/F+	65 E-/F+	66 E-/F+
	12	67 E-/F-	68 E-/F+	69 E-/F+	70 E-/F-	71 E-/F-	72 E+/F+

Key

Watering treatment
Control =
Flood 1 =
Flood 2 =

Flooded mesocosms
- Included
- Excluded

Fauna treatments
Earthworms
E- = Absent
E+ = Present

Dung fauna
F- = Absent
F+ = Present

Table S4.2: Summary of ANOVA (Type II) results for effects of earthworm species and flooding treatment on log-transformed total starting earthworm mass (g).

Variable	Total start mass (g)		
	Df	F	P
Flooding	2	1.31	0.280
Species	2	1565.47	**<0.001**
Flooding x Species	4	1.17	0.339
Residuals	42		

Dung fauna exclusion method

The dung fauna exclusion method failed to exclude dung insects fully. On average, control dung pats contained 21.6± 3.6 adult insects and 14.6± 4.1 larvae while exposed pats contained 22.3± 3.2 adult insects and 40.5± 5.7 larvae. The dung fauna exclusion method did not significantly reduce adult insect numbers (Fig. S4.2; $X^2 = 0.37$, $p = 0.55$) or biomass (Fig. S4.2; $F_{1,64} = 3.2$, $p = 0.08$) but did significantly reduce larva numbers (ratio = 0.36, $CI_{95\%}$ = [0.33, 0.40], $p < 0.001$), suggesting that egg−laying was reduced. Thirty-three dung fauna-containing mesocosms were retained for analyses (see *Methods*, Table S4.1, S4.3) from which a total of 735 adult insects and 1336 larvae (1301 Diptera + 35 Coleoptera) were collected, with Coleoptera larvae found in 19 mesocosms. Insect counts showed a wide range for both adults (μ = 22.27± 3.15, range = 1 — 62) and larvae (μ = 40.48± 5.73, range = 0 — 116).

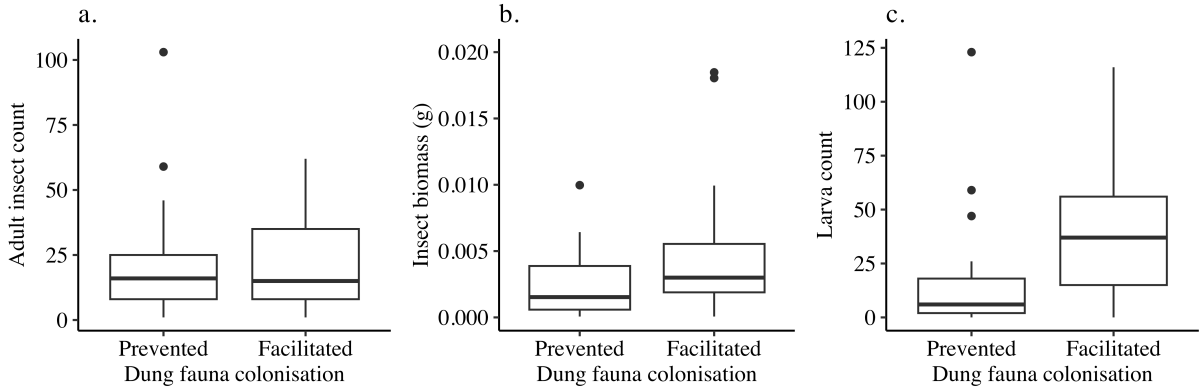


Fig. S4.2: Boxplots showing *a.* adult insect number, *b.* insect biomass, and *c.* insect larva counts across dung pats with colonisation excluded and open to insect colonisation. There were no significant differences between treatments in *a.* and *b.* ($p > 0.05$) but larva counts were significantly reduced when colonisation was prevented (Df = 1, $X^2 = 417.4$, $p < 0.001$). In *b.*, one outlier value of 0.079g has been excluded from the 'Facilitated' treatment group for ease of viewing the other data.

Table S4.3: Replicate numbers for treatment combinations after exclusion of flooded mesocosms. Analysis in the *Results* section uses only mesocosms with dung fauna absent. Analyses with the full dataset are presented in the *Appendix*.

Flooding treatment	Earthworms	Dung fauna	Replicates
Control	Absent	Absent	6
Control	Absent	Present	4
Control	Present	Absent	6
Control	Present	Present	6
Flood 1	Absent	Absent	4
Flood 1	Absent	Present	6
Flood 1	Present	Absent	6
Flood 1	Present	Present	5
Flood 2	Absent	Absent	6
Flood 2	Absent	Present	6
Flood 2	Present	Absent	5
Flood 2	Present	Present	6

Table S4.4: pairwise comparisons for wet dung mass across earthworm (E− = earthworms absent; E+ = earthworms present) and flooding treatment levels

Contrast	Difference	95% CI		P
		Lower	Upper	
E+ Control - E− Control	⁻100.58	⁻165.43	⁻35.73	**0.001**
E+ Control - E+ Flood 1	48.17	⁻12.67	109.00	0.183
E+ Control - E− Flood 1	⁻162.17	⁻220.17	⁻104.16	**<0.001**
E+ Control - E+ Flood 2	79.17	21.16	137.17	**0.003**
E+ Control - E− Flood 2	⁻178.33	⁻236.34	⁻120.33	**<0.001**
E− Control - E+ Flood 1	148.75	81.36	216.14	**<0.001**
E− Control - E− Flood 1	⁻61.58	⁻126.43	3.27	0.070
E− Control - E+ Flood 2	179.75	114.90	244.60	**<0.001**
E− Control - E− Flood 2	⁻77.75	⁻142.60	⁻12.90	**0.012**
E+ Flood 1 - E− Flood 1	⁻210.33	⁻271.17	⁻149.50	**<0.001**
E+ Flood 1 - E+ Flood 2	31.00	-29.83	91.83	0.630
E+ Flood 1 - E− Flood 2	⁻226.50	⁻287.33	⁻165.67	**<0.001**
E− Flood 1 - E+ Flood 2	241.33	183.33	299.34	**<0.001**
E− Flood 1 - E− Flood 2	⁻16.17	⁻74.17	41.84	0.954
E+ Flood 2 - E− Flood 2	⁻257.50	⁻315.50	⁻199.50	**<0.001**

Table S4.5: pairwise comparisons for dry dung mass across earthworm (E− = earthworms absent; E+ = earthworms present) and flooding treatment levels

Contrast	Difference	95% CI		P
		Lower	Upper	
E+ Control - E− Control	⁻18.92	⁻39.47	1.64	0.085
E+ Control - E+ Flood 1	7.43	⁻11.85	26.72	0.842
E+ Control - E− Flood 1	⁻15.83	⁻34.22	2.55	0.122
E+ Control - E+ Flood 2	19.33	0.95	37.72	**0.035**
E+ Control - E− Flood 2	⁻22.00	⁻40.38	⁻3.62	**0.012**
E− Control - E+ Flood 1	26.35	4.99	47.71	**0.009**
E− Control - E− Flood 1	3.08	⁻17.47	23.64	0.997
E− Control - E+ Flood 2	38.25	17.70	58.80	**<0.001**
E− Control - E− Flood 2	⁻3.08	⁻23.64	17.47	0.997
E+ Flood 1 - E− Flood 1	⁻23.27	⁻42.55	⁻3.98	**0.011**
E+ Flood 1 - E+ Flood 2	11.90	⁻7.38	31.18	0.429
E+ Flood 1 - E− Flood 2	⁻29.43	⁻48.72	⁻10.15	**0.001**
E− Flood 1 - E+ Flood 2	35.17	16.78	53.55	**<0.001**
E− Flood 1 - E− Flood 2	⁻6.17	⁻24.55	12.22	0.904
E+ Flood 2 - E− Flood 2	⁻41.33	⁻59.72	⁻22.95	**<0.001**

Table S4.6: contrast table for *Lumbricus terrestris* total mass change across flooding treatment levels.

Contrast	Difference	95% CI		P
		Lower	Upper	
Control - Flood 1	⁻4.44	⁻9.37	0.50	0.081
Control - Flood 2	⁻4.47	⁻9.18	0.24	0.064
Flood 1 - Flood 2	⁻0.03	⁻4.97	4.90	1.000

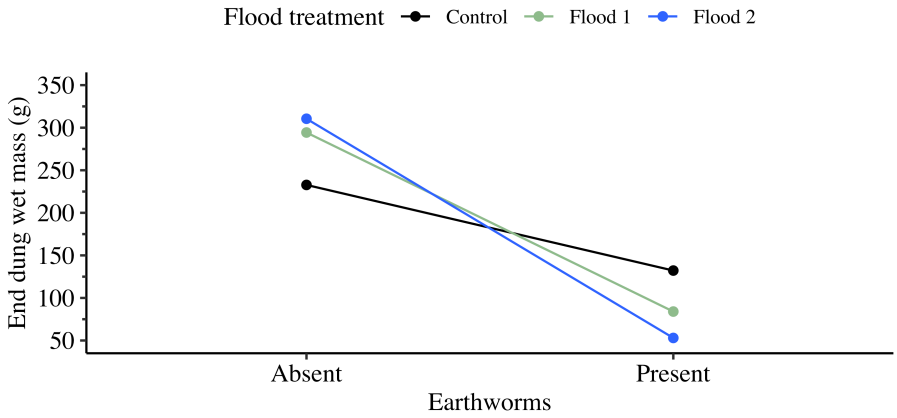


Fig. S4.1: Interaction plot showing effects of earthworms on end dung wet mass (g) across flooding treatment levels.

Table S4.7: contrast table for insect larva counts across earthworm (E− = earthworms absent; E+ = earthworms present) and flooding treatment groups. Ratios have been back-transformed from the log scale.

Contrast	Ratio	95% CI		P
		Lower	Upper	
E+ Control / E− Control	1.34	1.04	1.72	**0.015**
E+ Control / E+ Flood 1	3.79	2.68	5.35	**<0.001**
E+ Control / E− Flood 1	1.37	1.09	1.71	**0.001**
E+ Control / E+ Flood 2	6.73	4.48	10.12	**<0.001**
E+ Control / E− Flood 2	1.06	0.86	1.31	0.972
E− Control / E+ Flood 1	2.83	1.94	4.12	**<0.001**
E− Control / E− Flood 1	1.02	0.78	1.34	1.000
E− Control / E+ Flood 2	5.04	3.26	7.77	**<0.001**
E− Control / E− Flood 2	0.79	0.61	1.02	0.101
E+ Flood 1 / E− Flood 1	0.36	0.25	0.52	**<0.001**
E+ Flood 1 / E+ Flood 2	1.78	1.09	2.91	**0.011**
E+ Flood 1 / E− Flood 2	0.28	0.20	0.40	**<0.001**
E− Flood 1 / E+ Flood 2	4.93	3.25	7.48	**<0.001**
E− Flood 1 / E− Flood 2	0.78	0.62	0.97	**0.019**
E+ Flood 2 / E− Flood 2	0.16	0.10	0.24	**<0.001**

Table S4.8: contrast table for adult insect counts across earthworm (E- = earthworms absent; E+ = earthworms present) and flooding treatment groups. Ratios have been back-transformed from the log scale.

Contrast	Ratio	95% CI		P
		Lower	Upper	
E+ Control / E− Control	0.98	0.65	1.48	1.000
E+ Control / E+ Flood 1	1.11	0.74	1.66	0.977
E+ Control / E− Flood 1	0.56	0.40	0.78	**<0.001**
E+ Control / E+ Flood 2	1.05	0.72	1.53	0.999
E+ Control / E− Flood 2	0.85	0.59	1.22	0.808
E− Control / E+ Flood 1	1.14	0.73	1.77	0.965
E− Control / E− Flood 1	0.58	0.39	0.84	**<0.001**
E− Control / E+ Flood 2	1.07	0.70	1.62	0.998
E− Control / E− Flood 2	0.87	0.58	1.30	0.926
E+ Flood 1 / E− Flood 1	0.51	0.35	0.73	**<0.001**
E+ Flood 1 / E+ Flood 2	0.94	0.63	1.41	0.998
E+ Flood 1 / E− Flood 2	0.77	0.52	1.14	0.386
E− Flood 1 / E+ Flood 2	1.86	1.33	2.60	**<0.001**
E− Flood 1 / E− Flood 2	1.51	1.11	2.08	**0.002**
E+ Flood 2 / E− Flood 2	0.82	0.57	1.18	0.607

Table S4.9: contrast table for Diptera larva counts across earthworm (E- = earthworms absent; E+ = earthworms present) and flooding treatment groups. Ratios have been back-transformed from the log scale.

Contrast	Ratio	95% CI		P
		Lower	Upper	
E+ Control / E− Control	1.34	1.04	1.73	**0.015**
E+ Control / E+ Flood 1	3.76	2.66	5.32	**<0.001**
E+ Control / E− Flood 1	1.39	1.10	1.74	**0.001**
E+ Control / E+ Flood 2	6.73	4.46	10.16	**<0.001**
E+ Control / E− Flood 2	1.08	0.87	1.34	0.915
E− Control / E+ Flood 1	2.80	1.92	4.09	**<0.001**
E− Control / E− Flood 1	1.03	0.79	1.36	0.999
E− Control / E+ Flood 2	5.02	3.24	7.78	**<0.001**
E− Control / E− Flood 2	0.80	0.62	1.04	0.164
E+ Flood 1 / E− Flood 1	0.37	0.26	0.53	**<0.001**
E+ Flood 1 / E+ Flood 2	1.79	1.09	2.94	**0.011**
E+ Flood 1 / E− Flood 2	0.29	0.20	0.41	**<0.001**
E− Flood 1 / E+ Flood 2	4.85	3.18	7.40	**<0.001**
E− Flood 1 / E− Flood 2	0.78	0.62	0.98	**0.026**
E+ Flood 2 / E− Flood 2	0.16	0.11	0.24	**<0.001**

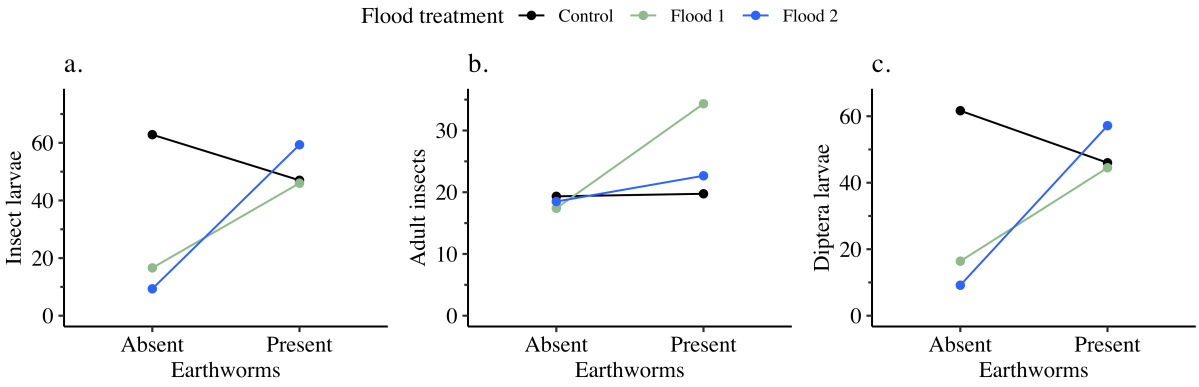


Fig. S4.3: Interaction plots showing effects of the flooding treatment and earthworms on *a.* Insect larva abundances, *b.* adult insect abundances, and *c.* Diptera larva abundances.

Appendix

This appendix presents analyses of the full dataset with dung fauna-excluded mesocosms included. With these included, there were 66 mesocosms in total with no significant differences in earthworm starting mass across flooding and dung fauna treatment groups (Table A4.1).

Table A4.1: Summary of ANOVA (Type II) results for effects of earthworm species, flooding treatment, and dung fauna on log-transformed total starting earthworm mass (g).

Variable	Total start mass (g)		
	Df	F	P
Flooding	2	0.26	0.769
Dung fauna	1	0.13	0.718
Species	2	2869.12	**<0.001**
Flooding x Dung fauna	2	1.09	0.340
Flooding x Species	4	1.14	0.343
Dung fauna x Species	2	0.23	0.797
Flooding x Dung fauna x Species	4	1.68	0.163
Residuals	84		

Full analysis methods

All data were analysed using R version 4.3.1 (R Core Team, 2023). Due to partial failure of the rain shelter, treatments in six mesocosms were compromised by rainwater and these were excluded from all analyses (Table S4.1, S4.3). Two excluded mesocosms were from the *Control* + earthworms absent + dung fauna present treatment group, two were from the *Flood 1* + earthworms absent + dung fauna absent treatment group, and one mesocosm was excluded from both the *Flood 1* + earthworms present + dung fauna present and *Flood 2* + earthworms present + dung fauna absent treatment groups. Type II Analysis of Variance (ANOVA) was performed for all response variables using the *car* package (Fox

and Weisberg, 2019) to account for differences in sample size across groups. For dung and soil response variables, the flooding, earthworm, and dung fauna treatments, and all possible interactions, were included in models. Earthworm response variables were analysed separately for each species with the flooding treatment, dung fauna treatment, and their interaction included in models. The dung fauna exclusion method was not fully successful (see *Results*); however, only exposed dung pat mesocosms were included in analysis of dung fauna response variables, with the flooding treatment, earthworm treatment, and their interaction, included as explanatory variables.

End dung wet mass, end dung dry mass, surface soil moisture content, and mean and total earthworm mass change were all analysed with ANOVA. Additionally, the effects of surviving earthworm number, flooding treatment, and their interaction, on end dung dry mass were analysed with Analysis of Covariance (ANCOVA) with non-significant interactions and variables removed from the model before reporting and interpreting regression slopes. Diagnostic plots of all models were inspected and end dung dry mass was log-transformed for ANCOVA analyses to better fit model assumptions. When factor variables or interactions had statistically significant effects on response variables, *post hoc* group comparisons were performed with the *emmeans* package (Lenth, 2023). Group differences were back-transformed to the response scale for interpretation and reporting for GLMs. All plots and figures were produced in R with the *ggplot2* package (Wickham, 2016).

Full analysis results

Dung decomposition and soil moisture

For both end dung dry mass (Fig. A4.1a) and wet mass (Fig. 4A.1b), there was no statistically significant three-way interaction among variables (Table 4A.2). However, earthworms significantly reduced end dung dry mass (difference = −26.1 g, $CI_{95\%}$ = [−31.4, −20.8], $p < 0.001$). For wet dung mass, there were significant two-way interactions between flooding and dung fauna, and earthworms and flooding (Table A4.2, A4.3, A4.4). Earthworms reduced end dung wet mass for all flooding treatments levels (Table A4.3) but had then greatest effect in *Flood 2* (difference = −244 g, $CI_{95\%}$ = [−180.8, −12.8], $p <$ 0.001), followed by *Flood 1* (difference = −180.0 g, $CI_{95\%}$ = [−134.3, −9.7], $p < 0.001$), and the lowest effect in *Controls* (difference = −118.0 g, $CI_{95\%}$ = [−73.6, 6.5], $p < 0.001$). In the presence of earthworms, end dung wet mass decreased from *Control* to *Flood 1* to *Flood 2* whereas the opposite trend was observed in the absence of earthworms (Fig. A4.1b, A4.2a). For the dung fauna and flooding interaction (Table A4.4, Fig. A4.1b, A4.2b), end wet dung mass was significantly higher, in the absence of dung fauna, in *Flood 1* than *Controls* (difference = 55.7 g, $CI_{95\%}$ = [5.1, 112.3], $p = 0.02$) and *Flood 2* than in *Controls* (difference = 56.6 g, $CI_{95\%}$ = [4.8, 108.4], $p = 0.02$) with no other significant pairwise differences (Table A4.4). Surface soil moisture content (Fig. A4.1c) was significantly increased with earthworms present (Table A4.2; difference = 5.08, $CI_{95\%}$ = [3.33, 6.82], $p < 0.001$) and was also significantly higher moisture than in *Controls* for both *Flood 1* (Table A4.3; difference = 4.53, $CI_{95\%}$ = [1.92, 7.14], $p < 0.001$) and *Flood 2* (Table A4.2; difference = 4.89, $CI_{95\%}$ = [2.35, 7.42], $p < 0.001$).

Table A4.2: Summary of ANOVA (Type II) results showing effects of earthworms, flooding, dung fauna, and their interactions on end dry dung mass, end wet dung mass, and soil moisture content.

Variable	Dung dry mass			Dung wet mass			Soil moisture		
	Df	F	P	Df	F	P	Df	F	P
Earthworms	1	99.31	**<0.001**	1	289.96	**<0.001**	1	35.88	**<0.001**
Dung fauna	1	0.53	0.469	1	0.08	0.785	1	0.85	0.361
Flooding	2	0.82	0.444	2	3.29	**0.045**	2	13.81	**<0.001**
Earthworms x Dung fauna	1	0.68	0.414	1	2.19	0.144	1	0.01	0.928
Earthworms x Flooding	2	2.70	0.077	2	8.36	**0.001**	2	0.14	0.873
Dung fauna x Flooding	2	1.56	0.220	2	3.51	**0.037**	2	1.36	0.265
Earthworms x Dung fauna x Flooding	2	1.42	0.250	2	2.52	0.089	2	1.95	0.152
Residuals	54			54			54		

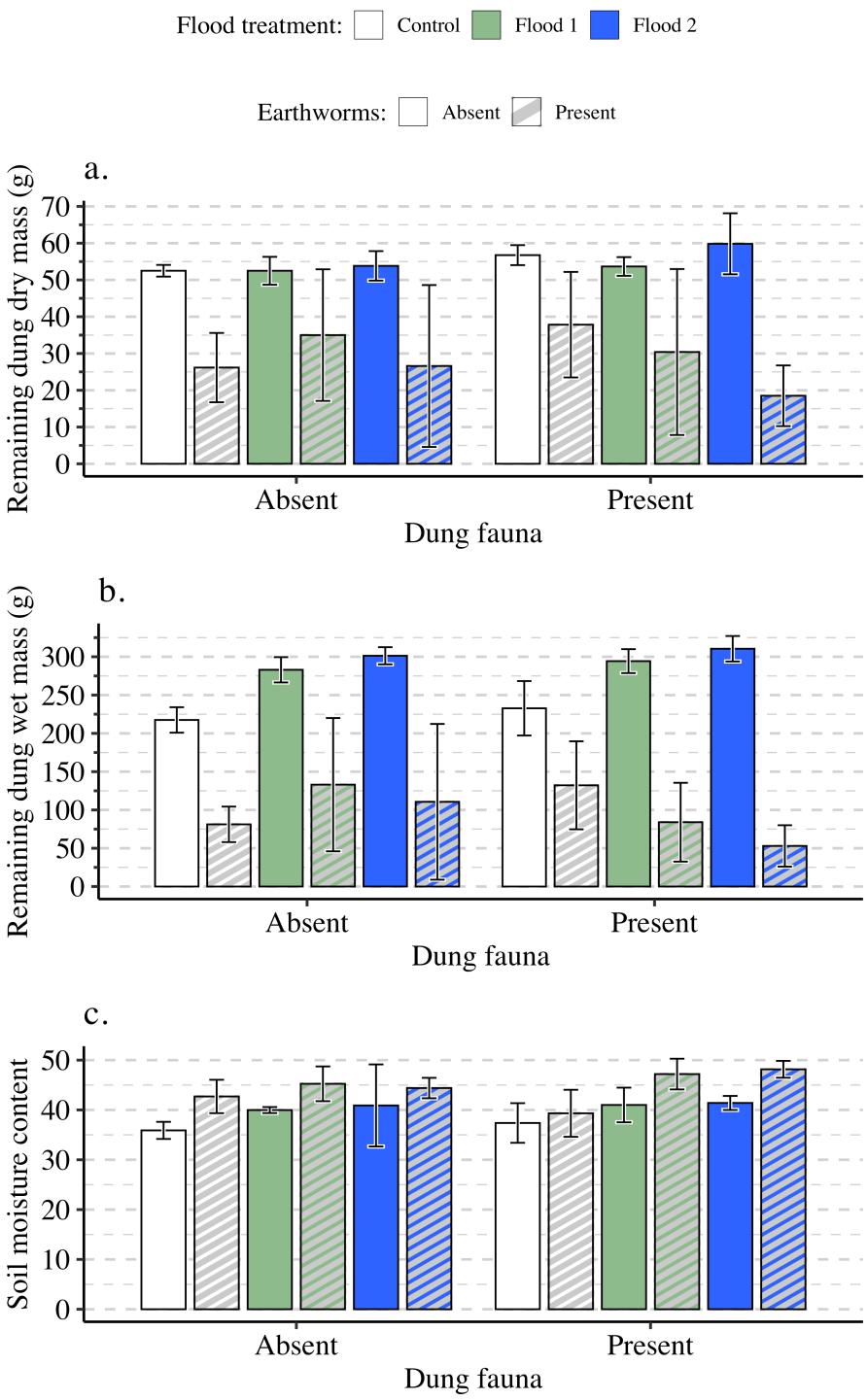


Fig. A4.1: Bar plots showing *a.* end dry dung mass, *b.* end wet dung mass, and *c.* surface soil moisture across flooding, earthworm, and dung fauna treatment groups. Bar heights show group means and error bars show 95% confidence intervals.

Table A4.3: pairwise comparisons for wet dung mass across earthworm (E− = earthworms absent; E+ = earthworms present) and flooding treatment levels (groups averaged over levels of dung fauna treatment)

Contrast	Difference	95% CI		P
		Lower	Upper	
(E+ Control) - E− Control	-118.46	-172.07	-64.85	**<0.001**
(E+ Control) - (E+ Flood 1)	-1.83	-53.62	49.96	1.000
(E+ Control) - E− Flood 1	-182.00	-235.61	-128.39	**<0.001**
(E+ Control) - (E+ Flood 2)	24.87	-26.92	76.66	0.716
(E+ Control) - E− Flood 2	-199.25	-249.79	-148.71	**<0.001**
E− Control - (E+ Flood 1)	116.62	61.84	171.41	**<0.001**
E− Control - E− Flood 1	-63.54	-120.05	-7.04	**0.019**
E− Control - (E+ Flood 2)	143.33	88.54	198.11	**<0.001**
E− Control - E− Flood 2	-80.79	-134.40	-27.18	**0.001**
(E+ Flood 1) - E− Flood 1	-180.17	-234.95	-125.38	**<0.001**
(E+ Flood 1) - (E+ Flood 2)	26.70	-26.31	79.71	0.673
(E+ Flood 1) - E− Flood 2	-197.42	-249.21	-145.63	**<0.001**
E− Flood 1 - (E+ Flood 2)	206.87	152.08	261.65	**<0.001**
E− Flood 1 - E− Flood 2	-17.25	-70.86	36.36	0.931
(E+ Flood 2) - E− Flood 2	-224.12	-275.91	-172.33	**<0.001**

Table A4.4: pairwise comparisons for wet dung mass across dung fauna (F− = dung fauna colonisation facilitated; F+ = dung fauna colonisation prevented) and flooding treatment levels (groups averaged over levels of earthworm treatment)

Contrast	Difference	95% CI		P
		Lower	Upper	
F+ Control - F− Control	33.13	-20.48	86.73	0.458
F+ Control - F+ Flood 1	-6.71	-61.49	48.08	0.999
F+ Control - F− Flood 1	-25.54	-82.05	30.96	0.764
F+ Control - F+ Flood 2	0.71	-52.90	54.32	1.000
F+ Control - F− Flood 2	-23.51	-78.29	31.28	0.801
F− Control - F+ Flood 1	-39.83	-91.62	11.96	0.223
F− Control - F− Flood 1	-58.67	-112.27	-5.06	**0.024**
F− Control - F+ Flood 2	-32.42	-82.96	18.12	0.416
F− Control - F− Flood 2	-56.63	-108.42	-4.84	**0.024**
F+ Flood 1 - F− Flood 1	-18.83	-73.62	35.95	0.911
F+ Flood 1 - F+ Flood 2	7.42	-44.37	59.21	0.998
F+ Flood 1 - F− Flood 2	-16.80	-69.81	36.21	0.935
F− Flood 1 - F+ Flood 2	26.25	-27.36	79.86	0.699
F− Flood 1 - F− Flood 2	2.03	-52.75	56.82	1.000
F+ Flood 2 - F− Flood 2	-24.22	-76.01	27.57	0.738

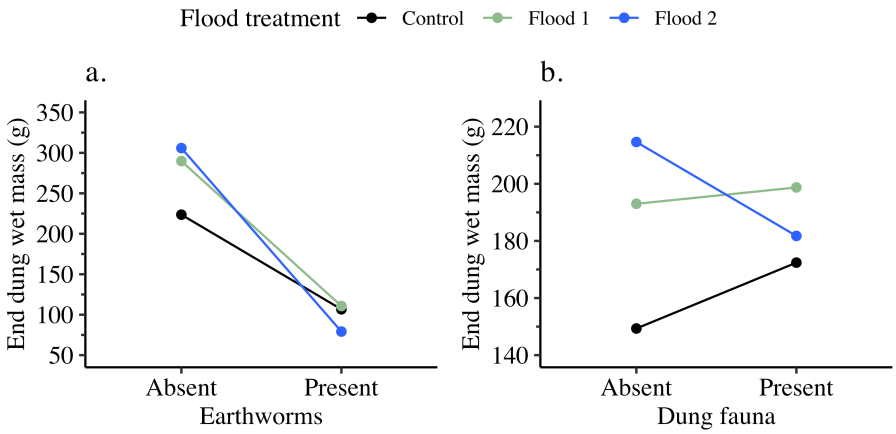


Fig. A4.2: Interaction plots showing effects of *a.* earthworms and *b.* dung fauna on end dung wet mass (g) across flooding treatment levels.

Earthworm performance

Survival and mass change

Thirty-four earthworm-containing mesocosms were kept for analysis (see *Methods*, Table S4.1, S4.3). Of the 476 earthworms added to these mesocosms, 403 earthworms were recovered alive at the end of the experiment, representing a survival rate of 84.7%.

Lumbricus terrestris survival (overall = 73.5%) was not affected significantly by the flooding or dung fauna treatments (Table A4.5; Fig. A4.3ba). Total *L. terrestris* mass decreased by 2.45 g ($CI_{95\%}$ = [4.00, 0.91], p = 0.003) with no significant differences between treatments (Table A4.5; Fig. A4.3b. However, mean mass increased in the presence of the dung fauna but decreased in controls (Table A4.5; Fig. A4.3c, difference = 1.53 g, $CI_{95\%}$ = [0.08, 2.97], p = 0.04).

Allolobophora chlorotica survival (overall = 89.2%) was significantly reduced in the presence of the dung fauna (Table A4.5; Fig. A4.3d, odds ratio = 0.31, $CI_{95\%}$ = [0.11,

0.88], $p = 0.028$). Total *A. chlorotica* mass increased by 0.34 g ($CI_{95\%}$ = [0.26, 0.41]) but increases were significantly lower in the presence of dung fauna (Table A4.5; Fig. A4.3e; difference = −0.19, $CI_{95\%}$ = [−0.32, −0.05], $p = 0.009$). Mean *A. chlorotica* mass increased by 0.088 g ($CI_{95\%}$ = [0.079, 0.096], $p < 0.001$) but was not affected by the flooding or dung fauna treatments (Table A4.5; Fig. A4.3f).

Dendrobaena veneta survival (overall = 83.8%) was significantly lower in the *Flood 2* treatment compared to *Controls* (Table A4.5; Fig. A4.3g; odds ratio = 0.16, $CI_{95\%}$ = [0.03, 0.84]. $p = 0.026$) and significantly higher in the presence of the dung fauna (Table A4.5; Fig. A4.3g; odds ratio = 4.06, $CI_{95\%}$ = [1.26, 13.10], $p = 0.019$). Total *D. veneta* mass decreased by 1.16 g ($CI_{95\%}$ = [1.82, 0.50]), but this loss was reduced in the presence of the dung fauna (Table A4.5; Fig. A4.3h; difference = −1.56 g, $CI_{95\%}$ = [−2.68, −0.44], $p = 0.008$) while mean mass change showed no overall change ($\mu = -0.08$ g, $CI_{95\%}$ = [−0.18, 0.01], $p = 0.9$) and was not affected by experimental treatments.

Table A4.5: Summary of ANOVA (Type II) results for effects of flooding, dung fauna, and their interactions on earthworm response variables. Analyses were performed separately for each species.

Species	Variable	Survival			Total mass change			Mean mass change		
		Df	X^2	P	Df	F	P	Df	F	P
Lumbricus terrestris	Flooding	2	0.23	0.890	2	0.26	0.769	2	0.55	0.582
	Dung fauna	1	0.28	0.598	1	0.77	0.388	1	4.30	**0.048**
	Flooding x Dung fauna	2	5.11	0.078	2	2.60	0.092	2	2.36	0.113
	Residuals	28			28			28		
Allolobophora chlorotica	Flooding	2	0.15	0.929	2	0.47	0.630	2	1.65	0.210
	Dung fauna	1	5.21	**0.023**	1	8.23	**0.008**	1	0.13	0.721
	Flooding x Dung fauna	2	1.54	0.464	2	0.93	0.408	2	0.53	0.595
	Residuals	28			28			28		
Dendrobaena veneta	Flooding	2	15.84	**<0.001**	2	3.03	0.064	2	0.92	0.410
	Dung fauna	1	18.57	**<0.001**	1	7.52	**0.011**	1	2.46	0.128
	Flooding x Dung fauna	2	5.54	0.063	2	2.74	0.082	2	0.82	0.451
	Residuals	28			28			28		

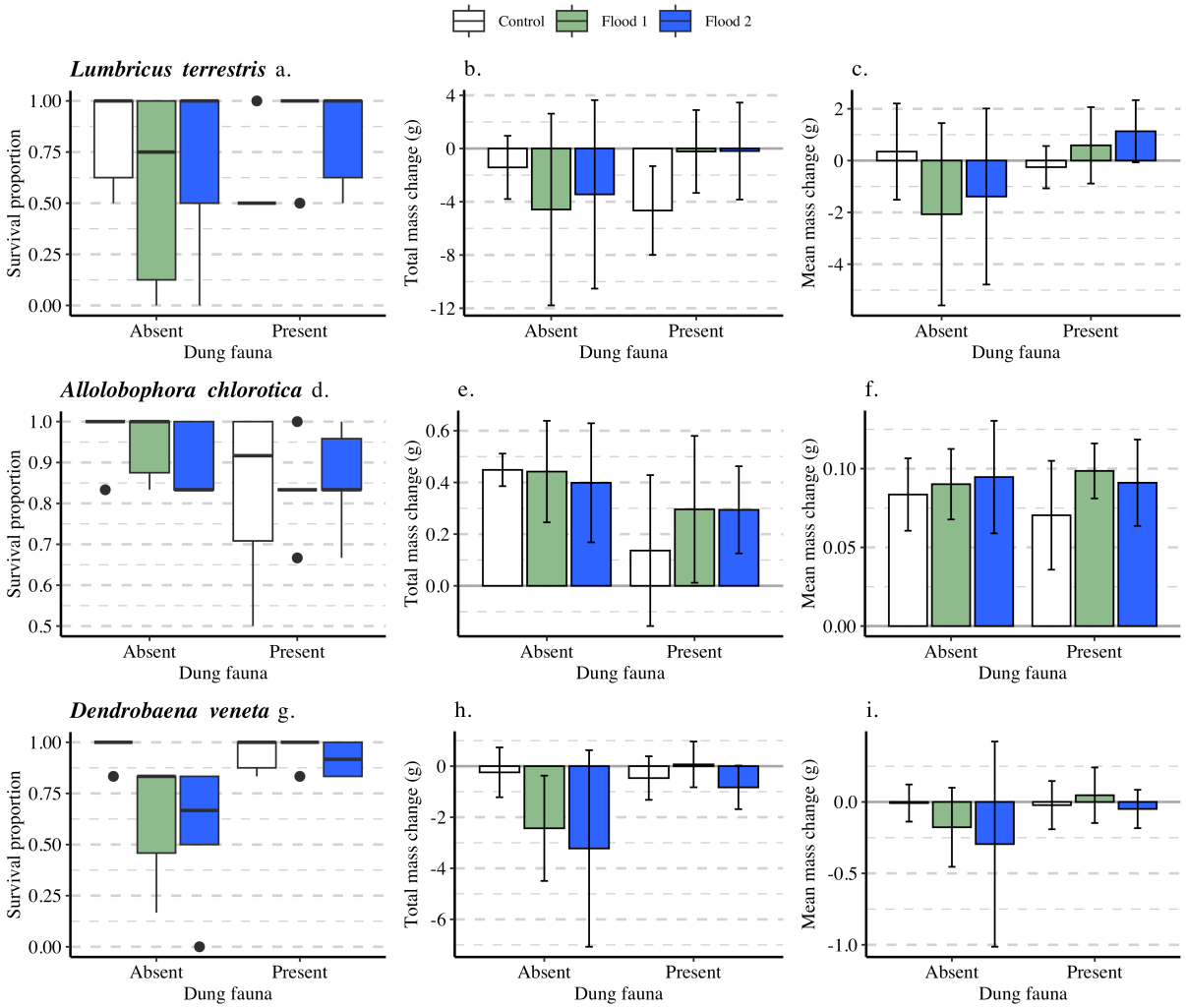


Fig. A4.3: Box plots showing survival and bar plots showing mean and total mass change of *Lumbricus terrestris* (*a.* - *c.*), *Allolobophora chlorotica* (*d.* - *f.*), and *Dendrobaena veneta* (*g.* - *i.*) across flooding and dung fauna treatment groups. Flooding treatments are represented by bar / box colour. For bar plots, bar heights show group means and error bars show 95% confidence intervals. For box plots, box outer horizontal lines show group interquartile ranges, central horizontal lines show group medians, and extended vertical lines show full data range for each group. Box plot points below medians show lower outlier data points (>1.5x interquartile range below 25th percentile) while points above medians upper outlier data points (>1.5x interquartile range above 75th percentile).

Effects of earthworm survival on dung decomposition

There was a significant negative effect of both surviving earthworm number (Fig. A4.4a; $b_{log} = -0.11$, $CI_{95\%} = [-0.21, -0.01]$, $p = 0.026$) and *L. terrestris* survival (Fig. A4.4b; $b_{log} = -0.43$, $CI_{95\%} = [-0.69, -0.16]$, $p = 0.002$) on log-transformed end dung dry mass (Table A4.6), but no effect of surviving *A. chlorotica* or *D. veneta* (Table A4.6; Fig. A4.4c, A4.4d). For both total surviving earthworm number and *D. veneta* survival, end dung dry mass was significantly reduced in *Flood 2* compared to other treatment levels (Table A4.6; Fig. A4.4a, A4.4d).

Table A4.6: Summary of ANCOVA (Type II) results showing effects of surviving earthworm number, flooding treatment, and their interaction on end dung pat dry mass. Separate models with survival of all earthworms and each individual species as explanatory variables were constructed.

Species	Variable	Df	F	P
All	Surviving earthworms	1	5.24	**0.030**
	Flooding	2	4.00	**0.030**
	Surviving earthworms x Flooding	2	0.27	0.767
	Residuals	28		
Lumbricus terrestris	Surviving earthworms	1	10.34	**0.003**
	Flooding	2	2.72	0.083
	Surviving earthworms x Flooding	2	0.57	0.572
	Residuals	28		
Allolobophora chlorotica	Surviving earthworms	1	0.40	0.535
	Flooding	2	2.35	0.114
	Surviving earthworms x Flooding	2	0.19	0.829
	Residuals	28		
Dendrobaena veneta	Surviving earthworms	1	3.72	0.064
	Flooding	2	3.82	**0.034**
	Surviving earthworms x Flooding	2	0.01	0.991
	Residuals	28		

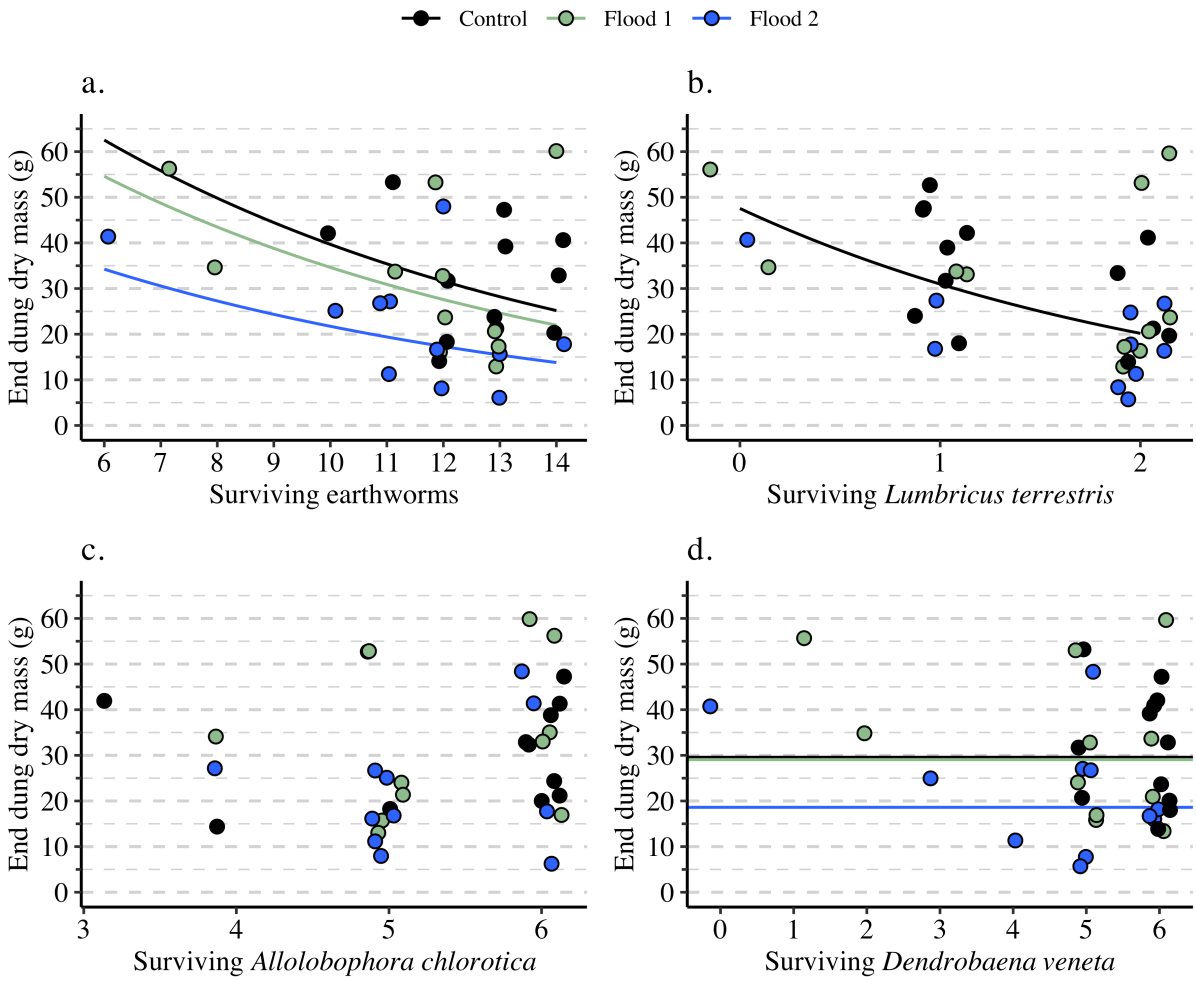


Fig. A4.4: Scatter plots showing correlation between end dry dung mass and *a.* total earthworm survival, *b. Lumbricus terrestris* survival, *c. Allolobophora chlorotica* survival, and *d. Dendrobaena veneta* survival. Lines of best fit are shown in *a.* and *b.* as regressions were statistically significant ($p < 0.05$). In *a.* multiple lines of best fit are displayed as the flooding treatment had a statistically significant effect ($p < 0.05$). Multiple horizontal lines are plotted in *d.* as there was a statistically significant main effect of flooding ($p < 0.05$) but no statistically significant effect of surviving *D. veneta* number ($p > 0.05$). Point and line colours represent flooding treatments (black = control, green = Flood 1, and blue = Flood 2). Lines of best fit in *a.* and *b.* have been back-transformed from the log scale. A jitter has been applied on the x axis for all points in all plots to reduce overplotting.

Discussion on challenges of the dung fauna colonisation treatment

This study attempted to facilitate natural colonisation of dung pats to sample a wide range of the insect dung fauna (Skidmore, 1991) and compare their performance to insect-free

controls, an approach that has been used previously to allow colonisation by dung flies (Kavanaugh and Manning, 2020). However, the exclusion treatment used in the present study was only partially successful: larva numbers were significantly reduced but adult insect numbers and biomass were similar between exposed and covered pats (see *Supplementary information – Dung fauna exclusion method*). Although dung insect larvae are the primary consumers of solid dung (Holter, 2016), the entry of adult insects into the control pats and mesocosms could be responsible for the lack of differences between the two dung fauna treatment levels. It is not clear if the larvae and adult insects found in exclusion pats originated from successful colonisation of the pats, or if they originated from eggs or larvae that were in the dung when it was collected from the field. Fresh dung (<24 hours old) was collected and mechanically homogenised before it was formed into artificial pats. This process should have minimised contamination risk of dung fauna exclusion pats. However, some eggs or larvae may have been present and persisted in the dung and continued development in the dung fauna excluded pats. Previous studies have typically introduced known quantities and compositions of dung insects, primarily adult dung beetles, into pats (Beynon et al., 2012b, 2012a; Manning et al., 2016, 2017b; Slade and Roslin, 2016). This approach has advantages as it ensures that all treatment pats will be 'colonised' and reduces chance of insects entering control pats, but experimental communities are often simple or monocultures. While natural colonisation treatments appear effective for dung flies, direct application of dung beetles may be necessary to ensure colonisation (Kavanaugh and Manning, 2020). A hybrid approach may therefore be beneficial for future studies: large sample pats could be used to collect many individuals and species which could then be divided up across experimental units, controlling for abundance or biomass. This would allow analysis of the effects of species richness,

diversity, and individual effects of rare species while also ensuring a clear difference between controls and ‘colonised’ pats.

Chapter 5: Effects of increased late summer temperatures, earthworms, and dung beetles on cattle dung decomposition

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Author contributions: MJT and FXJS designed and set up the experiment. MJT collected all data from the experiment, performed all data analysis, and wrote this manuscript. OTL provided feedback on experimental design, running, data collection, and analysis. Both OTL and FXJS provided multiple rounds of feedback on draft sections and complete drafts.

Status: In preparation for submission to a journal.

Abstract

The decomposition of cattle dung is an important ecosystem service performed by dung beetles and earthworms in UK pasture ecosystems. Rapid decomposition of cattle dung is important for maintaining pasture productivity, maximising grazing area for cattle, and reducing risks of infection with parasites. Earthworms and dung beetle larvae both feed directly on cattle dung so may compete for this resource; these interactions could have positive, negative, or neutral effects on cattle dung decomposition. Anthropogenic climate change is expected to increase both mean summer temperatures and the number of extreme

heatwave events. Both earthworms and dung beetles are ectothermic so these heatwaves may have strong effects on their performance (survival, growth, and reproduction), functioning, and ecological interactions. In a factorial microcosm experiment, earthworms (*Dendrobaena* veneta – presence or absence), dung beetles (*Acrossus rufipes* – presence or absence), and a warming treatment that simulated a heatwave (ambient conditions or artificially heated to raise mean temperatures by +6 °C for the duration of the experiment) were manipulated and effects on dung beetle and earthworm performance and cattle dung decomposition were recorded. Earthworms and the heatwave treatment additively reduced end dung dry mass: earthworms reduced end dung dry mass by 22.7% and the heatwave treatment reduced dung dry mass by 7.0%. Dung beetles did not perform well in either the ambient or heatwave treatments but there was a significantly reduced probability of recovering live larvae from heated microcosms compared to control microcosms, suggesting that reproductive performance was negatively affected by the heatwave treatment. Earthworms gained more mass in the presence of dung beetles, indicating that the activity of dung beetles on cattle dung may have positive effects on earthworm performance. These results suggest that cattle dung decomposition by earthworms can be robust to extreme heatwaves, albeit under controlled experimental conditions, and that functioning may increase as temperatures increase. However, poor performance of dung beetles, under both ambient and future warmed conditions, may have negative impacts on dung decomposition.

Keywords: Dung beetles, earthworms, heatwaves, climate change, cattle dung decomposition, pastures

Introduction

The UK contains ~9.6 million cattle, with a breeding herd of ~3.24 million individuals (Department for Environment, Food and Rural Affairs, 2023). The dung produced by these cattle suppresses plant growth (Williams and Haynes, 1995) and reduces effective grazing area as cattle avoid feeding near pats (Dohi et al., 1991), likely to avoid parasites (Seó et al., 2015). Individual cattle can produce up to ~0.68 m^2 of dung per day (MacLusky, 1960) and the combined dung produced by cattle in the UK could make up to 4.8% pasture unusable at any one time (Beynon et al., 2012b). Rapid decomposition of cattle dung is therefore important for cattle health and pasture productivity. Across temperate farmed landscapes in the UK, and more widely, this service is provided by both the insect dung fauna (Skidmore, 1991) and earthworms (Bacher et al., 2018).

The majority of insect-mediated dung decomposition in the UK is performed by dung beetles in the families Scarabaeidae (Aphodiinae and Scarabaeinae) and Geotrupidae (Jessop, 1986; Skidmore, 1991). Dung beetles utilise dung to feed their offspring (Holter, 2016) and can be divided into guilds based on morphology and behaviour (Floate, 2011; Hanski and Cambefort, 1991). The UK dung beetle fauna contains paracoprid (tunneller: Geotrupidae and *Onthophagus* spp.) and endocoprid (dweller: Aphodiinae) species, but no representatives of the teleocoprid (roller) guild. Through consuming and burying dung (Floate, 2011; Holter, 2016), dung beetles provide a range of ecosystem functions and services including dung decomposition (Beynon et al., 2015; Holter, 2016; Lee and Wall, 2006; Manning et al., 2016), reduction of cattle parasite abundance (Sands and Wall, 2017), improvement of soil drainage (Keller et al., 2022), and seed dispersal (Nichols et al., 2008). High functional diversity of dung beetles is required to maximise ecosystem

functioning, especially during periods of environmental stress (Beynon et al., 2012a; Manning et al., 2016, 2017b; Slade et al., 2007; Slade and Roslin, 2016).

Earthworms (Oligochaeta: Lumbricidae) are also important decomposers of cattle dung in pasture ecosystems (Gittings et al., 1994; Holter, 1977, 1979a, 1983; Kaartinen et al., 2013; Schon and Dominati, 2020). Earthworms are divided into ecotypes (Bouché, 1977, 1972) based on morphology and ecology and all groups are attracted to cattle dung and accumulate under dung pats (Bacher et al., 2018; Barth et al., 1994; Holter, 1983; Hughes et al., 1994; Knight et al., 1992). The high densities of earthworms burrowing under cattle dung pats can modify soil properties under pats (Bacher et al., 2020). Earthworms contribute to cattle dung decomposition by feeding on dung (Holter, 1979a; Kaartinen et al., 2013) and incorporate dung into soil through casting and burrowing (Hendriksen, 1997; Schon et al., 2020, 2017, 2015). While dung insects colonise dung pats soon after defecation (Sladecek et al., 2021, 2017), earthworms arrive later with their abundance increasing over multiple weeks (Bacher et al., 2020, 2018; Holter, 1983; Knight et al., 1992). The initial activity of dung insects may be important for promoting earthworm activity by 'priming' pats or increasing attractiveness of pats (Holter, 1983, 1977). Interactions between dung beetles and earthworms can have positive (Holter, 1977; Kaartinen et al., 2013) or negative (O'hea et al., 2010a) impacts on dung decomposition. However, in some cases combinations of dung beetles and earthworms may provide no more functioning than beetles (Xie et al., 2021) or earthworms (Hirschberger and Bauer, 1994) alone. High earthworm activity can decompose dung pats faster than dung beetle larvae can develop (Gittings and Giller, 1997), and consumption of dung be earthworms can reduce dung beetle larva mass (Xie et al., 2021), which may have negative effects on dung beetle populations and their functioning.

The performance of, and interactions between, dung beetles and earthworms may also be strongly influenced by climatic variables (Prather et al., 2013). Due to anthropogenic climate change, UK summer temperatures are expected to increase (Met Office, 2022) with increases to both maximum and mean temperatures (Patterson, 2023). Record high summer temperatures were recorded in the UK in 2022 (Kendon et al., 2023). Dung beetles are ectothermic and their performance is strongly influenced by temperature, with the historical distribution ranges (Robinson, 2013) and projected future distributions (Dortel et al., 2013) of European species being partially driven by climate. Increased temperature may have positive or negative effects on dung beetles as increased temperatures reduce adult survival and reproduction (Holley and Andrew, 2019a) as well as dung burial by tunneller and roller dung beetles (Holley and Andrew, 2019a, 2020). However, in other cases temperature increases can also increase dung removal by dung beetles (Gotcha et al., 2022) and dung beetle reproductive rates (Kirkpatrick and Sheldon, 2022), although offspring size may be reduced (Wu and Sun, 2012). Tolerance to warming varies both across and within species (Gallego et al., 2018; Gotcha et al., 2020; Holley and Andrew, 2019b) and many species show plastic responses to increased or more varied temperatures, including reduced metabolic rate (Carter and Sheldon, 2020), deeper burial of brood balls (Kirkpatrick and Sheldon, 2022; Mamantov and Sheldon, 2021), earlier laying and hatching of eggs (Wu and Sun, 2012), and phenological shifts (Cuesta et al., 2021). Exposure to increased temperatures can reduce tolerances to heat stress (Nyamukondiwa et al., 2018), and larvae exposed to increased temperatures can show reduced adult body size (Carter and Sheldon, 2020), so even non-lethal heat stress may have strong effects on dung beetle communities and functioning, although the overall effect of a heatwave on dung decomposition will depend on the composition of species present (Slade and Roslin, 2016).

Earthworms are also ectothermic animals and are strongly affected by temperature (Edwards and Arancon, 2022) and temperature fluctuation (Uvarov et al., 2011). Soil temperatures have a strong influence on earthworm growth rates (Daniel et al., 1996; Eriksen-Hamel and Whalen, 2006; Wever et al., 2001), reproductive rates (Boström and Lofs, 1996), and the survival of adults and cocoons (Johnston and Herrick, 2019; Presley et al., 1996; Richardson et al., 2009), with responses often being species-specific (Singh et al., 2019; Uvarov et al., 2011). In addition, temperature has strong effects on the activity of earthworms and the functions and services that they provide, including their burrowing activity (Perreault and Whalen, 2006), effects on plant nitrogen (N) uptake (Liu et al., 2024), soil gas emissions and N leaching (Marhan et al., 2015), and casting (Makoto et al., 2016). Increased temperatures can have negative effects on earthworm community abundance and species richness (Briones et al., 2009; Eisenhauer et al., 2014; Johnston, 2019), and increased temperatures can exacerbate the effects of other stressors such as heavy metals (Khan et al., 2007), tillage (Johnston et al., 2018), and pesticides; however, earthworm activities can maintain soil fauna richness during warming stress (Siebert et al., 2019).

While the effects of warming on dung beetles and earthworms have been widely investigated, the potential for warming to alter or disrupt the interactions between them is less well studied. The diversity of recorded responses to warming for both earthworms and dung beetles makes the effects of warming on the interactions between the two taxa, and potential effects on cattle dung decomposition in pasture ecosystems, difficult to predict. In this factorial microcosm experiment, the presence or absence of earthworms and dung beetles were manipulated under either ambient or warmed conditions to determine the effects of warming on earthworm performance, dung beetle performance, and cattle dung decomposition. It was predicted that both dung beetles and earthworms would increase

rates of dung decomposition, but that the two groups would interact, with dung beetles increasing the functioning of earthworms through a 'priming' effect. It was also predicted that the heatwave treatment would have a direct impact on dung decomposition and an indirect negative effect on dung decomposition through reducing the functioning of both earthworms and dung beetles. Finally, it was predicted that the heatwave treatment would alter the interaction between dung beetles and earthworms and their effects on dung decompositions due to differences in tolerance to increased temperatures of both groups.

Methods

Dung collection and experimental animals

This experiment was conducted in an outside grassy courtyard area at the John Krebs Field Station (Department of Biology), University of Oxford, near the village of Wytham, Oxfordshire, UK from August to October 2022. Fresh (<24 hours old) cattle dung was collected from a mixed-sex herd of Angus-Saler-Stabiliser cattle on an organic farm adjacent to the John Krebs Field Station on 24 August. The herd had not recently been treated with anthelminthic medication at the time of dung collection. The dung was homogenised by hand and then allocated to ~300 ml clear plastic containers (fresh pat mass = 310 g ± 10 g). The dung was stored in a freezer at −20 °C until use in order to kill any invertebrate larvae or eggs (Payne, 1927).

Acrossus rufipes (Linnaeus, 1758) (Coleoptera: Scarabaeidae: Aphodiinae) was used as a representative dung beetle in this experiment. This species has previously been called *Aphodius rufipes* (Linnaeus, 1758) and is commonly referred to as such in the literature, but this name is now considered a synonym of *Acrossus rufipes* (Linnaeus, 1758). Adult *A.*

rufipes were collected from Port Meadow, Oxfordshire, UK, on 26 August and kept in ventilated plastic containers filled with 15% gravimetric water content Kettering Loam Topsoil (soil details provided below). Dung beetles were stored in groups of ~30 individuals in an air-conditioned glasshouse until they were used in the experiment and were fed *ad libidem* with fresh cattle dung (collected from the same farm described above). *Acrossus rufipes* is a large (9 – 13 mm length) species (Jessop, 1986) that has been used in previous experiments examining earthworm and dung beetle interactions (Holter, 1977; O'hea et al., 2010a). The species is common in European pastures with a peak in activity during August (Holter, 1979b). Adults feed on the liquid portion of dung and particles <14 μm (Holter, 2000), while larvae are bulk feeders on solid material in dung (Holter, 1974, 1973) before they pupate under the soil surface (Holter, 1979b).

Due to extremely hot temperatures and limited rainfall during the study (Kendon et al., 2023), epigeic (surface dwelling) earthworms were in very low abundance in pastures around the John Krebs Field Station. Therefore, the commercially-available epigeic earthworm *Dendrobaena veneta* (Rosa, 1886) was used as a representative species. *Dendrobaena veneta* is a 'compost epigeic' earthworm and is not found in UK pastures; however, it is similar in morphology to other pasture-dwelling epigeic species (Bottinelli et al., 2020). *Dendrobaena veneta* has an average adult mass of 0.98 g and a body size of 50 – 80 x 5 – 7 mm (Domínguez and Edwards, 2011). Therefore, it is a similar size to other epigeic species found in pastures including *Lumbricus rubellus* Hoffmeister, 1843 (25 – 150 x 3 – 6 mm), *L. festivus* (Savigny, 1826) (48 – 110 x 3.5 – 6 mm), and *L. castaneus* (Savigny, 1826) (30 – 85 x 2 – 5 mm) but is larger than other species such as *Satchellius mammalis* (Savigny, 1826) (24 – 41 x 1.5 – 3 mm) (Bacher et al., 2018; Sherlock, 2016; Sherlock et al., 2018). *Eisenia fetida* (Savigny, 1826), another 'compost epigeic' earthworm species (Domínguez and Edwards, 2011; Katsiamides and Stürzenbaum, 2021),

has been used successfully in dung beetle and earthworm interaction experiments (O'hea et al., 2010a). Therefore, it was determined that *D. veneta* would be a suitable test species for this experiment. Juvenile *D. veneta* were purchased from Yorkshire Worms Ltd. (Holme-on-Spalding-Moor, Yorkshire, UK) and were stored in 20 L white polypropylene buckets, sealed with a nylon insect mesh lid, in 25% gravimetric water content Kettering Loam Topsoil (details below) in the same glasshouse as the dung beetles. Earthworms were fed *ad libidem* with dried and rewetted cattle dung (Lowe and Butt, 2005) collected from the same farm as used for the dung beetles.

Experimental set up

Microcosms (Fig. 5.1) were constructed from ~3 L white polypropylene buckets (height = 138 mm, diameter = 200 mm) with sealable lids. A single ~2 cm diameter hole was drilled into the base of each bucket to allow water drainage. A 3 x 3 cm square of 40 g m^{-2} nylon insect mesh was placed over the hole and secured with hot-melt adhesive to prevent earthworm escape. Microcosms were then part-filled with ~300 g of washed garden gravel (10 cm diameter), followed by ~2 kg of 25% gravimetric water content Kettering Loam topsoil (Boughton Loam Ltd., Kettering, Northamptonshire, UK). This is a sterilised sandy-loam topsoil (24% clay, 18% silt, 58% sand, pH 6.8) that has been used as a substrate for earthworms in previous experiments (Butt and Briones, 2011; Lowe and Butt, 2008a, 2007a) as well as for controlled culture of earthworms (Lowe and Butt, 2005). An ~18 cm diameter hole was cut from the centre of each bucket lid and the remaining ring-shaped lid was used to hold a layer of 40 g m^{-2} insect mesh over the top of the bucket to allow air flow while preventing earthworm and dung beetle escape. To prevent excess rain entering the buckets, water-resistant waxed paper plates were placed over the microcosms and held in place with bamboo skewers and small plastic pots filled with gravel. The plates

did not form a complete seal against the bucket lid so did not prevent air flow. On 2 September, the frozen dung pats were removed from the freezer and left to thaw for a few hours under ambient conditions. Once they were soft enough to remove from their tub, microcosm lids were removed and pats were gently squeezed out onto the soil surface of each microcosm and gently pressed down by hand to ensure contact with the soil and to make a flatter, rounder, shape (~5 cm height, ~10 cm diameter).

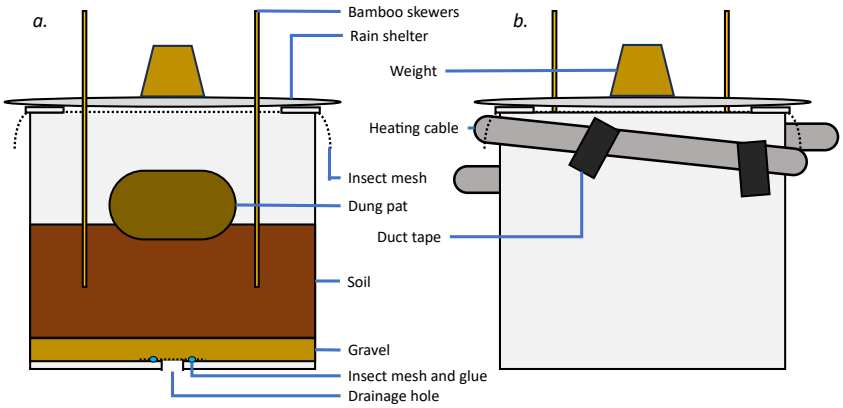


Fig. 5.1: Diagram of microcosms used in the experiment showing *a.* interior view and *b.* external view. Diagrams are not to scale.

Earthworms (absent or present), dung beetles (absent or present), and temperature (control or heated) were manipulated in a fully factorial experimental design with the eight treatment combinations replicated 15 times for a total of 120 microcosms. The microcosms were positioned in a semi-randomised arrangement. Microcosms were arranged in six rows of 20 which alternated between warmed and control treatments (Fig. S5.1). Each row contained five randomly ordered replicates of each of the four earthworm and dung beetle treatment combinations. Microcosms in each row were placed ~10 cm from one another with ~100 cm between each row. The heatwave treatment was applied with heated gardening cables, which were tested before being used. Three 20 m cables were used, with one cable used for each row of warmed microcosms. The heating cable was wrapped

around the upper rim of each warmed microcosm in a complete loop and secured in place with duct tape. The semi-randomised spatial arrangement of microcosms was necessary to prevent the heating cables touching themselves and overheating, which could have caused failure. Ten temperature data loggers (EasyLog EL-USB-2, Lascar Electronics, Whiteparish, Wiltshire, UK) were used to record the effects of the heatwave treatment on temperature. Five data loggers were randomly allocated to warmed microcosms, four to control microcosms, and one left in an open-topped container between the third and fourth row of microcosms (the centre of the experimental setup, Fig. S5.1) to record ambient temperatures. Microcosms were checked daily, and new duct tape was applied when necessary to ensure that heating cables remained fixed in place. Heating cables were checked daily by hand to feel if they were still outputting heat and were operational. All heating cables were fully operational for the duration of the experiment.

On 2 September, immediately after dung pats were added to microcosms, six adult *A. rufipes* were randomly added to each beetle-containing microcosm and were observed to burrow quickly into the dung pats. It was not possible to sex adults before adding them to microcosms but adding six randomly selected adult beetles to microcosms meant that there was a ~98.4% chance of including at least one female (assuming a 1:1 sex ratio in the *A. rufipes* population at the time of collection) so it would be expected that only 1 of the 60 beetle-containing microcosms to have contained solely males. Lids and mesh, that had been removed to add the dung pats, were then reapplied. *Dendrobaena veneta* were removed from the storage containers on 14 September, cleaned with tap water, and stored in darkness at 15 °C in an incubator for 48 hours to allow gut voidance (Fründ et al., 2010). Sixty clear plastic tubs were prepared with ventilated lids and were lined with moist tissue paper. Earthworms were removed from the incubator and haphazardly assigned to groups of six with total starting mass of ~1.4 g, corresponding to approximate masses of

earthworms expected for ~320 g of fresh dung from previous studies (Holter, 1983), before each group was randomly allocated to one earthworm-containing microcosm. There were no statistically significant differences in earthworm starting mass across heatwave and dung beetle treatment levels (Table S5.1). Earthworms were added to microcosms on 16 September, as earthworms accumulate at dung pats over time following defecation (Bacher et al., 2018; Barth et al., 1994; Holter, 1983). Microcosm lids were removed, and earthworms were quickly placed on top of the dung pat in their microcosms before lids were reapplied to reduce chance of dung beetle escape. Earthworms were visible through the mesh lids after being added to microcosms and were seen to burrow quickly into the dung and surrounding soil. Each microcosm then received 100 ml of tap water through the mesh lid.

Data collection and analysis

The experiment was ended after 34 days (20 days after adding the earthworms) on 6 October. For each microcosm, dung pats were removed, and any soil stuck to the base of the pats was carefully scraped off. Pats were then hand sorted for earthworms and dung beetle larvae and dung pat fresh mass was recorded. The soil in each microcosm was then hand-searched for earthworms and dung beetle larvae. Any dung beetle larvae collected from the soil or dung pats were stored in 70% ethanol while earthworms from each microcosm were cleaned with tap water and transferred to clear ventilated plastic tubs lined with moist tissue paper. Earthworms were returned to the incubator for 48 hours at 15 °C in darkness for gut voidance before being weighed (Fründ et al., 2010). Dung pats were dried in a drying oven at 70 °C until they reached constant mass. Dry dung pats were hand-sorted to remove any remaining soil before they were weighed.

Data were analysed in R version 4.3.1 (R Core Team, 2023). To compare dung decomposition across treatment groups, remaining dung pat dry mass and dung pat water content were analysed with Analysis of Variance (ANOVA), with earthworms (absence or presence), dung beetles (absence or presence), the heatwave treatment (control or heated), and all possible interactions included as explanatory factors. The correlation between remaining dung mass and earthworm mass change was analysed with Analysis of Covariance (ANCOVA), with dung beetles, the heatwave treatment, and their interactions included as additional explanatory variables.

For fauna performance, earthworm mean mass change was analysed with ANOVA, with beetles, the heatwave treatment, and their interaction included as explanatory factors. Dung beetle larva counts were analysed with Poisson Generalised Linear Models (GLMs), larvae mean mass and total mass were analysed with ANOVA, and the probability of a microcosm containing live beetle larvae was analysed with a binomial GLM. As live dung beetle larvae were only recovered from a small number of microcosms (see *Results*), separate analysis of larva mass and number was conducted with zero values included or excluded to identify the effects of the heatwave and earthworm treatment on the larvae that were recovered.

Residual plots for all ANOVA models were inspected and dung water content and earthworm mean mass change were log-transformed to better fit model assumptions. When variables or interactions had statistically significant effects, *post hoc* comparisons were performed with the *emmeans* package (Lenth, 2023), with group differences and confidence intervals back-transformed to the response scale for interpretation and reporting for log-transformed variables or GLMs. All plots were made with *ggplot2* (Wickham, 2016).

Results

Effects of the heatwave treatment

The mean temperature reading of the ambient data logger (not kept in a microcosm) was 15.72 °C. The mean temperature reading of the control data loggers was 15.52 °C ($CI_{95\%}$ = [15.20, 15.84]) and was not significantly different from the ambient mean (Df = 3, t = −1.995, p = 0.14). The heating cables significantly increased mean temperatures by ~6 °C (Fig. 5.2, Table 5.1) and temperatures increased more at night (19:00 – 07:00; mean = +6.3 °C, min = +7.8 °C, max = +5.6 °C, Table 5.1) than during the day (07:00 – 19:00; mean = +5.7 °C, min = +7.9 °C, max = +4.2 °C, Table 5.1). Heating cables significantly increased both maximum and minimum recorded temperatures but had a greater effect on minimum recorded temperatures (Table 5.1). The highest temperature reading in the experiment was 35.5 °C from a heatwave treatment microcosm. The highest recorded control treatment temperature was 31 °C and the highest recorded ambient temperature was 34 °C. Temperature ranges were reduced in the heatwave treatment for both the whole study period (estimate = −3.55 °C, $CI_{95\%}$ = [−5.59, −1.51], p = 0.004) and for average daily temperature ranges (estimate = −1.61 °C, $CI_{95\%}$ = [−2.19, −1.02], p < 0.001).

Table 5.1: Table showing group means (± SE) for maximum, mean, and minimum temperature readings (°C) from data loggers in control (n = 4) and heatwave (n = 5) treatments. Differences between treatment means, 95% confidence intervals for differences, and P values are also shown.

Variable	Time period	Group mean (±SE)		Difference [95% CI]	P
		Control	Heatwave		
Max temp	Day time	29.8± 0.8	34.0± 0.4	4.2 [2.2, 6.3]	**0.002**
	Night time	23.4± 0.1	29.0± 0.6	5.6 [4.0, 7.2]	**<0.001**
Mean temp	Day time	17.6± 0.1	23.3± 0.4	5.7 [4.7, 6.7]	**<0.001**
	Full day	15.5± 0.1	21.5± 0.4	6.0 [5.0, 7.0]	**<0.001**
	Night time	13.5± 0.1	19.8± 0.4	6.3 [5.3, 7.4]	**<0.001**
Min temp	Day time	5.9± 0.4	13.8± 0.6	7.9 [6.0, 9.8]	**<0.001**
	Night time	5.5± 0.3	13.3± 0.5	7.8 [6.3, 9.3]	**<0.001**

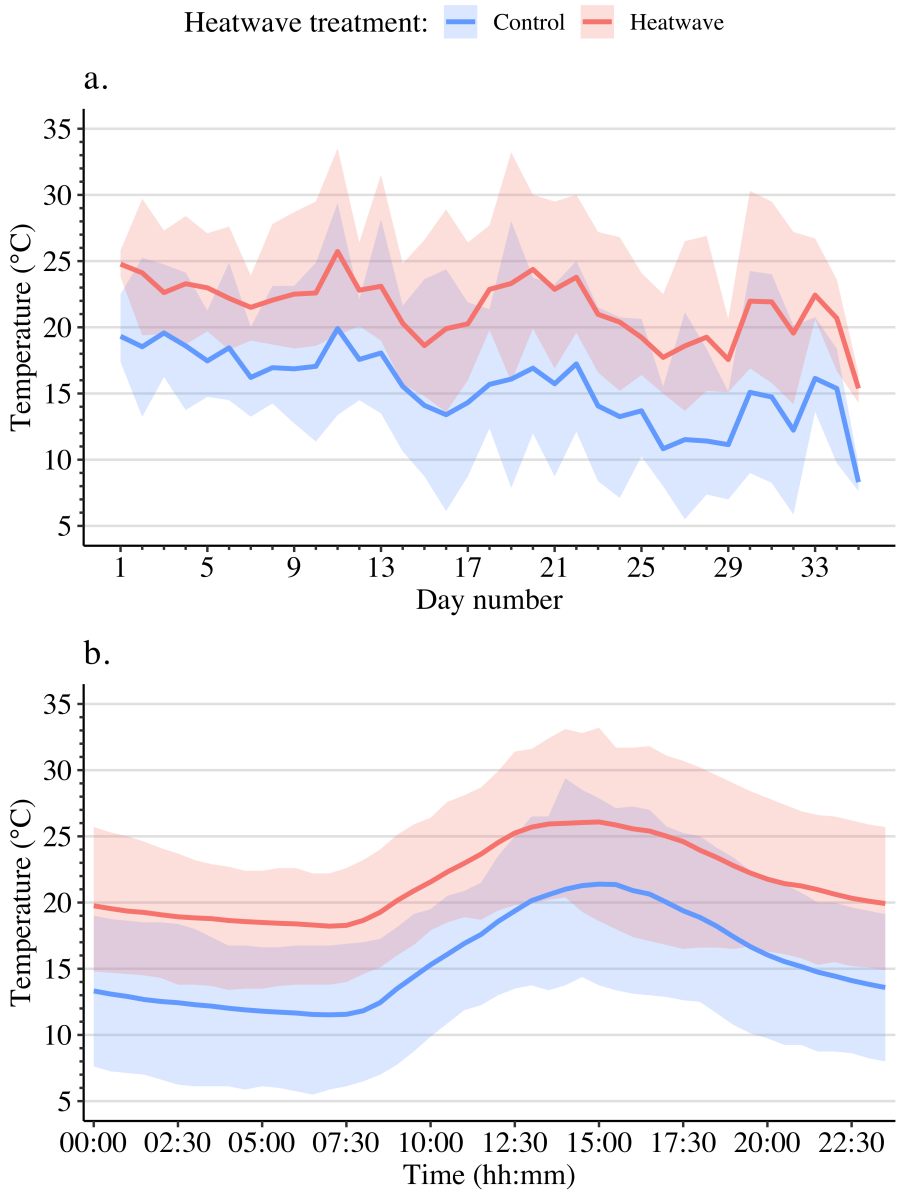


Fig. 5.2: Line plots showing data logger temperature recordings throughout the experiment for control (blue; lower line and ribbon, $n = 4$) and heatwave (red; upper line and ribbon, $n = 5$) microcosms. Temperatures were recorded every 30 mins. *a.* shows average daily temperature throughout the duration of the experiment from day 1 (2 September) to day 35 (6 October). Ribbons show mean maximum and minimum temperatures for each day. *b.* shows average temperatures over a 24-hour time period for the two treatments. Data were grouped by data logger and time point to produce an average temperature at each time point for the two treatments. Ribbons show the mean maximum and minimum temperature for each time point (mean of maximum value recorded by each data logger at each time point).

Dung decomposition

For remaining dung dry mass, there was no significant effect of dung beetles or any two-way or three-way interactions between the treatments (Table 5.2, Fig. 5.3a). However, end dung dry mass was significantly reduced by both earthworms (difference = 9.43 g, $CI_{95\%}$ = [8.13, 10.73], $p < 0.001$) and the heatwave treatment (difference = 2.66 g, $CI_{95\%}$ = [1.35, 4.00], $p < 0.001$).

For log-transformed dung water content, there was a statistically significant interaction between earthworms and the heatwave treatment (Table 5.2, S5.2, Fig. 5.3b) where *D. veneta* reduced dung moisture content in the heatwave treatment (ratio = 0.83, $CI_{95\%}$ = [0.74, 0.92], $p < 0.001$) but not in the control treatment. The heatwave treatment significantly reduced end dung moisture both in the absence (ratio = 0.85, $CI_{95\%}$ = [0.77, 0.95], $p = 0.001$) and presence of earthworms (ratio = 0.71, $CI_{95\%}$ = [00.64, 0.79], $p < 0.001$). There was no statistically significant three-way interaction between beetles, the heatwave treatment, and earthworms, two-way interactions involving beetles, and no main effect of dung beetles (Table 5.2, Fig. 5.3b).

Table 5.2: Summary of ANOVA results showing effects of the heatwave treatment, dung beetles, earthworms, and their interactions, on remaining dung dry mass (g) and dung moisture content.

Variable	End dung dry mass (g)			Dung moisture content		
	Df	F	P	Df	F	P
Heatwave	1	16.35	**<0.001**	1	74.65	**<0.001**
Beetles	1	1.19	0.278	1	0.11	0.737
Earthworms	1	206.03	**<0.001**	1	11.63	**0.001**
Heatwave x Beetles	1	1.35	0.247	1	0.32	0.573
Heatwave x Earthworms	1	0.05	0.828	1	10.02	**0.002**
Beetles x Earthworms	1	1.96	0.164	1	0.75	0.387
Heatwave x Beetles x Earthworms	1	0.57	0.453	1	0.79	0.376
Residuals	112			112		

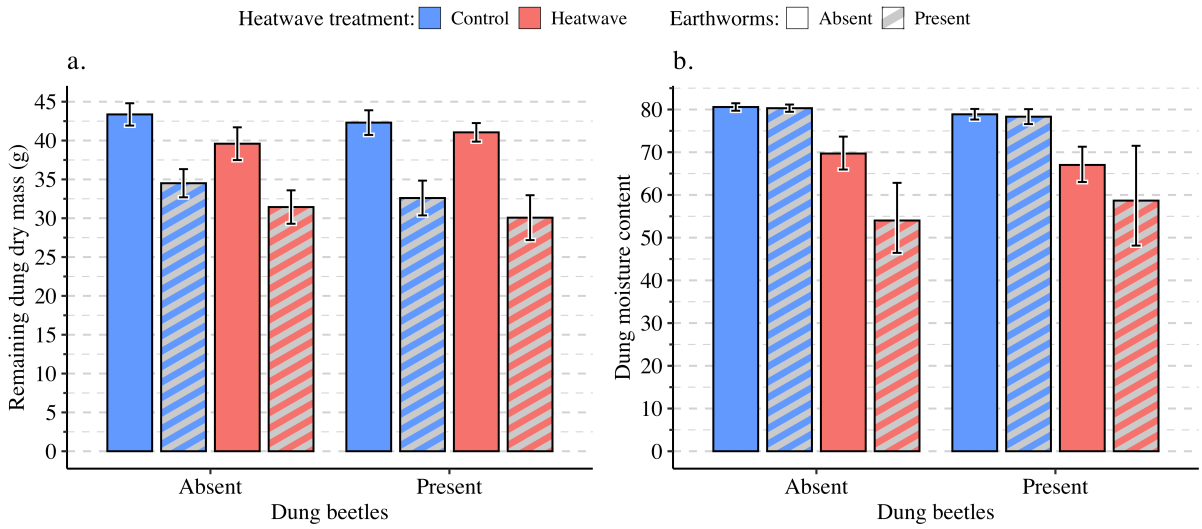


Fig. 5.3: Bar plots showing *a.* remaining dung dry mass (g) and *b.* dung moisture content across earthworm (absent = clear fill, present = stripe fill), dung beetle, and heatwave (control = blue, heatwave = red) treatment groups. Bar heights show group means and error bars show 95% confidence intervals. Group means and confidence intervals in *b.* have been back-transformed from the log scale.

Earthworm performance

Survival of *D. veneta* was extremely high, with 359 out of 360 earthworms recovered alive at the end of the experiment (99.7% survival). Overall, mean earthworm mass increased during the experiment by 0.36 g ($CI_{95\%}$ = [0.34, 0.38], $p < 0.001$). Mean mass change was log-transformed to better fit the assumptions of ANOVA and was significantly increased by both the heatwave treatment (Fig. 5.4a, Table 5.3, ratio = 1.19, $CI_{95\%}$ = [1.01, 1.24], p = 0.029) and the presence of dung beetles (Fig. 5.4a, Table 5.3, ratio = 1.21, $CI_{95\%}$ = [1.10, 1.34], $p < 0.001$) but there was no significant interaction between the two variables (Table 5.3).

There was a statistically significant negative correlation between mean earthworm mass change and remaining dung dry mass, where less dung was removed in microcosms where mean earthworm mass gain was lower (Fig. 5.4b, Table 5.4, $b = -37.23$, $CI_{95\%}$ = [−49.62,

−24.83], $p < 0.001$). There were no other statistically significant main effects or interactions between variables (Fig. 5.4b, Table 5.4).

Table 5.3: Summary of ANOVA results showing effects of the heatwave treatment, dung beetles, and their interaction on mean earthworm mass change

Variable	Mean worm mass change		
	Df	F	P
Beetles	1	15.07	**<0.001**
Heatwave	1	5.05	**0.029**
Beetles x Heatwave	1	0.02	0.901
Residuals	56		

Table 5.4: Summary of ANCOVA results showing correlation between earthworm mass change and remaining dung dry mass across heatwave and dung beetle treatment groups

Variable	Dung dry mass (g)		
	Df	F	P
Mean earthworm mass change	1	34.70	**<0.001**
Beetles	1	1.19	0.281
Heatwave	1	1.58	0.214
Mean earthworm mass change x Beetles	1	0.01	0.927
Mean earthworm mass change x Heatwave	1	0.28	0.600
Beetles x Heatwave	1	0.64	0.429
Mean earthworm mass change x Beetles x Heatwave	1	0.00	1.000
Residuals	52		

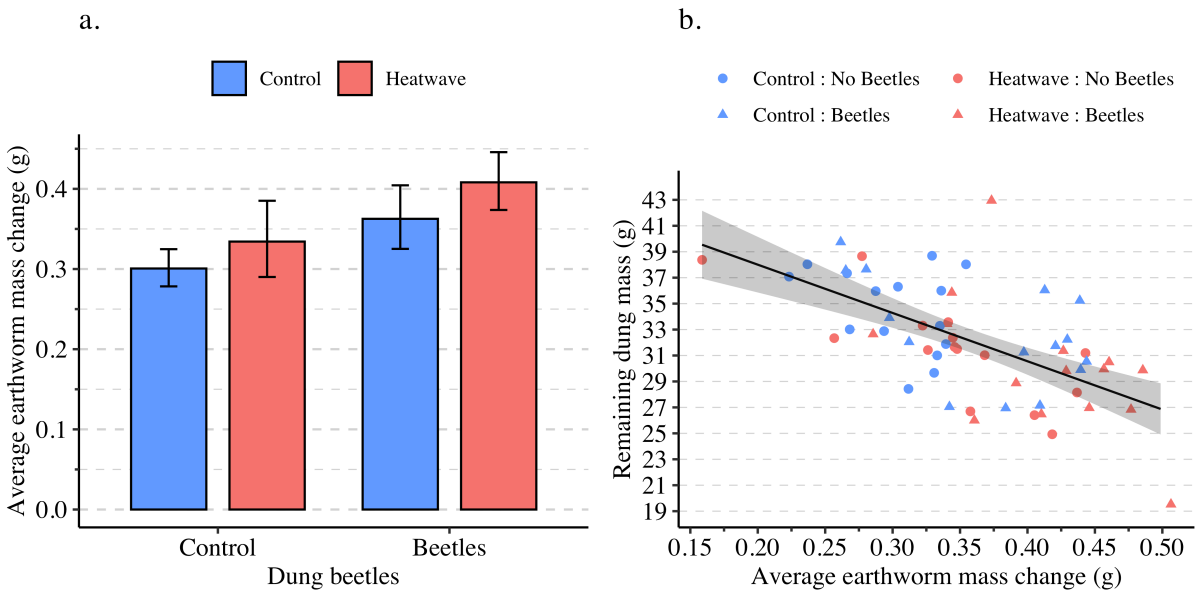


Fig. 5.4: *a.* Bar plot showing mean earthworm mass change across treatment groups. Bar heights show group means and error bars show 95% confidence intervals which have both been back-transformed from the log scale. *b.* Scatter plot showing correlation between mean earthworm mass change (g) and remaining dung dry mass (g). The line in *b.* shows the line of best fit and the shaded ribbon shows 95% confidence interval for the regression of end dung dry mass as a function of mean earthworm mass change.

Dung beetle larva performance

In total, 92 live *A. rufipes* larvae were recovered from 16 microcosms. For ambient microcosms, five control and seven earthworm-containing microcosms contained live larvae. For heatwave microcosms, two control and two earthworm-containing microcosms contained live larvae. When microcosms containing zero larvae were excluded (see *Methods*), there was no statistically significant effect of the heatwave treatment, earthworms, or their interaction on larva number and total mass (Table 5.5, Fig. 5.5a, 4b). However, when zero values were included, the heatwave treatment significantly reduced larva number (Table 5.5; ratio = 0.36, $CI_{95\%}$ = [0.22, 0.57], $p < 0.001$), total mass (Table 5.5; difference = −0.35 g, $CI_{95\%}$ = [−0.67, −0.04], $p = 0.030$), and the probability of a

microcosm containing live beetle larvae (Table 5.5; odds ratio = 0.23, $CI_{95\%}$ = [0.06, 0.84], p = 0.026) compared to controls.

Table 5.5 Summary of ANOVA results (Type II) showing effects of earthworms and the heatwave treatment on dung beetle larva number, mass, and the probability of finding live larvae in a microcosm. Larva number and mass analyses were conducted separately with zero values included or excluded.

Variable	Test	Earthworms			Heatwave			Earthworms x Heatwave		
		Df	Test statistic	P	Df	Test statistic	P	Df	Test statistic	P
Larva number (with 0s)	X^2	1	1.09	0.297	1	21.93	**<0.001**	1	1.21	0.272
Larva number (no 0s)	X^2	1	0.02	0.888	1	0.05	0.819	1	0.15	0.695
Total larvae mass (with 0s)	F	1,56	0.19	0.666	1,56	4.94	**0.030**	1,56	0.61	0.437
Total larvae mass (no 0s)	F	1,12	0.05	0.827	1,12	0.04	0.837	1,12	0.48	0.501
Pr. live larvae	X^2	1	0.38	0.540	1	5.68	**0.017**	1	0.18	0.670

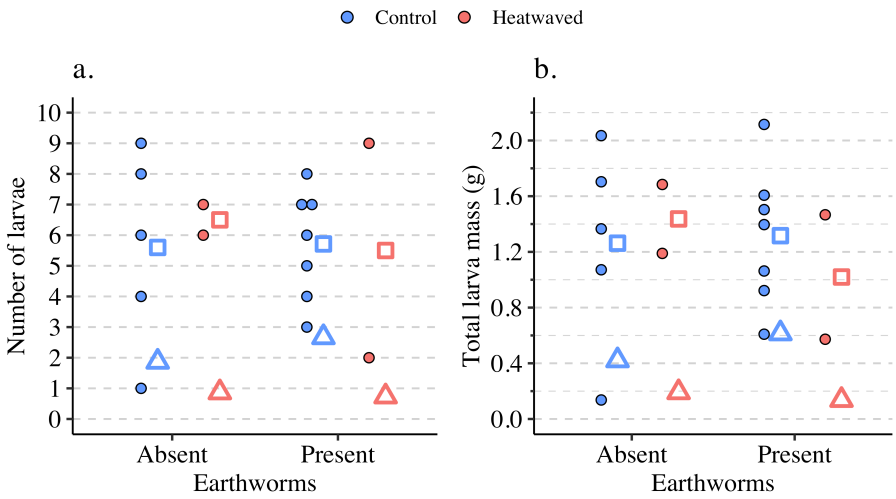


Fig. 5.5: Dot plots showing *a.* number of larvae recovered and *b.* total larva mass across earthworm and heatwave treatment levels. Zero values have been excluded from both plots for ease of viewing the other data (number of zero values for each treatment combination: Control + Earthworm absent = 10, Heatwave + Earthworms absent = 13, Control + Earthworms present = 8, Heatwave + Earthworms present = 13 for both plots). Hollow square shapes show treatment group means with zero values excluded. Hollow triangle shapes show treatment group means with zero values included.

Discussion

In this study, the heatwave treatment (+6 °C mean temperature, Fig. 5.2) and earthworms (*D. veneta*) had a significant additive positive effect on cattle dung decomposition (*i.e.,* the treatments reduced end dung mass compared to controls), while dung beetles (*A. rufipes*) had no significant effect (Fig 5.3). Both the heatwave and dung beetle treatments significantly increased earthworm mass gain (Fig. 5.4), suggesting that the activity of dung beetles improved the ability of the earthworms to process the dung. Dung beetle performance was generally low but was not affected by earthworms (Fig. 5.5). However, the heatwave treatment had a negative effect on beetle performance by reducing reproductive success across microcosms.

Limited effects of dung beetles on dung decomposition

In this study, there were limited effects of *A. rufipes* dung beetles on cattle dung decomposition. However, dung beetle reproductive performance was low across the whole experiment, with larvae only recovered from 16 of the 60 microcosms to which adults were added (Fig. 5.5). *Acrossus rufipes* adults feed on the liquid components of dung (Holter, 2016, 2000) while larvae are bulk feeders (Holter, 1974, 1973), so the low reproductive performance likely resulted in the limited effects of the dung beetle treatment on dung decomposition as it is the larvae that perform the majority of dung decomposition. In this experiment, the heatwave treatment significantly reduced the probability of a microcosm containing live beetle larvae, with only two of the 16 larva-containing microcosms being exposed to the heatwave treatment, suggesting that either reproduction, or larval survival, was reduced by the heatwave treatment. However, reproductive performance was also low in the control microcosms where live larvae were only found in 12 out of 30 (40%). Although it was not possible to sex beetles before adding them to microcosms, it was expected that only one of the 60 beetle-containing microcosms would have contained only males (see *Methods*). 2022 was the warmest year in the UK since 1884, with temperatures reaching 40.3 °C during a heatwave in July (Kendon et al., 2023), resulting in high temperatures in the control microcosms. Mean maximum daily temperatures in the control treatment were 29.8 °C ± SE 0.8 with a maximum recorded temperature of 31 °C (Fig. 5.2) which may have been too high for *A. rufipes* reproduction. *Acrossus rufipes* is active from May to October with a peak in activity in August (Holter, 1979b), and is a generalist species that feeds and breeds in both shaded and open environments (Roslin and Koivunen, 2001), suggesting that the species can tolerate high temperatures. However, the species is also nocturnal (Holter, 1979b), which may suggest reduced heat tolerance as nocturnal and crepuscular dung beetles show reduced

thermoregulation under heat stress compared to diurnal species (Gallego et al., 2018) and activity period predicts thermal maxima for Geotrupine and Scarabaeine species (Gotcha et al., 2020). Additionally, historic *A. rufipes* specimens collected in the Iberian Peninsula show a positive correlation between collection date and latitude, but no trend in temperature at the time of the collection, suggesting that the range of the species is shifting northwards due to rising temperature (Lobo et al., 2023). As egg, larvae, and emerging adult number of *Teuchestes fossor* (Linnaeus, 1758) can be reduced in exposed pats (faster drying and +2.67 °C mean temperature) compared to shaded pats (Vessby, 2001), it may have been the case that the extremely high temperatures of 2022 resulted in stressful conditions for *A. rufipes* in both the control and warmed treatments.

Earthworms and the heatwave treatment increased cattle dung decomposition

In this study, the heatwave treatment caused a significant reduction in remaining dung dry mass compared to controls (Fig. 5.3). Cattle dung contains large amounts of microbial biomass, on which adult dung beetles feed (Holter, 2016), and microbial activity can account for a large proportion of cattle dung mass loss during decomposition (Kaartinen et al., 2013). Increased microbial metabolism may have reduced dung pat end mass in warmed microcosms. *Dendrobaena veneta* also had a significant effect on dung decomposition (Fig. 5.3), as previously recorded in other studies (Holter, 1977; Kaartinen et al., 2013; Schon et al., 2017, 2015). The negative correlation between earthworm growth and remaining dung dry mass suggests that the earthworms were directly consuming the dung, with the dung acting as a suitable food source for growth (Fig. 5.4). Dried and rewetted cattle dung is a recommended food source for maximising earthworm growth rates (Lowe and Butt, 2005) and compost earthworms can consume large amounts of cattle

dung during vermicomposting (Domínguez and Edwards, 1997). Therefore, the positive effects of *D. veneta* on dung decomposition in the present study are unsurprising. Earthworms also reduced dung pat moisture content, but only in the heatwave treatment (Fig. 5.3). Earthworm activities can increase (Hodson et al., 2023) or decrease (Andriuzzi et al., 2015; Ma et al., 2021) soil moisture. In pasture systems, earthworms can disrupt dung pat crust formation and so accelerate weathering of pats (Holter, 1979a). Earthworms were found to have burrowed into the dung pats (Fig. S5.2) and produced large amounts of castings that formed a layer between the soil surface and remaining dung. This low-density layer of earthworm casts may have increased air flow around the remaining dung and contributed to increased drying in the heatwave treatment.

Dendrobaena veneta is a 'compost earthworm' (Domínguez, 2018) that was used in this experiment out of necessity due to low abundances of pasture earthworms around the John Krebs Field Station during the extremely hot and dry August of 2022 (Kendon et al., 2023). This species is similar in morphology, size, and ecology to species that naturally occur in pastures (Bacher et al., 2018; Sherlock, 2016; Sherlock et al., 2018) and other compost epigeic species such as *E. fetida* have been previously used in experiments investigating earthworm and dung beetle interactions (Bang et al., 2009; O'hea et al., 2010a). However, compost epigeic species are chosen for vermicomposting due to their high growth rates, high ability to process organic matter, and high robustness (Domínguez and Edwards, 2011). Therefore, this species may have been more able to tolerate the heatwave treatment than species that naturally occur in pastures. Further experimental work with other epigeic species, or species from other ecotypes, would provide further information on the effects of heatwaves on dung decomposition performed by earthworms.

The heatwave treatment increased earthworm performance

In this study, survival of *D. veneta* was extremely high, with only one individual out of the 360 added to microcosms unaccounted for at the end of the experiment. Additionally, earthworms gained mass in the experiment, with greater mass increases in the warmed treatment (Fig. 5.4). *Dendrobaena veneta* shows increased growth rates and cocoon production rates in 20 °C compared to 15 °C in culture and showed further increases at 25 °C (Fayolle et al., 1997). Individuals also reach maturity faster at 25 °C compared to 15 °C (Viljoen et al., 1992), suggesting that the strong heatwave treatment in the study reported here (which increased mean temperatures from 15.1 °C to 21.5 °C) may have moved temperatures closer to the optimum temperature for *D. veneta*. However, mean maximum temperatures in the day were increased from 29.8 °C to 34.0 °C with a maximum temperature recording of 35.5 °C in a heated microcosm (Fig. 5.2). For vermicomposting, optimum culture temperatures for *D. veneta* are 25 °C with a tolerance range of 15 – 25 °C (Domínguez and Edwards, 2011), but there appear to be few studies examining performance above 25 °C. *Eisenia fetida* and *Eisenia andrei* (Bouché, 1972), more commonly used vermicomposting species, also have temperature optima of 25 °C but wider tolerance ranges of 0 – 35 °C (Domínguez and Edwards, 2011). It may be the case that the narrower temperature ranges of *D. veneta* reflect less study, rather than true differences in tolerance, although substrate temperatures of 20 – 25 °C are thought to be optimal for most compost earthworms (Domínguez, 2018). When sampling microcosms, no earthworms were visible on the soil or pat surface and many individuals were found deeply burrowed into the dung pats (Fig. S5.2). Unfortunately, due to the small size of the microcosms and dung pats and relatively large size of the data loggers, it was not possible to record dung or soil temperatures during the experiment. Experimental warming treatments can increase dung pat temperatures, but not soil temperatures at depths of 12 cm

(Slade and Roslin, 2016). Additionally, dung pat temperatures can be higher than air temperatures 1 m above pats with pats >1 week old having higher temperatures than fresh pats (Greenham, 1972) and dung maximum temperatures exceed air maximum temperatures (Matthiessen and Palmer, 1988) so pat temperatures in microcosms may have exceeded air temperatures. However, exposed dung pats are warmed by solar radiation (Greenham, 1972) so pats may not have been heated as much as air in the shaded and enclosed microcosms of this experiment. Therefore, while air temperatures in the microcosms may have been stressful for this species, the soil and dung may have provided some a degree of microclimatic thermal buffering which may explain the beneficial effects of the heatwave treatment on earthworms in this study.

The tendency of increased temperatures and soil moisture to covary, with soil moisture reducing as temperatures increase, is likely to have a strong influence on earthworm performance. The effects of temperature on burrowing and surface-casting activities of epic-anecic *Lumbricus terrestris* L. and endogeic *Aporrectodea caliginosa* (Savingy, 1826) are influenced by soil moisture (Perreault and Whalen, 2006) and the growth rate of *A. caliginosa* increases as temperature increases, but only with sufficient soil moisture (Eriksen-Hamel and Whalen, 2006). Additionally, increased temperatures can exacerbate the effects of low soil moisture on *Aporrectodea tuberculata* (Eisen, 1874), resulting in increased mortality (Wever et al., 2001). *Eisenia fetida* growth and fitness are maximised with high moisture levels and intermediate temperatures, where high temperatures cause large fitness reductions when moisture levels are low (Presley et al., 1996). Therefore, earthworm effects on dung decomposition may be driven by a combination of temperature and soil moisture, rather than temperature *per se*. In this experiment, it was necessary to use sealed microcosms to prevent earthworm and dung beetle escape, and rain shelters were necessary to prevent microcosm flooding. This created a shaded environment with

reduced potential for water evaporation, maintaining soil moisture, and may explain the lack of negative effects of the heatwave treatment on earthworms in this study. *Satchellius mammalis* and *L. festivus*, epigeic species attracted to cattle dung in UK pastures (Bacher et al., 2018), both have ranges that are strongly influenced by annual temperature range and precipitation and are expected to shift northwards with climate change (Sherlock et al., 2022). Therefore, heatwaves and reduced summer precipitation may severely reduce the dung decomposition activity of these species in pastures.

Interactions between dung beetles and earthworms

Earthworms can have a strong influence on dung beetle performance. When earthworm activity is high, dung pats can be completely removed before 40 days which is faster than the development time of Aphodiine dung beetles, which could cause significant mortality (Gittings and Giller, 1997). Additionally, soil feeding endo-anecic earthworms such as *Aporrectodea nocturna* Evans, 1946 may feed on dung balls buried by tunneller dung beetles such as *Onthophagus yubarinus* Matsumura, 1937, reducing size of larvae (Xie et al., 2021). However, in other cases, the activities of surface feeding earthworms such as *Eisenia fetida* can have no effects on brood ball production by tunnelling dung beetles such as *Copris tripartitus* Waterhouse, 1875 resulting in no declines in dung beetle performance (Bang et al., 2009). In the present study, no effects of earthworms on dung beetle performance were recorded; however, this may be due to low dung beetle performance across the whole experiment. *Acrossus rufipes* is an endocoprid (dweller) species with larvae that feed within the dung (Holter, 1974) before moving into the soil to pupate (Holter, 1979b). If beetle reproductive performance had been higher in this experiment and more larvae were present in experimental pats, the high levels of

earthworm activities may have negatively impacted larval development by acting as an additional form of competition for the dung

Dendrobaena veneta earthworms gained more mass in the presence of dung beetles than in their absence in this study (Fig. 5.4). Earthworms typically arrive at dung pats later than dung insects (Barth et al., 1994) and can accumulate in large numbers (Bacher et al., 2020, 2018). Earthworm accumulation is typically hump shaped (Barth et al., 1994; Holter, 1983) as densities decline over time following a peak; however, abundances can be higher than surrounding soils in excess of 100 days (Knight et al., 1992). While the delayed arrival of earthworms may reflect lower mobility compared to dung insects, the later arrival of earthworms can also be triggered by the activity of dung insects (Holter, 1983). Earthworms accumulate more slowly when dung beetles are excluded and will preferentially feed from dung pats with dung beetles than those without (Holter, 1983). Additionally, Holter hypothesised that the actions of adult and larvae dung beetles might disrupt and loosen dung pat structure, allowing easier access for earthworms (Holter, 1983). Despite limited *A. rufipes* reproduction in the present study, tunnels into the dung pats were visible in dung beetle + no earthworm pats (*i.e.* created by activity of beetles, not earthworms), suggesting that the entry of beetles into pats might have facilitated earthworm entry into pats and feeding. In this experiment, microcosms were sealed, and cattle dung acted as the only food source available to the earthworms. Therefore, the increased earthworm growth in the presence of dung beetles suggests that activity of the dung beetles on the pats increased nutritional value of the dung for the earthworms and promoted faster growth. Alternatively, the dead beetles themselves may have acted as an additional food source for the earthworms in microcosms containing dung beetles. While earthworms are saprophages, soil mesofauna may also be ingested (Lee, 1985) and arthropod cuticle has been detected in the gizzards of several UK species (Piearce, 1978).

Conclusions

In this study, earthworms were the dominant drivers of dung decomposition, and their performance was increased by an extreme heatwave treatment of +6 °C, while dung beetles had little effect on dung decomposition, either on their own or in interaction with earthworms. Earthworms benefitted from dung beetle presence, even in cases when dung beetle reproduction was not successful, suggesting that the dung beetles may have 'primed' the pats for earthworm activity, or dung beetle carcasses acted as an additional food source for the earthworms. Therefore, this study indicates that earthworms can act as important decomposers of cattle dung, even when dung beetle performance is low, and that dung decomposition services provided by earthworms may be resistant to increased late summer temperatures.

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Supplementary information



Fig. S5.1: Arrangement of experimental microcosms. Microcosms are arranged in lines that alternate between heatwave and control treatments from left to right.



Fig. S5.2: Cross-section of experimental dung pat during data collecting. *Dendrobaena veneta* earthworms are visible, having burrowed into the pat.

Table S5.1: Summary of ANOVA results showing lack of differences in starting total earthworm mass (g) across dung beetle and heatwave treatments.

Variable	Start earthworm mass (g)		
	Df	F	P
Heatwave	1	0.44	0.508
Beetles	1	0.27	0.603
Heatwave x Beetles	1	0.02	0.882
Residuals	56		

Table S5.2: Pairwise contrasts across heatwave and earthworm treatment levels for dung moisture content. Contrasts and confidence intervals have been back-transformed from the log scale.

Dung moisture treatments	Pairwise difference		
	Ratio	95% CI	P
Earthworms x Control / No earthworms x Control	0.99	[0.89, 1.11]	0.998
No earthworms Heatwave / No earthworms x Control	0.85	[0.77, 0.95]	**0.001**
No earthworms Heatwave / Earthworms x Control	0.86	[0.77, 0.96]	**0.002**
Earthworms Heatwave / No earthworms x Control	0.70	[0.63, 0.78]	**<0.001**
Earthworms Heatwave / Earthworms x Control	0.71	[0.64, 0.79]	**<0.001**
Earthworms Heatwave / No earthworms Heatwave	0.83	[0.74, 0.92]	**<0.001**

Chapter 6: Effects of the earthworm *Allolobophora chlorotica* and rainfall extremes on growth and fruit production of Alpine Strawberry plants

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Status: In preparation for submission to a journal.

Abstract

In the UK, summer and autumn rainfall is predicted to decrease due to climate change. This reduction in rainfall could have strong negative effects on plant performance and fruit and flower production, with the potential to reduce crop yields, reduce pollinator resources, and alter plant-herbivore interactions. Additionally, drought could have negative impacts on the performance of invertebrates that influence plant growth. These invertebrates include earthworms (Oligochaeta: Lumbricidae), ecosystem service providers

with generally positive effects on plant growth but with performance and activity that is strongly influenced by soil moisture. In this pot experiment, the effects of the endogeic earthworm *Allolobophora chlorotica* (Savigny, 1826) on the fruit production and growth of alpine strawberry plants (*Fragaria vesca* L.) were examined across three watering treatments with a control treatment simulating mean summer rainfall, a reduced watering treatment simulating 50% of mean summer rainfall, and an increased watering treatment simulating 150% of mean summer rainfall, based on historic data from Oxfordshire, UK. Performance of both earthworms (reproduction and mean mass change) and strawberry plants (fruit number, fruit mass, and fruit quality) were significantly lower in the reduced watering treatment. Earthworm reproduction and strawberry fruit number and fruit mass were higher in the increased rainfall watering treatment compared to the control watering treatment. However, there were no significant effects of earthworms on strawberry plant vegetative growth or fruit production across any of the watering treatments. These results suggest that drier summers will have negative effects on both earthworms and plant performance and that, if multiple dry years occur in a row, earthworm populations and fruit production could be severely affected. The limited effects of earthworms on plant performance in this study suggest that earthworms may not mitigate these negative effects on plant performance.

Keywords: Strawberries, earthworms, drought, rainfall, fruit production, crop yields, climate change

Introduction

In the UK, future summer and autumn precipitation is predicted to decrease (Cotterill et al., 2023; Met Office, 2022). These changes to weather patterns could reduce plant performance and crop production (Farooq et al., 2009; Lipiec et al., 2013; Rial-Lovera et al., 2017; Seleiman et al., 2021). In addition, reduced rainfall can influence plant-invertebrate interactions, such as pollination (Kuppler and Kotowska, 2021; Phillips et al., 2018) and herbivory (Gutbrodt et al., 2011). Belowground invertebrates, such as earthworms (Oligochaeta: Lumbricidae), can have strong effects on plant responses to drought, influencing plant growth form and performance. Earthworms can increase plant growth (Van Groenigen et al., 2015) by increasing soil fertility through their casts (Van Groenigen et al., 2019), influencing the soil microbiota (Medina-Sauza et al., 2019), influencing soil water holding capacity (Hodson et al., 2023), and may stimulate plant growth signalling pathways (Puga-Freitas et al., 2012; Puga-Freitas and Blouin, 2015). Therefore, economic analysis suggests earthworms make significant contributions to global crop yields (Fonte et al., 2023). However, the growth (Lowe and Butt, 2008a; Wever et al., 2001), activity (Lee, 1985), and reproduction (Evans and Guild, 1948) of earthworms is strongly influenced by soil moisture. During periods of drought or decreased soil moisture, activity of endogeic earthworm species can cease as earthworms enter a period of dormancy termed aestivation (Lee, 1985). Soil moisture can also influence earthworm effects on plant growth. During droughts, earthworms can have both positive (Hodson et al., 2023; Johnson et al., 2011) and negative (Blouin et al., 2007) effects on plant performance and can both increase (Hodson et al., 2021) or decrease (Ma et al., 2021) soil moisture content. Earthworms influence plant growth form and resource allocation (Poveda et al., 2005; Scheu, 2003; Zaller et al., 2011), which could provide benefits for crop yields if the trait of interest (*e.g.* fruit mass or fruit number) is

beneficially affected by earthworms. In this way, earthworms may improve crop yields during periods of environmental stress.

This study investigated the effects of the endogeic earthworm *Allolobophora chlorotica* (Savigny, 1826) on plant growth and fruit yields of alpine strawberry (Rosaceae, *Fragaria vesca* L. cv. "Golden Alexandria") across three watering treatment levels that simulated average, increased, or decreased late summer rainfall in Oxfordshire, UK. It was predicted that plant performance (growth and fruit yields) would increase with increased simulated rainfall and decrease with reduced simulated rainfall. It was predicted that earthworm performance (mass change, survival, juvenile earthworm number) would follow the same pattern. It was also predicted that earthworms would have a beneficial effect on plant performance, consistent with effects reported elsewhere (Van Groenigen et al., 2015). However, it was predicted that the benefits conferred by earthworms would depend on the watering treatment. It was predicted that earthworms would provide a greater benefit with increase simulated rainfall, as earthworm activity would be enhanced by the increase soil moisture from this treatment, and a reduced benefit with decreased simulated rainfall due to declines in earthworm activity, increased earthworm mortality, or cessation of earthworm activity due to aestivation.

Methods

Experiment overview and study site

A fully factorial experiment was conducted that manipulated earthworms (presence or absence) in plant pots over three watering treatment levels (*control*, *wet*, or *dry*; described below), with each treatment combination replicated ten times for a total of 60 experimental

units. The experiment was conducted from 16 July – 18 December 2022. Part of the duration of the experiment (16 July – 21 October) was conducted in a grassy outdoor area at the John Krebs Field Station (Department of Biology), University of Oxford, near the village of Wytham, Oxfordshire, UK. This outdoor area contained raised garden beds with a variety of planted flowering vegetable crops (beans, courgettes, aubergines, alpine strawberry) in addition to naturally occurring dandelions (*Taraxacum* spp. Weber), daisies (*Bellis perennis* L.), and white clover (*Trifolium repens* L.). The later portion of the experiment (21 October – 18 December) was conducted in a research glasshouse at the same field station. The field station is adjacent to an organic cattle farm and close to Wytham Woods where 679 pollinator taxa were identified from surveys conducted from 2017 – 2020 (Falk, 2021). Additionally, several honeybee colonies (*Apis mellifera* Linnaeus, 1758) are maintained on site at the field station. Therefore, a range of pollinating insect species, and floral resources for pollinators, were present at the site which would have facilitated pollination of the study plant in this experiment (see below).

Study system

Alpine strawberry (Rosaceae, *Fragaria vesca* L. cv. "Golden Alexandria", supplier Unwins Seeds, Westland Horticulture Ltd., Dungannon, Tyrone, Northern Ireland, UK) was used as a representative plant species for this experiment. Alpine strawberry is a small herbaceous perennial plant that is popular with amateur growers (Chambers et al., 2018; Chambers, 2019). It is similar in morphology to *Fragaria* x *ananassa* (Weston) Rozier, a commonly grown horticultural crop, although *F. vesca* has a much wider flowering period. Like *F.* x *ananassa*, *F. vesca* produces compound fruit and, while flowers are partially wind pollinated, visitation from a range of insect species is necessary for pollination of all achenes to maximise fruit development (Chagnon et al., 1993). Incomplete pollination or

poor abiotic conditions can lead to fruit deformation and small size (Free, 1970), with complete developmental failure leading to a tiny and deformed fruit called a nubbin (Delaplane and Mayer, 2000). Strawberry marketing class is determined by size and form (Animal and Plant Health Agency and Department for Environment, Food and Rural Affairs, 2014), so fruit yields are dependent on both fruit number and fruit development.

Allolobophora chlorotica (Savigny, 1826) was chosen as a representative earthworm species. Earthworms are typically divided into three ecotypes based on morphology and ecology (Bouché, 1977, 1972). *Allolobophora chlorotica* has been described as an endogeic species (Bottinelli et al., 2020) or intermediate between ecotypes (Bouché, 1972), and forms temporary horizontal burrows near the soil surface (Brown, 1995; Capowiez et al., 2024; Lavelle, 1988). *Allolobophora chlorotica* is a hermaphroditic species which reproduces via cocoons that are deposited in the upper layers of soil (Butt, 1997). Cocoons consist of balls of hardened mucus containing albuminous fluid, ova, and spermatozoa in which offspring develop (Domínguez and Velando, 2013). During droughts, *A. chlorotica* can enter a period of dormancy called aestivation or diapause, where activity is halted to reduce water loss (Lee, 1985; Plum and Filser, 2005). *Allolobophora chlorotica* exists as a pink or green morph (Satchell, 1967b), although evidence suggests that this species could be a cryptic species complex with multiple pink and green phenotype lineages (King et al., 2008). For this experiment, only the pink morph was used due to differences in moisture preferences between the pink and green morphs (Lowe and Butt, 2007a) and because, although the two morphs can interbreed (Satchell, 1967b), cocoon viability is reduced in inter-morph breeding (Dupont et al., 2022; Lowe and Butt, 2008b).

Experiment setup

Experimental units were constructed from ~2 L (15 cm upper diameter) black polypropylene plant pots. Each pot had six drainage holes (~1 cm diameter) in the base. To prevent earthworm escape through these holes without preventing water drainage, two plastic mesh disks with 1 mm square holes were attached to the inside and outside of the pot based using hot-melt adhesive. The pots were open-topped, so adhesive hook tape (2 cm width) was applied around the upper rim of the plant pots to prevent earthworm escape (Lubbers and Van Groenigen, 2013). Sixty pots were part-filled with 200 g of washed 10 cm diameter gravel before being filled with a substrate made from a mixture of Kettering Loam Topsoil (Boughton Loam Ltd., Kettering, Northamptonshire, UK) and dried cattle dung milled to <2 mm. Kettering Loam topsoil is a sandy-loam soil (24% clay, 18% silt, 58% sand, pH 6.8) that has been used as a substrate for earthworms in previous experiments (*e.g.,* Butt and Briones, 2011; Lowe and Butt, 2008a, 2007) and is the recommended substrate for controlled culture of endogeic and anecic earthworms (Lowe and Butt, 2005). Fresh cattle dung (<24 hours old) was collected from a mixed-sex herd of Angus-Saler-Stabiliser cattle on an organic farm adjacent to the John Krebs Field Station and oven-dried at 70 °C prior to milling to <2mm (Lowe and Butt, 2003). Several bags of Kettering Loam soil (~15% gravimetric water content) were first homogenised and combined with the dried cattle dung at a ratio of 5 g of dry dung to 1 kg soil with an electric paddle mixer (1220 W corded mixer, Wickes PLC, Watford, UK). Tap water was then added to the soil and homogenised with the same paddle mixer in order to wet the substrate to ~25% gravimetric water content. Pots were filled with ~1800 g of the substrate in four stages: each pot received 250 g, 500 g, 750 g, and 300 g of substrate in four randomly ordered rounds. After filling, each pot was gently raised and dropped onto a

concrete slab several times from ~5 cm to evenly compact the soil across pots. The soil was then gently pressed by hand to form a flat uniform surface in preparation for planting.

Strawberry seeds were sown in the same research glasshouse in which the latter portion of the experiment was conducted (see below) on 3 May across three lidded 60-cell plug trays (2 x 2 cm cells) filled with Westland John Innes Seed Sowing Compost (Westland Horticulture Ltd., Dungannon, Tyrone, Northern Ireland, UK). Each cell received approximately six seeds that were gently pressed by hand to ensure good contact with the compost. Supplemental lighting from high pressure sodium bulbs was provided daily from 06:00 – 22:00 (16 hour light : 8 hour dark) for the first six weeks of growth. As seeds germinated and seedlings developed, seedlings were gradually thinned using forceps until each cell contained one seedling. Plug trays were stored in non-draining horticultural trays which were watered regularly until seedlings were ready for transplanting into the experimental pots. Strawberry seedlings were transplanted on 16 July. A ~5 cm deep hole was made in the centre of the substrate in each pot using a garden dibber. Strawberry seedlings (Fig. S6.1) were carefully removed from the plug tray and roots were washed with tap water to remove as much of the compost as possible. One seedling was then gently placed into the soil hole in a random order after which the soil around the hole was pressed gently by hand to ensure good contact between the roots and substrate. Each pot then received ~100 ml of tap water and any open flowers or unopened flower buds were removed.

Adult (visible clitellum) pink morph *A. chlorotica* were hand collected over several days in May 2022 from an outside grassy area at the John Krebs Field Station. As the pink morph of *A. chlorotica* may consist of multiple lineages (King et al., 2008), all earthworms were collected from the same location. Earthworms were collected from underneath weighted

polypropylene buckets that had been left for several weeks in the grassy area where they pressed down the vegetation. These buckets were lifted regularly and any pink morph *A. chlorotica* at the soil surface or amongst the compressed vegetation were collected. Earthworms were kept in groups of ~20 individuals in 3 L ventilated containers (24.5 x 18.5 x 7.5 cm) filled with a mixture of Kettering Loam topsoil and dried, milled, and rewetted cattle dung (as described above) for food (Lowe and Butt, 2005, 2003). Each container was filled with ~2 kg of soil and ~200 g of ~80% water content dung before water was added to reach 25% gravimetric water content, as recommended for *A. chlorotica* culture (Lowe and Butt, 2005). Earthworm containers were stored in the same glasshouse as used for the experiment prior to use. Earthworms were removed from their culture containers on 20 July. Earthworms were washed with tap water and transferred to empty ventilated containers lined with moist tissue paper and stored in the same glasshouse for 48 hours for gut voidance (Fründ et al., 2010). On 22 July, individual earthworms were randomly assigned to groups of three earthworms (for a density of 170 earthworms m^{-2}) with similar starting mass. These groups were then randomly assigned to earthworm-containing pots with no significant differences in earthworm starting mass across watering treatment levels ($p > 0.05$). Earthworms were placed on the soil surface of pots and observed to burrow quickly into the soil (Fig. S6.2).

Experimental protocol

The experimental pots were moved out of the glasshouse on 29 July and randomly arranged in a 6 x 10 grid under a 2 x 3 m garden polytunnel rain shelter consisting of a metal frame and 150 g m^{-2} transparent polyethylene cover (Fig. S6.3, S6.4, Pro-Tunnel GroZone Max, Smart Garden Products Ltd., Abingdon, Oxfordshire, UK) to act as a rain shelter. Polytunnel walls were rolled up to prevent overheating and allow access to

pollinating insects. The pots were initially placed on the ground under the polytunnel but, due to extremely high temperatures during July 2022 (Kendon et al., 2023), a raised bed was constructed where the pots were initially placed on 5 August (Fig. S6.5). This consisted of a ~2 x 3 m rectangle made of ~30 cm tall cement blocks that was filled in with a sandy loam topsoil (North Oxfordshire Topsoil Ltd., Banbury, Oxfordshire, UK). The pots were then sunk into this soil to prevent overheating for the remainder of the experiment. Four extra pots, the same as those used in the experiment and containing *A. chlorotica* but without strawberry plants, were placed adjacent to the experimental pots for the second week of the experiment and showed very high mortality of *A. chlorotica* after hand searching the soil for earthworms on 16 August. Therefore, on 17 August, additional *A. chlorotica* were prepared in the same way as described previously. On 19 August, all pots were moved to the glasshouse and each earthworm-containing pot received an additional two *A. chlorotica*, for a final density of 283 earthworms m^{-2}. Pots were returned to the raised bed on 23 August. The average starting earthworm mass (with five earthworms) was 1.28 g ± SE 0.01 g and there were no significant differences in starting mass over the watering treatment levels ($F_{2,27} = 1.03$, $p = 0.372$, treatments described below). Mean air temperatures in the polytunnel were 16.6 °C (SD = 6.6, min = −0.9 °C, max = 40.0 °C, recording dates = 5 August – 18 October 2022).

In addition to the earthworm treatment, pots were watered with one of three watering levels. Each earthworm and watering treatment combination was replicated ten times. Watering levels were based on historic rainfall data from Oxfordshire collected between 1970 and 2020 (Burt and Burt, 2019). Rainfall data for August and September were extracted and average mm of rainfall was calculated for each month. This was multiplied by the surface soil area of the pots (176.71 cm^2) to estimate rainfall in ml that the pot would receive in one month. This was then divided by nine (two watering events per

week) and rounded up to the nearest ml to provide the volume of water (in ml) per watering event for *controls*. A *dry* treatment was generated by halving this estimate while a *wet* treatment was generated by multiplying this estimate by 1.5. Therefore, all pots were watered twice per week: *controls* received 108 ml per watering event, *dry* pots received 54 ml per watering event, and *wet* pots received 162 ml per watering event. Pots were returned to the glasshouse on 21 October to allow any final fruit to finish development before the experiment was ended. *Fragaria vesca* has a perpetual flowering period, so would continue to produce new buds and flowers for the remainder of the experiment. Therefore, unopened buds and open flowers were removed on 31 October and plants were regularly checked until the end of the experiment with any new buds or flowers being removed as they developed. It was determined that, due to the small size of the rain shelter it would be necessary to move the experimental pots into the glasshouse to prevent them being compromised by increase rainfall levels in October to December. Pots received the same watering treatment as when outside when in the glasshouse. Mean glasshouse temperatures were 11.1 °C (SD = 4.2, min = 0.3 °C, max = 21.8 °C, recording dates = 20 November – 14 December 2022).

Strawberry fruits were harvested when mature (Fig S6.6; fully red in colour) throughout the duration of the experiment and weighed to record fresh mass. Strawberries were then dried at 70 °C and weighed. Strawberries were photographed and later classified according to UK Fresh Fruit and Vegetable Marketing Standards (Animal and Plant Health Agency and Department for Environment, Food and Rural Affairs, 2014) where fruits with either no imperfections, or minor deformities, were classified as Class 1, fruits with moderate damage, deformity, and discolouration were classified as Class 2, and fruits that were severely deformed or damaged were classified as Class 3.

Strawberry plants were harvested on 18 December. All plants were cut at the soil surface to harvest aboveground biomass. Soil was then cleaned from the roots with tap water. Strawberry plant shoots and roots were placed into individual paper bags and transferred to a drying oven at 70 °C until they reached constant mass and were weighed. Any immature fruits on plants were counted and dried at 70 °C. The total mass of dried immature and mature fruits was combined to calculate the total dry fruit (mature + immature) mass per plant. Shoot and root mass were used to calculate the shoot : root ratio. The shoot and root mass was summed and used to calculate the fruit : plant ratio (the ratio of total mature fruit mass to total dry plant biomass). The soil was then removed from each pot and hand searched for earthworms and numbers of both adult and juvenile *A. chlorotica* were recorded. Cocoons were present in the soil but could not be reliably collected so were not counted. Earthworms recovered from each pot were grouped, washed with tap water, and transferred to clear ventilated plastic tubs lined with moist tissue paper and stored in a 15 °C incubator in darkness for 48 hours for gut voidance (Fründ et al., 2010) before masses of all adults and juveniles were recorded on 20 December. All masses (fruits, plant parts, and earthworms) were recorded to 0.001 g precision using a OHAUS Pioneer PX323 microbalance (OHAUS Europe GmbH, Switzerland).

Data analysis

Data analysis was performed with R version 4.3.1 (R Core Team, 2023). Strawberry shoot mass, root mass, shoot : root ratio, total fresh fruit mass, mean fresh fruit mass, total dry fruit mass (mature + immature fruits), and the coefficient of variation of fresh fruit mass were analysed with Analysis of Variance (ANOVA), with earthworms, the watering treatment, and their interaction included as explanatory factors. Fruit number (both total mature and total mature + immature fruits) was analysed with Poisson Generalised Linear

Models (GLMs) and the proportion of mature strawberries in Class 1 and in Class 1 or 2 were analysed with Binomial GLMs, with the same explanatory variables as used in the ANOVAs described previously. Earthworm mean and total mass change were analysed with ANOVA, juvenile earthworm number was analysed with a Poisson GLM, and adult earthworm survival was analysed with a Binomial GLM with watering treatment included as the explanatory factor for all earthworm analyses. No juvenile earthworms were found in the *dry* treatment (see *Results*), so analysis of juvenile earthworm mass response variables (means and total mass) was only conducted across the *control* and *wet* treatments. Assumptions of ANOVA were checked by inspecting diagnostic plots and total dry fruit mass (mature + immature fruits), shoot mass, root mass, and shoot : root ratio were analysed on the log scale. Fruit : plant ratio and total wet fruit mass were analysed on the log+1 scale, as simple log transformation was not possible due to some plants failing to produce fruit. If variables or interactions had statistically significant effects, *post hoc* tests were performed with the *emmeans* package (Lenth, 2023), with group differences back transformed to the response scale for interpretation and reporting when data transformation was performed or data were analysed with GLMs. All figures were produced with *ggplot2* (Wickham, 2016).

Results

Earthworm performance

Overall mean *A. chlorotica* survival was 68.7% and median survival was 80%, with no significant differences in survival across watering treatments (Fig. 6.1a, Table 6.1). One *dry* earthworm-containing pot contained no adult earthworms. There were no significant differences in total earthworm mass change across treatments (Fig. 6.1b, Table 6.1), but

total mass significantly decreased during the experiment by an average of 0.48 g ($CI_{95\%}$ = [−0.34, −0.61], $p < 0.001$) per pot. Mean individual earthworm mass also declined (μ = −0.023 g, $CI_{95\%}$ = [−0.014, −0.031], $p < 0.001$) and earthworms lost significantly more mass in the *dry* treatment than in the *control* (Table 6.1; Fig. 6.1c; difference = 0.025 g, $CI_{95\%}$ = [0.003, 0.048], $p = 0.024$) or *wet* treatments (Table 6.1; Fig. 6.1c; difference = 0.025 g, $CI_{95\%}$ = [0.003, 0.048], $p = 0.025$). Twenty-four juvenile *A. chlorotica* were collected from 12 out of 30 earthworm-containing pots, with zero found in *dry* treatment pots (Fig. 6.1d – f). Mean juvenile mass did not differ significantly between *wet* and *control* treatments (Table 6.1; Fig. 6.1f), but both juvenile number (Fig. 6.1d, Table 6.1, ratio = 3.8, $CI_{95\%}$ = [1.42, 10.2], $p = 0.008$) and total juvenile mass (Fig. 6.1e, Table 6.1, difference = 0.034g, $CI_{95\%}$ = [−0.0004, 0.067], $p = 0.052$) were significantly higher in the *wet* treatment than in the *control* treatment.

Table 6.1: Summary of ANOVA results showing the effect of the watering treatment on earthworm response variables.

Response variable	Test	Watering treatment		
		Df	Test statistic	P
Earthworm survival	X^2	2	4.72	0.095
Total adult mass	F	2,27	2.09	0.143
Mean adult mass[a]	F	2,26	5.19	**0.013**
Juvenile earthworms	X^2	2	28.17	**<0.001**
Total juvenile mass[b]	F	1,18	4.31	0.052
Mean juvenile mass[abc]	F	1,10	0.00	0.964

[a]Replicates with zero earthworms found were excluded from mean mass analyses.
[b]*Dry* treatment pots were excluded from juvenile earthworm mass analyses as none contained juvenile earthworms.
[c]*Control* and *Wet* treatment pots with no juvenile earthworms were excluded from mean juvenile mass analysis.

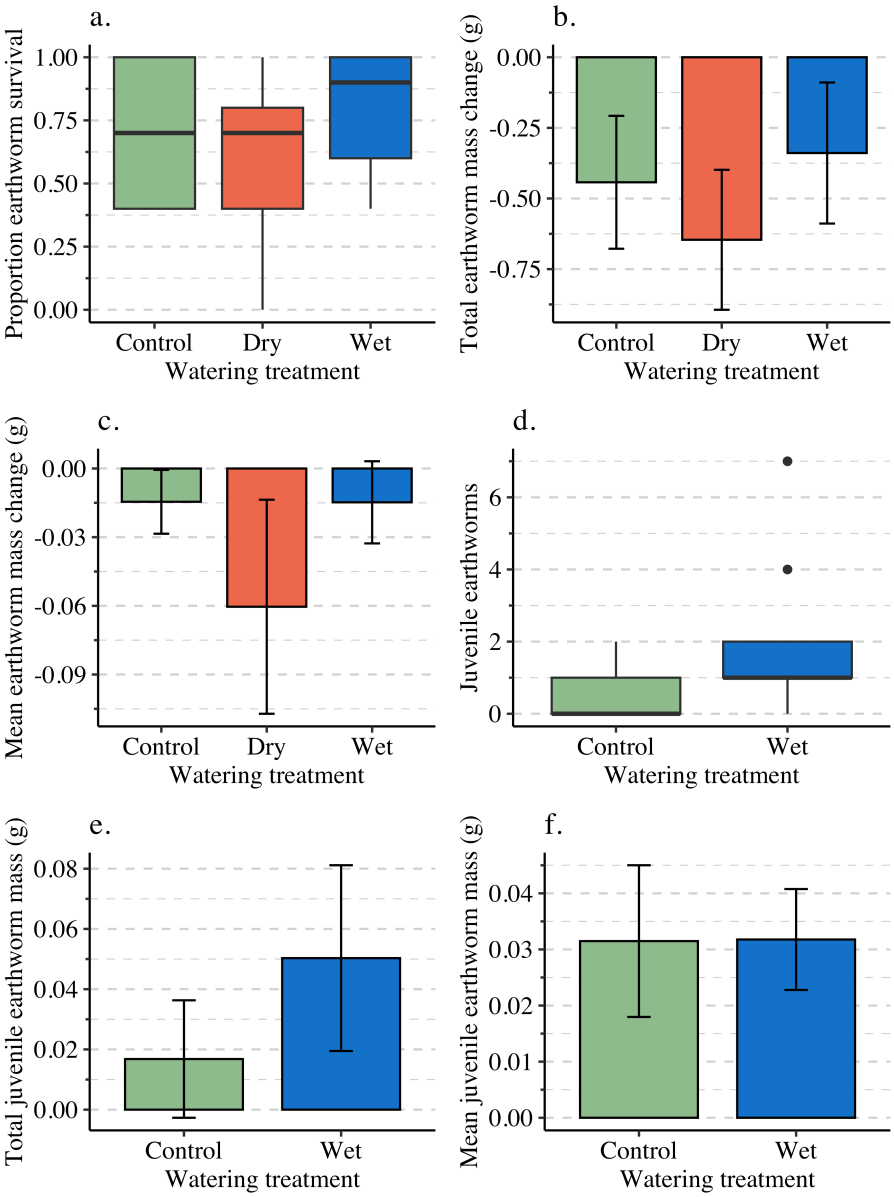


Fig. 6.1: Box and bar plots showing *a.* proportion of adult earthworm survival, *b.* total adult earthworm mass, *c.* mean adult earthworm mass, *d.* juvenile earthworm counts, *e.* total juvenile earthworm mass, and *f.* mean juvenile earthworm mass. For box plots, box outer horizontal lines show group interquartile ranges, central horizontal lines show group medians, and extended vertical lines show full data range for each group. Box plot points show outlier data points (>1.5x interquartile range above 75th percentile). For bar plots, bar heights show group means and error bars show 95% confidence intervals. The *dry* treatment is excluded from plots *d.* – *f.* as no juvenile earthworms were recovered from the *dry* treatment pots.

Plant performance

Fruit yields

In total, 554 mature strawberries were collected (Fig. S6.7). Mature strawberries were collected from 57 out of 60 plants, with one plant from each of the *dry* + earthworms present, *dry* + earthworms absent, and *control* + earthworms present treatments failing to produce mature fruit. When immature fruits were included, all plants successfully produced at least one fruit.

There were no statistically significant interactions between the watering treatment and earthworms for any of the fruit response variables measured, nor was there a significant main effect of earthworms for any variables (Table 6.2; Fig. 6.2). The watering treatment significantly affected all response variables except for the proportion of fruits in Class 1 and 2 (Table 6.2; Fig. 6.2f). Significantly more fruits (Fig. 6.2a) were harvested from the *wet* treatment than from the *control* (ratio = 1.80, $CI_{95\%}$ = [1.45, 2.24], $p < 0.001$) and *dry* (ratio = 7.45, $CI_{95\%}$ = [5.12, 10.86], $p < 0.0001$) treatments, and significantly more fruits were harvested from the *control* treatment than from the *dry* treatment (ratio = 4.13, $CI_{95\%}$ = [2.79, 6.12], $p < 0.001$). Both mean (Fig. 6.2b) and total (Fig. 6.2c) fruit mass was significantly higher in the *wet* treatment than in the *control* (*Mean mass*: difference = 0.24 g, $CI_{95\%}$ = [0.02, 0.47], $p = 0.03$. *Total mass*$_{(\log +1)}$: difference = 12.95 g, $CI_{95\%}$ = [9.35, 16.56], $p < 0.001$) and *dry* treatment (*Mean mass*: difference = 0.98 g, $CI_{95\%}$ = [0.75, 1.21], $p < 0.001$. *Total mass*$_{(\log +1)}$: difference = 22.94 g, $CI_{95\%}$ = [19.34, 26.54], $p < 0.001$), and higher in the *control* treatment than in the *dry* treatment (*Mean mass*: difference = 0.74 g, $CI_{95\%}$ = [0.51, 0.97], $p < 0.001$. *Total mass*$_{(\log +1)}$: difference = 9.99 g, $CI_{95\%}$ = [6.38, 13.59], $p < 0.001$). The coefficient of variation of strawberry fruit mass (Fig. 6.2d) was also significantly lower in the *wet* treatment than in the *dry* treatment (difference =

0.23, $CI_{95\%}$ = [0.07, 0.38], p = 0.002). The proportion of harvested fruits in class 1 (Fig. 6.2e) was significantly lower in the *dry* treatment than in *wet* (odds ratio = 0.33, $CI_{95\%}$ = [0.15, 0.73], p = 0.003) or *control* treatments (odds ratio = 0.40, $CI_{95\%}$ = [0.18, 0.91], p = 0.023). When immature fruits were considered in addition to mature fruits (Fig. 6.2g), the total number of mature and immature fruits was significantly higher in the *wet* treatment than in the *control* (ratio = 1.83, $CI_{95\%}$ = [1.50, 2.23], $p < 0.001$) and *dry* (ratio = 7.95, $CI_{95\%}$ = [5.59, 11.30], $p < 0.001$) treatments and higher in the *control* than in the *dry* treatment (ratio = 4.34, $CI_{95\%}$ = [3.00, 6.26], $p < 0.001$). Similarly, total dry mature and immature dry fruit mass (Fig. 6.2h) was significantly higher in the *wet* treatment than in the *control* (ratio = 2.99, $CI_{95\%}$ = [1.58, 5.63], p <0.001) and *dry* (ratio = 32.41, $CI_{95\%}$ = [17.19, 61.11], $p < 0.001$) treatments and higher in the *control* than in the *dry* treatment (ratio = 10.85, $CI_{95\%}$ = [5.76, 20.46], $p < 0.001$).

Table 6.2: Summary of ANOVA results (Type II) showing the effects of earthworms, the watering treatment, and their interaction on strawberry fruit response variables.

Response variable	Test	Earthworms			Water			Earthworms x Water		
		Df	Test statistic	P	Df	Test statistic	P	Df	Test statistic	P
Fruit number	X^2	1	0.58	0.444	2	245.33	**<0.001**	2	0.16	0.923
Mean wet mass[a]	F	1,51	0.01	0.929	2,51	57.81	**<0.001**	2,51	2.01	0.144
Total wet mass[b]	F	1,54	1.24	0.271	2,54	142.01	**<0.001**	2,54	0.28	0.754
Fruit CV[c]	F	1,47	1.52	0.223	2,47	6.61	**0.003**	2,47	0.54	0.587
Fruit Pr. class 1[a]	X^2	1	2.79	0.095	2	10.39	**0.006**	2	1.32	0.518
Fruit Pr. class 1 & 2[a]	X^2	1	0.00	0.979	2	4.80	0.091	2	0.69	0.709
Total fruit number[d]	X^2	1	0.10	0.756	2	306.56	**<0.001**	2	0.79	0.675
Total dry mass[de]	F	1,54	1.46	0.232	2,54	91.39	**<0.001**	2,54	0.35	0.707

[a]Pots with zero strawberries harvested were excluded from analysis of mean wet mass, proportion of fruits in class 1, and proportion of fruits in class 1 or 2.
[b]Analysis performed on the log +1 scale.
[c]Pots with zero or one strawberry harvested were excluded from analysis of Coefficient of Variation.
[d]Analysis included both mature and immature fruits.
[e]Analysis performed on the log scale.

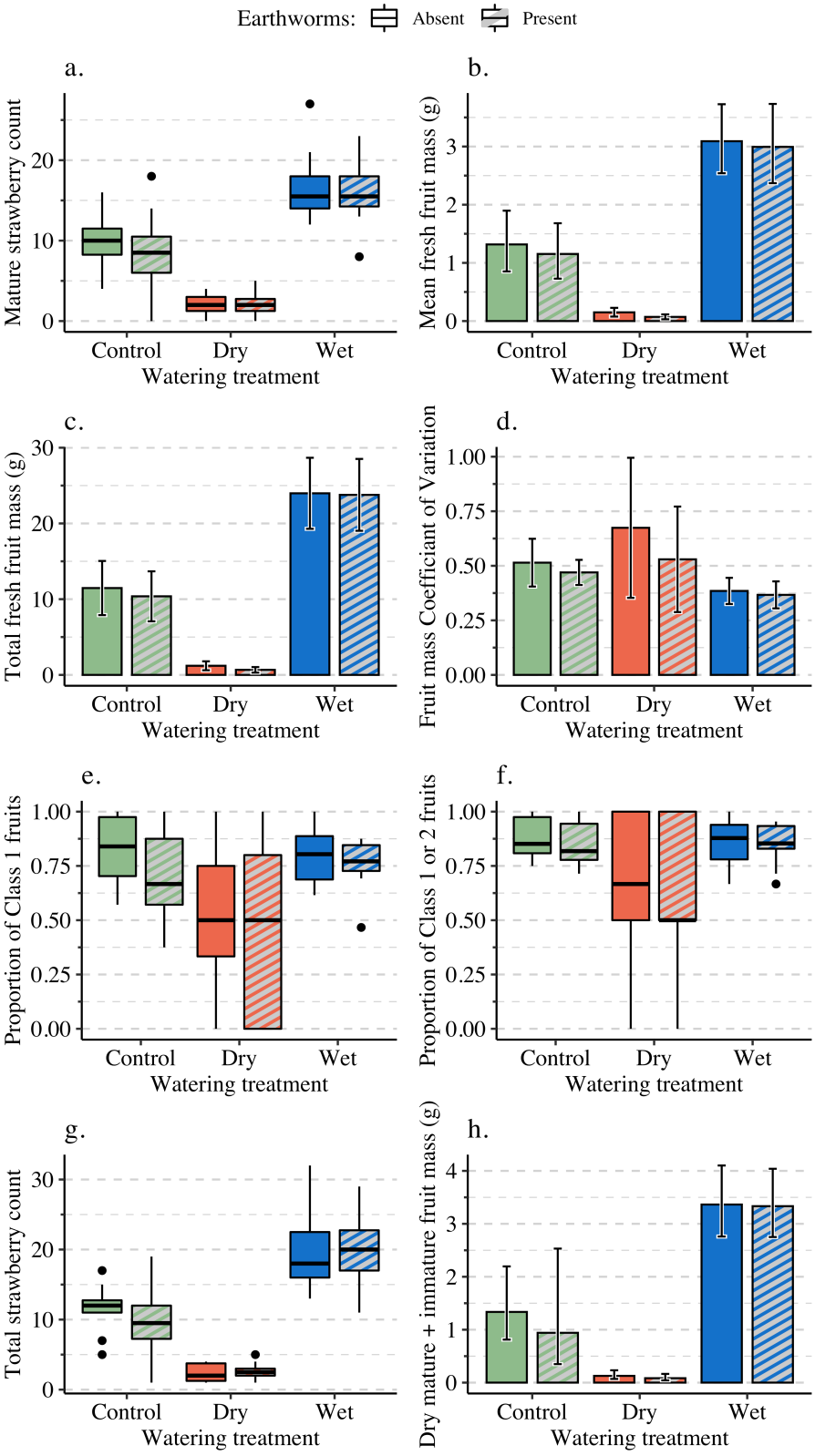


Fig. 6.2: Box and bar plots showing *a.* mature strawberry fruit counts, *b.* mean strawberry fruit mass (g), *c.* total strawberry fruit mass (g), *d.* coefficient of variation of strawberry fruit mass *e.* the proportion of fruits in Class 1, *f.* the proportion of fruits in class 1 or 2, *g.* the total mature + immature fruit count, and *h.* total dry strawberry fruit mass (g, immature + mature fruit) across earthworm (pattern fill) and watering (colour and plot labels) treatments. For bar plots, bar

heights show group means and error bars show 95% confidence intervals. Means and error bars in *c.* have been back-transformed from the log + 1 scale. For box plots, box outer horizontal lines show group interquartile ranges, central horizontal lines show group medians, and extended vertical lines show full data range for each group. Box plot points show outlier data points (>1.5x interquartile range below 25th percentile or >1.5x interquartile range above the 75th percentile for points plotted below and above boxes, respectively).

Plant growth

There was no significant interaction between the watering treatment and earthworms for any of the plant growth variables nor was there a significant effect of earthworms for any variables (Table 6.3; Fig. 6.3a – d),. However, all variables were significantly affected by the watering treatment (Table 6.3; Fig. 6.3a – d). Shoot mass$_{(log)}$ (Fig. 6.3a) was significantly higher in the *wet* treatment than in *control* (ratio = 1.14, $CI_{95\%}$ = [1.04, 1.26], p = 0.004) and *dry* treatments (ratio = 3.60, $CI_{95\%}$ = [3.28, 3.96], $p < 0.001$), and in the *control* treatment than in the *dry* treatment (ratio = 3.15, $CI_{95\%}$ = [2.87, 3.47], $p < 0.001$). Root mass$_{(log)}$ (Fig. 6.3b) was also significantly higher in the *wet* treatment than in the *control* (ratio = 1.48, $CI_{95\%}$ = [1.21, 1.80], $p < 0.001$) and *dry* treatments (ratio = 4.05, $CI_{95\%}$ = [3.32, 4.93], $p < 0.001$), and in the *control* treatment than in the *dry* treatment (ratio = 2.74, $CI_{95\%}$ = [2.25, 3.33], $p < 0.001$). Shoot : root ratio$_{(log)}$ (Fig. 6.3c) was significantly higher in the *control* treatment than in *wet* treatment (ratio = 1.29, $CI_{95\%}$ = [1.10, 1.52], p = 0.001). Fruit : plant ratio$_{(log +1)}$ (Fig. 6.3d) was significantly higher in the *wet* treatment than in the *control* (difference = 0.32, $CI_{95\%}$ = [0.15, 0.50], $p < 0.001$) and *dry* treatments (difference = 0.79, $CI_{95\%}$ = [0.62, 0.97], $p < 0.001$) and higher in the *control* treatment than in *dry* treatment (difference = 0.47, $CI_{95\%}$ = [0.30, 0.64], $p < 0.001$).

Table 6.3: Summary of ANOVA results showing the effects of earthworms, the watering treatment, and their interaction on strawberry plant response variables.

Response variable	Earthworms			Water			Earthworms x Water		
	Df	F	P	Df	F	P	Df	F	P
Shoot mass[a]	1,54	0.98	0.328	2,54	645.14	**<0.001**	2,54	0.49	0.614
Root mass[a]	1,54	0.89	0.350	2,54	155.59	**<0.001**	2,54	0.04	0.957
Shoot : root ratio[a]	1,54	2.95	0.092	2,54	7.33	**0.002**	2,54	0.02	0.976
Fruit : plant ratio[b]	1,54	0.70	0.407	2,54	61.77	**<0.001**	2,54	0.38	0.687

[a]Analysis performed on the log scale.
[b]Analysis performed on the log +1 scale.

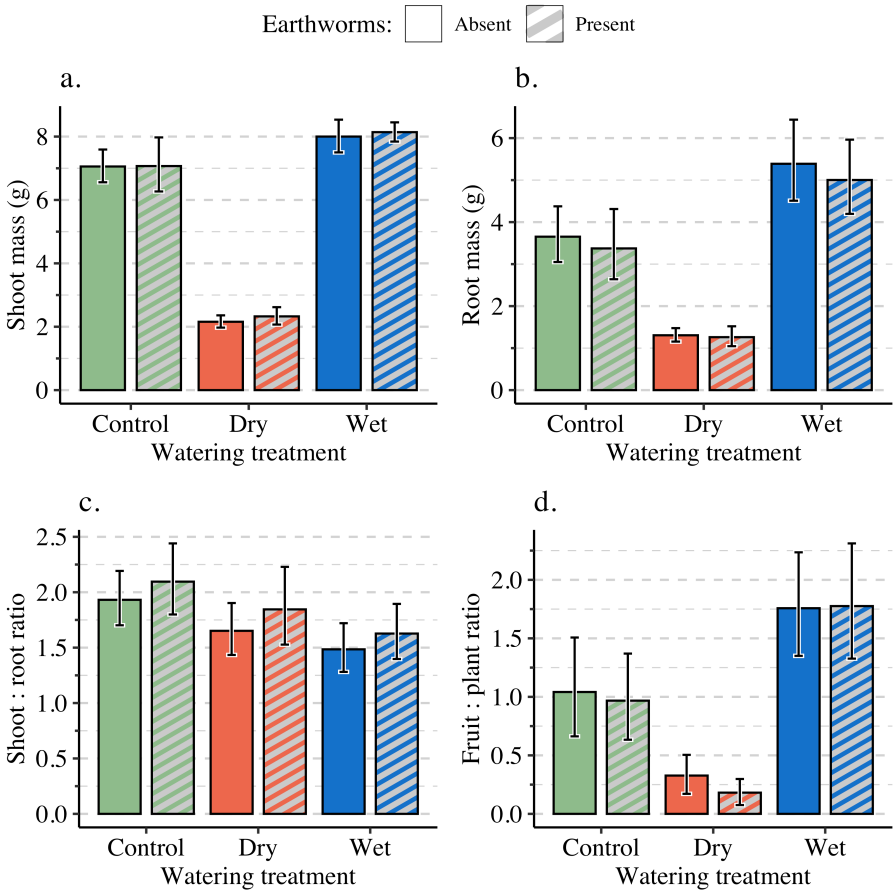


Fig. 6.3: Bar plots showing *a.* shoot mass, *b.* root mass, *c.* shoot : root ratio, and *d.* fruit : plant ratio across earthworm and watering treatment groups. Bar heights show group means and error bars show 95% confidence intervals. Confidence interval limits and group means in *a.*, *b.*, and *c.* have been back-transformed from the log scale. Error bars and confidence interval limits and group means in *d.* have been back transformed from the log + 1 scale.

Discussion

Although the benefits of earthworms for plant growth have been documented widely in previous studies (Scheu, 2003; Van Groenigen et al., 2015) and elsewhere in this thesis (Chapters 2 and 3), there were no significant positive or negative effects of *A. chlorotica* on *F. vesca* shoot growth, root growth, or fruit production in this study (Fig. 6.2, 6.3). The performance of the *A. chlorotica* (Fig. 6.1 – survival, mass change, and juvenile production) and *F. vesca* (Fig. 6.2, 6.3 – fruit yields and growth) were both significantly affected by the watering treatment. Both groups were severely impacted by the *dry* watering treatment and both groups showed higher performance with increased simulated summer rainfall. These results suggests that the endogeic earthworm *A. chlorotica* may be unable to ameliorate the negative effects of reduced summer rainfall on certain plants.

Effects of earthworms and watering treatment on plant performance

The watering treatment significantly influenced all measures of plant performance, with performance increasing from the *dry* to the *control* to the *wet* treatment (Fig. 6.2, 6.3). Previous work in the United States found that *F.* x *ananassa* cv. "Jewel" shoot mass can be reduced in years with reduced rainfall (Schattman et al., 2022). In addition, fruit yields of *F.* x *ananassa* cv. "Sachinoka" grown in a glasshouse increased as drip irrigation levels were increased (Yuan et al., 2004), while in other cases less frequent irrigation caused reduced fruit number and mass in cv. "Chandler" (Serrano et al., 1992). Reductions in substrate moisture reduced shoot, but not root, mass in cv. "Salut" (Klamkowski and Treder, 2006) and drought stress can cause declines in fruit yields and fruit quality indicators in multiple strawberry plant varieties (Adak et al., 2018). However, in other cases, while fruit masses are decreased, water stress can cause concentrations in flavour and health-promoting compounds in strawberry fruits (Terry et al., 2007). Additionally,

excessive rainfall can lead to fruit damage, reducing yields and fruit quality (Herrington et al., 2011). In this experiment, no evidence was observed of increased fruit damage in the *wet* treatment. This may have been because plants were watered carefully at the soil surface to prevent water splashing off leaves and out of the pots. Overall, the results of the present study suggest that increasingly dry summers could have strong negative impacts on strawberry plant performance and fruit production.

Although many studies have documented positive influences of earthworms on plant growth (Van Groenigen et al., 2015), *A. chlorotica* had no positive or negative effects on any of the measured strawberry traits in the present study (Fig. 6.2, 6.3). The initial earthworm density in the experiment was ~170 earthworms m^{-2} and was increased to ~283 earthworms m^{-2} when the additional two earthworms were added (see *Methods*). Mean earthworm survival (3.4 earthworms) was equivalent to ~192 earthworms m^{-2} and median earthworm survival (four earthworms) was equivalent to ~226 earthworms m^{-2}. These densities are higher than those reported from a range of habitats, although they are lower than values reported from temperate pastures (Fründ et al., 2010). As the effects of earthworms on plants can be increased at higher earthworm population densities (Van Groenigen et al., 2015), the high densities used in this experiment would be expected to have increased plant growth. Additionally, large amounts of earthworm castings were visible on the soil surface and throughout the soil when pots were sampled at the end of the study. Earthworm casts have higher levels of fertility than surrounding soil due to a combination of earthworms concentrating nutrients through preferential feeding and transforming nutrients through gut-mediated processes (Van Groenigen et al., 2019). However, despite the large amount of visible earthworm activity, there were no measured benefits for plant growth or fruit production. The soil used as the substrate in this experiment (Kettering Loam, described in *Methods*) is steam-sterilised by the

manufacturer, a process that would likely have removed much of the soil microbiota. As earthworms can influence the soil microbiota (Medina-Sauza et al., 2019), this sterilisation process may be partially responsible for the limited effects of earthworm on plant growth recorded here. The effects of earthworms on plant growth in pot experiments can increase over time (Pashanasi et al., 1992) and the direction of earthworm effects on plant growth may change at different time points (Doan et al., 2013). The soil in this experiment was homogenised by hand prior to being used to fill the experimental containers to ensure a consistent structure and evenly dispersed cattle dung as food for the earthworms. There may have been insufficient time for the earthworms to increase plant growth through their activities in the homogenised experimental soil. Future work in which earthworms 'pre-condition' soil before seeds are planted may result in pot experiments that are more field realistic. Additionally, natural earthworm communities consist of multiple species and the interactions between these species could influence plant growth in addition to overall earthworm abundance, as earthworm community composition can affect processes such as soil nitrogen dynamics (Sheehan et al., 2006), carbon cycling (Xia et al., 2011), and plant primary production (Andriuzzi et al., 2016). Despite the high densities of earthworms used in this experiment, including just one species may be a potential reason for the lack of effects on plant growth seen.

In a previous microcosm experiment, *Lumbricus terrestris* L. increased fruit yields of *F.* x *ananassa* (Infante et al., 2018). Infante *et al.* used 2.8 L plant pots, which were similar in size to the ~3 L pots used in this experiment, and two adult earthworms per experimental unit, which is lower than the five earthworms per unit in this experiment. Although Infante *et al.* did not report individual body masses, adult individual *L. terrestris* can weigh in excess of 3 g after reaching sexual maturity (Daniel et al., 1996) and earthworms continue growing upon reaching sexual maturity (Domínguez and Guimaraěs, 2024). Therefore, the

biomass of earthworms per container was much higher in the study of Infante *et al.* at ~6 g per 2.8 L compared to ~1.3 g per 3 L container in this experiment. The much higher individual body mass and total earthworm mass per container may explain the contrasting earthworm effects in the two experiments, as effects of earthworms on plant growth can increase with earthworm biomass (Brown et al., 1999). In addition to body size differences, *L. terrestris* and *A. chlorotica* represent different earthworm ecotypes (Bottinelli et al., 2020; Bouché, 1972; Capowiez et al., 2024). As an epi-anecic species, *L. terrestris* feeds on decaying plant matter at the soil surface and forms large, permanent, vertical burrows underneath a 'midden' of plant material and casts at the soil surface (Hoeffner et al., 2019; Stroud et al., 2016a). *Allolobophora chlorotica*, as an endogeic species, feeds on organic matter within the soil and forms smaller, temporary, horizontal burrows (Brown, 1995; Lavelle, 1988). These ecological differences may have a greater effect on plant performance than just body size. Future work comparing effects of earthworms in the same ecotype but with different body mass; for example, *A. chlorotica* and the slightly-larger *Aporrectodea caliginosa* (Savigny, 1826) (Sherlock, 2016; Sherlock et al., 2018), would allow examination of the effects of ecotype and ecology vs body size and mass.

Application of vermicompost to soils at 10 cm depth can stimulate soil microbes (Arancon et al., 2006) and increase vegetative growth and fruit yields of *F.* x *ananassa* cv. "Chandler" (Arancon et al., 2004; Singh et al., 2008). Vermicompost is produced from decomposition of organic material by 'compost earthworms' (Arancon et al., 2003; Domínguez, 2018) and can have strong positive effects on plant growth (Blouin et al., 2019), with benefits similar to those provided by earthworms (Van Groenigen et al., 2015). The strong beneficial effects of earthworms, and earthworm-derived vermicompost, on strawberry production in previous studies contrasts with the lack of effect of earthworms in

the current study. It may be that the relatively small-bodied earthworms used in our study did not have a strong enough effect on soil properties to influence plant growth over the short timescales of the experiment duration.

Effects of the watering treatment on earthworm performance

Earthworm survival (mean = 68.7%, median = 80%) was not significantly affected by the watering treatment, although the lowest survival rates were recorded in the *dry* treatment (Fig. 6.1). The high survival of earthworms in this experiment reflects the wide soil moisture tolerances of *A. chlorotica* (pink morph) which shows similar growth rates in soil with 21% and 29% moisture content, with faster growth than the green morph in dry soils (Lowe and Butt, 2007a), and is more common that the green morph in dry soils (Satchell, 1967a). *Allolobophora chlorotica* is an endogeic earthworm (Bottinelli et al., 2020) that burrows primarily near the soil surface (Capowiez et al., 2024) and, like many endogeic species, can enter aestivation or diapause, a period of dormancy during which an individual reduces water loss by emptying its alimentary canal and forming its body into a tight ball within a mucus lined chamber (Lee, 1985), with reduced response to chemical stimuli (Plum and Filser, 2005). However, water loss still occurs, and body mass losses of 50% for *Ap. caliginosa* (Bayley et al., 2010) and ~42% for *Hormogaster elisae* Alvarez, 1977 (Hormogastridae) (Díaz Cosín et al., 2006) have been recorded during aestivation. Earthworm mean mass decreased in the *dry* treatment compared to the *wet* and *control* watering treatment levels, suggesting that, although the earthworms in our study were largely able to survive in the *dry* treatment, this came at the cost of maintenance of body mass.

While cocoons were not counted, no juvenile earthworms were found in the *dry* treatment pots (Fig. 6.1). When comparing the *wet* and *control* treatment levels, significantly fewer

juvenile earthworms were found in the *control* treatment than the *wet* treatment. Earthworm reproduction is influenced by soil moisture: cocoon production of the endogeic species *Pontoscolex corethrurus* (Muller, 1856) (Glossoscolecidae) is increased at higher soil moistures (Hua et al., 2008) and cocoon production by *Ap. caliginosa* can be reduced during mild drought or halted completely in severe drought (Holmstrup, 2001). For *A. chlorotica*, both cocoon mass and production increase with soil moisture and are reduced at lower moistures, as individuals enter diapause, although excessive soil moisture also reduces cocoon production (Evans and Guild, 1948), suggesting that the *dry* treatment provided insufficient soil moisture for cocoon production in this experiment. Additionally, results of the present experiment showed no differences in mean juvenile mass between the *wet* and *control* treatments. Hatchling pink morph *A. chlorotica* growth rate is similar in soils at 21% and 29% gravimetric water content (Lowe and Butt, 2007a), but can be reduced at higher juvenile densities (Butt, 1997). Therefore, the *wet* treatment may have been more optimal for adult cocoon production, but the conditions of the *wet* and *control* treatments had little effect on the growth of the juveniles. Alternatively, conditions for juvenile growth may have been more optimal in the *wet* treatment, but increased competition from higher abundances reduced growth rates to the levels presented in the *control* treatment. The complete absence of cocoon production in the *dry* treatment suggests that *A. chlorotica* populations may be negatively affected in the future as dry summers become more common.

Conclusions

The results of this experiment suggest that the performance of the endogeic earthworm species *A. chlorotica* can be affected by rainfall and that, as future mean summer rainfall reduces, reproductive performance could be severely reduced with negative effects on

population maintenance. Despite widely reported beneficial effects of earthworms on plant growth, the earthworms used in this experiment had no effect on alpine strawberry fruit yields or growth, even in the *control* and *wet* watering treatments where earthworm performance was increased. Therefore, these results suggests that earthworms will be unable to ameliorate the negative effects of reduced rainfall on plant performance.

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Supplementary information



Fig. S6.1: Example strawberry seedling prior to being transferred to an experimental pot.



Fig. S6.2: Photo showing earthworms being added to soil surface of an experimental pot.



Fig. S6.3: Photo showing polytunnel rain shelter.



Fig. S6.4: Photo showing initial pot arrangement under rain shelter before construction of raised bed.



Fig. S6.5: Photo showing pot arrangement in the raised bed.



Fig. S6.6: Example of a mature strawberry (fully red) on one of the experimental plants prior to harvest.

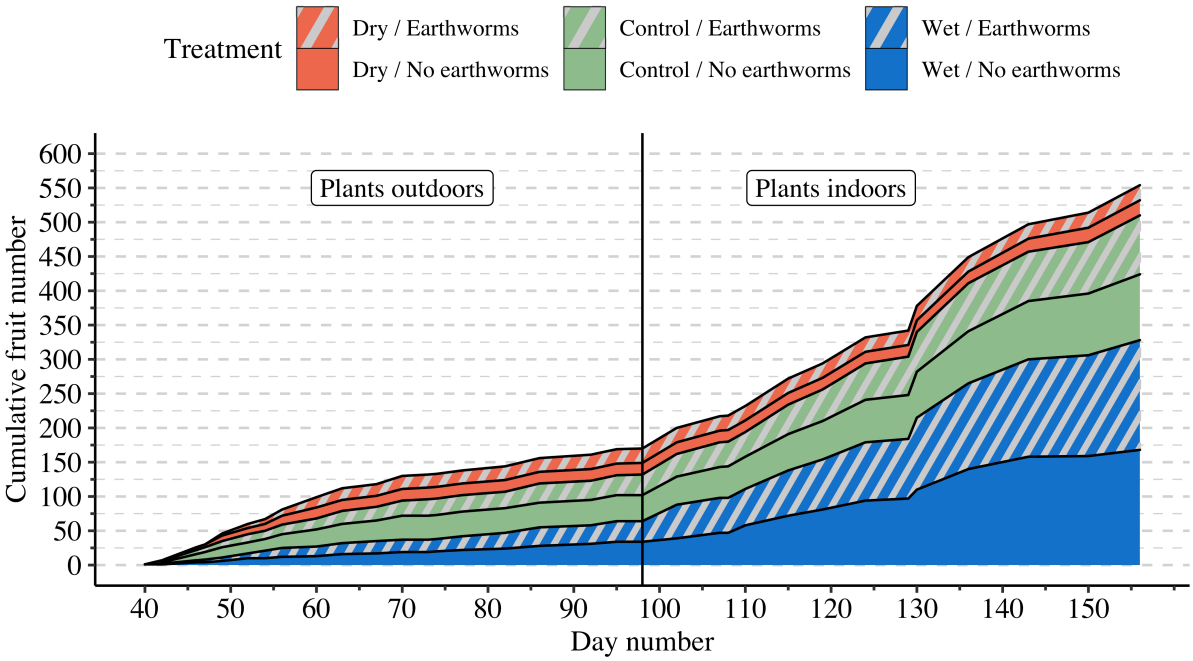


Fig. S6.7: Area plot showing cumulative strawberry fruit production across earthworm and watering treatment levels. The vertical black line indicates when plants were moved into a glasshouse from outdoors.

Chapter 7: General discussion

In this thesis I investigated the effects of earthworms on plant performance (Chapters 2, 3, and 6) and cattle dung decomposition (Chapters 4 and 5) across manipulative mesocosm, microcosm, and pot experiments. Generally, earthworms had a significant positive effect on the ecosystem service of interest (Chapters 2, 3, 4, and 5) although this was not always the case (Chapters 2 and 6).

Variable effects of earthworm on plant growth across experiments

The effects of earthworms on plant growth in different substrates and environmental contexts were explored across five experiments reported in Chapters 2, 3, and 6. The effects of earthworms on plant growth differed across these experiments. The 'compost epigeic' earthworm *Dendrobaena veneta* (Rosa, 1886) had a significant positive effect on plant growth in a commercial peat-free horticultural compost during drought and waterlogging stress (Chapter 3) and unstressed conditions (Chapter 2) with the magnitude of the effect depending on earthworm abundance in experimental microcosms under unstressed conditions (Chapter 2). However, in Chapter 2, *D. veneta* had no significant positive effect on plant growth in the other two horticultural composts tested and, in Chapter 6, the endogeic earthworm *Allolobophora chlorotica* (Savigny, 1826) had no significant effect on alpine strawberry growth in topsoil under ambient, 'dry', or 'wet' conditions.

The beneficial effects of earthworms on plant growth in soil are well known (Scheu, 2003; Van Groenigen et al., 2015) and the topsoil used as a substrate in Chapter 6 (Kettering Loam) is highly suitable for *A. chlorotica* growth, reproduction, and burrowing activity

(Lowe and Butt, 2007a, 2005). Therefore, the lack of beneficial effect of *A. chlorotica* in Chapter 6 is surprising as overall earthworm performance (survival, mass maintenance, and reproduction) was high, and signs of earthworm activity in the form of casts were clearly visible in earthworm-containing pots. Although earthworms generally have positive effects on plant growth, effects differ across plant taxa and crop types; for example, grasses and grain crops may be benefitted while legumes show no response (Van Groenigen et al., 2015). Additionally, soil type influences earthworm effects on plant growth with earthworms having positive effects in clayey and loamy soils but no overall effect in sandy soils (Van Groenigen et al., 2015). Kettering Loam is comprised of 24% clay, 18% silt and 58% sand so is classified as a sandy clay loam by texture (Natural England, 2008). This sandy texture may have been relevant for the lack of earthworm effects on plant growth in Chapter 6.

Potential benefits of compost earthworms in horticultural plant growth media

Much work has focussed on the effects of earthworms on plant growth in soils (Van Groenigen et al., 2015); however, in many gardens containers or horticultural nurseries plants are grown in soil-less horticultural substrates (Durham et al., 2018; Hentges et al., 2019; Relf, 2020). Vermicompost produced by the activities of compost earthworms (Domínguez, 2018) can increase plant growth (Blouin et al., 2019), and has been successfully used as an additive in horticultural growth substrates to increase yields (Arancon et al., 2003; Atiyeh et al., 2000b; Hidalgo et al., 2006; Zaller, 2007), although the potential benefits of direct earthworm application have received less attention.

In the UK, these potential benefits could be particularly important due to an upcoming ban on the sale of peat-containing composts for horticultural use in order to protect peatland (Department for Environment, Food and Rural Affairs, 2022, 2021). Peatlands provide

regulating, supporting, and cultural ecosystem services (Bain et al., 2011; Millennium Ecosystem Assessment, 2005; Whitfield et al., 2011) and cover ~12.2% of the UK, although only ~22% remain in a 'near-natural' condition with large areas degraded by human activities or conversion to arable land or woodland (Evans et al., 2017; Minasny et al., 2023). UK peatlands form part of the highly degraded Atlantic bog (68% degraded) and Nemoral-submeridional fen (53% degraded) regions within Europe (UNEP, 2022), with much of this degradation resulting from industrial peat extraction, which includes draining, drying, and milling peat to produce horticultural products (Alexander et al., 2008). Drained and degraded peatlands can release large amounts of carbon as CO_2 so protecting and restoring peatlands is important for limiting anthropogenic greenhouse gas emissions (Leifeld and Menichetti, 2018), hence the upcoming bans and regulation in England (Department for Environment, Food and Rural Affairs, 2022, 2021). Peat-free composts are widely produced and consist of a range of materials including wood, bark, or coconut coir among others (Bek et al., 2020; Holmes and Bain, 2023, 2021; Leyman and Aplin, 2007). However, members of the public are concerned about the performance of peat-free composts (Environment and Forestry Directorate, 2023) despite general public support on a ban of peat-containing composts (Department for Environment, Food and Rural Affairs, 2022).

In Chapters 2 and 3, the compost earthworm *D. veneta* provided large benefits to plant growth in soil-less peat-free horticultural composts (Fig. 2.2, 2.3, 3.4, 3.5, 3.5), consistent with their beneficial effects in soils (Van Groenigen et al., 2015) and the beneficial effects of vermicompost on plant growth (Blouin et al., 2019). Earthworm performance (survival, overall maintenance of body mass, and reproduction) across experiments was high and 860 of the 912 earthworms used across Chapters 2 and 3 were recovered alive (survival rate: 94.3%), with all 444 earthworms being recovered from non-stressed conditions (controls in

Chapter 3, all earthworms in the *New Horizon* compost in Chapter 2). Additionally, several instances of mating earthworms were found when collecting earthworms at the end of experiments and reproduction readily occurred in the tested substrates in Chapter 2, suggesting that the horticultural substrates were suitable for earthworm survival, growth, and reproduction. In Chapter 3, the beneficial effects of earthworm on radish plant growth were independent of both the waterlogging or drought treatments (Fig. 3.4, 3.5, 3.6), although crop yields were still reduced by both waterlogging and drought. Therefore, addition of earthworms into horticultural substrates may be an effective way to maintain yields and plant growth during times of environmental stress. These benefits could be particularly important in gardens containers maintained by amateur growers, which are small and require a large amount of irrigation and maintenance (Nagase and Lundholm, 2021), and are vulnerable to waterlogging and drought (Durham et al., 2018; Hentges et al., 2019; Relf, 2020).

Composting earthworms are widely distributed (Sims and Gerard, 1999) and are also commercially-available from a number of suppliers within the UK (Butt and Williams, 2010; Katsiamides and Stürzenbaum, 2021), where they are marketed for fishing bait, live reptile food, or for vermicomposting with earthworms of different size categories (by body mass) advertised (personal observation). Additionally, a number of UK companies sell ‘wormeries’ for small-scale vermiculture and home production of vermicompost (Butt and Williams, 2010). The ease of acquiring these earthworms, and their strong benefits on plant growth in a widely-sold and popular commercially-available compost (Bek et al., 2020; Holmes and Bain, 2023), provides additional support that compost earthworms could provide great benefits to growers.

One potential challenge of using earthworms in container gardens is earthworm escape. Despite using adhesive hook tape which can prevent escape of earthworms from open-topped containers (Lubbers and Van Groenigen, 2013), several *D. veneta* were seen crawling on the underside of lids shortly after being introduced to microcosms in both Chapters 2 and 3. Compost earthworms are highly mobile (Domínguez and Edwards, 2011) which may reduce their suitability for use in container gardens due to escape risks. This was particularly true in the flooding experiment of Chapter 3 where large numbers of earthworms escaped from microcosms during flooding events and a small number of individuals were found to have entered other microcosms. However, in the drought experiment of Chapter 3 and both experiments in Chapter 2, earthworm recovery rates were extremely high suggesting that, after burrowing into the substrate, the chance of escape was reduced. Incorporating earthworms into plant growth substrates before adding it to containers may reduce chance of escape and reduce stressful conditions experienced by the earthworms before they are able to burrow into the substrate. Alternatively, if earthworms are applied to the surface of the compost, adding a small amount of extra compost to cover the earthworms may be a good compromise to reduce chance of earthworm escape without increasing complexity of the substrate preparation for container filling.

Future directions for earthworms and plant growth

For experiments reported in Chapters 2 and 3 I utilised a single earthworm species, *Dendrobaena veneta*. However, there are a number of other species that are used in vermicomposting that are commercially available, including *Eisenia fetida* (Savigny, 1826), *E. Andrei* (Bouché, 1972), and *Bimastos rubidus* (Savigny, 1826) (=*Dendrobaena rubida* (Savigny, 1826)) (Domínguez and Edwards, 2011). These species differ in their

body size, growth rates, and moisture and temperature optima (Domínguez and Edwards, 2011), so the functioning of different species may be differently affected by environmental stress. Compost earthworms also show indeterminate growth and giant specimens of *Dendrobaena hortensis* (Michaelsen, 1890) and *E. andrei* of 9.1 g and 7.8 g, respectively, have been recorded from vermicomposting units (Domínguez and Guimaraěs, 2024). As some of the work in this thesis showed that relationships between earthworm abundances and plant performance can be non-linear (Chapter 2), future work and experiments investigating the application of different compost earthworm species, either alone or in combination, of different abundances and body masses or developmental stages, would be valuable to further investigate the potential of compost earthworms to improve plant performance in horticultural substrates.

All experiments in Chapters 2 and 3 used the same microcosm design, constructed from 20 cm high, 10 cm diameter grey Polyvinyl Chloride (PVC) piping (Fig. 2.1). Garden containers vary widely in size and shape and are made from a variety of materials including metals, terracotta, plastic, and fabric (Hentges et al., 2019). Different materials and colours can differ in thermal properties (Markham et al., 2011) and substrate water loss rates and irrigation requirements (Million and Yeager, 2022). These differences will likely also affect earthworm performance, the effects of earthworms on plant growth, and how these effects are altered by environmental variables and stressors. Testing the effects of earthworm on plant performance in containers of different materials, under both stressed and unstressed conditions, would be valuable to further examine the potential applications of earthworms in horticultural settings as the effects of container material on abiotic conditions may exacerbate (or ameliorate) the effects of environmental stressors on both earthworms and plants.

My experiments reported in Chapters 2 and 3 all used radish (*Raphanus sativus* L.) as a representative horticultural crop. Summer radishes are fairly small and grow quickly, with cv. "Cherry Belle" (the variety used in my experiments) attaining harvestable size in as little as 24 days (Kostka-Rick and Manning, 1993). However, many other vegetable crops are grown in containers in the UK, including other root vegetables such as carrots, radishes, and onions and other crops such as lettuces, peas, and beans (Royal Horticultural Society, 2024). These crops differ in their growth periods, nutrient requirements, and root anatomies so may be differently affected by earthworms. Future work focussing on different plant species would be useful to examine if the benefits of earthworms on food production for horticultural substrates are universal. Container gardens are also used to grow ornamental plants alone or in combination (Hillier, 1991). Earthworms can influence plant growth form (Poveda et al., 2005; Zaller et al., 2011) so may alter floral abundance and production of ornamental plants, influencing aesthetic properties and pollinator resources. Future experimental work examining effects of earthworms on these ornamental plant species would be valuable for measuring ecosystem services provided by earthworms beyond food production.

Effects of earthworms on cattle dung decomposition

Earthworms significantly reduced end dung mass in both Chapter 4 (*Lumbricus terrestris* L., *A. chlorotica,* and *D. veneta;* Fig. 4.2) and Chapter 5 (*D. veneta;* Fig. 5.3) of this thesis, consistent with their attraction to cattle dung (Bacher et al., 2020, 2018; Knight et al., 1992) and effects on cattle dung decomposition by feeding on, and burying, dung (Hendriksen, 1997, 1991b, 1991a; Holter, 1977; Kaartinen et al., 2013; Schon et al., 2020, 2017, 2015). The performance of earthworms in both experiments was robust to simulated flooding (Chapter 4; Fig. 4.4) and heatwave (Chapter 5; Fig. 5.4) stress. Earthworms

typically arrive at dung pats later than dung insects and it can take several weeks before earthworm abundances at dung pats reach their peak (Bacher et al., 2018; Knight et al., 1992). It has been suggested that dung insect activity may be necessary to 'prime' dung pats for earthworm activity either by increasing accessibility to earthworms or increasing attractiveness of pats to earthworms (Holter, 1983). Dung insect activity therefore might increase the effectiveness of earthworms for cattle dung decomposition. However, earthworm and dung insects typically show additive effects on dung decomposition (Holter, 1977; Kaartinen et al., 2013), or may show positive or negative interactions between earthworms and dung flies or dung beetles, respectively (O'hea et al., 2010a). In Chapter 5, *D. veneta* gained more mass in the presence of dung beetles but there was no interaction between the two taxa on dung decomposition (Fig. 5.4), suggesting that 'priming' effect could benefit earthworms without increasing ecosystem services. In Chapter 4, the presence of the insect dung fauna did not increase dung decomposition by earthworms (Chapter 4 – *Appendix*), although there were species-specific effects on earthworm performance, suggesting that earthworm and dung insect interactions may not be universal and may differ across earthworm species and ecotypes.

One potential caveat relevant to both chapters was the use of the compost earthworm *Dendrobaena veneta* instead of naturally-occurring pasture earthworms. However, *Eisenia fetida,* another compost earthworm, has been successfully used in earthworm and dung beetle interaction experiments (Bang et al., 2009; O'hea et al., 2010a). Additionally, measurements of burrowing activity suggest that *E. fetida* inhabits the 'litter dweller' functional group, which also includes *Satchellius mammalis* (Savigny, 1826), *Lumbricus rubellus* Hoffmeister, 1843, and *L. castaneus* (Savigny, 1826) (Capowiez et al., 2024), which are all species that are attracted to dung pats in pastures (Bacher et al., 2018). *Dendrobaena veneta* is similar in size, morphology, and ecology to *E. fetida* (Domínguez

and Edwards, 2011; Katsiamides and Stürzenbaum, 2021) so, like *E. fetida*, was a suitable proxy for pasture-dwelling epigeic species for the experiments reported in Chapters 4 and 5.

Challenges with experimental dung fauna treatments

Chapters 4 and 5 both manipulated the presence and absence of the insect dung fauna. In Chapter 4, artificial dung pats were colonised naturally by the insect dung fauna. In Chapter 5, adult *Acrossus rufipes* (Linnaeus, 1758) beetles were collected from the field and applied to artificial dung pats in microcosms. In both experiments, dung beetle performance was limited. In Chapter 4, few dung beetles were recovered from pats and emergence traps, suggesting limited colonisation and reproduction, and in Chapter 5 larvae were recovered from a minority of experimental dung pats, even in the unheated control microcosms. Additionally, the colonisation prevention method used in Chapter 4 was only partially successful so 'control' pats contained similar adult insect abundances to 'exposed' pats. Due to these challenges, the dung fauna treatment was excluded from main analyses in Chapter 4. These analyses were presented in the *Appendix* of Chapter 4.

Natural colonisation of dung pats has been used successfully in experiments for both dung beetles and earthworms (Kaartinen et al., 2013), although in some cases dung beetles must be introduced to experimental pats (Kavanaugh and Manning, 2020), which may explain the limited dung beetle activity in Chapter 4. Additionally, the dung pat colonisation and beetle collection periods were performed fairly late in the season for both experiments. Natural pat colonisation (Chapter 4) and dung beetle collection and introduction (Chapter 5) occurred in mid-September and late August, respectively. *Acrossus rufipes* is active until October with major activity peaks in late August to September (Hanski, 1980); however, the extreme summer temperatures of 2022 (when the experiment described in

Chapter 5 was conducted) may have stressed the beetles and resulted in this low performance in the experiment.

A major challenge with mesocosm and microcosm experiments is selection of species. The invertebrate dung fauna is complex and consists of coprophagous flies and beetles as well as parasitoid wasps, predatory beetles and flies, and other non-insect invertebrates (Skidmore, 1991). Additionally, many earthworm species with different functional behaviours are attracted to, and feed on, dung pats (Bacher et al., 2018). Deliberate selection, collection, and introduction of known species mixtures and abundances allows careful control of starting community composition but may miss potential species interactions or effects of individual species. Experimental communities often only consist of a few species and fully factorial community composition treatments may not be possible. Natural colonisation allows for a wider representation of the dung invertebrate community but large amounts of variability may make it difficult to examine effects of community composition, and taxa may differ in their ability to colonise pats depending on experimental and mesocosm design (Kavanaugh and Manning, 2020). Additionally, as the starting colonising community in each replicate is unknown, it may be difficult to determine if invertebrates recovered from experimental units at the end of experiments result from variation in colonisation, community interactions, experimental manipulations, or a combination of factors.

For future work, a combined approach of natural colonisation and deliberate introduction may be beneficial. Earthworms accumulate under dung pats at densities of up to 4000 individuals m^{-2} (Bacher et al., 2018). Sampling of natural pats, or large artificial pats, would allow for acquisition of large numbers of earthworms of multiple species. These earthworms could be divided into ecotypes based on colour, size, and morphology

(Bouché, 1972) and allocated to experimental pats. Such an approach would allow for manipulation of starting functional composition, abundance, and biomass and would allow for effects of individual species to be examined through identification of adult earthworms at the end of the experiment (assuming that earthworm survival and recovery rates from experimental units is high). Similarly, large artificial pats could be constructed to collect large numbers of dung beetles and flies of multiple species and functional groups that can be allocated to pats in the same way as the earthworms. In this way, future experiments may be able to make use of the benefits of both the natural colonisation and artificial community methods whilst limiting the downsides.

Future directions for earthworms and dung decomposition

In addition to environmental stress, the dung fauna may also be exposed to chemical stressors through application of anthelminthic medication to cattle. Residues persist in the cattle dung and can have severely negative impacts on dung insect survival, performance, and functioning (Beynon et al., 2012a, 2012b; Kavanaugh and Manning, 2020; Manning et al., 2018, 2017a, 2017b; O'hea et al., 2010b; Verdú et al., 2015). However, anthelminthic medications appear to have little negative effect on earthworm survival and life history (Svendsen et al., 2005, 2002) or attraction to dung pats (Scheffczyk et al., 2016; Svendsen et al., 2003), and may actually attract earthworms (Torkhani et al., 2011), although high concentrations may reduce earthworm fecundity (Römbke et al., 2010). Therefore, earthworms may be able to maintain dung decomposition when insect communities are perturbed by veterinary medication residues, although dung pats may be less attractive or accessible to earthworms without the activity of the insect dung fauna to 'prime' pats (Holter, 1983). Future work assessing how earthworms and dung insects interact during periods of chemical disturbance, and understanding how these interactions may be further

affected by climatic stressors, would be valuable for predicting potential negative effects on dung decomposition.

In both Chapters 4 and 5, earthworms had a much stronger effect on cattle dung decomposition than dung insects under both control conditions and during flooding or heating stress (see *Appendix* of Chapter 4 for analysis of effects of dung insects). However, drought stress might have very strong impacts on the effects of earthworms on dung decomposition and UK summer rainfall is predicted to decrease due to climate change, making this stressor more likely to occur (Met Office, 2022). In the UK, drought severity is expected to increase at higher levels of temperature warming (Hanlon et al., 2021). The experiment reported in Chapter 5 was conducted from August to September 2022 and it was necessary to use commercially obtained *D. veneta* instead of earthworms collected from pasture. Field abundances in pats in pastures around the John Krebs Field Station (Department of Biology), University of Oxford, near the village of Wytham, Oxfordshire, UK were extremely low, and it was not possible to collect enough earthworms for microcosms (personal observation). The summer of 2022 was a record-breaking summer for high temperatures in the UK (Kendon, 2022; Kendon et al., 2023), and the low abundance of earthworms in pastures suggests that climate change may severely reduce earthworm abundances and activity in pastures. Examination of the interaction between temperature, soil moisture, and dung moisture may provide a clearer view of how dung decomposition services by earthworms may be affected by a changing climate.

Future work with multiple stressors

A future direction for both the effects of earthworms on cattle dung decomposition and effects of earthworms on plant growth would be to examine the effects of multiple

stressors on earthworm-derived ecosystem functioning and services. When I conducted experiments investigating the effects of environmental stress on earthworm functioning in my thesis, I applied single stressors; *e.g.,* drought (Chapter 4), waterlogging / flooding (Chapters 4 and 5), and heating (Chapter 6). However, earthworms can be exposed to multiple stressors simultaneously in the field. For example, a prolonged heat wave and reduced rainfall may expose earthworms to both drought and heating stress. Environmental stressors may also co-occur with anthropogenic stressors such as pesticides, residues from veterinary medicines, pollution (*e.g.* by heavy metals) or mechanical disturbance through tillage. In some cases, these stressors may have synergistic detrimental effects on earthworms. For example, sublethal concentrations of copper in soils can increase mortality of *Aporrectodea caliginosa* (Savigny, 1826) exposed to drought (Friis et al., 2004), carbonyl pollution and flooding in combination can negatively affect *A. caliginosa* survival (Zorn et al., 2008), and simulations of *Lumbricus terrestris* L. population dynamics suggested negative relationships between increasing temperature or decreasing soil moisture and earthworm population size when deep tillage occurred but not during direct drilling (Johnston et al., 2018). In the cattle dung system, these multiple environmental stressors could influence performance of earthworms, the insect dung fauna, and alter how the two groups interact with one another. Future work examining potential synergistic effects of multiple stressors, both environmental and anthropogenic, on earthworm and dung insect interactions would be valuable for forecasting how dung decomposition services may be impacted in the future. Additionally, experimental work investigating effects of earthworms on plants during periods of simultaneous stressors would be valuable to determine how robust the benefits provided by earthworms to plants are to field-realistic perturbations.

Review of experimental approaches used in this thesis

All experiments reported in this thesis were small to medium scale experiments using discrete experimental units: mesocosms, microcosms, or pots. These experiments were run outdoors (Chapters 4, 5, and 6) or in an air-conditioned but not climate-controlled glasshouse (Chapters 2, 3, and 6). Although performing experiments under laboratory conditions would have allowed for additional control of experimental conditions, this would not have been suitable for the experiments reported in this thesis due to the space requirements of experiments. Running experiments at the microcosm / mesocosm scale was appropriate for the experiments focussing on potential benefits of earthworms when added to horticultural substrates (Chapters 2 and 3) as the experimental set up allowed me to produce experimental units that were similar to garden containers. Additionally, this scale worked well for the strawberry experiment (Chapter 6) as the relatively small size of the experimental units enabled the experiment to be set up under a rain shelter with open sides. This protected the experiment from rainfall and allowed manipulation of water levels received by the experimental units while also allowing pollinating insects to access the alpine strawberry plants used in the experiment.

Using sealed experimental units in the dung decomposition experiments (Chapters 4 and 5) was necessary to prevent exit of dung insects and earthworms or ingress of insects or earthworms from outside experimental units. In experiments reported elsewhere, earthworms have been shown to invade open-topped dung decomposition experimental mesocosms (*e.g.,* Schon et al. 2015) which may make effects of earthworms of dung decomposition challenging to determine. Therefore, the approach used in Chapters 4 and 5 allowed for quantification of the effects of earthworms on cattle dung decomposition by

providing an earthworm-free control which may not have been possible in an open mesocosm design or field-scale experiments.

Experiments in Chapters 2 and 3 were microcosm experiments using 20 cm height, 10 cm diameter, PVC cylinders as experimental units (Fig. 2.1). PVC cylinders have been used in a large number of previous earthworms experiments in different contexts (*e.g.* Hallam et al., 2021; Hoeffner et al., 2019; Svendsen and Weeks, 1997; Wurst et al., 2004), and they have advantages as they allow a high substrate depth to be provided in combination with a small footprint. This allows for efficient use of experimental space as a large number of units can fit into a relatively small area. In my experimental work, I used stainless steel vent clamps to seal the bottom of microcosms with insect mesh to contain the substrate. This method was effective as clamps could be easily applied or removed through tightening or loosening with a screwdriver. This approach also meant that microcosms could be rapidly assembled and disassembled without damaging the mesh or PVC piping, allowing reuse in later experiments which reduced financial cost and research waste. However, the screw tightening mechanism on each vent clamp protruded by ~2 cm. Therefore, the base of each microcosm was not fully circular which made arranging the microcosms in experiments challenging. For my experiments, this was not a problem as some space between microcosms was necessary to reduce chance of earthworms moving between microcosms. However, in the flooding experiment reported in Chapter 3, the protruding vent clamps often damaged the plastic bags used to line the experimental flood chambers, requiring regular replacement of bags. Overall, this method for microcosm construction was highly effective for the experimental work reported in Chapters 2 and 3 of this thesis.

Earthworm escape is a major challenge in microcosm and mesocosm experiments with open-topped experimental units. In some cases, earthworm escape can be very high (*e.g.* Zorn et al., 2008) and if these missing earthworms are not recovered or detected it may be difficult to determine if missing earthworms reflect escape or mortality. Following recommendations from the literature, I used a layer of 2 cm-width adhesive hook tape around the upper rim of microcosms to prevent earthworm escape (Lubbers and Van Groenigen, 2013) for the PVC cylinder microcosms used in Chapters 2 and 3 and plant pots used in Chapter 6. However, in both Chapters 2 and 3, *D. veneta* were seen to be able to cross the hook tape and escape the microcosms. Therefore, it was necessary to temporarily place lids over microcosms until the earthworms had burrowed into the substrate. Other than in the flooding experiment (Chapter 3, described above), earthworm recovery rates approached 100% across all experiments suggesting that the hook tape was sufficient to prevent escape after the earthworms had settled into the substrate. In an additional flood experiment using similar methods as used in Chapter 3 (not reported in this thesis), I recorded *A. chlorotica* earthworms in flood chambers after flooding that presumably also were able to cross adhesive hook tape. Therefore, future experimental work may need to employ additional means of preventing earthworm escape or ensure sufficient distance between experimental units to prevent movement between them by earthworms, particularly when investigating stressors that cause escape responses in earthworms such as flooding (Plum, 2005).

Experiments reported in Chapters 4, 5, and 6 all used topsoil as a substrate. The soil used in Chapters 5 and 6 was Kettering Loam Topsoil. This topsoil has been widely used in earthworm ecological (Lowe and Butt, 2002, 2007a), behavioural (Butt, 2002), and ecotoxicological studies (Elliston and Oliver, 2020). A major advantage of using this soil for my own experimental work was its proven track record of suitability as a substrate for

earthworms of all ecotypes (Butt and Lowe, 2011; Lowe and Butt, 2005). Additionally, the pre-sterilisation and screening (to 6 mm) of the soil by manufacturers reduced the risk of contamination with soil invertebrates and facilitated application of earthworm treatments to compare with earthworm-free controls. The highly standardised nature of the soil also ensured that starting conditions across microcosms were similar. However, the sterilisation and pre-screening of the soil may negatively impact the field-relevance of results. In Chapter 5, I used a commercially available, but unscreened and unsterilised, sandy loam soil as the substrate for experimental mesocosms. This approach had advantages for reducing financial cost as the soil was cheaper per kilogram, and using Kettering Loam for this experiment would have been financially prohibitive. However, this soil caused additional challenges as it was necessary to manually sieve the soil to remove soil macro invertebrates and homogenisation was necessary due to a lower level of consistency in the soil. This was a very time-consuming process and meant that large volumes of the purchased soil were sieved out and therefore unusable in the experiment. Although field collected soil is likely to provide the highest level of field relevance, the time requirements for collection, extraction and transport costs, and necessary space and equipment for preparation and homogenisation need to be carefully considered for future work. Although less field-relevant, utilisation of a widely-used and standardised base soil allow easier (and sometimes direct) comparison with results of other studies using the same substrate.

Conclusions

The experimental work presented in this thesis provides further support of the beneficial effects of earthworms on plant growth and suggests that the compost earthworm *D. veneta* may be a useful way to increase plant performance and crop yields in soil-less horticultural substrates (Chapter 2) and that these benefits may still be provided during periods of

environmental stress (Chapter 3). However, the lack of benefits provided by the endogeic earthworm *A. chlorotica* in topsoil (Chapter 6) suggests that earthworm benefits on plant growth are not universal. Additionally, this thesis demonstrates the importance of earthworms for cattle dung decomposition during periods of warming stress (Chapter 5) and flooding stress (Chapter 4). Earthworm services were maintained during periods of stress, suggesting that earthworms could be important in maximising pasture productivity as the climate changes during the Anthropocene.

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