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Cover: Earthworms (species unknown)
(photo Elsa María Arrázola Vásquez)

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Abstract

Soil compaction has a key role in shaping earthworm burrowing activity. Understanding the impacts of soil conditions (*e.g.* soil moisture, soil compaction) on earthworm burrowing is important to foresee the effects on soil functions driven by earthworm activity. This thesis investigated the effects of soil compaction on earthworm burrowing, using different earthworm species. Effects on burrowing rates and burrowing modes, as well as on energy costs for burrowing, were analysed. Also, the temporal dynamics of earthworm burrowing were examined by exposing earthworms to different initial soil conditions under semi-field conditions.

The effects of compaction were adverse, with a significant reduction in burrowing rates at higher soil compaction levels, from 13.5 cm d^{-1} to 5.8 cm d^{-1} and 6.5 cm d^{-1} to 3.6 cm d^{-1}, for *A. caliginosa* and *A. longa*, respectively. In compacted soils, soil ingestion was the main burrowing mode for earthworms. Earthworms tended to spend about 50% more energy to burrow in compacted soils, which could be detrimental in terms of their long-term energy requirements. An analysis of the temporal dynamics of earthworm burrowing under semi-field conditions showed that earthworm burrowing rates were in the range between 1 and 4 cm^3 d^{-1} while casting rates were between 1.3 and 3.3. cm^3 d^{-1} and the fraction of ingested soil increased towards autumn, after 24 weeks. The data suggest that burrowing is a function of the interaction effects of soil compaction and moisture level, with stronger negative impacts in moist-compacted soils.

This thesis found that soil compaction reduces significantly the burrowing rates and increases the energy costs of burrowing in earthworms. In addition, the temporal burrowing dynamics showed that even with different soil initial conditions, earthworms burrowing seems to be similar in their mean burrowing rates, mean casting rates and burrowing mode, after six months.

Keywords: bioturbation, *Aporrectodea caliginosa*, *Aporrectodea tuberculata*, *Aporrectodea longa*, soil ingestion, cast production

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Efectos de la compactación de suelos en la excavación y sus costos energéticos en diferentes especies de las lombrices de tierra

Resumen

La compactación de suelos tiene un rol clave que moldea la actividad de las lombices. Entender mejor los impactos de las condiciones del suelo (e.g. humedad o compactación del suelo) en la actividad de las lombrices de tierra es importante para comprender los posibles efectos en las funciones del suelo que son impulsadas por la actividad de las lombrices. En esta tesis se investigó los efectos de la compactación de suelos en diferentes especies de lombriz de tierra. Se analizaron los efectos en las tasas, modos y los costos energéticos asociados a la excavación de túneles. Asimismo, se analizaron las dinámicas temporales en la actividad de las lombrices, enfocadas en la excavación de túneles, en donde experimentos con diferentes condiciones iniciales de humedad y compactación, fueron expuestos a condiciones climáticas en el campo.

Los efectos de la compactación fueron adversos, con reducciones significativas en las tasas de excavación cuando la compactación aumenta, con valores de 13.5 cm d^{-1} a 5.8 cm d^{-1} y 6.5 cm d^{-1} a 3.6 cm d^{-1} , para *A. caliginosa* y *A. longa*, respectivamente. En suelos compactados, las lombrices tienden a gastar un 50% más de energía para excavar, lo cual puede ser perjudicial a largo plazo en los requerimientos energéticos de las lombrices.. El análisis de las dinámicas temporales de la actividad de las lombrices, en condiciones de campo (parcialmente), mostró que la excavación de túneles varía durante seis meses (otoño). Las tasas de excavación estuvieron en un rango de 1 a 4 cm^3 d^{-1}, las tasas de producción de turrículos oscilaron enre 1.3 y 3.3 cm^3 d^{-1} y la ingesta de suelo aumentó durante esta temporada. Los resultados apuntan a que la actividad de las lombrices está en función de la interacción de los efectos entre la compactación y los niveles de humedad en el suelo.

Esta tesis encontró que la compactación de suelos reduce significativamente las tasas de excavación y aumenta los costs energéticos para excavación en las lombrices. Adicionalmente, el análisis de las dinámicas temporales de la actividad de las lombrices indicó que aún cuando las condisiones del suelo son en un principion distintas, la actividad de las lombrices parece que es similar en sus tasas de excavación, tasas de producción de turrículos y el modo de excavación(ingesta de suelos, después de seis meses.

Palabras clave: bioturbación, *Aporrectodea caliginosa*, *Aporrectodea tuberculata*, *Aporrectodea longa*, ingestión de suelos, producción de turrículos

Dedication

To my mom and dad

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Arrázola-Vásquez, E. *, Larsbo, M., Capowiez, Y., Taylor, A., Sandin, M., Iseskog, D., Keller, T. (2022). Earthworm burrowing modes and rates depend on earthworm species and soil mechanical resistance. Applied Soil Ecology, 178, 104568.

II. Arrázola-Vásquez, E., Larsbo, M., Capowiez, Y., Taylor, A., Herrmann, A.M., Keller, T. (2023). Estimating energy costs of earthworm burrowing using calorimetry. (Submitted to European Journal of Soil Biology).

III. Arrázola-Vásquez, E., Larsbo, M., Capowiez, Y., Taylor, A., Iseskog, D., Keller, T. (2023). Temporal dynamics of earthworm burrowing under semi-field conditions as affected by soil compaction level. (Manuscript).

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* Corresponding author

The contribution of Elsa María Arrázola Vásquez to the papers included in this thesis was as follows:

I. Planned the study together with the co-authors. Performed laboratory work and data analysis. Prepared the first draft of the manuscript and worked on the final version with assistance from the co-authors.

II. Planned the study together with the co-authors. Performed earthworm sampling, laboratory work using a calorimeter and respirometer, and data analysis. Prepared the first draft of the manuscript and worked on the final version with assistance from the co-authors.

III. Planned the study together with the co-authors. Performed earthworm sampling. Fieldwork, image analysis (X-ray tomography) and data analysis were done together with the co-authors. Prepared the first draft of the manuscript and worked on the final version with assistance from the co-authors.

1. Introduction

Soil represents one of the largest pools of global biodiversity (FAO et al., 2020). Soil fauna is composed of a variety of organisms that range from microorganisms (*e.g.* bacteria, fungi) to soil vertebrates (*e.g.* moles) (Decaëns et al., 2006). Among soil macrofauna, earthworms are the most recognized group in temperate climates. Their impact on a variety of soil processes has been widely studied (Coleman & Wall, 2015) and they are considered key ecosystem engineers (Lavelle et al., 1997). Soil ecosystem engineers are organisms that can transform biotic or abiotic materials to make them available to other species, which then causes modifications in the habitat (Jones et al., 1994). The impacts of earthworm activity in the form of burrowing and cast production, usually known as earthworm bioturbation, on soil physical, chemical and biological properties are well described (Makeschin, 1997). These changes in soil properties and soil conditions, in turn, have effects on multiple soil processes, *e.g.* carbon cycling, soil structure formation, nutrient cycling and water regulation (Blouin et al., 2013; Bertrand et al., 2015; Creamer et al., 2022).

Earthworms are highly sensitive to changes in soil environmental conditions. Soil conditions, such as soil moisture and temperature, have a strong influence on the life cycle, activity and behaviour of earthworms. For instance, soil water restricts the life cycle of earthworms, although they can survive a certain level of desiccation (Roots, 1955). In fact, their survival is controlled by this factor and its interaction with temperature and soil texture (Booth et al., 2000). Temperature affects the burrowing and casting activities of earthworms, usually in interaction with soil water content (Gerard, 1967; Perreault & Whalen, 2006a; Kaneda et al., 2016). Moreover, it is one of the main factors affecting the seasonal activity of earthworms and, of their food supply (Nordström, 1975). Soil moisture and temperature are the main factors that can restrict the activity windows of earthworms, but they are not the only factors. Other soil properties, such as pH, soil texture, soil organic matter (SOM) content and soil compaction, also affect the life cycle, activity and behaviour of earthworms. In the case of compaction, the degree of compaction in agricultural soils is increasing, due to the use of heavier and heavier farm vehicles and intensive soil tillage (Batey, 2009). In the agricultural context, compaction is a controlling factor of earthworm abundance, burrowing mode, biomass and cast production (Boström, 1986; Pižl, 1992; Chan & Barchia, 2007). It has been shown that earthworms tend to avoid compacted soil (Söchtig & Larink, 1992) as well as adapt their burrowing modes (Dexter, 1978). However, there is little quantitative data on the impacts of soil compaction on earthworm burrowing rates, or on how and if earthworm burrowing modes change due to soil mechanical constrains.

The impacts of compaction on earthworm burrowing activity not only affect their burrowing modes or the length of created burrows but should also have an impact on their energy requirements. Establishing the energy budget (how energy resources are allocated) for an organism can provide information about the physiological rates of the organism as a function of fluctuating conditions in terms of climate and food (Sousa et al., 2010). In the bigger picture, understanding how different organisms acquire and use their energy makes it possible to connect different processes at different levels (*e.g.* populations and ecosystems) (Nisbet et al., 2000). While there is literature data about earthworm respiration rates as a function of temperature, providing information on the metabolic functioning, there is currently no information about the respiration or energy costs associated with burrowing (Yonemura et al., 2019).

Earthworm burrowing is a function of soil conditions (Le Bayon et al., 2017). Many studies have investigated the impacts of changes in soil conditions (*e.g.* temperature, moisture, compaction) on earthworm burrowing in laboratory conditions or within short periods (*e.g.* days). However, the results do not necessarily reflect the continuous changes that occur under field conditions. Not many studies have investigated the burrowing behaviour under field conditions. Some have measured changes in burrowing activity in the field, but not its temporal dynamics (Francis & Fraser, 1998). The few studies that have included continuous monitoring of temporal changes in earthworm burrowing for extended periods have been performed in laboratory conditions, *e.g.* that by Francis et al. (2001). To fully understand the temporal dynamics of earthworm burrowing activity, experiments must be carried out in field or semi-field conditions (*e.g.* use columns with sieved soil, but placed in the field), to assess how earthworms respond to natural fluctuations in soil conditions. This could also provide more accurate information about the possible effects of climate and soil management on the soil ecosystem functioning.

Earthworms are key actors in the proper functioning of soils, so studying the impacts of different environmental parameters on earthworm bioturbation is essential. For instance, identifying the effects of compaction on earthworm burrowing activity can provide further insights into their burrowing temporal dynamics as a function of mechanical resistance and possible consequences for soil processes. In addition, understanding how earthworms respond physiologically to compaction, in terms of energy requirements, is key to accurate modelling of how compaction affects earthworm activity and, directly or indirectly, their population and the ecosystem. In general, it is critical to identify possible effects on soil processes in which earthworms are involved (directly or indirectly) and to gain knowledge of their behaviour as a function of the temporal and spatial changes occurring in soil ecosystems. This information is needed for modelling future impacts under climate change scenarios, and as input to develop more sustainable management practices within agriculture.

2. Aim and Objectives

The overall aim of this thesis was to provide a better understanding of the impacts of soil compaction on burrowing by different earthworm species. The specific objectives of the work were to:

- Quantify burrowing rates and earthworm burrowing as a function of soil mechanical resistance for different earthworm species (Paper I).

- Measure the energy costs of earthworm burrowing as a function of soil compaction for different earthworm species (Paper II).

- Evaluate the temporal dynamics of earthworm burrowing for different compaction levels, under semi-field conditions (Paper III).

3. Background

3.1 Earthworm classification

Earthworms are classified based on their feeding, morphological and behavioural characteristics. The most commonly used ecological classification, developed by Bouché (1977), divides earthworms into three main groups, epigeic, endogeic and anecic (Fig. 1). The epigeic group consists of species that live in the organic soil layer, feed mainly on fresh litter, have small body sizes (1-4 cm), and have a weak muscular system due to no burrowing activity (Lee, 1985; Edwards & Arancon, 2022; Makeschin, 1997). The endogeic earthworms are inhabitants of the upper mineral soil profile, usually have medium body size (5-15 cm), have a moderately developed muscular system, and create an extensive horizontal burrowing network (Lee, 1985; Hendriksen, 1990; Edwards & Arancon, 2022; Makeschin, 1997). Earthworms in the anecic group move throughout the soil profile, feed mainly on litter in the organic soil layer and bring their food to deep soil horizons, have larger body sizes (10-30 cm) and a well-developed muscular system, and build vertical unbranching burrows (Lee, 1985; Edwards & Arancon, 2022; Makeschin, 1997).

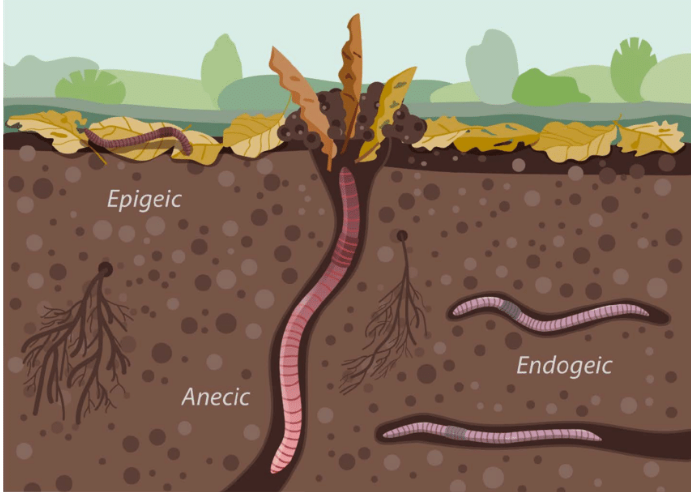


Figure 1. Epigeic, endogeic and anecic earthworms, and their feeding, burrowing and habitat within the soil profile. Source: (Eisenhauer & Eisenhauer, 2020)

This initial classification has been shown not to be completely accurate, and several attempts have been made to reclassify earthworms based on different characteristics such as exerted axial and radial pressure (Keudel & Schrader, 1999), burrowing behaviour (Felten & Emmerling, 2009) and body wall analysis (Briones & Álvarez-Otero, 2018). One of the most recent attempts was made by Bottinelli et al. (2020), who performed an analysis of 13 morpho-anatomical traits and concluded that the ecological classification of earthworm species should consider percentages of the three main ecological categories initially defined by Bouché (1977).

3.2 Earthworm bioturbation and soil processes

Rooting plants and burrowing animals can displace soil particles, and earthworms can mix soil through ingestion and egestion, this process is known as bioturbation (Meysman et al., 2006). Bioturbation influences several ecosystem processes and services (*e.g.* water supply, soil formation, climate regulation (Fig. 2)) which in turn generate impacts in a landscape context (Lavelle et al., 2006). Berke (2010) identified earthworms as bioturbators because their ecosystem effects include: disturbance by creating persistent or temporal biological and physical changes, the mix of materials and alteration of biogeochemistry. Earthworm impacts on different soil processes are diverse and are a consequence of the development of certain biogenic structures, such as burrows and casts, which constitute “earthworm bioturbation” (Piron et al., 2017). These two biogenic structures have different impacts on soil properties that change based on environmental conditions and species. For instance, the burrows refilled with cast can influence mesoporosity and water retention, which can be mainly done by endogeic earthworms. On the other hand, high cast volumes might influence biological activity in soils surrounding the casts or burrows, known as the drilosphere, primarily created by endogeic and anecic earthworms.

It is known that soil ecosystems without earthworms have lower annual bioturbation rates and that in cold-temperate areas in Europe, earthworms are the dominant organisms driving bioturbation (Taylor et al., 2018). Earthworm bioturbation depends on the presence of endogeic and anecic species because these organisms are the ones that burrow in the soil profile. Some authors, like Taylor et al. (2018), have calculated that mean bioturbation rates can be around 25 Mg dw ha^{-1} y^{-1}, ranging from 15 to 34 Mg dw ha^{-1} y^{-1} (topsoil of 20 cm depth and < 2mm fine soil ha^{-1}). Scheu (1987) found that cast production of *Aporrectodea caliginosa* was around 3.005 g dry wt m^{-1}, which is equivalent to 4.3 mm of the soil layer. Both studies calculated the bioturbation rates as a function of temperature changes in the field conditions.

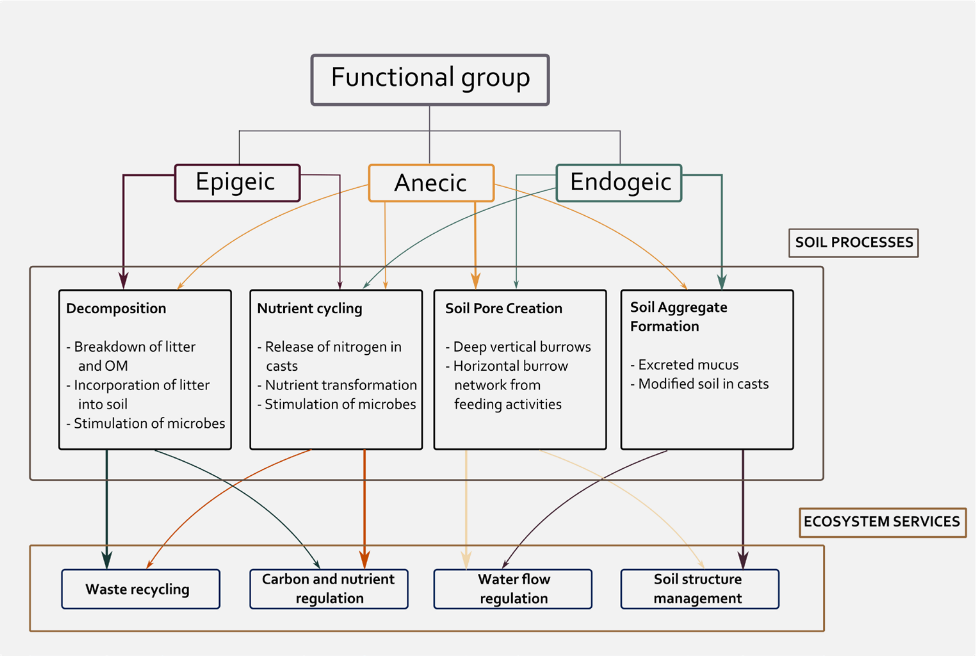


Figure 2. Earthworm functional groups and their involvement in different soil processes and ecosystem services. Adapted from Dewi and Senge (2015)

The impacts of burrows on soil processes have been widely studied and the importance of permanent burrows created by anecic earthworms (*e.g. Lumbricus terrestris*) or of the intricate burrow system created by some endogeic earthworms (*e.g. A. caliginosa*) for water infiltration and percolation is well documented (Bastardie et al., 2003; Ernst et al., 2009; Capowiez et al., 2014). In addition, the consumption of litter by epigeic (*e.g. Dendrobaea octaedra*) and anecic worms is key to the decomposition of organic matter and resource allocation (Jouquet et al., 2006). Earthworm burrows are considered hotspots of soil biological activity, as there is evidence of higher microbial activity compared with the surrounding bulk soil (Jégou et al., 2001; Savin et al., 2004). Earthworm casts have similar impacts as earthworm burrows on soil processes, such as stable aggregate formation (Buck et al., 2000) and high microbial activity with the presence of bacteria and actinomycetes (Martin & Marinissen, 1993). Casts can have high organic carbon content (Schrader & Zhang, 1997), higher nitrate and ammonium concentrations (Parkin & Berry, 1999; Clause et al., 2014) and higher available phosphorus than bulk soil (van Groenigen et al., 2019).

Earthworm casts have been shown to either increase or reduce soil erosion, depending on cast stability which will determine if casts are easily eroded or can act as a physical barrier to water (Le Bayon & Binet, 1999; Lipiec et al., 2015).

Although the impacts of earthworm activity on soil processes through the production of burrows and casts are generally well known (Piron et al., 2017), and the effects of different earthworm ecological groups are well described, their quantitative representation remains limited. Earthworms influence many soil biological, chemical and physical properties and processes, directly or indirectly. The creation of burrows and cast production directly influence the physical structure of soil through soil aggregation, bioturbation, soil fragmentation, macropore formation and bio-accumulation (Creamer et al., 2022). Soil processes can be associated with certain soil functions and sub-functions, which in turn are related to ecosystem services. The soil functions mediated by earthworms are (Creamer et al. (2022)): nutrient cycling (sub-functions: nutrient transformation, reallocation and assimilation), carbon and climate regulation (sub-functions: decomposition, resource allocation) and water regulation and purification (sub-functions: infiltration and percolation, water storage and biological retention). These soil functions are associated with three types of ecosystem services: production, support and regulation services (Adhikari and Hartemink, 2016; Lavelle et al., 2006).

3.3 Soil structure and earthworm activity

Soil structure is defined as the three-dimensional arrangement of solids and voids. It controls many soil processes and forms the habitat of soil organisms. Soil structure and soil organisms have a strong influence on each other because soil organisms can modify soil structure, but they are also limited by it (Elliot & Coleman, 1988). According to Lavelle (1988), the effects of earthworms on soil structure are dependent on earthworm activity and community composition.

Earthworms modify soil structure in different ways. Earthworms create burrows or galleries, which are macropores in the soil profile that improve water infiltration and soil aeration (Bottinelli et al., 2015). Findings by Jarvis

(2007) indicated that, depending on the earthworm species, biopores can be temporary or more long-term. In general, biopores created by endogeic earthworms tend to have a short turnover time, as the burrow systems are highly dynamic. In contrast, burrows created by anecic earthworms are more long-term as these tend to be re-used by the worm. The relevance of the burrowing network on certain soil processes is affected by several factors such as burrow density, continuity, tortuosity and length, and earthworm density (Capowiez et al., 2014).

Earthworm activity is known to have a significant influence on soil aggregation, but the dynamics of these effects are intricate (Marashi & Scullion, 2003). Earthworms aggregate soil directly when they produce cast. It has been suggested that the incorporation of soil organic matter (SOM) in casts can enhance the physical protection of SOM in microaggregates found within casts (Jongmans et al., 2001; Bossuyt et al., 2005). It has been shown that the presence of earthworms in soils increases the formation of macroaggregates (Bossuyt et al., 2005; Zangerlé et al., 2011) and a significant abundance of casts might improve water holding capacity of soils and promote plant growth (Lipiec et al., 2015). Nonetheless, earthworm casts can also contribute to soil erosion, particularly in compacted soils, due to more surface cast production in these soils, that when exposed to rainfalls is easily eroded, which can cause losses in nutrients *e.g.* N, P and K (Le Bayon & Binet, 1999; Shipitalo & Le Bayon, 2004; Jouquet et al., 2012).

Moreover, earthworms process plant residues and soil, and distribute organic matter, which in turn influences soil structure and organic matter dynamics (Jongmans et al., 2003). According to Martin et al. (1992), irrespective of their ecological feeding (litter or soil), earthworms feed on easily decomposable organic compounds rather than on bulk soil organic matter. Scullion & Malik (2000), found that earthworm activity helps with carbon stabilisation and physical protection of organic compounds. In addition, there is evidence that the walls of biopores in the subsoil have a higher carbon content than the bulk soil, indicating that earthworm burrowing activity can increase the carbon content in the subsoil (Hoang et al., 2017). The importance of soil organic carbon for soil physical properties have been widely studied, particularly its positive impact on aggregate stability (Chenu et al., 2000; Blanco-Canqui & Lal, 2004), and pore size distribution, particularly the 0.2-720 μm diameter classes) (Fukumasu et al., 2022).

3.4 Earthworm activity and soil abiotic properties and conditions

As mentioned previously earthworms are highly sensitive to changes in soil temperature and soil moisture, which are the main drivers of earthworm activity (*e.g.* reproduction, maturity). For instance, Berry & Jordan (2001), identified that soil moisture and temperature affected the growth (as specified by variations in weight) and development of the tuberculata pubertatis and clitellum (indicators of sexual maturity in adult worms) of *L. terrestris*. Booth et al. (2000) analysed the effects of soil texture, temperature and moisture on the growth of juvenile earthworms, concluding that all three factors play an important role in earthworm growth (as specified by variations in weight). Similarly, Eriksen-Hamel & Whalen, (2006), showed that soil temperature and moisture content affect the growth rate and activity of *A. caliginosa*. Following the same trend, a reduction in live weight (an indicator of growth) as a function of water suction has been found for *Aporrectodea longa*, without any changes in their weights at $\Psi < 60$ kPa (Kretzschmar & Bruchou, 1991).

Another important soil property that can influence earthworm burrowing is soil compaction. Compaction is a reduction of soil porosity, which will disturb the water and air movement in soils, potentially causing oxygen deficiency, and reducing water infiltration (Piearce, 1984; Söchtig & Larink, 1992). It is an important controlling factor for earthworm abundance and behaviour, as it negatively impacts earthworm density, locomotion, biomass and cast production (Boström, 1986; Pižl, 1992; Chan & Barchia, 2007). In an agricultural context, the degree of soil compaction varies due to management practices such as ploughing, seedbed preparation, machinery traffic, etc. There are diverse causes of why soil compaction is a threat to earthworm bioturbation, as it can diminish significantly the abundance of earthworms due to direct killing, damage of individuals or fewer mated adults (Piearce, 1984; Boström, 1986; Pižl, 1992; Crittenden et al., 2014).

Earthworm bioturbation, burrowing and casting production are severely limited when soil mechanical resistance is high. It has been found that there is a significant reduction in cast production when soil strength exceeds 250 kPa for *A. longa* (Kretzschmar, 1991). Also, a negative correlation has been observed between surface cast production and compaction levels for *A. caliginosa*, *Lumbricus rubellus* and *Allolophobora chlorotica* (Scullion & Ramshaw, 1988). The burrow length of *A. caliginosa* is reduced by half in soils with high density (Söchtig & Larink, 1992). The effects of soil mechanical restriction are closely related to soil water conditions. For instance, Kretzschmar (1991) found that in wet (matric potential, Ψ=-7kPa) and compacted soils (350 kPa), earthworm cast production might get restricted. Similar conclusions were made by Dexter (1978), who indicated that there must be an upper limit of soil strength where earthworms cannot burrow anymore.

3.5 Earthworm metabolism

To understand how an ecosystem functions, it is important to understand its energy flows (Phillipson & Bolton, 1976). Thus, it is necessary to know how energy is used and acquired by individual organisms, which can be done by studying the bioenergetics of different organisms (Phillipson, 1975). Analysis of the energy budget of an organism provides information on its development, reproduction and functional needs within the ecosystem (Meehan, 2006). Quantification of the energy budget can be done by analysing heat production or respiration rate (oxygen consumption or carbon dioxide (CO_2) efflux), representing direct or indirect calorimetry, respectively.

Most studies measure respiration rate, as it is an easy and commonly used methodology. As mentioned previously, some studies focus on the organism's metabolism or how it responds to different environmental conditions. For instance, Phillipson & Bolton (1976) measured the respiration rates of different earthworms species (*Dendrobaena rubida*, *Lumbricus castaneus*, *Octolasion cyaneum* and *Aporrectodea rosea*), which showed fluctuations in the CO_2 efflux (μL g^{-1} h^{-1}, gut free fresh weight) during the seasons. They also observed some differences in the respiration rates between litter-dwelling and soil-dwelling species. Uvarov & Scheu (2004) showed that respiration rates (CO_2 efflux) for mature *L. rubellus* (adults) increased with temperature (*e.g.* 10, 15 and 20°C). In an extensive study, Byzova (1965) calculated the mean oxygen consumption rate of different earthworm species of different ecological groups, for instance, *A. caliginosa*, *L. terrestris* and *D. octaedra*, showing that the ecological differences are connected to the differences in a metabolic level, and that respiration rates depend on weight. This dependence of the respiration rate on weight is stronger in earthworms with higher muscular activity. Moreover, in ecotoxicological studies (using different pollutants and heavy metals) respiration rates (O_2 or CO_2 measurements) are considered a suitable indicator of the metabolic response and indirect measure of the organisms' maintenance costs. This provides information on the possible effects of these stressors *e.g.* loss of population, biodiversity or habitat (Tang et al., 2016; Świątek & Bednarska, 2019; Anyanwu & Semple, 2021).

While respiration rate measurements provide good information on earthworms' metabolic responses to environmental stressors, it does not measure all the metabolic processes involved in organisms functioning, as an anaerobic process can also occur. Other methods have to be used for measuring energy without being restricted to aerobic processes such as isothermal calorimetry (direct calorimetry). Isothermal calorimetry is widely used in environmental microbiology, as it monitors heat flow produced at high precision in different biological processes. It is used for multiple assessments such as toxicity, biodegradation and risk assessment of soil pollutants (Rong et al., 2007; Braissant et al., 2010), functioning of the soil microbial community (Bölscher et al., 2020; Chakrawal et al., 2020; Dufour et al., 2022). Recently it has been used to quantify the compaction effects on root development (Colombi et al., 2019). Direct calorimetry is not commonly used for quantifying earthworm activity. A study used it for pollutants effects: Lamprecht (2013) measured heat production rates of earthworms after being exposed to pentachlorophenol (PCP) using a Calvin calorimeter. Wallwork (1973) measured calorific equivalents on annelids using bomb calorimetry to establish the nutritional status of a field population.

Although there is clear evidence of the physical, chemical and biological impacts of earthworm bioturbation, little is known about the energy requirements of earthworms in general, particularly for burrowing purposes. Only a few studies have attempted to quantify the energy budgets of earthworms. Bolton and Phillipson (1976) determined energy budgets for *A. rosea* (small immatures, large immatures, and adults) at 10 °C, obtaining values between 12.9 and 13.6 kJ g^{-1} dry weight. They also observed differences in assimilation for the three groups of individuals and concluded that ingestion may be driven by food quality rather than energy content. A study by Ruiz et al. (2015) using a plastic penetration model predicted that for a burrow length of 1 m and an earthworm with a mean radius of 1.2 mm and a normalized water content of 1, the energy expenditure was around 3.1 $x10^{-3}$ J (Ruiz et al., 2015). The main conclusion of the study was that the energy consumption of an earthworm when penetrating through soil could be around half of its total energy budget. Based on the current lack of information on earthworm respiration and the energy dynamics of earthworm movement (Yonemura et al., 2019), there is an opportunity to get improved quantitative data on earthworms' energy budgets associated with burrowing, which will allow a better understanding of earthworm metabolism.

4. Materials and Methods

4.1 Experimental designs

4.1.1 Earthworm burrowing as a function of compaction (Paper I)

Earthworm burrowing activity was monitored using a 2-D terrarium for two earthworm species (*Aporrectodea caliginosa* and *Aporrectodea longa*). Our focus was to study the impact of soil mechanical resistance on earthworm burrowing, therefore the earthworms were exposed to four different soil bulk densities (1.1, 1.3, 1.5 and 1.7 g cm^{-3}). For each soil bulk density, its corresponding soil mechanical resistance was measured, with mean cone penetration resistance values of 117, 579, 949 and 1068 kPa, respectively. The combination of earthworm species and soil mechanical resistance level was replicated five times, to achieve a total of 40 experimental samples (two earthworm species × four soil mechanical resistance levels × five replicates). Here the burrowing rates (cm d^{-1})were quantified, as well as the cast production and the ingestion factor (proportion of burrow that was created by soil ingestion).

For each sample, earthworm activity was recorded for three days with two surveillance cameras (LUPUSNET HD - LE 936 PLUS) on each side of the terrarium (Fig. 3). All the experiments were done inside a growth chamber (Model SED-41C8, Percival Scientific Inc.), and kept in darkness at a constant temperature of 15 °C and 60% relative air humidity, experiments were always started at around the same time of day.

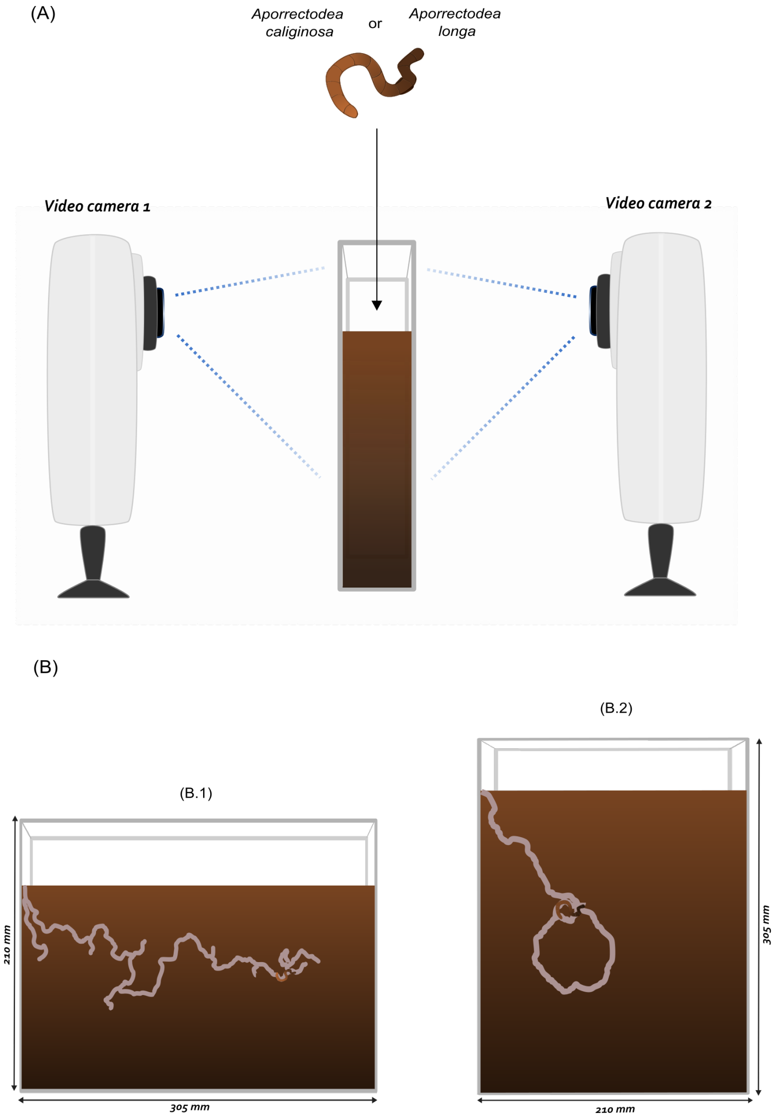


Figure 3. Experimental set-up for Paper I. (A) Lateral view of the experimental set-up. (B) Camera view of the experimental set-up, (B.1) for *A. caliginosa* and (B.2) for *A. longa*.

4.1.2 Energy costs of burrowing (Paper II)

Paper II aimed to quantify the energy burrowing costs of earthworms as a function of soil compaction For this purpose, two earthworm species (*Aporrectodea caliginosa* and *Aporrectodea tuberculata*) were exposed to two treatments, compacted (bulk density, ρ= 1.4 g cm^{-3}) and loose soil (ρ= 1 g cm^{-3}), at constant matric potential of -100 hPa. Two methodologies were used to establish the energy requirements during burrowing: a respirometer (RESPICOND 96) measuring CO_2 efflux and an isothermal calorimeter (TAM Air) measuring heat dissipation. Besides measuring samples with earthworms in the soil, two controls were used, one comprising soil without an earthworm and the other comprising an earthworm without soil (placed on a wetted filter paper).

For the respiration measurements, a total of 16 experimental units were used (two earthworm species × two compaction levels × four replicates). For the isothermal calorimeter measurements, a total of 12 experimental units were used (two earthworm species × two compaction levels × three replicates). In addition, there were measurements of soil-only and earthworm-only (earthworm on filter paper) (for details see Paper II). For both methodologies, measurements were conducted over 48 hours, at a temperature of 20 °C.

To quantify burrow volume and belowground cast production, which were then related to the energy costs, X-ray scanning was conducted for all experimental units, using the GE Phoenix X-ray scanner (v|tome|x 240) at the Department of Soil and Environment, Swedish University of Agricultural Sciences, Uppsala. The data of cast production and burrow volume was used to quantify the ingestion factor.

4.1.3 Temporal dynamics of burrowing activity under different soil moisture and compaction levels, in semi-field conditions (Paper III)

These experiments aimed to assess how the burrowing activity of earthworms evolved through time under semi-field conditions. For this purpose, different initial conditions were designed, with four treatments combining two initial soil moisture conditions (moist and dry) and two initial bulk densities (1.1 and 1.4 g cm^{-3}), making it a total of four treatments and five replicates per treatment. Two earthworms, one *Aporrectodea caliginosa* and one *Aporrectodea tuberculata* were placed in each repacked soil column (Diameter = 18.5 cm; Height = 25 cm). The columns were placed in the soil (the soil surface of the columns at the same level as the soil surface) in a small garden plot in Ultuna, Uppsala, Sweden, following the placement as shown in Fig. 4. The experiments were run for approximately six months (late June until early December 2021).

To quantify the temporal dynamics of burrowing activity, X-ray imaging was used in all experimental units, using the GE Phoenix X-ray scanner (v|tome|x 240) at the Department of Soil and Environment at the Swedish University of Agricultural Sciences, Uppsala. Soil columns were scanned three times during the six months. The columns were removed from the plot in early December (week 24), as it is the start of winter and earthworms are not active during this season. The scans were performed on three occasions during the experiments: after one week, after 8 weeks and after 24 weeks. The final scan (after 24 weeks), was done after 2 months the columns were removed (in early December), due to problems with the X-ray tomographer. During this time (2 months) all columns were placed in a cold room at a temperature of 3°C.

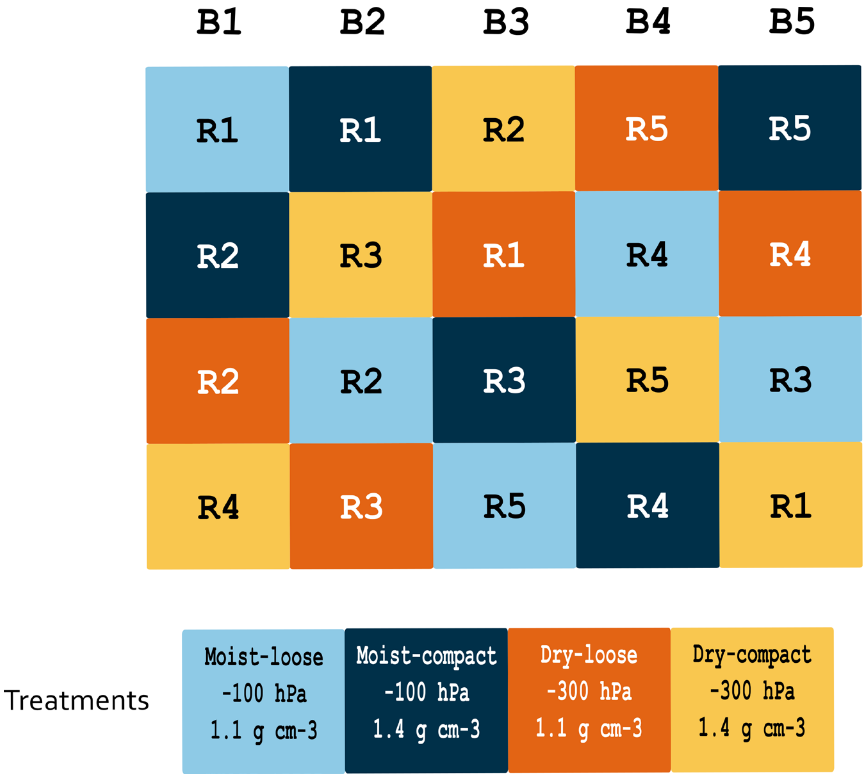


Figure 4. Experimental design of Paper III, with information about the treatments with the initial conditions of the columns, block (B1, B2, B3, B4, B5) and replicates (R1, R2, R3, R4, R5)

4.2 Earthworms

The earthworm species used in this thesis were sampled from arable fields in Sweden. In addition, the purpose was to assess if species of different ecological groups behaved differently under various compaction scenarios. Therefore three species were selected, *A. caliginosa*, *A. tuberculata* and *A. longa*, which are part of the endogeic (*A. caliginosa* and *A. tuberculata*) and epi-anecic (*A. longa*) ecological groups. The three species can be found in temperate zones and are widely used in earthworm ecology and physiology studies.

The earthworms used in all the papers were adults with visible clitellum. For Paper I, earthworms used were taken from a laboratory population kept at the Department of Ecology, Swedish University of Agricultural Sciences. For Papers II and III, all earthworms were collected from an arable field near Uppsala, Sweden. For all the experiments, the earthworm's guts were emptied before the start of the experiments and their weight was recorded.

4.3 Soil properties

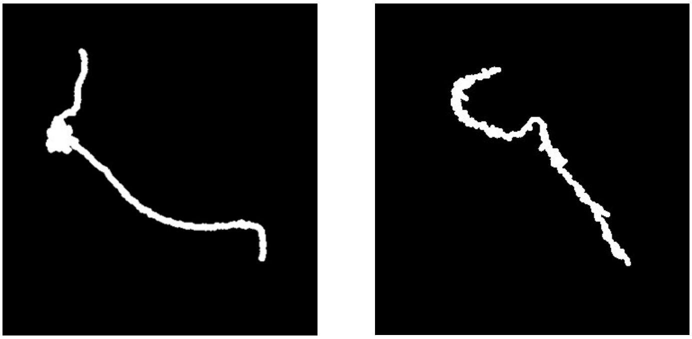
For all the experiments, the soil used was taken from the top 20 cm of an arable field near Uppsala, Sweden (59.9 N, 17.6 E). It has a silty clay loam texture (39.8% clay, 51.9% silt, and 8.3% sand). The soil organic carbon (SOC) content was 2.26%, and the pH was 5.9 (measured in a 1:2 ratio of soil: deionized H_2O). The soils were sieved to obtain 2 mm aggregates and then oven-dried for 24 hours at 105 °C. All the visible plant and root residues were removed before sieving.

For papers I and II, the soil was moistened to achieve a matric potential of -100 hPa. While for Paper III, the two initial matric potentials were -100 hPa and -300 hPa. The amount of water required was calculated based on soil water retention measurements carried out on soil cores placed on a tension plate using the same soil and considered all the desired bulk densities.

4.4 Image analysis

4.4.1 Time-lapse imaging (Paper I)

From the three-day recordings, an image was extracted every five minutes, to create a time-lapse image sequence. The geometrical distortion in the time-lapse sequence was corrected using bUnwarpJ (Arganda-Carreras et al., 2006) in ImageJ. To track the earthworm movement, the earthworm prostomium was followed and obtained the x and y coordinates. This was done by using the “Manual tracking” plugin in ImageJ (see example in Fig. 5). The coordinates allowed us to quantify different variables: burrow length, number of changes of direction of an earthworm (i.e. changing from forward to backward movement or vice versa), and total movement of the earthworms. The burrow network was recreated using the recorded coordinates, timestep by timestep, which allowed us to quantify the burrow length and total movement. All the calculations were done in R (R Core Team, 2018). In addition, manual measurements in the final images were done to verify total burrow length quantifications.



A. longa
(epi-anecic)

A. caliginosa
(endogeic)

Figure 5. Typical burrow systems for *A. longa* and *A. caliginosa* (Size: 349 × 380 pixels)

4.4.2 X-ray imaging (Papers II and III)

For the X-ray image analysis (Papers II and III) radiographs were taken for each experimental unit at a certain voltage, current and exposure time. Then a 3D image was constructed from the radiographs using the GE reconstruction software datos|x 2.1 RTM. The images had a certain voxel size in all directions, which corresponds to an image resolution. For the details of the number of radiographs, voltage, current, exposure time and voxel size see the Material and Methods sections of Papers II and III.

For both papers, the height of the volume was fixed in all replicates and varied depending on the treatment. For Paper II some replicates there were some collapsing burrows, and in Paper III in addition to collapsing burrows there was soil settling. Thus for the image analysis, a height was selected that varied among the treatments (in both papers) and scan times (in Paper III).

The soil surface was defined as the vertical slice where the soil covered more than 90% of the area. The X-ray image processing methodology was similar for both experiments in Papers II and III. ImageJ and plugins included in FIJI (Schindelin et al., 2012) and SoilJ (Koestel, 2018) were used. For all the radiographs a 3D median filter was applied with a radius of one to reduce noise in the images. The solids from the air were segmented using Otsu's method (Otsu, 1979). In both papers the earthworms had to be removed in some or all experimental units, to quantify the burrows, as well as the below-ground cast.

In Paper II, earthworms were found in some of the experimental units, so these were removed from the images as follows. The initial 3D image was adjusted for brightness/contrast, where grey values were set between 23000 and 35000. The resulting binary image was eroded and dilated (two times each), objects smaller than 5000 voxels were removed using the "objects counter" and the "earthworm holes" were filled with the 3D fill holes function. Finally, as the segmentation did not remove the entire worm, manual removal of the earthworms was done slice-by-slice. In addition, the below-ground cast had to be removed. For this purpose, a semi-automatic approach was used. The details on the process are in Paper II - Supplementary Material. Finally, the binary images of both the "soil" and the "cast" were combined, to get an image of the final burrow created. The SoilJ plugin was used to quantify the burrow volume, specifically the PoreSpaceAnalyzer. For the burrow length quantification, the average earthworm diameters were assumed for the species used, based on the literature.

In Paper III, the image analysis included the removal of both earthworms and the below-ground cast. For removing them, the same procedure as in Paper II was followed, the details are described in Supplementary Material-Paper II. The images were segmented again and the threshold was adjusted (four pixels), to remove the smallest pores, that did not burrow due to their size. Then, the final image was used for further analysis of the earthworm burrowing.

4.5 Statistical analysis

All statistical calculations, for the three papers, were performed using the R software (R Core Team, 2018) and for the figures, the ggplot2 package version 3.3.3 or version 3.4.1 were used. The packages used were the ‘nlme’ package (version 3.1-160) and the ‘emmeans’ package (version 1.8.4-1). In Paper I, a two-way analysis of variance (ANOVA) was done using earthworm species and soil mechanical resistance as factors, to analyse their single and interaction effects. For the burrowing rate data (non-normal), a non-parametric Kruskal-Wallis test was performed with soil mechanical resistance and species as factors. In addition, a Tukey test was done to make pairwise comparisons between soil mechanical resistance levels. Linear regressions were used to analyse how burrowing properties changed as a function of soil mechanical resistance. In Paper II, a simple linear model was performed using treatment and species as factors, without interaction effect. This was done for all the factors tested (see Paper II for more details), except for the heat dissipation dynamics that were only qualitatively described with no statistical analysis. In Paper III, a linear mixed model was used to evaluate the effects of soil moisture level, compaction level, time (scan time) and their interaction on different burrow factors. Further details of the fixed and random effects of the linear mixed model can be found in Paper III (Materials and Methods - Statistical Analysis).

5. Results

5.1 Earthworm burrow length and burrow volume reduce when soil compaction increases (Paper I and II)

The burrow length results obtained in Paper I showed that for *A. caliginosa*, there were significant differences between the lowest (117 kPa) and highest (1068 kPa) mechanical resistance tested (Table 1). In contrast, *A. longa* did not show significant differences between any of the resistance levels studied. However, it can be seen from Table 1 that for both species. there was a trend for both total burrow length and total movement to be reduced when mechanical resistance increased A similar trend was found in Paper II (Table 1), where both burrow volume and burrow length decreased in compacted treatments in comparison to loose treatments. For *A. caliginosa*, the decrease in the burrow volume was around 25%, whilst for *A. tuberculata* this decrease was around 22%. These experiments show that compaction negatively affects burrowing activity.

Table 1. Earthworm burrow length at all mechanical resistance levels in Paper I, and the burrow volume for both bulk densities used in Paper II. Standard error (SE).

		Burrow length (m)	
	Mechanical resistance	*A. caliginosa*	*A. longa*
Paper I	117 kPa	0.45 (0.04)	0.24 (0.05)
	579 kPa	0.27 (0.05)	0.22 (0.01)
	949 kPa	0.27 (0.05)	0.13 (0.03)
	1068 kPa	0.18 (0.03)	0.13 (0.02)

		Burrow volume (cm^3)	
	Bulk density	*A. caliginosa*	*A. tuberculata*
Paper II	1 g cm^{-3}	1.85 (0.12)	2.73 (0.35)
	1.4 g cm^{-3}	1.38 (0.13)	2.11 (0.20)

5.2 Earthworm burrowing rate decreases as a function of mechanical resistance and is species-dependent (Paper I)

With increasing mechanical resistance, there was a significant reduction in burrowing rates, as well as a significant difference between species (Fig. 6). The average burrowing rate for *A. caliginosa* showed a pronounced reduction from 13.5 cm d^{-1} at 117 kPa to 5.8 cm d^{-1} at 1068 kPa. *A. longa* showed a reduction in burrowing rate from 6.5 cm d^{-1} to 3.6 cm d^{-1}, at the highest and lowest resistance levels tested, respectively (Paper I). This means that for the endogeic species (*A. caliginosa*) the reduction in burrowing rate was around 57%, while for the anecic species (*A. longa*) it was about 45%.

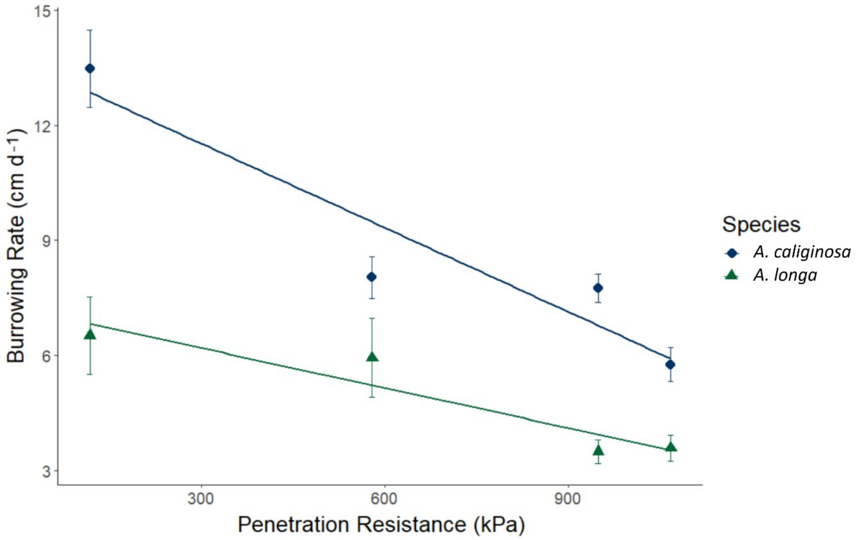


Figure 6. Earthworm burrowing rate (cm d^{-1}) as a function of penetration resistance (*A. caliginosa*: $R^2= 0.89$, $p < 0.01$; *A. longa*: $R^2= 0.89$, $p < 0.01$). Error bars indicate standard error ($n = 5$) (Paper I)

5.3 Earthworm burrowing modes are influenced by soil mechanical resistance (Paper I)

The effects of soil mechanical resistance on earthworm burrowing mode were more evident for *A. caliginosa* than *A. longa* (Paper I). For *A. caliginosa*, there was a shift in the burrowing mode from displacing soil to ingesting soil when creating burrows (Table 2). At lower mechanical resistance levels of 117 and 579 kPa, the ingestion factor was around 0.3, which means that 30% of the total burrow length created was done by soil ingestion. While at higher resistance levels of 949 and 1068 kPa, the ingestion factor values were around 0.6. In contrast, *A. longa* showed no clear changes in the burrowing modes when the resistance increased, as the ingestion factor values varied in a range between 0.42 and 0.78, with no clear relationship with the mechanical resistance. Similar behaviour was found for both *A. caliginosa* and *A. tuberculata* (Table 2, Paper II). On average, in the compacted treatments ($\rho= 1.4$ g cm^{-3}) the results showed that 73% of the burrow created were done using ingestion as their preferred burrowing mode versus 42% in loose treatments ($\rho= 1$ g cm^{-3}) for *A. caliginosa*. In contrast, *A. tuberculata* showed similar burrowing mode in both treatments, with 58%

and 60% of burrows created using ingestion in compacted and loose treatments, respectively (Table 2).

Table 2. Ingestion factor values for Paper I and Paper II. Standard error (SE)

		Ingestion factor	
	Mechanical resistance	*A. caliginosa*	*A. longa*
Paper I	117 kPa	0.34 (0.12)	0.58 (0.03)
	579 kPa	0.33 (0.04)	0.42 (0.05)
	949 kPa	0.61 (0.06)	0.78 (0.07)
	1068 kPa	0.66 (0.08)	0.58 (0.06)

		Ingestion factor	
	Bulk density	*A. caliginosa*	*A. tuberculata*
Paper II	1 g cm^{-3}	0.42 (0.08)	0.58 (0.20)
	1.4 g cm^{-3}	0.73 (0.12)	0.60 (0.11)

5.4 Respiration rates and energy costs are a function of compaction levels (Paper II)

The average values of the respiration rate for burrowing (Table 3) were always higher in *A. tuberculata* than in *A. caliginosa* (p = 0.04). In the compacted soils in both species, these values were also higher, for *A. tuberculata* by 0.8 times, and for *A. caliginosa* by 2.3 times. However, the differences seen due to compaction levels were not significant (p = 0.11). The possible effect of compaction level was also seen in the specific respiration rate (normalised with body mass) (Table 3), with higher values for compacted treatments vs. loose treatments (p = 0.04). But, it seems that the specific respiration rates of *A. tuberculata* and *A. caliginosa* were similar (p = 0.65).

The energy costs for burrowing followed a similar trend as the respiration rates (Table 3). On average the earthworms spend more energy for burrowing in compacted soils (~30 J d^{-1}) compared with loose soils (~20 J d^{-1}), for both species. But the compaction level did not statistically affect these values ($p = 0.352$), due to high variability. Also, these increases were similar between the species ($p = 0.783$). For the specific energy costs (energy costs normalized by body weight) (Table 3) on average there was an increase between 27-55% in different compaction levels, which varied between species. While statistically there was no significant effect of compaction level ($p = 0.423$), there was an indication that species might have different specific energy costs for burrowing ($p = 0.085$).

Table 3. Mean respiration rate and energy costs for burrowing, for *A. caliginosa* and *A. tuberculata*. Standard error (SE). (Respiration: *n* =6; Energy costs: *n* =4).

Soil compaction level	**Species**	**Total respiration rate (mg CO_2 d^{-1}) – burrowing R_B**	**Specific Respiration rate (mg CO_2 g^{-1} fw d^{-1}) – burrowing**	**Total energy costs (J d^{-1}) – burrowing R_B**	**Specific energy costs (J g^{-1} fw d^{-1}) – burrowing**
Compacted	*A. caliginosa*	0.43 (0.08)	1.15 (0.32)	30.34 (7.26)	57.12 (14.57)
	A. tuberculata	1.00 (0.23)	0.87 (0.24)	28.14 (11.09)	21.16 (11.01)
Loose	*A. caliginosa*	0.13 (0.21)	0.36 (0.43)	21.54 (13.49)	36.83 (23.63)
	A. tuberculata	0.55 (0.47)	0.38 (0.41)	18.34 (3.48)	16.61 (2.53)

5.5 Energy cost of burrowing and cast production are influenced by soil compaction (Paper II)

Preliminary theoretical calculations of the energy costs of burrowing in Paper I showed that in scenarios with higher soil penetration resistance, the energy required to create a burrow increased in both species studied (*A. caliginosa* and *A. longa*) and that these energy costs differed between the species. This trend was confirmed when energy costs and respiration rates were quantified in Paper II. The results showed that the energy cost per unit burrow volume (J cm^{-3}) in the compacted treatments compared with loose treatments was around 2.8-fold higher in *A. caliginosa* and around 2.1-fold higher in *A. tuberculata* (Fig. 7). Similarly, the energy costs per unit cast volume (J cm^{-3}) increased slightly more under soil compaction for *A. caliginosa* than for *A. tuberculata* (Fig. 7), which is supported by results from Paper I.

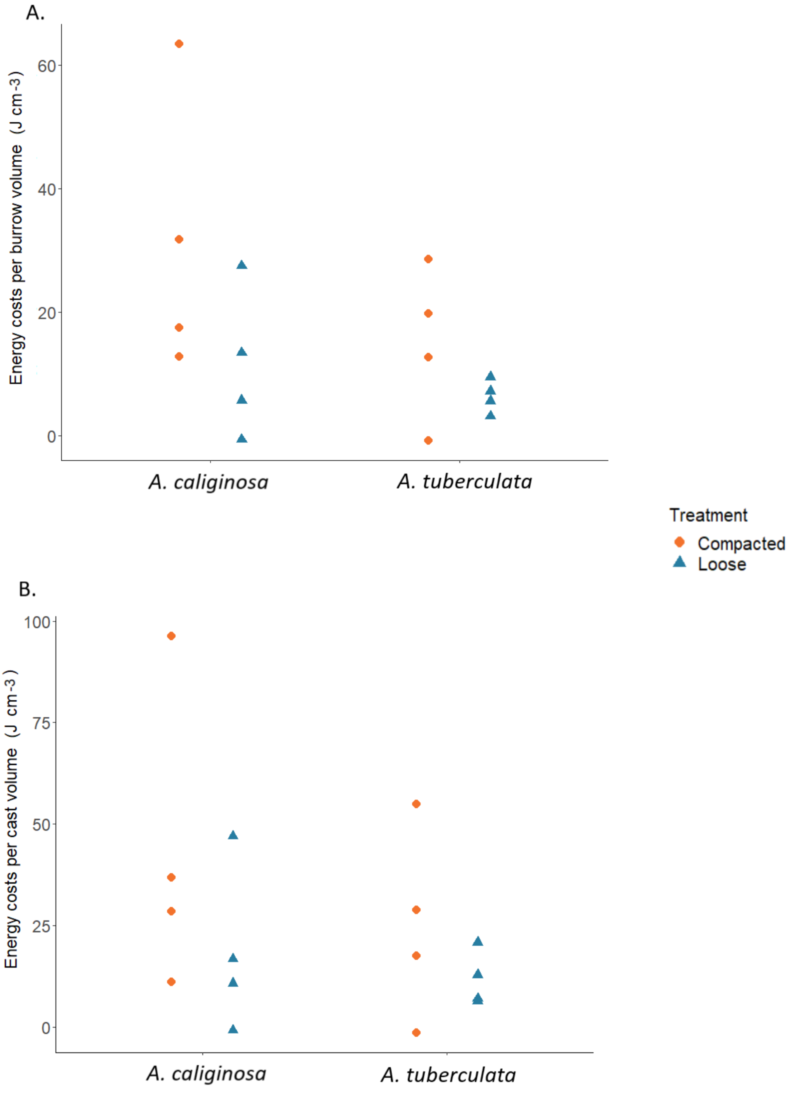


Figure 7. (A) Energy costs per burrow volume (J cm^{-3}), and (B) Energy costs per cast volume (J cm^{-3}) for *A. caliginosa* and *A. tuberculata* (Paper II)

5.6 Earthworms' heat dissipation and burrowing rate dynamics are affected by soil compaction (Paper I and II)

The burrowing rate dynamics of both *A. caliginosa* and *A. longa* showed reductions when the mechanical resistance increased (Paper I). The first few hours had much higher burrowing rates, but once the earthworm was inside the soil, the rate seemed to stabilise (Fig. 8). At lower soil mechanical resistance (117 kPa), there were more marked fluctuations and peaks in burrowing rates, while at higher mechanical resistance (1068 kPa), these fluctuations and peaks were less pronounced (Fig. 8).

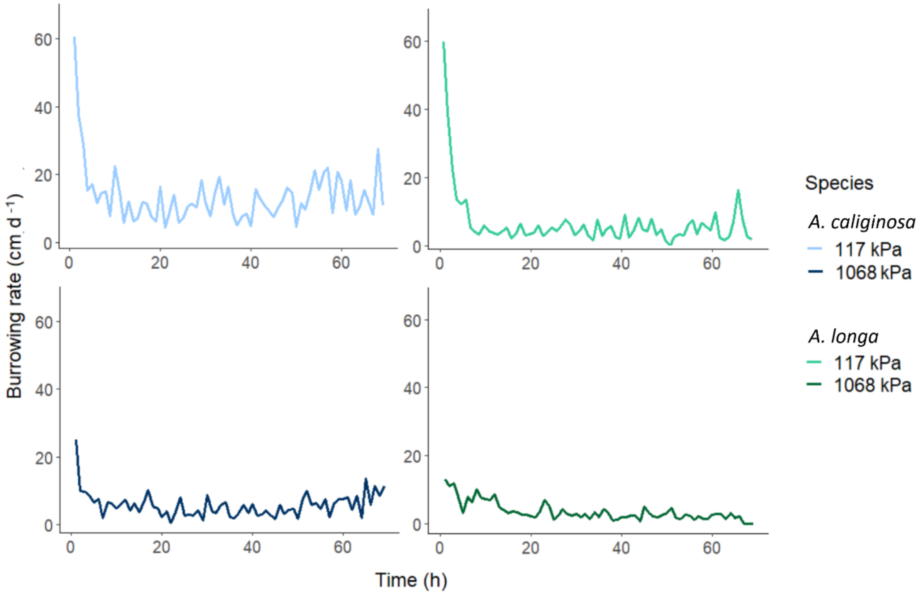


Figure 8. A. caliginosa and *A. longa* average burrowing rates (cm d^{-1}) dynamics at 117 kPa and 1068 kPa (Paper I)

The heat dissipation dynamics (Paper II) of *A. caliginosa* and *A. tuberculata* showed similar behaviour to the earthworm burrowing rates seen in Paper I, with evidence of fluctuations and activity peaks throughout the experiments (Fig. 9). The experimental data showed that both species had heat flows with greater fluctuations and peaks in compacted soil. In contrast, in loose soil, the heat dissipation appeared to differ between the species, with

A. caliginosa showing smaller fluctuations and *A. tuberculata* continuing to show greater fluctuations.

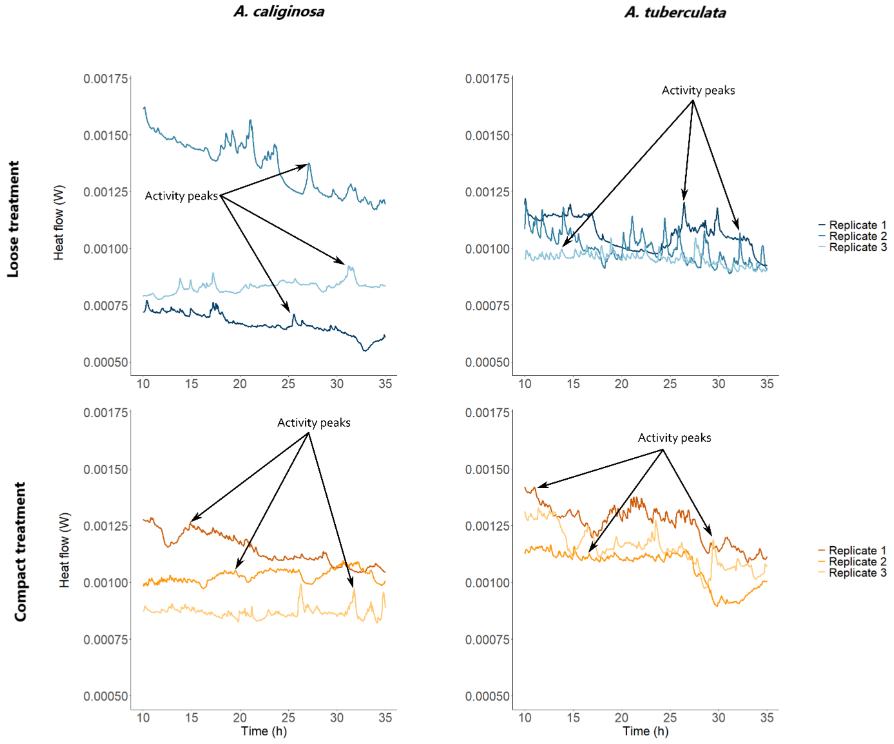


Figure 9. A. caliginosa and *A. tuberculata* heat dissipation dynamics in compacted and loose treatments (Paper II)

5.7 Burrowing rates decrease and the ingestion factor increases and stabilises over time, under semi-field conditions (Paper III)

The experiments were done in semi-field conditions and provided information on the dynamics of earthworm burrowing. The mean burrowing rates (cm^3 d^{-1}) (Fig. 10A) reduce after the initially higher levels, and after 24 weeks show only small fluctuations with time (Paper III). The final mean burrowing rates varied between 1.5 and 4 $cm^3\ d^{-1}$.

For the moist treatments, the burrowing rates were high at the beginning of the experiment and then decreased by around 60-80%. By the end of the experiment, burrowing rates in the moist treatments had slightly increased but there was a difference between the compaction levels, with a 20% and 64% increase for loose and compacted soil, respectively. In contrast, the dry treatments had very low initial burrowing rates but showed an increase at 8 weeks. In the dry-compacted treatment, burrowing rates continued to increase, by 51% between weeks 8 and 24. Whilst in the dry-loose treatment, the rates decreased by 24% between weeks 8 and 24. The linear mixed model (Supplementary Material in Paper III) showed that burrowing rates were affected by the interaction effects of soil moisture conditions and compaction levels ($p = 0.011$)

On average, ingestion factor values showed an increase at all time scans except for the moist-loose treatment (week 8) (Fig. 10B). The lack of increase in this treatment could be attributed to an increase in soil displacement (pushing aside soil) as the burrowing mode, instead of ingestion. By the end of the experiments, ingestion was the preferred burrowing mode and ingestion factor values were around 0.77-0.9, which indicates that 77-90% of the burrows were created by soil ingestion. The moist treatments showed slightly higher values than the dry treatments. Even though there was an increase with time in the ingestion factor, the linear mixed model (Supplementary material in Paper III), did not show any statistically significant effects of soil moisture levels, compaction levels or time.

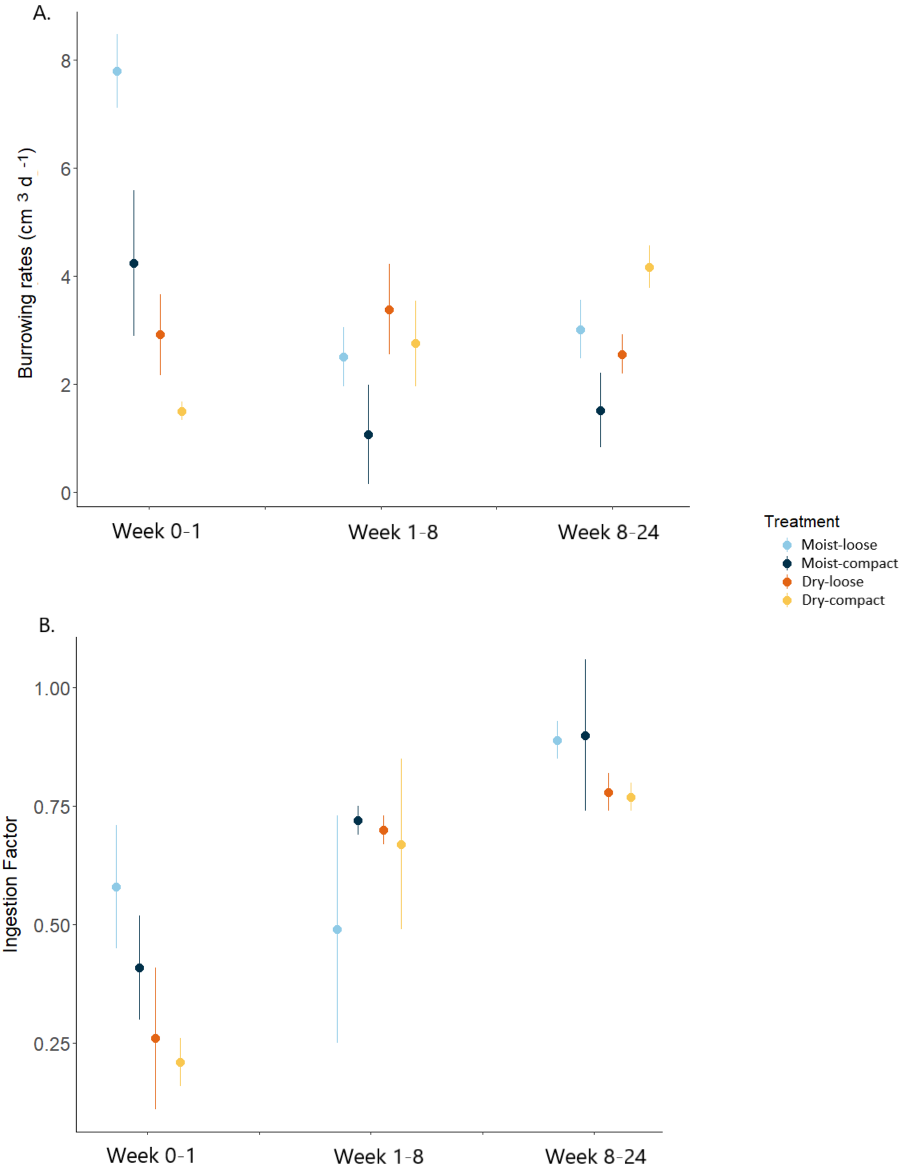


Figure 10. (A) Mean burrowing rates (cm^3 d^{-1}) and (B) Mean ingestion factor as a function of time. Error bars indicate standard error (*n*=5 or *n*=3). For the ingestion factor, in treatment "moist-compact" for week 8 (n=2) (Paper III)

5.8 Casting rates fluctuate over time under semi-field conditions (Paper III)

The mean casting rates were variable among all treatments with time (Fig. 11). The casting rate between weeks 1 and 8, for the moist soils reduced by around 50%, while for the dry soils, the casting rates increased around 2 and 7 times, in loose and compacted treatments, respectively. In the period, between weeks 8 and 24, the trends in the casting rate changed in all treatments. In the moist-compacted treatment, the casting rates continue to decrease reaching a mean value of 1.27 cm^3 d^{-1}. There is also a slight reduction in the mean cast rate for the dry-loose soil treatment from 2.43 cm^3 d^{-1} (weeks 1- 8) to 1.98 cm^3 d^{-1} (weeks 8 -24). In contrast, the casting rate in moist-loose treatment increases again with a mean value of 2.62 cm^3 d^{-1}. Finally, in the dry-compacted treatment, the casting rate continues to increase showing the highest casting rate with a mean value of 3.23 cm^3 d^{-1}. The linear mixed model showed a possible effect of compaction level (p = 0.05) in the casting rates.

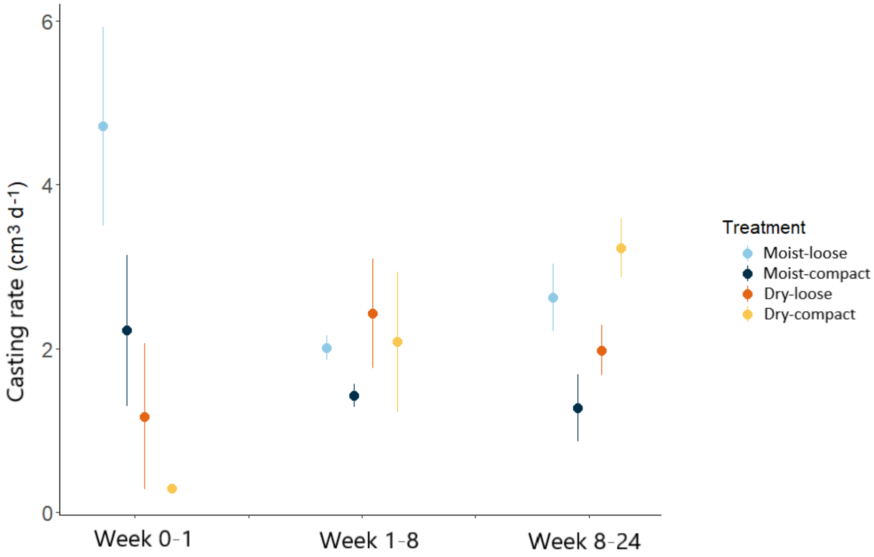


Figure 11. Casting rates (cm^3 d^{-1}) as a function of time. Error bars indicate standard error (n=5 or n=3). For the moist-compact treatment during week 8 (n=2) (Paper III)

6. Discussion

6.1 Earthworm burrowing rates and burrowing modes are a function of soil mechanical resistance and are species dependent

It is well-known that earthworm activity is hampered by soil compaction (Dexter, 1978; Söchtig & Larink, 1992) and that in general earthworms tend to avoid compacted areas (Stovold et al., 2004; Capowiez et al., 2009). One of the main impacts of compaction is a reduction of burrow development (burrow length or burrow volume). This was confirmed by our results (Papers I and II), which showed in line with previous studies, that burrow length and volume for different earthworms species decrease with increasing mechanical resistance or bulk density, which are common variables to assess soil compaction (Capowiez et al., 2021). Earthworm burrowing rates as a function of mechanical resistance show a steady decrease in burrowing rates as mechanical resistance increases (Paper I). This decrease in burrowing rates was seen for both species studied (*A. caliginosa* and *A. longa*) (Fig 6). For *A. caliginosa*, the average burrowing rate per day (cm d^{-1}) showed a reduction of around 57% between the lowest (117kPa) and highest (1068 kPa) mechanical resistance. The average burrowing rate by *A. longa* also showed significant reductions of 45% between these lowest and highest mechanical resistance values.

Our data also indicate that the effect of increasing mechanical resistance varies among the species, and hence it indicated that this impact is species-dependent. *A. caliginosa* and *A. longa* belong to different ecological groups of earthworms, which could explain the differences in the magnitude of impact. While the endogeic worms (*e.g. A. caliginosa*) tend to burrow and feed primarily in mineral soil, epi-anecic worms (*e.g. A. longa*) create a single burrow where they live and feed litter from the soil surface (Lee, 1985). In addition, the species show differences in physiology in terms of their muscle system. Earthworms have a hydrostatic skeleton and a muscular system with longitudinal and circular muscles (Lee, 1985). Keudel & Schrader (1999), showed that earthworm species generate different radial or axial stresses, which are related to the type of muscle that contracts in the movement. Also, they indicated that depending on the burrowing mode (*e.g.* cavity expansion or ingestion) a specific stress is more relevant, for instance, radial stresses are more important for cavity expansion. *A. caliginosa* exerts higher radial stresses than *A. longa* (Keudel & Schrader, 1999; Ruiz & Or, 2018), which may explain why the impact of soil compaction differed between these species. The difference in radial stress perhaps confers a biological advantage for *A. caliginosa* earthworms over *A. longa* in terms of higher activity in the soil, as they burrow more in the soil (Ruiz & Or, 2018).

6.2 Mechanical resistance changes earthworm burrowing modes

In compacted soils, earthworms cast more and ingestion becomes the main burrowing mode in these conditions (Dexter, 1978; Kemper et al., 1988; Beylich et al., 2010). The results obtained in this thesis support both statements, as the ingestion fraction increased with increasing mechanical resistance, as well as cast production per burrow length (g m^{-1}) (Paper I). However, as found for burrowing rates, the magnitude of the effect differed between *A. caliginosa* and *A. longa*. Whereas *A. caliginosa* shows an evident shift from cavity expansion to ingestion at mechanical resistance somewhere between 579 kPa and 949 kPa, for *A. longa* the ingestion factor was never less than 0.4 (Table 2). Considering their differences in radial stresses and the ingestion fraction values obtained, it was possible to conclude that *A. caliginosa* moves by cavity expansion, at lower resistance levels, and seems to be forced to shift to ingestion when the soil compaction levels increase. Therefore, it seems that cavity expansion is their preferred burrowing mode when soil compactions levels are not extremely high. By contrast, for *A. longa* ingestion seems to be the predominant burrowing mode independent of how soil compaction levels fluctuate, this could be related to their feeding and ecological traits, as they burrow to seek shelter and not for food. This implies that some species adapt their burrowing modes when compaction levels are too high, while others are not affected by it. This translates into the differences in the magnitude of the impact of mechanical resistance on earthworm burrowing activity (burrowing rates, burrow length or volume and cast production).

6.3 Energy costs for burrowing and cast production are influenced by compaction levels

Measuring respiration rates or heat dissipation provides information on the metabolism of organisms. Earthworms are highly sensitive to changes in temperature and soil moisture (Gerard, 1967; Wever et al., 2001). There is much evidence to show that earthworm respiration rate (metabolic rate) fluctuates during the year and varies between species, which could be related to differences in burrowing activity (Phillipson & Bolton, 1976). Our initial calculations of energy costs for burrowing (Paper I), showed that for *A. caliginosa* there was an increase in their daily energy costs (kJ d^{-1}) as mechanical resistance increased. In contrast, *A. longa* did not show any influence due to higher mechanical resistance levels. The results in Paper II showed a similar trend as Paper I. In these experiments (Paper II) both species had higher respiration rates (mg CO_2 g^{-1} fw d^{-1}) and energy costs (J g^{-1} fw d^{-1}) at higher compaction levels, which suggests an increase in the metabolic rates by earthworms.

In the same experiments (Paper II), the respiration rates and energy costs for burrowing (Table 3) of *A. caliginosa* and *A. tuberculata*, suggest that at higher compaction levels energy costs associated with burrowing increase. In addition, the energy costs per burrow volume and cast volume (Fig. 7) also indicate a similar tendency. Here, the energy costs to create a volume of burrow or cast increased around two-fold for both species after soil compaction. What this suggests is that earthworms are spending more energy on burrowing and need more energy to produce cast in compacted soils. This information coupled with the increase in the ingestion factor under high mechanical resistance or bulk density, makes us wonder about the possible implications on earthworm energy costs. Considering that earthworms are forced to ingest soil to burrow in compacted soils, this behaviour could involve a higher input of energy towards processing the soil rather than other metabolic processes of the organism. Thus this might affect the overall organisms' energy requirements. Moreover, based on the results obtained in Paper I, it could be assumed that the reduction of burrowing rates and the changes in burrowing modes as a response to the increase in mechanical resistance implies an adaptation of earthworms' burrowing depending on soil compaction levels, which could also hint that the overall energy requirements for earthworms in compacted soils are different from loose soils.

There are possible consequences for bioturbation, and therefore for the soil ecosystem, if soil compaction alters the energy requirement for earthworm burrowing. For the studied species, *A. caliginosa* and *A. tuberculata* (both endogeic), feeding on soils is their principal food source. If compaction reduces their burrowing activity, this forces them to ingest soil (even with low-quality C content) to fulfil their energy requirements. But, simultaneously they are spending energy to find a high-quality food source, that involves more burrowing. This continuous cycle of spending and processing energy in compacted soils, with low burrowing rates, might become detrimental to the earthworms' energy budget. This means that the energy allocation from food could go more to maintenance purposes rather than reproduction, growth or energy reserves (based on the energy budget model for adult earthworms in (Johnston et al., 2014)). As this energy budget depends on environmental conditions and the organisms' specific conditions (Nisbet et al., 2000; Johnston et al., 2014), upscaling the effect of compaction on a community level it is possible to speculate that organisms will have to compete for resources to compensate for the adverse impacts of compaction, which not only hampers the activity but also might hamper their access to food. As a result, there could be probability to see a reduction in earthworms' abundance, and in consequence, bioturbation and soil processes driven by earthworm activity could be significantly affected.

6.4 Earthworm burrowing appears to be a function of the interaction effects between soil moisture levels and compaction levels

The semi-continuous monitoring of earthworm burrowing under semi-field conditions (Paper III) provided valuable information about the temporal dynamics of earthworm burrowing activity. Analysis of the results in all the treatments provided a better understanding of changes in earthworm burrowing depending on different (initial) soil conditions and revealed some differences between the treatments.

The results show that earthworm burrowing rates decreased with time (Fig. 10A), and were significantly affected by the interaction of soil compaction and soil moisture level. During the first week of the experiment, differences in burrowing rates could be associated with the different initial conditions of soil moisture and compaction levels. The period during weeks 1 and 8 revealed differences in burrowing rates between treatments, where the burrowing rates in moist treatments (i.e. moist-loose and moist-compacted) plummeted (Fig. 10A). Finally, during weeks 8 and 24, burrowing rates in moist-loose treatment slightly increased, while they slightly decreased in the moist-compacted treatment. Earthworm burrowing rates were rather constant during the whole experiment in the dry-loose treatment. The burrowing rates for the dry-compact treatment were initially very low and then increased until the end of the experiment (Fig. 10A). Across all treatments, burrowing rates were between 1 to 4 cm^3 d^{-1} in our study, except for higher rates in the first week of the experiments where earthworms probably searched for shelter in the soil. Compared to the burrowing rates of adults of *Allolobophora rosea* used in a study by Bolton & Phillipson (1976), which were around 0.27 to 0.45 cm^3 d^{-1} at different temperatures (4 to 14 °C), burrowing rates in my experiment were higher, but *A. rosea* is much smaller than the species used in my experiment (0.225 g vs. 0.8 g (average body mass of *A. caliginosa* and *A. tuberculata*)) and the results include the burrowing of two individuals. The mean burrowing rates indicate that the macroporosity development (Results Paper III) in compacted soils was affected more when soils were moist, which might hint that soil compaction has a stronger effect in moist soils, however, this needs further analysis. While in loose soils, it seems that the interaction effect between compaction level and moisture might not be that significant, based on the results obtained.

The casting rates (Fig. 11) followed similar temporal trends as the burrowing rates. This can be explained by the rather high ingestion factor (Fig. 10B), which shows that soil ingestion was the main burrowing mode throughout the experiment. Thus, the increase in burrow volume was associated with an increase in cast volume. The differences between the mean casting rates during week one, and the casting rates of the subsequent time measured (between weeks one and eight) showed contrasting responses. In the dry treatments, casting rates increased, while for the moist treatments, casting rates decreased. In the moist-loose treatment, the reduction in casting rate was connected to a reduction in the ingestion factor, meaning that some burrows were created by cavity expansion rather than soil ingestion. Casting rates in the moist-compact treatment consistently decreased after week one until the end of the experiment. Low cast production in soils that are compacted and wet has been observed before in *Aporrectodea longa* worms where cast production plummeted in soils with a matric potential of -7 kPa and compaction of 350 kPa (dry bulk density: 1490 kg m^{-3}) (Kretzschmar, 1991). Contrastingly, the dry-compact treatment casting rates increased throughout the duration of the experiment. The mechanisms behind this behaviour are not clear, however, the obtained results seem to concur with a similar trend found by Kretzschmar (1991) which showed that the cast production at a matric potential of -40 kPa was higher at 350 kPa compaction than at 200 kPa (dry bulk density: 1410 to 1300 kg m^{-3}). The final mean casting rates obtained in the experiment were between 1.3 cm^3 d^{-1} and 3.3 cm^3 d^{-1}. This indicates that the soil turnover driven by earthworms (soil passing through the gut) might change depending on the soil conditions. For instance, the columns used and the mean casting rates obtained showed that in moist soils the soil turnover might take longer when compaction levels are higher (ca. ±3600 days) compared to lower compaction levels (ca.± 2000 days). The earthworm density in the columns, 2 individuals (ind.) per 0.027 m^2 corresponds to 74 ind. per m^2. Torppa & Taylor (2022) found earthworm densities for endogeic worms around 70 ind. per m^2 in fields using diverse crop rotations and different tillage systems (conventional, reduced or no tillage) in Uppsala, therefore, the earthworm density used in the columns was representative with the ones found in arable fields.

Finally, the ingestion factor did not show effects of any of the parameters (soil moisture levels and compaction levels) but it did increase with time (Fig 10B). This trend points out that soil ingestion seemed to be the preferred burrowing mode during the autumn season, and this could be to increase their energy reserves for the winter season, as many of the earthworms enter an obligatory diapause (Edwards & Arancon, 2022). In dry treatments, the ingestion factor was slightly lower than in moist treatments. Some authors have found that soil ingestion is a function of moisture levels, with less ingestion in very dry soils and more ingestion in wetter soils (Scheu, 1987; Hindell et al., 1994; Perreault & Whalen, 2006b), which agrees with the observations from my experiment during six months.

The analysis of the temporal dynamics of burrowing activity in this experiment provided information on the changes in burrowing rates, casting rates and ingestion factor of earthworms during 24 weeks. While many of the results were not necessarily conclusive, they provide hints that the earthworm burrowing activity is a function of the interaction effects of soil moisture and soil compaction. The results from the experiment called for a need to do more analysis of the temporal dynamics of earthworm burrowing in (semi-)field conditions, to be able to get more definitive results.

6.5 Methods for quantifying earthworm burrowing

In this thesis, different methods were used to quantify earthworm burrowing. Isothermal calorimetry was used to estimate the energy costs of earthworms, and as mentioned previously (Section 3.5) this instrument had not been used in earthworm ecology. One of the benefits of isothermal calorimetry is that it measures the heat dissipation of all metabolic processes occurring in the samples, which includes aerobic and anaerobic processes of the organisms or soil. Based on our results, the instrument used (TAM Air) was suitable to quantify the heat dissipation from earthworms burrowing with a very high resolution (time and precision) and sensitivity. The results obtained are comparable to the ones from the respirometer (See Paper II).

Another advantage of this instrument is the possibility to use it in a wide range of temperatures (*e.g.* 5 -90 °C), which will allow us to test earthworm activity within the temperature range where these organisms are active, between 5 and 20°C, depending on the species. One of the limitations of isothermal calorimetry is the size of the jar (*e.g.* 125 ml) constricting the burrowing activity of the earthworm. Also, it limits the use of earthworm species to a small size range, for instance, large earthworms (*e.g.* *L. terrestris*, *A. longa*) would be too restricted in their burrowing activity and therefore not all relevant ecological groups could be tested, leaving away many of the species with an ecological relevance. Finally, the time of the experiments cannot last too long, as the jars are hermetically sealed, limiting the oxygen supply.

Also the use of the X-ray CT analyses to quantify the below-ground cast production and the temporal dynamics of earthworm burrowing. This is a non-destructive methodology used widely in earthworm ecology to quantify earthworm burrow morphology and burrowing activity (Bottinelli et al. 2015). X-ray CT was very useful to monitor the temporal earthworm burrowing dynamics without destroying the soil columns. For instance, it was possible to quantify the below-ground cast production after each scan, which is otherwise difficult unless the samples are destroyed. However, the method has also its drawbacks. The X-ray scanner is not a common or easily accessible device, and not many institutions have one or have access to one. In addition, the costs of using the instrument are high. The transport of the samples from the field to the scanner takes time and becomes problematic as samples can be disturbed. In our case, the field was close to the campus, so the transport of the soil columns was easy, however, each column took approximately 30 minutes to scan, and depending on the number of columns the overall time to scan the samples could take several days. Finally, depending on the sample size the resolution of the images varies, the bigger the sample the lower the image resolution. In our case, the scanner malfunctioned during the COVID pandemic, which restricted access to technical support and in consequence delayed the measurements.

7. Conclusions and future perspectives

7.1 Conclusions

The temporal dynamics of earthworm burrowing activity as a function of soil conditions are not well known, in particular for soil compaction. This thesis reduced this knowledge gap by analysing the effects of soil compaction on earthworm burrowing, quantifying burrowing rates and identifying changes in earthworm burrowing modes (Paper I) and the energy costs of burrowing as a function of compaction (Paper II). Changes in earthworm burrowing activity, burrow creation and cast production under semi-field conditions were assessed over a prolonged period, with different initial soil moisture and compaction level conditions in different treatments (Paper III).

In Paper I, the reduction of burrowing rates and the burrow length under different compaction levels were quantified. In addition, it was found that earthworms change their burrowing modes, from a displacement of soils to ingestion, when they are exposed to higher compaction levels, information that was previously claimed but never quantified. In the same study, it was also established that earthworms' response (*A. caliginosa* (endogeic) vs. *A. longa* (epi-anecic)) to compaction is not the same for all species. The findings from Paper I, lead to questions regarding the effects of energy costs for burrowing on earthworms. Our initial estimates suggested an increase in energy costs due to higher compaction.

In Paper II, the initial assumption from Paper I, where energy costs for burrowing increased at higher compaction levels, was confirmed. Measurements of respiration rates and heat dissipation suggest that for both studied species, energy costs for burrowing increase with compaction, and energy costs to create a unit of burrow volume or a unit of cast are higher in compacted soil. This results in an evident impact on the energy budgets of the organisms, which could cause a reallocation of resources to prioritize burrowing instead of other expenses such as reproduction or energy reserves (from an adult earthworm perspective). While there was no strong statistical evidence of differences between species, a trend of an increase in energy costs for burrowing was seen in the endogeic earthworm, *A. caliginosa*, and it is possible to presume that in this ecological group soil compaction might have stronger negative impacts on their energy costs. However, further analysis should be done to confirm if these trends are indeed relevant, particularly for endogeic earthworms which feed mainly on soils. The study confirmed the suitability of the isothermal calorimeter as a reliable methodology to quantify metabolic rates (heat dissipation) of earthworm activity. This is a promising approach, as it provides a higher and more dynamic resolution of data, which includes respiration and other metabolic processes of the organisms, compared with other methodologies.

Finally, Paper III, allowed us to better understand how the temporal dynamics of earthworm burrowing change during an extended period in semi-field conditions. It was possible to identify that the main burrowing mode for earthworms during autumn was soil ingestion. That in moist-compacted soils the earthworm burrowing rates and casting rates reduce significantly compared to the other initial soil conditions. There is an apparent recovery in dry compacted soils, which needs further analysis. In general, the results indicated that earthworm burrowing activity is a function of the interaction effects between soil moisture levels and soil compaction levels.

7.2 Future perspectives

Based on the results of this thesis, I would like to postulate some general and more specific future perspectives. First, there is a need to focus on the continuous feedback between the abiotic factors, which are a function of weather conditions and might be affected by plant growth and soil management, soil structure and earthworm burrowing (Fig. 12). This analysis will allow to better understand the effects of anthropogenic activity (land use, soil management) and climatic conditions on earthworms and how they respond. Thus, it would provide more information about the changes in soil processes driven by their activity and how these change over time. Moreover, it will be possible to assess how the earthworms can modify their local conditions in the long term.

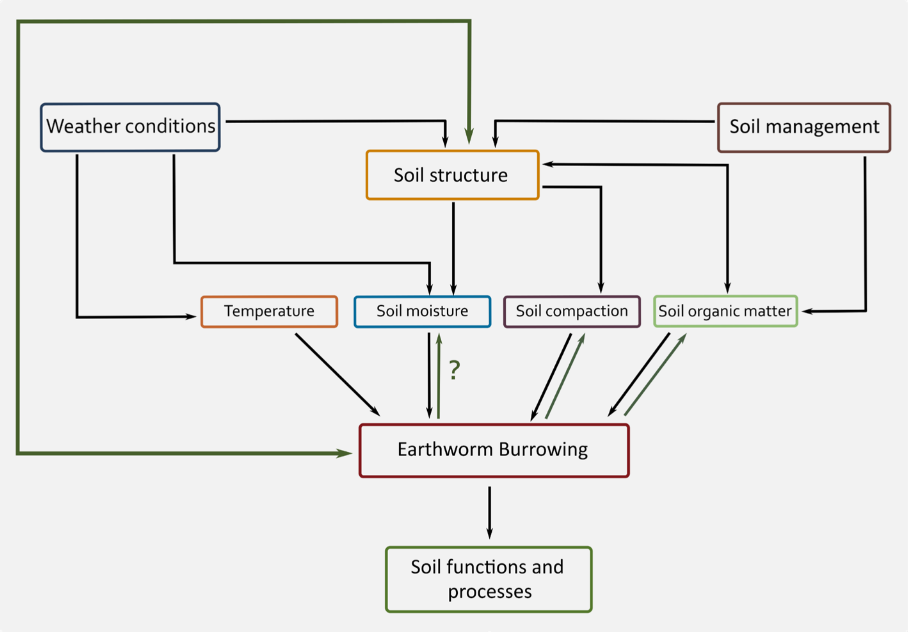


Figure 12. Feedback between weather conditions, soil structure and earthworm burrowing

In addition, to better understand the earthworm burrowing dynamics as a function of different biotic or abiotic factors, there is a need to analyse these dynamics from a species-dependent perspective, even from an ecological group-depending perspective, as analysing all existing species might be more challenging to achieve. The results from this thesis indicate that different earthworm species, even from similar ecological groups, are not responding the same way to external stressors. Nowadays there might be an underestimation of the effects of some soil conditions such as compaction, on the burrowing behaviour of relevant earthworm species, which in turn will also affect the accuracy of the information concerning soil functions or ecosystem dynamics.

There is a need for further studies that quantify the energy budgets of earthworms, as there is limited information. These quantifications could be done as a function of different environmental parameters. The energy quantification of burrowing has a key role in the understanding of earthworm burrowing dynamics and its possible implications on different soil processes and trophic interactions. Under the current scenario of climate change, it would be necessary to particularly focus on temperature and moisture regimes, to better understand their effects on earthworm energy budgets. Moreover, there is potential to use new techniques like isothermal calorimetry which provides data with high temporal resolution and, hence gives more information about burrowing dynamics (*e.g.* diurnal patterns). This will allow us a better understanding of the response of earthworms to stressors.

While there are plenty of studies done in the laboratory, under controlled conditions, it is necessary to refocus the experimental conditions done in earthworm research. I suggest that earthworm studies should be done more under semi-field conditions, where parameters like moisture and temperature are the natural weather conditions. This will allow us to have more realistic data on how earthworms behave in natural ecosystems. Also, these experiments need more frequent monitoring, because earthworm burrowing is continuously changing, perhaps a repetitive use of X-ray imaging analysis or an increase in the usage of DNA/RNA extraction techniques. DNA extraction techniques are useful to assess the presence of organisms in soils from family to species, (this is widely used in soil mycology) and could give an idea of the relative proportion of possible groups found in the samples. RNA techniques might be useful to identify the enzymes that are used by earthworms in certain soil processes. Both X-ray imaging and soil DNA/RNA extraction could allow us to monitor burrowing activity more frequently and with less disturbance.

The importance of improving the current information can be useful in the improvement of soil structure models or nutrient and carbon cycling models that currently do not have enough information about earthworm burrowing dynamics. In addition, increasing the knowledge of earthworm burrowing dynamics will allow us to better understand the impacts on soil structure, and feedback with soil functions, soil ecosystem functions and services.

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Popular science summary

Earthworms are important soil organisms that play a key role in different soil processes. They are referred to as ecosystem engineers due to their ability to modify soil structure and soil conditions. Earthworms have traditionally been grouped into three main ecological groups: epigeics, endogeics and anecics. Earthworms are highly sensitive to changes in soil environmental conditions where soil moisture and temperature are the principal drivers of their activity. However, soil organic carbon, pH, soil texture and soil compaction may also strongly influence their activity.

Soil compaction is a major threat to soil structure (i.e. the spatial arrangement of soil pores and particles) and functioning, and negatively affects soil organisms like earthworms. While there is a lot of information about the changes in earthworm burrowing activity as a function of soil moisture levels and temperature, little quantitative data are available on how earthworms are affected by soil compaction. Based on observations, it is widely accepted that an increase in soil compaction negatively affects earthworm burrowing. Nevertheless, quantitative information of the impacts of soil compaction on burrowing activity, burrowing rates and energy requirements for burrowing remain limited.

In this thesis, I measured earthworm burrowing as a function of soil compaction, considering also the probable different responses among earthworm species. In paper I, the burrowing rates were quantified at different compaction levels in two-dimensional terraria. The results showed that burrowing rates were reduced with increasing compaction. By using two earthworm species from different ecological groups, it was identified that earthworm species responded differently to increases in soil compaction.

In the second paper, I measured the energy costs for earthworm burrowing as a function of soil compaction. The results showed that the energy costs increased with soil compaction levels. In addition, the results suggested that the increase in the energy costs for burrowing are different between earthworm species. Finally, I was able to test a new methodology, i.e. isothermal calorimetry, which provided high-resolution data on energy costs for earthworm burrowing. The study demonstrated the potential of calorimetry in earthworm research.

Finally, in Paper III, I focused on the temporal dynamics of burrowing activity. The results showed that earthworm burrowing activity changed as a function of the interaction effects between soil moisture levels and compaction levels. In particular, it was found that high soil moisture and high compaction levels seem to be detrimental to earthworm burrowing. However, there is a need for further analysis to understand the complexity of these interactions.

This thesis provides a better understanding of how burrowing by earthworms is affected by soil environmental conditions, in particular soil compaction. I used customised experimental approaches and new methodologies such as time-lapse imaging, isothermal calorimetry and repeated X-ray imaging, and provided quantitative data on burrowing rates and energy costs for burrowing. I used different earthworm species and could show that the response to soil environmental conditions is species-dependent.

Populärvetenskaplig sammanfattning

Daggmaskar är viktiga markorganismer som spelar en nyckelroll i olika markprocesser. De kallas ekosystemingenjörer på grund av sin förmåga att förändra markstrukturen och markförhållanden. Daggmaskar har traditionellt delats in i tre ekologiska huvudgrupper: de som i huvudsak lever i markens förnaskikt (epigeics), de som lever i matjorden och gräver i alla riktningar (endogeics) och de som skapar mer permanenta vertikala gångar (anecics). Daggmaskar är mycket känsliga för förändringar i markens förhållanden där markfuktighet och temperatur är de främsta drivkrafterna för deras aktivitet. Halten av organiskt kol i marken, pH, markstruktur och markpackning kan dock också starkt påverka deras aktivitet.

Ur ett jordbruksperspektiv är markpackning ett stort hot mot markstrukturen (dvs. det rumsliga arrangemanget av markporer och partiklar) och markens funktion och påverkar markorganismer såsom daggmaskar negativt. Medan det finns mycket information om hur daggmaskar påverkas av markfuktighet och temperatur, finns lite kvantitativa data tillgängliga om hur daggmaskar påverkas av markpackning. I litteraturen kan vi hitta information om förändringar i daggmaskarnas grävningsaktivitet som orsakats av markfuktighet och temperatur, men kunskap om hur markpackning påverkar daggmaskarnas grävning är knapphändig. Vissa studier anger att en ökad grad av markpackning påverkar grävningsaktiviteten negativt men kvantifieringen av markpackningens effekter på grävningsaktiviteten och hur mycket energi de behöver för att gräva har varit begränsad.

I den här avhandlingen bestämde jag mig för att belysa olika frågor med fokus på daggmaskars grävningsaktivitet och hur de påverkas av olika grader av markpackning. Detta gjordes för olika daggmaskarter. Avhandlingen bygger på tre artiklar där den första fokuserade på kvantifieringen av olika daggmasksarters grävhastighet till följd av markpackning. Jag kunde identifiera att daggmaskarnas grävhastighet berodde på graden av markpackning. Genom att använda två arter från olika ekologiska grupper var det också möjligt att dra slutsatsen att markpackningens effekter varierade mellan olika arter.

I den andra artikeln, som byggde vidare på resultaten från den första artikeln, var hypotesen att markpackning förändrar daggmaskarnas energiåtgång vid grävning. Resultaten visade att energiåtgången vid grävning ökar med graden av markpackning, vilket indikerar en påverkan på daggmaskarnas energibehov. Dessutom antydde resultaten också att ökningen av energibehovet varierar för olika daggmaskarter. Jag kunde också testa en ny metod (isotermisk kalorimetri) som gav högupplösta data om daggmaskars energiåtgång vid grävning samt visade metodens potentiella användning i daggmaskforskning.

Slutligen, i den tredje artikeln, fokuserade jag på den tidsmässiga dynamiken i daggmaskars grävningsaktivitet genom att exponera daggmaskar från två arter för olika markförhållanden och övervaka deras aktivitet mer frekvent under naturliga väderförhållanden i sex månader. Jag kunde identifiera att daggmaskars grävning förändrades på grund av interaktionerna mellan markfuktighet och markpackning. Det verkar också som att väderförhållandena spelar en viktig roll. I synnerhet verkar kombinationen av höga fuktnivåer och höga packningsnivåer vara mer skadliga för daggmaskens grävning. Vi behöver dock ytterligare analyser för att bättre förstå dessa komplexa interaktioner.

Denna avhandling bidrog med kunskap som ger en bättre förståelse för daggmaskars grävningsaktivitet, samt dess dynamik till följd av markförhållanden. Avhandlingen undersökte framför allt hur markpackning påverkar daggmaskarnas grävningsaktivitet. Jag använde skräddarsydda experimentella metoder och nya metoder som till exempel isotermisk kalorimetri, och kvantifierade grävningshastigheter och energikostnader för grävning. Jag använde olika daggmaskarter och kunde visa att responsen på markens miljöförhållanden är artberoende.

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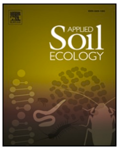

Earthworm burrowing modes and rates depend on earthworm species and soil mechanical resistance

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ABSTRACT

Earthworms drive multiple soil processes, but their specific impact on soil functions differs between earthworm species and ecological categories. A key challenge in modern agriculture is soil compaction due to heavy machinery, but we have limited quantitative knowledge about how the burrowing activity of different earthworm species is affected by compaction. Here, we address this question in a laboratory experiment with 2-D terraria, where we used *Aporrectodea caliginosa* (Savigny, 1826) and *Aporrectodea longa* (Ude, 1885) as representatives of two different ecological categories. We exposed both species to four different soil mechanical resistance levels and monitored their burrowing activity for three days. We quantified burrowing rates and cast production, assessed the burrowing mode, and estimated energy requirements as a function of soil mechanical resistance. The results showed that the burrowing rates of both earthworm species significantly decreased with increasing soil mechanical resistance, but that the impact was species-dependent and lower for *A. longa*. Earthworms changed their burrowing mode towards ingestion when soil mechanical resistance increased, and this shift was more prominent for *A. caliginosa* that primarily burrowed via cavity expansion (i.e. by pushing soil aside) at low soil mechanical resistance. We further show that energy requirement and cast produced per unit burrow length increased with soil mechanical resistance. Our study revealed significant and species-dependent adverse effects of soil mechanical resistance on earthworm burrowing, which in turn has consequences for many soil processes mediated by earthworms, such as water infiltration, soil aeration, nutrient cycling and soil organic matter turnover.

1. Introduction

Earthworm burrowing impacts soil drainage and aeration, soil structure stability, and the resulting macropore networks provide habitat for smaller soil organisms (Bolton and Phillipson, 1976; Francis et al., 2001). Burrowing behaviour is highly influenced by a range of soil properties and soil conditions, including soil bulk density. An increase in soil bulk density due to e.g. soil compaction has been shown to decrease earthworm abundance (Pižl, 1992; Crittenden et al., 2014), and reduce burrowing activity (Rushton, 1986; Capowiez et al., 2021) and cast production (Kretzschmar, 1991). Compaction generally leads to higher soil mechanical resistance, which increases the energy requirement of earthworms for burrowing (Ruiz et al., 2015) and reduces burrow lengths (Söchtig and Larink, 1992; Stovold et al., 2004). It has been found that compaction adversely affects earthworm activity, as well as soil functions and processes that are directly or indirectly mediated by earthworms (Beylich et al., 2010).

Earthworm species differ in their burrowing activity, and therefore, burrow characteristics and systems are also earthworm specific (Francis et al., 2001). Bastardie et al. (2005) discerned two classes of burrow systems with different morphological characteristics (e.g. connectivity, diameter, continuity and length), which were associated with different earthworm ecological categories or species. Using of this information, it is plausible that changes in soil conditions – e.g. soil mechanical resistance – impact different species differently. Thus, the effects on different soil processes vary, because specific soil processes are closely related to

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earthworm species or specific ecological groups, such as preferential flow through vertical macropores created by anecic species or aggregate production mediated by endogeic earthworms.

When earthworms move through the soil in their search for food or shelter, they can burrow either by ingesting (and egesting) soil, cavity expansion (i.e. pushing the soil aside), or by using and expanding existing cracks (Lee, 1985). It has been assumed that the preference for a certain burrowing mode (ingestion, cavity expansion or crack propagation) varies as a function of soil conditions. It is claimed that in compacted soil, earthworms shift their burrowing mode from cavity expansion to ingestion (Dexter, 1978). Nevertheless, it remains largely unclear if and how the burrowing mode changes depending on soil mechanical conditions. The burrowing mode likely impacts earthworm burrowing rates, as earthworms might burrow faster or slower depending on how they move through the soil. Moreover, we may expect that different burrowing modes require different amounts of energy (Beylich et al., 2010). Changes in earthworm energy requirements would not only affect burrowing rates, but also earthworm growth rates and population dynamics.

The importance of earthworm bioturbation for many soil processes is well recognized, nevertheless, there is a lack of information regarding how burrows are created (Capowiez et al., 2001). Understanding earthworm burrowing as a function of soil conditions is crucial to gain knowledge of how much soil is “processed” by earthworms under different soil conditions, to establish quantitative links between earthworm activity, available energy resources (i.e. soil organic carbon) and soil processes mediated by earthworms. Considering that soil compaction is a major threat to different soil functions, there is a need to assess how earthworm behaviour is affected by compaction at species level, and to quantify how compaction affects species-specific burrowing rates. Moreover, identifying how burrowing modes and energy requirements of earthworms vary with soil mechanical resistance can provide a better understanding of the temporal dynamics of earthworm burrowing.

The main objective of the present study was to quantify earthworm

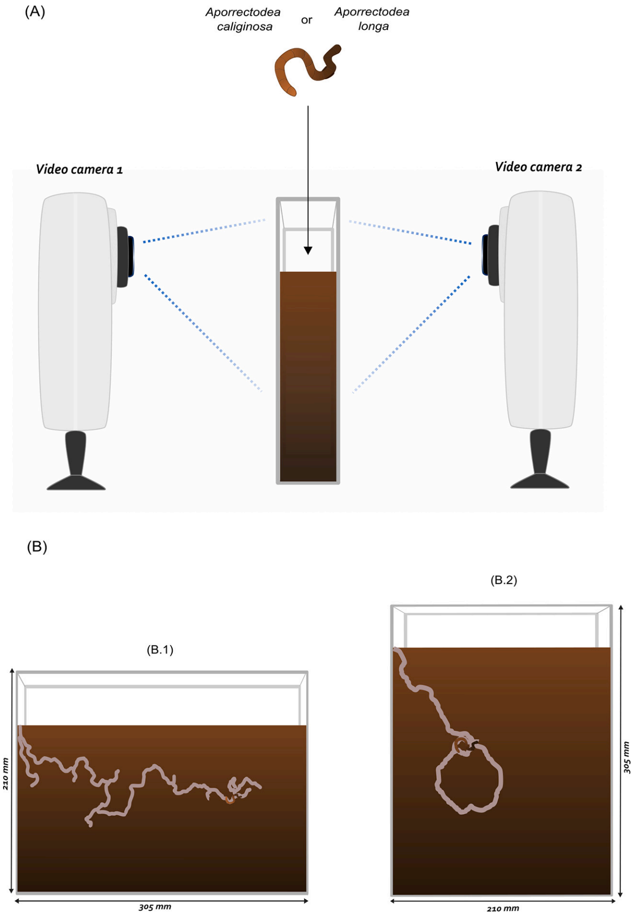

Fig. 1. (A) Lateral view of the experimental set-up. (B) Camera view of the experimental set-up, (B.1) for *A. caliginosa* and (B.2) for *A. longa*. The experimental set-up was located inside a growth chamber. Inside the growth chamber, where there was no light, a constant temperature (15 °C) and constant air humidity (60 %). Note that the figures are not drawn to scale.

burrowing rates as a function of soil mechanical resistance for an endogeic (*Aporrectodea caliginosa* (Savigny, 1826)) and an epi-anecic (*Aporrectodea longa* (Ude, 1885)) earthworm species, using a laboratory 2-D terrarium. Moreover, we determined the earthworm burrowing mode (ingestion versus cavity expansion) and cast production at different levels of soil mechanical resistance, and estimated the energy requirement for burrowing as influenced by soil mechanical resistance. We hypothesized that burrowing rates decrease and energy requirements increase with increasing soil mechanical resistance, that the burrowing mode is dependent on soil mechanical resistance, and that the two earthworm species are differently affected by compaction.

2. Materials and methods

2.1. Experimental design

This study considered two earthworm species and monitored earthworm burrowing activity using a 2-D terrarium. To study the impact of soil mechanical resistance on earthworm burrowing, the soil in the terrarium was prepared to represent different soil bulk densities. Each combination of earthworm species and soil mechanical resistance level was replicated five times. In total, we performed 40 experimental cycles (two earthworm species × four soil mechanical resistance levels × five replicates).

For each cycle, one earthworm was placed on the surface of the terrarium, and earthworm activity was recorded for three days with two surveillance cameras (LUPUSNET HD - LE 936 PLUS) on each side of the terrarium (Fig. 1). The 2-D terrarium was placed inside a growth chamber (Model SED-41C8, Percival Scientific Inc.), and kept at a constant temperature of 15 °C and 60 % relative air humidity. Inside the chamber, there was no light, and no diurnal temperature cycles were programmed. To ensure similar day rhythms for the earthworms, experiments were always started at around the same time of day.

The 2-D terraria were made from transparent Plexiglas® and had inner dimensions of 210 mm × 305 mm × 3 mm for *A. caliginosa* and 305 mm × 210 mm × 5 mm for *A. longa*; i.e. the orientation of the box was changed for the epi-anecic *A. longa* as shown in Fig. 1 as they tend to burrow more vertically than horizontally. The difference in thickness of the boxes is due to the difference in diameter between the two earthworm species; however we ensured that the “relative thickness” of the box (i.e. the ratio of earthworm diameter to terrarium thickness) was similar for both earthworm species (similar to Perreault and Whalen, 2006).

The terraria were carefully filled with soil (silt clay loam, see Section 2.2), layer by layer (seven layers in total), to ensure homogeneous soil bulk density within the terrarium. The experiment involved four different soil bulk densities (1.1, 1.3, 1.5 and 1.7 g cm^{-3}). For each soil bulk density, we measured its corresponding soil mechanical resistance using a micro-penetrometer (cone apex angle 15°, velocity 5 mm min^{-1}) in a similar apparatus as described by Ruiz et al. (2015). The mean cone penetration resistance was 117, 579, 949 and 1068 kPa for the four compaction levels. Since earthworms experience mechanical resistance rather than bulk density when they burrow, we correlated earthworm burrowing to soil mechanical resistance in this study.

In total, we used five individuals of each earthworm species. Every individual was used at all four compaction levels, with the sequence of compaction levels randomly assigned. Earthworms were left to rest in optimum conditions for at least two weeks before they were used again.

2.2. Soil properties and soil conditions

The soil used was taken from the top 20 cm of an arable field near Uppsala, Sweden (59°49′58.4″N, 17°42′13.2″E). The topsoil texture is silty clay loam (39.8 % clay, 51.9 % silt, and 8.3 % sand). The soil organic carbon (SOC) content was 2.26 %, and the pH was 5.9 (measured in a 1:2 ratio of soil: deionized H_2O). Visible plant and root residues were removed before the soil was sieved using a 2 mm mesh, and then oven-dried for 24 h at 105 °C. For the experiments, the soil was moistened to a matric potential of −100 hPa. The amount of water required was calculated on the basis of soil water retention measurements carried out on soil cores placed on a tension plate using the same soil and the four considered bulk densities.

2.3. Earthworms

Experiments were carried out with two earthworm species: *A. caliginosa* and *A. longa*. Earthworms are traditionally classified into three main ecological categories (Bouché, 1977): epigeic, endogeic and anecic. However, earthworm species do not always fit into one category, and therefore, we followed the recent categorization developed by Bottinelli et al. (2020), where *A. caliginosa* is considered 80 % endogeic, 4 % anecic and 16 % epigeic, and *A. longa* is considered 68 % anecic and 32 % endogeic. The two earthworm species used in this study thus represent different ecological strategies: endogeic vs. epi-anecic. Both species are among the most common species in agricultural fields in Sweden (Boström, 1995; Lagerlöf et al., 2002) and Scandinavia (Sveistrup et al., 1997; Rasmussen, 1999). Globally, *A. caliginosa* is found mostly in temperate zones of Europe and North America, but there are also records in Australia, Asia (e.g. China, Japan, Russia) and South America (e.g. Argentina, Chile) (GBIF Secretariat, 2021a). *A. longa* is primarily found in temperate zones of Europe, with some records in the east of North America (GBIF Secretariat, 2021b).

Adult earthworms were taken from a laboratory population kept at the Department of Ecology, Swedish University of Agricultural Sciences. Before the experiments, all earthworms were stored in a growth chamber at 15 °C. In immediate preparation for the experiments, earthworms were kept in Petri dishes with moist filter paper for 48 h to empty their guts, and then their body weights were recorded. The initial average body weight of *A. caliginosa* and *A. longa* was 0.51 g (standard deviation, SD = 0.15 g) and 1.93 g (SD = 0.41 g), respectively. The width on average was of 2.5 mm (*A. caliginosa*) and 4 mm (*A. longa*), while the length was 50 mm (*A. caliginosa*) and 120 mm (*A. longa*). The earthworms were placed inside the 2-D terraria and left to burrow for 69 h. After the experiments, the earthworms' guts were emptied again, and their final body weight was recorded.

2.4. Burrow metrics derived from image analysis

An image was extracted every 5 min from the original film, to create a time-lapse image sequence. The cushion effect (i.e. geometrical distortion) of the images was corrected using bUnwarpJ (Arganda-Carreras et al., 2006) in *ImageJ*. Tracking of earthworm movement was done using the “Manual tracking” plugin in *ImageJ*. For the tracking, x and y coordinates were obtained by following the movements of the earthworms prostomium in each image. The coordinates allowed us to quantify i) the burrow length, ii) the number of changes of direction of an earthworm (i.e. changing from forward to backwards moving, or vice versa), and iii) the total movement of the earthworms. The latter includes both burrowing (i.e. creation of new soil pore space) and movements in already existing burrows. Both burrowing and total movement were quantified by assuming that the earthworms moved along straight lines between coordinates on successive images. For the quantification of burrow length and total movement, the burrow network was recreated from the recorded coordinates, timestep by timestep. The straight line between the coordinates for consecutive time points was considered a new burrow if it ended outside of the existing network. If it ended inside the existing burrow, it was considered movement but not burrowing. The created line was then dilated to the approximate diameter of the earthworm. An earthworm was considered to have changed direction if the distance between the position of the prostomium at time points t and $t-1$ was larger than the distance between the positions at $t+1$ and $t-1$. These quantifications were done in R software (R Core

Team, 2018). Hourly averages were calculated for total earthworm movement, burrow length, and burrowing rate (i.e. increment in length divided by increment in time). Quantification of total burrow length was verified with manual measurements at the end of the experiments. The manual measurements were made with a ruler on the final images of the burrows.

2.5. Cast production

At the end of the experiments (i.e. after 69 h), all casts were collected from the surface and from within the burrows. Because the amount of cast for SOC analysis was not always enough for the 40 experimental cycles, we had to pool casts from some experiments, resulting in three replicates for SOC cast analysis. The samples were oven-dried and their weights were recorded. The SOC contents of the casts were measured by dry-combustion on a TruMac CN (LECO Corp.).

2.6. Estimation of burrowing mode

Based on cast production and burrow length, we estimated how much of the burrowing was done by ingestion and how much by cavity expansion. The volume of ingested soil ($V_{ingestion}$) was calculated as the product of the dry mass of all casts produced (m_{cast}) and the initial soil bulk density (ρ_{soil}). The total volume of the created burrows (V_{burrow}) was calculated from the burrow length (L_{burrow}) and the burrow radius (r_{burrow}) by assuming a cylindrical shape of the burrows. We define the "ingestion factor" ($f_{ingestion}$) as the fraction of ingested soil volume ($V_{ingestion}$) to total burrow volume (V_{burrow}):

$$f_{ingestion} = \frac{V_{ingestion}}{V_{burrow}} = \frac{m_{cast}}{\rho_{soil} \bullet L_{burrow} \bullet \pi \bullet (r_{burrow})^2} \tag{1}$$

An $f_{ingestion}$ of zero indicates that all burrows were produced by pushing the soil aside (i.e., burrowing by cavity expansion), while an $f_{ingestion}$ of one indicates that all burrows were created by ingestion.

2.7. Estimation of energy requirement

We estimated the energy requirement of earthworms from the amount (mass) of ingested soil (m_{cast}), the organic carbon content in the soil (i.e., before ingestion; SOC) and in the casts (i.e., after egestion; OC_{cast}), and by assuming an energy density (w) for soil organic carbon of 35 kJ per g of soil organic carbon (Bölscher et al., 2017). We assumed that SOC was initially uniformly distributed in the soil. The energy required by earthworms (E_{req}) is then given as:

$$E_{req} = E_{intake} - E_{cast} = m_{cast}(SOC - OC_{cast})w \tag{2}$$

2.8. Statistical analyses

The homoscedasticity and normality of the data were verified using Barlett and Shapiro-Wilk tests. The data, except the $f_{ingestion}$, were log-transformed due to variance heterogeneity and/or deviations from normality. Total burrow length, total earthworm movement, cast production, cast production per burrow length, $f_{ingestion}$, number of direction changes, energy requirement per day, and energy need per burrow length, were analysed with a two-way ANOVA with earthworm species and soil mechanical resistance as factors, to see if there was any interaction effect. In addition, analyses were also made separately for each species with a one-way ANOVA. For the analysis of the burrowing rate data, as it did not fulfil the homoscedasticity and normality assumptions even after log-transformation, a non-parametric Kruskal-Wallis test was performed with soil mechanical resistance and species as factors. Moreover, a Tukey test was done to make pairwise comparisons between soil mechanical resistance levels for total burrow length, total movement, number of direction changes, energy requirement per day, and energy need per burrow length. Linear regressions were used to analyse how burrowing properties changed as a function of soil mechanical resistance. All statistical calculations were performed using the R software (R Core Team, 2018), R stats package version 3.6.2. For the figures, the ggplot2 package version 3.3.3 was used.

3. Results

3.1. Characteristics of earthworm burrow systems

For *A. caliginosa*, the final burrow system after 69 h of burrowing displayed an intricate pattern with many dead-ends (Fig. 2). Visual inspection of the image sequences indicated this was due to an "exploratory behaviour" of *A. caliginosa*, meaning that the individuals "poked" the surrounding soil with the prostomium and sometimes moved backwards. In contrast, *A. longa* created a burrow system with one main burrow that did not have any dead-end branches. However, *A. longa* created more loops than *A. caliginosa*. Also, *A. longa* did not show the same degree of "exploratory behaviour" as *A. caliginosa*.

In general, *A. caliginosa* burrowed and also moved more than *A. longa*. However, this difference decreased with increasing soil mechanical resistance, and earthworm movement was similar for both species at the highest soil mechanical resistance level. The total length of created burrows was significantly different between earthworm species ($p < 0.01$, Two-way ANOVA test) and soil mechanical resistance levels ($p < 0.01$, Two-way ANOVA test), while the total earthworm movement was only significantly different between soil mechanical resistance ($p = 0.01$, Two-way ANOVA test) but not between earthworm species (Table 1). Note that not all earthworm movements resulted in the creation of a new burrow, and therefore, the total earthworm movement is always larger than the total burrow length. The total earthworm movement decreased significantly with increasing soil mechanical resistance for *A. caliginosa* ($p = 0.03$, Two-way ANOVA test), but not for *A. longa* ($p = 0.32$, Two-way ANOVA test). The number of direction changes decreased with increasing soil mechanical resistance (Table 1).

3.2. Burrowing rates

For both earthworm species, burrowing rates fell markedly after the first few hours of burrowing, and this trend was more pronounced at lower soil mechanical resistance levels (Fig. 3). At higher soil mechanical resistance levels, burrowing rates were more stable throughout the whole duration of the experiments (Fig. 3). Some fluctuations in burrowing rates were recorded throughout the experiments, but no diurnal cycles were observed.

Average burrowing rates differed significantly between earthworm species ($p < 0.01$, Kruskal-Wallis test). Burrowing rates of *A. caliginosa* were significantly higher than for *A. longa*, at all soil mechanical resistance levels ($p < 0.01$, Kruskal-Wallis test). For example, at the lowest soil mechanical resistance (117 kPa), the burrowing rate of *A. caliginosa* (13.5 cm d^{-1}) was more than twice as high as for *A. longa* (6.5 cm d^{-1}). Average burrowing rates significantly decreased with increasing soil mechanical resistance for both earthworm species ($p < 0.01$, Kruskal-Wallis test). Burrowing rates for *A. caliginosa* were more strongly reduced with increasing soil mechanical resistance than for *A. longa* (Fig. 3). The burrowing rates were reduced by 57 % and 45 % for *A. caliginosa* and *A. longa*, respectively, when the mechanical resistance increased from 117 kPa (lowest soil mechanical resistance) to 1068 kPa (highest soil mechanical resistance).

3.3. Cast production

Total cast production was different for the two earthworm species ($p < 0.01$, Two-way ANOVA test), but was not influenced by soil mechanical resistance ($p = 0.34$, Two-way ANOVA test; Fig. 4). On average, *A. longa* produced about twice as much casts per day than *A. caliginosa*, at all soil mechanical resistance levels. Although the amount of casts

A. longa

(epi-anecic)

A. caliginosa

(endogeic)

Fig. 2. Typical burrow systems for *A. longa* and *A. caliginosa* (size: 349 × 380 pixels).

Table 1
Mean values (SE) of total earthworm movement and total burrow length during 69 h for *A. caliginosa* and *A. longa* at four levels of soil mechanical resistance. Different letters within a row indicate significant differences between soil mechanical resistance levels ($p < 0.05$). $n = 5$ for all measurements.

		Soil mechanical resistance (kPa)			
		117	579	949	1068
A. caliginosa	Total movement (m)	1.78^{a} (0.12)	1.37^{ab} (0.20)	1.28^{ab} (0.19)	0.92^{b} (0.04)
	Burrow length (m)	0.45^{a} (0.04)	0.27^{ab} (0.05)	0.27^{ab} (0.05)	0.18^{b} (0.03)
	Number of direction changes	255^{a} (24)	224^{a} (37)	238^{a} (32)	163^{a} (9)
A. longa	Total movement (m)	1.42^{a} (0.24)	1.22^{a} (0.19)	0.97^{a} (0.24)	0.91^{a} (0.13)
	Burrow length (m)	0.24^{a} (0.05)	0.22^{a} (0.01)	0.13^{a} (0.03)	0.13^{a} (0.02)
	Number of direction changes	186^{a} (32)	126^{a} (27)	116^{a} (31)	103^{a} (11)

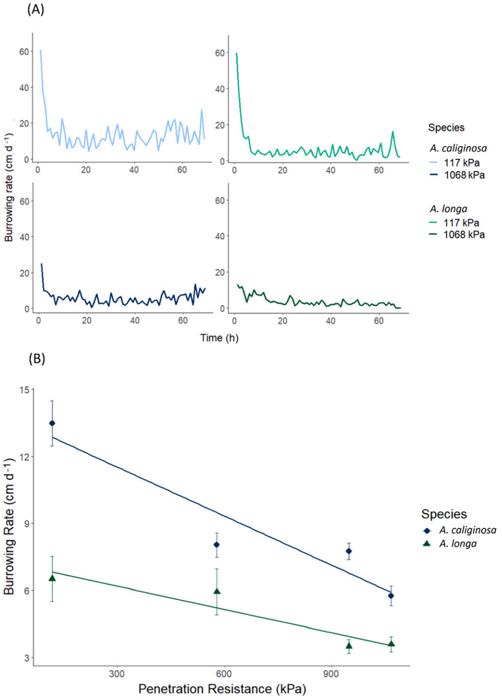

Fig. 3. (A) Burrowing rates as a function of time at two different levels of mechanical resistance for (left) *A. caliginosa* and (right) *A. longa*. (B) Average burrowing rate as a function of soil mechanical resistance (*A. caliginosa*: $R^2 = 0.89$, $p < 0.01$; *A. longa* = R^2: 0.89, $p < 0.01$). Error bars indicate standard error ($n = 5$).

produced was related to the length of the burrows created by the earthworms, we found that for both earthworm species, cast production per burrow length increased significantly ($p < 0.01$, Two-way ANOVA test) with soil mechanical resistance (Fig. 4). For both earthworm species, the cast produced per unit length of burrow doubled between the lowest and highest soil mechanical resistance level (Fig. 4).

3.4. Burrowing mode

In general, over the 69 h, burrowing by ingestion was more common for *A. longa* than for *A. caliginosa* (Fig. 5). For *A. caliginosa*, $f_{ingestion}$ (Eq. (1)) increased from 0.34 (about one-third of the burrows were created by ingestion and two-thirds by cavity expansion) at the lowest soil mechanical resistance (117 kPa) to 0.66 (two-thirds of the burrows were due to ingestion and one third due to cavity expansion) at the highest level of soil mechanical resistance (1068 kPa). For both earthworm species, there was a significant effect of soil mechanical resistance on the ingestion factor ($p < 0.01$, Two-way ANOVA test).

3.5. Estimates of energy requirement

The estimated energy requirement (Eq. (2)) per day revealed a

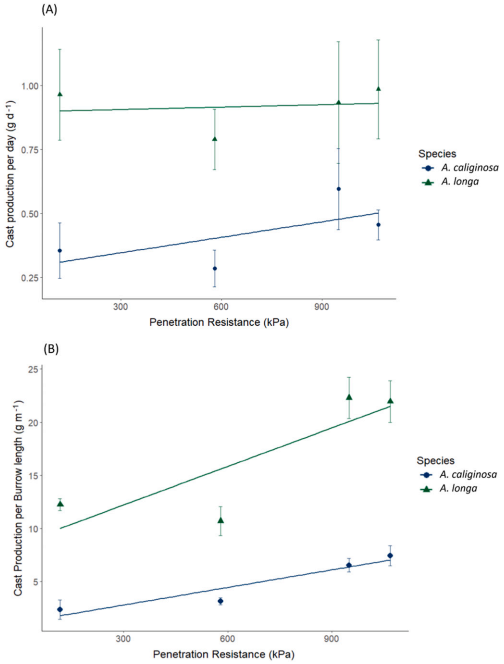

Fig. 4. (A) Cast production per day as a function of mechanical resistance (*A. caliginosa*: $R^2 = 0.89$, $p < 0.01$; *A. longa*: $R^2 = 0.89$, $p < 0.01$). (B) Cast production per metre of burrow as a function of soil mechanical resistance (*A. caliginosa*: $R^2 = 0.41$, $p = 0.22$; *A. longa*: $R^2 = 0.023$, $p = 0.92$). Error bars indicate standard error ($n = 5$).

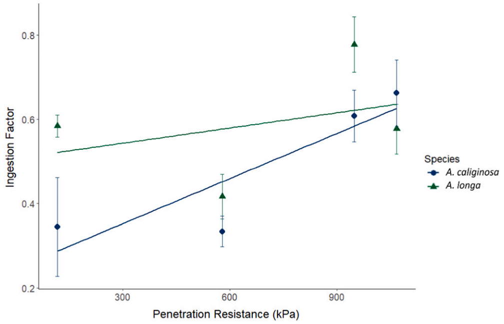

Fig. 5. Ingestion factor as a function of soil mechanical resistance (*A. caliginosa*: $R^2 = 0.78$, $p < 0.01$; *A. longa*: $R^2 = 0.12$, $p = 0.36$). Error bars indicate standard error ($n = 5$).

significant difference between earthworm species ($p < 0.01$, Two-way ANOVA test). On average, across all soil mechanical resistance levels, daily energy used by *A. longa* and *A. caliginosa* was 4.4×10^{-2} kJ d^{-1} (SD = 0.002) and 1.9×10^{-2} kJ d^{-1} (SD = 0.005), respectively. Our data show that the daily energy use increased by 10 % for *A. longa* and 80 % for *A. caliginosa* when soil mechanical resistance increased from 117 kPa to 949 kPa (Table 2). Energy demand at 1068 kPa was slightly lower than at 949 kPa for both earthworm species (Table 2). Also, there was no significant impact of soil mechanical resistance on energy requirement per day for either of the species (*A. caliginosa*: $p = 0.51$; *A. longa: p* = 0.97, Two-way ANOVA test).

Table 2
Mean values (SE) of energy requirement per day (kJ d^{-1}) for *A. caliginosa* and *A. longa* at four levels of mechanical resistance. Different letters within a row indicate significant differences between soil mechanical resistance levels ($p < 0.05$). n = 5 for all measurements.

	Soil mechanical resistance (kPa)			
	117	579	949	1068
A. caliginosa	0.015^a (0.015)	0.017^a (0.015)	0.027^a (0.009)	0.017^a (0.006)
A. longa	0.042^a (0.035)	0.045^a (0.029)	0.046^a (0.048)	0.042^a (0.032)

The energy used per burrow length for *A. longa* was four times higher (9.6×10^{-1} kJ m^{-1}) than for *A. caliginosa* (2.2×10^{-1} kJ m^{-1}). On average, the concentration of organic carbon of the casts was 2.12 % and 2.09 % for *A. caliginosa* and *A. longa*, respectively (the initial soil organic carbon concentration was 2.26 %; see Section 2.2). For both earthworm species, the energy requirement per metre burrow significantly increased with increasing soil mechanical resistance (*A. caliginosa: p* < 0.01; *A. longa*: $p = 0.02$, Two-way ANOVA test; Fig. 6).

4. Discussion

4.1. Burrowing behaviour differs between earthworm species

We observed a difference in burrowing behaviour between the two earthworm species. *A. caliginosa* showed a greater exploratory behaviour, i.e. many backwards and forward movements, maybe to find the most suitable spots for foraging. Being an earthworm species with primarily endogeic traits, the many direction changes probably reflect this search for better foraging spaces. However, this exploratory behaviour tended to decrease with increasing soil mechanical resistance, and under high soil mechanical resistance, we observed a significant reduction in the total movement for *A. caliginosa*. A balance between the energy costs and benefits of foraging exists (Stephens and Krebs, 1986), and therefore, the reduction in earthworm movement could mean that exploration for better foraging spots required more energy than could be afforded in soil with high mechanical resistance. *A. longa* showed fewer changes of directions compared with *A. caliginosa*, but a decrease in direction changes with increasing soil mechanical resistance was

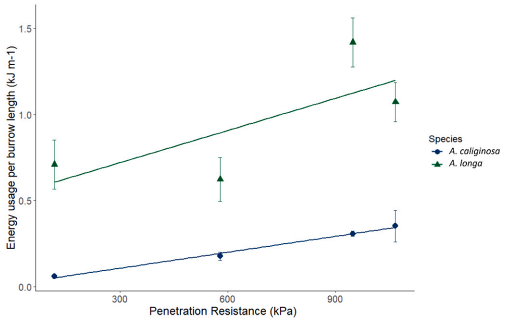

Fig. 6. Energy requirement per metre burrow length as a function of soil mechanical resistance (*A. caliginosa*: $R^2 = 0.99$, $p < 0.01$; *A. longa*: $R^2 = 0.53$, $p = 0.27$). Error bars indicate standard error ($n = 3$).

observed for both earthworm species. *A. longa* had little exploratory behaviour, reflecting a different ecological trait. As, an epi-anecic earthworm *A. longa* naturally feeds on the soil surface, while *A. caliginosa* being an endogeic earthworm forages within the soil (Bottinelli et al., 2020).

The final burrow systems of *A. caliginosa* were longer compared to those created by *A. longa*, and had many dead-end branches, as also reported by Capowiez et al. (2015). We observed that *A. longa* created “loops” in their burrow systems. This is somewhat contradictory to the results from Felten and Emmerling (2009) who reported that burrow systems of *A. longa* were branched. The reasons for the differences could be related to variations in experimental conditions (14 days duration and food provided in Felten and Emmerling (2009) vs. 3 days duration without food provided in our set-up), or reflect that earthworms can behave differently even though they belong to the same species. Our data demonstrate that the burrowing behaviour of an earthworm influences and shapes the characteristics of the burrow system.

4.2. Burrowing rate and burrowing mode are a function of soil mechanical resistance and differ between species

Earthworm burrowing rates decreased with increasing soil mechanical resistance, and the decrease was greater for *A. caliginosa* than for *A. longa* (Fig. 3). In their natural environment, *A. caliginosa* and *A. longa* show different inherent feeding behaviours: *A. caliginosa* burrows primarily to find food, while *A. longa* burrows for shelter and searches for food on the soil surface (Lavelle, 1988; Lowe and Butt, 2002). We expected this difference in feeding behaviour to be reflected in differences of burrowing rates, and this was confirmed by our results indicating higher burrowing rates for *A. caliginosa* than *A. longa*. Under favourable soil conditions, i.e. low soil mechanical resistance, earthworms are little restricted mechanically in their search for food within the soil, and this could be a reason for the higher burrowing rates when soil mechanical resistance is low. Moreover, the energy need for burrowing is lower at low soil mechanical resistance, so earthworms can “afford” to travel relatively long distances to find food.

Earthworms have a hydrostatic skeleton, filled with coelomic fluid and a muscular system composed of longitudinal and circular muscles (Lee, 1985). Depending on which of these muscles earthworms contract, either axial or radial pressure is created. Radial pressure is most relevant for burrowing by cavity expansion (i.e. pushing soil aside) because it allows the earthworm to penetrate the soil in front with lower axial pressure (Keudel and Schrader, 1999). The maximum axial and radial pressures that earthworms can exert vary among earthworms species. *A. caliginosa* can exert relatively high radial pressure but only relatively low axial pressure, while *A. longa* can exert axial pressures twice as high as *A. caliginosa* but can only exert relatively low radial pressures (Keudel and Schrader, 1999; Ruiz and Or, 2018). According to Ruiz and Or (2018), the higher pressures of *A. caliginosa* (endogeic) could provide a mechanical advantage and could extend the activity spectrum of these earthworms, in comparison to anecic worms (*A. longa*). These differences could explain why *A. caliginosa* (high maximum radial pressure) burrows via cavity expansion when soil mechanical resistance is low, while *A. longa* (low maximum radial pressure) burrows via ingestion even at low soil mechanical resistance. The shift from cavity expansion to ingestion for *A. caliginosa* when soil mechanical resistance increased could indicate that soil mechanical resistance exceeded the pressure limit of *A. caliginosa*.

The strong decrease in burrowing rate with increasing soil mechanical resistance, together with the switch in burrowing mode from cavity expansion to ingestion with increasing soil resistance for *A. caliginosa*, may indicate that burrowing by ingestion is slower than burrowing by cavity expansion. This is supported by the generally lower burrowing rates of *A. longa*, which had a higher $f_{\text{ingestion}}$. For *A. longa*, which did not significantly change its burrowing mode, the reduction in burrowing rate with increasing soil mechanical resistance was not as pronounced as for *A. caliginosa*.

We determined burrowing mode from the ratio of cast volume to burrow volume. Cast production depends greatly on the ecophysiology of earthworm species (Buck et al., 2000) and can provide insights into the burrowing mechanism of earthworm species. Our calculations of the ingestion factor (Eq. (1)) indicate that the burrowing mode i) is a function of soil mechanical resistance, and ii) varies between earthworm species. Some studies have found that surface cast production increases at higher compaction levels (Beylich et al., 2010), yet our results did not reveal any increase in absolute cast production (above and below ground) with increasing soil mechanical resistance. However, cast production per metre burrow increased with increasing soil mechanical resistance, for both earthworm species (Fig. 4). This was expected because earthworms have to ingest more soil to create a unit length of burrow at higher soil mechanical resistance levels. The $f_{ingestion}$ was larger than 0.5 at the two highest soil mechanical resistances for both earthworm species, which means that the predominant burrowing mode in compacted soils was ingestion. We observed a shift to ingestion of soil between 579 and 949 kPa (Fig. 5), which could indicate a possible threshold in this mechanical resistance range. Our findings are supported by Kemper et al. (1988) and Dexter (1978), who reported that earthworms move through compacted soil mainly by ingestion. Our data suggest that earthworm muscular build could play an important role in determining the burrowing mode of earthworms. Yet the exact reason for the change in burrowing mode remains unclear: it could be forced by mechanical constraints, or could burrowing by ingestion be more energy-efficient than burrowing by cavity expansion in highly compacted soils?.

4.3. Energy requirements per burrow length increased with soil mechanical resistance

We estimated an average daily energy requirement (Eq. (2)) of 1.9×10^{-2} kJ d^{-1} for *A. caliginosa* and 4.5×10^{-2} kJ d^{-1} for *A. longa*, averaged across all mechanical resistance levels. Our estimate for *A. caliginosa* is similar to the maintenance costs (including energy requirement for burrowing) obtained from the earthworm energy budget model presented by Johnston et al. (2014), which yields 2.4×10^{-2} kJ d^{-1} for *A. caliginosa* with 0.5 g body weight at a temperature of 15 °C. The higher absolute energy demand for *A. longa* might explain why *A. longa* had a higher $f_{\text{ingestion}}$, i.e. burrowed primarily via ingestion, already at low soil mechanical resistance levels – *A. longa* is in need of more energy (more food) than *A. caliginosa*.

The energy requirements to create a unit length of burrow doubled for *A. longa* when soil mechanical resistance increased from 117 kPa to 949 kPa, and for *A. caliginosa*, energy consumption per burrow length increased five-fold between 177 kPa and 1068 kPa soil mechanical resistance. This clearly shows that the energy to create a burrow of a certain length increases when the soil mechanical resistance increases, and this was found for both earthworm species. We suggest that analysing the energy requirements concerning different burrowing modes could be a subject for future research. Linking soil mechanical conditions, burrowing mode and energy requirement will allow prediction of earthworm bioturbation under various soil conditions.

In this study, we manipulated soil mechanical resistance by varying soil dry bulk density, while matric potential was kept at −100 hPa for all levels of soil mechanical resistance. For a given soil, mechanical resistance increases with increasing bulk density and decreasing matric potential (e.g. To and Kay, 2005). We could therefore expect similar effects on burrowing rates and energy requirements when soil mechanical resistance increases due to soil drying. However, soil moisture itself has additional direct impacts on earthworm well-being and therefore also on burrowing rates and maintenance costs, since earthworms need to keep their body surface moist by secretion of mucus (Edwards and Bohlen, 1996). Holmstrup (2001), performing laboratory experiments with *A. caliginosa*, showed that earthworms entered diapause at matric potentials <-200 hPa. In wet soil, oxygen could become limiting for earthworms (Chuang and Chen, 2008), especially in compacted soils where gas transport is restricted (e.g. Berisso et al., 2013).

It is expected that soil compaction and drier soil conditions increase in future due to a continued trend towards heavier farm vehicles and due to climate change, and this would increase soil mechanical resistance (Gao et al., 2012). Therefore, our results suggest the risk of a general decrease in earthworm burrowing together with an increased energy demand of earthworms in arable soil. In the long term, this would negatively affect many soil processes that are mediated by earthworms, including crop yield. The beneficial functions of earthworms for soil processes can only be maintained if soil management is adapted to both reduce soil compaction and increase soil organic matter in arable soils so that earthworms can meet their energy requirements and stay active under future more stressful environmental conditions.

5. Conclusions

We showed that burrowing rates of earthworms decrease with increasing soil mechanical resistance. Burrowing rates were reduced by half when soil mechanical resistance increased from about 100 to 1000 kPa. The impact of compaction on burrowing rates was species dependent, with *A. caliginosa* being more strongly affected than *A. longa*. Soil mechanical resistance also influenced the earthworms burrowing mode, and the impact was different for the two earthworm species studied. *A. longa* burrowed primarily by ingestion at all levels of soil mechanical resistance, while *A. caliginosa* burrowed by cavity expansion at low soil mechanical resistance and by ingestion at high levels of mechanical resistance. Further research is needed to reveal whether earthworms change their burrowing mode as a function of soil mechanical resistance to save energy, or whether they are forced to do so because they cannot exert the pressure needed for cavity expansion. We showed that the energy needed to create a unit length burrow strongly increased with increasing soil mechanical resistance. Our data could be used to parameterize simulation models of earthworm behaviour. The results demonstrate the impact of soil mechanical conditions on earthworm burrowing, and that this effect is species dependent. Further studies with different earthworm species are needed to better understand how soil mechanical conditions affect earthworm burrowing behaviour at the species and ecological group level.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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This thesis investigated and quantified the impacts of soil compaction on earthworm burrowing by using different earthworm species. Results indicated that earthworm burrowing is a function of soil compaction. At higher soil compaction levels, there is an increase in the energy costs associated with burrowing purposes. When exposed to natural climatic conditions and for extended periods earthworm burrowing, is affected by the interaction effects between soil compaction and soil moisture levels. The findings provide better insights into earthworm burrowing and its temporal dynamics as a function of soil compaction.

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