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Earthworm and arbuscular mycorrhizal fungal communities in agricultural soils

Management of key soil organisms for sustainable agriculture

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Cover: An earthworm and an arbuscular mycorrhizal fungus in agricultural soil
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Abstract

Intensive agriculture is detrimental to soil biodiversity and functioning. Promoting communities of key soil organisms, such as earthworms and arbuscular mycorrhizal (AM) fungi, may help improve agricultural sustainability by replacing inputs with ecosystem services. In this thesis, I explore ways to manage earthworm communities for improved soil functioning via adjusted agricultural practices, promotion of source habitats in the landscape, and inoculation. I also explore the effects of soil compaction on AM fungal symbiosis in wheat varieties, and test application of grassland soil as a method to increase AM fungal diversity in agricultural soils. I show that diverse earthworm communities and bioturbation can be promoted by reducing tillage intensity and total earthworm densities can be increased via diversifying crop rotations. Moist and fertile semi-natural grasslands with high small-scale habitat heterogeneity may serve to sustain earthworm diversity in agricultural landscapes. Inoculation with commercially obtained *Lumbricus terrestris* earthworms may help restore populations of this tillage sensitive species and improve wheat growth but the long-term establishment of these worms is uncertain. Application of grassland soil may increase AM fungal diversity in agricultural soils but this may not be reflected in AM fungal taxa colonizing wheat roots. I also show that soil compaction differently affects AM colonization in wheat varieties. Further research needs to identify which earthworm and AM fungal community properties best result in functional benefits under different conditions. Nevertheless, I show that earthworm and AM fungal communities can be promoted in various ways, individually or in combination, suggesting potential to enhance functional effects of these key organisms in agricultural soils.

Keywords: Lumbricidae, Glomeromycota, *Triticum aestivum*, ecological intensification, ploughing, crop diversity, soil biodiversity conservatio

Daggmaskar och arbuskulära mykorrhizasvampar i jordbruksmark

Sammanfattning

Intensivt jordbruk påverkar jordbruksmarkens funktion och biologiska mångfald negativt. Genom att främja samhällen av viktiga markorganismer, såsom daggmaskar och arbuskulära mykorrhiza (AM) svampar, kan man ersätta tillförsel av insatsmedel med ekosystemtjänster utförda av markorganismer vilket bidrar till ökad hållbarhet inom jordbruket. I denna avhandling studerar jag hur man kan främja daggmasksamhällen och nå bättre funktion i marken genom ändrade jordbruksmetoder, hänsyn till omgivande habitat i jordbrukslandskapet som kan fungera som källor av daggmaskar för spridning till åkerfält samt direkt tillförsel av daggmaskar i åkerfält. Dessutom studerar jag hur markpackning påverkar AM-symbiosen i olika vetesorter, och undersöker om det är möjligt att öka mångfalden av AM-svampar i jordbruksmark genom tillförsel av gräsmarksjord. Jag visar att daggmasksamhällena och deras bioturbation kan gynnas genom reducerad plöjning och mer mångsidigt växtföljd. Fuktiga och näringsrika gräsmarker med hög grad av heterogenitet på en liten skala kan bidra till att bibehålla mångfalden av daggmaskar i jordbrukslandskapet. Direkt tillförsel av individer av stor daggmask (*Lumbricus terrestris*), en art som är känslig för plöjning, kan hjälpa till att restaurera populationerna av denna art samt förbättra tillväxten hos vete, men det finns osäkerheter kring de tillförda daggmaskarnas långsiktiga överlevnad och förökning i fälten. Tillförsel av gräsmarksjord kan öka mångfalden av AM-svampar i jordbruksmark, men denna ökade mångfald reflekterades inte i de AM-svampar som fanns i vetets rötter. Vidare visar jag att effekten av markpackning påverkar koloniseringen av AM-svampar olika för olika vetesorter. Mer forskning behövs för att identifiera vilka egenskaper hos samhällena av daggmaskar och AM-svampar som ger de bästa funktionella fördelarna under olika förhållanden. Emellertid visar jag att daggmask- och AM-svampsamhällen kan gynnas på olika sätt, både enskilt och tillsammans, vilket tyder på att det går främja de positiva effekterna av dessa viktiga organismer i jordbruksmarken.

Nyckelord: Lumbricidae, Glomeromycota, *Triticum aestivum*, ekologisk intensifiering, plöjning, grödornas mångfald, bevarandet av markens biologiska mångfald

Lierot ja arbuskelimykorritsasienet maatalousmaassa

Tiivistelmä

Voimaperäinen maatalous on haitallista maaperäeliöstön monimuotoisuudelle ja toiminnoille. Maan avaineliöiden, kuten lierojen ja arbuskelimykorritsa- ali AM-sienten, tuottamat ekosysteemipalvelut voivat parantaa maatalouden kestävyyttä. Tutkin tässä väitöskirjassa tapoja hoitaa lieroyhteisöjä niiden tuottamien toimintojen vahvistamiseksi maatalousmenetelmiä mukauttamalla, säilyttämällä lähde-habitaatteja ja inokuloimalla. Tutkin myös maaperän tiivistymisen vaikutusta AM-symbioosiin eri vehnälajikkeissa ja kokeilen, voiko AM-sienten monimuotoisuutta maatalousmaassa lisätä levittämällä pelloille niittymultaa. Osoitan että maanmuokkauksen keventäminen tukee monimuotoisia lieroyhteisöjä ja bioturbaatiota ja että monipuolinen viljelykierto lisää lierojen kokonaismäärää. Kosteat ja viljavat luonnonlaitumet, joissa on vaihtelevasti mikrohabitaatteja, tukevat lierojen monimuotoisuuta maatalousympäristöissä. Syöteiksi myytävien *Lumbricus terrestris* -lierojen lisääminen peltomaahan voi lisätä tämän kyntöherkän lajin yksilömääriä ja tukea vehnän kasvua, mutta on epäselvää pystyvätkö nämä lierot muodostamaan uusiutuvia populaatioita. Niittymullan levittäminen voi lisätä AM-sienten monimuotoisuutta peltomaassa, mutta tämä ei välttämättä näy vehnän juurissa esiintyvien sieniosakkaiden lajimäärässä. Osoitan myös, että maan tiivistymisen vaikutus vehnän AM-symbioosiin riippuu lajikkeesta. Lisää tutkimusta tarvitaan, jotta voidaan määrittää mitkä liero- ja AM-sieniyhteisöjen ominaisuudet parhaiten lisäävät näiden eliöiden hyödyllisiä toimintoja eri olosuhteissa. Lierojen ja AM-sienten lajistoon peltomaassa voidaan siis vaikuttaa monin eri tavoin, mikä mahdollistaa näiden avaineliöiden toiminnallisen hyödyntämisen maatalouden kestävyyden parantamiseksi.

Avainsanat: Lumbricidae, Glomeromycota, *Triticum aestivum*, ekologinen voimaperäistäminen, kyntö, viljelykasvien monimuotoisuus, maaperäeliöstön monimuotoisuuden suojelu

Dedication

To my children and all PhD students who become parents while writing their thesis.

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. K. A. Torppa, A. R. Taylor (2022). Alternative combinations of tillage practices and crop rotations can foster earthworm density and bioturbation. Applied Soil Ecology, 175: 104460.

II. K. A. Torppa, J. Forkman, N. I. Maaroufi, A. R. Taylor, T. Vahter, M. Vasar, M. Weih, M. Öpik, M. Viketoft. Soil compaction affects arbuscular mycorrhizal symbiosis differently for spring wheat varieties with contrasting root traits (submitted manuscript).

III. K. A. Torppa, C. Castaño, A. Glimskär, H. Skånes, M. Klinth, T. Roslin, A. R. Taylor, M. Viketoft, K. E. Clemmensen, N. I. Maaroufi. Soil moisture and fertility drive earthworm diversity in north temperate semi-natural grasslands (manuscript).

IV. K. A. Torppa, J. Bengtsson, C. Castaño, M. Ittonen, N. I. Maaroufi, T. Vahter, M. Vasar, M. Viketoft, M. Öpik, A. R. Taylor. Complex crop and soil biological responses to field application of earthworms and arbuscular mycorrhizal fungi (manuscript).

Paper I (open access) is reproduced with the permission of the publishers.

The contribution of Kaisa Anneli Torppa to the papers included in this thesis was as follows:

I. Main author. Participated in planning the study in collaboration with AT. Coordinated the fieldwork and participated in it with help from AT and field assistants. Analysed the data. Wrote the manuscript with help from AT.

II. Main author. Planned the study with help from TV, NM, AT and MV in the experiment designed by MW. Conducted the fieldwork with help from field assistants. Analysed the data with the help from JF. Wrote the manuscript with the help from the co-authors.

III. Main author. Planned the study and conducted the fieldwork in collaboration with CC, AG, HS, AT, MV, KC and NM. Analysed the data with help from TR. Wrote the manuscript with help from the co-authors.

IV. Main author. Planned the study in collaboration with JB, AT and MV. Coordinated the fieldwork and participated in it with the help of an assistant. Analysed the data. Wrote the manuscript with help from the co-authors.

1. Introduction

1.1 Soil biodiversity and functions in agricultural soils

Soil biota is an integral part of soil and responsible for many soil functions that are vital to humankind (Bardgett & Van Der Putten, 2014). The diversity of life forms in the soil is immense, and consists of millions of microbial taxa and hundreds of thousands of species of protists and soil animals (Wall et al., 2001). Due to the huge diversity, large population sizes and small body sizes of most soil organisms, as well as the cryptic nature of soil as a habitat, studying soil biodiversity is difficult, and a large part of it is still unknown (Bardgett & Van Der Putten, 2014; Cameron et al., 2018; Graaff et al., 2019). Even for earthworms, one of the most studied groups of soil animals, only about one fourth of all species (~7000) are known to science (Orgiazzi et al., 2016; Phillips et al., 2017).

The soil biota drives a multitude of soil functions, such as decomposition, nutrient mineralization, soil structure formation, water regulation, and biological population regulation. These functions, in turn, are linked to important ecosystem services, such as plant production, climate regulation, water regulation, and biological control (Brussaard, 2012). Soil organisms contribute to soil functions in different ways depending on their size, movement, and feeding habits (Bardgett, 2005). Microorganisms, such as bacteria and fungi, are the primary actors in decomposition, breaking down organic compounds with their extra-cellular enzymes and releasing nutrients for plants. Soil microbes also include several plant-associated organisms, such as symbionts and pathogens, which play an important role in plant production and health. Soil microfauna, such as protists and nematodes, also includes pathogens, but are mostly important contributors to nutrient mineralization, via feeding on microbes and this way stimulating microbial turnover. Soil microfauna also regulates the flow of energy and nutrients via complex food web interactions. Together with microbes and organic matter, the smallest animals are food to larger soil invertebrates, such as springtails and mites (soil mesofauna), which are, in turn, eaten by even larger ones, such as centipedes and many insect larvae (soil macrofauna). The non-predatory soil meso- and macrofauna break up organic matter into smaller pieces, making it better available for smaller soil animals and the primary decomposers. The largest soil animals, such as earthworms, which are strong enough to move soil particles, are also important for soil structure and water properties. They also serve as an important prey for many below- and above-ground animals like moles and birds (Bardgett, 2005). Via their numerous interactions with each other, the soil environment, and above-ground biotas, soil organisms form complex entities, whose responses to perturbations are difficult to predict (Graaff et al., 2019).

Soil biodiversity is important for agricultural production due to its key role in several soil functions related to plant productivity (Barrios, 2007; Brussaard et al., 2007). At the same time, intensive agricultural management practices, such as extensive use of pesticides and mineral fertilizers, heavy soil cultivation, and monocultures drastically reduce soil biodiversity (Postma-Blaauw et al., 2010; Tsiafouli et al., 2015). The loss of soil biodiversity has been shown to impair soil functions like carbon and nitrogen cycling (de Vries et al., 2013; Graaff et al., 2019). While agricultural intensification has massively increased global food production since about the 1950's, it has done so at the cost of, among other things, soil biodiversity (Godfray et al., 2010; van der Putten et al., 2023). This, together with above-ground biodiversity loss and climate change, has further increased our dependence on high chemical and energy inputs (Bender et al., 2016; Bommarco et al., 2013; El Mujtar et al., 2019). The trend of decreasing soil biodiversity needs to be reversed in order to feed the growing population on the earth without compromising the adequacy of resources for future generations of both human and non-human life.

But what is required to support soil biodiversity and functions to enhance agricultural sustainability? Because soil systems are complex, the effects of specific actions on soil biota and the resulting functional changes are difficult to study, and almost always context dependent (Graaff et al., 2019). Despite of this, several studies, such as those mentioned in the previous paragraph (de Vries et al., 2013; Tsiafouli et al., 2015), have remarkably increased our understanding of these processes. We know, for example, that intensive agriculture is especially detrimental to large-bodied soil animals, like earthworms, while small-bodied soil animals, like nematodes, are less affected (Tsiafouli et al., 2015). We also know that, under intensive agricultural management, microbial communities become increasingly dominated by bacteria compared to fungi (De Vries et al., 2013). Thus, intensive agriculture seems especially detrimental to some of the key groups of soil organisms, such as earthworms and mycorrhizal fungi (Lavelle et al., 1997; Smith & Read, 2008). Studying how to manage communities of these specific groups of soil organisms could especially benefit agricultural sustainability and result in overall increased soil functionality via the key roles that these groups play in the ecosystem.

1.2 Key soil organisms for sustainable agriculture

To support earthworm and arbuscular mycorrhizal (AM) fungal communities for the benefit of agricultural production and sustainability, it is essential to know which characteristics of their communities to promote and how. Functional redundancy, i.e., the proportion of functionally similar species, is assumed to be high in soil communities (Nielsen et al., 2011). Thus, instead of aiming at high overall diversity, focusing on functional diversity, as well as keystone species or groups, may be a more fruitful approach for improving the biological functioning of the soil.

The principal way to promote functionally important soil organisms should be via identifying and adopting agricultural management practices that improve or maintain good living conditions for the target taxa (Schwartz et al., 2006). However, if the population densities of those taxa have been drastically reduced, or if they are completely lost from the soil due to long-term intensive management, restoration of their populations may require promoting re-colonizationg from surrounding landscape, or re-introduction via inoculation (Bender et al., 2016; 2017).

1.2.1 Earthworms

Earthworms (Crassiclitellata; Jamieson et al. 2002) are segmented worms that occur in most terrestrial parts of the world (Edwards & Arancon, 2022). They are considered ecosystem engineers which, via their feeding, burrowing and casting activities, also called ‘bioturbation’, profoundly affect soil chemical, physical and biological properties (Lavelle et al., 1997). Because of their major role in litter decomposition and transformation, nutrient mineralization, as well as soil structure formation and water regulation, earthworms are also intimately linked to the above-ground systems. They are important drivers of many ecosystem services, such as plant productivity and water regulation (Blouin et al., 2013). In soils where earthworms are present, yields are on average 25% higher than where they are not present, which has been attributed especially to their capacity to mineralize nitrogen (van Groenigen et al., 2014). However, earthworms may also provide disservices, such as increase greenhouse gas emissions from the soil (Lubbers et al., 2013).

Earthworms are commonly classified into ecological groups. The most commonly used ecological classification divides earthworms into three categories: epigeic, anecic and endogeic earthworms (Bouché 1972, 1977). Epigeic earthworms are relatively small, pigmented worms that live in and feed on litter, and typically do not burrow in the mineral soil. Anecic earthworms are large and partially pigmented, feed on litter, and make deep, semi-permanent burrows. Endogeic earthworms are unpigmented, live in the upper mineral soil feeding on more humified organic matter, and create more shallow and temporary burrows than anecics. Although not all earthworm species can be strictly assigned to one of the three categories, the classification helps understand responses of different earthworm species to disturbances and environmental variation (Bottinelli et al., 2020). The ecological categories have also been used to infer functional consequences of loss of certain species, but more functionally oriented approaches, such as trait-based analyses, may be more suitable for that purpose (Bottinelli & Capowiez, 2021).

The effects of agricultural management practices on earthworms are well-studied (e.g. Chan 2001; Pelosi et al. 2014a; Briones & Schmidt 2017; Corredor et al. 2023). Conventional tillage and other intensive soil cultivation practices are the most detrimental to earthworm diversity

(Briones & Schmidt, 2017). Conventional tillage reduces numbers of especially epigeic and anecic earthworms, as it destroys the habitat and buries the food resources for the epigeics and destroys the burrow systems of the anecics. Reduced organic matter inputs and low crop diversity are also detrimental to earthworms, as they result in reduced resource quantity and variability for earthworms (Abail & Whalen, 2018; Rodríguez et al., 2020; Schmidt et al., 2003), and may be especially harmful for endogeic species (Milcu et al., 2006). However, less is known about the context-dependency of how management affects earthworms. In areas with naturally low densities of epigeic and anecic worms, adjusting tillage is unlikely to help enhance their densities. Furthermore, agricultural management consists of a range of practices, which may have different and interdependent effects on earthworms (Pelosi et al., 2014). For example, certain pesticides may only harm earthworms when incorporated into deeper soil by tillage, and the effects of certain tillage practices on earthworms may be negligible when combined with enhanced organic matter inputs (Chan, 2001; Pelosi et al., 2014). Studying the effects of several types of practices in concert, preferably in different contexts, such as different soil types, is necessary for acquiring a more comprehensive picture of how earthworm diversity can be supported.

On a broader scale, environmental conditions, such as climate and soil type, and the regional species pool, determine earthworm species composition in agricultural fields (Decaëns et al., 2008). Thus, adjusting agricultural management practices will not increase earthworm diversity where it is naturally low due to other limiting factors, such as extreme temperatures, low pH, or very high clay or sand contents (Edwards & Arancon, 2022). Furthermore, if an earthworm species is lost from an agricultural field due to long-term intensive management, adjusting agricultural practices will help restore population density only if source populations for recolonization exist in the surrounding landscape (Nieminen et al., 2011). Due to the historically larger focus on above- rather than below-ground biodiversity, and the prevailing limitations in earthworm taxonomical knowledge, relatively little is known about earthworm ecology and distributions on the species level (Decaëns, 2010; Guerra et al., 2021; Phillips et al., 2020). This kind of knowledge would be crucial for determining the potential earthworm species diversity and composition in agricultural soils of a certain area, as well as for determining the conservation status of earthworm species (Guerra et al., 2021). Achieving this will require including earthworms in regular monitoring campaigns, as well as adopting a wider selection of tools, including molecular methods, for earthworm detection and species determination.

Due to long-term intensive agricultural management practices, certain species of earthworms may have completely disappeared from large areas. One such species could be the anecic *Lumbricus terrestris*, which is very sensitive to intensive tillage. Simultaneously, this species is considered especially important for soil functions due to its role in litter decomposition and translocation, as well as soil macropore creation and water infiltration (Andriuzzi et al., 2015; Huang et al., 2020). When there is little suitable habitat for *L. terrestris* left in the landscape, which could work as a source of re-colonization, re-introduction may be the only way to recover their populations. Feasible ways for farmers to re-introduce earthworm species need to be developed to allow using species re-introduction for the benefit of earthworm communities and earthworm-mediated soil functions.

1.2.2 Arbuscular mycorrhizal fungi

Arbuscular mycorrhiza (AM) is the most common type of mycorrhiza, found in more than 80% of land plant species, and the prevailing mycorrhizal type in grasslands and cultivated lands (Smith & Read, 2008). AM fungi (Glomeromycota; Tedersoo et al. 2018) are characterized by tree-like structures called arbuscules, which they form inside their host plants cells and through which the exchange of nutrients and carbon between the fungus and its host plant occurs (Smith & Read, 2008). Thus, like other mycorrhizae, AM are generally considered a mutualistic association, benefitting both the fungal partner and the plant. In addition to helping the host plant in nutrient uptake, AM fungi can protect their hosts from pathogens (Wehner et al., 2010), herbivores (Frew et al., 2022), and drought (Cheng et al., 2021). Furthermore, AM fungi have been shown to improve soil structure and water holding capacity, by binding soil particles together with their hyphal networks (Querejeta, 2017). Due to these key roles of AM fungi for the growth and health of their hosts, AM fungi are considered highly beneficial for agricultural production (Rillig et al., 2019).

As for many other groups of soil organisms, intensive agriculture is detrimental to AM fungi. Intensive tillage disturbs AM fungal hyphal networks, long fallow periods and crop monocultures reduce the access and diversity of AM fungal hosts, and high fertilizer inputs reduce the plant need to allocate carbon to AM fungal aided nutrient uptake (Bowles et al., 2017; Guzman et al., 2021; Vahter et al., 2022). As a result, AM fungal communities in intensively managed agricultural fields are typically low in diversity and dominated by few ruderal species that spread and re-grow their hyphae efficiently after disturbances (Chagnon et al., 2013; Verbruggen & Kiers, 2010). The reduced AM fungal diversity is likely to result in reduced ecosystem functioning, as it diminishes the AM fungal functional repertoire (Powell & Rillig, 2018), and the dominance of ruderal species may reduce crop AM fungal nutrient uptake due to their lower investment in extraradical hyphae (Hart & Reader, 2002).

Although AM fungal symbiosis is considered mainly mutualistic, the presence of AM fungi in the soil does not always benefit the plant (Johnson et al., 1997). Not all plant species form symbioses with AM fungi (e.g., most species of Brassicaceae do not; Cosme et al., 2018), and for those that do, the benefit depends on environmental conditions and the combination of plant and AM fungal species (Berger & Gutjahr, 2021). If the disadvantage of carbon cost to the plant becomes bigger than or equal with the benefit of nutrient gain, the AM symbiosis becomes parasitic or commensal (i.e., when the plant neither benefits nor suffers from the symbiosis; Johnson et al. 1997). Mutualistic AM symbiosis is especially common when nutrient availability in soil is low, as the benefit of nutrient uptake via AM symbiosis then becomes larger for the plant (Berger & Gutjahr, 2021; Verbruggen et al., 2013). Similarly, plant species and functional groups, that inefficiently take up nutrients with their roots, typically benefit more from AM fungal symbiosis than more nutrient-efficient ones (Unger et al., 2016; Wen et al., 2019). AM fungal symbiosis could also be more beneficial for plants when soil structural properties hamper root growth, such as if the soil is severely compacted, as it may be easier for the thin AM fungal hyphae to access nutrients in such condition. Breeding crop varieties that positively respond to AM fungal colonization has been suggested as another means to reduce the need for mineral fertilizers and other agricultural inputs. To reach that goal, however, the drivers of a mutualistic AM fungal symbiosis need to be better understood.

In addition to adopting AM fungal friendly agricultural practices, inoculation with AM fungi could help recover AM fungal communities in agricultural soils (Bender et al., 2016). Commercial AM fungal inocula, also known as “biofertilizers”, are expensive to produce, and mainly marketed for tree cultivation and horticultural purposes (O’Callaghan et al., 2022). For wide scale field application, the use of commercial AM fungal inocula is problematic due to uncertainties in their establishment, high context dependency of crop benefits, and unknown ecosystem consequences of potential spread of foreign AM fungal taxa (Hart et al., 2017), highlighted by the low reliability of product descriptions concerning their taxonomic content (Vahter et al., 2023). Indeed, it has been suggested, that commercial AM fungal inocula are mainly useful in horticulture on sterile soils, as well as in hydroponic and aeroponic cultivation, where native AM fungal communities may be completely lacking (Hart et al., 2017). For soil inoculation with AM fungi to be more widely applicable, its utility under different conditions has to be better assessed, and environmentally safe and cost-effective inoculation methods need to be developed.

2. Framework and objectives

In this thesis, I focus on the drivers of diversity and community structure of two functionally important groups of soil organisms in agricultural soils: earthworms and arbuscular mycorrhizal fungi. My main aim is to provide knowledge for supporting soil communities for enhanced soil functioning and, ultimately, for more sustainable agriculture.

The chapters of the thesis fall within three wider themes, which concern the problematics of supporting well-functioning soil communities from different perspectives. First, I explore agricultural management as a driver of earthworm and AM fungal diversity and function (papers I & II). Second, I investigate the role of semi-natural grasslands in preserving earthworm diversity in agricultural landscapes (paper III). Third, I explore the possibility of managing earthworm and AM fungal communities via inoculation (Paper IV).

My specific aims were to

- test the potential of a diversified crop rotation to mitigate the detrimental effect of intensive tillage on earthworm density and bioturbation (paper I),
- assess how soil compaction affects AM fungal symbiosis and nutrient uptake in spring wheat varieties (paper II),
- investigate the potential of semi-natural grasslands to preserve earthworm diversity in agricultural landscapes (paper III), and
- explore how farmers could feasibly increase earthworm and AM fungal diversity in their soils via inoculation (paper IV).

3. Methods

3.1 Study design

The data for my thesis originate from samplings in three field experiments (papers I-II and IV) and one regional scale field survey (paper III). For paper I, I characterized earthworm communities, bioturbation, and selected soil properties in a long-term field experiment comparing different tillage intensities within two crop rotations. For paper II, I examined AM fungal root colonization, community composition and wheat P uptake in a field experiment comparing the effect of soil compaction on different varieties of spring wheat. For paper III, I surveyed and molecularly identified earthworm communities from 28 semi-natural grasslands, and determined several soil, vegetation, management, and landscape variables potentially driving earthworm diversity and community structure. For paper IV, I conducted a field experiment at seven organic fields, where I manipulated earthworm and AM fungal communities via inoculation with earthworms and grassland soil.

All field experiments and the field survey were conducted in Uppland county, south-central Sweden. The annual mean temperature in the area is 6.5 ˚C and the average annual precipitation is 586 mm. The length of the growing season, defined as the average number of days with mean daily temperature higher than 5 ˚C, is 180–190 days (Swedish Meteorological and Hydrological Institute, Uppsala airport weather station, 1991–2020). Uppland is geologically variable, with a higher proportion of the area on fine sediments and organic soils in the west, and more moraine soils and bedrock outcrop in the east (The Geological Survey of Sweden, Quarternary Deposits Map). The most commonly cultivated crops in the area are winter wheat and spring barley (Jordbruksverket, 1993–2022).

3.1.1 Long-term experiment on tillage intensities in two crop rotations (Paper I)

To investigate whether a diverse crop rotation mitigates the detrimental effect of intensive tillage on earthworms and earthworm bioturbation, I used a long-term experiment managed by SLU, established in 2007 at the Säby experimental site in Uppsala. It compares different tillage intensities within simple and diverse crop rotations. The simple crop rotation consists of only cereals, wheat (*Triticum aestivum*, L.) and barley (*Hordeum vulgare*, L.), whereas in the diverse crop rotation, barley has been replaced by peas (*Pisum sativum*, L.) and oil seed rape (*Brassica napus*, L.). From the six tillage intensities of the experiment, I included three in the study: conventional tillage (CT, ploughing to 23 cm depth), reduced tillage (RT, cultivator 10-12 cm depth) and no tillage (NT, direct sowing). The design of the experiment is a split-plot with three replicate blocks, where different tillage intensities are applied within the two rotations. At the time of sampling, the crop in both rotations was wheat, and the preceding crops in the simple and diverse rotation were barley and peas, respectively. Sampling was conducted during three consecutive days in June 2017.

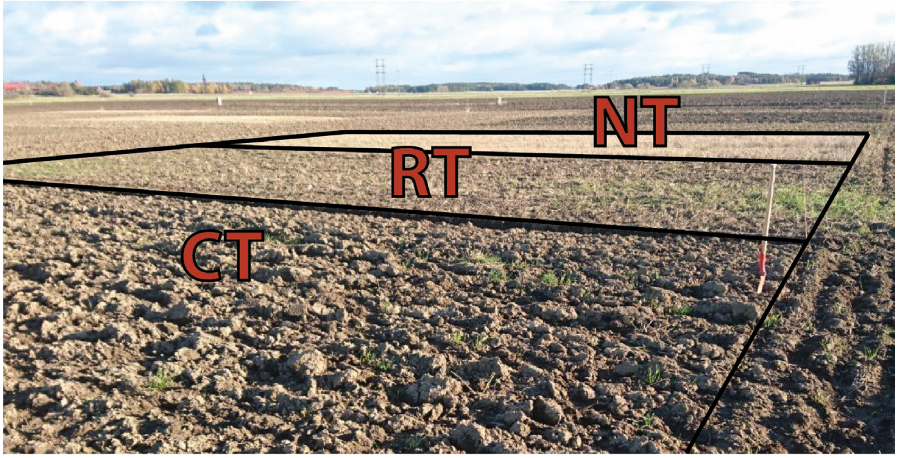

Figure 1. Soil surface in the three tillage treatments (CT, conventional tillage; RT, reduced tillage; and NT, no tillage) soon after ploughing in autumn 2018. The difference in the amount of crop residues on the soil surface is clearly visible (paper I). Photo: Kaisa Torppa

3.1.2 Field experiment on compaction effects on spring wheat varieties (Paper II)

To test whether soil compaction has an effect on AM fungal colonization in spring wheat, and if the effect varies between varieties, I sampled AM fungi in a field experiment comparing compaction effect on nutrient uptake in different spring wheat varieties (Liu et al., 2022ab; Weih et al., 2021). The experiment, located at the Säby experimental site in Uppsala, was run for two years (2018-2019), and the material for my study was collected during the second year of the experiment. The experimental design was a split-plot with four replicates, where the compaction treatment (compaction vs no compaction) was applied in the main plots, and the spring wheat varieties in the sub-plots. The soil was compacted using a front loader, and the compaction effect was verified via measurements of bulk density and penetration resistance (Liu et al., 2022ab). Of the nine varieties included in the experiment, I selected five, including old and new varieties used in both conventional and organic cultivation and with different root characteristics. The sampling was conducted at spring wheat flowering in early July 2019.

3.1.3 Field survey on earthworm communities in old semi-natural grasslands (paper III)

To investigate the drivers of earthworm diversity and community structure in semi-natural grasslands, I surveyed earthworm communities, and a range of environmental factors, in 28 grasslands in Uppland. The grasslands were selected based on previous studies as a set of semi-natural grasslands representative for the area (Pärt & Söderström, 1999; Söderström et al., 2001; Vessby et al., 2001), with varying levels of grazing, and sparsely located trees, shrubs and boulders. All grasslands were older than 150 years, and no cultivation practices had been applied since the end of the 1800s. At each grassland, sampling was conducted during the first two weeks of August in 2021, at three study locations 10 m apart from each other.

3.1.4 Field experiment on earthworm and AM fungal inoculation (paper IV)

To explore feasible ways for farmers to enhance earthworm and AM fungal diversity in their fields via inoculation, and to test the crop response to these, I performed a field experiment at seven organically managed fields in Uppland. Each field worked as a replicate, with a set-up of ten treatments. The treatments consisted of eight main treatments, which were fenced with 1×1 m metal frames, reaching down to 30 cm below and 20 cm above ground, to prevent earthworms from escaping, as well as two unframed controls. The eight framed treatments included all possible combinations of adding or not adding earthworms (EW+ and EW0), adding or not adding grassland soil (AMF+ and AMF0), and adding or not adding manure (MAN+ and MAN0). For the EW+ treatment, 30 individuals of commercially obtained *Lumbricus terrestris* individuals were added. For the AMF+ treatment, 0.9 litres of air-dried grassland soil that served as an AMF inoculum were added. A similar amount of gamma sterilized grassland soil was added in the AMF0 treatment to test if nutrients and organic matter contained in the added grassland soil had an effect on the crops. For the MAN+ treatment, 145 g of dried cow manure pellets were added, corresponding to the highest allowed level of P application in Sweden (22 kg per ha). The two unframed control treatments were set up to rule out an effect of the frame and of the sterilized soil on the soil communities and the crop. To test whether wheat varieties differ in their response to inoculation with earthworms and grassland soil, two varieties of spring wheat were sown as a sub-level treatment in all ten plots.

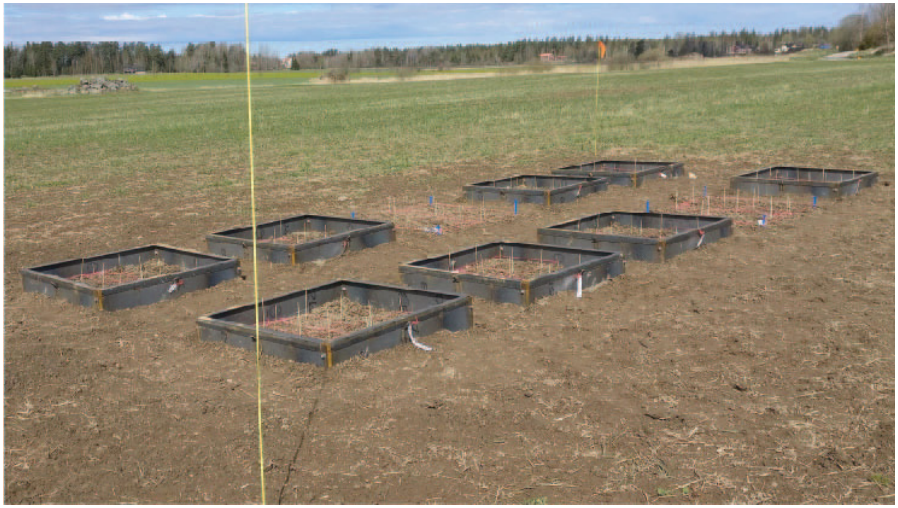

Figure 2. The experimental set-up after sowing in spring 2021, consisting of the eight framed main treatments and the two unframed controls (paper IV). Photo: Kaisa Torppa

3.2 Sampling and taxonomic determination of soil organisms

3.2.1 Earthworm density and community structure

To determine earthworm density and species composition (papers I, III and IV), I used a combination of hand-sorting and chemical extraction methodology. The same protocol was used in all studies where earthworms were included. For each sample, a pit of 30 cm (width) × 30 cm (lenght) × 20 cm (depth) was dug and all soil collected from the pit was hand-sorted for earthworms. For the chemical extraction, a solution containing allyl isothiocyanate (AITC; the chemical that gives mustard its bitter taste) was poured in each pit, to force individuals in the deeper soil layers to the surface (Zaborski, 2003). Each pit was observed for emerging earthworms for 30 minutes. All hand-sorted and chemically extracted individuals were rinsed with water and stored in ethanol for taxonomic determination.

Figure 3. (A) Earthworm hand-sorting at one of the old semi-natural grasslands (paper III). (B) An individual of *Lumbricus terrestris* emerging from the soil following application of the chemical extractant (AITC solution). (C) An *L. terrestris* individual. Photos: Nadia Maaroufi (A) and Kaisa Torppa (B and C).

Earthworms were morphologically determined in all studies using the key by Sherlock (2012). All adult individuals with a well-developed clitellum were determined to species level, whereas juveniles were determined to genus level. For paper III, all earthworm individuals, including adults and juveniles, were additionally determined using DNA barcoding. In this approach, DNA was extracted from each individual, the cytochrome oxidase subunit I (COI) gene was amplified using a mix of forward and reverse primers, and the purified PCR products were Sanger sequenced at Macrogen Europe B.V (Amsterdam, the Netherlands). After quality assessment of the sequences in Geneious 6.1.8 (Drummond et al. 2011), the sequences were assigned to species using the reference sequences in the Barcoding of Life Database (BOLD) and GenBank (NCBI).

3.2.2 AM fungal symbiosis

AM fungal symbiosis was characterized in terms of AM fungal community structure in roots and soil (papers II and IV), and by quantifying AM fungi in roots using different approaches. For paper II, AM fungal quantity in roots was characterized by AM fungal root colonization (% root length colonized) and biomass (concentration of the marker fatty acids PLFA 16:1ω5 and NLFA 16:1ω5). For paper IV, AM fungal quantity in the roots was determined using a quantitative PCR approach (AM fungal sequence counts per g root).

To determine AM fungal community structure in roots and soil, root and soil samples were collected from the experimental fields with a sterilized shovel. In the lab, the root samples were washed on a sieve, which was sterilized with bleach between each sample. Both root and soil samples were dried by storing them, within permeable bags, in a plastic bag containing silica gel. The DNA was extracted from the root and soil samples using specific extraction kits for each type of samples, and the small-subunit (SSU) ribosomal RNA gene was amplified using AM fungal specific primers. The purified samples were Illumina sequenced at Asper Biogene in Tartu, Estonia. The sequences were cleaned using the gDAT pipeline (Vasar et al., 2021), and assigned to AM fungal virtual taxa (VT) in the MaarjAM database (Öpik et al., 2010).

To determine AM fungal root colonization, part of the collected roots were air-dried and stained using trypan blue (Koske & Gemma, 1989). The proportion of root length colonized was determined using the magnified intersection method (McGonigle et al., 1990), where the stained roots are cut in one cm pieces, mounted on a microscope slide in a random order, and systematically observed with a microscope for AM fungal structures.

To determine AM fungal biomass in the roots, I used fatty acid profiling. After washing, a part of the roots collected for the community analysis was stored in -18 ˚C. Fatty acids were extracted and fractionated using the methodology in Bligh and Dyer (1959) and White et al. (1979) at the James Hutton Institute (Aberdeen, UK). As complementary indicators of AM fungal biomass in the roots, I used the concentration of both the PLFA 16:1ω5 and the NLFA 16:1ω5 (Lekberg et al., 2022; Olsson, 1999).

To quantify AM fungal structures in the roots, the number of AM fungal sequences per g of roots were determined using relative quantitative PCR (qPCR) approach (with some modifications to Voříšková et al. 2017).

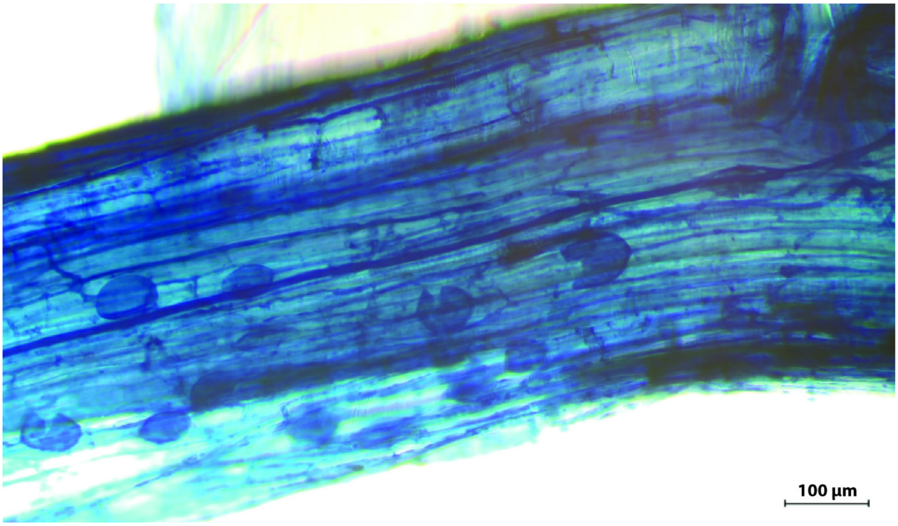

Figure 4. Stained AM fungal hyphae and vesicles (dark blue) inside a spring wheat root (paper II). Photo: Jane Oja

3.3 Environmental variables

In the different papers of my thesis, various environmental variables were measured for more complete understanding of the forces driving soil community change and crop response. Due to the experimental scale in papers I, II and IV, the moderating environmental variables were mainly soil related. In paper III, a wider scale of environmental variables were used, including soil, vegetation, management, and landscape structure.

3.3.1 Soil

Both earthworms and AM fungi are sensitive to soil structural and chemical variation, although in different ways, and agricultural management practices differently affect soil properties. Thus, the choice of soil variables measured in the experiments depended on the treatments and the organisms groups included in each paper.

In paper I, I was interested in whether the earthworm community changes, induced by tillage practices and crop rotations, were mediated via soil structural and chemical changes. Thus, soil organic matter content, soil moisture, and bulk density in the different treatment combinations were measured. The soil properties were selected based on previous knowledge of their importance for earthworm communities (Capowiez et al., 2009; Capowiez et al., 2021; Edwards & Arancon, 2022; van Vliet et al., 2007), and the potential of tillage and crop rotations to affect them (Bai et al., 2018; Hamza & Anderson, 2005).

In paper II, the changes in wheat AM fungal symbiosis could have derived from compaction-induced changes in the soil. Soil pH and P, NO_3 and NH_4 concentrations and volumetric contents were measured due to the potential effect of soil compaction on these variables, and their likely effect on AM fungal symbiosis. Soil bacterial biomass could also have responded to soil compaction, and was estimated by summing up nine commonly used PLFA biomarkers for bacteria from the soil. Soil bulk density and penetration resistance were measured as part of validation of the compaction treatment (Liu et al., 2022ab).

In paper III, the aim was to identify a range of factors driving earthworm density, diversity and community structure in old grasslands. To characterize the immediate living environment for earthworms, a number of soil parameters were measured. These parameters, hierarchically shaped by vegetation, management and landscape (see section 3.3.2), included organic matter content, C:N ratio, bulk density, water content and texture (clay, silt and sand content). We also determined the carbon and nitrogen isotopic ratios, $\delta^{13}C$ and $\delta^{15}N$, in soil organic matter. Higher values of $\delta^{13}C$ indicate plant drought stress (Klaus et al., 2016), and thus long term low water availability in the soil. Increased $\delta^{15}N$ in the soil reflects loss of inorganic N from the soil via denitrification and leaching, and is often correlated with increased N mineralization and soil fertility (Kahmen et al., 2008).

In paper IV, soil pH, N and P concentrations, texture, and organic carbon content were measured on the field level, to allow evaluating variation in soil communities between fields.

Figure 5. Soil bulk density and water content (paper I) were determined by taking a soil core of 5 cm diameter to 30 cm depth, dividing the core in three 10 cm pieces, weighing them field moist, drying them at 105 ˚C for 24 h and weighing them again. Photo: Mats Ittonen

3.3.2 Vegetation, management and landscape

In addition to the soil properties described in the previous section, we determined a range of vegetation, management and landscape parameters as potential drivers of earthworm communities in semi-natural grasslands (paper III). Soil, vegetation, management and landscape were considered to represent a hierarchy of environmental properties, where soil determines the immediate living environment for the earthworms, and landscape defines the wider scale variation in conditions, with cascading effects on management, vegetation, and soil.

Vegetation determines the quantity, quality, and variability of earthworm food resources (Piotrowska et al., 2013; Spehn et al., 2000). To describe these, we determined average vegetation height, % coverages of plant functional groups (grasses, legumes, other herbs, and mosses), and plant species richness within a circular area of three meter radius around the three study locations at each grassland.

Grassland management directly and indirectly affects vegetation and soil. The grasslands varied in the number and species of grazing animals, but were neither fertilized nor mowed. Thus, the only management related variable we determined was grazing pressure. As a basis for calculation, we used grazing animal unit, which describes the feed need of a grazing animal species relative to a dairy cow. Grazing pressure was calculated as the average of the five years preceding the sampling based on grazing animal unit, number of animals per year, size of the grazing area and the length of the annual grazing period. The data for the calculations were acquired from land managers.

Landscape properties, such as topography and soil type, influence habitat variability, land-use, and movement of organisms. We considered landscape especially from the perspective of earthworm habitat area and variability, determining the earthworm species pool in a certain area (Decaëns et al., 2008). To describe the extent of grassland habitat and the variability of habitat types around the sampling locations we determined the coverages of habitat types within different radii around them. This was done with the help of a GIS based classification BIOTOP SE (Skånes, in preparation), which combines data derived from aerial photos with property and land cover data from the various national databases (the Swedish Board of Agriculture, the Swedish Environmental Protection Agency, and the Land Survey). Based on the habitat coverages, we calculated the coverage of grassland habitat and habitat heterogeneity, which we defined as the Shannon diversity of habitat types within a certain radius. To select the radius for the calculations, we compared the Akaike information criterion (AIC) of the models testing the effects of the full set of uncorrelated environmental variables on earthworm density, with grassland coverage or habitat heterogeneity determined for different radii around the sampling locations. As expected for the low mobility of earthworms (Eijsackers, 2011), the final radius for both variables was small, 25 m and 20 m for grassland coverage and habitat heterogeneity, respectively.

3.4 Crop responses

In papers II and IV, I was interested in crop responses to the variation in soil organisms. In paper II, I explored the co-variation in AM colonization with P uptake and yield in different spring wheat varieties, and in paper IV, I tested whether addition of earthworms, grassland soil or manure, individually or in combination, changed nutrient concentrations or yield in spring wheat, and whether the potential changes depended on wheat variety.

For paper II, P concentration (g/kg), P:N ratio, P content (g/m^2) and yield were determined. P and N concentrations were analysed from the shoots at Agrilab, Uppsala, and P concentration was converted to P content based on shoot dry weight per m^2. The yield was determined by harvesting a 2 × 6 m area in the centre of each plot four months after sowing.

For paper IV, P and N concentration in wheat shoots at flowering, as well as grain mass per m^2 and average head biomass, as indicators of yield, were determined. The nutrient concentrations were determined from the shoots, associated to the roots that were collected for the AM fungal analyses, at Agrilab, Uppsala. Grain mass per m^2 was determined by harvesting the heads of the two wheat varieties separately from the respective 15 x 30 cm harvest areas in each plot. Average head biomass was calculated by dividing the total grain mass by the number of heads per harvest area.

4. Results and discussion

In this thesis, I explore and present ways to manage two functionally important groups of soil organisms, earthworms and arbuscular mycorrhizal fungi, with the aim to enhance the soil functions they provide and sustainably support agricultural production.

In paper I, I found reduced tillage intensity to be especially important for density of the large anecic earthworms and total bioturbation, while a diversified crop rotation was more important for endogeic earthworms and total earthworm density. In paper II, I showed that the effect of soil compaction on spring wheat AM colonization depends on the wheat variety, and may be larger for varieties that are characterized by root traits associated with poor P uptake. In paper III, I showed that earthworm density and diversity in semi-natural grasslands increase with higher long-term soil moisture conditions and soil fertility. In addition, DNA barcoding revealed nearly twice as many species as morphological species determination. In paper IV, I found support for the possibility to manipulate earthworm and AM fungal communities in agricultural fields via inoculation with commercially obtained *Lumbricus terrestris* earthworms and grassland soil. However, the consequences of these inoculations were context dependent and partly undesirable from the production perspective.

4.1 Varying effects of tillage and crop rotations on earthworm ecological groups and bioturbation

Diversification of a crop rotation mitigated the negative effect of intensive tillage on total earthworm density (paper I). The mitigation effect was mostly a result of endogeic earthworms benefitting from a diverse crop rotation. Anecic earthworm density and bioturbation were mainly determined by tillage intensity, and clearly highest under no tillage. Reduced tillage intensity may thus be necessary to maximize earthworm mediated soil functions. However, earthworm mediated soil functions can also be enhanced via increasing endogeic earthworms with the help of a diversified crop rotation, when withdrawing from intensive tillage is not feasible.

The differing responses of endogeic and anecic earthworms to tillage intensity and crop rotation are explained by the differences in the feeding and burrowing activity of these ecological groups (Briones & Schmidt, 2017). As endogeics feed in mineral soil without making permanent burrows, and anecics feed in the litter layer, using their deep burrow systems to avoid unfavourable conditions at the soil surface (Bouché 1977; Lavelle 1988), a stronger limiting effect of food resource availability on endogeics, and soil disturbance on anecics, can be assumed. Indeed, the detrimental effect of intensive tillage on anecic earthworms is well supported by previous studies (Chan 2001; Briones & Schmidt, 2017), and some authors have suggested, that agricultural management practices that affect organic matter inputs to the soil, such as organic amendments and crop rotations, may be especially important for endogeic earthworms (Simonsen et al., 2010; Ashworth et al., 2017).

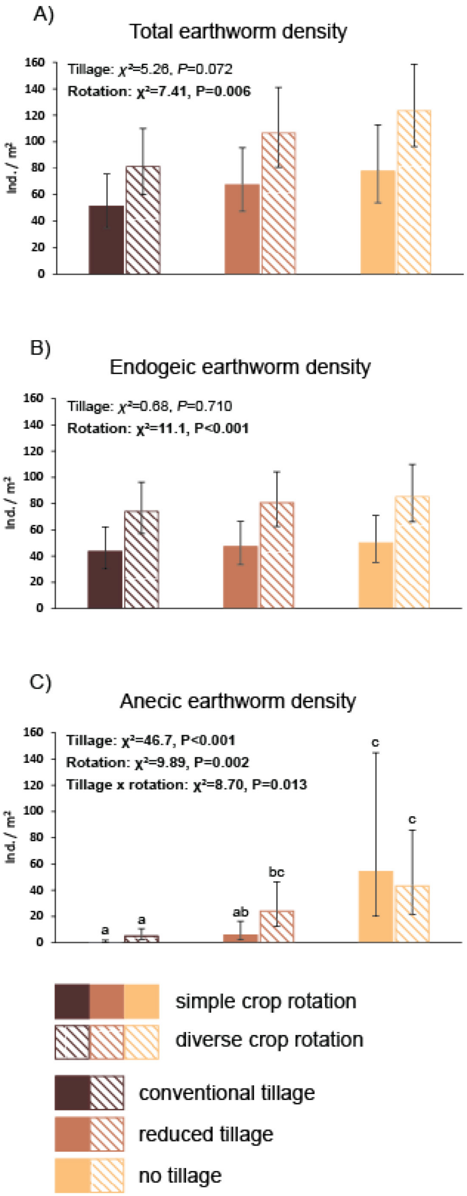

Figure 6. Estimated marginal means for A) total earthworm densities, B) densities of endogeic earthworms, and C) densities of anecic earthworms in the different treatments with 95% confidence intervals. For anecic earthworms (C), columns sharing the same letters are not significantly different (Tukey's HSD test, P > 0.05). χ2 and P values for the explanatory variables are presented, and the statistically significant (P < 0.05) variables are in **bold** font. Figure adapted from paper I.

In our study, the simple crop rotations only consisted of cereals, whereas the diverse crop rotation included oil seed rape and peas in addition to a cereal. As the preceding crop in the diverse crop rotation was peas, it is likely that the beneficial effect of the diverse crop rotation on total and endogeic earthworm density was largely due to the good quality residues, with low C:N ratio, of peas. Indeed, including a legume in a crop rotation has been shown before to increase earthworm densities (Hubbard et al., 1999; Rodríguez et al., 2020; Schmidt et al., 2003).

In our approach, bioturbation describes the amount of soil egested and transported by all earthworms in a certain area and time, and thus reflects the overall functional effect of the total earthworm community (Taylor et al., 2019). It does not differentiate between functional effect types, which vary between species and ecological groups (Huang et al., 2020; Sheehan et al., 2006). Despite of this, the high contribution of anecic earthworms to total bioturbation suggests a major contribution of this group to earthworm mediated soil functions. Although more experimental evidence of the functional roles of different earthworm ecological categories is necessary, it is likely that both reduced tillage intensity and crop rotation diversification are needed to maximize earthworm functional effects in the soil.

4.2 Compaction effect on wheat AM fungal colonization depends on the variety

Soil compaction increased AM fungal colonization in one of the spring wheat varieties, 'Alderon', whereas in the other four varieties colonization was the same in both compacted and non-compacted soil (paper II). 'Alderon' is characterized by root traits that indicate poor P uptake (Lynch, 2019), having the thickest, the fewest and the most deep-reaching roots of the tested varieties (Liu et al., 2022b). This suggests that a variety with roots that are poorly adapted for P uptake may be more dependent on AM fungal aided P uptake than more P efficient varieties, and that this pattern may be stronger in compacted soil. However, the pattern requires verification with other P inefficient varieties.

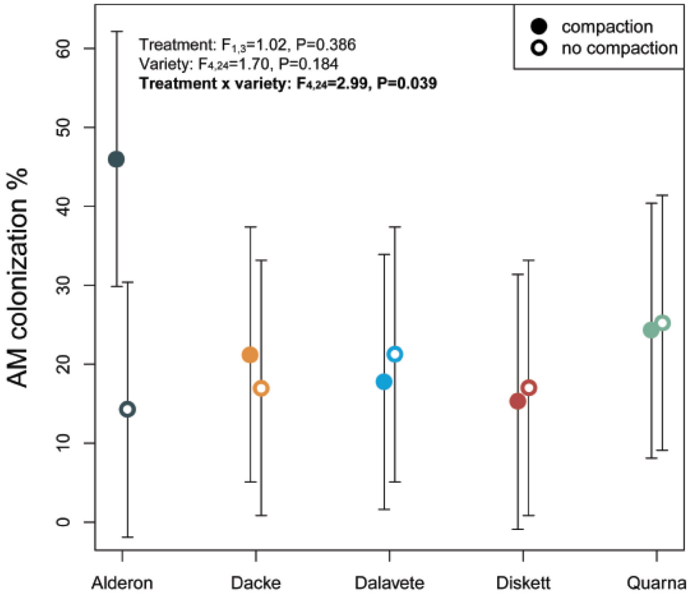

Figure 7. Estimated marginal means for AM fungal colonization in the roots of the five spring wheat varieties in the compaction (filled circles) and the non-compaction (empty circles) treatments with 95% confidence intervals. The figure is adapted from paper II.

P concentration and P:N ratio increased with AM fungal colonization in two of the varieties, ‘Alderon’ and ‘Diskett’. P:N ratio in the two varieties also increased with AM fungal biomass in the roots, and the total P content increased with AM fungal colonization and biomass in ‘Diskett’. This suggests that these two varieties are AM responsive, i.e., that they benefit from the symbiosis (Janos, 2007). However, the two varieties also differ from each other in many ways. While ‘Alderon’ is characterized by P inefficient roots, that may be more suitable for N uptake (i.e. deep roots; Lynch 2019), ‘Diskett’ has intermediate roots that likely work well for both P and N uptake. Thus, AM fungal responsiveness in ‘Diskett’ may be driven by something else than poor P uptake by the roots. Simultaneously, ‘Diskett’ is the most cultivated spring wheat variety in Sweden (Jordbruksverket, 2019), and especially appreciated among farmers due to its stable yields (Pernilla Vallenback, pers. comm.). Although more research is necessary to verify this, it could be speculated that the stable yields in ‘Diskett’ are due to complementary use of roots and AM fungal symbiosis in nutrient uptake, which would make the variety more tolerant to variable environmental conditions. Detecting AM responsiveness in ‘Diskett’ is also encouraging for breeding AM responsive crop varieties, as it suggests that AM mediated nutrient uptake does not need to come with increased AM dependency.

4.3 Earthworm communities in old semi-natural grasslands are driven by soil moisture and productivity

We detected high variation in earthworm density and diversity between semi-natural grasslands (paper III). Both earthworm density and diversity were highest in grasslands characterized by high soil moisture and low C:N ratio. Thus, moist and fertile semi-natural grasslands likely serve best for earthworm conservation and as source habitats for re-colonization of cultivated soils. This is a contrasting pattern compared to plants, for which dry, unproductive grasslands increase diversity and provide important habitat for species specialized to these conditions (Löfgren et al., 2020). This highlights the importance of a variety of grassland types being present in the landscape for biodiversity conservation, and suggests that different groups of organisms with varying ecological preferences, including soil organisms like earthworms, should be considered in conservation policies.

Earthworm diversity also increased with increasing small-scale habitat heterogeneity, and the proportion of epigeic earthworms increased with higher soil moisture and soil organic matter content. It is known from previous research, that habitat heterogeneity affects earthworm communities, and that earthworm species, due to habitat heterogeneity, typically show aggregated distribution patters (Decaëns et al., 2008; Decaëns & Rossi, 2001; Jiménez et al., 2014; Richard et al., 2012). It is also known that epigeic earthworms commonly dominate in habitats where litter quality or soil pH prevents endogeic and anecic earthworms from consuming the litter layer, such as boreal forests (De Wandeler et al., 2016). It is possible that when organic matter content is high in our study, litter quality is less suitable for endogeic and anecic species, although more research is necessary to verify this. This would explain the higher proportion of epigeics in these conditions, and why there is no increase in overall earthworm density and diversity with increasing soil organic matter content.

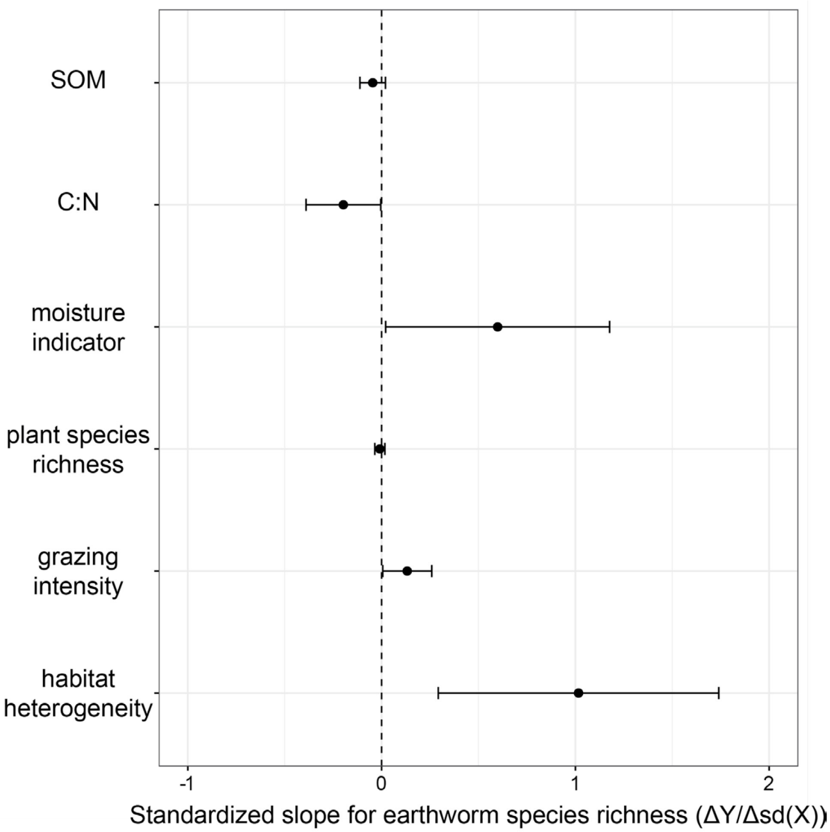

Figure 8. Effect size for the change in earthworm species richness in response to changes in SOM, soil C:N ratio, soil pH, the moisture indicator, plant species richness, grazing intensity, and habitat heterogeneity. Standardized slopes and 95% confidence intervals are shown. The effect sizes were calculated normalizing the variables by subtracting the mean and dividing by the standard error. The figure is adapted from paper III.

Nearly twice as many earthworm species were determined by DNA barcoding as when using morphological determination, due to the ability of DNA barcoding to determine also juvenile individuals and cryptic species. This suggests that when earthworms are morphologically identified, which still is the most commonly used method (but see Richard et al. 2010; Maggia et al. 2021; Lilja et al. 2023), earthworm species richness is often underestimated. Increasing the use of DNA barcoding in earthworm ecological and conservational studies would greatly improve their resolution, and enable determining distribution patterns and habitat preferences also for sparsely occurring species.

4.4 Complex crop responses to modified earthworm and AM fungal communities

We found it possible to modify earthworm and AM fungal communities in agricultural fields via inoculation with commercially obtained *L. terrestris* and with grassland soil. After one field season, the density of *L. terrestris* was higher when this species was added in spring and, depending on other treatments, soil AM fungal richness was higher when grassland soil was added (paper IV). However, we found no evidence of long-term establishment of the added earthworms, and application of grassland soil only increased soil AM fungal richness under the variety Diskett, when also earthworms and manure were added. Furthermore, application of grassland soil reduced AM fungal richness in wheat roots. Based on this study, we cannot give a mechanistic explanation for the observed patterns in AM fungal richness. Nevertheless, we suspect that application of grassland soil may have increased weed density and diversity, potentially with secondary effects on AM fungal richness in soil and wheat roots, via offering alternative hosts to AM fungal taxa (Kiers et al., 2011).

Inoculation with earthworms also affected AM fungi, and the grassland soil application affected earthworms, depending on the other treatments. Inoculation with earthworms reduced soil AM fungal richness under Quarna, when no manure and grassland soil was applied, and application of grassland soil reduced total earthworm density. Earthworms might have reduced AM fungal richness by feeding on their spores and hyphae. The effect of grassland soil application on earthworms could have derived from increased weed density, as high root density has been shown to be detrimental for earthworms (Eisenhauer et al., 2009). However, this needs to be confirmed with further research, where weeds are better monitored. The potentially negative effect of earthworms on AM fungi may compromise the synergistic effects of the two groups on crop performance. Thus, focusing on enhancing the diversity of one of the groups according to case-specific needs may be advisable.

Crop responses to inoculation with earthworms and application of grassland soil were context dependent. Inoculation with earthworms increased average head mass of the variety Quarna when earthworms were added together with manure. Earthworm addition together with manure but without grassland soil increased wheat P concentration at flowering, but earthworm addition together with both manure and grassland soil decreased wheat N and P concentrations. If application of grassland soil increased weeds, the reduced nutrient concentrations, when earthworms and grassland soil were applied together, could have derived from better competitive ability of weeds to take up nutrients mineralized by the worms. Application of grassland soil reduced total grain mass per m^2 and average mass per head, and increased wheat shoot N and P concentration at flowering. If the reduced grain and head mass indicate reduced wheat growth in general, the increased shoot nutrient concentrations may have been due to smaller plants, e.g., due to competition with weeds for something else than nutrients such as water or light.

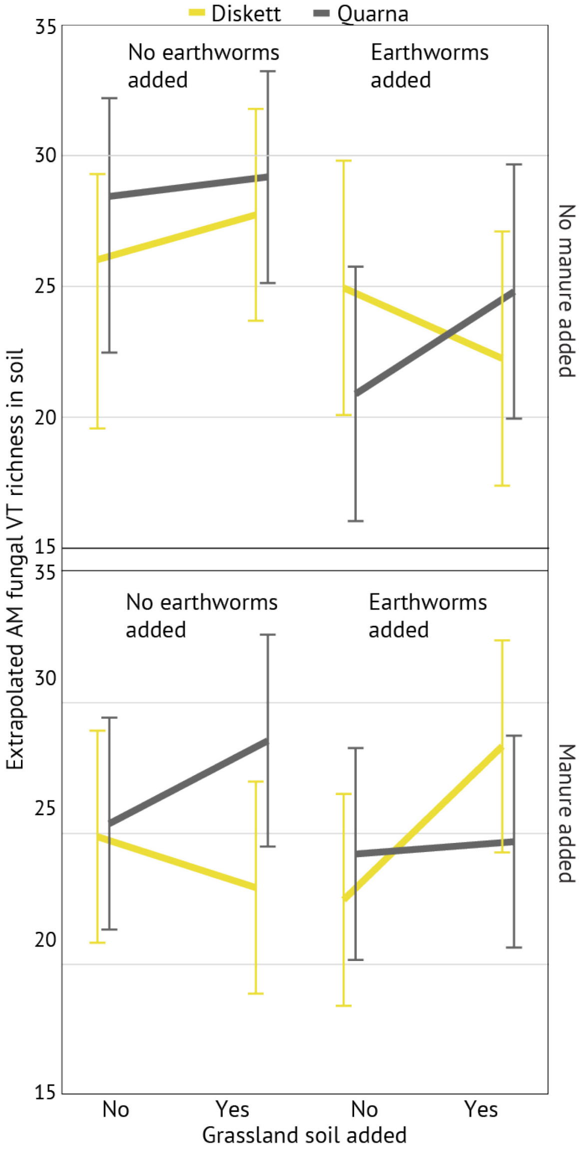

Figure 9. The four-way interaction effect of application of grassland soil, inoculation with earthworms, application of manure and variety on extrapolated richness of AM fungal virtual taxa in soil. The estimated marginal means and 95% confidence intervals are shown. The figure is adapted from paper IV.

5. Conclusions and recommendations

Supporting soil biodiversity for sustainable agriculture is a challenge due to the complexity of soil communities and their functional roles, and the context dependency of management effects on both of them. There are still large gaps in knowledge regarding taxonomy and ecology of soil organisms, functional roles of different species in different contexts, and the best practices for promoting beneficial soil functions in a targeted way. In this thesis, I have contributed to narrowing these knowledge gaps by studying management and functional roles of two key groups of soil organisms, earthworms and arbuscular mycorrhizal fungi.

In line with previous research (Briones & Schmidt, 2017; Chan, 2001; Pelosi et al., 2014a), I show that reduced tillage intensity may be necessary to maximize earthworm-mediated functions in agricultural soils (**paper I**). Simultaneously, increased quantity or quality of organic matter inputs, e.g., in the form of a diversified crop rotation, is highly beneficial for less tillage-sensitive species, which also provide important soil functions. To encourage farmers to support earthworm communities in their soils, it is important to highlight that there are different options for doing that. Thus, in addition to reduced tillage, more emphasis should be put on practices affecting organic matter inputs as means for farmers to support earthworm communities in their soils. From the research point of view, I recommend more emphasis on the mechanistic understanding of crop rotation effects on earthworms. Monitoring variation in earthworm communities at different stages of various crop rotations would be necessary to disentangle the roles of different crop species, and the persistence of their effects for earthworm communities, and to determine the optimal rotation for maximized earthworm mediated soil functions. Comparing single and combined effects of high and low-quality residue inputs on earthworms would provide useful information about the roles of resource quality and continuity for earthworms. To complement bioturbation as a quantitative estimation of earthworm functional importance, I also recommend more experimental field studies on the qualitative functional roles of earthworm species from distinct ecological categories, as this topic has mainly been studied in mesocosms, and the results may not be transferable to field conditions.

Reducing mineral nutrient inputs with the help of AM fungal symbiosis requires a better understanding of the drivers of AM responsiveness and nutrient uptake in globally important crops such as wheat (Berger & Gutjahr, 2021; Pellegrino et al., 2015). In **paper II**, I found two interesting results potentially related to the relationship between root traits and AM fungal responsiveness in wheat varieties. First, I found increased AM fungal colonization due to soil compaction in the variety 'Alderon', characterized by root traits indicating poor P uptake efficiency. Second, I found an indication of AM responsiveness, combined with intermediate root traits, that likely work for both P and N uptake, in the most cultivated spring wheat variety in Sweden, 'Diskett'. The first result suggests that P inefficient wheat varieties may be more dependent on AM fungal P uptake in compacted than non-compacted soil, likely due to limited access to soil P by their roots. The second result suggests that AM responsiveness in wheat does not necessarily indicate an increased AM dependence, which would be good news for the potential to breed AM responsive wheat varieties without compromising wheat nutrient uptake via roots. It also allows for speculation, whether sustained AM fungal responsiveness together with intermediate root traits in a wheat variety increases flexibility in nutrient uptake strategy under different conditions, which could be reflected in more stable yields. Due to the speculative nature but potentially high importance of both results, I recommend further research, where more varieties with similar characteristics are compared. If generalizable, the results provide important information about the factors driving AM fungal responsiveness in wheat, and suggest that AM fungal responsiveness should be considered in wheat breeding as a potential way to improve nutrient uptake under varying conditions.

In **paper III**, I provide important information for earthworm conservation by showing that higher soil moisture, soil fertility and small-scale habitat heterogeneity drive earthworm diversity in semi-natural grasslands. The pattern contrasts with plant diversity, as dry, unproductive grasslands serve as an important habitat for plant species adapted to those conditions. Our finding is in line with previous research, which has shown that below-ground and above-ground biodiversity do not always match (Cameron et al., 2019). It also highlights the need for better investigation of below-ground biodiversity, to be able to include it in conservation policies (Guerra et al., 2022, 2020; Zeiss et al., 2022). The results from **paper III** are also important because of the high resolution in species determination using DNA barcoding, which allowed determination of also juvenile earthworms and cryptic species. Our estimations of local earthworm species richness are more precise than in most earlier studies (but see Maggia et al. 2021; Lilja et al. 2023), which have relied on morphological species determination. Wider adoption of DNA barcoding for earthworm species determination would highly benefit earthworm ecological research, and be especially important for earthworm diversity studies, and monitoring of species distributions.

Manipulation of soil communities via inoculation may help to restore soil biodiversity and increase abundance of beneficial soil organisms where they have been severely reduced (Bender et al., 2016, 2017; Jouquet et al., 2014; Rillig et al., 2016). For the inoculation to result in crop benefits, a good understanding of the establishment of the added organisms and their relationship with the target crops are necessary. To avoid potential environmental risks associated with spreading organisms of foreign origin, interactions between the added organisms and the native biota should be studied, and native inocula should be used when possible (Hart et al., 2017; Jouquet et al., 2014). Furthermore, inoculation methods should be feasible and cheap to be adopted by farmers. As shown by the complex results from **paper IV**, achieving all these requirements may be difficult due to the complexity of soil systems. Based on **paper IV**, the biggest uncertainties for earthworm addition using commercially obtained *Lumbricus terrestris* are related to long-term establishment of these potentially locally adapted forms of the species, and the strong context dependency of the benefit of inoculation for crop yield. Although we managed to increase AM fungal richness in the soil via application of grassland soil, the consequences of doing this for crops and soil ecosystems are likely even more difficult to predict than those from earthworm addition. The highly context dependent effects of application of grassland soil on AM fungal diversity in soil and wheat roots, and on earthworm density cannot be mechanistically explained by this study. They could derive partly from increased weed density or diversity, but as weeds were not monitored in this study, their role in AM fungal benefit for crop species needs to be investigated by further research. The specific needs of crop species and varieties may often vary depending on the context, and the functional effects of earthworms and AM fungi differ from each other and depend on the composition of their communities (Milleret et al., 2009). Thus, increasing diversity of either group in a non-targeted manner is unlikely to lead to predictable crop benefits. I recommend more research on mediating factors of earthworm benefit on crop growth, the effect of weeds on AM fungal symbiosis in crops, and the relative importance of earthworm and AM fungal functions depending on the specific needs of different types of crop species.

Multiple ways exist to support earthworm and AM fungal communities in agricultural soils. Adopting less intensive agricultural management practices, such as reduced tillage and diverse crop rotations, is recommended as it preserves and increases soil biodiversity as a whole, resulting in overall healthier soils. When soil communities are severely degraded, however, adjusting agricultural management will only result in increased diversity, when source populations exist at realistic migration distances from the fields. Indeed, conservation of biodiversity in agricultural soils should include the landscape perspective to be successful. There is also potential for more targeted manipulation and use of functionally important soil communities for crop benefits. However, this requires better mechanistic understanding about the factors that affect soil biological and symbiotic functions and their effects on various crops.

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Popular science summary

Soil is home to millions of species of microorganisms and invertebrates. Via moving, feeding, and interacting with each other, soil organisms take care of important soil functions and constitute an integral part of healthy soils. They decompose dead organic material, make nutrients available for plants, and maintain good soil structure – all functions that are especially important for agriculture. At the same time, intensive agricultural practices, such as ploughing, high inputs of mineral fertilizers and pesticides, and low crop diversity, are detrimental to soil organisms, reducing their numbers and species diversity. This, in turn, leads to reduced soil functioning. Managing agricultural soils in a way that supports the life in soil could improve agricultural sustainability by replacing energy and chemical inputs with ecosystem services provided by soil organisms.

Some groups of soil organisms are considered especially beneficial for agriculture. Earthworms, for example, have such a large effect on soil chemical and physical properties that they are considered ecosystem engineers (species that modify the ecosystem in such a profound way that all organisms are affected). Via their feeding and movement, earthworms bioturbate, i.e., burrow in and mix the soil, shaping the living conditions of other soil organisms and regulating root growth conditions and nutrient availability for plants. Indeed, crop yields are on average 25% higher when there are earthworms in the soil compared to when they are lacking. Another important group of soil organisms is mycorrhizal fungi. These fungi live in a symbiosis with plants, i.e., they collaborate with plants by exchanging nutrients that they take up with their hyphal networks for plant photosynthetic carbon. In agricultural soils, the most common type of mycorrhizal fungi is arbuscular mycorrhizal (AM) fungi, which grow their hyphae inside their host plants cells, forming branched structures called arbuscules, where the nutrient to carbon exchange happens. In addition to enhanced nutrient uptake, these fungi can help their host plant by protecting them from drought, diseases and pests.

Supporting earthworm and AM fungal communities in agricultural soils could help reduce fertilizer and pesticide inputs and contribute to a more sustainable agriculture. However, this requires better understanding of which properties of earthworm and AM fungal communities to enhance and how, and in which conditions the community changes result in benefits for crops. In this PhD thesis, I have contributed to this understanding by exploring 1) how tillage and crop rotations can be adjusted to support earthworm communities and bioturbation, 2) whether soil compaction affects AM fungal symbiosis in different spring wheat varieties, 3) whether semi-natural grasslands serve to sustain high earthworm numbers and diversity in agricultural landscapes, and 4) whether earthworm and AM fungal communities in field soil can be manipulated by inoculating with commercially obtained bait earthworms and grassland soil.

I show that earthworm numbers in agricultural fields can be increased regardless of tillage intensity by including peas, or potentially another legume, in the crop rotation. However, it is necessary to combine a diverse crop rotation with reduced tillage to maximise total earthworm diversity and bioturbation, as certain functionally important earthworm species are very sensitive to ploughing. I also show that soil compaction, e.g., due to the use of heavy machinery, can affect AM fungal symbiosis in wheat but that the effect depends on wheat variety. In compacted soil, those wheat varieties that are not so efficient in taking up nutrients with their roots may be more dependent on AM fungal nutrient uptake than other varieties. I found earthworm numbers and species diversity to vary widely between semi-natural grasslands, and detected nearly twice as many species when they were determined based on their DNA than on their visible characteristics. Earthworm abundance and species diversity were highest in moist and fertile grasslands, and species diversity increased also with higher small-scale habitat variability. Semi-natural grasslands characterized by these properties would thus serve to sustain high earthworm diversity in agricultural landscapes. As earthworms are most often determined based on their visible characteristics, adopting DNA-based identification would greatly improve accuracy in earthworm diversity estimates. I also found it possible to manipulate earthworm and AM fungal communities in field soil by application of commercially obtained bait earthworms and grassland soil. Earthworm application also showed beneficial effects on wheat growth, but this largely depended on wheat variety and manure application. Furthermore, the increased AM fungal diversity in the soil due to application of grassland soil was not reflected in the AM fungal symbiosis with wheat or in wheat growth.

Multiple ways exist to support earthworm and AM fungal communities in agricultural soils. Adopting less intensive agricultural management practices, such as reduced tillage and diverse crop rotations, is probably the best way to do this as it likely increases soil biodiversity as a whole, resulting in overall healthier soils. However, there is also potential for more targeted manipulation of earthworm communities and AM fungal symbiosis for crop benefits. For example, breeding crop varieties that can flexibly use both their roots and AM fungal symbiosis for nutrient uptake under various conditions could increase nutrient uptake efficiency and yield stability. It may be possible to restore earthworm and AM fungal diversity by adding commercially obtained individuals of tillage sensitive species or grasslands soil with a rich AM fungal community. However, more research is necessary to ensure lasting effects of these additions without unwanted biological consequences, and to unravel which species are most important to promote for agricultural benefits in different situations.

Populärvetenskaplig sammanfattning

Miljontals arter av mikroorganismer och ryggradslösa djur lever i marken. Genom deras rörelse i marken, födointag och interaktioner mellan varandra, tar markorganismer hand on viktiga funktioner i marken och de är en viktig del av bördiga och välmående jordar. Markorganismerna bryter ned dött organiskt material, gör näringsämnen tillgängliga för växter och bibehåller en fördelaktig jordstruktur – funktioner som är särskilt viktiga för jordbruket. Men intensiva jordbruksmetoder, såsom plöjning, storskalig användning av mineralgödsel och bekämpningsmedel och låg diversitet av grödor, skadar markorganismerna, vars antal och artmångfald då minskar. Detta leder i sin tur till minskade markfunktioner och därmed ekosystemtjänster. Att bruka jordar på sätt som stödjer markorganismerna kan öka jordbrukets hållbarhet, då tillförsel av konstgjorda kemiska ämnen och energi ersätts av markorganismers ekosystemtjänster.

Vissa grupper av markorganismer är särskilt nyttiga för jordbruket. Daggmaskar, till exempel, anses vara ekosystemingengörer (arter som påverkar ekosystemet på så omfattande sätt att hela organismsamhället påverkas) p.g.a. sin påverkan på jordens biologiska, kemiska och fysikaliska egenskaper. Effekten av daggmaskarnas rörelse i jorden kallas bioturbation och påverkar levnadsförhållandena för övriga markorganismer samt reglerar förhållandena för växternas rottillväxt och näringsåtkomst. Skörden från grödor har visat sig vara i medeltal 25% högre i jordar med daggmaskar än i jordar utan daggmaskar. En annan viktig grupp av markorganismer är arbuskulära mykorrhiza (AM) svampar. Deras hyfer växer inuti celler i värdväxtens rötter och bildar förgrenade strukturer, arbuskler, i vilka växten och svampen utbyter kol och näringsämnen. Förutom att förbättra näringsupptaget, kan dessa svampar hjälpa sina värdväxter genom att skydda dem mot torka, sjukdomar och skadedjur.

Genom att gynna daggmaskar och AM-svampar kan man minska behovet av insatsmedel som mineralgödsel och bekämpningsmedel och, på så sätt, främja ett mer hållbart jordbruk. Detta kräver dock mer kunskap om vad som behöver främjas samt under vilka förhållanden som sådana åtgärder faktiskt gynnar grödorna. I denna doktorsavhandling har jag bidragit till detta genom att undersöka 1) hur plöjning och växtföljder skulle kunna förändras för att främja daggmaskar och bioturbation, 2) huruvida ökad markpackning påverkar AM-symbios i olika vårvetesorter, 3) om naturbetesmarker kan bidra till att bibehålla stor mängd och hög mångfald av daggmaskar i jordbrukslandskapet och 4) huruvida samhällen av daggmaskar och AM-svampar i åkerjord kan manipuleras genom att tillsätta kommersiellt tillgängliga daggmaskar som säljs för användning som fiskebete, respektive tillsätta jord från gräsmarker rika på AM-svampar.

Jag visar i min avhandling att individantalet av daggmaskar kan, oavsett plöjningsintensitet, ökas genom att inkludera ärt, eller möjligen andra baljväxter, i växtföljden. Men för att maximera diversiteten av daggamskar och deras bioturbation är det viktigt att kombinera varierade växtföljder med minskad plöjning, eftersom vissa daggmaskarter med viktig funktion i jorden är mycket känsliga för plöjning. Jag visar även att markpackning, som t.ex. orsakas av användning av tunga maskiner, kan påverka symbiosen mellan AM-svampar och vete, men att denna effekt är olika för olika vetesorter. I packad jord kan vetesorter med ineffektivt näringsupptag via rötterna bli mer beroende av näringsupptag via AM-svampar än vad andra vetesorter är.

I olika naturbetesmarker fann jag mycket varierande antal och artdiversitet av daggmaskar, och med hjälp av DNA-identifiering kunde nästan dubbla antalet arter identifieras jämfört med traditionella metoder baserade på synliga skillnader. Daggmaskars antal och artdiversitet var högst i fuktiga och näringsrika naturbetesmarker, och dessutom var artmångfalden högre i mer varierande gräsmarker, när variabiliteten mättes på för det mänskliga ögat liten skala. Naturbetesmarker med dessa egenskaper kan därmed hjälpa till att bibehålla daggmaskdiversiteten i jordbrukslandskapet. Oftast identifieras daggmaskar på basis av deras synliga egenskaper, men mina resultat visar att identifiering med hjälp av DNA kan öka noggrannheten markant gällande mångfalden av arter. Utöver detta, fann jag att det är möjligt att manipulera samhällen av daggmaskar och AM-svampar i åkerjord genom att tillföra kommersiellt tillgängliga fiskebetesdaggmaskar samt gräsmarksjord innehållandes AM-svampar. Tillförsel av daggmaskar kunde också gynna vetets tillväxt, men sådana effekter var beroende av vetesort och tillförsel av kogödsel. Den högre mångfalden av AM-svampar i marken efter tillförsel av gräsmarksjord reflekterades varken i AM-symbiosen mellan vetet och svampen eller i vetets tillväxt.

Det finns flera sätt att gynna daggmaskar och AM-svampar i jordbruksmarker. Att ta i bruk mindre intensiva jordbruksmetoder, såsom minskad plöjning och mer varierande växtföljder, är antagligen det bästa sättet, då det sannolikt ökar den biologiska mångfalden och därmed jordens hälsa som helhet. Det finns dock potential för mer riktad manipulation av daggmask- och AM-svampsamhällen för att gynna grödor. Till exempel skulle grödors näringsupptag och skördens stabilitet kunna ökas genom att man förädlar grödsorter som flexibelt kan utnyttja både sina rötter och AM-symbios för näringsupptag under olika förhållanden. Mångfalden av daggmaskar och AM-svampar skulle möjligen kunna restaureras med hjälp av tillförsel av relevanta daggmaskarter eller gräsmarksjord som är rik på AM-svampar, men mycket mer forskning krävs dels för att kunna säkerställa att tillförseln har bestående effekter utan oönskade biologiska konsekvenser, och dels för att reda ut vilka arter som är viktigast att nyttja för jordbruket i olika situationer.

Populaaritieteellinen tiivistelmä

Maaperässä elää miljoonittain mikrobeja ja selkärangattomia eläimiä, jotka ovat olennainen osa tervettä maata. Nämä maaperäeliöt huolehtivat maan tärkeistä toiminnoista liikkumalla ja syömällä maaperässä ja olemalla vuorovaikutuksessa toistensa kanssa. Ne hajottavat kuollutta eloperäistä ainesta, vapauttavat ravinteita kasvien käyttöön ja ylläpitävät maan mururakennetta – kaikki erityisen tärkeitä toimintoja maataloudelle. Samaan aikaan voimaperäiset maatalousmenetelmät, kuten kyntäminen, keinolannoitteet, torjunta-aineet ja vähälajiset viljelykierrot, ovat haitallisia maaperäeliöille, vähentäen niiden määrää ja lajirikkautta. Tämän taas on havaittu johtavan heikentyneisiin maaperän toimintoihin. Maatalouden kestävyyttä voitaisiin parantaa hoitamalla maata siten että se tukee maaperän eliöstöä, jolloin energian ja kemikaalien käyttöä voitaisiin mahdollisesti korvata maaperäeliöiden tuottamilla ekosysteemipalveluilla.

Tiettyjä maaperäeliöitä pidetään erityisen hyödyllisinä maataloudelle. Esimerkiksi lierojen maata muokkaavalla bioturbaatiolla on niin suuri vaikutus maan kemiallisiin ja fysikaalisiin ominaisuuksiin, että niitä pidetään ekosysteemi-insinööreinä. Liikkumalla ja ruokailemalla maassa, lierot muovaavat muiden maaperäeliöiden elinolosuhteita sekä säätelevät kasvien juurten kasvuolosuhteita ja ravinteiden saatavuutta. Kun maassa on lieroja, satojen on osoitettu olevan keskimäärin 25% korkeampia kuin lierojen puuttuessa. Toinen tärkeä maaperäeliöryhmä on mykorritsasienet. Nämä sienet elävät symbioosissa, eli tekevät yhteistyötä, kasvien kanssa vaihtamalla rihmastonsa avulla keräämiään ravinteita kasvien sitomaan hiileen. Maatalousmaassa tärkein mykorritsasieniryhmä on arbuskelimykorritsasienet. Nämä sienet ovat saaneet nimensä siitä, että ne muodostavat kasvien juurisolujen sisään haarautuvia rihmastoja eli arbuskeleita, joiden kautta ravinteet vaihdetaan hiileen. Tehostetun ravinteiden oton lisäksi nämä sienet voivat auttaa isäntäkasviaan suojautumaan kuivuudelta, taudinaiheuttajilta ja tuholaisilta.

Ravinne- ja torjunta-ainemääriä voitaisiin mahdollisesti vähentää tukemalla liero- ja arbuskelimykorritsasieniyhteisöjen tuottamia ekosysteemipalveluja. Tämä edellyttää kuitenkin parempaa ymmärrystä yhtäältä siitä, mitä liero- ja arbuskelimykorritsasieniyhteisöjen ominaisuuksia tulisi tukea ja miten niitä parhaiten tuetaan ja toisaalta siitä, missä olosuhteissa lierot ja arbuskelimykorritsasienet hyödyttävät tuotantokasveja. Olen tässä väitöskirjassa perehtynyt näihin teemoihin tutkimalla 1) millainen maanmuokkaus ja viljelykierto tukee lieroyhteisöjä ja niiden harjoittamaa bioturbaatiota, 2) vaihteleeko maan tiivistymisen vaikutus arbuskelimykorritsasymbioosiin vehnälajikkeiden välillä, 3) millaiset luonnonlaitumet tukevat parhaiten korkeita lieromääriä ja lierojen lajirikkautta maatalousympäristöissä, ja 4) voiko liero- ja arbuskelimykorritsasieniyhteisöjä muokata lisäämällä syöttimatoja ja niittymultaa peltomaahan.

Osoitan että lierojen määrää peltomaassa voi lisätä maanmuokkauksen voimakkuudesta riippumatta monipuolistamalla viljelykiertoa esimerkiksi hernekasvien avulla. Maanmuokkauksen voimakkuuden vähentäminen yhdistettynä monipuoliseen viljelykiertoon on kuitenkin tarpeen lierojen lajirikkauden ja bioturbaation maksimoimiseksi, sillä tietyt toiminnallisesti erityisen tärkeät lajit ovat herkkiä kyntämiselle. Osoitan myös, että maaperän tiivistyminen, esimerkiksi raskaasti koneistetun maatalouden seurauksena, voi vaikuttaa vehnän arbuskelimykorritsasymbioosiin, mutta sen vaikutus riippuu vehnälajikkeesta. Tiivistyneessä maassa sellaiset vehnälajikkeet, joiden juuret toimivat tehottomasti ravinteiden otossa, saattavat olla riippuvaisempia arbuskelimykorritsasymbioosin kautta saaduista ravinteista kuin muut lajikkeet. Osoitan myös, että lierojen määrä ja monimuotoisuus vaihtelevat paljon luonnonlaitumien välillä. Lieroja ja lierolajeja on eniten kosteilla ja viljavilla luonnonlaitumilla, ja lajimäärä lisääntyy myös mikrohabitaattien monimuotoisuuden lisääntyessä. Tällaiset laitumet siis todennäköisesti edistävät lierojen monimuotoisuuden säilyttämistä maatalousympäristöissä. Lisäksi lierolajien määrittäminen DNA:n perusteella auttoi tunnistamaan lähes kaksi kertaa niin paljon lajeja kuin määrittämällä lajit visuaalisesti ulkoisten ominaisuuksien perusteella. Visuaalinen määrittäminen on edelleen yleisin tapa tunnistaa lierolajeja, mutta DNA:han perustuva määrittäminen parantaisi huomattavasti lajimääräarvioiden tarkkuutta. Osoitan myös, että liero- ja arbuskelimykorritsasieniyhteisöjä voi muokata lisäämällä peltomaahan syöttimatoina myytäviä *Lumbricus terrestris* -lieroja ja niittymaata. Syöttimatojen lisäämisellä oli, vehnän lajikkeesta ja lannan lisäämisestä riippuen, suotuisa vaikutus vehnän kasvulle. Sen sijaan arbuskelimykorritsasienten lisääntynyt lajirikkaus peltomaassa niittymaan levittämisen seurauksena ei näkynyt vehnän arbuskelimykorritsa-symbioosissa eikä tukenut vehnän kasvua.

Peltomaan liero- ja arbuskelimykorritsasieniyhteisöjä voi tukea monin tavoin. Voimaperäisten maatalousmenetelmien korvaaminen esimerkiksi keventämällä maanmuokkausta ja monipuolistamalla viljelykiertoja on suositeltavin tapa, sillä se vaikuttaa suotuisasti koko maaperäeliöstön monimuotoisuuteen parantaen maaperän terveyttä kokonaisvaltaisesti. Myös kohdennetumpi lieroyhteisöjen ja arbuskelimykorritsasymbioosin muokkaaminen saattaa kuitenkin olla mahdollista. Esimerkiksi tuotantokasvien ravinteiden ottoa voitaisiin mahdollisesti parantaa ja satovaihtelua vähentää jalostamalla lajikkeita jotka voivat eri olosuhteissa käyttää joustavasti sekä juuriaan että arbuskelimykorritsasymbioosia ravinteiden ottoon. Liero- ja arbuskelimykorritsasienten monimuotoisuutta peltomaassa on mahdollista lisätä levittämällä pellolle syöttimatoina myytäviä lieroja ja niittymaata. Lisää tutkimusta kuitenkin tarvitaan vaikutusten keston ja mahdollisten haitallisten biologisten seurausten arvioimiseksi sekä tarkentamaan, millaisten lajien lisääminen on hyödyllistä missäkin tilanteessa.

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Alternative combinations of tillage practices and crop rotations can foster earthworm density and bioturbation

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ABSTRACT

Earthworms, which contribute to important soil functions, suffer from intensive agriculture. Their response depends among other things on the earthworm ecological group (anecic, endogeic, epigeic) and the combination of the applied farming practices. To advice on methodological adaptations that enhance earthworm-mediated soil functions, effects of different practices on earthworms need to be studied in concert. We investigated the effects of tillage intensity (conventional, reduced, no tillage) and crop rotation diversity (simple = wheat, barley; diverse = wheat, peas, oil seed rape) on earthworm density and community composition in a Swedish long-term experiment. Furthermore, we calculated annual earthworm bioturbation to quantify the effects of farming practices on earthworm functions. Total earthworm densities did not vary between the different tillage intensities, but were on average 58% higher in the diverse than in the simple crop rotation. The pattern was mainly due to the response of the most abundant endogeic earthworms, which were not affected by tillage intensity, but were nearly two times more abundant in the diverse than in the simple crop rotation. Densities of anecic earthworms were 17 times higher under no tillage than conventional tillage. Anecic earthworms also benefitted from a diversified crop rotation, but the response depended on tillage intensity. The level of bioturbation reflected the response of anecic earthworms, and was more than four times higher under no tillage, 549 g dw m^{-2} year^{-1}, than under conventional tillage. We conclude that highest earthworm bioturbation is best achieved with no tillage. However, earthworm densities and potentially bioturbation can be increased also by a diversified crop rotation, when reducing tillage intensity is not feasible.

1. Introduction

Earthworms (Annelida, Oligochaeta) are ecosystem engineers (Lavelle et al., 1997), which have a major effect on a range of important soil functions that are vital in agriculture (Blouin et al., 2013; Bertrand et al., 2015). Via their burrowing and casting activities earthworms enhance nutrient mineralization (van Groenigen et al., 2019), litter decomposition (Huang et al., 2020), and soil structure formation (Schon et al., 2017). Their presence in agroecosystems has been shown to aid in pest and disease control (Plaas et al., 2019) and increase yields by on average 25% (van Groenigen et al., 2014).

Agricultural methods can strongly affect earthworms, and earthworm densities in arable fields are generally lower than in pastures and permanent grasslands (Curry, 2004). The detrimental effect of conventional tillage practices such as moldboard and rotary ploughing on earthworms is well documented (Briones and Schmidt, 2017; Chan, 2001; Pelosi et al., 2014; van Capelle et al., 2012). However, how earthworms respond to intensive tillage depends on e.g. soil type, timing of the tillage operation and soil moisture conditions during tillage (Chan, 2001; Pelosi et al., 2014), as well as earthworm species and ecological group (Bouché, 1977). Conventional tillage is especially harmful for litter feeding earthworms, both the surface living epigeic earthworms and the deep-burrowing anecic earthworms (Briones and Schmidt, 2017), as ploughing moves litter to deeper soil layers, and destroys the system of permanent burrows inhabited by the anecics (Briones and Schmidt, 2017; Chan, 2001). Endogeic earthworms, which dwell and feed in upper mineral soil (Bouché, 1977; Lavelle, 1988), are relatively tolerant to intensive tillage, and may even benefit from incorporation of crop residues via ploughing (Chan, 2001). Although less intensive tillage practices exist (e.g. cultivator, chisel plough, direct sowing), which are less damaging for earthworms (Briones and Schmidt, 2017), conventional ploughing remains an important practice in many agricultural systems, such as organic farming (Casagrande et al., 2016), and for the cultivation of certain crops and soil types (Soane et al.,

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2012). For those systems, it is important to explore alternative ways to enhance earthworm densities and alleviate the negative effects of intensive tillage.

Agricultural practices that increase the quantity or quality of organic matter inputs to the soil have been shown to increase earthworm densities (Briones and Schmidt, 2017). Such practices include the use of organic fertilizers (Lapied et al., 2009), the application and incorporation of crop residues (Frazão et al., 2019a), planting especially leguminous cover crops (Roarty et al., 2017; Fiorini et al., 2022), and the use of leys (Jarvis et al., 2017) or legumes (Ashworth et al., 2017; Hubbard et al., 1999; Rodríguez et al., 2020) in crop rotations. These practices could alleviate the detrimental effect of intensive tillage on earthworms via an increase in food resources, and speed up the recovery of earthworm populations after tillage operations. However, in order to verify an alleviating effect of different types of organic matter inputs requires comparing the effects of these practices on earthworms with those of tillage intensity in a complete multifactorial design. Although such studies are few, some examples exist. For example, Melman et al. (2019) and Denier et al. (2022) did not find residue retention or cropping system (conventional, feed or biogas), respectively, to enhance earthworm densities under conventional tillage. On the other hand, Crotty et al. (2016) found that, in comparison to other forage crops, a legume (*Trifolium repens*) buffered the reduction in earthworm densities during the first year after conversion from forage to annual cereals both with conventional and no tillage, although the differences in anecic densities after conversion were not statistically significant between the preceding forage species. We are not aware of studies exploring whether other legume species alleviate the detrimental effect of tillage earthworms, or whether such buffering effect has long-term relevance.

Changes in the absolute and relative densities of earthworm ecological groups due to agricultural management are likely reflected in earthworm mediated soil functions both qualitatively and quantitatively (Pelosi et al., 2014). Especially the reduction of large anecic species due to intensive tillage (Briones and Schmidt, 2017) may drastically impair functions such as soil macropore formation (Pelosi et al., 2017; Krogh et al., 2021) and litter decomposition (Huang et al., 2020). However, studies quantifying the contribution of earthworms to soil functions in differently managed soils remain rare, because it is difficult to disentangle the direct causes for functional changes in agricultural soils. One way to demonstrate the overall functional effect of earthworms is to estimate earthworm bioturbation, i.e. the mass of soil translocated by earthworms in a certain area and time-period. Earthworm bioturbation by differently composed earthworm communities can be calculated for example with the help of species-specific egestion rates (Taylor et al., 2019). However, no study thus far has used this method to compare earthworm bioturbation in fields under different agricultural management.

In this study, we examined how tillage intensity and crop rotation diversity (cereal versus cereal/legume crop rotation) affect total earthworm densities, ecological group densities, community composition and bioturbation, which we use as a proxy for earthworm activity and function in the soil. Our main focus was on whether the effect of tillage intensity and crop rotation on earthworms and bioturbation depend on each other, and whether the earthworm ecological groups respond differently to the different management combinations. We also examined the effect of both management types on certain soil properties known to be meaningful for earthworms to explore the indirect drivers of earthworm community change under agricultural management. We specifically tested two hypotheses:

1. A reduction in earthworm density due to intensive tillage can be mitigated by including a legume in the crop rotation. The mitigation effect will be less pronounced for tillage sensitive anecic and epigeic earthworms than endogeic earthworms.
2. Tillage intensity, more than crop rotation, determines total earthworm bioturbation, which is due to the sensitivity of anecic species to intensive tillage and their large contribution to bioturbation.

2. Material and methods

2.1. Site characteristics and experimental design

Sampling was conducted between 12th and 14th June 2017 at the Säby experimental site in Uppsala, eastern Sweden (59°49′58″N 17°42′19″E). The sampled long-term experiment was established in 2007 and compares tillage methods of different intensities in two crop rotations. The climate of the region is humid continental with an annual mean air temperature of 6.7 °C and an annual mean precipitation of 547 mm during the past thirty years (Swedish University of Agricultural Sciences, Ultuna weather station, 1988–2017). During the sampling, the average temperature was 14.7 °C, and the mean rainfall was 1.1 mm day^{-1}. The soil at Säby is classified as Eutric Cambisol (Etana et al., 2009) and the soil texture is 23.3% clay, 52.2% silt, 24.5% sand (Arvidsson, 2010). The organic matter content of the soil at the start of the experiment was 4.0% (Arvidsson, 2010). Soil pH in the 30 cm topsoil, averaged over the sampled plots at the experimental site, is 5.56 (SD, standard deviation: 0.33).

The general experimental design is a split plot design with two crop rotations (simple, diverse) as main factors and tillage treatments as sub factors. Three tillage treatments were included in the study: conventional tillage (CT = moldboard ploughing, 23 cm depth), reduced tillage (RT = cultivator, 10–12 cm depth) and no tillage (NT = direct sowing). The simple crop rotation consists of winter wheat (*Triticum aestivum* L.) and spring barley (*Hordeum vulgare* L.), while the diverse crop rotation consists of winter wheat, peas (*Pisum sativum* L.) and oilseed rape (*Brassica napus* L.; Table 1; Supplementary Table S1). The combinations of the crop rotation and tillage treatments are replicated in three blocks with 9 × 21 m large plots (Supplementary Fig. S1). For all crops, residues were left in the field after harvest, and incorporated in the soil in the RT and CT treatments prior to seeding. During the sampling in 2017, i.e. 10 years after the start of the experiment, the crop in both crop rotations was winter wheat. The preceding crop (2016) in the diverse rotation had been peas, while in the simple rotation it was spring barley. Specific amounts of fertilizers and pesticides have been used for different crop species. On average, since the establishment of the experiment, the level of added N and S has been slightly lower and P and K slightly higher in the diverse than in the simple crop rotation. An overview of the applied fertilizers and pesticides in both rotations from 2007 to 2017 is presented in Supplementary Table S1.

2.2. Earthworm sampling

Four samples were taken per plot in the diverse crop rotation and, because of time constraints, two samples per plot in the simple crop rotation. For each sample, a hole of 30 cm (width) x 30 cm (length) x 20

Table 1
Annual crop species in the simple and the diverse crop rotation since the establishment of the experiment in 2007 until the sampling year 2017.

Year	Diverse rotation (DR)	Simple rotation (SR)
2007	Winter wheat	Winter wheat
2008	Peas	Barley
2009	Winter wheat	Winter wheat
2010	Spring oilseed rape	Barley
2011	Winter wheat	Winter wheat
2012	Winter wheat	Winter wheat
2013	Winter wheat	Winter wheat
2014	Spring oilseed rape	Spring barley
2015	Winter wheat	Winter wheat
2016	Peas	Spring barley
2017	Winter wheat	Winter wheat

cm (depth) was dug. The soil collected from the hole was immediately hand-sorted for earthworms. After this, 2.5 l of allyl isothiocyanate (AITC) solution, prepared according to the protocol by Zaborski (2003; 100 mg AITC per 1 l water), was poured in the hole, in order to collect worms deeper in the soil. Each hole was observed for protruding earthworms for 30 min. Collected individuals were rinsed on site in tap water and preserved in 99% ethanol.

The developmental stage of each earthworm was noted and all adult earthworms (indicated by the presence of a clitellum) were identified to species level (Sherlock, 2012). Biomass of the individual worms (g fresh weight, including gut content) was determined by weighing the worms after they had been rinsed in water for 5 min to remove the ethanol and gently dried with a tissue. The biomass was converted from fresh weight to dry weight assuming a water content of 80%, which was considered a realistic average for well-hydrated earthworms (Grant, 1955; Bayley et al., 2010). Adult worms were assigned to main earthworm ecological groups (epigeics, endogeics and anecics) based on Bouché (1977) and Bottinelli et al. (2020). For species representing intermediate ecological groups, such as *Allolobophora chlorotica* (epi-endogeic/intermediate) and *Lumbricus terrestris* (epi-anecic), the main category was used for simplicity (endogeic and anecic, respectively). Juveniles could only be assigned to either the genera *Allolobophora/Aporrectodea* or *Lumbricus*. Thus, percentages of adult individuals in the corresponding genera belonging to the different ecological groups in the complete dataset were used to assign juveniles to the different ecological groups accordingly. Total abundances and biomasses per sample were converted to densities and biomasses per square meter. All samples included severed parts of earthworms, which were not considered in the densities, as they could not be determined to species or converted to numbers of individuals. Thus, the data slightly underestimates natural community densities. How we handled the part biomass data is described in the following section.

2.3. Bioturbation

Average annual bioturbation in 2017, defined as the estimated dry weight of soil translocated via earthworm egestion per square meter, was calculated for each crop rotation/tillage treatment combination using the methodology described in Taylor et al. (2019). In short, the daily egestion rates (g dw faeces g^{-1} body dw day^{-1}) for the different ecological groups, determined in a laboratory experiment by Taylor and Taylor (2014) at 15 °C, were multiplied by the biomass of each earthworm ecological group in one quadrat meter and summed up to total bioturbation (g dw m^{-2} day^{-1}). It was not ideal to include the biomass of earthworm parts in the bioturbation calculation as it was not possible to determine parts to species or ecological groups to assign the egestion rates. However, there was some variation in the biomass of earthworm parts between the treatment combinations (4–41% of the biomass of the whole individuals). Therefore, bioturbation values were calculated twice for each sample, both excluding and including the part biomass, to account for possible discrepancies that could affect the results of the statistical analysis. For the latter values, the part biomass was divided into the different ecological groups based on the proportions of adult earthworm biomass in the respective groups. To calculate bioturbation for a full year, and to account for variation in earthworm egestion in response to temperature, bioturbation at 15 °C was adjusted to field temperatures during the sampling year assuming similar temperature dependency for egestion as for earthworm growth (Taylor and Taylor, 2014). For the field temperatures, we used mean monthly soil temperatures recorded at 10 cm depth at the SLU weather station at Ultuna, which is located approximately 3 km from the experimental site.

2.4. Soil parameters

To contribute to the discussion about the drivers of tillage and crop rotation effects on earthworms, we determined the variation of soil organic carbon (SOC) content, bulk density and water content in the different tillage/crop rotation treatment combinations. Two 30 cm soil cores were collected in each plot with a soil corer of 5 cm diameter, one for SOC and the other for bulk density and soil water content. Each core was divided into three pieces, representing the soil depths 0–10 cm, 10–20 cm and 20–30 cm, to examine differences in the three parameters between the soil depths. The samples were stored at 5 °C until processed. Total carbon content of the samples was determined using the dry combustion method (Elementar Vario El, Heraeus, Hanau, Germany). No inorganic carbon was detected after treatment with HCl, which means that total carbon content in the samples equals organic carbon content (Chatterjee et al., 2009). To determine soil bulk density and water content, the field moist soil samples were weighed, dried at 105 °C for 24 h, and re-weighed.

2.5. Statistical analyses

All statistical analyses were performed in R (version 3.5.3; R core team, 2019). To examine the effects of tillage intensity and crop rotation diversity on earthworm densities, and to determine whether the effect of one type of practice depended on the other, we used generalized linear mixed models (GLMM) from the package *glmmTMB* (Brooks et al., 2017). Models with a similar structure, with tillage intensity, crop rotation diversity and their interaction as explanatory variables, were used to test the effects of these factors on total, anecic and endogeic earthworm densities. Epigeic earthworms were collected in very low densities and were thus left out of the analyses. Three random factors were included in the models: replicate, and the interaction of replicate and crop rotation due to the nested experimental design, and the interaction of replicate, crop rotation and tillage (plot level) due to the pseudoreplication within plots. All models were checked for overdispersion and zero-inflation with the functions testDispersion and testZeroInflation from the *DHARMa* package (Hartig, 2020), and Poisson or negative binomial distribution, and zero-inflated model was applied accordingly (Supplementary Table S2). The significance levels of the effects were determined using the type III ANOVA in the package *car*, with contr.sum contrasts (Fox and Weisberg, 2019). Interaction terms with $P > 0.1$ were excluded from the final model. When an explanatory variable with more than two treatment levels had a significant effect, the differences between specific treatments were further analyzed with Tukey's HSD (Honest Significant Difference) post hoc test using the package *emmeans* (Lenth, 2020). *P*-values smaller than 0.05 are discussed as significant.

Differences in earthworm community composition were explored using multivariate methods in the *vegan* package (Oksanen et al., 2019). To test whether tillage intensity and crop rotation diversity separately or in interdependence affect earthworm community composition, a permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations and marginal effects of terms was performed, using the adonis2 function. We further examined community differences at the sample level by performing a non-metric multidimensional scaling (NMDS) ordination with Bray-Curtis dissimilarity, square root transformation and Wisconsin double standardization, using the package *metaMDS*. Good fit (stress = 0.05) was achieved with four dimensions. Tillage intensity and crop rotations were fitted onto the NMDS ordination using the envfit function, and when the treatment was significantly correlated with the NMDS axes, the different treatment levels were visualized as convex hulls around the sites (Fig. 1). Similarly, we further illustrated the responses of the earthworm ecological groups using the envfit function for the grouped earthworm densities, and projecting the vectors, that were significantly correlated with the NMDS axes (only anecics, see Section 3.3), as arrows on the NMDS diagram (Fig. 1). Earthworm juveniles belonging to the genera *Lumbricus* or either *Allolobophora/Aporrectodea* were treated similarly as separate species in both PERMANOVA and the NMDS.

To test whether total earthworm bioturbation (with and without the

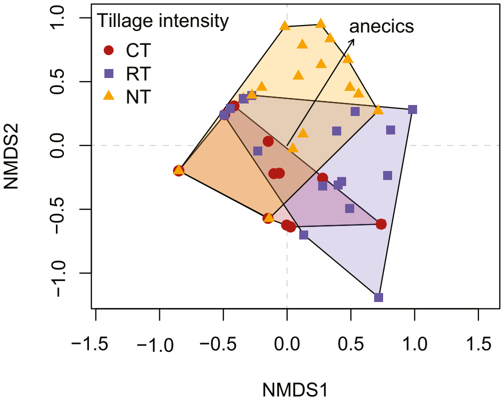

Fig. 1. The two first axes of a non-metric multidimensional scaling (NMDS) ordination presenting differences in earthworm community structure between samples (k = 4, stress = 0.05). Symbols of different shapes and colors and the associated polygons represent samples taken from the plots with different tillage treatments (CT = conventional tillage, RT = reduced tillage, NT = no tillage). Increasing anecic earthworm density is visualized with an arrow, as it was significantly correlated with the NMDS axes.

part biomass), SOC content, soil bulk density and soil water content varied between the tillage and the crop rotation treatments, we used general linear mixed models (LMM) in the package *lme4* (Bates et al., 2015). The explanatory and random variables were the same as for the earthworm models, except that for the soil parameters, we also included interactions of the main treatments and soil depth as an explanatory variable, to test whether the effects of the treatments varied between soil depths. Total bioturbation was ln-transformed and bulk density square root transformed to achieve linearity. Normal distribution and homoscedasticity of the residuals were graphically verified. Same procedure as for the earthworm models was used for determining the significance of the treatment effects as well as for testing differences between treatment levels. Tukey's HSD was also used to evaluate treatment effects on soil parameters averaged over the soil depths. The detailed structure of the models is presented in Supplementary Table S2.

3. Results

3.1. Earthworm densities and community composition

In total, we sampled 443 earthworm individuals, belonging to seven species, of which 90 were adults. The numbers of individuals per sample varied from one to 26, which corresponds to 11–286 individuals (ind.) m^{-2}. The majority of the collected earthworms belonged to the endogeic group (73.8%). Anecic earthworms occurred in intermediate numbers (24.0%), while epigeic earthworms were scarce (2.2%). The most common species was the endogeic *Allolobophora chlorotica* (Savigny, 1826), which in the adult stage was present in 11 of the 18 plots, with densities varying from 11 to 44 ind. m^{-2}. The six other species found were the endogeics *Aporrectodea caliginosa* (Savigny, 1826), *Aporrectodea rosea* (Savigny, 1826), and *Aporrectodea tuberculata* (Eisen, 1874), the epigeic *Lumbricus castaneus* (Savigny, 1826) and the anecics *Lumbricus terrestris* (Linnaeus, 1758) and *Aporrectodea longa* (Ude, 1885). Average densities and standard deviations of all species in the different treatments are presented in Supplementary Table S2.

3.2. Effect of tillage intensity and crop rotation on earthworm densities

The estimated marginal means (EMM) for the total earthworm densities ranged from 51 ind. m^{-2} under conventional tillage in the simple crop rotation to 124 ind. m^{-2} under no tillage in the diverse crop rotation. Total earthworm densities did not significantly differ between the tillage treatments, although there was an apparent increase in total earthworm density with reduced tillage intensity (Fig. 2A). Instead, total earthworm densities were on average 58% higher in the diverse than in the simple crop rotation (Fig. 2A; Table 2). The effect of crop rotation did not depend on tillage intensity.

Endogeic and anecic earthworms responded differently to tillage intensity and crop rotation. Estimated marginal means for endogeic earthworm densities ranged from 43 ind. m^{-2} under conventional tillage in the simple crop rotation to 85 ind. m^{-2} under no tillage in the diverse crop rotation. Endogeic earthworm densities were on average 71% higher in the diverse crop rotation than in the simple crop rotation, and the difference was significant (Fig. 2B; Table 2). Tillage had no effect on endogeic earthworm densities, and the effect of crop rotation did not depend on tillage intensity (Fig. 2B; Table 2).

Anecic earthworms responded significantly to both tillage intensity and crop rotation, and there was a significant interaction of the effects of the two factors (Table 2). More anecic earthworms were found in plots under no tillage than conventional tillage, regardless of the crop rotation (Fig. 2C). Under conventional tillage, anecic earthworm densities were marginally higher in the diverse than in the simple crop rotation (EMM: 4.98 and 0.34 ind. m^{-2}, respectively; $t_{43} = 2.97$, $P = 0.051$). However, there were no significant differences in anecic earthworm densities between the diverse and the simple crop rotation under no tillage (EMM: 43.2 and 54.1 ind. m^{-2}, respectively) and reduced tillage (EMM: 24.0 and 5.82 ind. m^{-2}, respectively).

3.3. Effects of tillage intensity and crop rotation on earthworm community composition

According to the PERMANOVA, both tillage intensity and crop rotation had a significant effect on earthworm community composition (Table 2). The effects of tillage and crop rotation were not interdependent, so the interaction term was not included in the final model. Tillage explained more of the variation than crop rotation ($R^2 = 0.15$ and $R^2 = 0.05$, respectively). Fig. 1 shows differences in earthworm community composition between samples as the two first axes of the NMDS ordination (k = 4, stress = 0.05). Tillage intensity showed significant correlation with the NMDS axes, so the tillage intensity associated with each sample was visualized with the shape and color of the sample point, and convex hulls were drawn around the sample points with the same tillage intensity applied. Similarly, anecic, but not endogeic, earthworm density was significantly correlated with the NMDS axes. This correlation is visualized with an arrow in the NMDS diagram that points in the direction of higher anecic earthworm density in the samples (Fig. 1). Even though the convex hulls largely overlap, the communities under no tillage appear distinct from those of the two tillage treatments. This seems to derive largely from the higher densities of anecic earthworms under no tillage, a pattern also supported by the GLMM results (Table 2).

3.4. Earthworm bioturbation

The models including and excluding biomass of earthworm parts yielded similar results. Thus, and for comparability to the density analyses, only the results for the models excluding the part biomass are presented here. The results including the part biomass are presented in Supplementary Fig. S2. The estimated marginal means for total bioturbation by earthworms from all ecological groups (excluding the part biomass) ranged from 98.0 g dw m^{-2} $year^{-1}$ in the plots under conventional tillage in the simple crop rotation to 742.2 g dw m^{-2} $year^{-1}$ in the plots under no tillage in the simple crop rotation (Fig. 3). Increasing

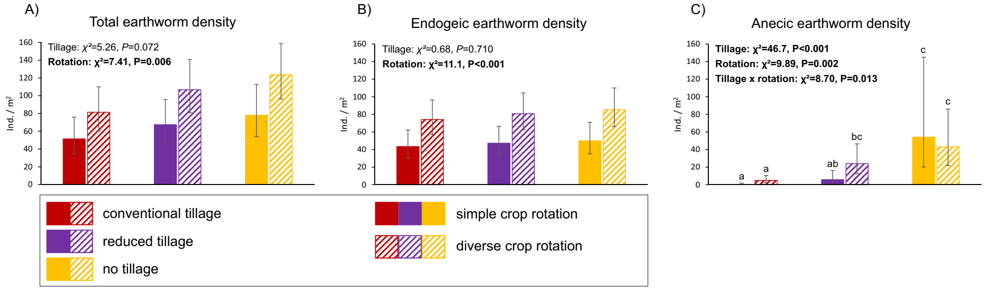

Fig. 2. Estimated marginal means for A) total earthworm densities and densities of B) endogeic and C) anecic earthworms in the different treatments with 95% confidence intervals. For anecic earthworms (C), columns sharing the same letters are not significantly different (Tukey's HSD test, $P > 0.05$). χ^2 and P values for the explanatory variables are presented, and the statistically significant ($P < 0.05$) variables are in **bold** font.

Table 2

Results of the generalized linear mixed models (GLMM), the general linear models (LM), and the permutational multivariate analysis of variance (PERMANOVA) evaluating the effect of tillage intensity, crop rotation and their interaction on endogeic, anecic and total earthworm density, total bioturbation, and earthworm community composition, respectively. Interaction term was only included in the final model when the *P*-value was smaller than 0.1 as in the cases of anecic earthworm density and total bioturbation. *P*-values with $P < 0.05$ are in **bold**.

	Tillage			Crop rotation			Tillage × crop rotation		
Earthworm density (ind./m²)	χ^2	*df*	*P*	χ^2	*df*	*P*	χ^2	*df*	*P*
Endogeic	0.73	2	0.694	6.84	1	**<0.009**			
Anecic	46.7	2	**<0.001**	9.89	1	**0.002**	8.70	2	**0.013**
Total	5.26	2	0.072	7.41	1	**0.006**			
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Total earthworm bioturbation (g dw/m²/yr)	5.56	2	**0.026**	1.40	1	0.349	3.35	2	0.081
	Pseudo-F	*df*	*P*	*Pseudo-F*	*df*	*P*			
Earthworm community composition	4.60	2	**<0.001**	3.24	1	**0.010**			

tillage intensity significantly reduced total bioturbation (Table 2). There were no differences in bioturbation between the crop rotations, and the effect of tillage did not significantly depend on crop rotation. When the interaction term was excluded from the model, the Tukey's HSD post hoc comparison revealed significantly higher bioturbation under no tillage than under conventional tillage ($t_{9.21} = 3.31$, $P = 0.022$).

Endogeic earthworms accounted for most of the bioturbation in the conventionally tilled plots in both crop rotations (99.4% and 97.0% in the simple and the diverse crop rotation, respectively) and in plots with reduced tillage in the simple crop rotation (94.9%). In plots with reduced tillage in the diverse rotation and plots with no tillage in the diverse and the simple crop rotation, the endogeic contribution was lower (58.2%, 32.2% and 25.5%, respectively). In anecic earthworms this pattern was reversed. Their contribution to total bioturbation was very low in conventionally tilled plots in both crop rotations (simple: 0.6%, diverse: 2.8%) and plots with reduced tillage in the simple rotation (4.6%). In plots with reduced tillage in the diverse rotation and no tillage in both crop rotations, the proportion of bioturbation carried out by anecic earthworms was much higher, accounting for 41.4 to 71.3% of the total bioturbation. Epigeics were estimated to contribute little to total bioturbation, values varying between 0.0% in conventionally tilled plots in the simple crop rotation and 3.0% in plots with no tillage in the simple crop rotation.

3.5. Soil parameters

Overall, average soil organic carbon content in the top 30 cm did not differ between the different tillage and crop rotation treatments. However, there were differences in soil organic carbon content between the different depths depending on the tillage treatment (Table 3; Supplementary Fig. S3). Typically, under conventional tillage, organic carbon content was evenly distributed throughout the soil profile, whereas under reduced and especially under no tillage, organic carbon content was high in the topsoil and was reduced in the deeper soil. There were no significant differences in soil organic carbon levels in the different soil depths between the two crop rotations.

Average soil bulk density and water content did not vary between the tillage and crop rotation treatments. Expectedly, both soil bulk density and water content increased with soil depth (Table 3), and this pattern did not depend on tillage or crop rotation.

4. Discussion

Our results from the multifactorial long-term experiment provide new evidence that a diversified crop rotation, which includes a legume, can alleviate the detrimental effect of conventional tillage on earthworms, and increase total and endogeic earthworm densities regardless of tillage intensity. Even though anecic earthworm densities were low under conventional tillage, we found that also their densities, and thus potentially bioturbation, can be increased by including a legume in the

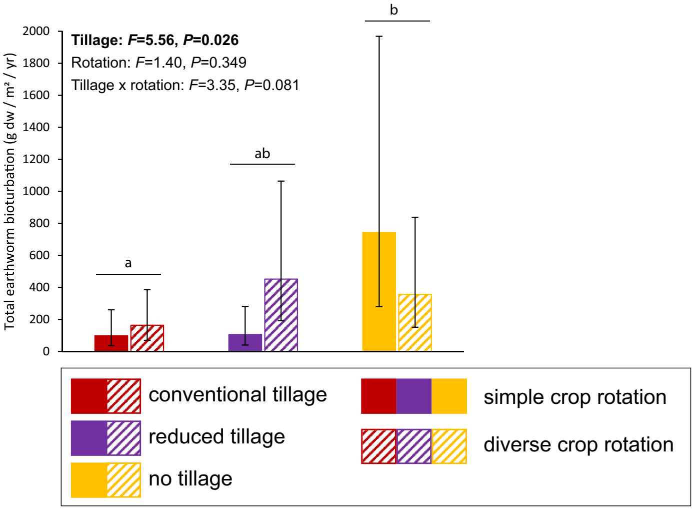

Fig. 3. Estimated marginal means for total bioturbation (g dw / m^2 / yr) in the different treatment combinations with 95% confidence intervals. Columns sharing the same letters are not significantly different (Tukey's HSD test, $P > 0.05$). χ^2 and P values for the explanatory variables are presented, and the statistically significant ($P < 0.05$) variables are in **bold** font.

Table 3

Results of the general linear models (LM) evaluating the effect of tillage intensity, crop rotation, soil depth and the interaction of tillage and soil depth on soil organic carbon content (SOC), soil moisture and bulk density. Interaction term was only included in the final model when the P-value was smaller than 0.1 as in the case of soil organic carbon. *P*-values with $P < 0.05$ are in **bold**.

	Tillage			Crop rotation			Soil depth			Tillage x soil depth		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
SOC	15.5	2	**<0.001**	0.002	1	0.967	0.57	2	0.572	13.0	4	**<0.001**
Soil moisture	2.56	2	0.127	4.10	1	0.180	6.22	2	**0.005**			
Bulk density	1.17	2	0.348	0.088	1	0.795	30.2	2	**<0.001**			

crop rotation. Even small increases in species densities can be meaningful for sustaining the species in the landscape, although restoring more functionally important anecic earthworm densities and bioturbation may require refraining from intensive tillage to allow population recovery.

4.1. Crop rotation matters more for endogeic earthworms than tillage intensity

Endogeic earthworms are generally considered to better tolerate intensive tillage than anecic and epigeic species (e.g. Chan, 2001; Briones and Schmidt, 2017). A meta-analysis by Briones and Schmidt (2017) showed that although endogeic earthworms responded negatively to conventional tillage, they were less affected than anecic and epigeic species. However, the response of endogeic earthworms to intensive tillage varies considerably between individual studies. The majority of studies, including ours, show similar densities of endogeic species in intensively tilled fields compared to fields with reduced or no tillage (Nuutinen, 1992; Pelosi et al., 2014), but some show lower densities (van Capelle et al., 2012), and some higher densities in intensively tilled fields (Baldivieso-Freitas et al., 2017; Boström, 1995). Some of this variation is likely due to species-specific responses, i.e. the composition of the studied earthworm community, and timing of sampling after the tillage event. For example, De Oliveira et al. (2012) found that densities of the endogeic species *Aporrectodea caliginosa* were more strongly reduced by conventional ploughing than those of the endogeic species *Aporrectodea rosea* immediately after tillage, but that the densities of both species recovered in 5–9 months. It has also been speculated that the response of endogeic earthworms to intensive tillage depends on conditions such as soil moisture during tillage, as many endogeic species can become inactive in deeper soil during dry periods, and should then be less affected by tillage (Faber et al., 2017). Different life history traits between species may also explain better tolerance and/or faster recovery of endogeic earthworms after soil disturbance but this topic remains little studied (De Lange et al., 2013). In addition, tillage may have indirect effects on endogeic earthworms via its effects on various soil properties. For example, endogeic earthworms are considered especially sensitive to an increase in soil bulk density (Capowiez et al., 2021) and a decline in organic matter content (Hoeffner et al., 2021), both of which are associated with tillage practices (Blanco-Canqui and Ruis, 2018).

In our study, the earthworm species composition was similar to and the average total densities were comparable to other studies investigating earthworm communities in agricultural soils in the same area (Lagerlöf et al., 2002, 2012). The most common species among adult individuals was *Allolobophora chlorotica.* This species is commonly classified as intermediate or epi-endogeic (Bottinelli et al., 2020): it does not create permanent burrows (Capowiez, 2000), and, although flexible in foraging depth, feeds mainly close to the soil surface (Le Couteulx et al., 2015). There is experimental evidence that, although *A. chlorotica* juveniles may benefit from *L. terrestris* middens for nutrition and burrows for movement (Lowe and Butt, 2007; Uvarov, 2009), *A. chlorotica* also competes with *Lumbricus* species for litter resources, *L. rubellus* often being the strongest competitor (Lowe and Butt, 2002). Simultaneously, *A. chlorotica* and *A. caliginosa* are also considered competitors (Uvarov, 2009). We found a large number of adult *A. chlorotica* under conventional and reduced tillage, but very few under no tillage, where L. *terrestris, Lumbricus* juveniles and the endogeic species *A. rosea* and *A. caliginosa* were more common. Our results match the experimental evidence for the patterns in interactions between these species. Furthermore, Lagerlöf et al. (2002) observed similar complementary density pattern between *A. chlorotica* and *A. rosea* in cultivated fields and their boundaries with different species dominating the two types of habitats in spring compared to autumn. We suggest that under no tillage, with more litter on the soil surface, *Lumbricus* species are better competitors and suppress the numbers of adult *A. chlorotica*. This may further release niche space for endogeic species, as reflected in higher numbers of *A. rosea* and *A. caliginosa*. However, we found many juveniles of both *Lumbricus* and *Aporrectodea/Allolobophora* under no tillage, which cannot be determined to species level using morphological features. Resolving whether the juvenile densities reflect that of the adults and verifying the complementary occurrences of the aforementioned species would require species determination using molecular methods such as DNA barcoding (Maggia et al., 2021).

Tillage can have both short-term and long-term effects on soil organic matter. In the short term, incorporation of crop residues via tillage, especially when grassland is converted to cultivated land (Wyss and Glasstetter, 1992), can strongly increase soil organic matter content. This increase in earthworm food resources in upper soil layers, that are easily accessible for endogeic earthworms, can greatly enhance endogeic earthworm densities (Boström, 1995). In the long term, intensive tillage reduces soil organic carbon (SOC, indicating soil organic matter) content in the upper soil (Meurer et al., 2018), which is likely to reduce endogeic earthworm densities (Hoeffner et al., 2021). In our study, we found differences in the depth distribution of SOC in the upmost 30 cm, with SOC content decreasing with increasing depth in no and reduced tillage and an even distribution of SOC down to 30 cm in conventional tillage. Higher SOC content in the top soil under no tillage may have been of importance to the surface feeding anecic earthworms. The average SOC content down to 30 cm was similar between the different tillage treatments. Frazão and colleagues found no response of endogeic earthworms to crop residue placement in a microcosm study (Frazão et al., 2019b), or to surface application versus incorporation of crop residues in the field (Frazão et al., 2019a). This suggest, that for endogeic earthworms, which move freely through the upper mineral soil, only the total soil organic matter content is important for population densities and not where SOC is located. Our finding of similar endogeic densities and the average SOC contents down to 30 cm in the different tillage treatments is consistent with this idea.

We showed that in the diverse crop rotation, which included peas, oil seed rape, wheat and barley, endogeic earthworm densities were on average more than 70% higher than in the crop rotation including only the two cereals. Several studies have found that crop rotations that include legumes, such as pea, soybean or white clover, enhance earthworm densities compared to continuous monocropping, and rotations with cereals only (Hubbard et al., 1999; Rodríguez et al., 2020; Schmidt et al., 2003). As legume residues serve as an easily palatable resource with high nitrogen content, it has been suggested that it is often the quality rather than the quantity of organic matter as a food resource that limits earthworm densities in arable soils (Curry, 2004). There is also evidence that increased inputs of high quality food resources such as manure (Simonsen et al., 2010) and legume residues (Ashworth et al., 2017), are especially beneficial for endogeic earthworms. In addition to our study, Crotty et al. (2016), Melman et al. (2019) and Denier et al. (2022) compared tillage to another type of agricultural management in a multifactorial experiment. Crotty et al. (2016) found that in comparison to other, mainly non-leguminous preceding forage species, only white clover alleviated the detrimental effect of conventional ploughing on all earthworm ecological groups. The latter two studies did not find residue retention (Melman et al., 2019) or cropping system (conventional, feed, biogas; Denier et al., 2022) to alleviate the detrimental effect of intensive tillage on earthworms. However, in the first case, the only crop species was corn (*Zea mays*), and in the latter, crop rotations in all systems included several legume species. Thus, in these studies, quality of the organic inputs may not have differed enough between the treatments for endogeic densities to diverge.

The best strategy to enhance endogeic and thus total earthworm densities is likely to both improve the quality and increase the quantity and continuous availability of their food resources. Earthworms seem to benefit especially from a crop rotation where crop species with low and high C/N ratio, such as legumes and grasses, alternate (Schmidt et al., 2003; Rodríguez et al., 2020). The benefit of such mixtures is assumed to be due to a combination of a good quality but fast decomposing and thus short term resource (legume), and a lower quality but slower decomposing and thus more continuously available source of nutrition (grass) (Rodríguez et al., 2020). In our long-term experiment, residue biomasses of the different crop species were not measured. Thus, we cannot determine whether quantity or continuity of residues also plays a role in driving the higher earthworm densities in the diverse crop rotation, in addition to the improved quality of organic matter from pea residues. We did not find differences in soil organic carbon content between the crop rotations, but this could be due to increased consumption of the previous year's residues by the larger earthworm community in the diverse crop rotation. It is, however, unlikely that including peas and oil seed rape in a crop rotation would increase the quantity of organic matter entering the soil, as those crop species have been reported to produce equal or lower biomass of residues than wheat (Soon and Arshad, 2002).

4.2. Reduction of earthworm bioturbation under intensive tillage reflects the response of anecic earthworms

We found that earthworm bioturbation, here defined as the dry weight of soil translocated via earthworm egestion per unit area and time (Taylor et al., 2019), is largely determined by tillage intensity. This is because under no tillage, the tillage sensitive anecic earthworms increase bioturbation on average by four times compared to conventional tillage, where bioturbation is solely due to the activity of endogeic earthworms. Similar results were obtained by Pelosi et al. (2017) who studied temporal dynamics in earthworm-macropores in different cropping systems using X-ray tomography. They found that in a non-ploughed living mulch cropping system, the volume and continuity of earthworm macropores was higher than in conventional and organic ploughed systems five months after ploughing, and that pore volume and continuity were correlated with anecic earthworm biomass. Unlike for anecic densities, we did not find higher bioturbation in the diverse compared to simple crop rotation under conventional tillage. The likely reason for this is that all anecic individuals collected from the conventionally tilled plots were juveniles and thus contributed less to bioturbation due to their small body size.

We decided to study tillage intensity and crop rotation effects on earthworm bioturbation, instead of e.g. earthworm biomass, because bioturbation better describes the functional importance of earthworms. Our way of calculating bioturbation allows easy quantitative estimation of the effect of earthworms on soil functioning. It tells about the quantity of soil that, in a certain amount of time, passes the earthworm gut and is then egested elsewhere improving fertility (van Groenigen et al., 2019) and changing soil aggregation (Zangerlé et al., 2011) at that location. As such, it extends the quantification of earthworm effect from physical to chemical soil properties, such as nutrient mineralization. Simultaneously, our method is a generalization, and more preciseness could be achieved by e.g. studying egestion rates of a wider variety of species and individuals in different life stages (juveniles vs adults). In addition, parameters affecting earthworm activity other than temperature should also be considered, such as soil moisture, soil compaction, and organic matter availability (Capowiez et al., 2021; Faber et al., 2017; Hoeffner et al., 2021). With these options for improvement, and by combining the method with the information that e.g. X-ray tomography can give about the effect of earthworms on soil porosity (Capowiez et al., 2015, 2014) and aggregate formation (Le Bayon et al., 2020), it would allow sophisticated comparisons of functional differences between earthworm species, and offer valuable information for modelling purposes (Meurer et al., 2020). Despite of these possibilities for methodological improvements in calculating earthworm bioturbation, our study gives valuable insight into the potential effect of different agricultural practices on earthworm functions.

4.3. Conditions for earthworms in cultivated soils can be improved in alternative ways

Anecic earthworms are often suggested to be especially important for soil structure through creation of continuous vertical macropores which improve water infiltration (Shipitalo and Le Bayon, 2004). The importance of anecic earthworms and their functions might further increase when extreme weather events like severe rains become more frequent due to climate change (Andriuzzi et al., 2015). However, the special role of anecic earthworms for soil structure has been surprisingly difficult to prove, and more evidence was recently called for by Lang and Russell (2020). In their meta-analysis, no significant effects were found of most studied earthworm species, including the well-studied anecic species *L. terrestris*, on soil porosity and bulk density. At the same time, there is evidence that endogeic earthworms can also be important for water infiltration (Capowiez et al., 2014), and both types of earthworms seem to be equally beneficial for crop growth (van Groenigen et al., 2014). Thus, we believe that the importance of endogeic earthworms in agricultural soils should not be overlooked, and that more research is needed on the functional roles of different earthworm species and ecological groups in agricultural soils. However, it is reasonable to assume that a more diverse community fulfills a greater range of functions (Tilman et al., 2014). In this view, agricultural practices that benefit earthworms with different functional roles should be favored. Based on our study, this would mean both a reduction in tillage intensity to increase anecic earthworms and a diversification of crop rotation to increase endogeic earthworms.

Our study underpins the importance of multifactorial experiments that allow examining interaction effects of different agricultural practices for agricultural research to be meaningful for farmers. Based on the outcome from such experiments, farmers are provided with a larger choice of methods for enhancing the abundance of functionally important soil organisms such as earthworms. Each agricultural field has an individual environmental context. Choices between alternative agricultural methodologies are necessary to give farmers the tools to sustainably improve soil fertility and yields according to local needs and conditions.

Data availability statement

The data that support the findings of this study are openly available in Dryad Digital Repository at https://doi.org/10.5061/dryad.3j9kd51m4 (Torppa and Taulor, 2022).

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2022.104460.

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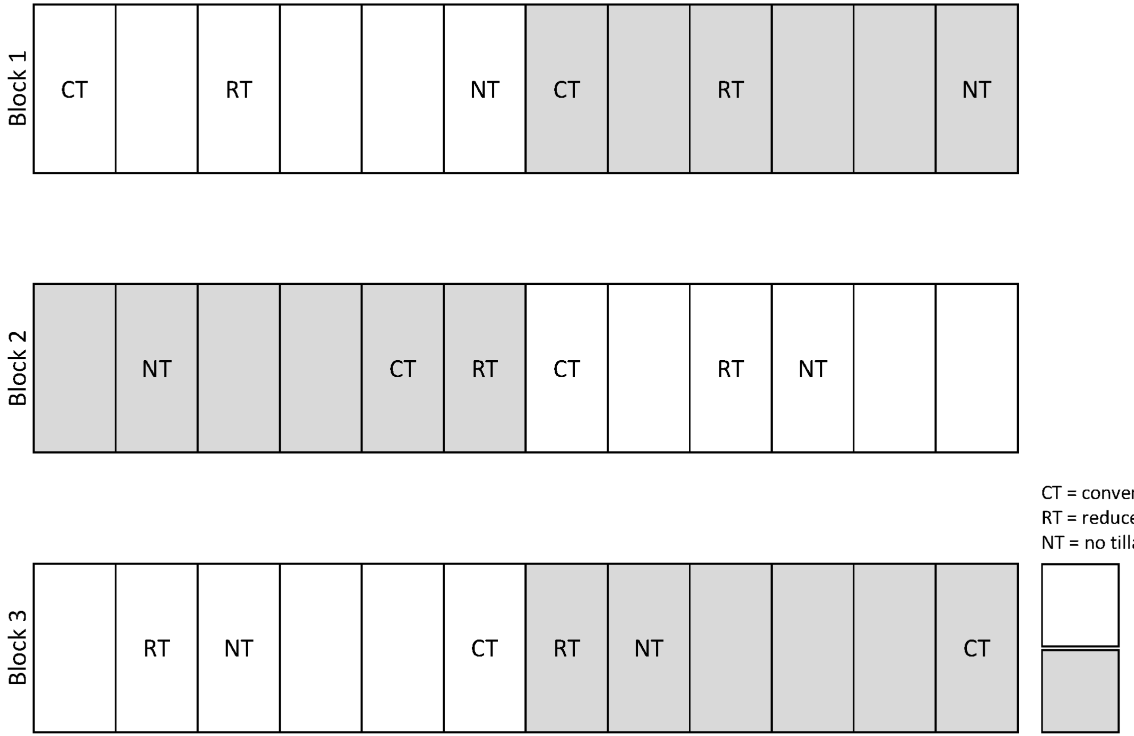
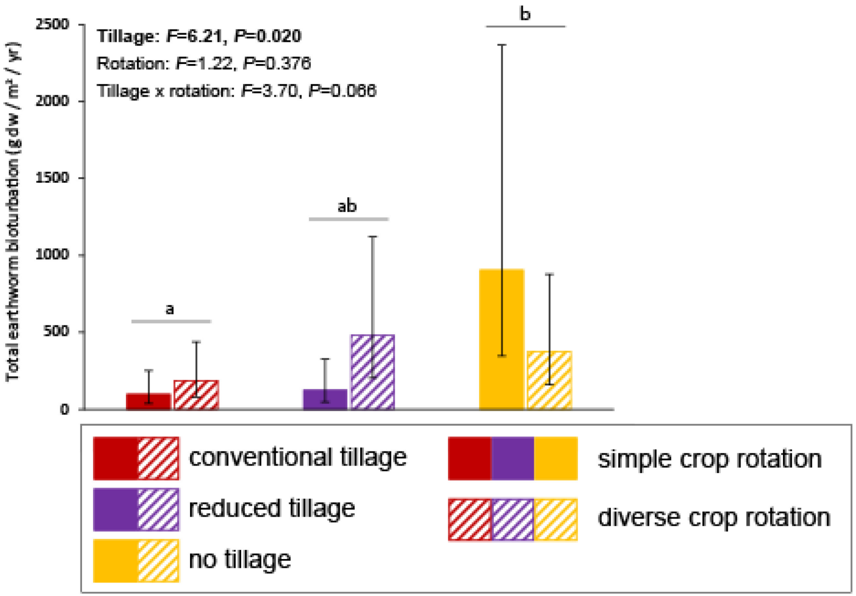
Supplementary Figures



CT = conventional tillage (plouging, 23 cm)
RT = reduced tillage (cultivator, 10-12 cm
NT = no tillage (direct sowing)

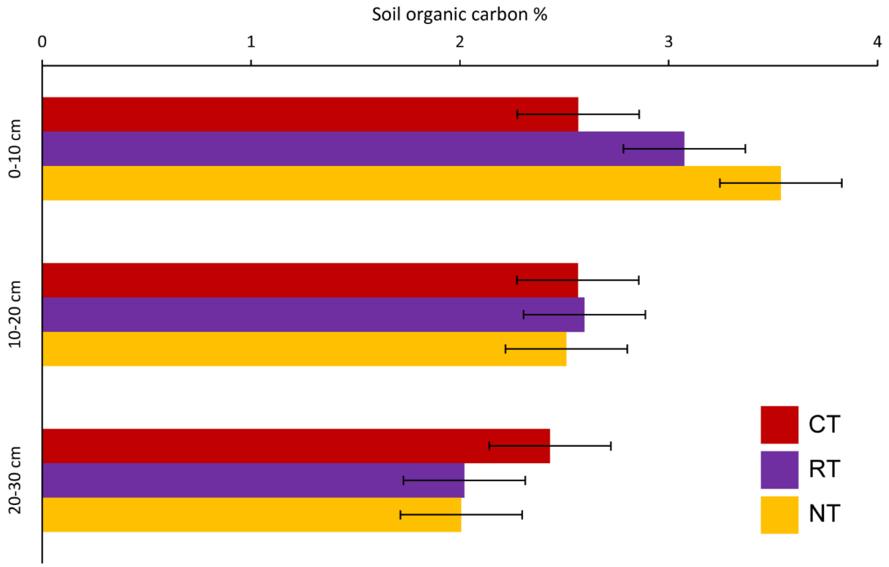
Supplementary Figure 1. Design of the long-term experiment. The blank cells are treatments that were not included in the study.

Supplementary Figures



Supplementary Figure 2. Estimated marginal means for total bioturbation (g dw / m2 / yr) including the part biomass in the different treatment combinations with 95% confidence intervals. Columns sharing the same letters are not significantly different (Tukey's HSD test, P>0.05). χ2 and P values for the explanatory variables are presented, and the statistically significant (P<0.05) variables are in bold font.

Supplementary Figures



Supplementary Figure 3. Soil organic carbon content (%) and the 95% confidence intervals in the different tillage treatments and soil depths. CT = conventional tillage, RT = reduced tillage, and NT = no tillage.

Supplementary Tables

Supplementary Table 1. Crop species and varieties, and applied fertilizers and pesticides in the two crop rotations after establishment of the experiment until the sampling year.

Year	Diverse rotation (DR)		Applied fertilizer (kg/ha/yr)				Pesticides					Simple rotation (SR)		Applied fertilizer (kg/ha/yr)				Pesticides				
	Crop	Variety	N	P	K	S	Product	Amount	Herbicide	Fungicide	Insecticide	Crop	Variety	N	P	K	S	Product	Amount	Herbicide	Fungicide	Insecticide
2007	winter wheat	Olivin	129	24		14	Ariane S	2.5 l	x			winter wheat	Olivin	129	24		14	Ariane S	2.5 l	x		
2008	peas	Fanst					Basagran	0.6 kg	x			barley	Minttu	80			15	Ariane S	1.75 l	x		
																		Event S	1.0 l	x		
2009	winter wheat	Olivin	124	23			Ariane S	2.5 l	x			winter wheat	Olivin	124	23			Ariane S	2.5 l	x		
2010	spring oilseed rape	Larissa	106	16			Matrigon	0.9 l	x			barley	Minttu	81	12			Ariane S	1.75 l	x		
							Select	0.5 l	x									MCPA	0.5 l	x		
							Karate	0.2 l			x											
							Mospilan	0.25 l			x											
2011	winter wheat	Olivin	120	18			Ariane S	2.2 l	x			winter wheat	Olivin	120	18			Ariane S	2.2 l	x		
							MCPA	0.5 l	x									MCPA	0.5 l	x		
2012	winter wheat	Olivin	122	18			Starane	1.2 l	x			winter wheat	Olivin	122	18			Starane	1.2 l	x		
							Harmony	12 g	x									Harmony	12 g	x		
2013	winter wheat	Olivin	135			20	Starane XL	1.2 l	x			winter wheat	Olivin	135			20	Starane XL	1.2 l	x		
							Harmony	12 g	x									Harmony	12 g	x		
2014	spring oilseed rape	Mahjong					Fox	1.0 l	x			spring barley	Tipple	90			9	Ariane S	2.0 l	x		
							Sumi-Alpha	0.6 l			x											
							Beta-Baythroyd	0.3 l			x											
							Select	0.35 l	x													
							Matrigon	0.9 l	x													
2015	winter wheat	Olivin	139	10	18		CDQ	20 g	x			winter wheat	Olivin	139	10	18		CDQ	20 g	x		
							Starane	0.4 l	x									Starane	0.4 l	x		
							MCPA	1.0 l	x									MCPA	1.0 l	x		
2016	peas	Onyx		22	43		Fenix	1.0 l	x			spring barley	Salome	81	6	10		Ariane S	1.8 l	x		
							Basagran	0.6 kg	x									MCPA	1.0 l	x		
							Select	0.35 l	x									Event S	1.0 l	x		
																		Proline	0.2 l		x	
							Acanto	0.2 l		x								Acanto	0.2 l		x	
2017	winter wheat	Julius	137	10	18		CDQ	0.2 g	x			winter wheat	Julius	137	10	18		CDQ	0.2 g	x		
							Starane 180	0.4 l	x									Starane 180	0.4 l	x		
							MCPA	1.0 l	x									MCPA	1.0 l	x		
							Topsin	0.3 kg		x								Topsin	0.3 kg		x	

Supplementary Tables

Supplementary Table 2. Mean density of earthworm species and ecological groups, and mean total earthworm density (ind. per m^2) and their standard deviations (SD) in the different tillage treatments (conventional tillage = mouldboard ploughing 23 cm; reduced tillage = cultivator 10-12 cm; no tillage = direct sowing) and crop rotations (SR = simple crop rotation, DR = diverse rotation). For a description of the two crop rotations see Table 1. The data presented in this table is raw data, and the values differ from the estimated marginal means from the statistical analyses.

		Conventional tillage, CT				Reduced tillage, RD				No tillage, NT			
		SR		DR		SR		DR		SR		DR	
	Ecological group[a]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Species													
Allolobophora chlorotica	endogeic	5.6	9.3	8.3	13.5	9.3	10.9	13.9	9.6	0	-	2.8	5.0
Aporrectodea longa	anecic	0	-	0	-	0	-	0.9	3.2	0	-	0	-
Aporrectodea caliginosa	endogeic	0	-	0	-	0	-	3.7	7.2	1.9	4.5	2.8	9.6
Aporrectodea rosea	endogeic	1.9	4.5	1.9	4.3	1.9	4.5	4.6	8.8	7.4	9.1	2.8	6.9
Aporrectodea/Allolobophora sp. (juvenile)		37.0	28.7	60.2	27.8	24.1	21.6	61.1	36.2	44.4	26.3	71.3	53.9
Aporrectodea turberculata	endogeic	0	-	0.9	3.2	3.7	9.1	1.9	6.4	1.9	4.5	0.9	3.2
Lumbricus castaneus	epigeic	0	-	0	-	0	-	0	-	3.7	9.1	0	-
Lumbricus sp. (juvenile)		0	-	4.6	10.0	5.6	6.1	19.4	15.1	38.9	57.8	35.2	26.7
Lumbricus terrestris	anecic	0	-	0	-	0	-	4.6	11.1	13.0	13.0	8.3	16.5
Ecological group													
Epigeics		0	-	0.4	0.9	0.5	0.5	1.7	1.3	7.1	14.1	3.1	2.3
Endogeics		43.9	25.0	70.4	34.2	38.5	22.8	84.3	42.0	54.9	31.8	79.5	53.5
Anecics		0.6	0.4	5.1	9.3	5.4	5.7	24.2	17.8	49.1	54.1	41.5	36.0
Total		44.4	25.3	75.9	41.3	44.4	23.3	110.2	43.0	111.1	94.0	124.1	72.1

[a] The ecological group was assigned to each species based on Bouché (1977) and Bottinelli (2020). In intermediate cases, the main category was used for simplicity.

Supplementary Tables

Supplementary Table 3. Specified model descriptions for the statistical analyses.

Model	Framework (R function)	Response variable	Explanatory variables				Random effects				Distribution	Link
1	GLMMs (glmmTMB)	Total earthworm density	Tillage	Rotation			Replicate	Replicate x Rotation	Replicate x Rotation x Tillage	Sample	Negative binomial (nbinom1)	Log
2	GLMMs (glmmTMB)	Endogeic density	Tillage	Rotation			Replicate	Replicate x Rotation	Replicate x Rotation x Tillage		Negative binomial (nbinom1)	Log
3	GLMMs (glmmTMB)	Anecic density	Tillage	Rotation	Tillage x Rotation		Replicate	Replicate x Rotation	Replicate x Rotation x Tillage		Zero-inflated negative binomial (nbinom2)	Log
4	LMMs (lme)	Total bioturbation (ln transformed)	Tillage	Rotation	Tillage x Rotation		Replicate	Replicate x Rotation	Replicate x Rotation x Tillage		normal	
5	PERMANOVA (adonis)	Earthworm community composition	Tillage	Rotation								
7	LMM (lme)	Soil organic carbon (0-10 cm, 10-20 cm, 20-30 cm)	Tillage	Rotation	Depth	Tillage x depth	Replicate	Replicate x Rotation	Replicate x Rotation x Tillage		normal	
9	LMM (lme)	Soil bulk density (square root transformed) (0-10 cm, 10-20 cm, 20-30 cm)	Tillage	Rotation	Depth		Replicate	Replicate x Rotation	Replicate x Rotation x Tillage		normal	
10	LMM (lme)	Soil water content (0-10 cm, 10-20 cm, 20-30 cm)	Tillage	Rotation	Depth		Replicate	Replicate x Rotation	Replicate x Rotation x Tillage		normal	

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Intensive agriculture reduces soil biodiversity and compromises the functions and ecosystem services provided by soil biota. This thesis explores ways to manage two functionally important groups of soil organisms, earthworms and arbuscular mycorrhizal fungi. Reduced agricultural management intensity together with preserving suitable habitat in the landscape are promising ways to promote earthworm diversity with potentially beneficial effects on agricultural sustainability. The benefits of more targeted manipulation of earthworm and arbuscular mycorrhizal fungal communities turn out highly context dependent.

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